Sustainable Agriculture

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Contents

Part I CLIMATE CHANGE

Part II GENETICALLY MODIFIED ORGANISMS

Part III BIODIVERSITY

Part IV ALTERNATIVE CONTROL

Part V ALTERNATIVE FERTILISATION

Part VI NEW FARMING SYSTEMS

Part VII POLLUTANTS IN AGROSYSTEMS

Agronomy for Sustainable Agriculture: A Review

Eric Lichtfouse, Mireille Navarrete, Philippe Debaeke, Véronique Souchère,
Caroline Alberola, and Josiane Ménassieu

Abstract *Sustainability rests on the principle that we must meet the needs of the present without compromising the ability of future generations to meet their own needs*. Starving people in poor nations, obesity in rich nations, increasing food prices, on-going climate changes, increasing fuel and transportation costs, flaws of the global market, worldwide pesticide pollution, pest adaptation and resistance, loss of soil fertility and organic carbon, soil erosion, decreasing biodiversity, desertification, and so on. Despite unprecedented advances in sciences allowing us to visit planets and disclose subatomic particles, serious terrestrial issues about food show clearly that conventional agriculture is no longer suited to feeding humans and preserving ecosystems. Sustainable agriculture is an alternative for solving fundamental and applied issues related to food production in an ecological way [Lal (2008) Agron. Sustain. Dev. 28, 57–64]. While conventional agriculture is driven almost solely by productivity and profit, sustainable agriculture integrates biological, chemical, physical, ecological, economic and social sciences in a comprehensive way to develop new farming practices that are safe and do not degrade our environment. To address current agronomical issues and to promote worldwide discussions and cooperation we implemented sharp changes at the journal Agronomy for Sustainable Development from 2003 to 2006. Here we report (1) the results of the renovation of the journal and (2) a short overview of current concepts of agronomical research for sustainable agriculture. Considered for a long time as a soft, side science, agronomy is rising fast as a central science because current issues are about food, and humans eat food. This report is the introductory article of the book Sustainable Agriculture, volume 1, published by EDP Sciences and Springer (Lichtfouse et al., 2009, this book).

Keywords Agronomy for sustainable development • Biodiversity • Climate change • Farming system • Food • Organic farming • Pest control • Pesticide • Soil • Sustainable agriculture • Water

Foreword

This article is dedicated to Ms. Josiane Ménassieu. Josiane was the Editorial Secretary of the journal Agronomy for Sustainable Development (ASD) from 2003 and retired in April 2008. The success of the renovation of the journal from 2003 to 2006 is mainly due to her intensive work. Her kindness was greatly appreciated by authors, peer-reviewers and Field Editors. Figure 1 shows a picture of the present that was offered to her by colleagues for her retirement.

1 The Journal Agronomy for Sustainable Development

Agronomy for Sustainable Development (ASD, agronomy-journal.org) is one of the seven journals of the French Institute of Agronomical Research (INRA,

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Fig. 1 *Coquelicots* painting by artist Agathe Rémy, who lives near Aix-en-Provence, France. *Coquelicots* is the French word for poppies

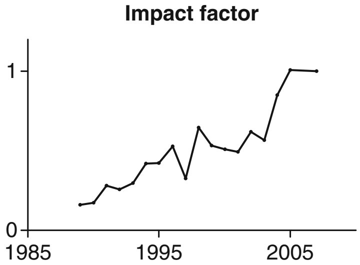

Fig. 2 Impact factor of the journal Agronomy for Sustainable Development (ASD). Note the increase in the impact factor from 0.566 in 2003 to 1.000 in 2007 (+77%). In 2006, the journal had two impact factors due to title change in 2005: 0.863 for the old title, Agronomie, and 0.306 for the new title, ASD. Those factors are not shown in the graph above because their calculation is different from that of normal impact factors. Specifically, the value for the old title is higher because it takes into account only older articles (2004) that thus have higher chances of getting cited (see scientific.thomsonreuters.com/isi for details)

international.inra.fr). The journal is managed by a collaboration of two INRA departments, the Department of Environment and Agronomy (www.inra.fr/ea) and the Department of Sciences for Action and Development (www.inra.fr/sad). Journal issues are produced by EDP Sciences (edpsciences.org). Our Editorial board collaborates with three Associate Editors and 32 Field Editors for manuscript peer-review. ASD publishes research and review articles. Submitted articles are first evaluated by a Pre-Selection Committee that declines about 50% of incoming manuscripts. Selected submissions are then sent to Field Editors for in-depth evaluation. The global rejection rate was 77% in 2006. The current impact factor (2007) is 1.000, ranking the journal 25/49 in the category Agronomy (Fig. 2).

ASD Journal was greatly reformed from 2003 to 2006. We changed topics from classical, production-oriented agronomy to sustainable and ecological agriculture (Lichtfouse et al. 2004; Alberola et al. 2008). We integrated social and economic sciences by setting up a collaboration between the INRA Department of Environment and Agronomy and the INRA Department of Sciences for Action and Development. Major journal topics currently include:

- Agriculture and global changes
- Agricultural production of renewable energies
- Ecological pest control and biopesticides
- Organic farming
- Genetically modified organisms in cropping systems
- Environmental impact on soil, water, air and biodiversity
- Risk assessment for food, ecotoxicology
- Decision support systems and companion modelling
- Social and economic issues of agricultural changes
- Innovation in farming systems
- Pollutants in agrosystem

Major journal changes implemented during the journal renovation are shown in Table 1. They include: the setting up of a pre-selection committee at the submission stage; an increase in the number of Field Editors from 14 to 32; novel topics focusing on sustainable agriculture (Lichtfouse et al. 2004); novel format instructions for more concise articles; a novel title (formerly *Agronomie)*; a switch from hardcopy to fully electronic managing; 100% of articles in English and a novel journal cover; and seven review reports produced per manuscript: three reports from Associate Editors at the pre-selection step, one report from the Field Editor, two reports from peer-reviewers and one report from the Editor-in-Chief; active commissioning of review articles by the Editor-in-Chief. As a consequence, we have observed several positive trends during the last few years (Table 1). The impact factor increased from 0.566 in 2003 to 1.000 in 2007 (+77%, Fig. 2). The rejection rate increased from 44% in 2003 to 77%

Table 1 Major changes in the journal agronomy for sustainable development (ASD) from 2003 to 2006

	Before renovation	Actual
Title	Agronomie	Agronomy for sustainable development
Field Editors	14	32
Pre-selection Committee	None	3 Associate editors
Topics	Conventional agronomy	Sustainable agronomy
Research article format	No size limit	Short articles
Language	93% English	100% English
Submissions per year	108	211
Pre-selection rejection (%)	0	50
Global rejection (%)	44	77
Research articles per year	79	44
Review articles per year	3	18
Impact factor	0.566	1,000
Acceptance delay	10.3 months	3.8 months
Article management	Hardcopy, post	100% electronic, pdfs
Article pdf downloads (/yr)	89,158	231,504
E-mail alert subscribers	417	1,307

in 2006. The number of submissions increased from 108 in 2003 to 211 in 2007 (+95%). The number of pdf article downloads at the ASD website increased from 89,158 in 2004 to 231,504 in 2007 (+160%). The number of subscribers to the free e-mail alert increased from 417 in 2004 to 1,307 in 2007 (+213%). The number of published review articles increased from 3 in 2005 to 13 in 2008 (+333%).

From 2005, review articles are published both in ASD and in the book series Sustainable Agriculture (Lichtfouse et al., 2009, this book). The first volume is issued in 2009. A call for review articles for the next volumes is posted on the ASD website (agronomy-journal.org). To conclude, the trends observed are very promising to encourage scientists to publish their best results in ASD. In the next section we discuss views on sustainable agriculture.

2 Sustainable Development and Sustainable Agriculture

The term "sustainable development" was first defined in 1987 by the Brundtland Commission, formally the World Commission on Environment and Development, solicited by the United Nations:

> *Sustainable development is development that meets the needs of the present without compromising the ability of future generations to meet their own needs.*

This concept was then enhanced by the United Nations Conference on Environment and Development at the Earth Summit, Rio de Janeiro, in 1992. From that time, sustainable development became a key issue in political and scientific bodies, e.g. the Intergovernmental Panel on Climate Change (IPCC, ipcc.ch), the Millennium Ecosystem Assessment (millenniumassessment.org) and more recently, the *Grenelle de l'Environnement* in France (legrenelle-environnement.fr). The concept of sustainable development is well accepted by a large public because it has defined global stakes, but is very vague about the practical ways of reaching those stakes.

Sustainable agriculture does not escape this weakness, as shown by many reports (Hansen 1996; Hansen and Jones 1996; Gliessman 1998; Gold 1999; Tilman et al. 2002; Boiffin et al. 2004; Dupraz 2005). Many authors and organisations worldwide give their own definition of sustainable agriculture. Some authors consider sustainable agriculture as a set of management strategies addressing the main societal concerns about food quality or environment protection (Francis et al. 1987). Other authors focus on the ability of agricultural systems to maintain crop productivity over the long term (Ikerd 1993). Other authors focus on one main factor of sustainability; for instance, flexibility, which is the adaptive capacity of agriculture to adapt to future changes (Gafsi et al. 2006). Overall, all authors agree on the occurrence of three approaches to the concept of sustainable agriculture: environmental, economic and social approaches. In other words, agricultural systems are considered to be sustainable if they sustain themselves over a long period of time, that is, if they are economically viable, environmentally safe and socially fair. Beyond this ideological definition, the practical issue is to build operational solutions to reach global goals. This is a challenging task because the stakeholders do not agree on the criteria to measure the sustainability of a farming system, and on how to balance those criteria. Many indicators have indeed already been produced to evaluate sustainability.

The link between sustainable agriculture and sustainable development is not obvious (Legrand et al. 2002). Sustainable agriculture could involve two approaches (Boiffin et al. 2004). The first approach is that agriculture should sustain itself over a long period of time by protecting its productive resources, e.g. maintaining soil fertility, protecting groundwater, developing renewable energies and finding solutions to adapt farming systems to climate change. This first approach considers the farming system as a closed area. The second approach is to consider that agriculture also has to contribute to the sustainability of large territories and social communities. Accordingly, agriculture should help urban areas to manage wastes, e.g. by recycling urban sewage sludge, developing rural employment, and offering a rural landscape for urban people. This second approach has wider goals and does not separate rural and urban areas. To conclude, the vagueness of the concept of sustainable development and sustainable agriculture is a strength because it does not restrict the research field too much, and, in turn gives freedom to scientists to explore wide, unknown domains.

3 Future Sustainable Farming Systems

After the Second World War, the development of conventional farming, or "industrial farming," was promoted in order to increase sharply food production worldwide. This social aim led to extensive use of pesticides, fertilisers and water, and to fast crop rotations and monoculture. Positive effects on yield were rapidly counterbalanced by negative environmental impacts such as soil erosion, groundwater pollution, river eutrophication, excessive water use, and the development of weeds and diseases resistant to chemical control. Industrial farming and other industrial activities have indeed led to the presence of pesticides and persistent organic pollutants in soils, water, air and food (Lichtfouse and Eglinton 1995; Lichtfouse 1997a; Lichtfouse et al. 1997, 2005b).

Today, to reach economic profitability, environmental safety and social fairness, farming systems should use fewer inputs and resources without drastically reducing yields. As the population is forecasted to increase to 9 billion in the next 50 years, it is necessary to maintain a high level of food production. Nonetheless, farming systems should also meet food quality policies enforced by national and international policies. This issue is particularly relevant given the occurrence of pesticide residues in food products because consumers and environmental associations are concerned about a possible new sanitary crisis. Pesticide use by farmers is thus widely criticised. On the other hand, decreasing pesticide use may lead to negative effects such as toxin risk in food (Le Bail et al. 2005). To reach more sustainable practices, several strategies are described in the literature. Those strategies involve various changes, from simple adjustment of the crop management sequence to fundamental changes at the farming system level. For example, MacRae et al. (1989) proposed the following framework based on efficiency – substitution – redesign (ESR). In the following section we describe three stategies to reach sustainable agriculture: the substitution strategy, the agroecological strategy and the global strategy.

3.1 Level 1: The Substitution Strategy

This level refers to the substitution logic, meaning that existing farming systems are only slightly adapted, but not fundamentally altered (Altieri and Rosset 1996). For instance, toxic chemicals and mineral fertilisers (NPK) should be replaced by compounds that are less pollutant, less persistent in soil and less energy-consuming. Applying biopesticides and growing genetically modified plants should decrease both pest development and the use of toxic pesticides. Growing symbiotic N legumes instead of applying costly, energy-consuming N fertilisers should also improve sustainability. Here, research is usually done at the plot level, which is the most common level for agronomists. Collaborations with scientists studying elementary processes, such as geochemists, pathologists and biologists, should be fostered. The substitution logic should be effective for a short time period because it allows a substantial reduction of chemical treatments. However, it may be not be efficient in the long run due to the appearance of pest resistance following the use of biopesticides, for instance.

3.2 Level 2: The Agroecological Strategy

The principle of the agroecological strategy is to build innovative technical scenarios relying on biological regulations in an integrated crop production scheme. This strategy involves applying ecological concepts and principles to the design, development and management of sustainable agricultural systems. Promoting biodiversity in agrosystems provides ecological services such as nutrient cycling, soil structuration and disease control. Biodiversity can be enhanced by cultural practices such as intercropping, rotation, agroforestry, composting and green manuring. Recent studies also address new issues in integrated pest management by combining the use of biological, physical, cultural and genetic control measures (Gurr et al. 2004). Increasing biodiversity by crop rotations (combination in time), intercropping (combination in space) and varietal mixtures has been suggested as an alternative to chemicals (Vandermeer et al. 1998). At this level, agronomy should interact with landscape ecology, because spatial variations in the landscape may be used for pest management. The productivity of farming systems should be increased by developing ecological principles and adapting them to farming systems. The agroecological strategy thus requires the enlargement of the experimental scale. Experimental scenarios should not be designed at plot level, but at the scale of larger territories. Therefore, investigations need a much better understanding of interactions of living organisms at plot and larger levels. They also require input from other disciplines such as ecology and geography.

3.3 Level 3: The Global Strategy

The principle of the global strategy is to solve agricultural issues at the global scale, by rethinking its relation to society. Indeed, most failures of intensive agriculture are closely linked to its economic model. There are fundamental contradictions among several aims assigned to agriculture. For instance, producing more and cheaper food products without polluting soils; and producing fruits and vegetables without pesticide residues and without visual pest damage appear to be unrealistic aims. Therefore, the global strategy relies on rethinking the role of agriculture in our society, as shown by new trends in agroecology (Gliessman 2006). This approach considers that sustainability cannot be solely reached by farming systems, but should also involve the food system, the relations between farms and food consumption, and the marketing networks. For example, authors studying the relationships between production and marketing highlighted the interest in alternative food networks focused on local production (Lamine and Bellon 2008). The global strategy thus requires interdisciplinary work with scientists from various sciences such as agronomy, ecology, sociology, economics and politics.

4 Agronomical Research for Sustainable Agriculture

Agronomy was first defined as the science of crop production. It was mainly focused on the study of relationships between climate, soil, cultural practices and crop yield and quality. Agronomy therefore integrates sciences such as biology, chemistry, soil science, ecology and genetics. Agronomists then enlarged their studies to the individuals performing the cultural practices, namely farmers. This approach raised new issues on the modelling of farmers' practices, and on the consequences of farmers' choices on crop production. Agronomists further analysed environmental impacts of farmers' practices. More recently, they have also studied how agriculture could benefit from the environment and ecosystems to improve crop production, thus leading to the concept of "ecological services."

To study crop production, agronomists have to integrate highly complex sciences that rule farming systems at very different spatial and temporal scales. Agronomists also have to cope with a high environmental variability. As a consequence, results obtained in an experimental field may not be reproducible in another field due to slight – possibly unknown – variations in soil and climate factors. Therefore, a key point of agronomical investigations is to define the validity domain of each finding. Concerning the integration of agricultural practices, a key point is to enlarge the classical scales of crop production studies, "plant and plot," to scales that are meaningful for the farmers, such as combination of plots and farm territory, and even larger scales. In a way, agronomy is a science of complexity aimed at integrating knowledge at various spatial levels from the molecule to the living organism, the farming system and the global scale. Thus, agronomy appears more and more to be the science relevant for global issues because it integrates knowledge from various sciences at various spatial scales. Considered for a long time as a soft, side science, agronomy is rising fast as a central science because current issues are about food, and humans eat food.

The systemic dimension is essential because in the next few decades most improvements of farming systems will rely on enhancing positive interactions among various parts of farming systems. To build sustainable farming systems, agronomists will not only have to assess the direct effects of techniques on a crop, but also the indirect effects on the whole ecosystem such as biodiversity changes, water pollution and soil erosion. The economic and social consequences of the new farming systems should also be evaluated with a pluridisciplinary approach with economists or social scientists. Therefore, sustainable agriculture fosters the development of multidisciplinary studies that associate agronomy with ecology, economics, sociology and geography (Lichtfouse et al. 2004). Meynard et al. (2006) identified four different ways to design innovative agricultural systems for sustainable development:

- Inventing new farming systems, breaking off with the current ones;
- Identifying and improving farming systems built by the local stakeholders;
- Giving tools and methods to stakeholders to improve their own systems or evaluate those proposed by scientists;
- Identifying the economic, social and organisation conditions that may help the actors to adopt alternative farming systems.

These approaches raise several new issues for agronomical scientists. For instance, it is not clear whether solutions will be found either by only a slight adaptation of research practices or by a sharp change in experimentation and modelling. Studying new spatial scales that show heterogeneous areas such as field margins will be a challenge. The integration of long-term changes in farming systems, such as soil organic matter turnover (Lichtfouse 1997b) and sewage sludge pollution (Lichtfouse et al. 2005a), should also be investigated and modelled because concepts of resilience and flexibility are relevant. It should also be noted that some farmers already have an accurate expertise in sustainable practices. Some are even ahead of research and are experimenting with new systems for, e.g. organic farming. Here, the issue for the agronomist is to build effective methods to gather local knowledge, to check findings and eventually to redesign experiments. Innovative agricultural systems will benefit from a close collaboration between scientists and farmers.

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Soils and Sustainable Agriculture: A Review

Rattan Lal

Abstract Enhancing food production and supporting civil/engineering structures have been the principal foci of soil science research during most of the nineteenth and the first seven or eight decades of the twentieth century. Demands on soil resources during the twenty first century and beyond include: (1) increasing agronomic production to meet the food needs of additional 3.5 billion people that will reside in developing countries along with likely shift in food habits from plant-based to animal-based diet, (2) producing ligno-cellulosic biomass through establishment of energy plantations on agriculturally surplus/marginal soils or other specifically identified lands, (3) converting degraded/desertified soils to restorative land use for enhancing biodiversity and improving the environment, (4) sequestering carbon in terrestrial (soil and trees) and aquatic ecosystems to off-set industrial emissions and stabilize the atmospheric abundance of CO_2 and other greenhouse gases, (5) developing farming/cropping systems which improve water use efficiency and minimize risks of water pollution, contamination and eutrophication, and (6) creating reserves for species preservation, recreation and enhancing aesthetic value of soil resources. Realization of these multifarious soil functions necessitate establishment of inter-disciplinary approach with close linkages between soil scientists and chemists, physicists, geologists, hydrologists, climatologists, biologists, system engineers (nano technologists), computer scientists and information technologists, economists, social scientists and molecular geneticists dealing with human, animal and microbial processes. While advancing the study of basic principles and processes, soil scientists must also reach out to other disciplines to address the global issues of the twenty first century and beyond.

Keywords Biofuels • Climate change • Food security • Soil functions • Sustainable agriculture • Waste management • Water resources

1 Introduction

Goals of soil management during the nineteenth century and the first half of the twentieth century, when world population was merely 38% of the 2006 level, was to maintain agronomic productivity to meet the food demands of two to three billion inhabitants. Demands on soil resources are different of a densely populated and rapidly industrializing world of the twenty first century. In addition to food supply, modern societies have insatiable demands for energy, water, wood products, and land area for urbanization, infrastructure, and disposal of urban and industrial wastes. There is also a need to alleviate rural poverty and raise the standard of living of masses dependent on subsistence farming. In addition, there are several environmental issues which need to be addressed such as the climate change, eutrophication and contamination of natural waters, land degradation and desertification, and loss of biodiversity. To a great extent, solutions to these issues lie in sustainable management of world's soil resources (Fig. 1), through adoption of agronomic techniques which are at the cutting edge of science.

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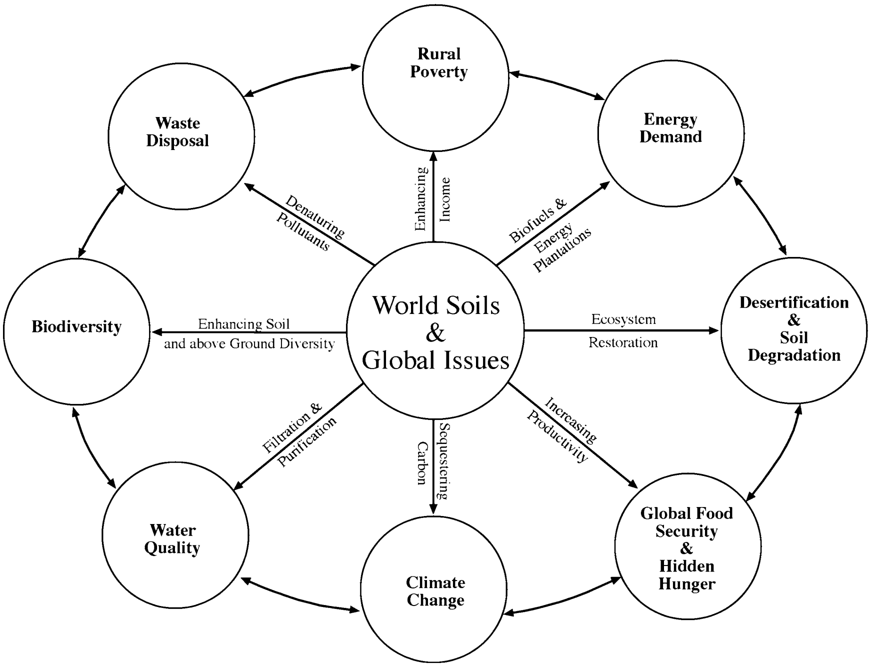

Fig. 1 World soils and global issues of twenty first century

2 Advancing Food Security

The world population of merely 0.2 billion during the biblical era increased by only 0.11 billion (to 0.31 billion) during the next 1,000 years by 1000 AD. However, the population increased 20 times to 6 billion during the next 1,000 years by 2000 AD. The world population is projected to reach 9.4 billion by 2050 and 10 billion by 2100 (Fischer and Heilig 1997; Cohen 2003). The most remarkable aspect of the future population dynamics is the fact that all of the projected increase by about 3.5 billion will occur in developing countries of Asia (mostly South Asia) and Africa (mostly sub-Saharan Africa). These are also the regions where soil resources are limited in extent (per capita), fragile to natural and anthropogenic perturbations, and prone to degradation by the projected climate change and the increase in demographic pressure. Thus, any future increase in agronomic/food production will have to occur through vertical increase in production per unit area, time and input (e.g., nutrients, water, energy) of the resources already committed to agriculture. It is in this context that developing and identification of some innovative methods of soil management are crucial to feeding the world population of 10 billion. These methods must minimize losses by delivering nutrients and water directly to the plant roots during the most critical stages of crop growth. Degraded and desertified soils must be reclaimed through enhancement of the soil organic matter (SOM) pool, creation of a positive elemental budget with balanced supply of all essential nutrients, effective control of soil erosion by water and wind, restoration of soil structure and tilth through bioturbation, and enhancement of activity and species diversity of soil fauna and flora. Soil management techniques must be chosen to ensure: (1) liberal use of crop residues, animal dung and other biosolids, (2) minimal disturbance of soil surface to provide a continuous cover of a plant canopy or residue mulch, (3) judicious use of sub-soil fertigation techniques to maintain adequate level of nutrient and water supply required for optimal growth, (4) an adequate level of microbial activity in the rhizosphere for organic matter turnover and elemental cycling, and (5) use of complex cropping/farming systems which strengthen nutrient cycling and enhance use efficiency of input. Identification, development and validation of such innovations must be based on modern technologies such as GIS, remote sensing, genetic manipulations of crops and rhizospheric organisms, soil-specific management, and slow/time release formulations of fertilizers. Increase in crop yields must occur in rainfed/dry farming systems which account for more than 80% of world's croplands. Breaking the agrarian stagnation/deceleration in sub-Saharan Africa must be given the highest priority by soil scientists and agronomists from around the world. While expanding irrigated agriculture is important, crop yields have to be improved on rainfed agriculture in Asia and Africa, by conserving or recycling every drop of rain, and by not taking soils for granted.

3 Biofuels

In comparison with the stone age or bronze age, the industrial era (1750–2050) will be referred to the carbon (C) age or carbon civilization by future generations from 2100 AD and beyond (Roston 2008). The use of fossil fuel, since the onset of industrial revolution ~1750, has drastically disturbed the global C cycle with the attendant impact on the climate change and the increase in earth's temperature along with change in rainfall amount and distribution. The present civilization is hooked on C, and is in need of a big time rehabilitation. Breaking the C-habit will require development of C-neutral or non-carbon fuel sources, and both soil science and agronomy have a major role to play in this endeavor. Not only the recommended agricultural input (fertilizers, pesticides, tillage methods, irrigation) must be efficiently used, the future energy demands will eventually be met by non-carbon fuel, most likely hydrogen. The latter can be generated from biomass produced through appropriate land uses and judicious cropping/farming systems. In the meanwhile, modern biofuels (ethanol, biodiesel) can play an important role in minimizing emissions of greenhouse gases and reducing the rate of increase of atmospheric concentration of CO_2 (Brown 1999; Cassman et al. 2006). Converting grains (e.g., corn) to ethanol is rather an inefficient method of energy production, and grains are and will remain in high demands as food staple for humans and feed for livestock and poultry.

Crop residues are also being considered as a source of energy (Somerville 2006; Service, 2007). Indeed, 1 Mg (1t) of lignocellulosic residues is equivalent to 250–300 L of ethanol, 15–18 GJ of energy, 16×10^6 kcal or 2 barrels of diesel (Lal 2005; Weisz 2004). The energy return on investment (EROI) for grain-based ethanol is low. Furthermore, crop residues (of corn, wheat, barley, millet, rice) must be used as soil amendment/mulch to control erosion, conserve water and replenish the depleted SOM pool through soil C sequestration, and restore degraded soils and ecosystems (Wilhelm et al. 2004). Crop residues must not be considered a waste, because they have multifarious but competing uses including conservation of soil and water, cycling of nutrients, enhancement of the use efficiency of fertilizers and irrigation water, and above all, as a food of soil organisms which are essential to making soil a living entity. Using crop residues for production of biofuels is "robbing Peter to pay Paul" and all that glitters is not gold, not even the green gold. The price of harvesting crop residues (such as from the US Corn Belt) will be severe soil and environmental degradation (dust bowl), because there is no such thing as a free lunch. It is, thus, important to identify dedicated crops which can be grown

Table 1 Species for establishing biofuel plantations

English name	Botanical name
1. Warm season grasses	
• Switch grass	*Panicum virgutum* L.
• Big blue stem	*Andropogan gerardi*, Vitnam
• Indian grass	*Sorghastrum nuttans* L. Nas
• Blue giant grass	*Calanagrostis canadensis* Michx Bean L.
• Guines grass	*Panicum maximum* L.
• Elephant grass	*Pennisetum perpereum* schm.
• Kallar/Karnal grass	*Leptochloa frscha* L.
• Molasses grass	*Melinis minutiflona*
• Reed canary grass	*Phalaris arundinacae* L.
• Cord grass	*Spartina pectinata* Link.
2. Short rotation woody crops	
• Popalar spp	*Populus* spp.
• Willow spp	*Salix* ssp.
• Mesquite (Velayti Babul)	*Prosopis juliflora*
• Miscanthus	*Miscanthus* spp.
• Black locust	*Robinia pseudoacacia* L.
• Birch	*Onopordum nervosum*
3. Halophytes	
• Pickle weed	*Salicornia bigelovii*
• Salt grass	*Distichlis palmeri*
• Salt brushes	*Atriplex* spp.
• Algae	*Spirulina geitleri*
• Cyanobacteria	*Cyanobacteria* spp.
4. Drought tolerant trees	
• Gum tree	*Eucalyptus* spp.
• Leucaena (Subabul)	*Leucaena leucocephala*
• Casurinas	*Casurina equisetifolia*
• Acacia	*Acacia* spp.
• Teak	*Tectona grandis*
• Cassia	*Casia siamea*

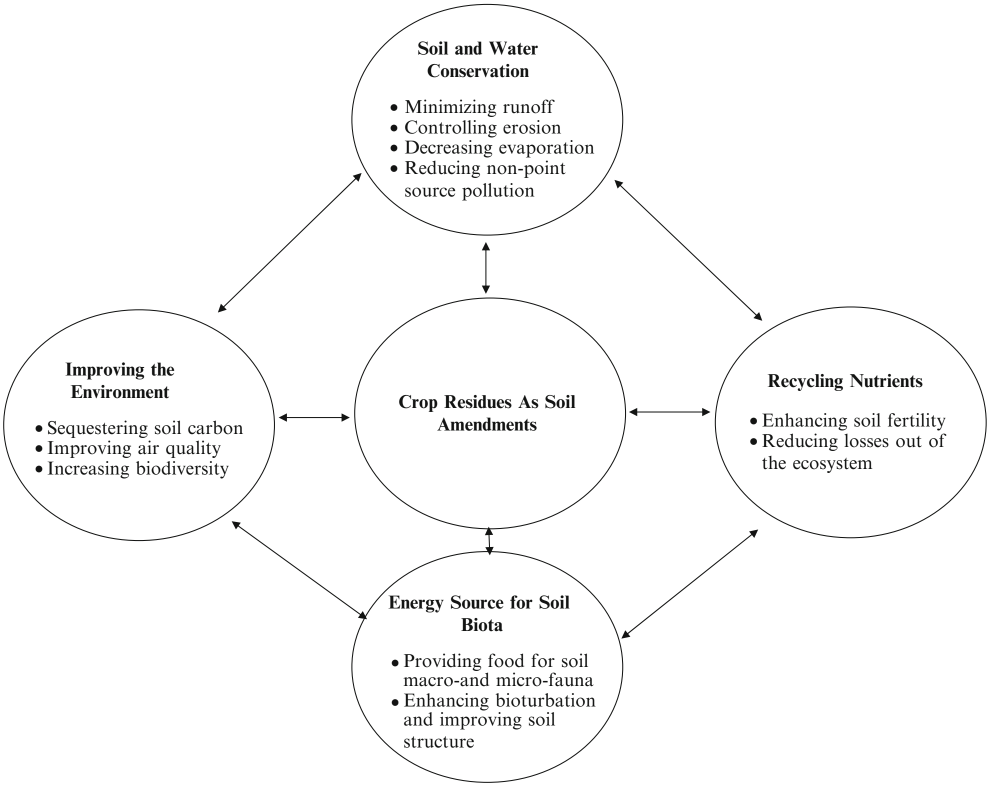

Fig. 2 Site and eco-system specific effects of crop residue management on soil and environment quality must be assessed in relation to improvement in soil quality and sustainable use of natural resources

to establish biofuel plantations (Table 1). Furthermore, new lands (agriculturally marginal/surplus soils; and degraded, disturbed and polluted soils) must be identified to establish appropriate biofuel plantations. In addition to providing the lignocellulosic biomass for conversion to ethanol, establishment of biofuel plantations on degraded soils would also lead to soil C sequestration and enhance soil quality and the ecosystem services that it would provide. The EORI of biofuel production system must be carefully assessed through a comprehensive life cycle analysis. In addition to establishing managed biofuel plantations, lignocellulosic biomass can also be harvested from natural vegetation growth on abandoned/set aside or fallowed land (Tilman et al. 2006). The issue of using crop residues for cellulosic ethanol production must not be resolved on the basis of short-term economic gains. The rational decision must be based on the long-term sustainable use of natural resources (Figs. 2 and 3). Indeed, the immediate needs for fuel must not override the urgency to achieve global food security, especially for almost 1 billion food-insecure people in Africa and Asia. If the crop residues harvested for celunol production are not returned as compost (with enhanced plant nutrients such as N, P, K), the long-term adverse impacts on soil quality (such as has been the case in severely degraded soils of sub-Saharan Africa and South Asia due to perpetual removal of crop residues) will jeopardize global food security and set-in-motion the soil degradation spiral with the attendant impact on social unrest and political instability (Fig. 4).

4 Waste Disposal

The importance of soil for the safe disposal of ever-increasing industrial and urban wastes cannot be over-emphasized. The municipal solid wastes generated in the US doubled between 1970 and 2003 (USEPA 2006), as is also the case in western Europe and developing economies, in addition, there are wastes of animal and poultry industry, and food

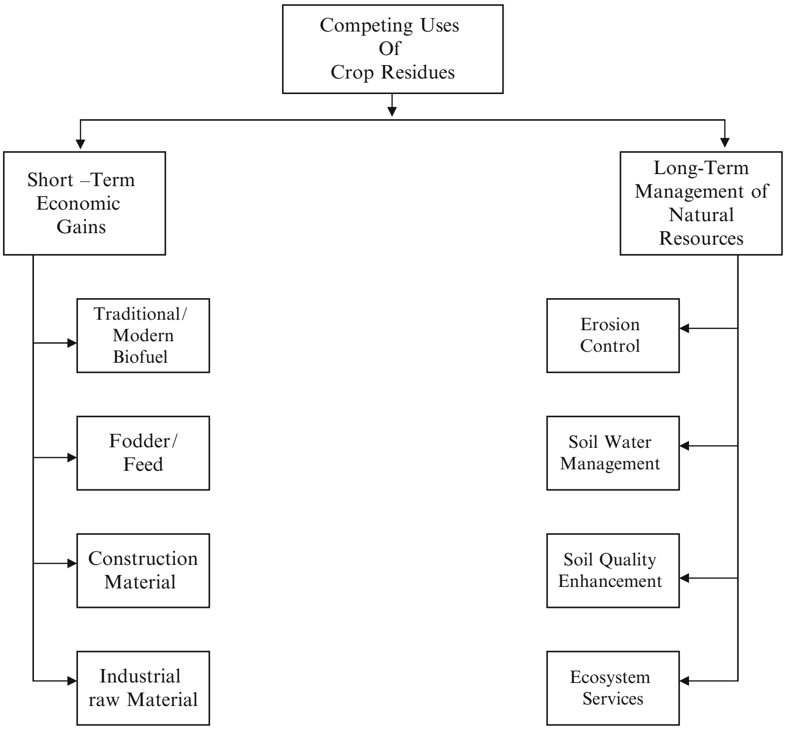

Fig. 3 An objective assessment of short-term economic gains vs. long-term and sustainable use of natural resources important to the decision-making process for competing uses of crop residues

processing plants and restaurants. These wastes containing biosolids which can be used as energy source (either for direct combustion or conversion to methane or ethanol), and as soil amendment, or both. The by products of biosolids used for production of methane gas or ethanol must be composted and used as soil amendment.

Soils of appropriate characteristics (e.g., good drainage, absence of impermeable layer in the vadoze zone, high activity and species diversity of macro and micro-organisms in the surface layer, highly aggregated and stable structure) is also a natural biomembrane which must be used to filter and denature/biodegrade industrial pollutants. Carefully chosen soils and the underlying parent material/geologic strata are being used as a repository for nuclear wastes (e.g., Yucatan mountain range in the southwestern US). Although questionable in terms of effectiveness and economic cost, geologic strata are also being used/considered for storage of industrial CO_2 emitted from point sources (Schrag 2007). The importance of soil as a medium for waste disposal is bound to increase with increase in population and industrialization, and soil scientists must be pro-active in this emerging field of great significance. Similarly, agronomists must be very actively involved in phytoremediation of polluted soils by using plants to denature industrial pollutants.

5 Farming Carbon

Carbon sequestration in terrestrial ecosystems (e.g., soils, trees, wetlands), and improving soil quality so that soils can be a net sink for CH_4 and release less N_2O, is an important issue which must be addressed by soil scientists, crop scientists, agronomists, foresters, and wetland ecologists. While understanding the processes which impact the ecosystem carbon pool and fluxes is important, soil scientists and agronomists must liaise with economists and policy experts to develop a methodology for trading of carbon credits so that it can be traded like any farm commodity (e.g.,

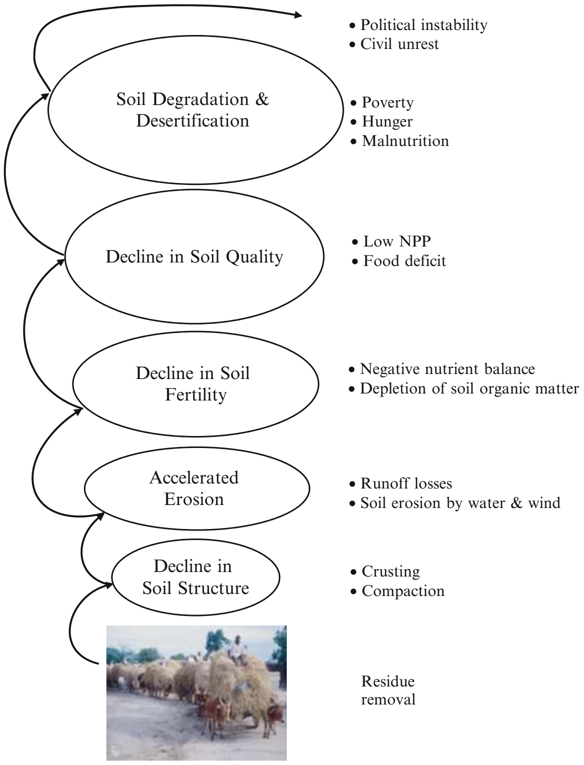

Fig. 4 The classic collapse

corn, millet, poultry). Similar to C sequestered in trees, methodology must be developed to trade C in soils (Breslau 2006; Brahic, 2006). In addition, emissions of CH_4 and N_2O can be converted to CO_2 equivalent, and also traded. Trading C credits can provide another income stream for farmers, and provide the much needed incentives to invest in soil improvements (e.g., erosion control, fertility management, irrigation).

Restoration of degraded soils and ecosystems is an important facet intimately linked to soil C sequestration. Soil degradation and desertification, biophysical processes driven by socio-economic and political factors, are severe problems in developing countries of South Asia and sub-Saharan Africa (Oldeman 1994). Restoration of eroded/degraded soils, through land use conversion via afforestation and conversion of degraded croplands to improved and well managed pastures, will lead to terrestrial C sequestration (in soils and trees) as an ancillary benefit. Soil degradation through land misuse, soil mismanagement, and excessive consumption of water through flood irrigation that leads to salinization and inundation are luxuries that the land-starved and the water-scarce world cannot afford, not anymore.

There is a strong link between soil restoration, carbon sequestration, food security (Lal 2006) and biodiversity (Fig. 5). Improvement of soil quality, gradual and a slow process as it may be, is caused by an increase in the terrestrial C pool. The latter is also linked with biodiversity, water quality and micro and mesoclimate, and emission of greenhouse gases into the atmosphere. Understanding interactive mechanisms, especially those which link processes in soil with those in atmosphere and hydrosphere through biosphere, are of a high priority for soil scientists and agronomists. In addition to quantifying these processes, soil scientists and agronomists must also communicate their findings to policy makers such as the US Congress, European Parliament, and the UN organizations. Through their interactive research outlined above, soil scientists must provide the basic information which is needed to bring together three UN Conventions (i.e., UNFCCC, UNFCBD, and UNFCDC). While providing crucial information on biodiversity, desertification control and climate change to strengthen cross linkages among three UN Conventions, soil scientists can also build bridges to link these organizations with the noble UN Millenium Development Goals of cutting poverty and hunger in half. In agricultural economy, which involves two-thirds to three-fourths of the rural population, increasing agronomic productivity and providing another income stream for farmers through trading of C credits are important strategies to advance food security while alleviating poverty and improving the environment. Generating income through trading of soil/terrestrial carbon credits may be the entry point or the handle to break the vicious cycle of soil degradation-low yields-poverty – hunger-severe soil degradation. It is a truly win-win-win strategy that deserves a serious attention of the world community.

6 Water Resources

In addition to fertility and nutrient supply, agricultural productivity will be constrained by lack of water resources, whose severity will be exacerbated by frequent and severe drought stress due to the projected climate change. Whereas agriculture is the largest consumer of water, the competition from industrial and

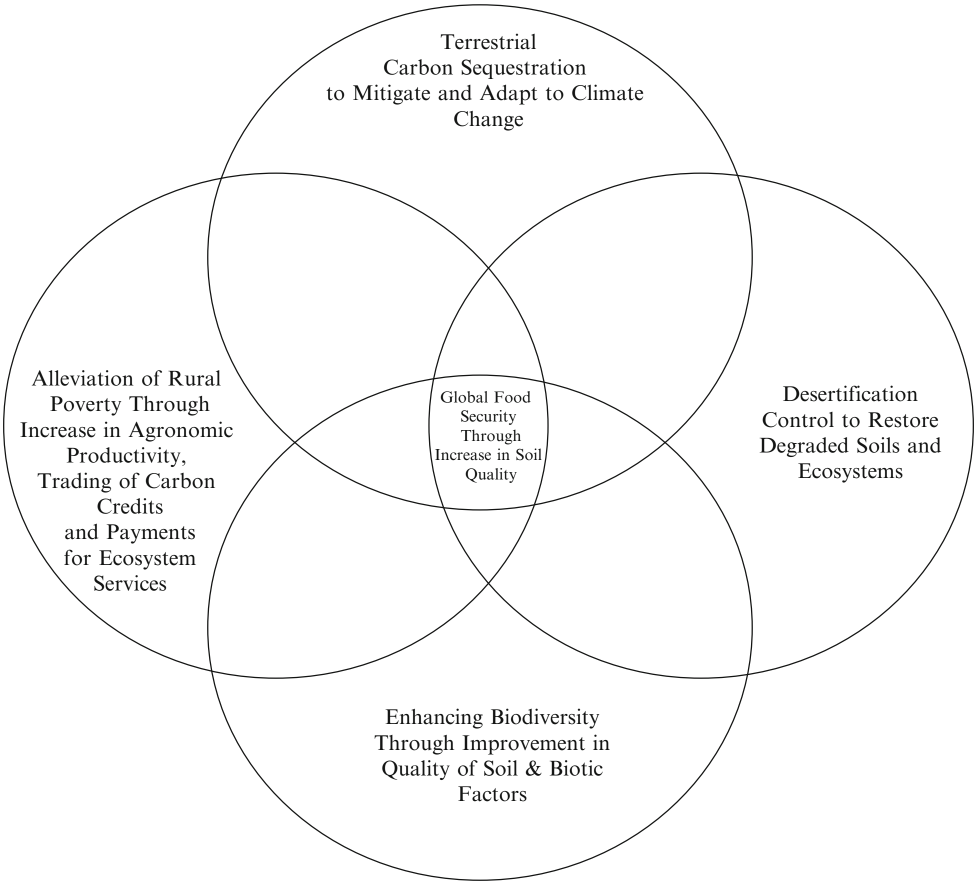

Fig. 5 A positive and synergistic interaction between desertification control, biodiversity improvement, climate change mitigation, and food security. The latter is improved through improvement in soil quality, increase in availability of water resources, strengthening of elemental cycling, and enhancement of bioturbation in the rhizosphere. Soil scientists and agronomists must be actively pursuing quantification of these synergistic effects

urban uses is increasing with increase in demographic pressure and rapid industrialization (Moldan 2007; Gleick 2003; Kondratyev 2003; Johnson et al. 2001). The scarcity of fresh water is exacerbated by non-point and point source pollutions (Tilman et al. 2006), and will be further aggravated by likely shift in diet in developing economies (e.g., China, India) from plant-based to animal-based products (Clay 2004). In this regard, improved understanding of soils and agronomic processes which enhance water use efficiencies is highly relevant and extremely critical. Soil scientists and agronomists need to work closely with plant breeders to develop genetically engineered plants which have high productivity per unit consumption of water, with irrigation engineers to reduce losses of water during conveyance and delivery, with micrometeorologists to reduce losses from soil evaporation, with hydrologists to economically and effectively recycle water drained into the sub-soil or ground water, and with municipalities of large urban centers to develop techniques of recycling waste water for irrigation and aquifer recharge. Replacing flood irrigation with subirrigation or drip irrigation techniques is a high priority.

7 Reaching Out

The traditional functions of soil have been: (1) the medium for plant growth, (2) foundation for civil structures, and (3) source of raw materials for industry. During the twenty first century and beyond, functions of soil must be expanded to include the following: (1) mitigation of climate change through C sequestration in terrestrial and aquatic ecosystems, (2) purification of water through filtration and denaturing of pollutants, (3) disposal of urban and industrial wastes in a way that these do not contaminate water or pollute air, (4) store germ plasm including that of microbes which can be used to combat diseases, (5) archive human and planetary history, (6) support being a reactor of chemical and physical processes, and (7) provide a strategic entity in national and international affairs to give peace a chance.

The concept of "sustainable agriculture" needs to be revisited in the context of the need for increasing productivity in developing countries which will entirely inherit the future increase in population of 3.5 billion by the end of the twenty first century. With reference to the densely populated countries of Asia and Africa, sustainable agricultural practices are those which: (1) maximize productivity per unit area, time and input of fertilizers, water and energy, (2) optimize the use of off-farm input, (3) increase household income through increase in production, trading of carbon credits, off-farm employment, and value addition of farm produce, (4) improve quality and quantity of fresh water resources at the farm level, (5) provide education opportunities especially for women, (6) create clean household cooking fuel for the rural population to improve health of women and children and spare animal dung and crop residues for use as soil amendments, and (7) address concerns of the farm family especially food security until the next harvest. It is a fact that indiscriminate use of chemicals, excessive tillage and luxury irrigation have degraded soils, polluted waters and contaminated air. The problem is not with the technology. It has been the over-fertilization, overuse of pesticides, excessive application of irrigation because of free water, unnecessary plowing, complete removal of crop residues along with uncontrolled grazing, and the use of animal dung for household fuel rather than soil amendment that have caused the problems.

Access to adequate and balanced food and clean drinking water are the most basic human rights which must be respected. Political stability and ethnic conflicts are caused by hunger and the desperateness created by it. Thus, the concept of sustainable agriculture must be based on the simple fact that agricultural ecosystems are only sustainable in the long-term if the outputs of all components produced balance the inputs into the system. Whether the required amount of input (nutrients) to obtain the desired yield is supplied in organic rather than inorganic form is a matter of availability and logistics. Plants cannot differentiate the nutrients supplied through the organic or synthetic sources. The important question is of supplying nutrients in adequate quantity and when needed to produce enough food to meet the needs of 6.5 billion people now and 10 billion by the end of the century. In some cases, in vicinity of large livestock or poultry farms, organic manures may be available. In other cases, massive intervention through fertilizer use has no practical alternatives in a world of growing population. In some cases traditional breeding is acceptable, in others the natural process of gene manipulation may have to be accelerated through techniques of genetic engineering. The strategy is to use the technology prudently and with utmost objectivity and rationality. Transgenic plants can be grown on degraded and salt-affected soils to produce biomass for biofuels, and to alleviate biotic and abiotic stresses on dryland agriculture. If effective, why not?

While those holding the neo-Malthusian views will again be proven wrong through adoption of already proven and emerging technologies for sustainable management of soil resources, soil scientists and agronomists cannot undertake these serious issues all by themselves. These are far reaching and complex functions that soil scientists may take lead in but must develop close cooperation with other disciplines. While advancing and improving the knowledge of basic processes, soil scientists must also work with geologists, hydrologists, climatologists, biologists, chemists, physicists, computer scientists, nano technologists, system engineers, economists and political scientists to address these emerging issues of the twenty first century. The key strategy is to reach out to other disciplines while strengthening and advancing the science of soil and its dynamics in an ever changing physical, social, economic and political climate. Agriculture, implemented properly, is an important solution to the issue of achieving global food security but also of improving the environment. The agricultural history of 10–13 millenia has taught us that the motto of modern civilization must be "In Soil We Trust".

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Soils and Food Sufficiency: A Review

Rattan Lal

Abstract Soil degradation, caused by land misuse and soil mismanagement, has plagued humanity since the dawn of settled agriculture. Many once thriving civilizations collapsed due to erosion, salinization, nutrient depletion and other soil degradation processes. The Green Revolution of the 1960s and 1970s, that saved hundreds of millions from starvation in Asia and elsewhere, by-passed Sub-Saharan Africa. This remains the only region in the world where the number of hungry and food-insecure populations will still be on the increase even by 2020. The serious technological and political challenges are being exacerbated by the rising energy costs. Resource-poor and small-size land-holders can neither afford the expensive input nor are they sure of their effectiveness because of degraded soils and the harsh, changing climate. Consequently, crop yields are adversely impacted by accelerated erosion, and depletion of soil organic matter (SOM) and nutrients because of the extractive farming practices. Low crop yields, despite growing improved varieties, are due to the severe soil degradation, especially the low SOM reserves and poor soil structure that aggravate drought stress. Components of recommended technology include: no-till; residue mulch and cover crops; integrated nutrient management; and biochar used in conjunction with improved crops (genetically modified, biotechnology) and cropping systems, and energy plantation for biofuel production. However, its low acceptance, e.g., for no-till farming, is due to a range of biophysical, social and economic factors. Competing uses of crop residues for other needs is among numerous factors limiting the adoption of no-till farming. Creating another income stream for resource-poor farmers, through payments for ecosystem services, e.g., C sequestration in terrestrial ecosystems, is an important strategy for promoting the adoption of recommended technologies. Adoption of improved soil management practices is essential to adapt to the changing climate, and meeting the needs of growing populations for food, fodder, fuel and fabrics. Soil restoration and sustainable management are essential to achieving food security, and global peace and stability.

Keywords Biochar • Conservation agriculture • Food security • Integrated nutrient management • No-till farming • Soil degradation • Soil restoration • Sustainable development

1 Introduction

Global estimates of food-insecure populations of 825 million (Lobell et al. 2008) to 850 million (Borlaug 2007) have increased to >1 billion in 2009. Regional estimates of the food insecure population include 263 million in South Asia (SA), 268 million in China and Southeast Asia, 212 million in sub-Saharan Africa (SSA), 60 million in South and Central America and the Caribbean, and 50 million in other regions of the world. Contrary to the United Nations' (UN) Millennium Development Goals of cutting hunger by half by 2015, the number of food-insecure populations in the world will increase. The stock of food grains in

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the world in 2007–2008, the lowest in decades, was only 75 million tons for milled rice and 105 million tons for corn in early 2008 (USDA-FAS 2008). An estimated 75% of the world's poor (<$2 per day income) live in rural areas and depend directly or indirectly on agriculture (FAO 2006). Food prices are rising (Normile 2008), leading to riots in 30 countries around the globe (Brown 2008; Hoyos and Blas 2008). Share of family income spent on food is estimated at 10% in the USA, 20% in Brazil, 30% in China, 50% in Kenya and 65% in Bangladesh (Hoyos and Blas 2008). Thus, the world's poor are under great stress, and increase in food prices is a threat to global peace and stability. There is a shortage of good quality soil to bring about the desired increase in food production. Soil fertility decline is an important factor (Sanchez 2002; Sanchez and Swaminathan 2005; IFDC 2006) that cannot be ignored. Indeed, agriculture requires more land, water and human labor than any other industry (FAO 2007; Kiers et al. 2008). Several studies have documented that the potential of genetically modified (GM) crops is appropriate in some contexts, unpromising in others, and unproven in many more (Kiers et al. 2008). The potential of GM crops remains unfulfilled, especially for the subsistence farmers of SSA and SA, where crop yields are strongly constrained by the severe problems of soil degradation and desertification. Furthermore, policy-makers are torn between allocating resources to food security and biofuel production. The objective of this article is to deliberate the need for a soil-based approach to enhance and sustain agronomic production and eliminate world hunger and malnutrition.

2 Prehistoric Farming Techniques and Soil Degradation

The literature is replete with prehistoric agriculture and its impact on soils and the environment of the Middle East, and in the valleys of the Nile, Yangtze, Indus and others. However, little is known about prehistoric farming and soil quality in other sites of ancient cultures. Yet, maintaining soil fertility and agronomic productivity have been serious issues facing humanity ever since the dawn of settled agriculture. For example, it has long been recognized that catenal processes result in large fertility contrasts between summit/shoulder slopes and footslope landscape positions (Scholes 1990), and that these contrasts were accentuated by the activities of prehistoric cultures such as addition of charcoal to create the "terre preta de Indio" (Mann 2002). Two important soil-related constraints faced by prehistoric farmers, drought stress and nutrient depletion, were alleviated by use of the innovative technologies of supplemental irrigation and nutrient recycling, respectively.

Irrigation and water management played a major role in the so-called "hydric civilizations". In addition to the well-known use of irrigation in Asia (Knozer 2000; Premathilake 2006; Groenfeldt 1991; Andrianov 1989; Knauss 2000) there are examples of using irrigation in the prehistoric Americas (Park 1983; Lange 1992). Central America has been the cradle of agriculture in the new world (Clement and Horn, 2001; Goman and Byrne 1998). In the Lake Titicaca Basin, covering the Andean Highlands of Peru and Bolivia, archaeological investigations have shown a massive landscape modification throughout prehistory to intensively cultivate marginal lands. These modifications, over 82,000 ha of land area, included raised beds and large earthen platforms to improve drainage, increase soil fertility by recycling nutrients and improve the micro-climate (Erickson 1992). In the Pampa de Chaparri hyper-arid region of Peru, prehistoric irrigation canals and furrowed fields have been preserved for 500 years (Nordt et al. 2004). On Easter Island (Rapa Nui), Chile, cultivation of yams (*Dioscorea* spp.) and taro (*Colocasia esculenta*) began in the twelfth century and continued through the fifteenth century (Stevenson et al. 2006).

Archaeological evidence of early irrigation systems has also been discovered in southeastern Arizona. Palacios-Fest et al. (2001) identified several stages of water management in the Santa Cruz River Valley of Southeastern Arizona in the prehistoric era. These researchers reported that prehistoric people first operated their irrigation system in a simple mode involving diversion of ephemeral flows following storms, and later in a complex mode involving diversion of perennial flows. Berger (2004) reported the use of canal irrigation from 1,600 years to 800 years ago (400 AD to 1200 AD) in the flood plains of Salt River in Phoenix, Arizona. Masse (1981) used aerial photographs to reveal complex and extensive remains of ancient Hohokam irrigation systems in the Salt River Valley near Tempe, Maricopa County, Arizona. These systems were probably constructed and used between 850 AD and 1450 AD, and consist of over 2,100 km of canals in the north and south of the Salt River.

Nutrient depletion was also a major problem in settled agriculture, except in the flood plains where soil fertility was renewed by alluvial deposition. Prehistoric depletion of soil nutrients was documented after centuries of indigenous agriculture in Hawaii. Hartshorn et al. (2006) observed that farmers began growing dryland taro and sweet potato (*Ipomea batatas*) using a digging stick on the leeward slopes of East Maui beginning approximately 500 years ago. Centuries of this extractive farming lowered concentrations of Ca^{2+} (49%), Mg^{2+} (28%), Na^{+} (75%), K^{+} (37%) and P (32%) in cultivated compared with uncultivated soils. Similar to the problem of accelerated water erosion faced by the Mayan culture in Central America, wind erosion was a serious constraint faced by the prehistoric farmers of the Kalaupapa field system, Molakai Island, Hawaii (McCoy and Hartshorn 2007). In New Zealand, there is strong evidence of prehistoric cultivation of four introduced Polynesian plants: bottle gourd (*Lagenaria siceraria*), paper mulberry (*Broussonetia papyrifera*), sweet potato and taro (Horrocks et al. 2004). These data identify combinations of early Polynesian crops, including both field – and tree – cropping systems, and provide evidence of prehistoric taro cultivation in the South Island. Lepofsky (1995) observed the prehistoric agricultural production and human-induced environmental changes in the Society Islands dating back to the seventh to tenth century AD. Valleys with the greatest arable potential were cultivated earlier than less preferred sites. Similar to the prehistoric civilizations, the problem of nutrient depletion has aggravated the food insecurity in SSA even during the twenty-first century (Smaling et al. 1993; Henao and Baanante 2006; IFDC 2006).

Increase in population, because of the transformation of the hunter–gatherer system into settled agriculture, necessitated development of an “ard” or a prehistoric plow (Highman et al. 1981; Lal et al. 2007). Introduction of the plow exacerbated the problem of soil erosion and depletion of soil organic matter (SOM). The plow-induced soil degradation plagued mankind even during the prehistoric era. Accelerated soil erosion had a devastating effect (Bunney 1990) throughout the Middle East and the Central American Mayan culture (Diamond 2004). With the world’s population of 6.7 billion in 2008 and projected to be 9.5 billion by 2050, the issue of producing adequate and nutritious food, the basic human right, is more important now than the challenges faced by sparsely-populated prehistoric farmers.

3 Constraints to Transforming Traditional Agriculture in Sub-Saharan Africa

The SSA remains the only region in the world where the number of hungry and malnourished populations will still be on the increase even by 2020 (Rukuni 2002). While other regions have improved per capita food availability since the 1970s, food production and availability have perpetually declined in SSA. It is both a technological and a political/economic challenge, and cannot be ignored any longer. Agrarian stagnation in SSA has defied numerous attempts at transforming subsistence agriculture, even with due consideration to issues related to biophysical constraints and the human dimensions challenges (Otsuka 2006; Vermeer 1983; Nieuwoudt and Vink 1989). Traditional agricultural practices in SSA have been addressed in relation to soil degradation (Chokor and Odemerho 1994), soil nutrients and SOM depletion (Stromgaard 1991; Dakora and Keya 1997; Sanchez and Leakey 1997; Nye and Greenland 1958), soil pests (Hillocks et al. 1996a,b), pest management (Abate et al. 2000), and plant defense mechanisms (van der Westhuizen 2004). Among commonly promoted strategies for achieving food security in SSA are: cooperative regionalism (Ugwuanyi and Obinne 1998), drought management (Hubbard et al. 1992), improvement of roots and tuberous crops such as cassava (*Manihoc esculenta*) (Prudencio and Alhassan 1994), use of indigenous knowledge (Oniang’o et al. 2004), integrated food systems (Hulse 2004), macroeconomic and public policy (Rukuni 2002), structural adjustment programs (Amalu 2002), multiple livelihood strategies of women farmers using micro-enterprises (Gladwin et al. 2001), political economy of urban population (Sutherland et al. 1999), income-generating employment (Duncan 1998; McCalla 1999) and policy framework (Van Rooyen and Sigwele 1998).

Innovative technologies have been successful in improving agriculture in Asia, especially in China and India. Important among these innovations to intensify traditional agricultural systems are ecological agricultural techniques involving more input of skills, knowledge and labor (Ellis and Wang 1997; Xu 2004; Shi 2002; Battershill and Gilg 1998), integrated farming systems (Li and Min 1999), improved germplasm/transgenic plants grown with efficient systems (Xu and Jeffrey 1995; Soleri et al. 2005) and diversification of farming systems (Short 1997). Yet, repeated attempts at adoption of improved technologies have been met with modest success in SSA, where food insecurity remains a major issue. The agrarian stagnation in SSA has defied all approaches and strategies. The stubborn problem will be solved only when Africans (scientists, farmers, policy-makers and the public at large) collectively resolve to solve it in a manner pertinent to the site-specific situations. SSA must enhance, restore and prudently manage soil and water resources to improve and sustain soil quality. Soil-related constraints to be addressed in SSA and other developing countries are accelerated erosion, SOM depletion, drought stress and soil nutrient management.

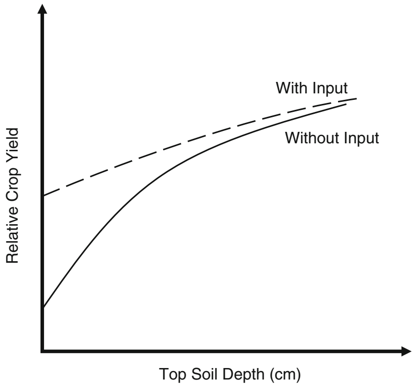

Fig. 1 Relative effects of differences in topsoil depth due to differences in severity of soil erosion on relative crop growth and yield with and without off-farm input. Adverse effects of erosion on crop growth are marked by the use of off-farm inputs. Inputs include fertilizers, manure and mulch

4 Crop Yield and Soil Erosion

Accelerated erosion is an important factor adversely affecting sustainability of cropping and farming systems. Erosion is more serious in the tropics than in temperate climates because of the prevalence of fragile soils of high erodibility, harsh climates of high erosivity, and predominately resource-poor farmers who cannot afford to adopt conservation-effective measures. Thus, adverse effects of erosion on agronomic productivity are more severe in Africa, Asia and the Caribbean than in the USA, Europe and Australia. Erosion affects crop yields and agronomic productivity both directly and indirectly. Directly, it reduces the effective rooting depth and available water and nutrient retention capacities. Indirectly, it decreases use efficiency of inputs and increases the amount of fertilizers, water and energy needed to produce the same yield. Erosion has both on-site and off-site impacts. On-site, it reduces seed germination, stand establishment, and plant growth and vigor. Off-site, through runon and deposition, it increases risks of inundation, pesticide damage, and seedling burial.

Experimental data on erosional impacts on yield of crops in soils of the tropics are scanty. Synthesis of available data (Lal 1987, 1998; den Biggelaar et al. 2003a,b) indicates stronger adverse effects on farms that do not use off-farm inputs than on those under intensive management. In general, application of fertilizers and soil amendments masks the adverse impacts of accelerated soil erosion (Fig. 1). In Nigeria, Salako et al. (2007) reported 65–75% reduction in crop yield with 25-cm removal of topsoil when no fertilizers or manure were applied. However, productivity of eroded soils was restored more effectively by the application of manure than by use of chemical fertilizers. Similar experiments by Oyedele and Aina (2006), also conducted on Alfisols in Western Nigeria, indicated that grain yield of maize (*Zea mays*) decreased from $3.2\,\text{Mg}\,\text{ha}^{-1}$ under control to $0.1\,\text{Mg}\,\text{ha}^{-1}$ where 20 cm of topsoil was removed. Maize yield decreased exponentially with decrease in the depth/thickness of the remaining topsoil. Drastic reduction in maize grain yield on eroded soil was attributed to the low physical and chemical quality of the exposed sub-soil. Field experiments conducted in the West African Sahel indicated that the grain yields of pearl millet (*Pennisetum glaucum*) were severely reduced on eroded and unmulched compared with uneroded and mulched soils (Michels and Bielders 2006). Furthermore, millet dry matter yield tripled with P application, and increased by a factor of 13.5 when additional N was applied. These researchers observed that the high P availability was the key factor to reversing decline in crop yields on erosion-affected soils, and manuring was more effective than mulching with straw. Conversely, some studies have documented the beneficial effects of adopting conservation-effective measures on crop yields. For example, installation of stone bunds in large areas of the Tigray Highlands in Northern Ethiopia have shown that average sediment accumulation behind 3- to 21-year-old stone bunds is 58 Mg ha^{-1} per year (Nyssen et al. 2007). Consequently, there was an average increase in grain yield of 53% on the lower parts of the landscape in the vicinity of the stone bunds compared with the central and upper parts. Similar positive effects on crop growth and yields have been reported with regards to the use of conservation tillage and mulching. Long-term effects of no-till (NT) farming in conjunction with crop residue mulch are due to improvements in SOM and decrease in soil erosion (Scopel et al. 2005).

Effects of accelerated erosion on trends in crop growth and yield in intensively managed soils under commercial farming practices are similar to those of the tropics, but the magnitude of the adverse effects on crop yield is small (den Biggelaar et al. 2003a,b). Experiments conducted on an eroded prairie landscape in the USA indicated that wheat (*Triticum aestivum*) yields were lowest on soils subject to tillage erosion, and were about 50% of those on uneroded or depositional sites (Papiernik et al. 2005). The data from field experiments, used to validate the results of modeling studies, are in accord with those of experimental studies with regards to the adverse effects of accelerated erosion on soil quality (Izaurralde et al. 2007). The fact that increase in risks of soil erosion hazard due to climate change would have similar adverse effects on crop growth and yield (Zhang 2005) necessitate planning for the use of adaptive measures, especially in the tropics and sub-tropics where the climate change impacts would be more drastic (Cline 2007), and resource-poor small land-holders do not have the capacity to adapt to the abrupt climate change.

The fate of erosion-displaced soil carbon is also a debatable issue that needs to be addressed through long-term research conducted on a watershed scale. Some researchers argue that soil erosion is a source of atmospheric CO_2(Lal 2003). In contrast, others hypothesize that C transported into aquatic ecosystems is a major sink (Van Oost et al. 2004, 2007), and may account for the so-called missing or fugitive CO_2. Resolving this issue is important to enhance the scientific understanding of the complex global C cycle and its impact on the projected climate change.

5 Soil Organic Matter and Crop Yield

Regardless of the farming system, e.g., traditional, commercial or modern and innovative, maintenance of soil organic matter (SOM) above the critical level (Aune and Lal 1998) is essential to sustaining productivity and minimizing the risks of soil and environmental degradation (Lal 2006a,b). There are numerous benefits of increasing the SOM concentration and pool on enhancing ecosystem services and improving the environment (Fig. 2). The key factor is

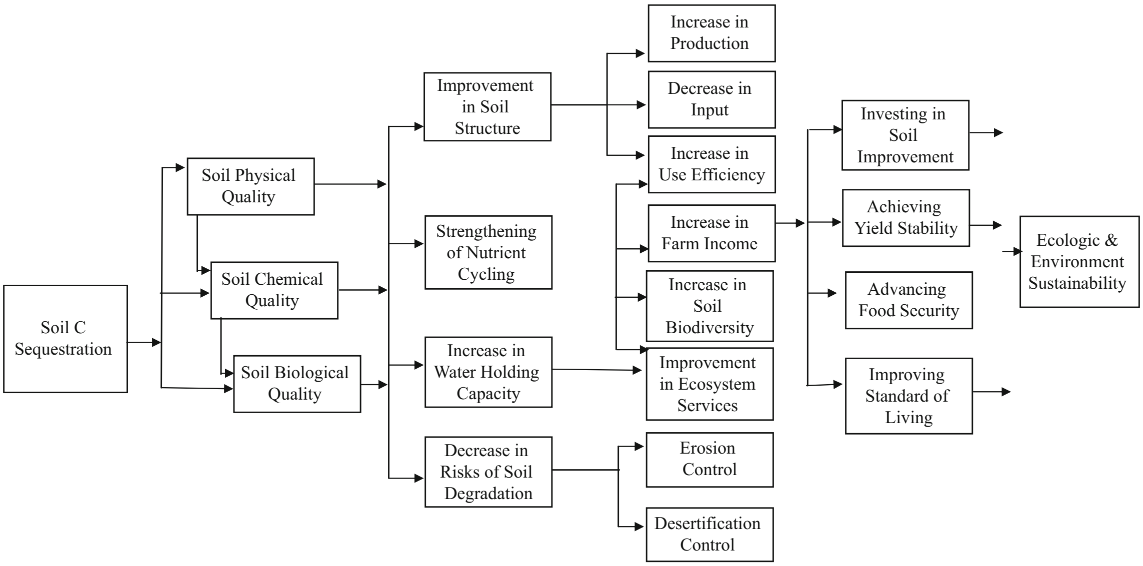

Fig. 2 The importance of soil carbon sequestration in ecosystems and environmental sustainability

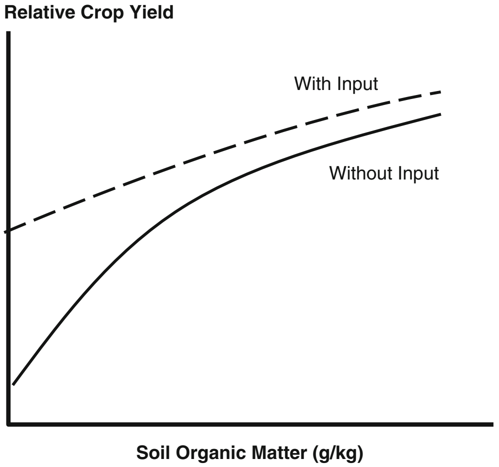

Fig. 3 Relative effects of differences in soil organic matter concentration in the root zone on crop growth and yield. Similar to the effects of accelerated soil erosion, decline in crop yield due to reduction in soil organic matter is more without than with application of fertilizers and other inputs

the improvement in soil quality with the attendant positive impact on soil processes and properties (Lal 2004). Similar to the effect of erosion, adverse effects of decline in SOM concentration on crop yields are also more severe without than with application of fertilizers and soil amendments (Fig. 3). These generalized trends are supported by the data from experiments conducted on representative soils and cropping systems in diverse agroecoregions of sub-Saharan Africa (Pieri 1991).

The data from a field experiment conducted in Burkina Faso, West Africa, indicated that optimum SOM concentration and crop performance results from a judicious combination of the use of organic/biosolids and inorganic fertilizers (Ouedraogo et al. 2007). In the Sudano-Sahelian zone of Burkina Faso, Mando et al. (2005a,b) observed that application of manure enhanced SOM concentration and increased sorghum (*Sorghum bicolor*) grain yield by 56–70%. Furthermore, grain yield was positively correlated with the particulate organic matter (POM) fraction. A long-term field experiment in semi-arid Kenya, designed to assess the sustainability of cereal/legume intercropping, monitored the relationship between SOM concentration and crop yield. Grain yield of sorghum improved with application of manure, whose residual effect lasted for several years (Kihanda et al. 2006). In Morocco, Bessam and Mrabet (2003) reported that conversion of plow tillage (PT) to no tillage (NT) farming sustained crop yields by enhancing the SOM pool. The mean rate of soil C sequestration over the 11-year period was 0.66 Mg ha^{-1} per year upon conversion from PT to NT farming.

Positive effects of enhancing the SOM pool on crop yield and agronomic sustainability have also been reported from experiments conducted in Asia. Shibu et al. (2006) synthesized the available literature on modeling SOM dynamics in the rice (*Oryza sativa*)-wheat (*Triticum aestivum*) system for different management scenarios, with impact on crop yield. Swarup et al. (2000) documented the positive effects of SOM on crop yields in India. In the Philippines, Manguiat and Rocamora (2004) reported that application of bio-organics significantly increased the average yield of six crops by 98–153% over the 3-year period. Some studies in South America have also documented the beneficial effects of improving SOM on crop yield (Pupiro et al. 2004). In Colombia, Basamba et al. (2006), observed that increase in maize (*Zea mays*) grain yield with adoption of conservation tillage was due to increase in SOM concentration. In the Pampas of Argentina, Quiroga et al. (2006) observed that the grain yield of barley (*Hordeum vulgare*) was strongly influenced by the improvements in soil quality caused by increase in SOM concentration. In Sao Paulo, Brazil, Silva et al. (2006) reported the beneficial effects of increase in SOM on growth and yield of radish (*Raphanus rativus*). In Cuba, biomass yield of leucaena (*Leucaena leucocephala*) increased with increase in application of worm humus or compost.

Similar to the tropics, there is also strong evidence about the positive effects of increasing SOM concentration on crop yields in North America and Europe (Lal 2004, 2006a,b). A strong and positive impact of applying sphagnum peat on the yield of potato (*Solanum tuberosum* L.) grown on a sandy soil was reported by Li et al. (2004). In Canada, Malhi et al. (2006) observed that practices which improve SOM concentration also enhance crop yields. The data from a study with long-term application of manure on tomato (*Solanum lycopersicum*) indicated similar yields and soil fertility status to applying inorganic fertilizers (Moccia et al. 2006). Martin-Rueda et al. (2007) documented the positive impacts of reduced tillage and crop rotations on SOM concentration and agronomic yield in some soils of Madrid, Spain. In Denmark, Thomsen and Sorensen (2006) observed that both grain yield and N uptake were highest on soil with the highest SOM level. Long-term field experiments in Estonia showed that rotation and tillage systems with positive impact on SOM concentration also increased and sustained crop yields (Viil and Võsa 2005).

6 Irrigation and Fertilization Management

The Green Revolution of the 1970s, which saved hundreds of millions from starvation in Asia and South/Central America, by-passed sub-Saharan Africa (SSA). In Asia, the Green Revolution was ushered in by adoption of input-responsive varieties, grown on irrigated soils with liberal use of fertilizer, especially the heavily subsidized nitrogen. It is probable that agricultural land area could double in SSA (and West Asia) by 2050, and may also increase by 20–25% in the Asia-Pacific region and the zone of tropical rainforest in South America and Southeast Asia. Yet there is no substitute for intensification of agriculture on the existing cropland. Intensification implies cultivating the best soils with the best management practices (BMPs) to produce the optimum sustainable yield so that marginal lands in fragile ecosystems, e.g., steep lands, tropical rainforest, can be saved for nature conservancy. It is in this context that the importance of expanding cropland area under irrigation by using small-scale projects, judicious and prudent use of chemical fertilizers, and adoption of genetically improved (GM) crops cannot be over-emphasized. Adoption of these options of agricultural intensification can be easily quadrupled and sustained at the high level of production. Ecosystems utilized by humans can only be sustained if the outputs are balanced by inputs. The latter may be of organic or inorganic origin, because plants cannot differentiate the nutrients supplied through the organic or inorganic sources. It is a question of logistics and availability rather than of the natural or synthetic origin. In addition to these inputs, conversion of traditional hoe-based and plow-based methods of seedbed preparation to NT farming is also essential. The promises of NT farming, used in conjunction with crop residue mulch and cover cropping as integral components of complex cropping systems, can be realized by addressing the challenges (biophysical and socio-economic) that it faces.

7 Promise and Challenge of No-Till Farming

Agronomic techniques to improve soil quality (Table 1) include NT farming and crop residue management, nutrient management and use of biochar. Plowing and other mechanical methods of seedbed preparation are redundant if weeds can be controlled chemically or biologically, seeds can be sown through the crop residue mulch, and fertilizers can be applied without incorporation into the soil. In this regard, NT farming, used in conjunction with crop residue mulch and cover crops, has numerous advantages including conservation of soil and water, saving in time and energy, improvement in SOM concentration, increase in soil biotic activity and decrease in weather-related impacts on crop yields. It is in this regard that the importance of the judicious management of crop residues cannot be over-emphasized. Therefore, planning for SOM management requires the data on the amount of crop residues produced under different cropping systems (Johnson et al. 2006).

Application of NT farming for erosion control and moisture conservation under row crop production in soils of West Africa was documented by long-term experiments conducted in Nigeria (Lal 1976, 1989). The potential of judiciously using crop residues as a basis

Table 1 Components of sustainable soil management system for advancing food security in the tropics

Farming Operation / Objectives	Recommended Management Practices
1. Seed bed preparation	No-till farming, crop residue mulch
2. Rotations	Legume-based crop rotations, agroforestry
3. Water management	Mulch farming, water harvesting and recycling, efficient irrigation methods
4. Fertility management	Manuring, BNF, biochar, slow release formulations of fertilizers, nano-enhanced
5. Erosion control	No-till, agroforestry, cover cropping, conditioners
6. Soil biotic activity	No-till, manuring, mulch farming
7. Enhancing SOM pool	Complex crop rotations, no-till, agroforestry, biochar application, manuring and biosolids

of SOM management was also reported by Shittu and Fasina (2006), who observed that residue mulching improved crop yields in Western Nigeria. Incorporation of a fallow (no cropping) period in the rotation cycle also improved SOM concentration and enhanced soil quality in Burkina Faso (Bostick et al. 2007). In the Mediterranean climate of north Africa, Masri and Ryan (2006) observed that incorporation of medic (*Medicago sativa*) and vetch (*Vicia faba*) increased the SOM pool compared with continuous wheat. In India, Mandal et al. (2007) concluded that the SOM level can be sustained with annual application of 2.9 Mg of biomass-carbon per hectare through manure and other biosolids. Ghosh et al. (2006) also observed that the groundnut (*Arachis hypogea*) – wheat systems contributed more carbon through root biomass than groundnut – chickpea (*Cicer arietinum*) systems, and recommended that additional crop residues along with fertilizers are essential to maintaining SOM levels on Vertisols in Central India. In northeastern China, Xu et al. (2006) reported that SOM concentration increased for Ustepts with application of crop residues and fertilizers. In El Batan, Mexico, Govaerts et al. (2006, 2007) observed that NT farming with crop residue mulch was essential to enhancing SOM and improving soil quality. In western Mexico, Scopel et al. (2005) also observed significant yield benefits of growing NT maize under the semi-arid conditions. In southern Brazil, De Bona et al. (2006) and Bayer (2006) observed that NT farming along with high input systems are needed to counter-balance the higher SOM decomposition rates in a sub-tropical Acrisol. In a clayey Oxisol of Brazil, Razafimbelo et al. (2006) reported the management of sugarcane (*Sacchrum rotundum*) residue (burning vs. no burning) had a strong impact on SOM dynamics.

A large body of literature available on the impact of crop residue management and NT farming in the USA and Brazil (West and Post, 2006) is not reviewed in this report. Thus, a few recent examples are discussed herein. Venterea et al. (2006) assessed the effects of rotational (biennial) tillage on SOM dynamics under corn-soybean (*Glycine max* L.) rotation, and concluded that biennial chisel plowing in the upper mid-west USA can enhance C storage in soil, reduce fuel costs and maintain yields compared with intensive annual tillage. Long-term effects of tillage and crop residue management in the sub-Arctic region of Alaska were assessed by Sparrow et al. (2006). They observed that adoption of reduced tillage can improve soil quality and conserve SOM, but long-term NT may not be feasible because of the weed problem and progressive buildup of crop residues on the soil surface in the cold regions. In the northern Great Plains region, Sainju et al. (2006a–c) assessed tillage and crop rotation effects and concluded that reduced tillage and increased cropping intensity enhance the SOM pool. Furthermore, use of hairy vetch (*Vicia villosa Roth*) and rye (*Secale cereole*) biculture was effective in sequestering more C than monocultures or no cover crop. A long-term study in central Texas, USA, indicated that NT associated with enhanced cropping intensity and N fertilization increased SOM and N pools (Dou et al. 2006). Also in the hot climate of Texas, Zibilske and Bradford (2007) observed that SOM accumulation may be stimulated by growing cover crops with higher polyphenolic contents and restricting soil – O_2 availability with NT farming. In Michigan, USA, Kravchenko and Thelen (2007) observed that use of winter wheat residue decreased the amount of plant-available N and increased grain moisture and test weight of corn grains at harvest. In California's Mediterranean climate, Veenstra et al. (2007) concluded that conservation tillage alone does not accumulate or stabilize more C than conventional tillage. Therefore, addition of cover crop biomass is essential to increasing total soil C accumulation. Incorporating cover crop in the rotation cycle is even more significant for the cotton (*Gossipium hirsutum*)-based systems in Alabama to enhance SOM and improve soil quality (Paudel et al. 2006). In addition to the amount of chemical composition of crop residues, accumulation of SOM in soil using higher residue-producing conservation systems and manure is also scale-dependent. On the basis of their data from a 9-ha field in Alabama, Terra et al. (2005) observed that the potential to sequester C in degraded soils in the southeastern USA may be higher than previously expected. In the Colombia Basin of Washington state, Cochran et al. (2007) studied the SOM dynamics in a semi-arid shrub-steppe ecosystem recently converted to irrigated agriculture. They concluded that cultivation, crop residue incorporation and dairy manure compost amendments contributed to increase in the total soil C pool. Johnson et al. (2007) tested the hypothesis that SOM decomposition is a function of biochemical composition when all other variables are constant, e.g., particle size, temperature and moisture. Variation in biochemical composition was created by selecting residues of five species including alfalfa (*Medicago sativa* L.), corn, cuphea (*Cuphea viscosissi-ma Jacq*), soybean and switchgrass (*Panicum virgatum* L.). A stepwise multivariate regression indicated that chemical recalcitrance slows root decomposition and explained why roots contribute more C to the SOM pool than surface residues. Thus, root activity is also an important factor in total CO_2 production (Chen et al. 2005). In the mid-South USA, Brye et al. (2006) studied SOM dynamics in a wheat-soybean double crop system for a range of wheat residue management scenarios. Their data showed that SOM, along with total N and C concentrations, increased with NT at one of the two locations in east-central Arkansas but not in the other. Brye and colleagues concluded that in a wheat-soybean double-crop production system in a relatively warm and wet environment, numerous soil properties can be improved with NT when crop residues are left unburnt. Measurements, made on peat soils in Ohio (Elder and Lal 2008a,b) and Florida (Gesch et al. 2007), indicated that respiration-induced subsidence can be reduced by conversion to NT farming. In Ohio, the rate of SOM buildup in NT soil increased with application of manure (Wang et al. 2006; Hao et al. 2002), and with retention of crop residue mulch (Blanco-Canqui and Lal 2007, 2007b, 2008).

Similar to the data from the USA, the positive impact of residue mulch and NT farming on the SOM pool have been reported by several studies in Canada (Malhi et al. 2006; Campbell et al. 2007; Janzen 2006; Wang et al. 2006; Liang et al. 2005; Singh and Malhi 2006; Gregorich et al. 2006). These conclusions of improvements in soil quality are also supported by the data from Europe and Australia. Positive impacts of improving cropping intensity and eliminating tillage on increasing the SOM pool were also observed in Brazilian Cerrado by In addition to cropping intensity, the impact of the climate gradient (temperature and moisture regimes) is also important to SOM and N dynamics (Ortega et al. 2005). Similar to the environmental problems of burning sugarcane residues in Brazil and Colombia, there is a strong interest in a system of “green can trash blanketing” (GTCB). In addition to improving SOM, there may also be advantages in saving of fertilizers over a long time (Robertson and Thorbun 2007).

The data from a long-term experiment in north-eastern Italy showed that residue incorporation enhanced the SOM pool at the mean rate of $0.17\,\mathrm{Mg\,C\,ha^{-1}y\,a^{-1}}$ (Lugato et al. 2006). Morari et al. (2006) also document the SOM dynamics under NT farming in north-eastern Italy. In semi-arid northern Spain, Bescansa et al. (2006) observed that conservation tillage improved the plant-available water capacity and increased crop yield during the dry years.

A critical analysis of the studies reviewed above, and others reported elsewhere, show that the effects of NT systems and crop residue management on the net gain of the soil C pool depend on a range of complex and often interacting factors. It is, therefore, highly challenging to generalize the results. The information available to date can be summarized as follows:

(a) Ecosystem services: Conversion of PT and other reduced tillage or conservation tillage systems, along with use of crop residue mulch and cover crops, provides numerous ecosystem services regardless of the biophysical and socio-economic environment. Important among these are: erosion control, water conservation, reduction in non-point source pollution, savings in time and energy and other inputs, stabilization of crop yields against drought, and decrease in the C footprint of the agricultural systems.

(b) Crop yields: Effects of conservation tillage and residue mulching on crop yields are variable and depend on many factors. Important among these are soil use history, soil quality at the time of conversion to NT farming, soil properties and profile characteristics, crop species (e.g., cereal, legume, root and tubers), climate, amount and composition (C to N ratio) of crop residues, the rate of N application, effectiveness of the seed drill in cutting through the crop residues and establishing seed-soil contact, drainage conditions, soil temperature at the time of seeding and during seed germination and seedling establishment, and the duration since conversion to NT farming. Some reduction in crop yields may occur in soils that have been recently converted to NT, and also in soils with persistent weeds and incidence of other pests (stem borer, slugs, etc.). Despite reduction in crop yields, the net benefit may still be higher with NT farming because of lower costs (e.g., diesel, machinery, fertilizers).

(c) Soil C pool: It is widely recognized that conversion of PT to NT leads to C sequestration in soils (Campbell et al. 2007; Sa et al. 2001; West and Post 2002; Vagen et al. 2005). These are some concerns about the hidden C costs (Schlesinger 2006), and the net C gains. Despite the low C footprint, it is also

possible that the total soil C pool in NT is either equal to, or in some cases even lesser than, that in the PT soil. In most cases, there is an increase in the soil C concentration in the surface 0–20 cm depth in NT compared with the PT soil. In others, however, the PT soil may have more C in the sub-soil than in the surface layer (Fig. 4) (Blanco-Canqui and Lal 2008; Baker et al. 2007). Location of crop residue in the soil (surface vs. deep incorporation) can strongly affect its decomposition (Coppens et al. 2006). Soil texture, initial soil C pool and internal drainage also play an important role in determining the net C gains with regards to the tillage system (Puget and Lal 2005). There exists a strong interaction between availability of N and the soil C pool. Addition of N may increase the soil C pool in N-deficient soils (Jaycinthe et al. 2002; Jagadamma et al. 2008), or decrease it while enhancing mineralization in others (Khan et al. 2007). Soil aggregation plays an important role in retention and turnover of root-derived C (De Gryze et al. 2006). The overall goal of managing the soil C pool is to create a positive C budget in the soil by increasing input more than the output (through moderation of erosion, mineralization and leaching), and increase its mean residence time (MRT).

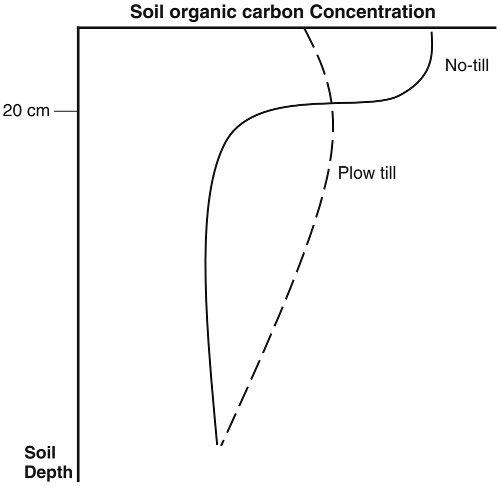

Fig. 4 Schematics of carbon profile in some soils managed by no-till and plow till

Despite the promise of NT farming in revolutionizing agriculture, its adoption rate is disappointingly low, especially so in developing countries of the tropics where it is needed the most. Total cropland area under NT farming is less than 100 Mha or merely 6% of the world's cultivated area (Derpsch 2007). Most of the NT farming is practiced by large-scale commercial farmers in the USA, Brazil, Argentina, Canada, Australia, Chile, Paraguay, etc. The lack of adoption of this important innovation by small land-holders is attributed to a range of biophysical and socio-economic factors (Table 2). The adoption of this innovation can be facilitated only if these constraints are effectively addressed. The issue is not that NT farming does not work; the goal is to make it work by alleviating constraints through site-specific and adaptive research conducted under on-farm conditions and with a participatory approach.

Table 2 Biophysical, socio-economic, political and cultural factors affecting the adoption of no-till farming in developing countries

Biophysical	Economic	Social	Political
1. Soil type (texture, clay, minerals)	1. Competing uses of crop residues	1. Land tenure	1. Policy interventions
2. Climate (drought), rain amount and distribution, temperature	2. Availability of inputs (herbicides, seed drill, fertilizers)	2. Mind set	2. Infrastructures
3. Terrain (slope gradient)	3. Yield reduction	3. Community participation	3. Institutional support
4. Weeds	4. Small size farm	4. Gender and social equity	4. Subsidies or lack thereof
5. Insects and pathogens	5. Lack of credit	5. Inappropriate demonstration techniques	5. Lack of political leadership, willpower and vision
6. Nitrogen immobilization	6. Price control	6. Lack of innovative platform	
7. Susceptibility to erosion			
8. Soil health at the time of conversion to NT			

8 Integrated Nutrient Management

Similar to the C budget, the nutrients removed from the soil must also be replaced. Nutrient depletion, with the attendant adverse impacts on crop yield, occurs when the nutrient removal (harvest, erosion, leaching and volatilization) exceeds the nutrient input: recycling, biological nitrogen fixation (BNF), animal manure, fertilizers, runon and aerial deposition. Nutrient depletion by indiscriminate mining through extractive farming has adversely impacted crop yields in SSA, SA and elsewhere in developing countries (IFDC 2006; Tan et al. 2005). There is a need to adopt management strategies which create and sustain positive nutrient and C budgets in managed ecosystems.

9 Soil Fertility Management

Judicious management of soil, water and plant nutrients is one of the strategies to adapt to climate change. Nutrient depletion and imbalance in soil adversely affect crop growth and yield, and are serious issues in soils of SSA (IFDC 2006; Smaling and Dixon 2006) and elsewhere in Asia, Central America and the Caribbean. Tan et al. (2005) estimated that globally nutrient depletion occurs at the rate of 18.7 kg ha^{-1} yr^{-1} of N, 5.1 kg ha^{-1} yr^{-1} of P and 38.8 kg ha^{-1} yr^{-1} covering 59%, 85% and 90% of harvested area in 2000. Tan and colleagues estimated the global annual nutrient deficit at 5.5 Tg of N, 2.3 Tg P, and 12.2 Tg K, causing a total production loss of 1,136 million tons of food grains. Soil nutrient depletion is attributed to lack of or insufficient use of fertilizers, unbalanced fertilization, and losses caused by erosion, leaching, volatilization and weeds. Increasing the input of plant nutrients into the ecosystem is crucial to creating a positive nutrient budget. Nutrients may be applied from inorganic or organic sources (Goulding et al. 2007). Nitrogen is the most limiting factor in crop production, and its use efficiency remains low because of the severe losses caused by volatilization and leaching (Eickhout et al. 2006). Nitrogen management is closely related to the soil C pool and its dynamics, and soils of the tropics are highly depleted of their soil C pool because of extractive farming practices used for centuries and millennia.

Using INM techniques is important for enhancing and sustaining soil fertility (INM: integrated nutrient management). INM involves combined use of mineral and organic fertilizer sources along with the adoption of legume-based, tree-based and animal-based farming systems. Several studies conducted in sub-Saharan Africa (SSA) and South Asia (SA) have documented the long-term and positive impacts of using INM techniques for improving soil fertility (Alemu and Bayu 2005; Smaling and Dixon 2006). The use of fire must be minimized because of numerous adverse impacts on ecosystem processes (Shriar 2007). The direct link between anthropogenic emissions and atmospheric abundance of CO_2 (Houghton 2002, Broecker 2007), necessitates adoption of mitigation strategies (Gleason et al. 2005), along with afforestation (Clawson 1979) and restoring prairie wetlands. There are numerous strategies of mitigating climate change (Fig. 5). Improving soil quality through C sequestration (King et al. 2004) is one of these options.

10 Biochar

Application of biochar (charcoal or black C) to soil can improve soil fertility. Ever since the discovery of "terra preta do Indio" (Indian Black Earth) in the Amazon (Sombroek et al. 2003; Marris 2006; Lima et al. 2002; Rumpel et al. 2006), there is a growing interest in using biochar as a soil amendment and for sequestering C and improving soil quality. There are several mechanisms by which application of biochar can enhance soil quality: (1) increase in soil's cation exchange capacity, (2) decrease in losses of nutrients by leaching, runoff and volatilization, (3) increase in soil's microbial activity that accentuates soil's resilience, (4) increase in soil structure and water retention capacity, (5) increase in buffering against soil acidification, and (6) reduction in emission of CH_4 and N_2O (Fowles 2007). A pot experiment with three rates (10, 50 and 100 Mg ha^{-1}) of biochar showed that application of biochar without application of N fertilizer had no impact on the yield of radish (*Raphanus sativus*) grown in a degraded Alfisol. However, higher yield increases were observed with increasing rates of biochar in the presence of N fertilizer, indicating an increase in N fertilizer use efficiency (Chan et al. 2007). A field experiment in Manans, Brazil, showed that charcoal (11 Mg ha^{-1}) significantly improved plant growth and doubled grain production only if applied in conjunction with NPK fertilizers. The highest crop yield of

$12.4\,\mathrm{Mg\,ha^{-1}}$ was obtained with application of poultry manure. Similarly, there are a range of soil processes that increase C sequestration through application of biochar: (1) it is a relatively stable/recalcitrant C with long residence time, and (2) it is translocated into the sub-soil away from the zone of natural and anthropogenic perturbations. Furthermore, black C is also translocated into the ocean (Dickens et al. 2004; Schmidt 2004). Therefore, it is argued that charcoal can be used in a climate-neutral manner whereby one mole of CO_2 emitted can be balanced by one mole of CO_2 sequestered (Seifritz 1993). Air-dried wood can be used to produce charcoal as per equation (1):

$$\begin{aligned} &CH_{1.44}O_{0.66}(\text{dry wood}) + 0.43O_2(\text{from air}) \\ &\quad \text{soot}\ 0.6C(\text{charcoal}) + 0.4CO_2(g) + 0.72H_2O. \qquad (1) \end{aligned}$$

The process shown in equation (1) can produce 750 kg of charcoal from 3 Mg of air-dried wood, with 60% of C in the wood converted into charcoal (Seifritz 1993). Charcoal conversion efficiency of wood is about 50%. A coal-fired power plant of 1 MW h power plant produces 6,800 Mg of CO_2 or 1,855 Mg of C. The CO_2 thus released can be sequestered in 1,130 ha ($11.3\,\mathrm{km^2}$) plantation of poplar. Therefore, biochar sequestration in terrestrial ecosystems is considered a viable option of enhancing soil fertility while mitigating climate change (Lehmann et al. 2006; Laird 2008). Despite its potential, three issues which remain to be addressed are (1) the logistics of producing a large quantity of biomass for making biochar to be used at high rates of application on soils at 10–20 $\mathrm{Mg\,ha^{-1}}$ or more, (2) loss of humus or the soil organic carbon (SOC) pool because of the removal of biomass for charcoal (Wardle et al. 2008), and (3) possible increase in mineralization of the SOC pool due to soil application of biochar (Shneour 1966, Wardle et al. 2008).

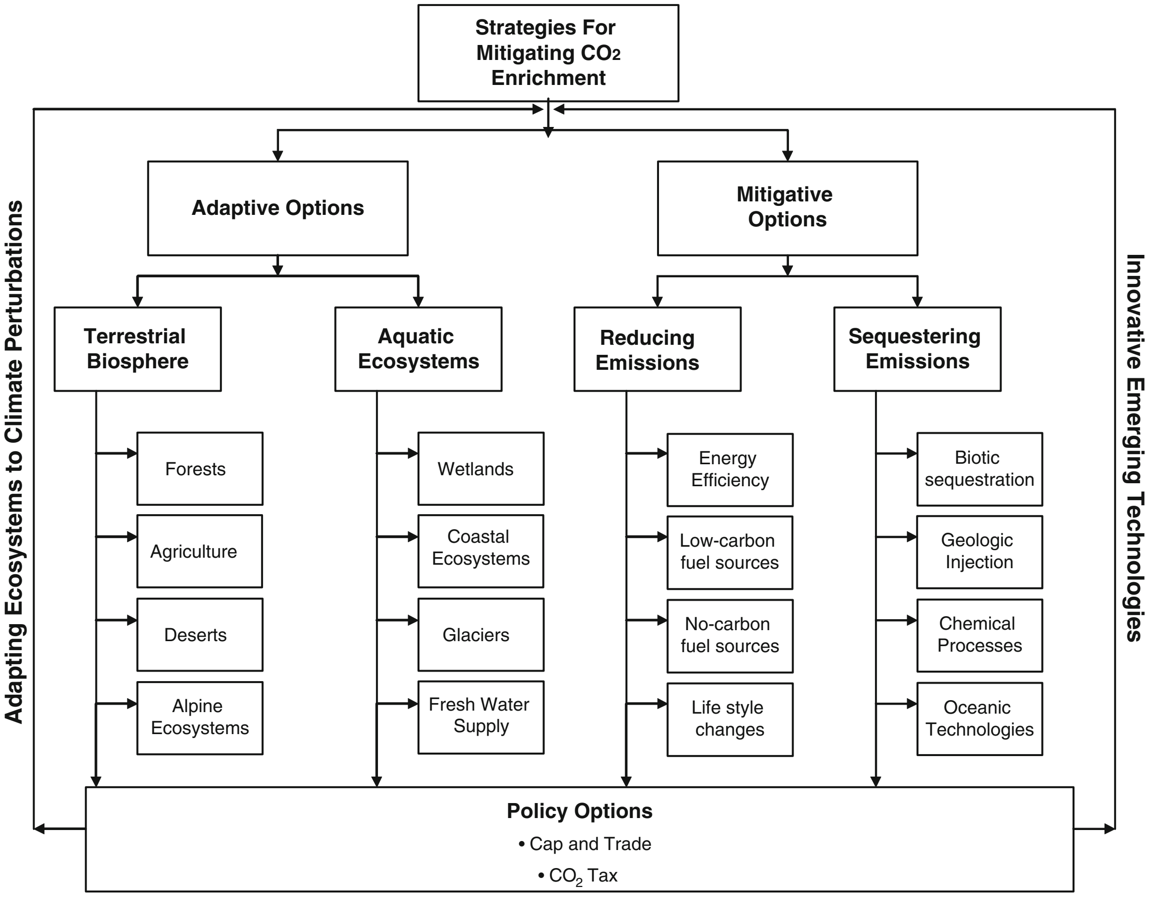

Fig. 5 Technological options for adapting to and mitigating atmospheric abundance of CO_2

11 Crop Yields and Agronomic Input

Strategies for improving soil quality may be built upon traditional knowledge, but must strongly rely on proven scientific innovations (Lal 2007). With the world average crop yield of milled rice at 4 $\mathrm{Mg\,ha^{-1}}$, the yield is about 3 $\mathrm{Mg\,ha^{-1}}$ in India compared with 8.5 $\mathrm{Mg\,ha^{-1}}$ for the USA. Similarly, for the world average yield of 5 $\mathrm{Mg\,ha^{-1}}$ for corn, the grain yield is 2 $\mathrm{Mg\,ha^{-1}}$ in India compared with 9.5 $\mathrm{Mg\,ha^{-1}}$ in the USA.

Crop yields in developing countries are strongly related to input, especially of fertilizer and irrigation. The data in Fig. 6 show that fertilizer use has drastically increased in all regions, except in SSA. Yields of crops in India have increased with increase in fertilizer use (Fig. 7), especially in irrigated wheat and rice. Because of low fertilizer use, and degraded/depleted soils, crop yields in countries of SSA are extremely low, as is shown by the data in Fig. 8 for Nigeria, Fig. 9 for Senegal, Fig. 10 for Uganda, Fig. 11 for Ghana and Fig. 12 for Kenya. Fertilizer response also depends on the cropland area under irrigation. Similar to trends in fertilizer use, cropland area under irrigation is also low in Africa (Fig. 13). Out of the total world irrigated area of about 275million hectare (Fig. 14), that in Africa is about 5 million hectare, or <2% of the world total.

In the long run, therefore, it is important to make these inputs available to minimize the adverse impacts of weather and soil degradation, and advance food security. There is no reliable substitute for the judicious use of inputs. Improved germplasm cannot extract water and nutrients from degraded/depleted soils where these do not exist. Therefore, making water and nutrients available at the critical time and in appropriate forms is essential to obtaining high yields. Growing improved varieties can help, but these are not substitutes for the essential inputs.

The scientific challenge lies in (1) understanding soil processes, (2) characterizing and mapping soil resources, and (3) predicting soil behavior under a variety of land uses and management scenarios (Miller and Wali 1995). The strategy is to make economic-agricultural development congruent with ecological, social and political realities, use and conserve indigenous genetic resources, and restore degraded soils and ecosystems (Miller and Wali 1995). Using the ecological footprint, Kitzes et al. (2007) and Hazell and Wood (2007) proposed two scenarios to balance human demands and ecosystem supply: (1) managing the consumption of food, fiber and energy,

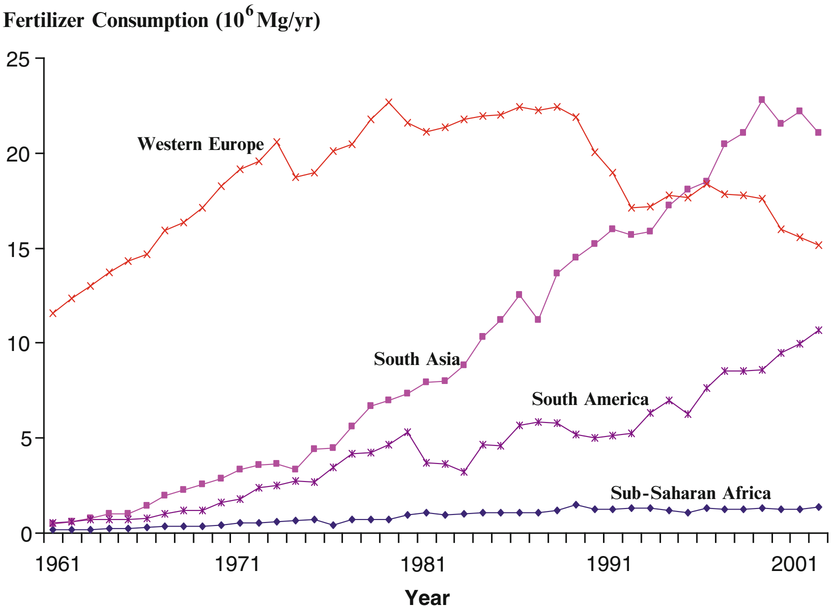

Fig. 6 Trends in regional and national fertilizer use (reported from IFDC 2004)

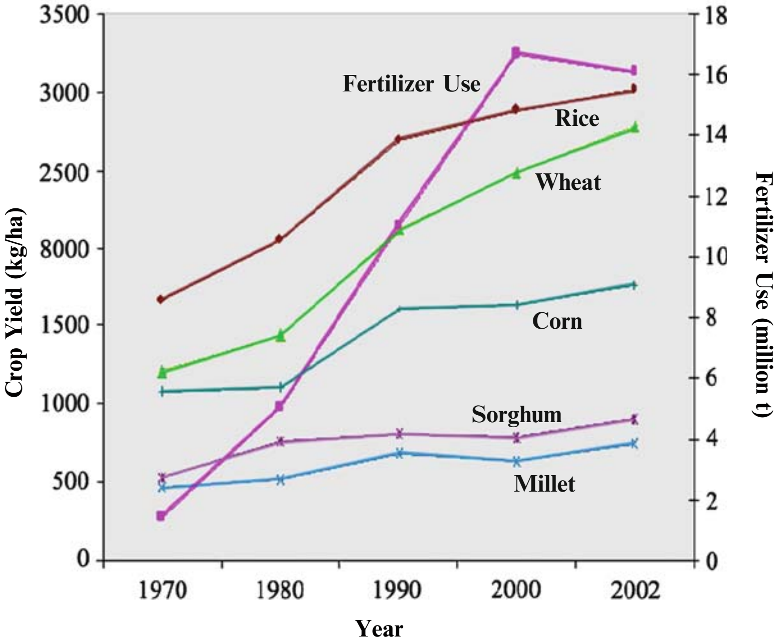

Fig. 7 Trends in fertilizer use and crop yields in India (data compiled from FAO Production Yearbooks, and IFDC 2004)

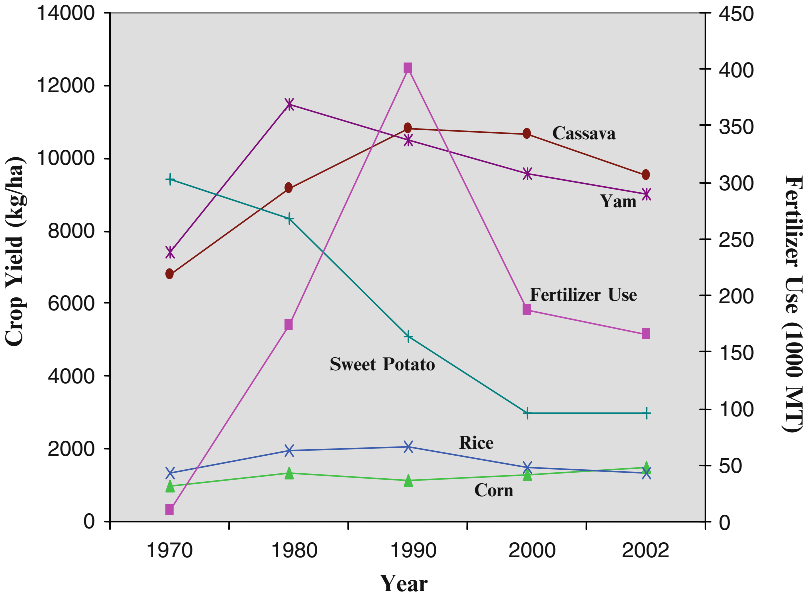

Fig. 8 Trends in fertilizer use and crop yields in Nigeria (data compiled from FAO Production Yearbooks)

and (2) maintaining and increasing the productivity of agricultural ecosystems. It is important to understand the linkages between human needs, agriculture and the environment. The strategy is to develop agricultural systems which balance the positives and negatives of farming and to protect the production capacity and wellbeing of the land (Pollock et al. 2007). Several technological options relevant to achieving these goals include: agrobiodiversity (Thrupp 2000), conservation agriculture (Hobbs et al. 2007) and social/political factors which determine farmers' interest in adopting recommended practices (Shriar 2007).

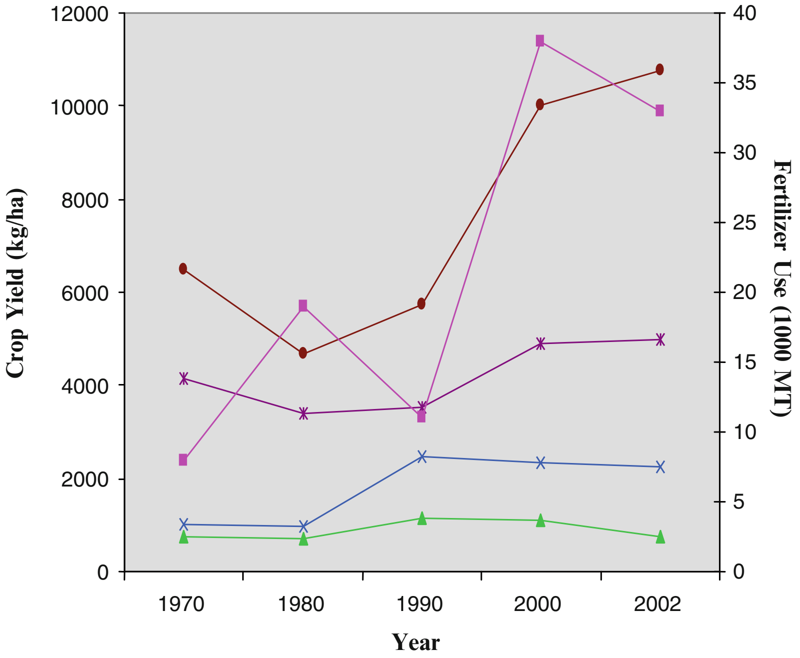

Fig. 9 Trends in fertilizer use and crop yields in Uganda (data compiled from FAO Production Yearbooks)

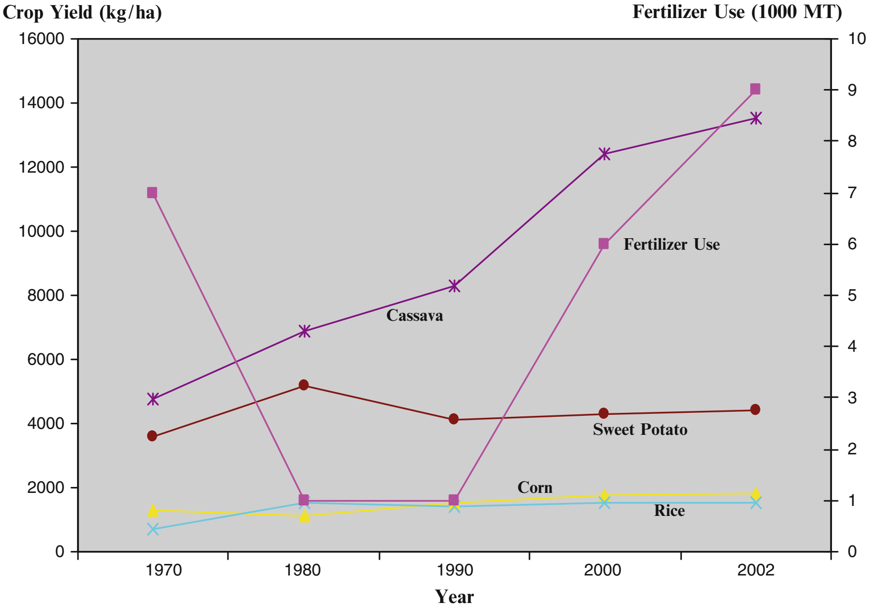

Fig. 10 Trends in fertilizer use and crop yields in Senegal (data compiled from FAO Production Yearbooks)

Recommended practices are those that enhance eco-efficiency or the sustainable use of resources in farm production and land management (Wilkins 2007; Pretty 2007).

In addition to advancing food security, soil C management is also important for controlling the abundance of CO_2 in the atmosphere (Dyson 1977). While the precious natural resource (SOM pool) competes with biofuel for alternate uses of crop residues (Jenny 1980) and for improving nutrient flow to plants (Janzen 2006), its judicious management is important for sustainable use of soil resources.

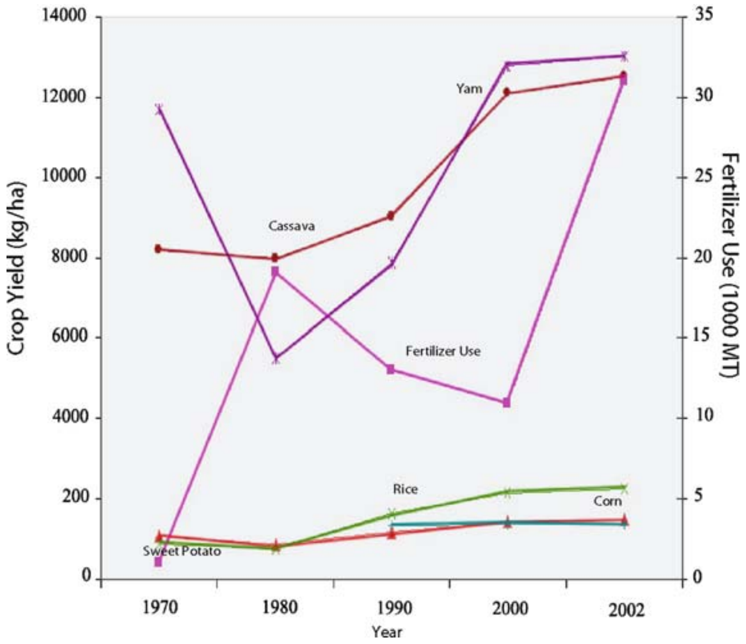

Fig. 11 Trends in fertilizer use and crop yields in Ghana (data compiled from FAO Production Yearbooks)

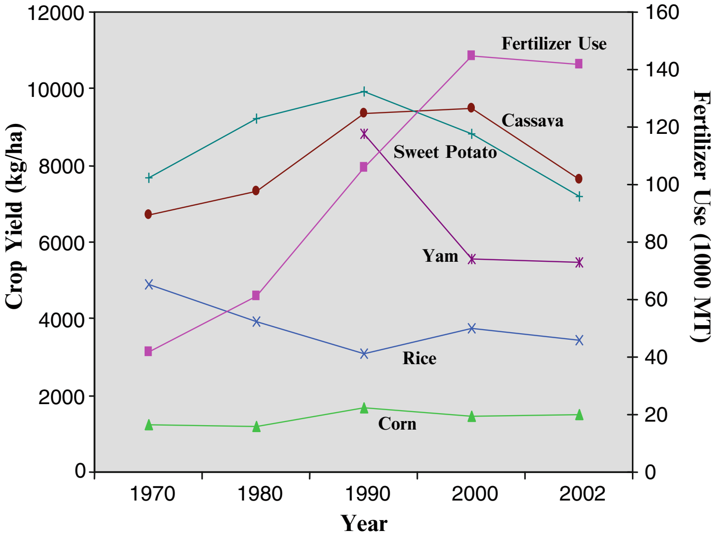

Fig. 12 Trends in fertilizer use and crop yields in Kenya (data compiled from FAO Production Yearbooks)

12 Climate Change and Food Security

Three biophysical factors which need to be addressed are soil quality, water availability or drought stress, and climate change. There is a strong interaction among these factors. For example, adverse effects of soil degradation and drought stress are exacerbated by differences in the amount and distribution of rainfall, and increase in temperature, especially during the flowering stage of crop growth. Adverse effects of soil erosion may also be exacerbated by climate change (Meadows 2003). Over and above the effect of extreme events, equally important is the impact of more gradual changes as well as

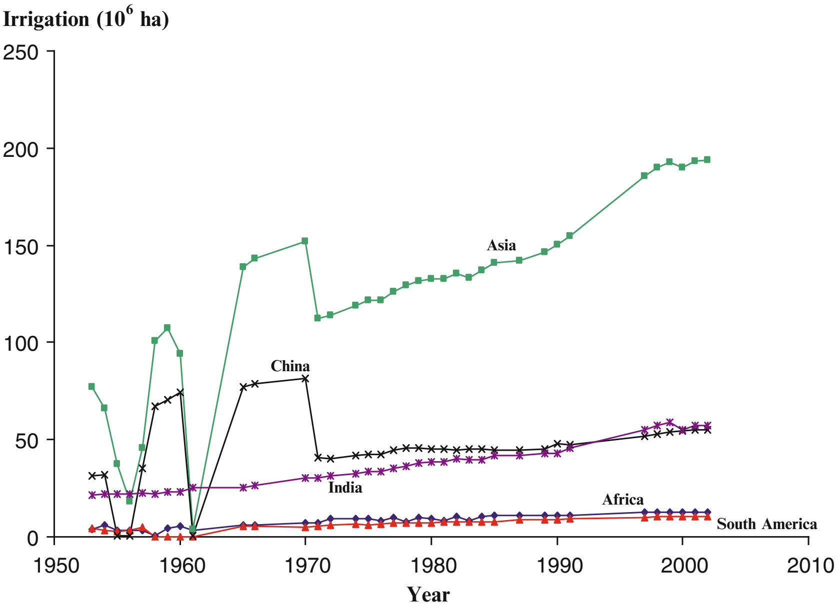

Fig. 13 Regional and national trends in irrigated land area (data compiled from FAO Production Yearbooks)

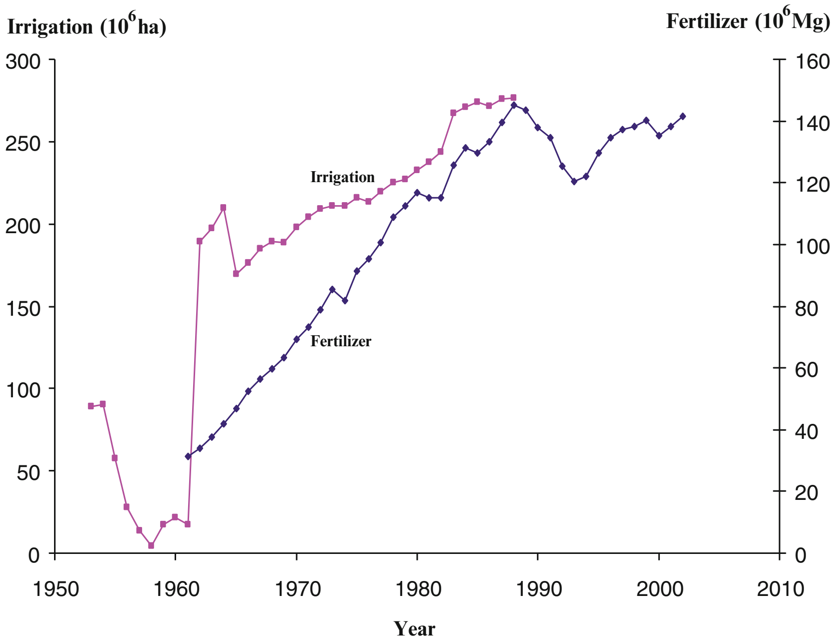

Fig. 14 Trends in global fertilizer use and irrigated agricultural land area (data compiled from FAO Production Yearbooks, and IFDC 2004)

interaction with social and economic factors (Vogel 2005). The effect of governance and economic development cannot be ignored. In some cases, however, there may be a positive effect of increase in atmospheric concentration of CO_2 on grain yield. Walker and Schulze (2006) observed that doubling of CO_2 and increase in rainfall by 10% may increase maize grain yield by 200–1,000 kg ha^{-1} depending on the use of manure and chemical fertilizers, respectively.

Land-use conversion and soil cultivation can be both a source and sink of atmospheric CO_2 (IPCC 2007). Adoption of sustainable practices can make the world's soils a major C sink. Soil C sequestration can off-set fossil fuel emissions and mitigate climate change. Soil C sink capacity is about $78 \pm 12\,\mathrm{Pg}$, equal to the historic C loss from the world's soils (Lal 1999, 2006a,b). The land-based C sink may be decreasing (Canadell et al. 2007), probably due to soil/ecosystem degradation. The rate of C sequestration depends on soil and crop management, and soil type and climate (Lal 2004; IPCC 2000), and can be as much as 1 Pg C per year for the next several decades (Pacala and Socolow 2004). Increase in the soil C pool is important for improving soil quality, and enhancing agronomic production (Lal 2003, 2006a,b). It is in this context that C sequestration in soils is deemed a win-win situation. While advancing food security through increase in use efficiency of input in soil of improved quality, it also accentuates numerous ancillary benefits (e.g., water quality, biodiversity).

Most productivity models, however, have indicated adverse effects of climate change on agronomic production in Africa. Parry et al. (2005) predicted that the region of greatest risk to decline in food production is SSA. Furthermore, the impact of climate change on risk of hunger depends on the degree of economic development, with the most adverse effect on low-income and poorly developed communities. Kurukulasuriya et al. (2006) conducted a survey of 9,000 farmers across 11 countries in SSA. They observed that farm revenues are likely to fall with warming for dryland crops and livestock. In contrast, revenues may rise for irrigated crops. In general, irrigated farmers are less sensitive to climate change than dryland/rainfed farmers. Verdin et al. (2005) reported that Ethiopia will need special attention because of persistent dryness and the positive trends in the Indian Ocean sea surface temperature. Gbetibouo and Hassan (2005) observed a distinct shift in farming practices and patterns due to climate change. These include a shift in the crop calendar and growing seasons including disappearance of some crops from climate-sensitive regions. Jones and Thornton (2003) estimated an overall reduction of only 10% in maize production by 2055, equivalent to losses of \$2 billion per year. However, the impact will be highly variable among regions.

13 Biofuel and Food Security Conundrum

Increasing energy demand is a global issue. Modern biofuels, bioethanol and biodiesel produced from grains (corn, soybean) or biomass (crop residues and biosolids from energy plantations such as switch grass, poplar, willow, etc.) are important for the future of sustainable energy (Goldemberg 2007; Goldemberg et al. 2004), but are also intimately linked with food security. One of the major issues is the source of feedstock for cellulosic ethanol (Somerville 2006; Kennedy 2007). Maintenance of SOM being closely linked to the soil application of crop residues, it has been widely recognized that it is either humus or ethanol (Jenny 1980) but not both. Soil application of crop residues is extremely important for soil quality (Wilhelm et al. 2004; Blanco-Canqui and Lal 2007, 2008). It is in this context that Tilman et al. (2006) proposed low-input high-density grassland biomass as a potential source for biofuel feedstock. Despite its importance and the urgency to replace fossil fuel by alternative energy sources, the impact of using grains or crop residue on food security must be objectively and critically addressed. In addition to the direct impact, food security is also impacted by competition for land, water and nutrients, to establish biofuel plantations. It is the question of a sustainable use of limited resources in a world of a growing population of 6.7 billion in 2008, projected to be 7 billion by 2012 and increasing at the rate of 1.3% per year.

14 Conclusion

The food crisis of 2008 is attributed to numerous interacting factors. Important among these are drought and soil degradation, both of which are especially severe in SSA and South Asia (SA). Then, adverse effects on crop yields are aggravated by high energy costs and poor support services. With predominantly small-sized land-holders, who do not have the resources to purchase the much-needed input, crop yields are low and highly dependent on weather. Weather-dependence of crop yields can only be reduced by improving soil quality, its waterholding capacity and overall soil fertility.

The resource-poor small land-holders in economically less developed regions of the tropics may be more vulnerable to climate change than large-scale commercial farms in developed economies of temperate regions. Therefore, making agriculture less susceptible to climate change implies development of irrigation, establishment of conservation-effective techniques, making fertilizers and soil amendments available to farmers, and development of farming/cropping systems which are less vulnerable to declining effective rains and warming temperatures.

Several promising technologies for restoration of degraded ecosystems and sustainable management of soil resources have existed since the 1960s and 1970s (e.g., NT farming, mulching, growing cover crops and integrated nutrient management). However, these proven technologies have not been adopted, especially in the developing countries of SSA and SA. Creating another income stream for farmers, through payments for ecosystem services (e.g., C sequestration, water quality, biodiversity) may be an important strategy to promote adoption of improved technology (Flugge and Abadi 2006). Involving farmers in the decision-making process, in choosing and implementing technological packages, is another important consideration.

Soils must not be taken for granted. While adoption of improved varieties is important, agronomic production can neither be improved nor sustained unless soil quality is restored and maintained. Maintaining soil quality and water resources at optimal level is essential to realizing the potential of improved varieties. Degraded soils do not respond to other inputs unless their physical, chemical and biological quality is restored. Even the GM crop varieties cannot extract water and nutrients from soil where they do not exist. Agronomic management of soil and water must go hand-in-hand with improved germplasm.

Seeds of the second Green Revolution will be sown in improved and restored soils which have a favorable soil moisture regime, optimal soil structure, and positive carbon and nutrient budgets.

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Denitrification at Sub-Zero Temperatures in Arable Soils: A Review

Rebecca L. Phillips

Abstract Nitrogen (N) in agricultural fertilizers is denitrified by soil bacteria when oxygen is limited, which effectively removes plant-available N from the soil to the atmosphere. Reported denitrification rates range from 0 to 239 $\mathrm{kg\,N\,ha^{-1}\,yr^{-1}}$, and, depending upon environmental conditions and management, may reduce the amount of N available for crop growth by 27%. Denitrification in soils also results in emissions of nitrous oxide (N_2O), which is a recognized pollutant that contributes to stratospheric ozone destruction and radiative forcing in the troposphere. Practitioners of sustainable agronomy aim to improve plant N-use efficiency and reduce emissions of the greenhouse gases by synchronizing N application and plant nutritional requirements. However, it is difficult to predict denitrification rates during and after the growing season based on current knowledge. High rates are consistently reported in irrigated cropping systems following heavy applications of fertilizer-N, but few studies report denitrification during the dormant season. Denitrification in winter may represent a significant sink for fertilizer-N in cropping systems, but further research at sub-zero soil temperatures is needed. Here, the three factors required for microbial denitrification: limited O_2 availability, electron donors and electron acceptors, are reviewed based on soil research performed both above and below 0°C. Gaps in the knowledge of denitrification rates in cropping systems, particularly when soils are frozen, are identified. Sustainable management of N in cropping systems such as greater N-use efficiency and lower greenhouse gas emissions could be advanced by greater understanding of denitrification in winter.

Keywords Fertilizer • Nitrogen • Nitrous oxide • Sub-zero temperatures

1 Introduction

The greatest agronomic uncertainty in balancing the nitrogen (N) budget of agricultural landscapes is the rate of denitrification, which converts plant-available N into gaseous N (Galloway et al. 2004). Specifically, it is not known when denitrification in the rooting zone reduces the availability of N to crops or the magnitude of N losses via denitrification. Current average N-use efficiency in cropping systems (% recovery of applied N) is reported to range from 30 to 50% (Cassman et al. 2002). A major reason for low N-use efficiency is the loss of gaseous-N from agricultural soils worldwide (Davidson and Seitzinger 2006). Denitrification may transfer up to 27% of agricultural N back to the atmosphere (Bouwman et al. 2005). However, spatial and temporal heterogeneity in denitrification rates, lack of quantitative data and inconsistencies between laboratory vs. field measurements contribute to uncertainties in the rate of denitrification, despite decades of research (Davidson and Seitzinger 2006).

Achieving synchrony between N supply and crop demand without sacrificing yield or protection of the environment requires greater knowledge of denitrification rates, yet knowledge of denitrification during the dormant season is limited. In many cases, gaseous-N

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losses at sub-zero soil temperatures are unknown or considered negligible. Consequently, wintertime N losses are rarely considered in crop fertilization recommendations. The body of evidence suggests microbes are physiologically active when soils are frozen (Clein and Schimel 1995; Mikan et al. 2002; Price and Sowers 2004; Rivkina et al. 2000), calling for agronomists to question what is known about the physicochemical and biological properties of soil below 0°C. Microbial emissions of N_2O and N_2 occur at sub-zero soil temperatures (Phillips 2007; Röver et al. 1998), but processes controlling denitrification rates in frozen soils are currently unknown. Gaps in the knowledge below 0°C need to be filled because there may be unforeseen opportunities for conservation of fertilizer-N and for reductions in greenhouse gas emissions during winter.

Denitrification is a ubiquitous process, occurring globally in both terrestrial and aquatic ecosystems (Davidson and Seitzinger 2006). This review focuses on denitrification through the process of anaerobic microbial respiration known to occur in sub-oxic soil microsites (Myrold and Tiedje 1985; Parkin 1987) specifically in cropped soils. Other, non-respiratory pathways observed in aquatic systems (e.g., chemodenitrification, aerobic ammonium oxidation) are outside the scope of this review (Hulth et al. 1999; Kuypers et al. 2005). Three fundamental factors are required for anaerobic microbial denitrification: (1) suboxic or anoxic conditions (herein referred to anoxic), (2) electron donors (herein referred to as organic C), and (3) electron acceptors nitrite (NO_2^-) or nitrate (NO_3^-), (herein referred to as NO_3^-). Each factor is reviewed separately with respect to denitrification when soil temperatures are greater or less than 0°C. Research that is necessary to unravel how denitrification might occur at sub-zero soil temperatures is proposed.

2 Denitrification Overview

Denitrification is classically defined as the microbial oxidation of organic matter, where NO_3^- is the terminal electron acceptor. It is a heterotrophic process of anaerobic respiration conducted by facultative bacteria using oxidized forms of N to accept electrons when O_2 is limited (Firestone et al. 1980). The end product is N_2, but some intermediate compounds (such as N_2O and NO) may also be produced, depending upon environmental conditions (Firestone and Davidson 1989). The primary factor controlling the rate of denitrification is O_2 availability because in sub-oxic conditions (<0.2 mg O_2 L^{-1}), some facultative microbes that normally use O_2 as an electron acceptor will use NO_3^- (Firestone et al. 1980; Seitzinger et al. 2006). Sub-oxic, as defined here, is three orders of magnitude lower than the density of O_2 in moist air (290 mg O_2 L^{-1}). Numerous laboratory incubation studies indicate that, for similar soils incubated at a constant temperature, denitrification rates can be manipulated by varying percent water-filled pore space (%WFPS), electron donors, and electron acceptors (Firestone and Davidson 1989; Myrold and Tiedje 1985; Sexstone et al. 1988). Numerous field studies indirectly point to these factors by reporting how differences in drainage (O_2 status), soil organic matter form and quantity (electron donors), and fertilization form and application (electron acceptors) alter rates of denitrification (Aulakh et al. 1984, 2001; Hofstra and Bouwman 2005). Accordingly, for a given soil and temperature, the kinetics of denitrification can largely be explained by these three factors.

An intermediate gaseous product of denitrification, N_2O, has received a great deal of attention (Bouwman et al. 1995; Davidson et al. 2000; Dobbie et al. 1999; Dobbie and Smith 2003; Jungkunst et al. 2006) because of its importance in the processes of ozone destruction and radiative forcing (Prather et al. 2001). The global warming potential of N_2O is nearly 300 times greater than CO_2 by mass (Forster et al. 2007). Two soil microbial processes, nitrification and denitrification typically produce this gas, so N_2O fluxes measured at the soil surface are not necessarily products of denitrification. Losses resulting from complete reduction of NO_3^- to N_2 are rarely measured because the large atmospheric background of N_2 makes it analytically difficult to detect small increases in N_2 from denitrification (Davidson and Seitzinger 2006).

While knowledge is growing with respect to cropping systems and surface fluxes of N_2O in winter (Kaiser et al. 1998; Maggiotto and Wagner-Riddle 2001; Wagner-Riddle et al. 1997), measurements of total gaseous losses of N via denitrification in frozen soils are lacking. Observed fluxes of N_2O at the surface of frozen soils suggest microbial denitrification may occur at sub-zero soil temperatures (Röver et al. 1998) but specific mechanisms are unknown. One question is: how much total N ($N_2O + N_2$) is from denitrification below 0°C and how much is degassed from accumulated products of denitrification prior to freezing?

Current knowledge of how management influences denitrification rates is largely garnered from experiments conducted during the growing season and at laboratory temperatures. Many field and laboratory experiments are conducted by manipulating the three factors required for denitrification (O_2 status, organic C, NO_3^-). For example, greater denitrification rates are found in fertilized cropped soils at high %WFPS (Barton et al. 1999; Hofstra and Bouwman 2005) because O_2 diffusion is restricted under saturated conditions and the proportion of soil volume that is anoxic increases (Sexstone et al. 1985; Smith 1980). In some cases, organic C and N additions will increase denitrification rates (Burford and Bremner 1975; Myrold and Tiedje 1985; Paul et al. 1993). Fertilizer-N and residue inputs might fuel denitrification in frozen soils at high moisture levels during the winter, but empirical studies are needed to estimate N transformation rates and controls in frozen soil. Filling these knowledge gaps during the winter will improve agronomic recommendations, with potentially positive economic and environmental benefits.

3 Water-Filled Pore Space in Frozen Soil

Application of soil %WFPS (1) as a proxy for soil O_2 status is fundamental to current models of denitrification (Firestone and Davidson 1989).

$$\%\mathrm{WFPS} = (\theta_v/\phi) \times 100 \tag{1}$$

where

θ_v = percent volumetric water content, $\theta_m \times \rho_b$

ϕ = percent total porosity = $(1 - \rho_b/\rho_p) \times 100$

θ_m = percent gravimetric water content

ρ_b = soil bulk density ($\mathrm{mg\,m^{-3}}$)

ρ_p = soil particle density ($\sim 2.65\,\mathrm{mg\,m^{-3}}$).

It is a well accepted approximation that at soil %WFPS >70 (where water is liquid), gaseous N emissions are the result of microbial denitrification (Bateman and Baggs 2005; Davidson 1991), although exact %WFPS values vary with soil mineralogy. It is less clear how %WFPS influences microbial denitrification when soil water is transformed to ice. Effects of freezing on the soil physical environment may influence rates of microbial denitrification at sub-zero soil temperatures.

Calculation of soil %WFPS becomes less tractable at sub-zero soil temperatures because the majority of liquid water becomes ice, rendering changes in bulk density (Kay et al. 1985), hydraulic conductivity (Pikul and Allmaras 1985), pore space volume (Loch and Kay 1978), and water content (Pikul et al. 1989). Liquid water in frozen soil is mobile (Pikul and Allmaras 1985) and flows along unfrozen liquid water channels (Edwards and Cresser 1992), which change in thickness as temperature decreases (Anderson and Hoeckstra 1965). Formation of ice pushes soil particles apart to increase soil pore size (Loch and Miller 1975), and ice lenses forms to create additional pores (Kay et al. 1985). The percent of water occupying soil pores is not constant because liquid water content and soil pore space are not constant at sub-zero soil temperatures.

The presence of both ice and unfrozen water in soil could enhance denitrification at oxic/sub-oxic interfaces controlled by the thermal gradient. Oxic/sub-oxic interfaces facilitate transport of oxidized forms of N from oxic to sub-oxic zones (Seitzinger et al. 2006). If these interfaces are present in frozen soil, then the amount of ice vs. unfrozen water could influence denitrification rates. However, the presence of oxic/sub-oxic interfaces is not likely to remain static in frozen soil because the amount of unfrozen water, the thickness of the water films, the size of transport channels, and hydraulic conductivity are controlled by soil temperature (Hoeckstra 1966; Pikul and Allmaras 1985). The temperature gradient continuously transforms ice to films of water (Kay et al. 1985), potentially creating sites for denitrification at oxic/sub-oxic interfaces.

Freezing also induces changes in soil structural stability (Bullock et al. 1988; Lehrsch et al. 1990), which interacts with water and temperature to affect soil pore space. Liquid water is replaced by ice lenses that weaken soil aggregates (Bullock et al. 1988; Edwards and Cresser 1992; Lehrsch et al. 1990). As the frost front moves into the soil and the majority of soil water is transformed to ice, soil cohesion is lost to shearing forces (Bullock et al. 1988). Slightly soluble chemicals precipitate at the surface of soil particles. As the thermal gradient vacillates diurnally and seasonally, ice crystals collapse and return to unfrozen water. As freezing progresses deeper into the soil, water also migrates upward towards the freezing front to increase water content near the surface (Hoeckstra 1966). Soil aggregates frozen at high water contents (>15% v/v) will be more strongly affected by freezing than soil aggregates frozen at lower water contents (Lehrsch et al. 1990), with greater loss of aggregate stability and cohesion across a wide range of soil series (Bullock et al. 1988).

Disruption to the soil matrix as a result of freezing, as described above, is complex and dynamic. Freezing affects soil volume, migration of water, aggregate stability, precipitation of solutes, ice crystal formation, and ice crystal collapse – all of which could alter soil O_2 status. Microbes can remain physiologically active when films of unfrozen soil water are present (Mikan et al. 2002; Priemé and Christensen 2001; Rivkina et al. 2000), and denitrification has been measured in soils at –2 °C (Dorland and Beauchamp 1991; Phillips 2007). Initial %WFPS at freezing may be fundamental to understanding interactions between soil physics and soil microbial activity, but diffusion of O_2 may be limited by other factors. Current %WFPS thresholds for denitrification (Firestone and Davidson 1989) in frozen soil may need adjustment and/or other potential factors (e.g., thermal gradient, ice-filled pore space) considered.

4 Soil Organic Carbon in Frozen Soil

Organic C often limits denitrification in cropped soils at soil temperatures above 0°C (Beauchamp et al. 1989; Burford and Bremner 1975; McCarty and Bremner 1993; Sainz Rozas et al. 2001), but it is uncertain how organic C influences denitrification below 0°C. Alternatively, studies of aerobic microbial respiration below 0°C suggest soil organic C can limit microbial activity (Feng et al. 2007; Michaelson and Ping 2003; Priemé and Christensen 2001; Schimel and Clein 1996) and that aggregate disruption from freezing releases potentially mineralizable C to microbes when soils thaw (Christensen and Tiedje 1990; Priemé and Christensen 2001). Greater microbial respiration observed following freeze-thaw cycles (Christensen and Tiedje 1990; Mikan 2002; Teepe et al. 2004) may be linked to soil organic C (Breitenbeck and Bremmer 1986; Mikan 2002; Schimel and Clein 1996; Skogland et al. 1988), since freeze-thaw events positively influence amounts of small, hydrophilic compounds (Michaelson and Ping 2003) and phospholipid fatty acids (Feng et al. 2007). Suggested mechanisms for C availability following freezing and thawing include rupture of cellular membranes in microbial biomass (Skogland et al. 1988), the release of organic matter previously bound in aggregates (Christensen and Christensen 1991), and exposure of fresh reactive surfaces (Edwards and Cresser 1992). In addition, thawing may enhance the availability of C required for anaerobic respiration through the collapse of ice crystals and diffusion of substrate to anoxic microsites.

Rarely are effects of freezing on microbial activity separated into components of C availability vs. physical soil disturbance. Reported pulses of aerobic microbial respiration in frozen soils may be due to disturbance from aggregate disruption (Edwards and Cresser 1992), release of organic C (Christensen and Christensen 1991; Myrold and Tiedje 1985; Schimel and Clein 1996), changes in the form of organic C (Feng et al. 2007), or to changes in the diffusion of gases or solutes in frozen soil. The causative factor of respiratory activities (physical disturbance, substrate amount/form, diffusion) likely contributes to the magnitude and duration of observed pulses. This may be critical to denitrification questions in agronomy because physical disturbances from freezing can be modified, to some extent, with tillage and residue management. Diurnal and seasonal soil temperature extremes are modulated and depth to frost is reduced with standing stubble (Pikul et al. 1986), with mulch application (Kohnke and Werkhoven 1963), and with reduced tillage (Kay et al. 1985). On the other hand, carbon can limit denitrification (McCarty and Bremner 1993), and addition of plant residues can promote denitrification activity (Aulakh et al. 1984; McCarty and Bremner 1993). Laboratory studies using soils from no-till cropping systems point to greater soil C as the reason for higher denitrification rates, compared to conventional tillage (Aulakh et al. 1984; Liu et al. 2007; van Bochove et al. 2000). Understanding and quantifying effects of management on denitrification in frozen soil require separation of physical disturbance (loss of aggregate cohesion and stability) from release and transport of organic C potentially bound in soil aggregates. From here, if organic C is limiting denitrification, both tillage and residue management recommendations could be balanced to potentially reduce denitrification rates.

5 Nitrogen in Frozen Soils

Effects of fertilizer-N on denitrification have been studied extensively (see review by Hofstra and Bouwman 2005). Fertilized soil at high %WFPS will promote facultative anaerobic bacteria to reduce N oxides and consume organic matter (Firestone and Davidson 1989; Mulvaney et al. 1997). The magnitude and duration of NO_3^- additions varies with soil texture, pH, climate, crop, management, etc (Jungkunst et al. 2006; Kaiser et al. 1998; Nieder et al. 1989; Sainz Rozas et al. 2001). It is generally accepted that cropped soils with high rates of fertilizer-N inputs generally exhibit higher denitrification rates than soils not receiving fertilizer-N additions (Aulakh et al. 2001; Barton et al. 1999; Jarvis et al. 1991; Kaiser et al. 1998). Manures and animal slurries amendments also enhance denitrification rates (Calderón et al. 2004; Ginting et al. 2003; Lessard et al. 1996; Mogge et al. 1999; Paul et al. 1993; Petersen 1999). The proportion of fertilizer-N denitrified in crop fields varies widely across soil series and climates (see review by Nieder et al. 1989), with 2.5% reported in Colorado, USA (Mosier et al. 1986) and 60% reported in Denworth, UK (Colbourn et al. 1984).

The effect of fertilizer-N application on denitrification rates in cropped soils during the winter is less known, particularly when soils are frozen. Field studies instead have reported significant emissions of N_2O (Goossens et al. 2001; Maggiotto and Wagner-Riddle 2001; Ruser et al. 2001; Wagner-Riddle et al. 1997). Nitrous oxide studies are labor intensive, and microbial activity in frozen soil is often assumed to be negligible; consequently N_2O flux data collected at the surface of frozen soil are rare (Phillips 2007; Röver et al. 1998). Evidence of greater microbial N_2O production in fertilized soil during winter suggests that denitrification may occur in anoxic soil microsites at low soil temperatures. Manure amendments were found to increase N_2O flux and denitrification in the field and in soil cores (30 cm depth) incubated at sub-zero soil temperatures (Phillips 2007), but further research is needed to determine the geographic prevalence of denitrification in frozen soils, and how form and timing of fertilizer-N application might alter gaseous N losses in winter.

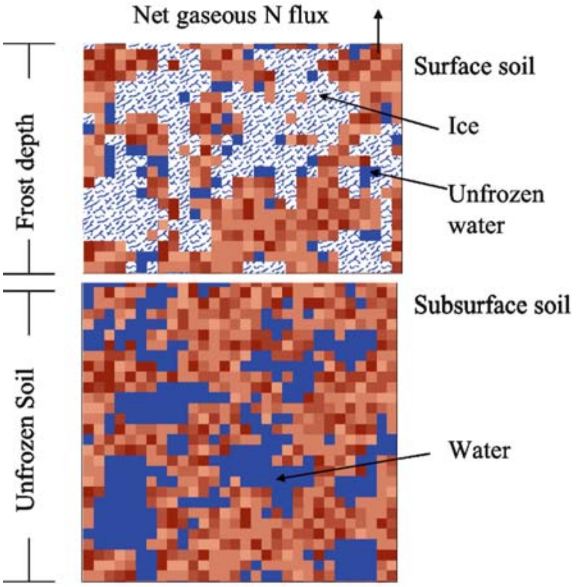

Fig. 1 Conceptual illustration of a soil column where ice and unfrozen liquid water exist in surface soil (frozen soil depth varies with frost depth) and liquid water exists in subsurface soil. *Brown* represents soil, *blue* represents water, and *white with blue* represents ice

Determining the location of denitrification in the soil profile is also paramount to understanding N management in winter (Fig. 1). Denitrification in the soil profile could occur (1) below the freezing front in subsurface soil, (2) in the frozen surface soil, (3) at the interface between frozen/unfrozen soils, or (4) throughout the profile in both frozen and unfrozen soil. In each case, the distance denitrification products would need to travel upward to the surface would significantly affect emissions at the surface because there may be opportunities for complete reduction of NO or N_2O to N_2 as they migrate from below the freezing front to the surface. Further, as suggested earlier, denitrification controls in the frozen surface soil are likely different from denitrification controls in the unfrozen subsurface. Snow cover, residue cover, and tillage will reduce the frozen soil depth; consequently, understanding the vertical distribution of denitrification activity within the soil profile will point to how management might influence N emissions at the soil surface by manipulating soil temperature.

6 Conclusion

What is not known about denitrification in cropped soils at sub-zero soil temperatures far exceeds what is known. Physical differences induced by soil freezing suggest use of %WFPS as a proxy for soil aerobic status is insufficient for predicting heterotrophic anaerobic respiration below 0°C. Organic C may limit aerobic microbial respiration below 0°C, calling into question if organic C might also limit anaerobic respiration. If so, management practices implemented in autumn (e.g., residue incorporation, compost or fertilizer-N application) could promote N and C losses via denitrification, with important agronomic implications (Fig. 2). Moreover, observed fluxes of N_2O in fertilized cropped soils during the dormant season point to potential losses of fertilizer-N inputs via denitrification, but total annual N losses need quantification. The agronomic importance of timing and form of fertilizer-N during the growing season is well known. Less known is how post-season N application influences the N-budget and plant-available N the following spring. Denitrified N losses from cropping systems at sub-zero soil temperatures may or may not amount to a significant portion of the N budget. However, the preponderance of the evidence suggests denitrification should not be considered negligible without further investigation.

A number of agronomic research questions have been raised with respect to the three factors required for denitrification (limited O_2, organic C, NO_3^-), a few of which are summarized below.

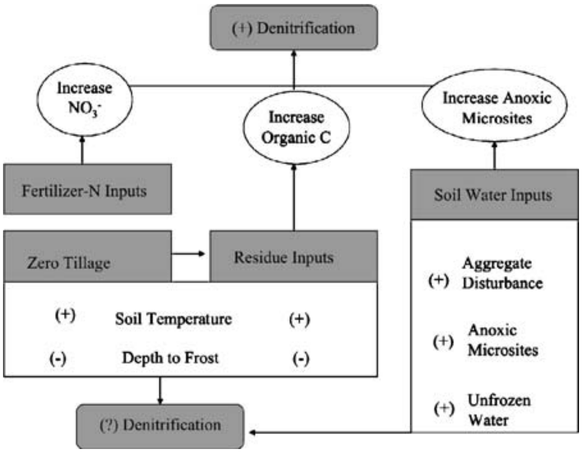

Fig. 2 Summary of potential crop management effects on denitrification in frozen soil. Positive effects are indicated with (+) and negative effects by (−). Some of the indirect effects of management on denitrification in soil, designated by (?), are unknown

- How much N is denitrified during the off-season, particularly when soils are frozen?
- How does water content below 0°C influence denitrification compared to above 0°C?
- What is the O_2 status of frozen soil and how does this change with the advancement of the freezing front?
- How does migration of water below 0°C influence denitrification?
- Is denitrification in frozen soil limited by organic C? How available is organic C to microbes below 0°C?
- How is substrate transport affected by soil comprised of ice and unfrozen soil water? How available are solutes to microbes in frozen soil?
- How is fertilizer-N transformed in frozen soil? At what point in the N-cycle is N transformation inhibited by freezing temperatures?
- Does the type of fertilizer-N applied to crop fields (e.g., compost, urea, anhydrous ammonium) influence denitrification in winter?
- Do plant residue fermentation products enhance denitrification below 0°C?
- Does denitrification occur in both the frozen soil near the surface and in the unfrozen subsurface soil?

The evidence indicates microbial denitrification occurs during the winter in previously-cropped soils at sub-zero soil temperatures, and the potential exists for some mediation with management. However, management studies should be preceded by basic knowledge of how frozen soil conditions alter soil O_2 status and anaerobic transformation of NO_3^- to gaseous N_2O and N_2. From there, questions of soil pH, texture, residue quantity and quality, fertilizer-N form and timing, etc., can be more parsimoniously addressed. Potential economic and climate change implications warrant continued, mechanistic research.

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Re-Thinking the Conservation of Carbon, Water and Soil: A Different Perspective

Thomas Francis Shaxson

Abstract Sustaining soil productivity requires continuing actions of soil organisms on organic materials for optimizing of soil porosity and of movements of roots, water and gases in the root-zone. Soil is more quickly formed and self-renewed from the top downwards than only by slow additions from the bottom upwards. Loss of porosity diminishes soil's infiltration capacity and water-holding potential. Factors that provide insufficient organic substrates for soil organisms and that unduly accelerate oxidation of soil organic matter hinder the self-recuperation of soil and facilitate 'Stage-1' loss of carbon from within soil aggregates. They predispose the soil to lose rapidly even more carbon, in particulate form, through 'Stage-2' losses during consequent processes of runoff and erosion. Forms of land use and management are advocated that favour the functioning of soil-inhabiting organisms, including plants, such that carbon's capture in photosynthesis is increased, its usefulness in the soil as a rooting medium is prolonged, and its subsequent immobilization in the process of sequestration ameliorates the rate of increase of carbon dioxide concentration in the global atmosphere.

Keywords Carbon sequestration • Porosity • Self-recuperation • Soil-organisms • Sustainability

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1 Introduction

> After seven decades of conservation programs that have consumed millions of dollars of economic resources and vast quantities of human resources, soil erosion and subsequent degradation of water resources remain serious environmental issues within the United States. (Napier, 2001)

The same may be said of other countries, in particular those covered by the Intertropical Convergence Zone where high temperatures and unstable masses of moist air lead to heavy storms and high intensities of erosive rainfall (Pereira, 1989). In places where farmers' capacities to manage the soil are insufficient or inappropriate to maintaining it, soil productivity is in decline, or its maintenance is increasingly costly, and the land's use less profitable. However, if even the wealthy USA, which has invested so much over so many years, has not solved this problem, then it is not surprising that other countries which adopted a similar paradigm have also not managed to prevent the degradation of their soil and water resources.

Superimposed on this broad concern is a worry, being addressed at this Colloquium, that carbon selectively removed during erosion processes may be contributing to carbon dioxide in the atmosphere and thus to global warming, adding urgency to the need to find solutions to ongoing degradation of soil and water resources. The deliberately provocative question is therefore implied: "Is 'erosion control' (however defined) the best approach for solving these problems?"

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2 Some Assumptions and Their Consequences

Many of the relevant facts about land degradation and soil erosion had been identified, and their implications assessed, by the 1940s and 1950s (Bennett, 1939; Stallings, 1957). However, in many countries, Governments' subsequent selective interpretations for purposes of developing policies and strategies to minimise land degradation and maintain soil productivity appear to have missed key points and misplaced important emphases.

Pronouncements about accelerated erosion's effects on soil productivity appear to have rested in part on some unquestioned but doubtful assumptions, which *seem to have led* also to some more or less inadequate approaches to controlling erosion. These have been typified by widespread primary reliance, in many Governments' programmes in past years, on physical conservation works such as terraces, bunds, and silt traps. The evidence of this is plain to see on huge areas of cultivated land across many countries of the world. Such works have failed to solve the problem on their own and the problem of carbon loss through soil erosion by water was probably unnecessarily severe. The difficulty appears to arise from a too-narrow an emphasis on soil erosion control and too limited an application of insights from other disciplines in the development of better means for improving and sustaining the resilience and productivity of soils in the face of severely erosive climatic events.

Through much of the earlier literature and policies on soil erosion and its control, common assumptions appear to have included:

(a) That productivity decline is commonly caused by soil erosion, and the relation between the two ought to be definable by knowing the quantities of soil lost: therefore measure soil loss and attempt to predict productivity changes under different scenarios
(b) That soil erosion is some sort of a force in its own right, capable of destroying land: therefore it has to be combated
(c) That runoff is the prime factor in erosion: therefore runoff must be controlled
(d) That soil productivity depends largely on its chemical constituents, and these – as well as clay and organic materials – are selectively eroded: therefore fertilizers must be added to counterbalance such losses in order to maintain/raise yields (Bridges et al., 2001; Doolette and Magrath, 1990)

This line of thinking became to an extent hallowed over time not only by respect for those who first paid serious attention to trying to solve the problems of land degradation on a large scale, but also by repetition of only selected aspects of what these pioneers had propounded. The approach, as applied in practice, has led to much dedicated research and many kilograms of reports; but it also tended to 'tramline' thinking and action towards ever-more detailed investigation of the same parameters, and the application of more refined forms of the same erosion-control technologies. It has not fostered much lateral thinking about the subject as a whole.

So, the common response has been to 'fight erosion' with 'erosion control', which has often translated, primarily through government policies, programmes and projects, into the construction of physical works to control and divert runoff and contain the soil it carries. For many, 'soil conservation' and 'erosion control' appeared almost synonymous. Even with financial inducements, farmers have not responded to this approach very enthusiastically, and these approaches have not often been widely adopted on the one hand nor, on the other, is there much evidence that these physical works, on their own, have improved the quality of the soil (even though they may have slowed the rate of gross soil loss). The justifications for recommending such an approach are further diminished by the difficulty that quantitative erosion-productivity relationships for most soils are not known (Eswaran et al., 2001; Sonneveld., 2002), although such information for a number of soils in tropical and subtropical regions has been clarified in recent years (Stocking et al., 2002; Stocking and Tengberg 1999; Tengberg and Stocking 2001).

A result has been that many conservation enthusiasts tended in the past to assume they were correct in their analyses of land degradation and that farmers were resistant in not sharing the faith or favouring the recommendations made. The only way to improve this unsatisfactory situation appeared to be for governments to pressure farmers more strongly, and/or reward them more generously, until they readily complied. However, the reason for non-adoption by farmers is more likely to have lain in the frustration they felt at not being able to get advice that is relevant to their problem of falling productivity: this implies a need for advice that can be seen to be effective and that is both feasible and of net benefit – or at least of no net disbenefit – to their farming enterprises.

The danger has been that, without adequate proof that such an approach can solve problems, governments might become disillusioned and reduce support for seemingly unproductive investments in 'SWC' (soil and water conservation), as occurred in, for example, the case of Lesotho in 1990 where continuing support for an aid programme for soil conservation was withdrawn (author's experience).

While there has been developing a growing awareness, over the last three decades in particular (Hudson, 1981) of a wider range of ways of addressing the problem, it has still been necessary to point out recently that "there is a need to re-think what we are doing, rather than re-fashioning dated concepts" (Hannam, 2001).

This paper contributes to this necessary re-thinking.

3 Views from a Different Vantage-Point

3.1 Some Anomalous Results

There exists a small number of 'inconvenient' results of erosion's effects on productivity that open a mental door to another perception. Some researchers have recorded certain instances, in research plots and in farmers' fields, where yields have risen rather than fallen after significant erosion has occurred (e.g. in Malaysia [Morgan, 1995], Papua/New Guinea [Kerr, personal communication], and Australia [Sanders, personal communication]. An example of such a condition of the soil is illustrated for example in Lesotho [Shaxson and Barber, 2003]). Though the number of such recorded instances is very small it would be unwise to discard such, apparently anomalous observations as mistakes or as special 'exceptions which prove the rule' because, in fact, they are important pointers to an unacknowledged but significant reality.

These odd observations indicate that the condition of a previously subsurface layer was better for rooting than that of the former surface layer; it was exposed at the surface (before the next season's crop was sown) when the topmost layer had been stripped off through erosion (Shaxson, 1997). This is in direct contrast with the more-common situation, where the subsurface layer that has been newly exposed by erosion is poorer for root growth than that which formerly covered it].

3.2 Different Considerations

Reassessment of some already-known facts allows some alternative interpretations of the assumptions a, b, c, d noted above:

(a) *Difference between yields* before and after soil erosion is more clearly related to differences between in-situ characteristics of the soil as a rooting environment before and after erosion than to the quantity of soil removed (Shaxson and Barber, 2003). The quantity and chemical quality of eroded soil provides an inadequate explanation of soil-productivity decline. Three-dimensional pore spaces and interlinkages of soil particles, organic matter and soil micro-organisms are important for good root-growth and function in the surface layers of soil, as also at sub-surface levels (FAO., 2001; Wild, 1988). While this is acknowledged in the recent study of erosion:productivtiy relations (Stocking and Tengberg, 1999) their damage and loss is more attributed to the erosion process itself, rather than damage to them being perceived as a precondition for accelerated erosion and runoff.

A more-effective strategy to maintain soil productivity should therefore emphasise protecting the soil surface and favouring the improvement of the complex integrity of the soil which remains in place into a good condition for the growth and functioning of roots. In this instance, this is a matter of changed emphasis rather than changed approach: emphasise the accelerated protection and improvement of what remains in situ (positive view) more than regret that which has been lost (negative view).

(b) *Undue erosion* is a foreseeable ecological consequence of changes (often management-induced) in relations between the components of the natural environment both above and below ground – geology, topography, vegetation, hydrology, soil, fauna and flora, all under the influence of climate, gravity, and the effects of people's actions.

From soil not adequately protected from erosive raindrop impact the active movement of eroded soil during a rainstorm is visible manifestation that this adjustment is happening. It may be occurring as a result of a detrimental change in condition of the surface soil, resulting in the ecosystem at that place changing from one level of metastable equilibrium to another, often (but not necessarily always) of lower productivity (Downes 1982).

If this is true, then soil erosion itself is not the first cause of destabilization and soil loss. The predisposing cause is a disturbance to the balance among environmental factors, which is often provoked by people's damage to the soil's cover and its three-dimensional architecture through inappropriate management.

(c) *Runoff control measures* offer barriers/diversions in the lateral dimension, after water has begun to flow down-slope. They have no effect on the prior impact of erosive raindrops falling in the vertical dimension, which cause splashing of soil particles, hammering of the surface, and its interstitial sealing by filtered-out fine particles, with loss of infiltration capacity through the first few millimeters of the soil surface and rapid saturation, thereby provoking runoff at the air/soil interface. If the porous condition of the surface can be maintained and improved by the interpolation of a permeable organic cover between rain and the soil surface, infiltration rates can remain surprisingly high, with little or no partition of the rainfall into runoff.

In this context physical cross-slope works against runoff are 'blunt instruments' for keeping the root-zone intact and in place, and not-very-effective substitutes for (though useful complements to) adequate amounts of cover, and good water-stability of the soil aggregates, against damaging effects of rainfall.

(d) *Soil productivity*, as expressed through plant growth, inheres in the dynamic interactions between its physical × chemical × biologic × hydric constituents that define the soil as a rooting environment, and not merely in a reservoir of useful chemicals (Squire, 1990; Wild, 1988). Both plant roots and soil moisture need to be explicitly identified as constituents, otherwise they tend to get overlooked when hidden within the 'biologic' and 'physical' segments.

Soil moisture at plant-available water potentials is a key control of soil productivity. The effect of its inadequacy on plant growth-functions is almost immediate – within hours or days rather than weeks – not only after the transpiration rate is diminished by slowed water transfer from soil to roots, but also as stressed plants are revived when rainwater re-enters the desiccated root-zone.

A range of pore-sizes enables water to be held in the soil at a range of water-potentials (in kPa) between Field Capacity and Wilting Point which is available to the transpiration stream of plants under the influence of evaporative demand (Allan. and Greenwood, 1999). Pores which are large enough to allow free drainage to below the root-zone allow excess rainwater to pass downwards towards the groundwater.

Components of any soil productivity-enhancing strategy should therefore include (a) whatever is necessary to ensure that rainwater can enter the soil without avoidable hindrance, (b) that the soil is maintained in an appropriately-porous physical condition to retain high proportions, or all, of its soil moisture at low tensions, so that plants can retrieve it readily, and (c) that unproductive water loss by direct evaporation from the soil surface is minimized.

3.3 Soil Porosity and Biological Activity

Soil porosity based on water-stable aggregates is of primary importance in the joint consideration of both soil productivity and soil erosion, because it moderates the movement of water, gases and roots within the soil.

In undisturbed conditions, both the physical processes of wetting, drying and weathering, and – under favourable conditions such as in the forest floor, prairie grasslands, well-managed pastures, and other managed situations rich in organic materials – the effects of biological activity contribute significantly to the build-up and maintenance of soil porosity. This is a consequence of (a) the activities of micro-organisms such as bacteria and fungi in transforming organic materials into humic gums, which cause soil particles to clump-together into irregularly-shaped aggregates, within and between which are the voids which form useful soil pores; (b) the expansion and subsequent decay of roots which leave tubes of various diameters in which organic materials have been disintegrated and transformed; (c) burrowing activities of mesofauna such as termites, worms, and other soil-inhabiting fauna. Water acceptance is very high, a situation less often found under conventional tillage systems than under residue-based no-till systems.

The literature abounds in references to soil organic matter, but the same seldom refer to the parallel necessity for the presence of active soil organisms which can effect its transformations. If the soil is inimical to their activity – too low in organic matter, too hot, too dry, too acid etc. – soil-benefitting transformations do not take place. In the author's experience in Lesotho, for instance, maize-stalks and leaves that had been ploughed-under 3 years previously re-appeared unaltered (and thus of no benefit to soil structure) when the field was again ploughed because there was no biological activity in the soil.

The combination of sufficient organisms, organic materials, water, and nutrients in soil provides for the continuing resurgence of biological activity from year to year. Maintaining a good soil-architecture – a soil with the pore spaces and structural stability that is most conducive to biological productivity – depends on (a) not losing it in the first place, and (b) if it has become damaged, its rapid recuperation. This latter can only be achieved by the microbially-induced transformations of organic matter to form the aggregates that both contain and bound the spaces in the soil, where life goes on, where waters and air move. On the one hand there must be self-perpetuating populations of living organisms to effect such transformations, and on the other there must be permanent or recurrent supplies of organic materials as a sufficient substrate for their activities. These may be variously provided by roots themselves, by the retention of residues from previous crops, and by transporting-in of organic materials from elsewhere as raw or composted additions.

In the majority of agricultural situations (in crop-land, pasture-land, forest-land, range-land), the key factors for avoiding (rather than controlling) runoff and erosion are surface cover and soil porosity. Both depend on living organisms, plants and other soil inhabitants, and their proper husbandry, which thus contributes to extending the useful life of carbon in complexes within the ecosystem and to preventing its premature return back to the atmosphere.

3.4 Tillage and the Loss of Soil Pores

Ploughs and discs and even hand hoes (Shaxson, 1999) through their capacity to shatter aggregates, excessively aerate the soil leading to high rates of organic-matter oxidation, and can cause severe subsurface compaction.

Tillage provokes not only gross physical alteration to soil-architecture but also results in accelerated oxidation of dead organic matter by soil organisms, with untimely release of respired carbon dioxide back to the atmosphere (Mrabet et al., 2001; Reicosky, 2001; Stewart, personal communication).

Exposure of unprotected soil to direct solar radiation also results in breakdown of the complex organic materials which give coherence and stability to soil aggregates, resulting in collapse of soil architecture, loss of pore-spaces and densification of the soil.

This tillage-induced oxidation can quickly negate the carbon-accumulating effects of photosynthesis, thereby short-circuiting and hindering the possibility of any subsequent processes of carbon sequestration by more-profound immobilization.

Repeated oxidation induced by tillage has often also resulted in net depletion of earlier-accumulated soil organic matter (as in chernozems in C. Asia, and other 'over-worked' soils across the world. The process occurs more rapidly in warmer climatic zones of the world.

In different situations some or all the carbon fixed by plants may therefore not persist beyond the first year in the soil – next year's tillage can result in loss of some/most/all the carbon fixed by photosynthesis in the previous year. The benefits of rotational cover-crops and/or rotational pastures in improving soil conditions can be more or less completely undone again by any tillage which subsequently actively incorporates them back into the soil (Hudson, 1981).

Loss of pore spaces from the soil, whether due to compaction, collapse, pulverization, oxidation of organic matter, or interstitial sealing, represents loss of useful voids in the soil matrix, hindering or even preventing exploration by root-hairs and root-tips, expansion of maturing roots, movements of water and gases and, from the moist boundaries of these spaces, the absorption of nutrients (McGarry, 2002).

These spaces are comparable with rooms in a building: all the important activities take place within the voids, not within the structural materials themselves. Their loss is comparable to the effects of demolishing a building: the mass of the rubble, glass, steel beams and other construction-materials is the same, but the value of those materials has vanished because the useful spaces of the architecture have disappeared. Loss of pore-space in the soil diminishes the value of the physical component of productivity.

This damage to soil architecture, at the surface and below, results in quicker saturation of affected soil horizons and an increased likelihood of early onset of runoff.

The improvement of soil by getting organic matter *back* into the profile is best achieved by soil-inhabiting organisms. Their energy comes free of charge and their actions tend, directly or indirectly, to improve the porosity of the soil. Heavy farm equipment's physical effects cannot emulate, nor even simulate, organisms' biological effects in improving soil condition.

3.5 Towards Sustainability – Prolonging the Usefulness of Resources

Throughout the history of agriculture, soil conditions have been modified by people's activities, and native vegetation widely substituted by other plants capable of producing higher yields and/or different plant products of greater use to people. Ideally, the substituted systems of use and management should be at least as stable and biologically sustainable in the face of the range of anticipated recurrent weather conditions as the native ecosystems they have supplanted.

The GAMMA Project of the universities in Montreal defined 'conservation' as 'prolonging the usefulness of resources' (Downes, 1978). In the context of this paper, resources of carbon, water and of life itself (expressed in the forms of e.g. soil-inhabiting plants and organisms) can properly be included in the development of this capacity.

Schrödinger indicated that, "metaphorically, the most amazing property and capacity of life is its ability to move upstream against the flow of time" (Lovelock, 1988) with the capacity to assemble complex energy-rich materials against the otherwise opposite entropic tendency of breakdown to simpler units, which is accompanied by dissipation of energy as time progresses. This capacity of life provides a common thread which interconnects both concepts and dynamic aspects of 'ecosystems', 'soil health', 'resilience' (of both soils and plants), 'sequestration and combination of carbon', 'self-recuperation capacity' (of ecosystems and their living components), and 'sustainability'.

Improvement of the soil as a rooting environment leads to more carbon-capture by plants, increasing the cycle of mutual benefit. It also favours retention of this carbon within the soil, and further prolongs its usefulness by increasing the net amounts which are subsequently sequestered in relatively-immobile forms. The concomitant sustaining of soil quality and of water-holding and water-transmitting capacities can greatly diminish the volumes of surface flow of excess water, and hence most or all losses of organic-matter particulate fragments in erosional runoff.

Better management of landscapes therefore contributes significantly not only to increased stability and productivity (of water and of plants) in agricultural and other ecosystems (Haigh and Gentcheva-Kostadinova, 2002; Shaxson and Douglas, 2004) but also, and simultaneously, to lessening the quantities and detrimental effects of carbon dioxide gas in the global atmosphere.

Managing an organic-rich agriculture ensures the regular addition of organic materials to the soil surface, by crop residues and cover-crops, manures etc. These protect the soil surface, provide food for the soil organisms and raw materials for transformation by them, and keep humic materials already within the soil in conditions that are shielded from ultra-violet solar radiation, which is capable of breaking chemical bonds in organic molecules.

Results from unirrigated residue-based No-till (NT) systems for a range of crops in Brazil (Landers, 1998), from mulching experiments in many situations – (e.g. on young tea in Malawi [Tea Research Stations, 1963] and from NT wheat in central Italy [Pisante, personal communications] and Morocco [Mrabet, 2002] in areas of annual rainfalls ranging from about 2,000 mm to 300 mm), show that improved surface-cover conditions – by diminishing direct insolation of the soil surface – prolong the usefulness of both rainwater and carbon in the soil. This enabled longer duration of early growth of young plants of tea (a perennial crop), and of duration of grain-filling of durum wheat (an annual crop), by delaying the onset of growth-inhibiting moisture stress when rainless conditions set in during and at the end of a rainy season (Shaxson and Douglas, 2004).

To obtain the full carbon-capturing effects of 'no-till' agricultural systems, it is essential, as a first task, to bring the soil to good condition for water-acceptance (possibly involving subsoiling etc. to break any earlier induced compacted layers at or below the surface). Then the retention of crop residues in the field becomes the means of ensuring net additions of organic matter, which are gradually and progressively incorporated by the soil biota into the soil matrix of the root zone and below. Such systems have the characteristics of (a) continually adding to the soil surface the raw materials for biological transformation into soil organic matter and (b) reducing rates of oxidation of the same organic matter by precluding unnecessary mechanical disturbance of the soil and thereby avoiding its excess oxygenation.

These aspects of the continuity of biological activity and of self-recuperation over time are fundamental to the sustainability of chosen land uses. These results were formerly achieved by 'resting' the soil (allowing/enabling biological self-recuperation of the soil) between phases of intense production. In traditional tropical agricultural systems, 'bush fallow' periods of maybe as long as 50 years were used in long rotational cycles with crops and pastures (Wrigley, 1961; Critchley et al., 1992).

Given today's increasing pressures of population, many small-holders' declining farm size and persistent poverty, a major challenge is to achieve the same degree of sustainability by simulating fallows' restorative effects very much more quickly. Even in rotational copping systems which involve tillage, three options (preferably used together, for synergy) can be outlined:

- Increase the soil's biological capability for recuperation – assist more organic activity.
- Reduce the time during which the soil suffers damage – rotate crops at shorter rather than longer intervals (Hudson, 1981).
- During the period of suffering damage, reduce the severity of its impact: use equipment, pasture-management etc., which is least-damaging to soil in optimum root-favourable condition, in preference to practices capable of causing adverse mechanical disturbance (Shaxson, 1993).

3.6 Soil as a Renewable and Self-Renewing Resource

Many consider soil to be, in practical terms, a non-renewable resource. This perception relates to the slowness with which a soil's deep parent materials are weathered into root-usable materials. However, in situations where organic matter and organisms accumulate on and within the upper horizons of the soil, the rooting-zone is enriched and increased (Shaxson, 1981). Further, it is possible that organic acids moving down from the surface may raise the rate of 'weathering' of mineral particles and so liberate nutrient ions within the root-zone.

Soil can be thus be said to be formed top-down as a recurrent process, almost independent of deep weathering (Wild, 1988) (Fig. 1).

Based on this understanding, soil restoration and development can be actively implemented in agricultural systems, more rapidly than with unimproved fallow periods, by using residue-based zero-tillage systems, as in Brazil (Derpsch, 2001; Landers, 2001a). By the same token, former thinning of soil-depth by

Fig. 1 Progressive top-down formation of the rooting environment through plants and other soil-inhabiting organisms contributing and transforming organic matter, combining with the effects of chemical weathering in the development of a soil. (Kasupe, Malawi, T.F. Shaxson)

erosion may also be reversed by improved forms of soil management that encourage the accumulation of organic matter in the soil surface layer. In different farming situations in a number of countries in the tropics and sub-tropics this is dramatically achieved also with the occasional/rotational use of e.g. *Mucuna* spp. as a dense clambering smother-crop across field-surface (Shaxson, 1999; Stocking, 2003). This provides excellent control of weeds, thick and complete cover to the soil, and large quantities of nitrogen-rich biomass whose fallen leaves produce excellent litter beneath, as a food-source for the soil biota, leading to increase in the soil's content of organic matter. In other situations, various legumes such as *Tephrosia vogelii* and Vetches (*Vicia* spp.) are used to achieve similar improvements in soil quality (Shaxson and Barber, 2003). This accumulation of carbon, with associated improvements in the physical and chemical qualities of a soil, is generally accompanied by a rising potential for increased productivity.

3.7 A Biological Definition of Soil

Many scientists include soil biology as a defining characteristic of a soil. A weathered layer that has no soil is called regolith, or sediment if it has been transported across the environment. A true soil is a biologically constructed zone at the interface between rock and atmosphere. It is reasonable, therefore to argue that soil should be valued more for the dynamics and diversity of its living components, which benefit plant-production, than only for non-living pedological characteristics such as its arrangement of horizons, range of mineral-particle sizes, and/or parent materials.

Arguably, society might may take better care of soil if it were considered less as an inorganic physical unit of mineral particles, air, water and nutrient ions that happens to contain life, but more descriptively as a living system, a complex and dynamic subsurface ecosystem of diverse living organisms (including plant roots), non-living organic matter, and biologically-transformed organic/humic products, which inhabits, modifies and interpenetrates an inorganic mix of mineral particles, air, water and nutrient ions, and which changes dynamically over the fourth dimension of time.

4 Land Husbandry Influences

4.1 Effects of Good Land Husbandry

Principles of good land husbandry (Shaxson, 1993; Downes et al., 1997) are shown in Table 1a,b. In the field they are well illustrated by an increasingly large number of Brazilian farmers, for instance, who have developed integrated residue-based farming systems with minimal soil disturbance whose total area has grown from around 1,000 to more than 14 million ha in 30 years (Pieri et al., 2002). These systems specifically pay attention to improving the protection and sustainability of soil productivity (Fig. 2). They combine commercial crops, legumes and cover-crops with no-tillage and direct drilling in rotational systems of farming. The principles of soil protection and improvement may be extended to managed pasture-based systems also. Benefits include, among others: greater yields and their stability in the face of unpredictable vagaries of weather/rainfall; improved conditions of soil architecture accompanied by greater water-holding capacity; lowered costs of production; release of space and time for diversification of crops and of people's activities; much-reduced soil erosion and surface runoff; more-reliable and increased stream flow; reduced floods and related infrastructure damage; reduced costs of maintenance of rural roads and of water-treatment for urban consumption (Landers, 1998, 2001; Mrabet et al., 2001).

Increased attention to prolonging the usefulness of carbon (in organisms and organic matter) on and in the soil is being shown, by e.g. the Brazilian experiences with residue-based NT systems, also to have positive hydrological effects – via benefits to soil porosity as well as to water storage, not only as soil moisture but also to groundwater. The results are (a) to diminish the duration of, and damage to plant production by, infrequent but significant periods of drought; (b) to make streamflow more regular and prolonged, whose corollary is to lessen the frequency, duration and severity of floods, following uncommon amounts of rainfall (Landers, 1998).

Conventional soil bulk-density measurements alone may not adequately explain observed positive improvements in water function because soils under residue-based NT systems are also penetrated by scattered but significant large diameter wormholes and root tubes which may not be adequately sampled.

Table 1a Better land husbandry – altering some technical perceptions

Newer view	Older view
1. Chief causes for concern are (a) decline of land's in-situ productive potentials, and (b) insufficiency of soil moisture.	1a. The primary cause for concern was with quantities lost of soil particles and water.
2. Improving and managing soil to ensure optimum rainwater absorption and retention will have more sure and widespread effects on plant production than only constructing physical cross-slope works to catch or direct runoff water and soil already on the move.	2a. It was commonly assumed that cross-slope physical conservation works would result in significant increases in yield, by holding back soil, water and nutrients in narrow bands across the slope.
3. Accelerated runoff and erosion are foreseeable ecological processes, and *consequences* of other aspects of land degradation.	3a. Accelerated runoff and erosion were visualised as primary active *causes* of land degradation.
4. Post-erosion yields at any site after erosion are closely related to the quality of soil which still remains in situ.	4a. It was generally assumed that decline in yields post-erosion could be related closely to quantities of water, soil particles and plant nutrients lost in the erosion process.
5. Rainfall's erosivity can be minimised by breaking the force of large raindrops by ensuring some form of cover over the soil surface.	5a. Erosivity of rainfall was usually implicitly assumed to be an unalterable feature of each rain event.
6. Soil's erodibility is increased or diminished over time by effects of management of the soil.	6a. Erodibility of a soil series was assumed to be an inherent characteristic of that series.
7. More intensive use of land at a particular site – such that it (a) improves soil architectural conditions by favouring soil-organic transformations and minimising tillage-damage, and (b) increases density, duration and frequency of cover over the soil – can improve rather than diminish conservation-effectiveness of the particular use.	7a. If at a particular site the land use was 'too intensive' for the Land Use Capability classification of that site, it was recommended to reduce the land use intensity until it matched that permitted for that Class.
8. Increased production of plant parts – with improvements in soil architectural conditions, and in the amounts of cover over the soil – is an effective way of achieving conservation of water and soil as a consequence of better husbandry within the farm production system.	8a. It was usually insisted that soil conservation be done/implemented before yields could rise.
9. Because the land system is dynamic, maintaining its capacity to continue producing what we want requires its active and conservation-effective management over time, at the same time as any re-allocations of land uses and imposition of any necessary physical works.	9a. It was implied that land would be least subject to erosion when its uses are allocated across the land in accordance with maps of 'Land Use Capability Classification', and treated with types and layouts of physical and biological conservation measures.
10. Solving problems of low productivity and of erosion and runoff requires an inter-disciplinary approach to match the inter-relatedness of the problems' causes. etc.	10a. It was assumed that soil conservation required a mono-disciplinary specialist approach, independent of other specialisations, and needing separate institutional arrangements. etc.

Table 1b Better land husbandry – altering some socio-economic perceptions

Newer view	Older view
11. Farm families have their own observations and perceptions about land degradation, and other views of the reality than those of non-farm agriculturists/specialists: they should be allowed to judge what is best in their situation.	11a. Specialists' perceptions of the land degradation problems and solutions were presumed to be the correct ones: outsiders should judge what is best.
12. The rural community, and the development of its abilities to manage its own environment, is the most appropriate focus of development assistance.	12a. Land conservation, production and economic efficiency have usually been proposed as the primary foci for development assistance.
13. Resource-poor small farmers have considerable knowledge about their environments, and make rational decisions about allocation of their resources within the 'envelopes' of constraints within which they make those decisions; the challenge is to lessen constraints and improve the shape of the 'envelope'.	13a. It was implicitly, or even explicitly, assumed that small resource-poor farmers are by nature conservative, irrational and ignorant of good land use; the task was to change farmers' rationality.

Table 1b (continued)

Newer view	Older view
14. Rural families ultimately decide what will be done on the land, and whether it would be in their interests to change according to recommendations; resource-poor small farmers are more vitally concerned than any outsider to maintain their lands' productivity in both the short and long term.	14a. Governments assumed that they decide what would be done on the land, as they assumed they had a greater long-term concern to maintain productivity and halt land degradation than do small farmers with (supposedly) short-term time-horizons.
15. To get conservation-effective agriculture improved, it is important to start from where people are now, assist them to do better what they are already trying to do, and remove constraints that inhibit their doing better.	15a. Adoption of recommended changes and innovations were promoted as being essential for getting agriculture moving.
16. A community, and the land it occupies and uses, is the optimum focus for village planning, and for integrating inputs of various 'disciplines'.	16a. The topographic catchment/watershed, with the people it contains, was stated to be the logically optimum unit for planning, and for demonstrating the effects of technical recommendations.
17. 'Participation' signifies technical advisers participating with farm families in helping people to identify and rank their most important problems, to decide what do about them, to implement decided actions, and to monitor the outcomes.	17a. 'Participation' was commonly taken to mean 'the people participating in implementing plans', devised by outsiders, which are considered good for them.
18. Advisory workers should be promoters of dialogue and of two-way information-transfer, catalysts of interactions, and facilitators of interchange and of farmers' well-informed actions.	18a. Extension workers were trained as demonstrators and one-way transmitters of information to farm families, in a process of transfer of technology'.
19. Until they have proved themselves to the satisfaction of individual farmers, technical advisers have very low credibility at the outset of their interactions with farm families. etc.	19a. Technical advisers armed with scientific knowledge assumed themselves to be 100% credible from the outset. etc. (Downes et al., 1997).

Fig. 2 From this farmer's own comparative field trial, the clod of soil on the right represents the farm's soil conditions after many years of conventional tillage with disc equipment and removal of crop residues. After less than 5 years of no tillage plus direct-drilling through the retained crop residues, the clod on the left shows the major improvements in organic-matter content and in soil porosity that have been achieved as a result. (Ponta Grossa, Brazil, T.F. Shaxson)

4.2 Effects of Poor Land Husbandry

By contrast, poorly managed systems – which by overgrazing, fire or excessive tillage, for example, allow or encourage breakdown of the complex compounds of carbon ('Stage-1 carbon-loss') in porous soil aggregates – thus pre-dispose the land to lose yet more carbon, as particles of litter and other organic remains, in subsequent processes of erosion and runoff ('Stage-2 carbon-loss').

Concentrating attention only on the Stage-2 carbon-loss (as in 'erosion-control' work) fails to take sufficient account of the effects the preceding Stage-1 loss of carbon from the ecosystem. This sidelines the very serious and far-reaching consequences of poor land husbandry, which include the increasing exposure of the soil surface, decline in soil-structural stability, diminution of soil porosity, lowering of productivity, and consequent increases in occurrence and severity of runoff, erosion, and water stress in plants (Fig. 3).

Fig. 3 Stage-2 carbon loss has occurred due to erosion, following Stage-1 carbon loss via oxidation of organic matter and associated degradation of soil architecture, compounded by compaction due to conventional disk-tillage, over only a few years. (São João de Aliança, Brazil, A.W. Bell)

4.3 The Need for Better Land Husbandry

All farmers who depend on the land try to look after it ('husband' it) in one way or another – some better, some worse. Land degradation and loss of productivity (of both plants and of water) is a usual and widespread result of poor land husbandry. Writings about land husbandry in English (e.g. Shaxson et al., 1989; Chinene et al., 1996) are paralleled by those in French (Roose E., 1996). Improvements in land husbandry are necessary to move from the 'poor' condition (still all-too-common) to the 'good' condition, and then to sustain it. It should aim to assist the plants and other organisms of the chosen agro-ecosystem to optimize between themselves the dynamic relations between the physical × biologic × hydric × chemical components of the soil's productivity, aided by farmers' decisions and actions.

5 Conclusions

The points discussed above appear to accord more closely with agro-ecological realities in the field than do some of those provided by the earlier common paradigm which had been widely accepted for so long. There are, therefore, a number of implications which should alter the balance of emphases in research, training and advisory work, as well as in the policy framework within which they would more-effectively foster better land husbandry leading to biologically-sustainable land uses.

5.1 Implications for Research

Through reading potentially relevant technical literature and reinterpreting the basic research data which is reported there (as well as re-examining one's own understanding of field experiences), it may be found that much of the detail needed to fill in the picture of sustainable organic-rich agriculture, as sketched above, already exists.

However, additional experimentation may be needed to disentangle the real effects of improved soil-moisture conditions in the three dimensions of space and the fourth dimension of time from those of erosion control itself, with respect to their comparative effects on plant growth. There are indications that some of the plant-growth benefits attributed to 'erosion control' are in fact attributable to benefits of additional soil moisture due to the measures used, such as cross-slope trash lines '*fanya juu*' terraces, and conservation banks, where runoff has accumulated locally along upslope sides of the banks, and thus had more time to soak in than where runoff had been diverted along a cross-slope shallow gradient (Hudson, 1992; Hellin and Haigh, 2002; Shaxson, 1999).

Research is needed to determine, in specific situations of cropland, pasture, rangeland, forest land, what proportion of declining production of biomass (at a constant, not rising, input-cost) is due to (a) insufficiency of plant nutrients (as commonly supposed) and/or to (b) root-impedance and soil-moisture deficiency following loss of soil porosity by whatever cause.

The contention that the difference between soil conditions before and after erosion provide a better explanation for yield-difference than the quantity of soil eroded' needs investigation, with a view to resolving the uncertainty about the 'grey box' of soil loss/yield loss relationship (Stocking and Tengberg, 1999; Stocking et al., 2002).

In view of the great need to extend plant growth into rainless periods and dry seasons, emphasis in plant-breeding may be directed to selection for root systems better capable of exploring soils for stored moisture that can freely enter the transpiration stream.

5.2 Implications for Training and Advisory Work

While the difficulties of implementing such an approach and strategy may be considerable in various socio-cultural and agro-climatic situations, notably in small-farmer and subhumid and dryland areas, the ecological principles remain valid in all situations. The challenge is to assist farmers to devise appropriate means of putting them into harmonious practice – using the resources of rainwater, soil, organisms, organic materials, and the energy available to themselves as farmers – to their better advantage and in ways that are simultaneously productive, sustainable and conservation-effective.

This implies the need for training advisory staff in the principles and practice of better land husbandry, in both its agro-ecological and socio-economic aspects, building on, expanding and, where necessary, remoulding knowledge they already have so as better to fit those realities. It is important to appreciate and show the two-way linkages between components at micro-scale (root hairs, soil pores, bacteria, ecosystems etc.) and those at macro-scale (weather, landscape, land use systems, institutions, etc.). A key need in such training is to match the ecology of agro-environmental situations being considered with the ecology of disciplines that teaching staff need to deploy for their training activities.

Moving to organic-rich systems of agriculture with much-improved soil-water relations greatly reduces the hazard of soil erosion at a given place, because the soil is better protected against raindrop damage and is more porous and absorptive. Therefore the technical 'erosion-hazard' class of a particular land unit – commonly assigned VIII-I from 'least safe' to 'most safe' (Shaxson et al., 1977) can be up-graded (e.g. from hazard-class IV to III, etc.), indicating greater flexibility of safe use and a wider range of suitable land-use types which could safely be allocated. By this means the 'marginality' of lands which are increasingly being brought under tillage by small resource-poor farmers can be modified by improving their organic quality and reducing their hazards of being eroded out of production. This would help to resolve the dilemmas encountered when attempting to classify land as 'non-arable' which is already covered with people already making arable use of it, as is often the case on steeplands in the tropical regions (Shaxson, 1999).

5.3 Implications for Policy

Within governments, relatively independent departmental policies which at present are variously aimed at 'soil conservation', 'the environment', 'agriculture' etc. need an overarching agro-ecological policy framework that interconnects the concerns they have in common. These include soil conditions, biomass production, erosion, flooding, sedimentation, irrigation, and related matters, which are all linked through their common features of sustainability of organic potentials, soil porosity and water use efficiency. The basis for such a framework should be a concern to encourage, develop and support systems of land use and soil management that are actively 'pro-biotic' with respect to life in the soil, at the same time discouraging those approaches of the past, which have allowed soils to degrade by default and inappropriate management, and which, in this sense, have turned out to be somewhat 'anti-biotic'.

5.4 A Valid Perspective

The perspective outlined in this paper appears valid for two main reasons:

(a) It suggests some credible alternatives to some commonly-held but doubtful assumptions.

(b) It offers a positive approach to enhancement of resources' value, agricultural sustainability, environmental improvement, and carbon sequestration, in contrast to the negative attitudes surrounding the difficulties of controlling soil erosion and the loss of carbon in runoff.

Prolonging the usefulness of carbon in living organisms and non-living residues in the soil also favours the formation, improvement and self-sustaining of its productivity as the rooting environment, as well as prolonging the usefulness of water within the soil and as streamflow. It thus contributes to the ongoing cyclical capability of plants and the associated soil biota to sequester carbon from the air.

Carbon captured from the atmosphere, in plants and other soil-inhabiting biota, is a key feature of effective strategies not only to minimise further erosional damage of productive soils, but also to recover, recuperate, and improve and maintain the resilience and productivity of those already damaged (Fig. 4).

Fig. 4 Residues from the previous crop provide multiple benefits for soil as the rooting environment: (a) cover which protects it against high-energy raindrop impact, high temperatures and ultra-violet solar radiation; (b) organic substrate for feeding soil-inhabiting organisms; (c) organic gums contributing to formation and stability of soil aggregates for soil porosity; (d) complex organic molecules increasing the cation exchange capacity; (e) recycling of plant nutrients. Avoiding Stage-1 loss of this carbon on and in the soil upholds its integrity and usefulness, minimizes risks of Stage-2 losses by soil erosion, and thus improves and sustains the soil's productivity. (Maize direct-drilled through wheat straw from the previous crop at Chapecó, Brazil. T.F. Shaxson)

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Cropping Systems, Carbon Sequestration and Erosion in Brazil: A Review

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Abstract Soils represent a large carbon pool, approximately 1,500 Gt, which is equivalent to almost three times the quantity stored in terrestrial biomass and twice the amount stored in the atmosphere. Any modification of land-use or land management can induce variations in soil carbon stocks, even in agricultural systems that are perceived to be in a steady state. Tillage practices often induce soil aerobic conditions that are favourable to microbial activity and may lead to a degradation of soil structure. As a result, mineralization of soil organic matter increases in the long-term. The adoption of no-tillage systems and the maintenance of a permanent vegetation cover (Direct seeding Mulched based Cropping system [DMC]), may increase carbon levels in the top-soil.

In Brazil, no-tillage practices (mainly DMC), were introduced approximately 30 years ago in the south in Paraná state, primarily as a means of reducing erosion. Subsequently research has began to consider the management of the crop waste products and effects on soil fertility, either in terms of phosphorus management, as a means of controlling soil acidity, or determining how manures can be applied in a more localised manner. The spread of no-till in Brazil has involved a large amount of extension work. The area under no-tillage is still increasing in the centre and north of the country and currently occupies ca 20 million hectares and covering a diversity of environmental conditions, cropping systems and management practices.

Most studies of Brazilian soils give rates of carbon storage in the top 40 cm of the soil of 0.4–1.7 t C ha^{-1} per year, with the highest rates in the Cerrado region. However, caution must be taken when analysing DMC systems in term of carbon sequestration. Comparisons should include changes in trace gas fluxes and should not be limited to a consideration of carbon storage in the soil alone if the full implications for Global Warming are to be assessed.

Keywords Brazil • Fluxes • Greenhouse gas • Mulch • No-tillage • Stocks

Résumé Les sols constituent le plus gros réservoir superficiel de carbone, environ 1,500 Gt C, ce qui équivaut à presque trois fois la quantité stockée dans la biomasse terrestre, et deux fois celle de l'atmosphère. Toute modification de l'usage des terres et, même pour les systèmes agricoles à l'équilibre, toute modification de l'itinéraire technique, peut induire des variations du stockage du carbone dans les sols. Les pratiques de labour favorisent souvent une aération du sol, qui est propice à l'activité microbienne et peuvent conduire à une dégradation de la structure. Il en résulte sur le moyen et long terme une minéralisation accrue de la matière organique du sol. Du fait de l'absence (ou limitation) des travaux du sol (No-tillage) et d'un maintien d'une couverture végétale permanente (DMC), les systèmes de semis direct favoriseraient la séquestration du carbone et limiteraient l'érosion.

Au Brésil, l'apparition du semi-direct dans la Région Sud, au Paraná date du début des années 70. Un des objectifs majeurs de l'époque était la lutte contre l'érosion, puis les recherches se sont développées

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vers la gestion des résidus de récolte et leur effet sur la fertilité, que ce soit pour la gestion du phosphore, le contrôle de l'acidité ou la localisation des engrais. Cette pratique, qui a pris une grande extension et continue de s'accroître dans le centre et le nord du pays, occupe actuellement entre environ 20 millions d'hectares avec une très grande diversité de milieux, d'agrosystèmes et d'itinéraires techniques.

Au Brésil, la plupart des auteurs donnent des vitesses de stockage du carbone dans des sols sous semis-direct allant de 0.4 à $1,7\,\mathrm{t\,C\,ha^{-1}}$ par an pour la couche 0–40 cm, avec les taux les plus élevés pour la région centrale du Cerrado. Mais certaines précautions sont nécessaires lors de la comparaison, en terme de séquestration du carbone, des systèmes de semis direct avec les systèmes labourés. Les comparaisons ne doivent pas se limiter au seul stockage de carbone dans le sol, mais doivent prendre compte les changements dans les émissions de méthane et d'oxyde nitreux qui sont des puissants gaz à effet de serres.

Mots clés Brésil • Flux • Gaz à effet de serre • Mulch • Semis-direct • Stocks

1 Introduction

Concerns about global warming and increasing atmospheric greenhouse gases concentrations (CO_2, CH_4, and N_2O) have led to questions on the role of soils as a source or sink of carbon (Houghton, 2003). Excluding carbonated rocks, soils constitute the largest surface carbon pool, approximately 1,500 Gt, equivalent to almost three times the quantity stored in the terrestrial biomass and twice the amount stored in the atmosphere. Therefore, any modification of land use or land management can induce changes in soil carbon stocks, even in agricultural systems in which carbon is perceived to be in a steady state (Lal et al., 1997; Six et al., 2002).

No-tillage is presumed to be the oldest system of soil management. In some parts of the tropics, No-tillage is still practiced as part of slash-and-burn agriculture. After clearing an area of forest, by controlled burning, seed is placed directly into the soil. However, as mankind developed more systematic agricultural systems, cultivation of the soil became an accepted practice as a means of preparing a more suitable environment for plant growth. Paintings in ancient Egyptian tombs portray farmers tilling their fields using a swing-plough and oxen, prior to planting. Indeed, tillage as symbolized by the mouldboard plough became almost synonymous with agriculture (Dick and Durkalski, 1997). No-tillage can be defined as a crop production system where soil is left undisturbed from harvest to planting except for fertiliser application.

In the southern part of Brazil, no-tillage was developed in response to soil erosion problems and declining levels of land productivity under "conventionally" tilled systems. The underlying land management principles that led to the development of no-tillage systems were, prevention surface sealing caused by rainfall impact, achievement and maintenance of an open soil structure and reduction of the volume and velocity of surface runoff. Consequently, the no-tillage strategy was based on two essential farm practices: (1) not tilling and (2) keeping soil covered at all times. The particular no-tillage system considered in this paper is referred to as 'a direct seeding mulch based cropping system' (referred here as DMC).

Farming methods that use mechanical tillage, such as the mouldboard plough, secondary tillage tools for seedbed preparation or disking for weed control, can cause soil carbon loss by several mechanisms: (1) by disrupting soil aggregates, which protect soil organic matter from decomposition (Karlen and Cambardella, 1996; Six et al., 1999), (2) by stimulating short-term microbial activity through enhanced aeration, resulting in increased net release of CO_2 and other gases to the atmosphere (Bayer et al., 2000a, b; Kladivko, 2001) and (3) by mixing fresh residues into the soil where conditions for decomposition are often more favourable than on the surface (Karlen and Cambardella, 1996; Plataforma Plantio Direto, 2003). Furthermore, conventional tillage can leave soils more prone to erosion, resulting in further loss of soil carbon (Lal, 2002). DMC practices, however, reduce soil disturbance and often result in a significant accumulation of soil carbon (Sá et al., 2001; Schuman et al., 2002) and consequently a reduction of gas emissions, especially CO_2 (Lal, 1998; Paustian et al., 2000). Furthermore, in Brazilian conditions, the possibility of an earlier seeding date with direct seeding often enables a second crop cycle with a commercial or cover crop. Consequently, more biomass is returned to the system each year. Despite this, there is considerable evidence that the main effect in terms of carbon storage, of no-tillage systems, is seen in the topsoil with little overall effect at deeper layers (Six et al., 2002).

The objective of this paper is to provide a synthesis of the effects of DMC on carbon sequestration and erosion in Brazil.

2 The Expansion of No-Tillage in Brazil

The history of DMC started in the South of Brazil. The first scientific no-tillage experiment was conducted in 1969 by the Federal University of Rio Grande do Sul in the southern part of the country on an area of 1 ha, however the trial was interrupted by the accidental destruction of the no-tillage seeding machine after the first seeding (Borges Filho, 2001). Several other studies were set up at the beginning of the 1970s in Parana state near Londrina and Ponta Grossa (Borges Filho, 2001; Sá et al., 2001; Six et al., 2002). The effectiveness of no-tillage systems in controlling soil erosion and reducing costs encouraged farmers in Parana State to take up the practice. This uptake corresponded with the release of modern herbicides, such as glyphosates, in the 1970s, which made no-tillage systems easier to manage. During the crop year 1974/75, DMC systems were adopted by approximately 235 farms (representing ca 16,500 ha of cultivated land) in Parana State. Until the end of the 1970s the spread of DMC was slow and limited to Parana and Rio Grande do Sul, mainly due to a lack of technical assistance and a lack of studies demonstrating the advantages of DMC. At the beginning of the 1980s, producers began to organize themselves into associations to promote DMC, the most well known being the "Clube da Minhoca" (literally meaning "The Earthworm Club") and the "Clubes Amigos da Terra" (The Friends of the Soil Clubs) (Borges Filho, 2001).

In the Cerrado regions (the Central area of Brazil covered mainly by Savannah), DMC was imported from the South at the beginning of the 1980s. However, the systems had to be adapted somewhat in order to be applicable to this area. Winters in the Cerrado region are dryer and hotter than in the South and summers are hot and humid, inducing a rapid rate of crop residues decomposition. One of the first trails involving DMC was carried out in 1981 at Rio Verde in Goiás State, by Eurides Penha. Penha seeded 200 ha of soybean on soybean residues from the previous crop. The year after, another farmer tried DMC systems with soybean and maize in Santa Helena de Goiás a neighbouring city (Borges Filho, 2001). In the beginning, the expansion of DMC was slow and only really began to accelerate at the end of the 1980s. During the cropping year 1991/92 the area under DMC in the Cerrado region was approximately 180,000 ha representing 13.3% of the total land under DMC in Brazil. From the early 1990s onwards, the rate of expansion of DMC in the Cerrado region became faster than in the rest of Brazil (Fig. 1).

At present, approximately 63 million hectares are under no-tillage systems throughout the world, with the USA having the largest area (∼21.1 Mha) (Derpsch, 2001). The 20 million hectares covered by DMC in Brazil (Febrapdp, 2004) make the country the second largest adopter of the practice in the world. The expansion of DMC in Brazil, has taken place not only as a result of the conversion from conventional tillage in the southern region (72%), but also following the clearing of natural savannah in the central-west area (28%). More recently, due to the high profit margins involved, ranchers in the Amazon region have started to convert old pastures to soybean/millet DMC systems. During the cropping year 2000/01 DMC systems dominated in the Southern region (Parana – 5 Mha, Rio Grande do Sul – 3.6 Mha and Santa Catarina – 1 Mha) and the Cerrado region 4.9 Mha, and was also important in Mato Grosso do Sul (1.7 Mha) and São Paulo State (1 Mha).

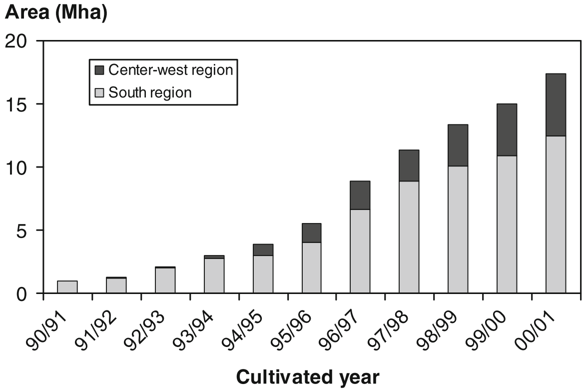

Fig. 1 Cultivated area under DMC systems in Brazil. (Adapted from Febrapdp, 2004)

3 Carbon Sequestration

For the purpose of this review the term ‘carbon sequestration’ is used according to the definition given by Bernoux et al. (2006). “Soil carbon sequestration”, for a specific agro-ecosystem in comparison with a reference one, should be considered as the result (for a given period of time and portion of space) of the net balance of all greenhouse gases, expressed in $C{-}CO_2$ equivalent or CO_2 equivalent, computing all emissions sources at the soil-plant-atmosphere interface, and also all the indirect fluxes (gasoline, enteric emissions, . . .). When comparing a DMC system with a conventional tillage system this means that not only is carbon storage taken into account, but the resulting greenhouse gases fluxes such as N_2O and CH_4 at the field and farm level are also taken into account.

3.1 No-Tillage, Conventional Tillage and Carbon Storage

Lindstrom et al. (1998), reported that globally, conservation or reduced tillage can store 0.1–1.3 t C ha^{-1} yr^{-1} and could feasibly be adopted on up to 60 percent of arable lands. These estimates depend on continued use of conservation tillage. Use of intensive tillage or mouldboard ploughing can negate or offset any gains made in carbon sequestration.

Changes in soil carbon stocks under no-tillage have been estimated in earlier studies for temperate and tropical regions. Cambardella and Elliott (1992), showed an increase of 6.7 t C ha^{-1} in the top 20 cm in a wheat-fall rotation system after 20 years of no-tillage, compared with conventional tillage. Reicosky et al. (1995) reviewed various publications and found that organic matter increased under conservation management systems with rates ranging from 0 to 1.15 t C ha^{-1} yr^{-1}, with highest accumulation rates generally occurring in temperate conditions. Lal et al. (1998), calculated a carbon accumulation rate of 0.1–0.5 t C ha^{-1} yr^{-1} in temperate regions. For the tropical west of Nigeria, Lal (1997) observed a 1.33 t C ha^{-1} increment during 8 years under no-tillage as compared to the conventional tillage of maize, which represents an accumulation rate of 0.17 t C ha^{-1} yr^{-1}. More recently, a review by Six et al. (2002) reported that in both tropical and temperate soils, a general increase in carbon levels ($\approx 325 \pm 113$ kg C ha^{-1} yr^{-1}) was observed under no-tillage systems compared with conventional tillage.

In the tropics, specifically in Brazil, estimates of the rate of carbon accumulation have generally been restricted to the two main regions under DMC (the south and central west). In the southern region, Sá (2001) and Sá et al. (2001) estimated a greater accumulation rate (0.8 t C ha^{-1} yr^{-1} in the 0–20 cm layer and 0.9 t C ha^{-1} yr^{-1} in the 0–40 cm layer) after 22 years under DMC compared to the same period under conventional tillage. The authors mentioned that accumulated carbon was generally greater in the coarse ($>20\,\mu$m) than in the fine ($<20\,\mu$m) particle-size-fraction, indicating that most of this additional carbon is weakly stable. Bayer et al. (2000a, 2000b), found a carbon accumulation rate of 1.6 t ha^{-1} yr^{-1} for a 9-year DMC system compared with 0.10 t ha^{-1} yr^{-1} for the conventional system in the first 30 cm layer of an Acrisol, in the southern part of Brazil. Corazza et al. (1999) reported an additional accumulation of approximately 0.75 t C ha^{-1} yr^{-1} in the 0–40 cm soil layer due to no-tillage, in the Cerrado region located in the centre-west. Estimates by Amado et al. (1998, 1999) indicated an accumulation rate of 2.2 t ha^{-1} yr^{-1} of soil organic carbon (OC) in the first 10 cm layer. Other studies considering no-till systems carried out in the central-western part of Brazil (Castro Filho et al., 1998, 2002; Lima et al., 1994; Peixoto et al., 1999; Resck et al., 2000; Riezebos and Loerts, 1998), reported soil carbon accumulation rates due to no-tillage, varying from 0 to 1.2 t C ha^{-1} yr^{-1} for the 0–10 cm layer.

More detailed accumulation rates are reported in Table 1. Rates are organized by region and are derived from published and unpublished material. In the Cerrado region carbon accumulation rates vary from 0.4 to 1.7 t C ha^{-1} for the 0–40 cm layer, which is similar to the range found in the Southern region (−0.5 to 0.9 t C ha^{-1}). Mean rates of carbon storage were similar among “Cerrado” (0.65 t C ha^{-1}), “South” (0.68 t C ha^{-1}), and “Other” (0.60 t C ha^{-1}) regions, when the soil surface layer was considered (0–20 cm). More variability was found in the Southern region (−0.07 to 1.6 t C ha^{-1}) for the 0–20 cm layer, than in the other regions. However, it is important to mention that these mean values aggregate different soil and crop types and the variability is high. For instance,

Table 1 Carbon storage rates (accumulation following conversion of a conventional tillage system to DMC) in DMC systems in Brazil

Place	State[a]	Succession or dominant plant[b]	Reported soil classification	Clay (%)	Layer (cm)	Duration (year)	Rate (t C/ha)	Sources
Cerrados region								
Planatina	DF	S/W	Latossol (Oxisol)	40–50	0–20	15	0.5	Corazza et al. (1999)
					0–40	15	0.8	
Sinop	MT	R – S/So – R/So – S/M – S/E	Latossol (Oxisol)	50–65	0–40	5	1.7	Perrin (2003)
Goiânia	GO	Rice/soya	Dark red Latossol		0–10	5	0.7	Ud
Rio Verde	GO	M or S/Fallow S/M or So or Mi	Red Latossol	45–65	0–20	12	0.8	Scopel et al. (2003)
Not specified	?	M or S	Dark Red Latossol (Oxisol)	>30	0–40	16	0.4	Resck et al. (2000)
South region								
Londrina	PR	W/S	Oxisol		0–10	22	0.31	Machado and Silva (2001)
					0–20	22	0.25	
					0–40	22	−0.17	
Londrina	PR	S/W – S/L –M/O	Red Latossol		0–20	7	0.5–0.9	Zotarelli et al. (2003)
Londrina	PR	S/W/S or M/W/M or S/W/M	Oxisol Typic Haplorthox		0–10	14	0.4[d]	Castro Filho et al. (1998)
					0–20		0.2[d]	
Londrina	PR	S/W/S or M/W/M or S/W/M	Oxisol Typic Haplorthox		0–40	21	0[c]	Corazza Filho et al. (2002)
Ponta Grossa	PR	(S or M)/(O or W)	Oxisol Typic hapludox	40–45	0–40	22	0.9	Sá et al. (2001)
Tibagi	PR	(S or M)/(O or W)	Oxisol Typic hapludox	40–45	0–40	10	−0.5	Sá et al. (2001)
Tibagi	PR	M/W – S/O – S/O	Red Latossol Oxisol	40–45	0–10	22	1.0[d]	Venzke Filho et al. (2002)
Tibagi	PR	M/W-S/O-S/O	Red Latossol Oxisol	42	0–20	10	1.6	Siqueira Neto (2003)
Toledo	PR	S/O	Haplic Ferrasol		0–10	3	−0.68[d]	Riezebos and Loerts (1998)
		S/O	Haplic Ferrasol		0–10	10	0.37[d]	
Passo Fundo	RS	W/S	Oxisol		0–10	11	0.59	Machado and Silva (2001)
					0–20	11	−0.07	
					0–40	11	0.29	
Passo Fundo	RS	W/S	Red Latossol Typic hapludox	63	0–30	13	0[c]	Sisti et al. (2004)

(continued)

Table 1 (continued)

Place	State[a]	Succession or dominant plant[b]	Reported soil classification	Clay (%)	Layer (cm)	Duration (year)	Rate (t C/ha)	Sources
		W/S-V/M			0–30	13	0.4	
		W/S-O/S-V/M			0–30	13	0.7	
Passo Fundo	RS	W/S	Red Latossol Typic hapludox	63	0–10	11	0.3	Freixo et al. (2002)
					0–20	11	0[c]	
					0–30	11	0[c]	
		W/S – W/M	Red Latossol Typic hapludox		0–10	11	0.4	Freixo et al. (2002)
					0–20	11	0.2	
					0–30	11	0[c]	
Santa Maria	RS	M and Mu/M	Ultisol	15	0–20	4	1.3	Amado et al. (2001)
Eldorado do Sul	RS	M/G	Podzólico vermelho escuro		0–17.5	5	1.4[d]	Testa et al. (1992)
		M/La			0–17.5	5	0.6[d]	
		O/M			0–17.5	5	0.2[d]	
Eldorado do Sul	RS	O + V/M + C	Clay loam Acrisol Typic Paleudult	22	0–17.5	9	0.84	Bayer et al. (2002)
Eldorado do Sul	RS	O/M	Clay loam Acrisol Typic Paleudult	22	0–30	9	0.51	Bayer et al. (2000b)
		O + V/M + C			0–30	9	0.71	
Eldorado do Sul	RS	O + V/M + C	Clay loam Acrisol Typic Paleudult	22	0–17.5	12	1.26	Bayer et al. (2000)[a]
Lages	SC	M or S/W or O	Cambissol		0–20	8	1.0	Bayer and Bertol (1999)
Other regions								
Campinas	SP	S or C/M	Rhodic Ferralsol Typic Haplorthox	60	0–20	3	0.8[de]	De Maria et al. (1999)
					0–20	8	0.4[de]	
Sete Lagoas	MG	M/B	Dark red Latossol Typic Haplustox		0–15	10	0[c]	Roscoe and Buurman (2003)
					0–45		0[c]	

[a] PR = Parana, RS = Rio Grande do Sul, DF = Distrito Federal, SC = Santa Catarina, SP = São Paulo, MT = Mato Grosso, GO = Goiás, MG = Minas Gerais

[b] Dominant succession: W = Wheat (*triticum aestivum*), S = Soybean (*Glycine max*), So = Sorghum (*Sorghum vulgaris*), R = Rice (*Oriza sativa*), E = *Eleusine coracana*, O = Oat (*Avena sativa*), V = Vetch (*Vicia sativa*), M = Maize (*Zea mays*), B = Beans (*Phaseolus vulgaris*), Mu = Mucuna (*Stizolobium cinereum*), C = cowpea (*Vigna unguiculata*), L = Lupine bean (*Lupinus angustifollios*), La = Lablabe (*Dolicbos lablab*), G = Guandu (*Cajanus cajan*)

[c] 0 means that the difference was not significant

[d] Calculated using an arbitrary soil bulk density of $1.2\,\mathrm{g\,cm^{-3}}$

[e] Value reported for OM, C = OM/1.724, ud = unpublished data from Metay

the mean value of 0.68 t C ha^{-1} for the South region was obtained averaging 15 observations (Table 1) and the associated standard deviation is 0.54 t C ha^{-1}.

Some studies performed in Brazil reported that organic carbon contents under DMC and conventional systems can be very similar (Corazza et al., 1999; Freixo et al., 2002; Roscoe and Buurman, 2003; Sisti et al., 2004). Sisti et al. (2004) reported that the soil under native vegetation (measured in areas neighbouring the experimental site) had a high carbon and nitrogen content (37 g C and 3.1 g N per kg soil) in the first 5 cm depth. Carbon and nitrogen content declined to approximately half these values at 10–15 cm layer. The carbon concentration in the top 5 cm of soil was considerably higher in all three rotations managed with DMC compared with the conventional system, although not as high as under the native forest. Machado and Silva (2001) showed decreases in SOC of 23.4% and 47.8%, respectively, at 0–5 cm depth for DMC and conventional tillage systems, when compared to an adjacent non-cultivated area. The study was carried out on an Oxisol in the south of Brazil, following 11 years of soybean–wheat cultivation. However, the authors also found SOC at the 0–40 cm to be the same as the forest soil for both DMC and conventional tillage.

Another important point when comparing soil carbon stocks in DMC and conventional systems is to avoid comparing superficial layers such as 0–5 cm and 0–10 cm. In conventional systems involving tillage, homogenisation occurs in the first 20 cm and thus a superficial layer cannot be compared directly without bias.

3.2 Fluxes of Other Greenhouse Gases

Increasingly, attention is being focused on the relationship between atmospheric carbon dioxide concentrations and carbon concentrations in soil. Carbon dioxide makes the largest contribution to climate change simply because of its abundance in the atmosphere compared with other greenhouse gases. Changes in farming practices, such as altering tillage intensity and crop rotations, may alter carbon sequestration in the soil and thereby help to alleviate carbon dioxide accumulation in the atmosphere. However, fluxes of other greenhouse gases such as N_2O and CH_4 at the field and farm level may be altered by DMC. Six et al. (1999) reported that CH_4 uptake increased ($\approx 0.42 \pm 0.10$ kg $C{-}CH_4$ ha^{-1} yr^{-1}) and N_2O emissions increased ($\approx 2.91 \pm 0.78$ kg $N{-}N_2O$ ha^{-1} yr^{-1}), in temperate soils, under no-tillage when compared to conventional tillage. These increased N_2O emissions lead to a negative global warming potential of the DMC system when expressed on a $C{-}CO_2$ equivalent basis. Global warming potentials are measurements of the relative radiative effect of a given substance (in this case CO_2) compared to another substance and integrated over a determined time period. For example 1 kg of CH_4 is as effective, in terms of radiative forcing, as 23 kg of CO_2. On a carbon or nitrogen mass basis, 1 kg of $C{-}CH_4$ is equivalent to 8.36 kg of $C{-}CO_2$ and 1 kg of $N{-}N_2O$ to 126.86 kg $C{-}CO_2$. The authors studied other changes induced by no-tillage and concluded that "from an agronomic standpoint no-tillage is beneficial, but from a global change standpoint more research is needed to investigate the interactive effects of tillage, fertilizer application methodology and crop rotation as they affect carbon accumulation, CH_4-uptake and N_2O-fluxes, especially in tropical soils, where data on this matter is still lacking". This is particularly true for the N_2O fluxes when legume crops are used as cover-crops or green fertilizer, as some studies tend to show that N_2O emissions may be enhanced as a result (Flessa et al., 2002; Giller et al., 2002).

Few results have been published regarding N_2O emissions in tropical regions. Pinto et al. (2002) showed low NO and N_2O emissions, low nitrification rates and the majority of inorganic N to be in the form of $NH_4{}^+$, all indicative of a conservative N cycle in the Cerrado. Passianoto et al. (2003) suggested that no-tillage regimes will result in lower CO_2 emissions than degraded pastures, but higher N_2O and NO emissions in Amazonia and that the addition of N fertilizer stimulates N_2O and NO emissions. A recent study (Metay, 2004), compared the production and emission of N_2O from two treatments: conventional tillage and DMC (no-tillage and direct sowing in the cover crop after weed-killer application). The main crop was rice (*Oriza sativa*) and the cover crop a fodder grass (*Brachiaria*) with a legume (*Crotalaria*). The experiment was established at "Embrapa Arroz e Feijão" field experiment station, in Santo Antonio de Goiás (Goiás State, Brazil) in 2002–2003. Data on climate, soil temperature, soil mineral nitrogen, soil moisture and soil carbon sequestration rates were monitored for more than 1 year as potential determinants of the greenhouse gases emissions. Twelve chambers in each of the treatments were used to measure greenhouse gases fluxes. Fluxes were measured twice a week. Chambers were sealed for 2 h to allow gases to accumulate. The chamber atmosphere was sampled five times during this period in 13 ml vacuum container tubes that were previously purged of other gases. N_2O fluxes were calculated by linear interpolation.

N_2O concentrations in the soil atmosphere were determined using permanent gas samplers inserted into the soil at various depths (10, 20 and 30 cm). The results demonstrated that N_2O emissions were very low (<1 g ha^{-1} day^{-1}) for both systems. Peaks of N_2O were observed after fertilization. N_2O is produced mainly by denitrification, which may be explained by low NO_3^- levels in soils and a $<60\%$ water filled pore space (WFPS) within the soil for the majority of the time. Low WFPS under these crops can be caused by evaporation at high temperature (more than or equal to 25 °C). However, measurements of gas concentrations in soil showed that the production of N_2O is reasonably prolific (concentrations of 1–30 times the atmospheric concentration). This suggests that N_2O is produced but cannot diffuse to the soil surface, either because denitrification is complete and N_2 is produced or because the N_2O is nitrified before diffusing. Microporosity in the upper layer needs to be studied further to better understand the diffusion and compaction conditions in these soils (Yamulki and Jarvis, 2002). Samples were taken immediately after fertilization (November, December and January). After fertilization, a higher variability in fluxes may occur, which agrees with the general principle that fertilization increases the emissions of N_2O due to a higher availability of organic N (Weitz et al., 2001). Further measures of potential denitrification are necessary to better understand the capacity of this particular soil to produce and emit N_2O.

Six et al. (2004) recognized that few studies have reported CH_4 flux differences between DMC and no-tillage systems and that all those that have, have found a significant enhancement of CH_4 uptake with the adoption of DMC (on average 0.6 kg ha^{-1} yr^{-1}). Preliminary results obtained in the Cerrado region near Rio Verde confirm this observation. CH_4 fluxes were analysed in November 2003 and January 2004 in 3 DMC systems aged 9, 11 and 13 years respectively, and a conventional tillage system. Preliminary results showed CH_4 absorption for all treatment but higher absorption in the DMC systems. CH_4 sinks in the conventional tillage were 3.8 and 4.8 g $C{-}CH_4$ ha^{-1} day^{-1} in November and January respectively, whereas they varied between 8–16 g $C{-}CH_4$ ha^{-1} day^{-1} in November and 7.3–14.3 g $C{-}CH_4$ ha^{-1} day^{-1} in January in the DMC systems. In order to obtain a complete picture, data need to be collected throughout the entire cropping cycle. Only then can any general conclusions be drawn.

3.3 Carbon Budgets at the Farm Level

Several studies in Brazil have shown that no-tillage systems are fuel-efficient compared to conventional systems. For example, Landers (2001) reported fuel consumption by a farmer cooperative in Planaltina (Goías State) (covering 2,270 ha) over a 6-year period. During this period the land was converted from 100% conventional tillage, in the agricultural year 92/93, to 100% no-tillage in the agricultural year 1997/98. The total number of hours that tractors were used was 10,630 in 1992/93 and 5,135 in 1997/98, showing a decrease in fuel consumption of ∼50%. Landers (2001) also noted that the number of machine operators was reduced to almost half, freeing up previous machine operators for employment in new farm enterprises. Studies on pesticide use in conventional tillage and DMC systems and resulting on farm and off farm emissions of greenhouse gases, are lacking, highlighting an area that needs further investigation.

4 Erosion under No-Tillage and Conventional Tillage

Land degradation, caused by water induced soil erosion is a major threat to sustainable agricultural land use, causing serious and costly environmental deterioration. Pimentel et al. (1995) estimated the world-wide cost of soil erosion to be ca US$ 400 billion per year. According to Laflen and Roose (1998), water induced soil erosion is a threat to the long-term sustainability of mankind in all regions of the globe. In the tropics, Lal (1995) estimated that the total transport or movement of carbon, displaced by soil erosion is 1.59 Pg yr^{-1}. According to Lal, this estimate ranges from a low of 0.80 Pg yr^{-1} to a high of 2.40 Pg yr^{-1}. However, only a fraction of soil moved from its original place is transported out of the watershed. The delivery ratio for tropical watersheds may be as low as 10%. This implies that as much as $0.16\,\mathrm{Pg\ C\ yr^{-1}}$ may be transported out of tropical watersheds with a range of $0.08–0.24\,\mathrm{Pg\ C\ yr^{-1}}$.

In Brazil, almost every region has problems related to soil erosion. According to De Maria (2004), no-tillage reduces runoff and soil loss by approximately 70% and 90% respectively. In order to reduce soil erosion rates, some Brazilian farmers have adopted appropriate farming systems, such as the use of cover crops, mixed crop rotations and conservation tillage. Conservation tillage systems have been developed as an alternative to conventional mouldboard ploughing, to reduce not only water but also wind erosion and to maintain and/or increase soil organic carbon contents (Six et al., 2002). These practices manage litter and crop residues with minimum and no-tillage. Keeping a mulch of crop residues protects the soil surface against raindrop impact, decreases evaporation, increases water storage, reduces production costs and slows down decomposition of soil organic carbon (Rosell and Galantini, 1997).

It has been reported (Bajracharya et al., 1998; Lal, 1995, 1997) that while deposition of eroded soil does not necessarily lead to the direct accumulation of carbon, it is likely to increase the overall sequestration of soil organic carbon by leading to an accumulation of organic material which has a greater potential to be converted into the stable form of soil organic carbon. Depositional and non-eroded areas increase potential accumulation of soil organic carbon possibly by providing favourable conditions for aggregation. Carbon accumulation in soil seems to occur within aggregates. The above authors concluded that erosion is likely to lead to a gradual depletion of soil organic carbon by exposing the stable carbon pool in micro aggregates in the soil surface and the subsoil, to degradative processes by disrupting macro aggregates and removing of successive layers of soil.

5 Summary and Conclusion

Caution must be taken when analysing DMC systems in term of carbon sequestration. Comparisons should not be limited solely to carbon storage in the soil but should include a consideration of associated trace gas fluxes. Associated fluxes of methane and nitrous oxide may change the final balance of $C{-}CO_2$ equivalents, based on the global warming potential of each gas. Most preliminary results tend to indicate that the adoption of DMC in Brazil is a promising strategy for mitigating carbon emissions to the atmosphere. Since the 1970s, DMC systems have been taken up by farmers in Brazil for a variety of reasons irrespective of carbon sequestration benefits. Therefore the use of DMC systems in Brazil is a good potential strategy for mitigating greenhouse gases emissions as the uptake of DMC is not dependent on carbon markets or political incentives.

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Influence of Land Use on Carbon Sequestration and Erosion in Mexico: A Review

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Abstract To reduce the impact of human activities on soil erosion and to increase C sequestration a series of alternative systems have been tested in recent years on hillside agriculture in Mexico. Among other systems conservation tillage (CT) and intercropping staple crops and fruit trees (MIAF) have been successful. Since accumulation of C occurs in those systems, it is concluded that the rate of C entrance to the soil-plant exceeds the rate of C exit. The identification and understanding the structure of such a systems, its components, and the role of each one of these components, is fundamental to intervene them in order to enhance reduction of soil erosion and C sequestration. A summary of experiences on this subject collected in Mexico is presented in the present paper. The more striking finding are: hillside agricultural system can store, and probably sequester, as much C as secondary native forestry systems and soil erosion and C losses are small when proper management systems are applied.

Keywords Soil carbon • Erosion • Climate change • No tillage • Conservation tillage • Soil N • Legumes

1 Introduction

Mexico's population increased from 25 to approximately 100 million inhabitants in half a century, but the country surface is the same, near 200 million hectares. Only 11% of the total land is apt for farming only 16% of this is prime arable land suitable for high-input agriculture. Six million hectares are irrigated, but water is one of the most serious limiting factors for present and future agriculture. The rest of the land is mostly either located on steep-slope terrain or in marginal semiarid conditions inhabited by just over 3 millions farmers (INEGI, 1998; Tiscareño et al., 2000). The shortage of farmland has resulted in increasing aggression to native forest and in a constant increment of steep slopes being cultivated. As a consequence, temperate and tropical forests have experienced a reduction of 30% and 75% since 1960, respectively. According to the World Resources Institute (2002), Mexico ranks among countries having the highest annual rates of native forests losses.

Agriculture is practiced in the four ecological macro-regions recognized in Mexico: an arid and semiarid region (<500 mm annual rainfall) covering approximately one-half of the national territory, a dry tropical region (900–1,200 mm, with seasonal rainfall) that occupies one-fourth of the surface and the remainder area (13% and 8%) is covered by the temperate hilly areas (600–900 mm) and the humid tropics (>1,200 mm), respectively (Claverán, 2000). Accelerated soil erosion affects 80% of Mexico's land (Maass and García-Oliva, 1990) and nearly 535 million tons of soil is lost annually (SEMARNAP, 1997). According to Maas and García-Oliva (1990), more soil has been lost during the last 40 years than in the past four centuries. Concurrent surface and gully erosion from deforestation and inappropriate cultivation of non-irrigated land have been identified on 65–85% of the land (Bocco and García-Oliva, 1992). The erosion is aggravated by the topographic and weather conditions

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of the country, which presents extreme variations in altitude (sea level to more than 5,000 m) and climate conditions ranging from desert to tropical humid forest (<200 mm to $>2{,}000$ mm rainfall).

Land degradation and erosion are common features in most of the agricultural land in Mexico, particularly on hillside agriculture along the sierras that criss-cross the country, but mainly in the southern part where rainfall is abundant. The main types of land degradation and percentage of land affected are presented in Table 1 (CONAZA, 1994). Biological degradation is also caused by lost of the top soil and excessive cropping. The former ranges from less than 10 Mg ha^{-1} up to 200 Mg ha^{-1} (CONAZA, 1994) and seriously reduces productivity. Water erosion is closely associated to slopes higher than 10% and management practices tending to maintain the soil without protection when the rainy season starts. Eolic erosion is more common under arid and semi-arid conditions. Losses of approximately 140 Mg ha^{-1} of soil have been reported for eolic erosion (Amante, 1989; Osuna, 1991). The average soil loss in the country is approximately 2.8 Mg ha^{-1} (Figueroa and Ventura, 1990). The biological erosion is the second largest degradation process after water erosion in Mexico and it represents the rate of organic matter mineralization. Approximately 80% of the territory is affected by biological degradation. Organic matter degradation is more probable to occur in areas closer to coastlines and less probable in the semiarid and arid zones. Both soil degradation processes are closely related to C sequestration capacity. Soil conservation practices as well as agronomic practices have been introduced to reduce soil degradation. These practices help to retain the soil C in their storages and may contribute to increase the rate of C sequestration.

Table 1 Land degradation in the Mexican territory (CONAZA, 1994)

Type of land degradation	Fraction of the territory affected (%)
Water erosion	85
Wind erosion	60
Leach bases	15
Physical degradation	20
Biological degradation	80
Salinity	20
Sodification	15

2 Soil Carbon and Carbon Sequestration

Appropriate tillage techniques were viewed in the past as soil erosion mitigation tools. However, today they are also viewed as a means to increase soil C sequestration. Soil organic C (SOC) plays a key role in the global C cycle and acts as a sink for atmospheric CO_2. This pool can be managed to increase the terrestrial SOC pool. But the potential of the ecoregions to sequester C varies with soil type and depth, climate, land use, and management. Soil tillage methods without soil inversion, like no-tillage systems, help to increase SOC pool (Lal, 1984, 1989). Conversely, soil management practices leading to soil inversion and disturbance may result in a decrease of the SOC content. The SOC is an important contributor to soil quality. Because of these reasons SOC has come under increased attention as a possible method to store C and reduce future increases in atmospheric CO_2 concentration (Kern and Johnson, 1993). However, limited information has been published in Mexico on the effect of conservation tillage (CT) on soil C (Báez, 2001; Etchevers, 2002; Salinas et al., 2001; Sandoval, 1997; Velásquez and Pérez, 2001). A brief summary of some relevant case studies recently published is presented.

2.1 Soil Carbon and Soil Management. Mega-Environment 2 Case Study

Table 2 shows the effect of conventional, zero tillage and previous crop residues management on soil organic matter (SOM), SOC, soluble-C, Kjeldahl N and C/N after 5 years in a Phaeozem representative on Mega-Environment 2 (Highlands Valleys of Mexico, Kenya, Ethiopia) (Sandoval, 1997, Etchevers, 2002). The data given are the average value of eight different rotations including maize, wheat, and vicia. Zero tillage influenced SOC, soluble-C, and N, in the first 40 cm of the profile. The largest effects of tillage systems were observed on SOC and soluble-C in the 0–5 cm depth increment. Zero tillage treatments accumulated more SOC than conventional tillage in the superior soil layers after 5 years. But more SOC was accumulated in the 0–20 cm depth increment of conventional tillage than zero tillage. This was attributed to the effect of surface soil being plowed under. Surface soil contains more root and plant residues than

Table 2 Effect of soil tillage and residues management on soil organic matter (OM), total soil organic C (C), soluble-C (Csol), Kjeldahl-N (NKj) and C/N after 5 years of treatment (Sandoval, 1997)

	Indicator[a]				
Main treatments	OM (%)	C (%)	Csol Abs	Nkj (%)	C/N
	0–5 cm depth				
Zero tillage	2.3a	1.32a	0.309a	1.12a	11a
Conventional tillage	1.8b	1.05b	0.250b	0.10b	11a
With residues	2.1a	1.23a	0.311a	0.12a	11a
Without residues	2.0a	1.15b	0.256b	0.11a	11[a]
	5–10 cm depth				
Zero tillage	1.9a	1.09a	0.233a	0.10a	0.11a
Conventional tillage	1.8a	1.07a	0.247a	0.10a	0.11a
With residues	2.0a	1.14a	0.265a	0.10a	0.11a
Without residues	1.8a	1.04a	0.222b	0.10a	0.11a
	10–20 cm depth				
Zero tillage	1.6b	0.95b	0.218a	0.09b	11a
Conventional tillage	1.9a	1.09a	0.226a	0.10a	11a
With residues	1.8a	1.06a	0.234a	0.10a	11a
Without residues	1.7a	1.00a	0.213a	0.09b	11a

[a] Different letters after the number indicates significant differences. Comparison must be made between zero and conventional tillage and between with and without residues

the underlying soil. When this is plowed under it increases the SOC in the lower layer. Carbon could be retained in deeper layers for longer periods than the C in the soil surface. Zero tillage resulted in more water being retained in the upper 5 cm of the soil profile (Sandoval, 1997).

2.2 Carbon Stocks in Different Land Use Systems in Hillside Conditions in Mexico

An example of carbon content in the above-ground, root and soil components under different land use systems are presented in Table 3 (Etchevers, 2002; Martínez, 2002).

The highest C stock (above-ground+roots+soil) was found in the Mixe watershed (306 Mg ha^{-1}) and the lowest in the Cuicateca (84 Mg ha^{-1}). However C stocks associated to different land uses (secondary native forest, permanent agricultural crops, annual and mixed annual + fruit trees crops) did not differ much within the watersheds. The hypothesis that under hillside conditions the agricultural systems accumulate as much C as secondary native forest systems was confirmed. In a similar manner it was observed that C stored in the under-ground portion of the systems was higher than the stored in the above-ground one. In general, more than 90% of the C was stored in the soil in the agricultural systems and less than 90% in the secondary native forestry.

A trend to store a major proportion of the C in the above-ground portion of the system was observed as the secondary forestry vegetation grew older. Annual increment of C in the system including fruit trees was approximately 1–2 Mg ha^{-1} year^{-1}. C stocked in the soil depends more on the quality of residues and moisture conditions than on the age of the system. The analysis of the vertical distribution of C in the under-ground showed that C percentage diminished with depth. Approximately 60% of C was concentrated on the first 50 cm of the soil profile (Acosta et al., 2001, 2002), however a great spatial variability of the C was observed within small distances in both the experimental and observation plots (Vergara et al., 2004).

Table 4 shows the amount of C that can be captured in weeds, stubble, and peach and coffee trees of living walls and barrier systems (PLW<30), CT and traditional tillage (TT), in the two experimental micro-watersheds (Mazateca and Mixe). Weeds can introduce between 1 and 2.5 Mg ha^{-1} yr^{-1} of C to the system, while C in crop residues may add between 2 and 4 Mg ha^{-1} yr^{-1} of C. Part of this C is rapidly

Table 3 Organic C in the above-ground, root and soil (0–105 cm) components in land use systems prevailing in three regions of Northern Sierra, Oaxaca, Mexico (Etchevers, 2002)

	Natural systems			Agricultural systems							
Component				Permanent		Mixed		Annual			
						(Mg ha^{-1})					
						Mazateca					
	LF	AF15	AF10	CA	PA	Plw[a]	Plw[b]	CT[a]	CT[b]	TT[a]	TT[b]
Above-ground	99.5	46.3	31.0	34.5	5.4	6.1	3.5	6.1	3.5	3.2	1.8
Root	3.3	2.3	4.1	4.8	1.4	1.5	2.9	2.3	4.3	2.0	5.5
Soil	152	156	240	148	174	158	128	266	273	235	195
Total	255	205	275	187	181	166	135	274	281	240	202
						Cuicateca					
	QF			PR		Plw	Plw[2]	CT[2]	CT[2]	TT[2]	TT[2]
Above-ground	37.6			2.2		4.3	3.4	4.2	3.8	3.3	2.7
Root	14.4			6.1		0.7	1.0	1.9	1.2	0.6	0.6
Soil	45			91		63	113	66	49	57	65
Total	97			99		68	117	72	54	61	68
						Mixe					
	AC10	AC7	AC2	CA		Clw		CT	TT		
Above-ground	25.0	24.1	9.9	11.2		5.6		3.1	4.8		
Root	7.8	5.1	3.8	4.0		1.9		2.8	2.9		
Soil	120	169	119	160		266		278	298		
Total	153	199	133	175		273		284	306		

LF = liquidambar forest; AF10 and AF15 = alnus forest of 10 and 15 years old; PA = pasture, Plw = peach living walls: CT and TT = conservation tillage and traditional tillage; QF = quercus forest; AC2, AC7, AC10 = acahuales of 2, 7 and 10 years old; CA = café; Clw = coffee living walls

[a] >30

[b] <30 = slope percentage

Table 4 C capture by residual weeds and stubble measured after harvesting the maize and C sequestered by fruit trees in various management systems (Etchevers, 2002)

	Weed C		Stubble C		Peach trees C Inc. year^{-1}
Plot	2001	2002	2001	2002	
			(Mg ha^{-1})		
			Mazateca		
Plw[a]	1.3	1.6	4.3	4.3	1.9
CT[a]	0.6	1.3	3.2	3.2	
TT[a]	1.0	1.1	3.3	1.9	
			Cuicateca		
Plw[b]	1.6	2.1	2.3	2.3	0.9
CT[b]	1.2	2.5	2.2	2.2	
TT[b]	1.4	1.4	1.8	1.9	
			Mixe		
Clw	0.9	2.4	2.8	2.8	1.3
CT	1.0	1.6	3.4	3.4	
TT	0.7	1.9	2.9	2.9	

Plw = peach living walls; Clw = coffee living walls; CT and TT = conservation tillage and traditional tillage

[a] >30

[b] <30 = slope percentage

mineralized but some remains in the soil contributing to the soil C pools. High density peach trees plantations used as living walls to prevent erosion in hillside conditions can sequester between 1 and 2 Mg ha^{-1} yr^{-1} of C during the first years after planting. This rate of C sequestration is considered comparable to rates exhibited by forestry systems (Galinski and Küppers, 1994).

Total C and dissolved-C losses due to erosion under hillside conditions in a micro-watershed of the Northern Sierra of Oaxaca, are presented as an example in Table 5 (Martínez, 2002). C lost in the soil sediments and in the water run-off was rather small in spite of the high slopes (ranging from 20% to 60%) and the rainfall (between 2,000 and 2,200 mm). Soil water infiltration was very high.

The C in the soil sediments and water run-off plots was very small in spite of the high slopes. The insignificance of the C lost can be explained by the low rate of erosion and high rate of water infiltration. Traditional

Table 5 Soil C losses in surface run-off in Mazateca Watershed (Martínez, 2002)

Land use[a]	Rainfall (mm)	Run-off (L)	Erosión (kg ha^{-1})	C (ppm)[b]	
				Soil	Water
Coffee	2 215	305	2.4	20	67
Maize-traditional (L)	2 282	373	2.3	11	12
Maize + peach intercrop. (L)	2 245	162	1.5	27	5
Slash-and-burn	2 353	446	6.3	15	6
Pasture	2 349	1878	88.7	11	4
“Acahual”	2 365	244	1.4	39	9
Maize-traditional (H)	2 020	618	11.5	12	10
Maize + peach intercrop. (H)	2 024	205	1.1	14	4

[a] H and L refer to high and low locations within the watershed
[b] Values are too small to be expressed in kg ha^{-1}

land use like slash-and-burn, may not be as aggressive on soil C as is generally considered.

2.3 Carbon Accumulation in Recovered Hardened Volcanic Materials (“Tepetates”)

Báez (2001) presents information on the evolution of C concentration on hardened volcanic material (tepetates) after their physical and chemical properties were amelioration (Fig. 1). The C content of the original material is near nil. Accumulated C (sequestered) is a clear function of management and time. Large extensions of these materials could be habilitated and subjected to conservation practices to capture atmospheric carbon in Mexico (Báez et al., 2002).

2.4 Management Effects on Soil Carbon Accumulation

Salinas et al. (2001) and Velázquez (2001) observed that zero tillage and preserving the crop residues in the surface resulted in more organic C accumulation in the top soil. The work of these authors was conducted in volcanic soils in the state of Michoacán. Zero tillage in both cases resulted in a good alternative to increase C sequestration by soils (Salinas et al., 2001; Velázquez et al., 2001).

3 Soil Erosion

Most information on soil erosion losses has been obtained from measurements conducted in small run-off plots. However, large watershed and models have been also studied. Variables studied on the small plots ranges from crops to soil management systems. Plots have been installed in dryland regions as well as on irrigated areas. Three contrasting conditions have been selected in the present paper: dryland agriculture, volcanic soils and hillside.

3.1 Soil Erosion Under Rainfed-Semiarid Conditions. the Aguascalientes Study Case

Table 6 shows the soil lost under various crops in a region with marked differences in seasonal precipitation (Osuna, 1997). Average rainfall was 587 mm yr^{-1}

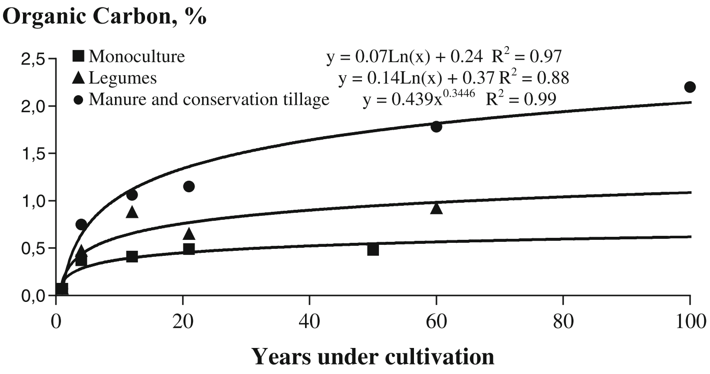

Fig. 1 Organic C accumulation under different management systems in indurate volcan[1]ic material (“tepetate”) conditioned for agriculture (Baez et al., 2002)

Table 6 Soil losses under different crops in a rainfed region. A 4-year-period average (El Llano, Aguascalientes, Mexico) (Osuna, 1997)

Crop	Soil loss ($Mg\ ha^{-1}$)	Relative loss
Maize	12.7	0.39
Beans	8.9	0.28
Wheat	1.5	0.05
Screen	203	0.07
Check (permanent fallow)	32.3	

Table 7 Soil lost under different maize management systems in a rainfed region. A 4-year-period average (El Llano, Aguascalientes, Mexico) (Osuna, 1997)

Soil management	Soil loss ($Mg\ ha^{-1}$)	C coefficient	Maize yield ($Mg\ ha^{-1}$)
Disc plow, no weeding	5.0	0.08	1.70
Grade, no weeding	64.9	0.93	1.41
Chisel, no weeding	33.1	0.55	1.63
Zero tillage	26.3	0.44	1.10

and had high erosive potential. Maize and beans are crops that require frequent weeding and showed higher soil losses than wheat, a crop that protects the surface because of its higher plant population. The effect of soil losses when maize was managed under different management practices is shown in Table 7. Under certain very specific conditions soil losses can be reduced by the use of appropriate type of plow and zero tillage was not the best option. Maintaining an adequate soil cover appears to be as important as not cultivating the soil. In another series of experiments conducted under similar conditions of restricted rainfall no advantages of the zero tillage over conventional tillage were observed (Jasso, 1997). These results meant that conservation tillage it is not always and appropriate technique recommended for C sequestration (Osuna, 1997).

3.2 Soil Erosion in Volcanic Landscapes. the Pátzcuaro Basin Study Case

Andisols are easily erodible soils under dry or wet conditions due to its poor structure. In Pátzcuaro, where the small landholders grow annual crops under steep-slope conditions soil erosion and nutrient losses are common features. Conservation tillage seems to be an appropriate technology to solve the above-mentioned problems (Tiscareño et al., 2000).

Cropping systems, which use plow and disk on an 8% slope produced high erosion. Soil losses averaged $3.2\ Mg\ ha^{-1}\ yr^{-1}$ in conventional tillage and approximately $0.3\ Mg\ ha^{-1}\ yr^{-1}$ in no-till plots. Reduction of storm water runoff (76%) with mulched no-till systems becomes a key factor to reduce sediments and promote infiltration and deep-water percolation. Soil moisture retention was also higher (53% in the first 150-mm soil layer) under the latter conditions. Carbon sequestration should be encouraged by conditions created in no till treatments.

3.3 Soil Erosion in Hillside Slopes. The PMSL (Oaxaca) Study Case

How soil management affects the water run-off, soil erosion and C losses was studied in three experimental watersheds of Oaxaca (Martínez, 2002). Table 8 shows an example of the annual run-off, soil erosion and related parameters corresponding to various soil management systems in one of the experimental watersheds.

Run-off in most conditions were very low in spite of high precipitation recorded (1,000–2,000 mm) and slopes ranging from 20% to near 50%. The values of the run-off coefficient (the relation between rainfall and run-off) allows to conclude that most of the water infiltrates and does not run-off. The treatments with the lowest run-off were the maize and fruit trees intercropping. Sediments concentration in run-off was very low as well as soil erosion not withstanding the slope conditions and the management systems. Storage C losses under this conditions were extremely low.

4 Conservation Tillage

Zero tillage farming is a pre-hispanic practice probably dating back to 5,000–9,000 years ago. Seeds were planted after slashing and burning the native vegetation and periods in between slash-and-burn cycles were

Table 8 Water run-off, run-off coefficient and soil erosion in the Mazateca Watershed (Martínez, 2002)

Treatment	Rainfall (mm)	Run-off (L)	Run-off coeff.	Conc ($g L^{-1}$)	Erosion ($g lote^{-1}$)	Erosion ($kg ha^{-1}$)
Coffee	2 215	305 (6.1)[a]	0.0028	0.0392	12	2.4
Maize-traditional (L)	2 282	373 (7.5)	0.0033	0.0301	11	2.3
Maize + peach intercrop. (L)	2 245	162 (3.3)	0.0014	0.0446	7	1.5
Slash-and-burn	2 353	446 (8.9)	0.0038	0.0700	31	6.3
Pasture	2 349	1,878 (37.6)	0.0160	0.2261	443	88.7
"Acahual"	2 365	244 (4.9)	0.0021	0.0282	7	1.4
Maize-traditional (H)	2 020	618 (12.1)	0.0061	0.0931	57	11.5
Maize + peach intercrop. (H)	2 024	205 (4.10)	0.0020	0.0279	6	1.1

2.4 H and L refers to high and low locations within the watershed

[a] () Run-off values in mm

long enough to allow the secondary vegetation to grow back to near its original state (Figueroa and Morales, 1992). Soil erosion was rather negligible during that period. Today farmers make an average of ten passes of machines over their fields in the traditional mechanized system (Anonymous, 2002). FIRA (2000) estimates that in the year 2000 there were 850 thousand hectares under conservation tillage in Mexico, i.e. approximately 4% of the cultivated surface, mostly in the central states of Michoacán, Jalisco and Guanajuato. This surface is small when compared with figures given for other Latin America countries. Our interest in this subject is due to its close relationship with C sequestration and sustainable agriculture (Pieri, 2001, 2002a, b).

To cope with the intensive rate of degradation conservation tillage practices have been conducted in Mexico (Claverán, 2000; Claverán and Rulfo, 2001; Claverán et al., 1997; INIFAP, 2000; RELACO, 1997; Velásquez et al., 1997;), as well as in others Latin America countries (RELACO, 1993, 1995, 1997, 1999). CIMMYT has been one of the most active institutions in conducting research on conservation tillage in Mexico and Central America (Buckles and Erenstein, 1996; Erenstein, 1997, 1999a, b; Erenstein and Cadena, 1997; Sayre, 2000; Sayre et al., 2001; Scopel, 1997a, b; Scopel and Chávez, 1997; Scopel et al., 2001; Soule, 1997; van Nieuwkoop et al., 1994), however little reference is made to C sequestration and soil erosion losses. A similar situation occurs in theses work conducted at the Colegio de Postgraduados (García, 1994; Muñoz, 1993; Magallanes, 1999; Navarro, 1998; Pérez, 1996; Román, 1993; Uribe, 1997; Sandoval, 1997; Tapia, 1999; Vidal, 1994). Experimental results have shown significant advantages of zero tillage over either conventional or minimum tillage. Over a 100 experiments conducted during a 5-year period showed that zero tillage reduced the erosion rate by nearly 80% in maize crops, and by nearly 95% in wheat crops with respect to conventional tillage (Osuna, 1997; Velásquez, 1997). Under moderate slope conditions (8%) there was a considerable reduction of soil erosion in Andosols with zero tillage (90–60% reduction) as compared to conventional tillage (Tiscareño et al., 1997). In cultivated lands on steeper slopes in southern Veracruz, Uribe (1998) determined that 27 kg of soil were lost per each kilogram of maize produced under conventional tillage; under zero tillage the loss was reduced to less than 1 kg.

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Rhizodeposition of Organic C by Plant: Mechanisms and Controls

Christophe Nguyen

Abstract During their life, plant roots release organic compounds in their surrounding environment. This process, named rhizodeposition, is of ecological importance because (1) it is a loss of reduced C for the plant, (2) it is an input flux for the organic C pool of the soil and (3) it fuels the soil microflora, which is involved in the great majority of the biological activity of soils such as the nutrient and pollutant cycling or the dynamics of soil borne pathogens, for example. The present review first examines the mechanisms by which major rhizodeposits are released into the soil: production of root cap cells, secretion of mucilage, passive and controlled diffusion of root exudates. In a second part, results from tracer studies (43 articles) are analysed and values of C flux from the plant root into the soil are summarized. In average, 17% of the net C fixed by photosynthesis is lost by roots and recovered as rhizosphere respiration (12%) and soil residues (5%), which corresponds to 50% of the C exported by shoots to belowground. Finally, the paper reviews major factors that modify the partitioning of photoassimilates to the soil: microorganisms, nitrogen, soil texture and atmospheric CO_2 concentration.

Keywords Carbon • Rhizodeposition • Rhizosphere • Tracer studies

Résumé **La rhizodéposition de C organique par les plantes: mécanismes et contrôles** Au cours de leur vie, les racines des plantes libèrent des composants organiques dans leur environnement proche. Ce processus, nommé rhizodéposition, est d'importance écologique car (1) c'est une perte de C réduit pour la plante, (2) c'est une flux d'intrant pour la réserve en C organique du sol et (3) il alimente la microflore du sol, qui est impliquée dans la grande majorité de l'activité biologique des sols tels que par exemple le cycle des éléments nutritionnels et des polluants ou encore les dynamiques des éléments pathogènes apportés par le sol. La présente revue examine en premier lieu les mécanismes par lesquels les rhizodépôts majeurs sont libérés dans le sol: la production de cellules de la coiffe racinaire, la sécrétion de mucilage, la diffusion passive et contrôlée d'exudats racinaires. En second lieu, les résultats de traceurs (43 articles) sont analysées et les valeurs de flux de C allant de la racine de la plante au sol sont synthétisées. En moyenne, 17% du C net fixé par la photosynthèse est perdu par les racines et il est restitué dans la respiration de la rhizosphère (12%) et dans les résidus de sol (5%), ce qui correspond à 50% du C exporté par les pousses vers le sous-sol. Enfin, l'article répertorie les facteurs principaux qui modifient la répartition des photoassimilats vers le sol: microorganismes, azote, texture du sol et la concentration en CO_2 de l'atmosphère du sol.

Mots clés Carbone • Rhizodéposition • Rhizosphère • Traçage

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1 Introduction

During their life, plant roots release organic compounds in their surrounding environment. This phenomenon is now being studied for more than one century. Indeed, the very complete book of Krasil'nikov (1961), reports that root excretion was first evidenced in 1894 by Dyer, who observed the excretion of acidic compounds from the roots of plants. Then, numerous workers identified sugars, organic and amino acids and other compounds in the nutrient solution in which different plants were grown. Krasil'nikov (1961) reported that as early as in 1927, Minima observed that root excretions of organic compounds by lupine, bean, corn, barley, oat, and buckwheat cultivated in Knop's nutrient solution, were maximum during the fourth week of growth. Afterwards, these excretions decreased and stopped altogether with plant growth. In the beginning of the twentieth century, it was already estimated that root-released compounds yielded 0.6–27% of the plant dry weight and studies also demonstrated that greater amounts of substances could be obtained if the nutrient solution was replaced (Krasil'nikov, 1961).

The release of organic compounds by living plant roots referred to as rhizo-deposition (Shamoot et al., 1968) is a process of major importance that is still subject of investigations for several reasons. Firstly, rhizodeposition is an input of organic C into the soil. The soil is the second largest C compartment (1.5×10^{12} t C) after oceans (3.8×10^{13} t C) and before atmosphere (7.5×10^{11} t C) and plant biomass (5.6×10^{11} t C) (estimates from (Schlesinger and Andrews, 2000)). Each year, it is estimated that 7.5×10^{10} t of C return to the atmosphere due to soil respiration. Considering that in average, shoots export to belowground about half of the C fixed by photosynthesis (Lambers, 1987), it is of major importance to determine how much of this flux enters the soil organic C pool. This is particularly relevant if the soil is expected to sequestrate C in response to elevation of atmospheric CO_2.

Secondly, rhizodeposition represents a loss of energy for the plant. At first sight, the release of organic C from roots into the soil might figure as a lost pool of reduced C that does not contribute to dry matter production. However, it is well established that rhizodeposits stimulate the biological activity in the rhizosphere, which have important positive feedbacks for the plant such as enhancing of nutrient availability for instance (Jones and Darrah, 1996). However, we still have no idea on the efficiency of rhizodeposition. In other words, would a plant gain extra advantages in terms of mineral nutrition for example, if it would deposit more C into the soil? The response to this question is fundamental with respects to outlooks aimed at engineering the rhizosphere.

In the past decades, many studies have focussed on rhizodeposition. Authors have concentrated on determination of C flows from plant roots to soil and on factors that affected them. Results have been reviewed at regular interval (Grayston et al., 1996; Hale and Moore, 1979; Kuzyakov and Domanski, 2000; Rovira, 1969; Whipps, 1990). Briefly, these articles outlined that: (1) plant roots are able to release a wide range of organic compounds, (2) there is a great degree of uncertainty about the amounts and the quality of organic C deposited in soil conditions; this comes from the major difficulty to estimate root-derived C in the presence of microorganisms that rapidly assimilate rhizodeposits, (3) in soil conditions, many factors are assumed to alter both the amount and the nature of the C compounds released from roots but little is known about how these factors operate.

Our knowledge of rhizodeposition is too much incomplete. As a result, the effective outputs of research on rhizodeposition are lacking despite virtual outputs are potentially numerous such as manipulating C flow to the rhizosphere to alter the microbial dynamics and the related processes (nutrient cycling, organic matter dynamics, pollutants bioavailability, soil-borne pathogen and inoculants dynamics, etc.).

Consequently, two areas of investigations could be suggested. On one hand, new methodologies have to be developed to obtain more reliable estimates of rhizodeposition under various environmental conditions. On the other hand, if a major goal is to manipulate rhizosphere processes through plant ecophysiology and through the quantity and the quality of rhizodeposits, it is necessary to obtain more information about the different mechanisms by which C is lost by roots as well as their regulations by plant genetic and by environment. The present article concentrates on that latter point. It first reviews literature related to the mechanisms by which major rhizodeposits (in terms of quantity) are deposited into the soil: sloughing-off of root cap cells, secretion of mucilage, passive diffusion of root solutes (exudation) and senescence of epidermal and cortical cells. The article then examines tracer studies (^{14}C) to summarize the main factors that are assumed to affect rhizodeposition. It is attempted to relate their effects to the aforementioned mechanisms of C release from roots. Finally, some outlooks are proposed for future investigations on rhizodeposition.

2 Mechanisms of Release of Organic C from Living Roots

2.1 Sloughing off of Root Border Cells

Apical meristems of plant roots are covered by a group of cells arranged in layers, the root cap, the surface of which sloughs-off as the root tip wends its way through the soil (Barlow, 1975). In mature branched roots, the entire cap itself can be lost as the results of pathogen attacks or as part of a normal developmental process as it was observed in field-grown maize (Varney and McCully, 1991). The cap initials generate cells that are displaced from the inner zone towards the periphery of the cap where they slough-off. During their transit through the cap, the cells first differentiate into statocytes, i.e. gravity-perceiving cells and then into cells able to secrete mucilage (Sievers and Hensel, 1991). The separation of cells from the periphery of the cap can easily be observed under a microscope for numerous plant species. In field-grown maize, the detached cells were found alive at some distance from the root tip (Vermeer and McCully, 1982), which indicates that border cells are still viable several days after their separation from the root. Among plants belonging to ten families, the viability of border cells after they separate from the root was demonstrated to be of 90% or higher in most cases except in the *Compositae* sunflower and *Zinnia* for which most of the border cells are dead when they detached from the cap (Hawes, 1990). Furthermore, in pea, detached cap cells exhibit different gene expression than that of attached cap cells (Brigham et al., 1995). It is suggested that they play a significant role in engineering the rhizosphere ecology (Hawes et al., 1998) and therefore, the term border cells was proposed instead of the original denomination "slough off cap cells" (Hawes, 1990). The suggested functions of root border cells are numerous: decrease of frictional resistance experienced by root tips (Bengough and McKenzie, 1997), regulation of microbial populations in the rhizosphere by attracting pathogens and preventing them from damaging root meristem and by promoting growth gene expression in symbiotic microorganisms (Hawes, 1990; Hawes et al., 1998, 2000; Zhao et al., 2000), protection against heavy metal toxicity such as aluminium (Miyasaka and Hawes, 2001).

In maize seedlings, the number of cells in the cap ranges from 3,900 to 20,900 (Clowes, 1976). It decreases with root age due to the reduction in the width of root apices (Clowes and Wadekar, 1988). In laboratory experiments, the cap removed artificially is regenerated in 1–9 days (Barlow, 1975; Clowes, 1976; Sievers and Hensel, 1991). The maximum number of cells released daily from the cap is very variable ranging from a dozen in tobacco to more than 10,000 for cotton and pine but it is conserved at the species level (Hawes et al., 2000). In maize, the daily production of cap cells increases from 356 cells day^{-1} at 15°C to a maximum of 3,608 cells day^{-1} at 25°C and declines to 851 cells day^{-1} at 35°C (Clowes and Wadekar, 1988). The production of border cells by roots growing in soil is poorly understood. In laboratory experiments, it has been demonstrated that environmental conditions experienced by root tips strongly influence border cell production. For example, atmosphere with high CO_2 and low O_2 partial pressure inhibits border cell separation in pea during germination whereas later in development, it increases the total number of border cells that accumulate over time (Zhao et al., 2000). The mechanical impedance experienced by maize roots creates friction that is decreased by the sloughing of root cap cell (Bengough and McKenzie, 1997). Consequently, in maize seedling grown in compacted sand the number of shed cap cells increases exponentially with the penetration resistance from 1,900 cells day^{-1} (56 cells mm^{-1} root elongation) for loose sand (resistance to penetration: 0.29 MPa) to 3,200 cells day^{-1} (750 cells mm^{-1} root elongation) for compacted sand (resistance to penetration: 5.2 MPa) (Iijima et al., 2000) (Table 1). The authors estimated that this corresponded to 1.5 and 2.6 μgC per day, respectively.

There are evidences that the number of border cells is also controlled at the genetic level. In pea, the separation of cells from the cap has been shown to be closely correlated to the expression of an inducible gene coding for a pectinmethylesterase, which is thought to solubilize cell wall polymer (Wen and Hawes, 1999). Furthermore, there are evidences that cap cells synthesized a factor that accumulates

Table 1 Production of root cap cells and mucilage by roots of different plant species

References	Plant	Nature of C	Amount	Units	Comment
Iijima et al. (2000)	*Zea mays*	Root cap cells	1,900	cells/day	Seedling grown in sand: resistance to penetration = 0.3 MPa. For calculations, the root cap cell is considered as a cylinder with a length of 80 μm and a diameter of 21 μm, a density of 1 g/cm^3 and a dry matter/fresh matter ratio of 0.072 (Iijima et al., 2000)
			1.52	μg C/day/root	
		Root cap cells	3,200	cells/day	Same conditions as above except the resistance to penetration = 5.2 MPa
			2.56	μg C/day/root	
Newman (1985)	*Zea mays*	Root cap cells	7	μg DM/mg DM of root growth	
			2.8	μg C/mg DM of root growth	Calculated assuming a C content of root cap cells of 40%
	Convulvus arvensis	Root cap cells	4	μg DM/mg DM root growth	
Hawes et al. (2000)	*Pinus gossypium*	Root cap cells	10,000	cells/day	
McLeod (1976)	*Vicia faba*	Root cap cells	420–636	cells/day	
Chaboud and Rougier (1991)	*Zea mays*	Mucilage	34	μg DM/mg DM root growth	
Vancura et al. (1977)	*Zea mays*	Mucilage	11–17	μg DM/mg DM root growth	Growth in axenic nutrient solution for 28 days
	Triticum aestivum	Mucilage	29–47	μg DM/mg DM root growth	Growth in axenic nutrient solution for 25 days
Bowen and Rovira (1973)	*Triticum aestivum*	Root cap cells + mucilage	3.2–6.4	μg DM/mg DM root growth	
Samsevitch (1965)	*Triticum aestivum*	Root cap cells + mucilage	700	m^3/ha	Calculated from the size of the droplet at the root tip
	Zea mays	Root cap cells + mucilage	1,250	m^3/ha	Calculated from the size of the droplet at the root tip
Griffin et al. (1976)	*Arachis hypogea*	Root cap cells + mucilage	0.13–0.27	mg MS/plant/day	Growth in axenic nutrient solution for 2 weeks
		Root cap cells + mucilage	0.15	% of root C	

extracellularly and inhibits the production of new cells by the cap meristem without inhibiting cell mitosis in the root apical meristem (Hawes et al., 1998). Hence, the cap turnover is stopped unless the factor is diluted or unless cells from the periphery are shed. Therefore, in soil, it can be assumed that the production of cap cells is favoured on one hand by rain, irrigation and the soil microporosity, which all facilitate the diffusion of the inhibitor away from the cap and on the other hand, by frictional forces that shed the cells from the root tip.

2.2 Secretion of Mucilage by Roots

A mucilaginous layer has been frequently observed on the root surface of many plants (Oades, 1978) and more particularly at the root tip where it can form a droplet in the presence of water (Samsevitch, 1965). There is no clear evidence that the epidermis and the root hairs secrete mucilage (Peterson and Farquhar, 1996). In Sorghum, Werker and Kislev (1978) reported small drops of mucilage secreted by root hairs in addition to a fibrillar mucilaginous layer secreted by the epidermal cells. However, the mucilaginous layer observed on these parts of roots may derived from the mucilage secreted by the root cap (Vermeer and McCully, 1982), from the degradation of epidermal cell walls (Foster, 1982) or may be synthesised by rhizosphere microorganisms (Rovira et al., 1979). However, for most of the plants examined, the mucilage is secreted by the outer layers of the cap cells (Paull and Jones, 1975; Rougier, 1981) and it can be seen at the root tip of several plants (Miki et al., 1980).

The mucilage is composed of polymerised sugars and of up to 6% proteins (Bacic et al., 1987; Rougier, 1981). The major sugars identified are arabinose, galactose, fucose, glucose, xylose (Bacic et al., 1987; Knee et al., 2001). In maize, the root cap polysaccharide has a molecular weight greater than 2.10^6 daltons, a density of $1.63\,\mathrm{g\,cm^{-3}}$ (Paull et al., 1975), a C content of 39% and a C/N ratio of 64 (Mary et al., 1993).

The initiation of mucilage synthesis takes place in the endoplasmic reticulum and completes in the Golgi saccules. The slime is transported to the plasmalemma by the Golgi vesicles. The mucilage is discharged between the plasmalemma and the cell wall by exocytosis (Morre et al., 1967; Rougier, 1981). All these processes are energy-dependant. The passage through the cell wall is not systematic and the mucilage can accumulate at the inner wall surface. It is assumed that if both the degree of hydratation of the mucilage and the cell tugor are sufficient, the slime moves passively through the cell wall and forms a droplet at the root tip (Morre et al., 1967). The passage trough the cell wall is probably due to an increase in the permeability of the middle lamella (Lynch and Staehelin, 1995). Under controlled conditions, the formation of the droplet follows a 3–4 h cycle (Morre et al., 1967). However, in these laboratory experiments, the saturated moisture and the periodic complete removal of the mucilage might probably have increased the droplet formation. In soil, it can be assumed that conditions might not be as favourable to the production of such an important amount of polysaccharide.

The properties of the mucilage secreted by the root cap have been extensively studied. The $\mathrm{COO^-}$ groups of the mucilage can bind to cations and in particular, those fixed to clay (Guckert et al., 1975; Jenny and Grossenbacher, 1963). Consequently, soil structure is affected and stability of aggregate is generally increased (Czarnes et al., 2000; Traore et al., 2000). Heavy metals also bind to root cap slime (Morel et al., 1986) and this may play a significant role in the protection of the root tip against their toxicity (Miyasaka and Hawes, 2001).

The root cap mucilage is able to hydrate extensively. Fully hydrated mucilage has a water content of 100,000% of dry weight but such a hydration is only obtained in the presence of free water (McCully and Boyer, 1997). Indeed, in mucilage collected on nodal roots of maize, the water content (% of dry weight) increases only up to 450% when the water potential of the mucilage increases from -11 MPa to -0.01 MPa (McCully and Boyer, 1997). Thus, unless the soil is saturated with water, the root cap mucilage appears as a dry coating over the apex and does not form a droplet as it is often observed in vitro (McCully and Sealey, 1996; Sealey et al., 1995). Furthermore, the surfactant and viscoelastic properties of the mucilage (Read and Gregory, 1997) might favour the adhesion of root cap cells to the soil particles and hence, their separation from the cap as the root tip moves trough the soil. This process is consistent with the rhizosheath observed on roots of grasses (Vermeer and McCully, 1982; Watt et al., 1994). The sheath consists in soil + mucilage and living border cells tightly adhering to the root. The mucilage originates both in the root cap and in microbial syntheses (Watt et al., 1993). The sheath is not observed just behind the root tip because the epidermis of this area has a thick complex surface on which mucilage does not adhere (Abeysekera and MacCully, 1993; McCully, 1999). The rhizosheath may function like a biofilm involved in plant nutrition and may have an important role in resistance to drought (Watt et al., 1993).

The formation of the rhizosheath from root cap mucilage suggests that its mineralization by microorganisms is reduced or very slow. In vitro, root mucilage can readily be utilised by rhizosphere bacteria as a sole source of carbon (Knee et al., 2001). Furthermore, in a laboratory experiment, Mary et al. (Mary et al., 1993) demonstrated that maize mucilage incubated in soil was mineralised at 45% of the added C within 2 weeks. However, in the rhizosphere, mucilage mineralization may be delayed by the preferential use by microorganisms of root exudates, which are more readily available and by the protection of mucilage due to its adsorption to the soil matrix (Sollins et al., 1996).

The amounts of mucilage synthesized in vitro ranges from 11 to 47 μg MS/mg MS root growth (Table 1). However, these quantities were determined from roots grown in water or in nutrient solution, which increases the outward diffusion of the mucilage from the periplasmic region and probably stimulates the biosynthesis of the slime (Sealey et al., 1995). Consistently with this, the estimation of the quantity of mucilage produced in soil based on the size of the droplet surrounding the root cap in vitro might be overestimated: 700 and 1,250 m^3/ha for wheat and maize, respectively (Samsevitch, 1965) (Table 1). At present time, the amount of mucilage produced in soil remains unknown.

2.3 Root Exudation

Excretion of organic compounds from roots was first reported as early as the end of the nineteenth century. In 1894, Dyer demonstrated the release of acidic substances from roots of barley, wheat and others (Krasil'nikov, 1961). The biochemical nature of compounds excreted by roots demonstrates a wide variety: simple and complex sugars, amino acids, organic acids, phenolics, alcohols, polypeptides and proteins, hormones, enzymes (Curl and Truelove, 1986; Grayston et al., 1996; Neumann and Römheld, 2000). In the literature, the meaning of the term "exudation" may differ significantly. Sensu stricto, exudates were first defined as low molecular weight compounds diffusing passively from intact cells to the soil solution (Rovira et al., 1979). However, "root exudates" is often used to describe more generally the low molecular compounds released from roots regardless of the process by which they are deposited into the rhizosphere. The main low molecular weight compounds released passively from roots are sugars, amino acids and organic acids. They diffuse passively from the cytoplasm that is commonly three orders of magnitude more concentrated than the soil solution (mM vs μM, respectively) (Neumann and Römheld, 2000). For example, in maize roots, average concentrations are 86 mM for sugars (Jones and Darrah, 1996), 9.5 mM for amino acids (Jones and Darrah, 1994) and 10–20 mM for organic acids (Jones, 1998). The lipid bilayer of the plasmalemma is a barrier to free diffusion of solutes because its permeability is reduced, especially for charged compounds compared to neutral molecules. However, the protons excreted by the H^+-ATPase provide an electrochemical gradient for the diffusion of anions (Jones, 1998). Transient defects in the plasmalemma can also significantly increase its permeability as suggested for amino acids (Chakrabarti and Deamer, 1992).

Membranes of plant cells bear sugar and amino acids proton-coupled ATPase transporters that mediate assimilate imports into cells (Bush et al., 1996). Hence, it is not surprising that in vitro, plant roots are able to actively take up sugars and amino acids from a solution (Jones and Darrah, 1994, 1996; Schobert and Komor, 1987; Soldal and Nissen, 1978; Xia and Saglio, 1988). The consequence of this influx on net exudation may be important in axenic nutrient solution but in soil, the evidences are less obvious. Indeed, microorganisms are also very efficient competitors for the uptake of sugars and amino acids (Coody et al., 1986; Jones, 1999; Nguyen and Guckert, 2001; Vinolas et al., 2001). The injection of labelled compounds into the rhizosphere indicated that plant capture was of minor importance compared to microbial uptake of glucose and of charged or uncharged amino acids (Nguyen et al., 2002; Owen and Jones, 2001; Schobert et al., 1988). Therefore, it is not known if the plant can tune the net exudation in non-sterile soil by altering the influx of sugars and amino acids.

In maize, the spatial examination of exudation indicates a greater efflux of solutes close to the root apex (McCully and Canny, 1985; McDougall and Rovira, 1970). This does not seem to relate to variability in the plasmalemma permeability nor to the spatial repartition of transporters, which is uniform along maize roots (Jones and Darrah, 1994, 1996). The greater exudation behind the root apices is consistent with the concentration gradients of sugars and amino acids inside the root (Jones, 1998) and with the diffusion through the apoplast of sugars from the phloem to the apical meristems (Jones, 1999), diffusion that is supported by experimental and theoretical evidences (Bret-Harte and Silk, 1994).

The amount of C exuded has been expressed in a wide range of units. Table 2 gives some estimates reviewed from the literature for plants cultivated in nutrient solution. Due to the re-sorption of exudates by plant roots, these values have to be considered with caution if extrapolations to soil conditions are aimed. It can also be seen that the proportion between sugar, amino acids and organic acids vary greatly, especially between sugars and organic acids. The relative proportions of sugars and amino acids exuded reflect quite correctly the relative concentrations of root tissues for these solutes.

Besides the passive diffusion of solutes, plants are able to response to environmental conditions by altering their excretion of organic compounds. For example, in response to environmental nutrient stress such as P or Fe deficiencies, anion channel proteins, embedded in the plasmalemma, increase significantly the passive efflux of carboxylates (malate, citrate, oxalate) whose complexing properties facilitate nutrient mobilization by the plant (Jones, 1998; Neumann and Martinoia, 2002; Neumann and Römheld, 2000). The chelating properties of organic acids are also a central mechanism involved in rhizosphere detoxification as demonstrated in aluminium tolerant plants (Barcelo and Poschenrieder, 2002; Gaume et al., 2001; Ma et al., 2001). Apart from organic acids, many other compounds are released by plant roots in response to environment. The most studied are phosphatases excreted by roots in P-stressed plants (Gaume et al., 2001; Miller et al., 2001), phytosiderophores released in Gramineous plants and which are involved in micronutrient acquisition (Crowley, 2000; VonWiren et al., 1996) and some phenolics such as flavonoids, which play an important role in symbiosis establishment (Werner, 2000). A comprehensive review covering these compounds is available in (Neumann and Römheld, 2000). There are numerous evidences that both the amount and the nature of root exudates are very variable according to the physiological status of the plant and to the plant species (Fan et al., 2001; Grayston et al., 1996; Neumann and Römheld, 2000). Therefore, it can be assumed that the controlled release of particular exudates in response to sensed environmental stimuli is probably a major mechanism that allow the plant to face unfavourable rhizosphere conditions such as nutrient deficiencies, toxicities or proliferation of pathogenic microorganisms.

2.4 Senescence of Root Epidermis

Behind the root tip, epidermal cells differentiate either into hair cells (trichoblast) or non-hair cells (atrichoblat). Root hairs are involved in anchorage, in water and nutrient uptake and in symbiosis (Hofer, 1991; Peterson and Farquhar, 1996). In the past recent years, extensive research detailed the genetic control of root hair development, especially in *Arabidopsis* (reviewed in (Gilroy and Jones, 2000; Schiefelbein, 2000)). From a study carried out by Dittmer (Dittmer, 1949) on 37 species belonging to 20 angiosperm families, the size of root hairs is quite constant within a given species but is very variable between species. Root hairs are typically 80–1,500 μm long and have a diameter of 5–20 μm. The root hair zone is in average 1–4 cm long (Hofer, 1991). Literature gives evidences that root hair density is also very variable between plants: 1–180 hairs mm^{-1} of root, 70–10,800 hairs cm^{-2} of root (Table 3).

Furthermore, environment strongly influences root hair development. For example, low levels of minerals, especially P and nitrate (Jungk, 2001; Ma et al., 2001), mechanical constraint, low O_2 partial pressure or high temperatures stimulate root hair formation. Similar effects can be observed when roots are exposed to ethylene which suggests that ethylene could be involved in the regulation of root hair development by environmental factors (Michael, 2001).

There is little information about the lifespan of root hairs. Based on the loss of the nucleus, it was estimated that the longevity of root hairs was 2–3 weeks in wheat, barley and maize (Fusseder, 1987; Holden, 1975). However, microscopical examinations indicate some cytoplasm lyses in 4 days old hairs in maize (Fusseder, 1987). Thus despite the cell wall can persist for several weeks or months (Hofer, 1991), the life span of root hairs is probably shorter i.e. 2–3 days. If root hairs are considered as cylinders that have a dry weight:fresh weight (DW:FW) ratio of 0.072, a density equal to 1 g cm^{-3} and a C content of 40% DW, the calculation for a hair density of 50 hairs mm^{-1} root indicates that small hairs (80 μm of length, 5 μm of diameter) correspond 2.2 ngC mm^{-1} root whereas large

Table 2 Quantities of C in root exudates of different plant species

References	Plant	Amount	Units	Compounds	Comments
Barber and Gunn (1974)	*Hordeum vulgare*	76–157	µg/plant/day	Exudates	Depending on mechanical constraint, 21 days of growth
		0.2–0.4	%root DM/day	Exudates	Calculated from original data
		5–9	%root DM		
Haggquist et al. (1984)	*Brassica napus*	16–21	µgC/plant/day	Total C	Sterile and non sterile roots, calculated from original data
In Hale and Moore (1979)	*Acer saccharum*	2.7–6.7	%root DM/day	Exudates	Defoliated-control, calculated from Smith (1971)
	Agropyron smithii	0.01	%root DM/day	Reducing sugars	Defoliated/control, effect of temperature, calculated from Bokhari and Singh (1974)
Kraffczyk et al. (1984)	*Zea mays*	0.03–0.06	%root DM/day	Sugars	Sterile and non sterile roots, 23 days of growth
		0.03–0.04	%root DM/day	Organic acids	Idem
		0.001	%root DM/day	Amino acids	Idem
		0.02–0.03	%root DM/day	Sugars	Three levels of K tested, nitrate + ammonium, 23 days of growth
		0.01–0.07	%root DM/day	Organic acids	Idem
		0.0005–0.0007	%root DM/day	Amino acids	Idem
		0.001–0.002	%root DM/day	Sugars	Three levels of K tested, nitrate, 25 days of growth
		0.016–0.019	%root DM/day	Organic acids	Idem
		0.0004–0.001	%root DM/day	Amino acids	Idem
Prikryl and Vancura (1980)	*Triticum aestivum*	121–153	µg C/cm root growth	Exudates	Sterile, nutrient solution: 2 or 4 day replacement
		196–226	µg C/mg DM root growth	Exudates	Sterile non sterile nutrient solution: 2 day replacement
		576–1,174	µg C/mg C root growth	Exudates	Nutrient solution: 2 day replacement, sterile-inoculated with *Pseudomonas putida*
				Exudates	
Jones and Darrah (1993)	*Zea mays*	0.1–1.2	% root DM/day	Exudates	Calculated from original data, Sterile, no or daily changes of nutrient solution, 10 day culture
		1.22	µgC/root tip/h	Exudates	Standard values for model simulation
		0.24	µgC/cm of root/h	Exudates	Idem

hairs (1,500 µm of length, 20 µm of diameter) are equivalent to 680 ng C mm^{-1}root. Medium size hairs (500 µm of length, 10 µm of diameter) correspond to 56 ngC mm^{-1}root. Theoretically, these amounts of C should be deposited into the soil after the hair death. However, to our knowledge, it is unknown if the cytoplasm material is released into the soil or recycled within the root tissue.

Table 3 Root hair density in different plant species

References	Plant	Root radius (cm)	Root hairs (mm^{-1} root)	Root hairs (cm^{-2} root)	Root hair length (mm)
Gahoonia et al. (1997)	*Triticum aestivum*	8.50×10^{-3}	38	7,115	1.27
	Triticum aestivum	8.00×10^{-3}	25	4,974	0.74
	Triticum aestivum	7.50×10^{-3}	24	5,093	0.49
	Hordeum vulgare	8.50×10^{-3}	30	5,617	1.1
	Hordeum vulgare	7.50×10^{-3}	27	5,730	0.52
	Hordeum vulgare	7.50×10^{-3}	31	6,578	1
	Hordeum vulgare	8.00×10^{-3}	30	5,968	0.64
Föse (1971) in Jungk (2001)	*Spinacia oleracea*	1.07×10^{-2}	71	10,561	0.62
	Brassica napus	7.30×10^{-3}	44	9,593	0.31
	Lycopersicon esculentum	1.00×10^{-2}	58	9,231	0.17
	Triticum aestivum	7.70×10^{-3}	46	9,508	0.33
	Allium cepa	2.29×10^{-2}	1	69	0.05
	Lolium perenne	6.60×10^{-3}	45	10,851	0.34
	Phaseolus vulgaris	1.45×10^{-2}	49	5,378	0.2
Masucci et al. (1996)	*Arabidospis thaliana*		53–63		
Bouma et al. (2000)	*Elymus pycnathus* (L.)				
	Main root		44		0.37
	First-order branching		7		0.32
	Second-order branching		3.5		0.32
	Puccinellia maritima (L.)				
	Main root		20		0.51
	First-order branching		11		0.47
	Second-order branching		5		0.5
	Spartina anglica (L.)				
	Main root		21		0.17
	First-order branching		10		0.24
	Second-order branching		5		0.25
Wulfsohn et al. (1999)	*Agropyron cristanum*				
	Main root		71		0.19
	Branchings		181.6		0.153

Despite it is not a general rule, there are numerous reports that cells from the root epidermis senesce (Curl and Truelove, 1986). For instance, in maize, the senescence is extensive proximal to the region where the late metaxylem matures (Wenzel and McCully, 1991). The senescence can even concern cortical cells. The nuclear staining with acridine orange pointed out that senescence of cortical cells concerns the old parts of the roots but some works also indicated the absence of nucleus in the cortex of young roots in cereals (Fusseder, 1987; Henry and Deacon, 1981; Holden, 1975). However, the impermeability of the cell wall to the stain may cause an artefact that biases the evaluation of the cell vitality (Wenzel and McCully, 1991). Thus, it would be necessary to gain more information about (1) the life span of the root epidermis (including root hairs) and of the root cortex in soil conditions, (2) the fate of the intracellular content of the senescing root cells.

2.5 Relative Proportion of Rhizodeposits

Due to the very different units used to express the quantities of C from rhizodeposits, comparisons are difficult. However, from Table 1, it is reasonable to estimate that border cells represents 1–3 μg C mg^{-1} DM of root growth, or 1.5–2.5 μg C day^{-1} $root^{-1}$. In average, mucilage ranges between 2 and 20 μg C mg^{-1} dry matter (DM) of root growth, assuming a C content of 39% DM. In comparison, Table 2 indicates mean exudation values of 150 μg C mg^{-1} DM of root growth, 0.2–7% root DM/day. These calculations suggest that exudation releases almost 10–100 times more carbon than border cells and mucilage. As calculated previously, the death of root hairs with a medium size and density would deposit 56 ng C mm^{-1} root. If root hair decay concerns 1 cm of root per day, which is reasonable, the amounts deposited are 3 orders of magnitude less than exudation.

3 Factors Affecting C Fluxes to the Rhizosphere

Factors affecting the release of C from roots into the soil are numerous and have been extensively reviewed e.g. (Curl and Truelove, 1986; Grayston et al., 1996; Hale and Moore, 1979; Rovira, 1969; Whipps, 1990). The literature points out that the total amounts of organic C deposited in the rhizosphere can vary greatly according to the plant ecophysiology. This can be explained as follows. Both the environment and the plant genetics and physiology can influence (1) the flux of C from each root to the rhizosphere, which is related to the root functioning and (2) the size and the morphology of the overall root. Therefore, any attempt to model rhizodeposition will have to consider the plant ecophysiology. The aim of that part of the paper is to examine tracer studies to analyse the main factors that affect rhizodeposition. To reach that goal, we have analysed the partitioning of net fixed C between the plant-soil pools from ^{14}C tracer experiments. The main factors examined are plant age, microorganisms, soil texture, soil nitrogen and atmospheric CO_2 concentration. There are of course numerous other factors that alter rhizodeposition. They were not detailed in the present study because no sufficient data from ^{14}C-labelling experiments were available. Among them are light intensity (Hodge et al., 1997), photoperiod (Todorovic et al., 1999), temperature (Martin and Kemp, 1980), soil pH (Meharg and Killham, 1990a), anoxia (Meharg and Killham, 1990c), defoliation (Holland et al., 1996; Paterson and Sim, 2000).

Tracer experiments have been chosen because studies are numerous and because the expression of results in terms of partitioning coefficients of net fixed C is a common basis for the majority of articles. Indeed, in non-tracer experiments, the comparison between studies is difficult or impossible because the classification of rhizodeposits is not uniform among articles (soluble, insoluble, sugars, total C, etc.) and because results are expressed in a wide range of units (Toal et al., 2000). Among tracer experiments, labelling of photoassimilates with ^{14}C is the most commonly used technique to study C flow to the rhizosphere. Others isotopes have also been used for labelling experiments such as ^{11}C and ^{13}C and C flows to the soil can also be studied using natural abundance of ^{13}C. All these techniques have been reviewed elsewhere e.g. (Kuzyakov and Domanski, 2000; Meharg, 1994; Morgan and Whipps, 2000) and will not be detailed here.

The present study examines experiments in which plants shoots were exposed to ^{14}C except (Palta and Gregory, 1997) in which ^{13}C was used as tracer. The exposition of shoots to the tracer was either as a pulse (few minutes to several hours) or as a permanent exposition from germination until sampling. These two procedures refer to as pulse or continuous labellings. Briefly, pulse labelling experiments are very useful to obtain information on C fluxes in relation to the plant ecophysiology but due to the brief exposition of the plant to the tracer, this technique fails to provide reliable C budgets, which can be assessed by continuous labelling. Moreover, on a technical point of view, continuous labellings differ from pulse-chase experiments in that they are cumbersome, expensive and hardly applicable in field situations (Meharg, 1994). However, Warembourg and Estelrich (Warembourg and Estelrich, 2000) compared 298 h and 78 day long labellings in *Bromus erectus*. They concluded that reliable estimations of C fluxes to the rhizosphere can be obtained from an intermediate strategies consisting in repeated short-term labellings of a few days each.

The tracer experiments reviewed for the present study expressed results as ^{14}C-partitioning coefficients, i.e. percentages of the net fixed C allocated to C compartments. The compartments are shoot and root C, CO_2 from rhizosphere respiration (root respiration + the rhizomicrobial respiration i.e. microbial respiration derived from rhizodeposition) and C in soil residues. The respective partitioning coefficient are SHOOT, ROOT, RR, RES. We have also investigated partitioning of ^{14}C between belowground compartments as percentages of labelled C exported by shoots. These partitioning coefficients are ROOTBG, RRBG and RESBG. Articles for which ^{14}C partitioning was not complete were discarded. Data were analysed with SAS V 8.02 for Windows (Microsoft), The SAS Institute Inc., Cary NC, USA.

3.1 Data Overlook

There were 43 articles examined. A given article presents as many sets of ^{14}C-partitioning coefficients as experiments/treatments. For example, an article that examines the effect of nitrogen fertilization (+N, −N) and of elevated CO_2 (elevated, ambient) will provide four sets of coefficients. Hence, the total number of coefficient sets analysed was 237 (Table 4). There are more data for pulse labellings than for continuous labellings (137 sets of coefficients vs 100, respectively) despite the number of articles analysed are comparable, around 20. Whatever the labelling procedure, the maximum contribution of an article to the total number of coefficient sets is 12–26% (data not shown).

The data point out that tracer experiments focus on a restricted number of plants (Table 4). There are 1.5 times more data for annual plants than for perennials. Among annuals, *Triticum aestivum* and *Hordeum vulgare* and *Zea mays* represent 88% of the coefficient sets and among perennials *Lolium perenne* represents more than 35% of the coefficient sets. More than 65% of the coefficient sets concern *Lolium perenne* and *Bromus erectus*. Data on *Bromus erectus* were drawn from a single article. Examination of plant age indicates that the great majority of the data concerns juvenile stage of development (Table 5). In continuous labelling experiments, the mean is 37 days and the median 28 days. In pulse labelling studies, the mean is 146 days and the median is 87 days but the coefficient of variation for the mean is two times greater that that in continuous labellings. The difficulty to maintain an atmosphere with a constant $^{14}CO_2$ activity and constant CO_2 concentration (Warembourg and Kummerow, 1991) can greatly contribute to explain the fact that continuous labelling focussed on younger plants compared to pulse-chase studies. Indeed, in pulse labelling experiments, late development stages such as flowering and grain filling have been investigated (Keith et al., 1986; Meharg and Killham, 1989; Swinnen and Van Veen, 1994).

Table 6 outlines that the partitioning coefficients from continuous labelling studies are normally distributed except the RES and RESBG coefficients (^{14}C in soil residues), the distributions of which are skewed to low values (Data not shown). This means that the majority of the data are low and few coefficients extend to greater values. The root sampling procedure may provide a possible explanation for the non-normality of these coefficients. Indeed, it is very difficult to separate, by hand picking/sieving, the fine roots from the soil and a variable proportion of them may be left in the soil, increasing artificially the values of RES and RESB coefficients. Moreover, the washing of roots extracts some soluble ^{14}C, which can also overestimate labelled soil residues (Swinnen et al., 1994). In pulse labelling experiments, none of the partitioning coefficients are normally distributed (Table 6). The distribution of PA coefficients has a low Kurtosis (data not shown). For the other coefficients, once again, data are skewed to the low values. The non-normality of the distribution of the partitioning coefficients in pulse-chase experiments can be explained by the non-standardization of the labelling procedures. Among the studies, both the exposition of shoots to $^{14}CO_2$ and the chase period are very variable in length (Table 5). The length of the labelling ranges from 20 min to 720 h (data not shown) with a median of 6 h and a mean of 108 h. The length of the chase period, that is the time elapsed between the labelling and the sampling, is probably the key point that affects assimilates partitioning. In the articles reviewed, the chase period ranges from 30 min to 504 h (data not shown) with a mean and a median equal to 145 h and 48 h, respectively (Table 6). This indicates that in general, the chase period is short, which could lead to an incomplete partitioning of ^{14}C and to an overestimation of the ^{14}C recovered in shoots and an underestimation of C flows to belowground. This is supported by the greater mean SHOOT coefficient in pulse-labelling studies compared to continuous labelling studies (64 vs 57, Table 6). Conversely, a long chase period may increase the labelled carbon retrieved in the rhizosphere respiration and decrease the ^{14}C in the soil residues.

The mean partitioning coefficients for SHOOT, ROOT, RR and RES determined from pulse and continuous labelling experiments are 64%, 20%, 12% and 5% and 57%, 22%, 14%, 7%, respectively. This indicates that shoots export almost half of the net fixed C to belowground (Lambers, 1987). In average, among the net C allocated belowground, half is retained in root tissues, a third is lost as root + rhizomicrobial respiration and more than 15% is retrieved as soil residues. It is interesting to note that in pulse-chase experiments, SHOOT coefficients for annual plants are greater than that of perennials, the contrary being observed for ROOT and RR coefficients. There are also differences between perennials and annuals for the belowground budget. Further investigations are needed to explore if these results are representative of a different strategy of assimilates partitioning to the soil between annual and perennial plants (Warembourg and Estelrich, 2001).

Table 4 Number of partitioning coefficient sets reviewed for different plant species. A partitioning coefficient set consists in the percentages of the tracer allocated to shoots, roots, rhizosphere respiration and soil residues

				Partitioning coefficient sets			
Annual (A)/ perennial (P)	Plante	Labelling	References	Number	Total/species	% of total relative to labelling	% of total
A	*Triticum aestivum*	C	Barber and Martin (1976); Billes et al. (1993); Liljeroth et al. (1994); Martin (1977); Martin and Kemp (1980); Merckx et al. (1985, 1986); Swinnen and Van Veen (1994)	45		56.3	
A		P	Gregory and Atwell (1991); Keith et al. (1986); Palta and Gregory (1997); Paterson et al. (1996); Swinnen et al. (1994); Swinnen et al. (1995); Whipps (1984); Whipps and Lynch (1983)	30	75	49.2	53.2
A	*Hordeum vulgare*	C	Barber and Martin (1976); Johansson (1992); Whipps (1984); Whipps and Lynch (1983); Zagal (1994)	13		16.3	
A		P	Gregory and Atwell (1991); Jensen (1993); Swinnen et al. (1995)	13	26	21.3	18.4
A	Zea mays	C	Helal and Sauerbeck (1984, 1986); Liljeroth et al. (1994); Martens (1990); Merckx et al. (1987); Whipps (1985)	15		18.8	
A		P	Holland et al. (1996); Kisselle et al. (2001); Todorovic et al. (2001)	8	23	13.1	16.3
A	*Bromus madritensis*	P	Warembourg and Estelrich (2001)	8	8	13.1	5.7
A	*Brassica napus*	C	Zagal (1994)	2		2.5	
A		P	Shepherd and Davies (1993)	1	3	1.6	2.1
A	*Lycopersicon esculente*	C	Whipps (1987)	2	2	2.5	1.4
A	*Pisum sativum*	C	Whipps (1987)	2	2	2.5	1.4
A	*Medicago truncatula*	C	Crawford et al. (2000)	1		1.3	
A		P	Crawford et al. (2000)	1	2	1.6	1.4
			Total continuous labelling experiments	**80**			
			Total pulse labelling experiments	**61**			
			Total	**141**			
P	*Lolium perenne*	P	Domanski et al. (2001); Meharg and Killham (1989, 1990a–c); Paterson et al. (1996, 1999); Rattray et al. (1995)	23		30.3	

(continued)

Table 4 (continued)

Annual (A)/ perennial (P)	Plante	Labelling	References	Partitioning coefficient sets Number	Total/species	% of total relative to labelling	% of total
P		C	Gorissen et al. (1996); Hodge et al. (1997); Van Ginkel et al. (1997); Zagal (1994)	12	35	60.0	36.5
P	*Bromus erectus*	P	Warembourg and Estelrich (2001)	28	28	36.8	29.2
P	*Castanea sativa*	P	Rouhier et al. (1996)	8	8	10.5	8.3
P	*Trifolium repens*	P	Todorovic et al. (1999)	5	5	6.6	5.2
P	*Festuca arundinacea*	C	Gorissen et al. (1996)	4	4	20.0	4.2
P	*Pinus taeda*	P	Reid et al. (1983)	4	4	5.3	4.2
P	*Populus tremuloides*	P	Mikan et al. (2000)	4	4	5.3	4.2
P	*Festuca pratensis*	C	Johansson (1992a, 1993)	3	3	15.0	3.1
P	*Cynodon dactylon*	P	Paterson et al. (1996)	2	2	2.6	2.1
P	*Lolium multiflorum*	P	Henry et al. (in press)	2	2	2.6	2.1
P	*Bouteloua gracilis*	C	Dormaar and Sauerbeck (1983)	1	1	5.0	1.0
			Total pulse labelling experiments	**76**			
			Total continuous experiments	**20**			
			Total	**96**			

C = continuous labelling, P = pulse labelling

Table 5 Age of plants and labelling characteristics in the tracer experiments reviewed

	Continuous labelling experiments Annual (A)/ perennial (P)	N[a]	Mean	Median	CV of mean[b]	Pulse labelling experiments N[a]	Mean	Median	CV of mean[b]
Age (days)	A	80	31.7	24.0	51.2	60	97.5	82.5	68.2
	P	20	56.0	59.0	31.1	66	190.5	93.0	97.5
	Total	100	36.6	28.0	52.1	126	146.2	86.5	101.9
Chase (h)	A					60	230.6	92.0	96.5
	P					72	73.6	48.0	78.1
	Total					132	145.0	48.0	120.0
Length of labelling (h)	A					60	60.1	1.8	237.8
	P					72	148.2	76.0	105.8
	Total					132	108.2	6.0	144.6

[a] Number of partitioning coefficient sets

[b] Coefficient of variation of the mean (%)

The SHOOT coefficient is significantly and negatively correlated to all of the belowground coefficients (ROOT, RR and RES) (Table 7). The correlations are stronger in pulse-chase experiments, which can be related to the greater temporal resolution of pulse labelling compared to continuous labelling. Whatever the kind of labelling, the ROOTBG, coefficient is significantly correlated positively to RRBG and to RESBG. This suggests a strong link between rhizodeposition and the metabolic activity of roots. This is consistent with the mechanisms involved in the release of C from roots. Indeed, an important exportation of photoassimilates from the shoots to the roots is expected to maintain the solute gradient between the root tissue and the soil solution and so, to favour the passive diffusion of root exudates into the soil. Moreover, a rapid root growth should increase the number of border cells and mucilage deposited into the soil as the result of frictional forces experienced by the foraging root apices.

Table 6 ^{14}C budget for tracer studies of C translocation into the soil. Results are expressed as percentages of the net ^{14}C fixed

	Labelling[a]	Annual (A)/ perennial (P)	N[b]	Mean	Maximum	Minimum	Median	CV of mean[c]	Pn[d]
SHOOT	C	A	80	56.6	78.9	34.8	55.2	16.8	
	C	P	20	58.2	86.4	22.0	60.1	26.9	
	C	**All**	**100**	**57.0**	**86.4**	**22.0**	**55.6**	**19.2**	**0.302**
	P	P	76	56.5	99.1	18.8	56.8	33.9	
	P	A	61	72.8	97.4	25.6	73.9	25.8	
	P	**All**	**137**	**63.7**	**99.1**	**18.8**	**65.8**	**32.3**	**0.003**
ROOT	C	A	80	21.2	37.5	3.9	21.8	37.1	
	C	P	20	27.1	40.4	9.1	28.9	33.8	
	C	**All**	**100**	**22.4**	**40.4**	**3.9**	**23.4**	**37.7**	**0.336**
	P	P	76	24.0	55.0	0.1	21.8	60.5	
	P	A	61	13.8	55.9	0.4	10.4	95.3	
	P	**All**	**137**	**19.5**	**55.9**	**0.1**	**16.1**	**75.9**	**<0.0001**
RR	C	A	80	14.5	26.1	0.1	15.3	44.5	
	C	P	20	10.1	22.0	0.5	9.3	60.7	
	C	**All**	**100**	**13.6**	**26.1**	**0.1**	**13.9**	**48.5**	**0.050**
	P	P	76	14.3	57.0	0.5	14.2	66.6	
	P	A	61	8.9	35.7	0.9	6.2	81.2	
	P	**All**	**137**	**11.9**	**57.0**	**0.5**	**10.9**	**75.3**	**<0.0001**
RES	C	A	80	7.7	30.4	1.2	6.4	78.2	
	C	P	20	4.5	23.0	1.9	3.6	100.1	
	C	**All**	**100**	**7.1**	**30.4**	**1.2**	**5.3**	**83.1**	**<0.0001**
	P	P	76	5.1	16.0	0.0	4.6	72.6	
	P	A	61	4.5	20.7	0.1	3.5	95.2	
	P	**All**	**137**	**4.9**	**20.7**	**0.0**	**4.2**	**82.2**	**<0.0001**
ROOTBG	C	A	80	48.4	76.8	10.0	51.2	28.2	
	C	P	20	66.0	84.8	40.3	67.3	16.9	
	C	**All**	**100**	**52.0**	**84.8**	**10.0**	**55.2**	**28.7**	**0.181**
	P	P	76	53.9	90.8	1.6	57.9	40.6	
	P	A	61	44.1	86.2	11.2	42.3	48.0	
	P	**All**	**137**	**49.6**	**90.8**	**1.6**	**52.1**	**44.5**	**0.006**
RRBG	C	A	80	34.0	79.3	0.2	36.2	41.9	
	C	P	20	22.6	45.1	3.6	24.8	46.4	
	C	**All**	**100**	**31.7**	**79.3**	**0.2**	**33.4**	**45.0**	**0.024**
	P	P	76	34.2	86.4	1.2	29.2	64.4	
	P	A	61	35.6	63.8	7.0	37.6	41.0	
	P	**All**	**137**	**34.9**	**86.4**	**1.2**	**33.1**	**54.7**	**0.015**
RESBG	C	A	80	17.6	69.4	2.4	15.3	68.0	
	C	P	20	10.9	29.5	4.3	8.8	57.6	
	C	**All**	**100**	**16.3**	**69.4**	**2.4**	**14.4**	**69.9**	**<0.0001**
	P	P	76	11.8	35.6	0.1	11.9	61.1	
	P	A	61	20.2	69.9	1.1	19.6	77.1	
	P	**All**	**137**	**15.6**	**69.9**	**0.1**	**13.9**	**79.5**	**<0.0001**

RR and RES are %^{14}C allocated to rhizosphere respiration and soil residues, respectively

The suffix BG is used when partitioning coefficients are expressed as percentages of ^{14}C allocated to belowground

[a] C = continuous labelling, P = pulse labelling

[b] Number of partitioning coefficient sets

[c] Coefficient of variation of the mean (%)

[d] Test for the normality of the distribution (Shapiro-Wilk test). Probability associated to the null hypothesis of normality of the distribution

Data from continuous labelling experiments indicate that the RRBG and RESBG coefficients are significantly negatively correlated ($r = -0.32$). Hence, this might reflect the fact that according to the studies, a variable fraction of the rhizodeposits are mineralised by the microorganisms, which consequently alters symmetrically the RESBG and RRBG coefficient. If this hypothesis is valid, this means that the rhizomicrobial

Table 7 Pearson correlation coefficients between partitioning coefficients in tracer studies. The value in italic is the probability associated with the null hypothesis Rho = 0

Labelling[a]		SHOOT	ROOT	RR	RES
C	SHOOT	1			
C	ROOT	−0.675	1		
		<0.0001			
C	RR	−0.433	−0.117	1	
		<0.0001	*0.2459*		
C	RES	−0.417	−0.043	−0.141	1
		<0.0001	*0.6731*	*0.1614*	
P	PA	1			
P	RAC	−0.857	1		
		<0.0001			
P	RR	−0.648	0.217	1	
		<0.0001	*0.011*		
P	RES	−0.515	0.257	0.341	1
		<0.0001	*0.0024*	*<0.0001*	
Belowground budget					
		ROOTBG	RRBG	RESBG	
C	ROOTBG	1			
C	RRBG	−0.696	1		
		<0.0001			
C	RESBG	−0.452	−0.324	1	
		<0.0001	0.001		
P	ROOTBG	1			
P	RRBG	−0.782	1		
		<0.0001			
P	RESBG	−0.535	−0.008	1	
		<0.0001	0.9239		

RR and RES are %^{14}C allocated to rhizosphere respiration and soil residues, respectively
The suffix BG is used when partitioning coefficients are expressed as percentages of ^{14}C allocated to belowground
[a] C=Continuous labelling, P=Pulse labelling

respiration contributes significantly to the rhizosphere respiration. In pulse labelling experiments, no such correlation is observed probably because of the variability of the length of the chase period.

3.2 Factors that Affect the Partitioning of ^{14}C-Assimilates to the Soil: A Quantitative Approach

3.2.1 Methods for Calculations

The following methods were applied to appreciate the effect of a factor on the partitioning of ^{14}C between plant-soil C pools. Be a given factor F tested at n levels, the relative variation (RV) in a partitioning coefficient 'PC' was calculated as $RV = (PC_n - PC_{n-1})/PC_{n-1}$. The level 'n' of the factor F was always high relative to the level $n - 1$. For example, if an article reports on the effect of N fertilization tested at $N1 < N2$ levels, the relative variation in partitioning of ^{14}C to shoots was calculated as follows: $RV_{SHOOT} = (SHOOT_{N2} - SHOOT_{N1})/SHOOT_{N1}$. The same calculations were performed for the other partitioning coefficients. Hence, a positive RV indicates that the factor increases the partitioning of assimilates to the compartment considered. If a second factor was studied such as the concentration of atmospheric CO$_2$, applied at two levels $L1 < L2$, the effect of nitrogen was calculated at the two levels of CO$_2$: $(SHOOT_{N2L1} - SHOOT_{N1L1})/SHOOT_{N1L1}$ and $(SHOOT_{N2L2} - SHOOT_{N1L2})/SHOOT_{N1L2}$. If a factor was tested at more than two levels $L1 < L2 < L3 \ldots < Ln$, the effects were calculated relative to two subsequent levels: Ln vs $Ln - 1$, $Ln - 1$ vs $Ln - 2 \ldots L2$ vs $L1$.

For each factor investigated, the relative variation coefficients RVs were classified according to the labelling procedure (continuous or pulse). Then, the maximum, the minimum, the median, the mean and its coefficient of variation were calculated. The normality of the RVs distributions were tested by the Shapiro-wilk test. If normalilty was accepted (at alpha $= 5\%$), the Student t test was used to test the null hypothesis: mean $= 0$, otherwise the non-parametric sign test was applied to test the null hypothesis: median $= 0$.

3.2.2 Plant Age

The data from pulse-chase experiments summarized in Table 8 clearly demonstrate that plant age significantly influences C partitioning of photoassimilates between plant-soil compartments. As the plant gets older, less carbon is partitioned to belowground. Data being non normally distributed, medians are examined. They are −43% to roots, −28% to rhizosphere respiration and −20% to soil residues (Table 8). The variability is very important as illustrated by the coefficients of variation. This is not surprising because the effect of age are more marked for young plant than for older ones, which is not taken into account in the calculations. No clear significant effect of age on C partitioning between belowground compartments can be observed. The medians of ROOTBG, RRBG and RESBG suggest that C allocated to belowground is less retained in roots when plant age increases. The partitioning coefficients from continuous labelling experiments do not evidence this pattern due to their low temporal resolution and due to the fact that very young plants were considered in these studies (37 day old in average, Table 5). Thus, the decline in C inputs into the soil with plant age is related to the decrease of assimilates partitioning to roots, which is particularly marked for annual plant (Keith et al., 1986; Swinnen and Van Veen, 1994; Swinnen et al., 1995).

3.2.3 Microorganisms

Only eight experiments are reported here for continuous labelling experiments and one in case of pulse labelling study (Table 9). This does not mean that the effects of microorganisms have not been investigated, but here, we only consider soil or sand culture experiments. In the literature, due to the difficulty to sterilized soil microcosms efficiently, the great majority of works investigating the influence of microorganisms on rhizodeposition have been performed in nutrient solution e.g. (Lee and Gaskins, 1982; Meharg and Killham, 1991).

The results indicate that microorganisms strongly increased the ^{14}C partitioned to the rhizosphere. In average, in non-sterile cultures, the RR and SOIL coefficients are significantly increased of +249% and +37%, respectively (Table 9). The same effects are observed for belowground budget. Belowground, less labelled C is partitioned to roots (−10%) whereas ^{14}C in rhizosphere respiration and in soil residues increased of +199% and +24%, respectively, but the variation of RESBG is not significant. Despite the small number of articles considered here, there are strong evidences that microorganisms increase greatly the partitioning of assimilates to the rhizosphere. There are several possible explanations. First, in non-sterile conditions, roots can establish symbiosis with mycorrhizal fungi. Mycorrhizae represent a significant sink for plant assimilates (Leake et al., 2001; Wu et al., 2002) since up to 30% of the photoassimilates can be allocated to the symbiotic fungus (Nehls and Hampp, 2000). Consequently, fungal respiration could explain the greater allocation of labelled C to rhizosphere respiration whereas growth of extraradicular hyphae and the hyphal C exudation contribute to a large extend to the plant-derived carbon retrieved as soil residues (Högberg and Högberg, 2002; Johnson et al., 2002; Sun et al., 1999). On the other hand, non-symbiotic rhizosphere microorganisms take up and assimilate soluble low molecular weight compounds released passively from root and hence, they maintain the C gradient between the internal root tissues and the soil solution. Furthermore, rhizosphere microflora can synthesize enzymes or metabolites that can alter the integrity of root cells or the permeability of their membrane. Finally, root morphology can be modified directly by phytohormones produced by rhizosphere microorganisms or indirectly by the changes in nutrient availability resulting from microbial processes. Consequently, any changes in root branching pattern would be expected to have significant consequences on root exudation, which can be more important at the root apices, such as in maize for instance (Jones and Darrah, 1996). Besides quantitative aspects of root exudation, both free and symbiotic microorganisms change the quality of root exudates. For example, Pinior et al. (Pinior et al., 1999) demonstrated that exudates from non mycorrhizal roots of cucumber stimulate hyphal growth of the mycorrhizal fungi *Gigaspora rosea* and *Gigaspora intraradices* whereas exudates

Table 8 Effect of plant age on labelled C partitioning between plant and belowground compartments. Effects are expressed as relative variations, see the text for explanations about the calculation of the effects

					Relative Variation(%)							
Factor	Labelling[a]	References		N[b]	Mean	CV of mean	Max	Med	Min	Pn[c]	Pt[d]	Pm[e]
Age (days)												
	C	Liljeroth et al. (1994); Merckx et al. (1985, 1986, 1987); Martens (1990); Whipps (1985, 1987); Zagal (1994)	SHOOT	21	4	503	59	4	−33	0.376	0.373	
Min = 14	C		ROOT	21	4	1,165	157	−8	−51	0.001		0.664
Max = 76	C		RR	21	15	266	111	5	−35	0.043		0.664
Mean = 39	C		RES	21	−11	−381	81	−19	−68	0.033		0.064
Median = 41	C		ROOTBG	21	5	937	182	−2	−63	<0.001		1.000
CV = 39	C		RRBG	21	16	211	132	2	−20	<0.001		0.007
	C		RESBG	21	−9	−464	95	−21	−65	0.075	0.335	
	P	Gregory and Atwell (1991); Jensen (1993); Keith et al. (1986); Meharg and Killham (1990b); Palta and Gregory (1997); Reid et al. (1983); Rouhier et al. (1996); Swinnen and Van Veen (1994); Swinnen et al. (1995); Warembourg and Estelrich (2001)	SHOOT	45	17	196	103	8	−57	0.013		0.000
Min = 28	P		ROOT	45	−26	−224	250	−43	−76	<0.001		0.000
Max = 600	P		RR	45	−1	−10,764	311	−28	−85	<0.001		0.008
Mean = 151	P		RES	45	18	596	374	−20	−93	<0.001		0.542
Median = 106	P		ROOTBG	45	−6	−527	143	−14	−55	<0.001		0.096
CV = 93	P		RRBG	45	26	288	293	10	−65	<0.001		0.291
	P		RESBG	45	39	203	312	24	−77	<0.001		0.096

RR and RES are %^{14}C allocated to rhizosphere respiration and soil residues, respectively

The suffix BG is used when partitioning coefficients are expressed as percentages of ^{14}C allocated to belowground

[a] C = continuous labelling, P = pulse labelling

[b] Number of partitioning coefficient sets

[c] P associated to Shapiro-Wilk test for normality

[d] P associated to Student test for location Mu = 0 for data normally distributed

[e] P associated to the non parametric sign test for location Median = 0 for data non normally distributed

from roots colonized by *Gigaspora rosea* inhibited further root colonization by *Glomus mossae*. Therefore, the soil microflora strongly modifies root exudation, which in turn alters both the size and the structure of microbial populations in the rhizosphere (Brimecombe et al., 2000; Grayston, 2000).

3.2.4 Soil Texture

Here, we report on experiments that compared plants grown on soils differing in their clay and loam contents. It is important to note that the range of the clay content reported here is low, from 2% to 15% (Table 10) due to the difficulty to sample the roots in soils with high clay contents. An increase in clay and loam content of soil alters greatly the partitioning of ^{14}C. Significantly more labelled C is retained aboveground and less is allocated to roots (mean variation = +15% and −25%, respectively, Table 10). Both the global and the belowground ^{14}C budget indicate that partitioning of ^{14}C to rhizosphere respiration and to soil residues are also increased but these effects are not significant due to the work of Whipps and Lynch (Whipps and Lynch, 1983) that indicated surprisingly low values for RR in the soil with a light texture. The increase in C loss from root in soil with increasing clay and loam contents is not surprising because numerous soil properties, which favour microbial activity and nutrient cycling, are related to the clay content: water retention, organic matter stabilization, high cation exchange capacity, for example. Thus, the suggested stimulation of rhizodeposition in relation to clay and loam contents of soil could be explained by some

Table 9 Effect of soil microorganisms on labelled C partitioning between plant and belowground compartments. Effects are expressed as relative variations, see the text for explanations about the calculation of the effects.

					Relative variation (%)							
Factor	Labelling[a]	References		N[b]	Mean	CV of mean	Max	Med	Min	Pn[c]	Pt[d]	Pm[e]
Microorganisms												
Non sterile vs sterile												
	C		SHOOT	8	−4	−316	21	−5	−24	0.841	0.400	
	C	Barber and Martin (1976); Martin (1977); Todorovic et al. (2001)	ROOT	8	2	918	31	7	−22	0.524	0.767	
	C		RR	8	249	112	658	157	−15	0.086	0.040	
	C		RES	8	37	175	181	24	−16	0.017		0.727
	C		ROOTBG	8	−10	−110	7	−14	−21	0.246	0.037	
	C		RRBG	8	199	119	598	114	−18	0.097	0.049	
	C		RESBG	8	24	271	169	−7	−24	0.005		0.727
	P		SHOOT	1	−8		−8	−8	−8			
	P	Whipps and Lynch (1983)	ROOT	1	−26		−26	−26	−26			
	P		RR	1	37		37	37	37			
	P		RES	1	886		886	886	886			
	P		ROOTBG	1	−38		−38	−38	−38			
	P		RRBG	1	14		14	14	14			
	P		RESBG	1	723		723	723	723			

RR and RES are %^{14}C allocated to rhizosphere respiration and soil residues, respectively

The suffix BG is used when partitioning coefficients are expressed as percentages of ^{14}C allocated to belowground

[a] C = continuous labelling, P = pulse labelling

[b] Number of partitioning coefficient sets

[c] P associated to Shapiro-Wilk test for normality

[d] P associated to Student test for location Mu = 0 for data normally distributed

[e] P associated to the non parametric sign test for location Median = 0 for data non normally distributed

Table 10 Effect of soil texture on labelled C partitioning between plant and belowground compartments. Effects are expressed as relative variations, see the text for explanations about the calculation of the effects

				N[b]	Relative variation (%)							
Factor	Labelling[a]	References			Mean	CV of mean	Max	Med	Min	Pn[c]	Pt[d]	Pm[e]
Soil texture (% of clay/loam)												
	C		SHOOT	11	15	130	42	11	−16	0.455	0.029	
Min = 2/10	C	Gorissen et al. (1996); Merckx et al. (1985, 1986); Whipps and Lynch (1983)	ROOT	11	−25	−118	44	−22	−66	0.195	0.018	
Max = 15/71	C		RR	11	3,479	181	19,500	24	−44	<0.001		1.000
Mean = 9/29	C		RES	11	67	185	233	6	−72	0.025		1.000
Median = 13/12	C		ROOTBG	11	−19	−98	6	−12	−51	0.340	0.007	
CV = 71/83	C		RRBG	11	4,789	184	27,158	45	−38	<0.001		0.549
	C		RESBG	11	77	169	320	24	−61	0.136	0.079	

RR and RES are %^{14}C allocated to rhizosphere respiration and soil residues, respectively

The suffix BG is used when partitioning coefficients are expressed as percentages of ^{14}C allocated belowground

[a] C = continuous labelling, P = pulse labelling

[b] Number of partitioning coefficient sets

[c] P associated to Shapiro-Wilk test for normality

[d] P associated to Student test for location Mu = 0 for data normally distributed

[e] P associated to the non parametric sign test for location Median = 0 for data non normally distributed

differences in fertility and in microbial activity. Besides, the effect of the soil texture on C fluxes to the rhizosphere can also be explained by the physical properties of the soil. Indeed, soil texture is interrelated with bulk density and porosity and the resulting mechanical impedance has been reported to increase rhizodeposition (Boeuf-Tremblay et al., 1995; Groleau-Renaud et al., 1998). On a theoretical point of view, the mechanical impedance in soils with a fine texture should promote the sloughing-off of root cap cells. Root exudation may also be favoured by the small size of soil pores, which increases the surface of the root that is covered by soil aggregates and which consequently facilitate mass flow diffusion of solutes. Hence, both experimental data and theoretical considerations support the relevancy of considering soil texture when investigating of C fluxes to the rhizosphere.

3.2.5 Soil Nitrogen

Soil nitrogen is a major factor that can severely limit plant growth and therefore, the effect of N fertilization on C fluxes to the rhizosphere is a highly relevant question. We summarized 9 data sets for continuous labelling experiments and 19 for pulse-chase studies, the latter being mainly related to an experiment on *Bromus erectus* (Warembourg and Estelrich, 2001) (Table 11). All data sets indicate that when plant are N fertilized, there is a highly significant decrease of labelled C partitioning to roots (−14% and −35% for continuous and pulse labellings, respectively) and conversely, an increase of ^{14}C retrieved in shoots (+11% and 36% for continuous and pulse labellings, respectively) (Table 11). The coefficients of variation are not excessive. The effect is more marked in pulse-chase experiments. This is consistent with the low root:shoot ratio of N-fertilized plants, which is commonly observed experimentally and which is well described by the functional equilibrium theory (Farrar and Jones, 2000). The global ^{14}C budget does not point out a significant effect of nitrogen on C allocation to rhizosphere respiration and to soil residues whatever the labelling procedure. However, the belowground budget is altered by N fertilization, in pulse-chase experiments. Indeed, the percentages of ^{14}C in rhizosphere respiration and in the soil residues are both significantly increased by nitrogen fertilization (mean of increase is +25% and +82%, respectively). This suggests that relative to C exported by shoots to belowground, N fertilisation increases rhizodeposition. This hypothesis has some theoretical basis. Indeed, if rhizosphere microorganisms are in competition with plant roots for mineral N (Kaye and Hart, 1997), a supply of nitrogen would be expected to stimulate microbial growth and consequently to increase the flux of passive exudation. Moreover, nitrogen deficiencies were reported to affect root morphology by reducing the branching (Baligar et al., 1998), which may have significant consequences on the production of mucilage and the release of root cap cells and on exudation. Thus, despite there are clues indicating that N fertilization increases the percentage of belowground C that is released from roots, the overall effect of N fertilization on rhizodeposition is difficult to predict because in parallel, nitrogen stimulates the total plant growth and photosynthesis and reduces the percentage of photoassimilates that are allocated belowground.

3.2.6 Atmospheric CO_2 Concentration

The elevation in atmospheric CO_2 concentration consecutive to the use of fossil C has raised the question as to C fluxes to the rhizosphere would be modified. This is of particular importance for understanding nutrient cycling and C sequestration in soil under elevated atmospheric CO_2. We report here on 24 data sets related to ^{14}C distribution within the plant and to the soil under elevated CO_2 (Table 12). Pulse and continuous labellings are equally represented. Studies concerned both herbaceous plants (ryegrass, wheat, maize) and trees (aspen and chestnut, data not shown). There is no clear effect of elevated CO_2 on the partitioning of assimilates to shoots and roots. However, in continuous labelling experiments, the ^{14}C retrieved in the rhizosphere respiration is significantly increased under elevated CO_2 (+36%). This is consistent with the data reviewed by Zak et al. (2000), which evidence an increase in soil and microbe respiration under elevated CO_2. Apart from that, the data do not indicate a clear effect of elevated CO_2 on C partitioning to the rhizosphere. This is not surprising because atmospheric CO_2 is not a factor directly connected to the rhizosphere. Any effect of atmospheric CO_2 enrichment on rhizodeposition is through plant growth in contrary to factors such as the soil texture or the presence of microorganisms that act more directly on the release of C from roots. Soil nitrogen can be considered as intermediate because it stimulates both the growth of plant and the growth of microorganisms. Hence, elevated CO_2 can alter the partitioning of assimilates to the rhizosphere through several mechanisms such as a change in plant structure, itself depending on the plant species (Pritchard et al., 1999), a modification of the root to shoot ratio (Rogers et al., 1994), an alteration in root morphology, a nutrient stress due to the stimulation of plant growth, etc.

Table 11 Effect of soil nitrogen on labelled C partitioning between plant and belowground compartments. Effects are expressed as relative variations, see the text for explanations about the calculation of the effects

				N[b]	Relative variation (%)							
Factor	Labelling[a]	References			Mean	CV of mean	Max	Med	Min	Pn[c]	Pt[d]	Pm[e]
Soil nitrogen (mg/kg)												
Soil content or applied as fertilization												
	C	Billes et al. (1993); Johansson (1992a);	SHOOT	9	11	78	27	9	0	0.470	0.005	
Min = 0	C	Liljeroth	ROOT	9	−14	−79	0	−13	−32	0.712	0.005	
Max = 505	C	et al. (1994);	RR	9	−6	−186	12	−8	−21	0.795	0.145	
Mean = 152	C	Merckx et al.	RES	9	−15	−253	78	−28	−39	0.001		0.070
Median = 73	C	(1987); Van	ROOTBG	9	−2	−380	10	−1	−24	0.059	0.453	
CV = 118	C	Ginkel et al.	RRBG	9	7	127	18	9	−7	0.429	0.045	
	C	(1997)	RESBG	9	−3	−1,330	99	−20	−30	0.001		0.508
	P	Henry et al.	SHOOT	19	36	96	109	23	−7	0.032		0.001
Min = 38.5	P	(in press);	ROOT	19	−35	−84	38	−39	−84	0.006		0.001
Max = 970	P	Mikan et al.	RR	19	−4	−1,028	96	−1	−64	0.210	0.677	
Mean = 694	P	(2000);	RES	19	38	209	236	31	−69	0.292	0.052	
Median = 750	P	Warembourg	ROOTBG	19	−16	−167	67	−25	−42	<0.001		0.001
CV = 32	P	and Estelrich	RRBG	19	25	156	90	29	−49	0.717	0.012	
	P	(2001)	RESBG	19	82	111	260	50	−51	0.262	0.001	

RR and RES are %^{14}C allocated to rhizosphere respiration and soil residues, respectively

The suffix BG is used when partitioning coefficients are expressed as percentages of ^{14}C allocated to belowground

[a] C =continuous labelling, P = pulse labelling

[b] Number of partitioning coefficient sets

[c] P associated to Shapiro-Wilk test for normality

[d] P associated to student test for location Mu = 0 for data normally distributed

[e] P associated to the non parametric sign test for location Median = 0 for data non normally distributed

4 Outlooks

Tracer experiments are very useful tools for investigating C fluxes from plant roots to the soil because they allow separating root-derived C from the C of the native soil organic matter. With such techniques, investigations on rhizodeposition can be performed on plants growing in soil including the microflora, which is more realistic than experiments in nutrient solution. In counterpart, in soil, it is difficult to estimate the fraction of rhizodeposits that is mineralised by microorganisms and consequently, the amount of C released from root cannot be determined in a reliable manner. The partitioning of rhizosphere respiration between root and microbial contributions is of particular importance if rhizodeposition is investigated to understand processes that are mediated by microbes. In that case, it is essential to evaluate how much energy is available to microorganisms to predict microbial growth in the vicinity of roots. Several attempts have been made to evaluate the rhizomicrobial contribution to rhizosphere CO_2 (Cheng et al., 1993; Helal and Sauerbeck, 1989; Johansson, 1992a; Kuzyakov et al., 1999; Todorovic et al., 2001; VonWiren et al., 1996) but at present time, none of them is fully satisfactory because all these studies rely on strong assumptions that are difficult to test. As an alternative, metabolic activity (growth, maintenance) of rhizosphere microbes can be determined or compared between different treatments to investigate its relationships with root activity (Nguyen et al., 2002; Soderberg and Baath, 1998). However, quantification of root-derived C fluxes in non sterile soil is undoubtedly a key point that needs further investigations and methodological developments for aiming at engineering the rhizosphere to manage nutrient and pollutant cycling or to control soil borne pathogens.

Rhizodeposits cover a wide range of compounds that have very different characteristics in terms of interactions with the soil matrix, availability to microbial assimilation, chemical properties, etc. Moreover, the release of root C into the root environment originates

Table 12 Effect of atmospheric CO_2 on labelled C partitioning between plant and belowground compartments. Effects are expressed as relative variations, see the text for explanations about the calculation of the effects

					Relative variation (%)							
Factor	Labelling[a]	References		N[b]	Mean	CV of mean	Max	Med	Min	Pn[c]	Pt[d]	Pm[e]
Atmospheric CO_2 (ppm)												
	C	Billes et al.	SHOOT	12	−6	−158	13	−10	−18	0.460	0.051	
Min = 350	C	(1993); Gorissen	ROOT	12	21	201	113	5	−23	0.032		0.774
Max = 800	C	et al. (1996); Van	RR	12	36	132	107	40	−61	0.734	0.024	
Mean = 633	C	Ginkel et al.	RES	12	−2	−2,459	126	−11	−50	0.005		0.388
Median = 700	C	(1997); Whipps	ROOTBG	12	5	461	54	−2	−22	0.022		0.146
CV = 24	C	(1985)	RRBG	12	24	201	113	16	−69	0.996	0.112	
	C		RESBG	12	−13	−280	90	−20	−45	0.003		0.039
	P	Paterson et al.	SHOOT	12	−3	−505	25	−4	−28	0.490	0.507	
Min = 350	P	(1996, 1999);	ROOT	12	10	315	87	7	−28	0.041		0.774
Max = 720	P	Rattray et al.	RR	12	2	2,656	115	−5	−87	0.304	0.899	
Mean = 477	P	(1995); Rouhier	RES	12	43	169	215	19	−23	0.024		0.388
Median = 450	P	et al. (1996);	ROOTBG	12	−3	−307	15	−3	−22	0.811	0.284	
CV = 32	P	Mikan et al.	RRBG	12	−4	−1,269	129	−9	−86	0.053	0.790	
	P	(2000)	RESBG	12	21	166	99	8	−17	0.087	0.061	

RR and RES are %^{14}C allocated to rhizosphere respiration and soil residues, respectively

The suffix BG is used when partitioning coefficients are expressed as percentages of ^{14}C allocated to belowground

[a] C = continuous labelling, P = pulse labelling

[b] Number of partitioning coefficient sets

[c] P associated to Shapiro-Wilk test for normality

[d] P associated to student test for location Mu = 0 for data normally distributed

[e] P associated to the non parametric sign test for location Median=0 for data non normally distributed

in various mechanisms (i.e. passive diffusion of solutes to the soil solution, active secretion of molecules, senescence of root tissues), the distribution and intensity of which are not homogenously distributed along the root. This complexity is well illustrated by the great difficulty to propose a nomenclature for the rhizodeposits. Consequently, the composition of C released from roots is virtually extremely variable. Indeed, the composition of rhizodeposits depends on the relative proportion of each category (exudates, secretion, senescing tissues) as well as of the intrinsic composition of each of these categories. For example, nutrient or toxicity stress is know to significantly increase the concentration of organic acids in root exudates (Jones, 1998). It is thus crucial to investigate in more detail the mechanisms by which root C is released into the soil. For example, the production of root cap cells and mucilage has been extensively studied in vitro, under experimental conditions that probably increase the phenomena. Very little is known about environmental control of rhizodeposition by root apices in soil conditions. If the mucilage sticks to the root cap even at soil water potentials close to 0.01 MPa, the continuous production of slime and the release of root cap cells might not be as important as suggested by laboratory investigations. There is also great debate as to determine whether the plant does have a control on the amount of C that passively diffuse to the soil solution. The ATPase transporters, which can actively reabsorb solutes in vitro, provide a mechanism by which the root can virtually modulate exudation. However, does the plant regulate the flux of exudates by controlling the number of these transporters and their activity? Research aimed at understanding the regulation of these proteins is particularly relevant. Indeed, these transporters would offer the opportunity to manipulate the flow of exudation both in term of quantity by over expressing or inhibiting the transporters synthesis and in term of quality by acting specifically on target transporters and thus on changing the exudation flux of a particular compound.

The spatial heterogeneity of rhizodeposition along a root segment outlines the need to link investigations on rhizodeposition to the root. From a theoretical point of view, the branching pattern, which determines the number of apices, would be expected to have significant effect on the number of slough-off root cap cells as well as on mucilage production and on the release of exudates if their diffusion is more important at the root tips as it was observed in maize. Moreover, the exudation, the release of border cells and the senescence of epidermis is proportional to the root radius. Hence, it is necessary to determine if in soil root morphology has indeed significant effects on rhizodeposition.

The "rhizosphere effect" observed experimentally for numerous soil processes mediated by the microflora is frequently related to the greater microbial abundance at the soil-root interface compared to bulk soil. Therefore, a major goal for investigations on rhizodeposition is to predict microbial growth in the root environment (Blagodatsky and Richter, 1998; Darrah, 1991, 1991; Newman and Watson, 1977; Toal et al., 2000). In the last decade, the development of techniques to establish microbial fingerprints evidenced that the structure of rhizosphere communities was both physiologically and genetically different from that of bulk soil and different between plant species. The relationships between size of the microflora, structure of microbial communities and functions performed by them is far from being elucidated and there is a relevant need to investigate the factors that determine the structure of microbial communities in the rhizosphere. Among them, rhizodeposits have been demonstrated to be relevant (Benizri et al., 2002; Griffiths et al., 1999). Since growth of soil microbes is generally limited by availability of C, it can reasonably be assumed that the dynamics of microbial community structure might derive from the competitive ability of rhizosphere microorganisms with respects to the amount of available C. On the other hand, plant roots are "chemical factories" that synthesize a wide variety of secondary metabolites (Bais et al., 2001), which are biologically active and which might orient the dynamics of microbial communities. Root-microbe interactions might not only be governed by trophic competition between microorganisms for rhizodeposits and by sophisticated signalling involved in symbiosis process. Allelopathy, which can play a significant role in the dynamics of plant community structure, might also contribute to determine the structure of rhizosphere microbial communities. The chemical diversity of secondary metabolites released into the rhizosphere is probably large and rhizodeposition of such compounds offers an exciting area of investigations and additional outlook to the use of the plant for engineering the rhizosphere.

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Environmental Costs and Benefits of Transportation Biofuel Production from Food- and Lignocellulose-Based Energy Crops: A Review

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Abstract Transportation biofuel production in the United States is currently dominated by ethanol from the grain of maize and, to a much lesser extent, biodiesel from soybeans. Although using these biofuels avoids many of the environmentally detrimental aspects of petroleum-based fossil fuels, biofuel production has its own environmental costs, largely related to fossil fuel use in converting crops to biofuels and crop cultivation itself, including ecological damages caused by nitrogen and phosphorus fertilizers, pesticides, and erosion. A new generation of biofuels derived from lignocellulosic sources offers greatly reduced environmental impacts while potentially avoiding conflicts between food and energy production. In particular, diverse mixtures of native prairie species offer biomass feedstocks that may yield greater net energy gains than monoculture energy crops when converted into biofuels, while also providing wildlife habitat and enriching degraded soils through carbon sequestration and nitrogen fixation. Ultimately, as demand for both food and energy rise in the coming decades, greater consideration will need to be given to how land can best be used for the greater benefit of society.

Keywords Biodiesel • Bioenergy • Biomass • Carbon • Ethanol • Greenhouse gas • Maize • Prairie

1 Introduction

Oil, coal, and natural gas currently supply around 90% of global energy use (Energy Information Administration 2006). Rising energy prices, energy security concerns, long-run supply, climate change, environmental degradation, and impacts on human health are among the many concerns raised by this overwhelming reliance on fossil fuels (Ezzati et al. 2004; Schröter et al. 2005; Hansen et al. 2006; McMichael et al. 2006; Stern 2006a,b). These problems have spawned efforts to develop renewable energy sources such as solar (Hoffert et al. 2002; Shinnar and Citro 2006), wind (Lenzen and Munksgaard 2002; Hoogwijk et al. 2004; Archer and Jacobson 2005), hydrogen (Deluga et al. 2004; Jacobson et al. 2005), and biomass (Larson 2000; Hamelinck and Faaij 2006; Herrera 2006). Although renewable energy sources have promise, three important questions need to be resolved before society can count on them as a sustainable energy supply. First, how much energy can renewable sources provide, and will this amount significantly reduce fossil fuel use while meeting rising energy demands to support a growing and increasingly affluent world population (Berndes et al. 2003; Hoogwijk et al. 2003; Meyers and Kent 2003; Dorian et al. 2006; Sims et al. 2006; de Vries et al. 2007)? Second, can renewable energy be supplied at a reasonable cost? Third, to what degree will alternative energy sources reduce environmental damage relative to fossil fuel use (Chow et al. 2003; Keith et al. 2004)?

Here I explore one aspect of renewable energy, namely the environmental consequences of producing the biological materials used as feedstocks for the transportation biofuel industry in the United States.

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I focus this review on the possible benefits of transitioning biofuel production from crops traditionally cultivated for food to those developed as environmentally beneficial bioenergy sources. I first evaluate the current state of biofuel production by assessing various environmental aspects of the two predominant US biofuels, maize grain ethanol and soybean biodiesel. I then investigate the advantages that a second generation of transportation biofuels, derived primarily from lignocellulosic biomass, can provide over these first-generation food-based biofuels.

2 US Biofuel Production From Food Crops

In the following section, I explore the potential for the two dominant biofuels in the United States, maize grain ethanol and soybean biodiesel, to offset fossil fuel use, and then discuss various environmental impacts of their production and use.

2.1 The Current State of US Biofuel Production

Fig. 1 Volunteer maize in a field of soybeans, indicative of the dominant crop rotation in the midwest US (Jason Hill)

The United States transportation biofuel market is dominated by domestically-produced ethanol derived from the grain of maize (*Zea mays* ssp. *mays*) (Fig. 1). To produce ethanol, starch from maize kernels is broken down into sugars, which are then fermented and distilled. The remainder of the kernel is commonly processed into distiller's dry grain with solubles (DDGS), which serves as a high-quality animal feed (Spiehs et al. 2002; Lumpkins et al. 2004). The other major US transportation biofuel is soybean (*Glycine max*) biodiesel, which displaces petroleum diesel. In biodiesel production, soybeans are crushed to separate the oils from the meal, which is used primarily as a protein source in animal feed. The oils are then converted to biodiesel and glycerol via a transesterification reaction with the addition of catalysts and alcohol reagents (Van Gerpen 2005; Haas et al. 2006; Meher et al. 2006).

Hill et al. (2006) examine the degree to which these two biofuels displace fossil fuels in the US transportation sector. In 2005, approximately 4.0×10^{10} kg of maize were used to produce 1.5×10^{10} L of ethanol in the US, and the oil from approximately 1.3×10^{9} kg of soybeans was used to generate 2.6×10^{8} L of biodiesel. In terms of each fuel's gross energy yield, these volumes of maize grain ethanol and soybean biodiesel have offset 1.7% and 0.1% of US gasoline and diesel use, respectively. Since fossil fuels are used both on farms and at conversion facilities to produce these biofuels, however, these gross energy values do not reflect the total "new energy" they contribute. The fossil energy invested in producing each of these biofuels must be subtracted from the gross energy yield to calculate the net energy yield. This fossil energy expenditure comes mainly from the petroleum diesel used to power farm equipment and tractor-trailers for transportation, the natural gas burned to provide process heat at the conversion facility, and the coal combusted to produce electricity. Maize and soybean production also require agrichemicals, barns, tractors, and other farm machinery that in turn require energy for their manufacture. Biofuel production requires the labor of farmers and factory workers who, with their families, consume energy in a variety of forms. Given current agricultural practices and biofuel industrial conversion standards, the production of both of these biofuels yields more energy than in the fossil fuels to produce them, with maize grain ethanol and soybean biodiesel yielding 25% and 93% more, respectively. Therefore, the US net energy offset in 2005 by producing maize grain ethanol was approximately 0.3% of gasoline use and 0.05% of diesel use from soybean biodiesel.

Whether maize grain ethanol returns more energy than is invested in its production has long been a source of debate, stretching back decades (Chambers et al. 1979). A comparison of recent, independent estimates of its net energy balance reveals two key areas of disagreement (Fig. 2). First, studies have varied the energy input boundaries for the life cycle of ethanol production, most notably in categories concerning energy expenditures to produce capital requirements such as farm equipment and conversion facilities. These input categories are rightfully included in net energy balance analyses because farm equipment is used directly in biofuel crop production and biofuel production facilities would not be built were it not for biofuel production itself. Second, there is variation in the estimates of the specific energy inputs themselves, both for widely-accepted categories and those less commonly included. Using current, well-supported, public data on farm inputs and ethanol production plant efficiencies resolves many of these discrepancies (Farrell et al. 2006; Hill et al. 2006).

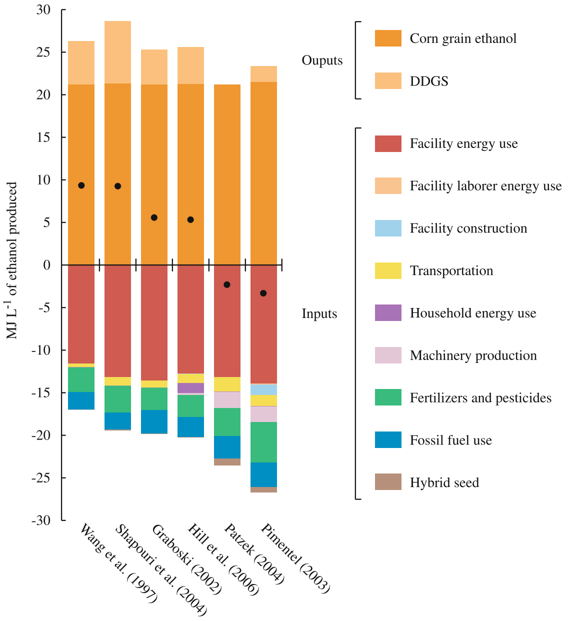


Fig. 2 The net energy balance of maize grain ethanol as estimated by six recent studies, most recently by Hill et al. (2006). All 11 input and output categories are ordered as they are shown in the legend, but some are so small as to be imperceptible. Only the estimate of Hill et al. (2006) includes all 11 categories. The estimated net energy balance (the sum of the outputs minus the sum of the inputs) from each study is shown by the placement of a *black dot*

Several environmental benefits come from replacing fossil fuels with maize grain ethanol and soybean biodiesel. Displacing petroleum-derived transportation fuels with biofuels avoids the negative effects of oil drilling, refining, and combustion. Further, the CO_2 released when combusting plant-derived biofuels was removed from the atmosphere during crop growth whereas burning fossil fuels introduces "new" CO_2 into the atmosphere, thus contributing to global warming. Therefore, a biofuel produced from crops grown with conventional farming practices, which lead to essentially no soil carbon sequestration (Robertson et al. 2000), would be carbon neutral were it not for the fossil fuels combusted in biofuel production. Even if carbon neutral, however, biofuel production may not be global climate change neutral. Biofuel production from maize and soybeans may increase emissions of nitrous oxide (N_2O), a potent greenhouse gas, from maize and soybean croplands. Under current farm and biofuel industry production standards, maize grain ethanol releases approximately 12% fewer greenhouse gases than gasoline, while soybean biodiesel releases approximately 41% less greenhouse gases than diesel because of lower farm and conversion facility fossil energy requirements (Hill et al. 2006). Farrell et al. (2006) reported a similar 18% savings for maize grain ethanol while noting that shifting conversion facility fossil fuel use from natural gas, as is commonly used, to coal would lead maize grain ethanol to be a net source that is approximately 2% greater than gasoline. These estimates assume that the cropland used to produce these biofuels is in equilibrium for carbon loss and gain. Converting land from any use that has a net sequestration of carbon (e.g., intact ecosystems or certain lands in conservation reserve) to crop production for biofuels would decrease this greenhouse gas savings and might cause the biofuel to release more greenhouse gases than the fossil fuel it replaced.

Biofuel production can introduce other negative environmental consequences that do not occur with fossil fuel production, namely those directly associated with crop production and conversion of these crops to biofuels. Here, the environmental effects of maize and soybean production are rightfully ascribed to the biofuels derived from them. Typical cultivation practices employed in major maize and soybean producing states use 7 g and 0.1 g of nitrogen (N) fertilizer per MJ of energy gained in producing maize grain ethanol and soybean biodiesel, respectively (Hill et al. 2006). Similarly, 2.6 g and 0.2 g of phosphorus (P) fertilizer are applied per MJ of energy gained in producing maize grain ethanol and soybean biodiesel, respectively. Eutrophication from N and P of agricultural origin moving to surface and ground water (Powers 2005) leads to loss of diversity (Carpenter et al. 1998; Suding et al. 2005), changes in aquatic ecosystem structure and function (Smith et al. 1999), drinking water contamination (Socolow 1999), and water quality degradation including the anoxic zone in the Gulf of Mexico (McIsaac et al. 2002; Dodds 2006). In addition to these fertilizers, 0.1 g and 0.01 g of pesticides are applied per MJ of energy gained in producing maize grain ethanol and soybean biodiesel, respectively. For maize, approximately 36% of this amount is atrazine, 23% acetochlor, 16% metolachlor, and 8% glyphosate, and around 82% of pesticide application to soybeans is glyphosate (United States Department of Agriculture, 2003, 2005). Also, both maize and soybean farming cause erosion and sedimentation (Johnson et al. 2006). Water availability is also of concern both for crop irrigation in drier climates and for converting feedstock conversion to biofuel (Berndes 2002; Oki and Kanae 2006).

2.2 Impacts of Increasing US Biofuel Production

Both maize grain ethanol and soybean biodiesel are currently used primarily as fuel additives rather than as biofuels themselves. When blended at low levels with gasoline or diesel, ethanol serves as an oxygenate, helping engines meet the emission requirements of the US Clean Air Act of 1990 (Fernandez and Keller 2000; Hansen et al. 2005). Maize grain ethanol production is growing rapidly due to state mandates for replacing methyl *tert*-butyl ether (MTBE), a gasoline oxygenate that pollutes groundwaters, federal production subsidies and incentives (e.g., a \$0.14/L federal volumetric ethanol excise tax credit), and a tariff on importing ethanol from foreign sources (\$0.14/L). Biodiesel blended into diesel substantially reduces tailpipe emissions of many criteria pollutants including carbon monoxide (CO), oxides of sulphur (SO_X), hydrocarbons (HC), and particulate matter (PM) (Wang et al. 2000; Nabi et al. 2006).

Both maize and soybean prices rose in 2006 as a result of increased biofuel demand, with prices for maize doubling between 2005 and the beginning of 2007. As demand for alternative fuels continues to rise, competition between using these crops for food and fuel purposes will become more pronounced. Currently, about 50% of the US maize crop is used to feed livestock, while the remainder is processed for human consumption, exported, or fermented into ethanol (United States Department of Agriculture 2006). Likewise, 90% of domestically-produced soybean meal is used for livestock feed (United States Census Bureau 2006a), and soybean oil constitutes 80% of US fat and oil consumption (United States Census Bureau 2006b). As a consequence of increased ethanol demand, more acreage is expected to be planted to maize at the expense of other crops, namely soybeans (FAPRI 2006). However, changing the 2 year maize and soybean rotation that is predominant in the US Midwest to continuous maize not only increases total fertilizer and pesticide use, but also decreases soil quality and yield (Karlen et al. 2006). Still, utilizing even substantial portions of US maize and soybean production would have but a minor effect on domestic energy markets. Devoting all US maize and soybean production to ethanol and biodiesel production would yield just 12% and 6% of US gasoline and diesel demand in terms of gross energy, respectively, with net energy gains of just 2.4% and 2.9% (Hill et al. 2006).

3 Maximizing the Environmental Benefits of Current Biofuels

Both government mandates for biofuel use and development of a domestic biofuel production industry based on maize grain ethanol and soybean biodiesel have established these two biofuels as the dominant renewable transportation alternatives in the near-term.

Efforts at various stages of their production and use can be made to maximize their environmental performance.

The environmental performance of current biofuels can be augmented by utilizing more sustainable crop production practices that increase resource use efficiency and integrate enlightened management practices (Tilman et al. 2002; Cook 2006). These include reduced or no-till cultivation (West and Post 2002; Kim and Dale 2005a; Grandy et al. 2006), organic (Drinkwater et al. 1998; Kramer et al. 2006) and more efficient (Matson et al. 1998; Crews and Peoples 2005) fertilization, and the use of cover crops (Kim and Dale 2005b). Although it has not been firmly established, applying conservation tillage to agricultural lands currently farmed under conventional tillage may sequester carbon in soils (West and Post 2002; Johnson et al. 2005), perhaps leading to one of seven "stabilization wedges" needed to stabilize atmospheric CO_2 emissions if adopted on a global basis (Pacala and Socolow 2004). Reduced erosion and decreased farm fossil fuel use for soybean farming in recent years (i.e., between the major biodiesel life cycle analyses of Sheehan et al. (1998) and Hill et al. (2006)) is largely due to fewer passes over land with farm implements and greater adoption of reduced tillage practices, in part attributable to widespread planting of soybeans genetically modified for glyphosate resistance (Cerdeira and Duke 2006). This transition to glyphosate-dominated soybean herbicide use is also associated with lower environmental damage from pesticide toxicity (Nelson and Bullock 2003), although many long-term ecological consequences of genetically modified organisms are as yet unrealized (Andow 2003).

Other biofuel feedstocks include waste cooking oils and fats (Zhang et al. 2003; Cvengroŝ and Cvengroŝová 2004) and residues from forest industries (Parikka 2004). Crop waste (i.e., that lost during handling, storage, and transport between farms and households) and agricultural residues (i.e., the crop biomass remaining after the consumable portion is removed) also provide attractive raw materials for biofuel production (Gallagher et al. 2003; Kim and Dale 2004). While using crop waste has the benefit of avoiding the conflict between food and fuel uses for the crops themselves, using agricultural residues with sensitivity to environmental concerns maximizes the use of additional products generated via high-input, intensive farming. In the Midwest US, residual maize stover can be harvested and combusted directly or converted to ethanol (Aden et al. 2002; Hoskinson et al. 2006) in a process akin to fermenting the sugars in sugarcane to ethanol while burning the residual bagasse to supply process heat and electricity (Borrero et al. 2003; De Olivera 2005; Botha and von Blottnitz 2006). Stover removal may reduce soil organic carbon storage, reduce productivity, and increase soil erosion, however (Linden et al. 2000; Hooker et al. 2005; Dolan et al. 2006; Johnson et al. 2006), thus requiring careful consideration of stover removal rates (Wilhelm et al. 2004). Using stover as a valuable coproduct of maize production also raises the possibility of tapping extant maize genetic diversity for desirable energy characters such as higher cellulose fractions or a perennial habit (Cox et al. 2006). Even if breeding for such characteristics leads to some degree of grain yield loss, such hybrids may prove economically viable depending on stover prices in a biofuel market.

Although both maize grain ethanol and soybean biodiesel are valuable biofuel additives, neither can do much to displace fossil fuels, and devoting any amount of these crops to biofuels has a disproportionately large effect on food markets. Given that current biofuel production is limited and that which is available comes at a considerable environmental price (De Oliveira et al. 2005), it is prudent to consider how biofuels can best be integrated into transportation fuel supplies. For example, Kim and Dale (2006) conclude that, under biofuel supply constraints and current vehicle fuel efficiencies, ethanol used in an E10 blend (10% ethanol and 90% gasoline by volume) provides greater environmental benefits in criteria pollutant release than an E85 blend (85% ethanol and 15% gasoline by volume). Similarly, the potential for soybean biodiesel to displace diesel use is limited, but diesel blends with as little as 1–2% biodiesel provide essential lubricity lost by the removal of sulphur in ultra-low sulphur diesel formulations (Hu et al. 2005; Knothe and Steidley 2005). Blending available biofuel stocks at low levels into conventional fuels might maximize their environmental benefits, therefore, especially in light of current supply constraints.

Employing less intensive cropping methods, using agricultural wastes and residues, and properly integrating biofuels into conventional supplies as fuel additives rather than fuel substitutes serve to minimize the negative environmental consequences of current biofuel production. However, making biofuels that will be both environmentally superior to fossil fuels and displace significant quantities of fossil fuel use will require exploration of plant resources other than those that have been domesticated and bred primarily for their food, feed, or forage value. In doing so, there even is the prospect of utilizing and improving degraded and marginal lands on which food crop production is neither economically viable nor environmentally sound.

4 Alternate US Biofuel Feedstock Production Methods

Growing recognition of the limited ability of food crop-based biofuels to offset fossil fuel use has increased awareness that a variety of new energy feedstocks will be needed if plant-based biofuels are to make any sort of significant impact on alleviating domestic reliance upon conventional transportation fuels. Increased attention is being given to lignocellulosic biomass as the preferred feedstock for the second generation of biofuels (Schubert 2006). In the following section, I provide a brief overview of how lignocellulosic biomass can be used to supply transportation energy, the various energy crops that are being developed, and the potential for these biofuels to offset fossil fuel use. I follow this with more detailed consideration of how diverse mixtures of native prairie species in US grasslands can provide a sustainable supply of biofuel feedstock while simultaneously improving degraded lands and providing habitat for wildlife.

4.1 Biofuels from Lignocellulosic Biomass

Lignocellulosic biomass, which consists of the cellulose, hemicellulose, and lignin compounds found in plant cell walls that comprise the bulk of herbaceous and woody vegetative tissues (McKendry 2002), provides a valuable and versatile feedstock for the production of a variety of biofuels (Huber et al. 2006). It can be combusted directly to provide electricity, itself an emerging transportation fuel, and process heat (Mann and Spath 2001; Demirbaž 2003; Robinson et al. 2003; Mani et al. 2006; Qin et al. 2006). Biomass can also be converted to ethanol through enzymatic hydrolysis of the cellulosic fractions into sugars (Foyle et al. 2006) followed by fermentation of these sugars as in maize grain ethanol production, with the lignin fractions being burned to provide heat and electricity (Lynd et al. 1991, 2002; Wyman 1999; Hamelinck et al. 2005). Biomass can also be gasified to produce hydrogen (Zhang et al. 2004; Kumabe et al. 2007; Ptasinski et al. 2007), electricity, synthetic hydrocarbons such as gasoline and diesel through subsequent Fischer–Tropsch synthesis (Spath and Dayton 2003; Wang et al. 2005; Zwart and Boerrigter 2005), or other biofuels such as dimethyl ether (Semelsberger et al. 2006). Other valuable products may also be generated in such "biorefinery" streams (Wyman 2003; Montgomery 2004; Ragauskas et al. 2006). New technologies for producing biofuels from biomass are rapidly emerging, including the development of engineered yeast for increased ethanol yields (Alper et al. 2006), utilization of new microorganisms for ethanol production (Seo et al. 2005), pretreatments for cellulosic digestion (Mosier et al. 2005), fuel cells for converting sugars directly to electricity (Chaudhuri and Lovley 2003), and catalysts for more efficient conversion of biomass to syngas (Salge et al. 2006).

Various plant species are currently used or are being developed for biomass production. Unlike maize and soybeans, which are annuals, lignocellulosic bioenergy crops are typically perennials, including both woody species such as willows (*Salix* spp.) (Volk et al. 2004, 2006; Keoleian and Volk 2005), poplars (*Populus* spp.) (Husain et al. 1998; Tuskan et al. 2006), and other hardwoods (Geyer 2006), and herbaceous species such as switchgrass (*Panicum virgatum*) (Parrish and Fike 2005; Samson et al. 2005; Fike et al. 2006), big bluestem (*Andropogon gerardii*) (Hallam et al. 2001), reed canarygrass (*Phalaris arundinacea*) (Lewandowski et al. 2003), and Miscanthus (*Miscanthus* spp.) (Clifton-Brown et al. 2004; Heaton et al. 2004). Of these, switchgrass has received particular attention, having been chosen by the US Department of Energy's Bioenergy Feedstock Development Program as a model energy crop due to its high biomass yields, broad geographic range, efficient nutrient utilization, low erosion potential, carbon sequestration capability, and reduced fossil fuel input requirements relative to annual crops (McLaughlin and Walsh 1998; McLaughlin and Kszos 2005).

Lignocellulosic biomass can be produced with significant environmental advantages over food-based crops, but it is not without potential problems. Particular care must be taken when selecting species for use as biofuel crops, for example, as many of the traits leading to the success of bioenergy crops, such as C_4 photosynthesis, long canopy duration, lack of pests and diseases, and rapid spring growth, are also associated with invasiveness potential (Raghu et al. 2006). Many lignocellulosic crops can be grown with low agrichemical and fossil fuel inputs, but intensive cropping practices may also be employed with high or even excessive fertilizer and pesticide inputs (Fike et al. 2006; Parrish and Fike 2005). Converting land from annual crop production into stands of perennial grasses in the conservation reserve program (CRP) has restored the ability of these soils to sequester carbon (Gebhart et al. 1994), but although carbon can also be sequestered in switchgrass stands managed for maximizing biomass production with high levels of nitrogen fertilization (Frank et al. 2004; Liebig et al. 2005), release of N_2O into the atmosphere may significantly offset the greenhouse gas mitigation potential of such lands (Conant et al. 2005). The spatial pattern of lignocellulosic crop production can also have a large impact on wildlife habitat and biodiversity preservation (Cook et al. 1991; Leemans et al. 1996; Green et al. 2005).

Even though the current contribution of lignocellulosic biofuels from both crop residues and dedicated energy crops to the US transportation energy supply is negligible, the potential exists for them to rival or surpass crop-based biofuels. Perlack et al. (2005) recently estimated that 6.8×10^{10} kg of maize stover can currently be sustainably harvested in the US. Assuming a demonstrated ethanol yield of $0.255\,\mathrm{L\,kg^{-1}}$ of biomass (Sheehan et al. 2004), this would provide 1.7×10^{10} L of ethanol, slightly greater than 2005 US ethanol production from maize grain, plus an additional electrical energy equivalent of 1.6×10^9 L of ethanol to be sold back to the grid. This would provide enough energy to offset 2.2% of US gasoline use, and assuming an average net energy balance ratio of 5 for lignocellulosic ethanol production (Hammerschlag 2006), the net contribution would be 1.8%, greater than current the net contribution of maize grain ethanol (0.3%). According to Milbrandt (2005), planting every acre of land currently in the CRP into switchgrass would yield approximately 7.6×10^{10} kg of biomass. This would provide approximately 1.9×10^{10} L of ethanol and 1.8×10^9 L of ethanol energy equivalent electricity, or enough to offset 2.5% of gasoline use with a net contribution of 2.0%. In addition to greater net energy gains than maize grain ethanol, both maize stover ethanol and ethanol from switchgrass grown on lands not currently in production would have the benefit of avoiding competition with food markets for biofuel feedstocks.

4.2 The Promise of Prairies

Energy crops, both food-based and lignocellulosic, are typically cultivated as monocultures, but enhanced environmental, energetic, and economic benefits may be realized by growing biomass in polycultures (Fig. 3). Tilman et al. (2006) recently demonstrated the value of biodiversity in biofuel production from grassland biomass (Fig. 4). They reported that annual production of native prairie plant biomass increased

Fig. 3 Blackeyed susan (*Rudbeckia hirta*), wild bergamot (*Monarda fistulosa*), and big bluestem (*Andropogon gerardii*) in a diverse restored prairie in Minnesota, USA (Clarence Lehman)

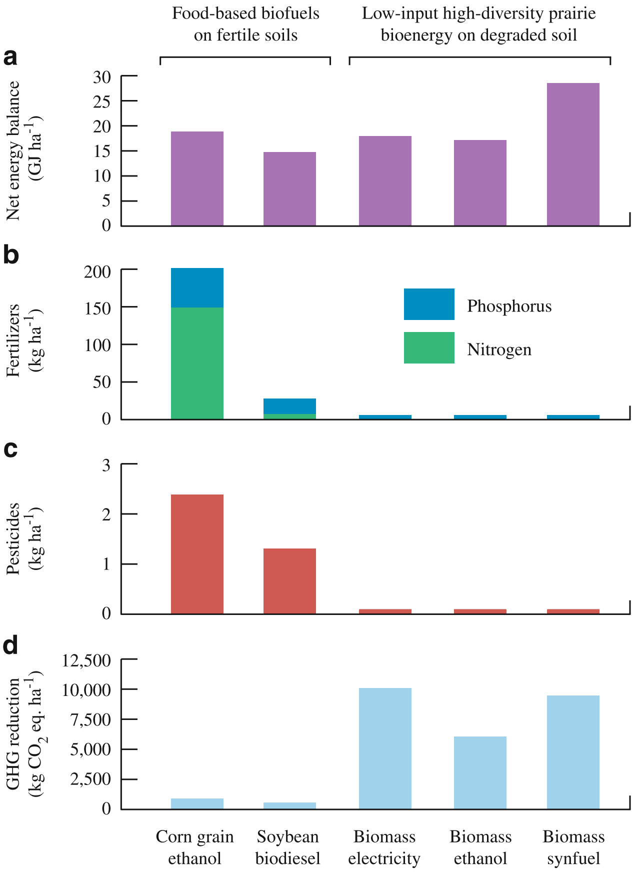
Fig. 4 An aerial view of the biodiversity experiment at cedar creek natural history area in Bethel, Minnesota, USA, reported in Tilman et al. (2006). The 9 m × 9 m plots are planted to either 1, 2, 4, 8, or 16 species randomly drawn from a set of native prairie plants (David Tilman)

with species diversity, with plots planted to 16 species yielding 238% more aboveground biomass than plots planted to a single species on average. Not only did more diverse plots become increasingly more productive over time relative to less diverse plots, but they also provided greater stability in year-to-year yield. Even though this experiment was conducted on degraded land, converting the biomass from the highly diverse plots to ethanol would generate a net energy gain of 17.8 GJ ha^{-1}, comparable to the average yield of 18.9 GJ ha^{-1} for maize grain ethanol produced on fertile farmland (Fig. 5a). In addition, whereas maize grain ethanol yields 25% more fossil energy than invested in its production, producing ethanol from the highly diverse prairie biomass harvested in this experiment would yield 440% more.

The environmental benefits of prairie biofuels are numerous. Unlike maize and soybeans, a prairie requires little or no fertilizer inputs. Nitrogen, which is cycled more efficiently in prairies than in cultivated maize cropland (Brye et al. 2001), can be supplied by native legumes. Phosphorus and other nutrients would need to be supplied only at low levels due to both efficient use in prairie plants and translocation of many elements to root systems late in the season before aboveground biomass is harvested (Fig. 5b). Unlike maize and soybean cropland, an established prairie requires no herbicide or insecticide application as it resists invasion from plants, pathogens, and herbivorous insects (Fig. 5c). This encourages diverse ecosystems, reduces input costs, and provides a valuable form of insurance to farmers (Heal et al. 2004). Harvesting a prairie also mimics natural burning, which is necessary for keeping out invading woody species, which can reduce soil carbon storage (Jackson et al. 2002). A prairie can provide habitat for wildlife, and biomass harvest can be timed to occur only after birds have fledged (Murray et al. 2003; Roth et al. 2005; Semere and Slater 2007). Restoring prairie for biofuel use can produce a valuable energy feedstock while offering valuable ecosystem services (Clergue et al. 2005; Foley et al. 2005). These ecosystem services include pollinator habitat for service to nearby crop fields (Greenleaf and Kremen 2006) and mitigation of agricultural runoff from traditional farming by reducing flow volumes and increasing nutrient use opportunity (Huggins et al. 2001), akin to similar services provided by wetlands (Hey et al. 2005).

One of the most vital ecosystem services provided by a diverse prairie is its ability to serve as a substantial carbon sink, reducing atmospheric carbon and improving degraded land. Approximately 1/3 of the total prairie plant biomass is above ground and available for harvesting each year, but the other 2/3 below ground continues to grow, sequestering carbon and supporting a rhizosphere that also decreases atmospheric carbon (Six et al. 2006). In total, about 4.4 Mg $\text{ha}^{-1}\,\text{yr}^{-1}$ of CO_2 are sequestered each year in the Cedar Creek prairie, far exceeding the 0.3 Mg $\text{ha}^{-1}\,\text{yr}^{-1}$ of CO_2 released when combusting the fossil fuels used to produce biofuels from the aboveground biomass. Therefore, as the carbon released when combusting the biofuel was initially sequestered from the atmosphere in the aboveground biomass itself, biofuels from prairie grasses are "carbon negative" (Fig. 5d). On the other hand, with respect to atmospheric carbon, both maize grain ethanol and soybean biodiesel are "carbon positive," creating a net release of greenhouse gases, albeit less than fossil fuels they displace. Intensive farming has led to massive carbon loss in soils (Huggins et al. 1998), and the ability of diverse prairies to sequester carbon and build

Fig. 5 Comparison of energetic and environmental aspects of biofuels produced from food-based crops and low agricultural input, highly diverse prairie biomass. Biofuels produced from biomass include electricity, ethanol, and synfuel hydrocarbons. Greenhouse gas (GHG) reductions are estimated relative to the fossil fuels that each of the biofuels displaces. Adapted from Tilman et al. 2006



soils (McLauchlan et al. 2006) can restore fertile land and increase its value (Daily 1995; Lal 2004).

Implementing large-scale biofuel production from diverse prairie biomass will require consideration of various practical and economic factors. First, supplies of both native grass and forb seed are limited, and quantities sufficient to plant available lands will take many growing seasons to produce. Second, various technical aspects of utilizing biomass of diverse species for biofuel production are unknown, although recent studies have considered both the digestibility (Weimer and Springer 2006) and combustion (Florine et al. 2006) of diverse grasses. Third, as with all lignocellulosic biomass sources, development of an infrastructure for transporting biomass to biofuel production facilities will be critical (Atchison and Hettenhaus 2004; Kumar and Sokhansanj 2006; Morrow et al. 2006). Fourth, a subsidy and incentive policy will be needed to foster adoption of lignocellulosic biomass, much as was done to encourage, and is still required for, the current generation of food-based biofuels (Tyson 2005). Such a policy might allow for harvesting prairie biomass for biofuels production on land in set-aside programs (e.g., CRP and CSP lands) while still receiving subsidy payments. Any such policy could be tailored to encourage management practices benefiting environmental concerns (Walsh et al. 2003) and outdoor recreation (Sullivan et al. 2004). A US carbon trading market that rewards farmers for conservation-friendly practices might also provide sufficient monetary incentive for prairie biomass farming (McLaughlin et al. 2002; Schneider and McCarl 2003; Kurkalova et al. 2004).

The demonstrated potential for producing biofuels from diverse mixtures of prairie species raises many related questions. How, for example, do interactions among species compositions and management practices affect both productivity and ecosystem services in grasslands (Camill et al. 2004; Guo 2006), especially when restored and managed specifically for biofuel production? What are the relative benefits of planting fertile farmland to prairie rather than food crops for biofuel production? Can prairie biomass production strategies be combined with grazing opportunities for mutual benefit? How will grassland productivity respond to global warming (De Broeck et al. 2006)? With the positive relationship between biodiversity and ecosystem productivity now firmly established (Hooper et al. 2005; Cardinale et al. 2006), are other native flora also suitable for biofuel production while maintaining a healthy, functioning ecosystem?

5 Conclusion

The shift to automobiles and airplanes marked the end of the era when transportation biofuel consisted mainly of the hay fed to horses, the ordinary diets of pedestrians, and wood used to power many steamboats and locomotives. As petroleum began to meet our transportation energy needs, agricultural practices focused more on those crops consumed by humans or fed to livestock and poultry. The recent surge in interest for using biological material to offset petroleum use has wed together food and transportation energy concerns once again. This presents both challenges and opportunities. Conflict over using crops such as maize and soybeans for food and biofuels will increase as demands for both end products rise in the future. Demand for agricultural products may very well be the major cause of future nonclimatic global change (Tilman et al. 2001). In the near term, gains in conservation and efficiency can have much greater effect on slowing climate change than even radical shifts in agricultural practices (Jackson and Schlesinger 2004). In the long term, this linking together of food and fuel markets in a time of increasing awareness of the benefits of sustainability will allow us to reevaluate current land use and implement strategies that lead to truly sustainable food and biofuel supplies (Robertson et al. 2004; Robertson and Swinton 2005; Reijinders 2006). The actual benefits of this shift will be realized more fully when biofuel production no longer relies upon fitting our energy production into our current agricultural system but rather adapting our agricultural practices in an environmentally sensitive manner to supply both our food and energy needs.

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Grasslands for Bioenergy Production: A Review

Enrico Ceotto

Abstract The promise of low-input high-diversity prairies to provide sustainable bioenergy production has recently been emphasized. This review article presents a critical discussion of some controversial points of using grasslands to produce bioenergy. The following issues are addressed: proteins vs. biofuels; reactive nitrogen emissions; biodiversity; and effective land use. Two major disadvantages in deriving bioenergy from grasslands are identified: (1) marginal lands are displaced from their fundamental role of producing meat and milk foods, in contrast with the rising worldwide demand for high-quality food; and (2) the combustion of N-rich grassland biomass, or by-products, results in emission of reactive N into the atmosphere and dramatically reduces the residence time of biologically-fixed nitrogen in the ecosystems. Nitrogen oxides, released during atmospheric combustion of fossil fuels and biomass, have a detrimental effect on global warming. Since intensively managed crops on fertile soils need to be cultivated to fulfil the dietary needs of populations, the potential role of inedible cereal crop residues in providing bioenergy merits consideration. This might spare more marginal land area for forage production or even for full natural use, in order to sustain high levels of biodiversity. Owing to the complexity of terrestrial systems, and the complexity of interactions, a modeling effort is needed in order to predict and quantify outcomes of specific combination of land use at higher integration levels.

Keywords Biodiversity • Bioenergy • Effective land use • Grassland • Proteins vs. biofuel • Reactive nitrogen emissions

1 Introduction

Plants have the unique ability to convert the incoming flux of solar energy, a renewable form of energy, into useful biomass, in the form of food, feed, and fuel. However, in order to fully exploit the potential of crops for transforming solar energy into dry matter, crops need to be supplemented with fossil energy, either directly through soil tillage or pumping irrigation, or indirectly through the application of energy-intensive industrial fertilizers and pesticides (Pimentel 1992). Consequently, modern agricultural systems are strongly dependent on fossil energy and therefore are vulnerable to the caprices of world fuel prices, and are also contributing to the rise in carbon dioxide (CO_2) and other greenhouse gases in the atmosphere (Mannion 1997). Until the early 1900s, most of the energy used by human societies was derived from agriculture and forests. Even the first petrol and diesel engines were initially designed to run on ethanol and peanut oil, respectively (Collins and Duffield 2005). From 1920 petroleum increasingly replaced vegetable oil, starch and cellulose as a feedstock for energy and industrial products (Morris and Ahmed 1992). By the early 1970s, the energy crisis stimulated a renewed interest in producing energy from crop biomass. In addition, evidence indicates that this massive use of fossil energy has increased the concentration of CO_2 and other greenhouse gases in the Earth's atmosphere.

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This has also become a concern because of the potential long-term influence on global climate change (IPCC 1996).

Some authors have concluded that the energy generated from plant biomass is close to “carbon neutral” because the CO_2 released in processing is the same as that captured by the plant by photosynthesis, while preserving the C stored for millenia in fossil reserves (Sims et al. 2006). In contrast, others have raised major ethical and environmental points: energy crops compete on fertile soil with food and feed production (Pimentel 1991; Giampietro et al. 1997); and when natural land is converted into arable energy crops, increased pollution from fertilizers and pesticides, increased soil erosion, and decreased biodiversity can result (Pimentel 2003).

Besides arable crops, grasslands can contribute to energy needs. Aiming to offset fossil fuels, prairie biomass can produce heat and electricity through direct combustion, or can be converted into transportation biofuels such as biodiesel, ethanol and methanol (Barnes and Nelson 2003). Boylan et al. (2005) reported an encouraging pioneer experience of co-firing grasses (i.e., *Panicum virgatum* L., *Cynodon dactylon* L. Pers. and *Festuca spp.*) in an existing coal-fired plant: about 10% of the energy from biomass was successfully achieved. Recently, Tilman et al. (2006) and Hill (2007) reported intriguing results on biofuel derived from low-input high-diversity grassland. In essence, a well-balanced mixture of 16 native prairie plant species, including C3 and C4 grasses, legumes, forbs and woody species, produced 238% more above-ground biomass than plots sowed to a single species. The net energy gain (i.e., output–input) for conversion of biomass into electricity, ethanol and synfuel was very close to that of maize (*Zea mays* L.) grain converted into ethanol, with major gains on output to input ratios. The basic strategies underlying their experiment are the following:

- Use of legumes as a primary route of nitrogen in the ecosystems, in order to avoid the use of the fossil energy-intensive industrial nitrogen fertilizers.
- Use of a diverse range of native prairie plant species to gain high efficiency in exploiting light, water and nutrient resources, and to achieve stability in yields.

In emphasizing the outcomes of their experiment (conducted in Cedar Creek, Minnesota, USA), they pointed out that biofuel derived from low-input high-diversity grassland neither displaces food production nor causes loss of biodiversity. In this review, I present a critical analysis of some controversial issues regarding the use of prairies for producing renewable energy. In particular, I pose a deliberately provocative question: it is worthwhile to displace forages from their traditional role of feeding animals, and consequently mankind, for the purpose of producing energy? To address this question, the following issues are discussed: (1) proteins vs. biofuel; (2) reactive nitrogen emissions; (3) biodiversity; and (4) effective use of land resources: intensification vs. extensification.

2 Proteins vs. Biofuel

“The primary form of food is grass. Grass feeds the ox: the ox nourishes man: man dies and goes back to grass again; and so the tide of life, with everlasting repetition, in continuous circles, moves endlessly on and upward, and in more senses than one, all flesh is grass.” Quote from an address of John James Ingalls, Senator of Kansas from 1873 to 1891. Cited by Barnes et al. (2003).

The assertion of Tilman et al. (2006) and Hill (2007) that biofuel derived from low-input high-diversity grassland does not displace food production is not compelling. Indeed, grasslands and forages play an important role in agriculture because they contribute to human food supply through animal production. Herbivores, notably domesticated species, have the unique ability to convert low-quality plant proteins into first-class meat and milk products (Fig. 1).

Humans in their adult state are unable to synthesize 8 of the 20 different amino acids required for the synthesis of the body proteins, either at all, or at sufficient levels to fulfil growth and maintenance (Follett and Follett 2001). These amino acids, referred to as essential amino acids, must be necessarily obtained from food sources and include: leucine, lysine, isoleucine, methionine, phenylalanine, threonine, tryptophan, and valine. In addition, during infancy histidine is also required (Follett and Follett 2001). Animal proteins contain adequate amounts of all essential amino acids and are easily digestible. In contrast, plant proteins are deficient in at least one essential amino acid, usually lysine for cereals, methionine and cysteine for legumes, and are also less easy to digest (Smil 2002).

Fig. 1 Prairie biomass is edible by domesticated herbivores, and thereby is converted into high-quality milk and meat foods. Grazing herds directly utilize the forage, displacing fossil fuel for hay harvesting and transportation (photo Enrico Ceotto)

Yet, an additional aspect should be considered: ruminants combine the ability to digest cellulose-rich plant biomass with the ability to convert low-quality plant proteins into high-quality animal proteins. This allows the use of large areas of marginal land, unsuitable for cultivation of arable crops, for meat and dairy production (Loomis and Connor 1992; Mannion 1997).

From the standpoint of energetics, meat and dairy products are not a good bargain: when grassland primary production is converted into animal products, most of the solar energy captured by the plants is lost as entropy (Mannion 1997; Stiling 1999); from 19 to 188 MJ of feed energy are required to produce 1 MJ of animal protein energy (Pimentel 1992). Therefore, the energy efficiency of the conversion is very low. In particular, beef production is an inherently less energy-efficient way to produce proteins through animal feeding than milk production, because the animals have high metabolic rates, combined with long gestation and lactation periods (Smil 2002). Pimentel and Pimentel (2003) pointed out that the meat-based American diet requires much more land, fossil energy and water resources compared with a lacto-ovo-vegetarian diet. Thus, it is tempting to imagine that there would be much more energy available from agriculture if we were all vegetarian. In practice, this is irrelevant. In fact, a diet rich in meat and dairy products is perceived as a symbol of prosperity, therefore vegetarianism will not likely be a voluntary choice for the majority of the population, either in rich or poor countries (Smil 2002). Giampietro (2004) pointed out that the technical changes in the agriculture of developed countries have been driven by the demand for higher nutritional quality of the diet, rather than the need for increasing the energy supply of the diet. The opposite is true for typical diets of developing countries, where the pressure to harvest more dietary energy from cultivated areas in the form of cereals is overwhelming. Nevertheless, as soon as poor countries ameliorate their standard of life, there is an increasing demand for beef, beer, and dairy products. This implies a higher cereal consumption per capita, thus increased fertile land requirement. Green et al. (2005) reported that:

- In developed countries, the meat production per capita is about 75 kg per person, data for the year 2000, albeit with a trend of slight decline from 1990 to 2000.
- In developing countries, in contrast, the meat production per capita is about 20 kg per person, data for the year 2000, with a trend of steady increase from 1980 to 2000.

This is in good agreement with Wilkins (2001), who highlighted different current pressures for developed and developing countries:

1. In developed countries, concern about the adverse effects on health of consumption of saturated animal fats, coupled with little population growth, has decreased the demand for ruminant products; concern by society for environmental pollution has increased strongly, and new market opportunities have arisen from demand for “natural” production systems.
2. In developing countries, high rates of population growth, coupled with aspirations for a better diet, have increased the overall global requirement for food.

Smil (2002) pointed out that the actual protein intake is excessive in industrialized countries and is inadequate for hundreds of millions of people in poor countries. The economic development and changing lifestyles in developing countries, particularly in China, are causing a rising demand for meat and dairy products worldwide (Smith et al. 2007). As Lal (2007) recently pointed out, access to adequate and balanced food sources, along with safe drinkable water, is the most basic human right that must be respected. Therefore, there is considerable need for increasing animal production on marginal lands, and prairies might provide a substantial contribution. Rather than convert abandoned and degraded agricultural land into prairies for biofuels, conversion into productive pastures would provide much more significant benefits to humankind. Moreover, research has indicated that grass feeding reduces the ratio of omega-6 to omega-3 fatty acids in meat and milk; yet, conjugated linoleic acid (an anti-carcinogen) in milk is also much increased with grazing (Wilkins 2001; Wilkins and Vidrih 2000). Consequently, it is apparent that, even in terms of quality of products, grasslands have the potential to provide important services to society.

3 Reactive Nitrogen Emissions

Nitrogen, along with carbon, is one of the most essential elements for life. However, many ecological problems arise when nitrogen is separated from its common partner carbon (Keeney and Hatfield 2001). Nitrogen oxides, released during the combustion of fossil fuels and biomass, have a detrimental effect on global warming (Moomaw 2002).

As highlighted earlier, one key strategy of the experiment planned by Tilman et al. (2006) was to exploit legumes as a main route of nitrogen in the ecosystems, with the purpose of avoiding the use of energy-intensive industrial nitrogen fertilizers. I do not disagree that this is an ecologically-sound strategy of nitrogen input, but I argue that the subsequent fate of fixed nitrogen must be taken into account. In fact, as Russelle et al. (2007) pointed out, one questionable point of the Cedar Creek experiment is that a substantial part of the energy gain for the conversion process of low-input high-diversity grasslands biofuel appears to come from combustion of biomass itself or by-products. In fact, regardless of whether biomass was co-fired with coal to generate electricity, converted into ethanol + electricity, or converted into synfuel + electricity, the critical point is that all nitrogen contained in harvested dry matter returned quickly to the atmosphere via combustion. This implies a dramatic reduction of the residence time of the biologically-fixed nitrogen. In contrast, in grazed grasslands, nitrogen, along with other plant nutrients, is recycled back into the soil via manure and urine (Barker and Collins 2003; Jarvis 2000; Wedin and Russelle 2007). The residence time of nitrogen can be centuries in unmanaged grasslands and decades in grazed grassland (Galloway et al. 2003).

Leaves and stalks of grassland plants contain 10–20 $\mathrm{N\,g\,kg^{-1}}$ dry matter, and rise to about 30 $\mathrm{g\,N\,kg^{-1}}$ dry matter in the case of legumes. Such nitrogen contents are quite high if compared with cereal straw (5 $\mathrm{g\,N\,kg^{-1}}$) and wood (3–5 $\mathrm{g\,N\,kg^{-1}}$). When biomass is burned to generate energy, nitrogen oxides (NO_x), a mixture of nitric oxide (NO), and nitrogen dioxide (NO_2), are released from two different pathways. The first is called thermal production and comes from the direct reaction of nitrogen and oxygen gas at high temperature ($N_2 + O_2 = 2NO$). The second is the oxidation of organic nitrogen compounds during pyrolysis at high temperature ($X\text{-}CH_2\ NH_2 + 3O_2 = CO_2 + 2H_2O + NO_2 + X$). Ozone, a substantial absorber of infrared radiation, is formed by NO_2 itself and NO_2 in the presence of volatile organic compounds. This leads to undesired feed-back: ozone is formed readily in a warm atmosphere, and is itself a greenhouse gas that promotes further warming (Moomaw 2002).

Co-firing grasses with coal resulted in lower CO_2, sulfur dioxide (SO_2) and metals emissions, whilst nitrogen oxide (NO_x) emissions remained unchanged (Boylan et al. 2005). Therefore, it is advisable to generate renewable energy from biomass containing very low nitrogen per unit weight.

From the standpoint of reducing reactive nitrogen emissions, the use of maize grain ethanol appears to be a convenient solution. In fact, the by-product of the ethanol industry is distillers' dried grains with solubles, with about 30% crude protein and 11% crude fat (Belyea et al. 2004). Owing to their high nutritional value, distillers' dried grains with solubles are used mostly to feed dairy and beef cattle, but are also suitable to be added to pig and poultry feed (Shurson et al. 2004). This implies that all the nitrogen, along with fat, is recycled as an animal feed and only starch is used for bioenergy. Since part of the biomass is used to feed animals, the current claim that maize grain ethanol threatens food security and might lead to starvation in poor people in developing countries appears to be exaggerated. The same can be asserted for soybean (*Glycine max,* (L.) Merr.) biodiesel production: soybeans are crushed to separate oil from the meal, which is not combusted but rather used as a high-value protein source for feeding animals (Hill et al. 2006). Yet another point of strength for both maize grain ethanol and soybean biodiesel merits highlighting: the recycling of nitrogen, phosphorous, and potassium within agricultural systems via manure has a substitution value for displacing the use of industrial fertilizers (Ceotto 2005).

If ligno-cellulosic biomass has to be used for co-firing with coal, then straw and stover appear to be the most convenient feedstock. Since more than one-half of the dry matter produced by grain crops has no direct human nutritional value, crop residues have the potential to provide a strategic source of biofuels (Smil 1999). Owing to their low nitrogen content, crop residues are poorly suited for animal feeding, except for maintenance of dry stock and as a fiber adjuvant for distillers' dried grains with solubles. On the other hand, they are well suited to be burned to obtain energy, associated with little reactive nitrogen emissions (Fig. 2). The use of cereal residues for energy generation certainly does not threaten global food security. On the contrary, an additional income derived from crop residues has the potential to stimulate farmers to produce more cereals. Nevertheless, a pitfall is just around the corner: crop residues play a crucial role in maintaining or increasing soil organic matter, a key condition for sustainable land use. Therefore, a crucial question arises: what is the fraction of crop residues that could be collected from the field without depleting soil organic matter and increasing soil erosion? Graham et al. (2007), referring to maize stover production in Iowa/Minnesota, concluded that about two-thirds could be collected without detrimental effects, while others have recommended lower amounts (Wilhelm et al. 2004). If reduced tillage and crop rotations including forages are adopted, it is likely that a higher fraction of straw and stover could be used for bioenergy without detrimental effects. Still, the potential contribution from ley farming merits consideration: the alternation between grassland and arable cropping leads to accumulation of soil organic matter during the grass phase, which then breaks down during the arable phase, supplying nutrients that sustain crop yields (Wilkins 2001). Moreover, integration of perennial pasture and grain crops leads to major environmental benefits in terms of insect and weed disruption, improved water-use efficiency and reduced soil erosion (Sulc and Tracy 2007).

Fig. 2 About one-half of dry matter produced by grain crops is in the form of inedible biomass. Owing to their low nitrogen content, crop residues are poorly suited for animal feeding, and well suited to be burned to obtain energy. Thus, crop residues have the potential to provide a strategic source of biofuels (photo Enrico Ceotto)

Finally, on marginal lands, grazing herds might directly utilize the forage, therefore displacing fossil fuel for hay harvesting and transportation. Admittedly, cattle herds are a source of CH_4, NH_3, N_2O, and NO_x emissions, so they can negatively affect global climate change (Freibauer and Kaltschmitt 2001; Asner et al. 2004). However, agricultural systems are inherently complex, and land-use choices entail rarely, if ever, "win-win" solutions. Some unintended trade-offs are inevitable, therefore "small loss-big gain" or "win-lose" solutions are good compromises for balancing human needs and ecosystem services (Defries et al. 2004).

4 Biodiversity

Tilman et al. (2006) asserted that biofuel derived from low-input high-diversity grassland does not cause losses in biodiversity. Nevertheless, as Russelle et al. (2007) pointed out, they burned the plots, except for a narrow strip that was cut for biomass measurements. Thus, it seems likely their results do not properly represent a harvested system. Yet, their assertion is certainly true when prairies are compared with arable soils, but it is controversial if biofuel production is compared with pastures. In fact, two oak species, *Quercus macrocarpa* and *Quercus elipsoidalis,* were included in the list of 16 planted species, but the annual burning management did not allow survival of woody species in multi-species plots. On the contrary, grazing systems may lead to woody encroachment in the long term (Asner et al. 2004), with major advantages for both biodiversity and the C sink in above- and belowground biomass. Stuth and Maraschin (2000) suggested that grazing may reduce the competitive ability of grasses and allow woody plants to invade at faster rates; however, the reverse is also true: grazing may determine harsher environmental conditions at soil level, owing to less vegetation cover. Therefore, they postulate that the primary influence of grazing is the reduction of fuel loads, and therefore the occurrence of fire events, which may indirectly favor the diffusion of woody species.

On the other hand, high fertilizer applications and intensive grazing adversely affect biodiversity (Wilkins 2001). Therefore, areas managed for high levels of biodiversity are likely to produce low yields of herbage with low feeding value (Tallowin and Jefferson 1999). In principle, if the target of food and forage production is met by small areas managed for high agricultural yields, then vast areas could be managed for biodiversity. In practice, things are more complex: research is required to determine the best size and connectivity between land uses in order to achieve a successful biodiversity management (Wilkins 2001; Green et al. 2005); pressure by society, deriving from tourism and recreational use of rural areas, should also be considered in land-use planning at higher integration levels (Wilkins 2001). However, from the standpoint of tourism and recreational use, it seems likely that grazed grasslands are at least as attractive as prairies managed for bioenergy production.

5 Effective Land-Use Resources

5.1 Historical Overview

In prehistoric times people obtained food by collecting plant material and hunting animals. One hunter-gatherer individual had to collect about 33 MJ in the form of food every day to assure survival in his/her family unit (Loomis and Connor 1992). At that time at least 1.5 km^2 of land (i.e., 150 ha) were required to provide food for one person (Faidley 1992). Grazing grasslands were vital to prehistoric people a long time before herbivores were domesticated (Barnes and Nelson 2003). On a geological scale, agriculture is a recent development and dates back only 10,000–12,000 years. Shifting cultivation was one of the first agricultural practices, in which portions of land are cleared and burned to allow periodical cultivation of cereals. Shifting cultivation of forest lands supports a population of about 7.7 people km^2, about 13 ha per person (McCloud 1998). The transition of shifting cultivation to subsistence farming did not increase productivity per hectare. In the Middle Ages, cereal yields in central Europe remained at about 1,000 kg ha^{-1} (Loomis and Connor 1992). About 200 kg ha^{-1} was required for seeding the subsequent year; about 400 kg $\mathrm{ha}^{-1}$was required for feeding animals and to produce beer; the remaining 400 kg $\mathrm{ha}^{-1}$was little more than the dietary need of the farmer who did the work (McCloud 1998).

Cereal yields were doubled to 2,000 kg ha^{-1} from the 1600s to the mid-1700s. This revolution was introduced by livestock farming, in which cereals were rotated with clover and grasses for feeding animals, and manure and urine was returned to cropland (McCloud 1998). The development of industrialized agriculture began in the early 1950s, when the use of industrial nitrogen fertilizers allowed spectacular yield increases. The Haber–Bosch process was initially used for producing explosives, but after the second World War, the production of industrial fertilizers had the consequence that humanity no longer had to rely on biological nitrogen fixation and limited natural resources of nitrogen fertilizers (Trewavas 2002). Global cereal production has doubled in the past 40 years, and in addition to undeniable benefits, industrial agriculture has added substantial and environmentally detrimental amounts of reactive nitrogen, and phosphorus, to terrestrial and aquatic ecosystems (Tilman et al. 2002).

Penning de Vries (2001) indicated that with current yield levels, from 0.05 to 0.5 ha of land is necessary to produce the food an average human being consumes. This wide range depends on whether a strictly vegetarian or meat-based diet is considered. Yet, if all energy for human use (transportation, heating, and cooking) were generated by energy crops, every individual would need from 0.2 to 2.0 ha of land. In the meantime, availability of land is becoming increasingly scarce due to land degradation, expanding urban and residential areas, and pressure from other human activities.

5.2 Wildlife-Friendly vs. Land-Sparing Farming

As far as agriculture management is concerned, there are contrasting schools of thought on how to couple the solution of environmental problems with the fulfilment of dietary needs of an increasing world population. As Green et al. (2005) and Balmford et al. (2005) pointed out, two different sorts of land-use suggestions predominate in the literature: wildlife-friendly farming, whereby agricultural practices are made as benign as possible to the environment, at the cost of productivity per unit area, with increased pressure to convert marginal land to agriculture; and land-sparing farming, in which productivity per unit area is increased to potential levels and pressure to convert land to agriculture is consequently decreased, at the cost of higher risk of environmental pollution from smaller areas and threat to wildlife species on farmland. A long-lasting debate exists about the role of legumes. Some authors (e.g., Crews and Peoples 2004; Drinkwater et al. 1998) suggest that sustainable land use would be greatly improved by using legume crops as a main source of nitrogen inputs. In contrast, others claim that the pressures to utilize crop plants that can fix nitrogen must be balanced against the equally important objective of achieving optimal utilization of solar energy per unit area. In this view, Sinclair and Cassman (1999) contend that the increasing food demand from the human population already exceeds the low carrying capacity of legume-dependent cropping systems. The industrial synthesis of ammonia provides the means of survival of about 40% of humanity; only one-half of today's population could be sustained by prefertilizer farming with a strict vegetarian diet (Smil 2000). Preindustrial agricultural systems rely solely on solar energy, but this implies low productivity per hectare, per hour of labor and per worker; thus, a dramatically lower standard of living (Fig. 3).

In contrast, industrialized agricultural systems are relatively highly dependent on fossil energy, but they allow more land area to be devoted to non-agricultural purposes and assure a better quality of life for human populations (Fig. 4).

Achieving a more judicious use of fossil energy is a major challenge for science in agriculture, as well as in urban and industrial systems. However, low-input agriculture is not the obvious solution for the problem. In fact, if the productivity per unit area is lowered, larger areas of non-cultivated land must be converted into arable soil. De Wit (1979) pointed out that the most sensible use of fossil energy in agriculture is achieved when the highest yields per hectare are obtained from as small an acreage as possible by highly skilled farmers. Loomis (1983, p. 367) agreed, pointing out that the simplest strategy for efficient use of limited resources is generally intensive cropping: "a system that comes rapidly to complete cover and extends the cover for the full growing season without limitation by nutrients, diseases and pests." This implies less energy use

Fig. 3 Plowing scene of the early 1930s. These preindustrial agricultural systems rely solely on solar energy. Nevertheless, their low productivity per hectare, per hour of labor and per worker imply that: (1) more natural land has to be converted to arable crops to fulfil a production target; (2) more people have to work in agriculture with a dramatically lower standard of living (Painting by Franco Serafini 1992)

Fig. 4 Industrialized agricultural systems are relatively highly dependent on fossil energy, but they allow more land area to be devoted to non-agricultural purposes and assure a better quality of life for both agricultural and non-agricultural workers (photo Enrico Ceotto)

per unit of product, and more land available for other purposes. In this view, I would suggest that land-use decisions should be made on a higher spatial scale, involving a full weighting of benefits and trade-offs on fertile and marginal areas. An intriguing viewpoint was advocated by Giampietro et al. (1992): to assess the land area necessary to produce 1 kg of maize grain, we could consider: (1) only the area under maize cultivation; (2) we could also include the area of fallow land required at farm level to allow sustainable production; (3) we could also include the space requirement to produce the external inputs applied to the crops; (4) finally, we could even consider the space of wild ecosystems needed to preserve the stability of the environment. As the scale of observation is enlarged, it is increasingly evident that the higher the productivity per land area, the larger the land space that can be exploited for other purposes. Trewavas (2001) estimated that without pesticides, irrigation or fertilizers, current food production would only be achieved by plowing up an extra 2,000 Mha, with cutting down of forests and dramatic destruction of wilderness.

5.3 Low-Input High-Diversity Prairies vs. Intensive Land Use

Tilman et al. (2006) emphasized that annual biomass production of native prairies increased with species diversity, with plots sowed to 16 species yielding 238% more aboveground biomass than plots sowed to a single species. The appealing inference that such mixtures of species provide a solution for effective land use is not justified (Grace et al. 2007). Indeed, the low-input high-diversity grassland average biomass production was 3,700 kg ha^{-1} yr^{-1}, equivalent to a gross energy output of 68.1 GJ ha^{-1} yr^{-1}. With intensive management, an annual pasture production in temperate regions commonly reaches 15,000 kg ha^{-1} yr^{-1}, corresponding to 255 GJ ha^{-1} yr^{-1}(Loomis and Connor 1992). Therefore, 1 ha of intensively managed grassland can provide the same production as 3.74 ha of low-input high-diversity grassland. A well-fertilized and irrigated maize crop commonly produces about 22,000 kg ha^{-1} $\text{yr}^{-1}$of aboveground biomass, one-half of which is grain and the other half is stover. The energy content of maize stover is about the same as grassland hay (i.e., 18 MJ kg^{-1}). Thus, 1 ha of maize grown for grain produces, as a by-product, an amount of biomass and energy about threefold that provided by low-input high-diversity prairies. Therefore, it is quite evident that the well-balanced mixtures of 16 plant species, including grasses, legumes and other forbs, cannot overtake the biophysical constraints imposed by nutrients, mostly nitrogen and phosphorous, and water limitations.

In order to assess the effectiveness of fossil energy use in agriculture, it is worthwhile to highlight some relationships between fossil carbon released and carbon assimilated by the crops.

Schlesinger (1999) indicated a factor of 1.436 moles of CO_2-C released per mole of nitrogen when accounting for the full carbon cost of nitrogen fertilizer, including manufacture, transport, and application. When 1 kg of nitrogen is supplied to a field crop, about one-half is incorporated in aboveground crop biomass. The other half is accumulated in the soil nitrogen pool, transferred to the atmosphere as NH_3, NO_x, N_2O, or N_2, or lost to aquatic ecosystems in the form of nitrate (Galloway 2005). Since the average nitrogen content of cereal crops is about 1.1% of dry matter, the uptake of 0.5 kg of nitrogen allows production of 45.5 kg dry matter with a 44% carbon content, corresponding to 20 kg C (Fig. 5). Thus, the carbon released for the industrial production of 1 kg nitrogen is about 7% of the net assimilation of a cereal crop, i.e., $1.436/20 = 0.07$. The carbon assimilation of a crop growing under non-limiting production conditions amply makes up for the fossil fuel-derived CO_2 emissions necessary to sustain its growth (Ceotto 2005).

In the literature, there are many articles reporting thorough energy balances of land-use systems (Hill 2007; Loomis and Connor 1992; Pimentel 2003; Tilman et al. 2006). Nevertheless, they normally contain evaluations and comparisons among three or four specific case studies, and extrapolation to other agricultural systems are hardly, if ever, possible.

6 Conclusion

There are two major disadvantages in deriving bioenergy from grasslands: (1) marginal lands are displaced from their fundamental role of producing meat and

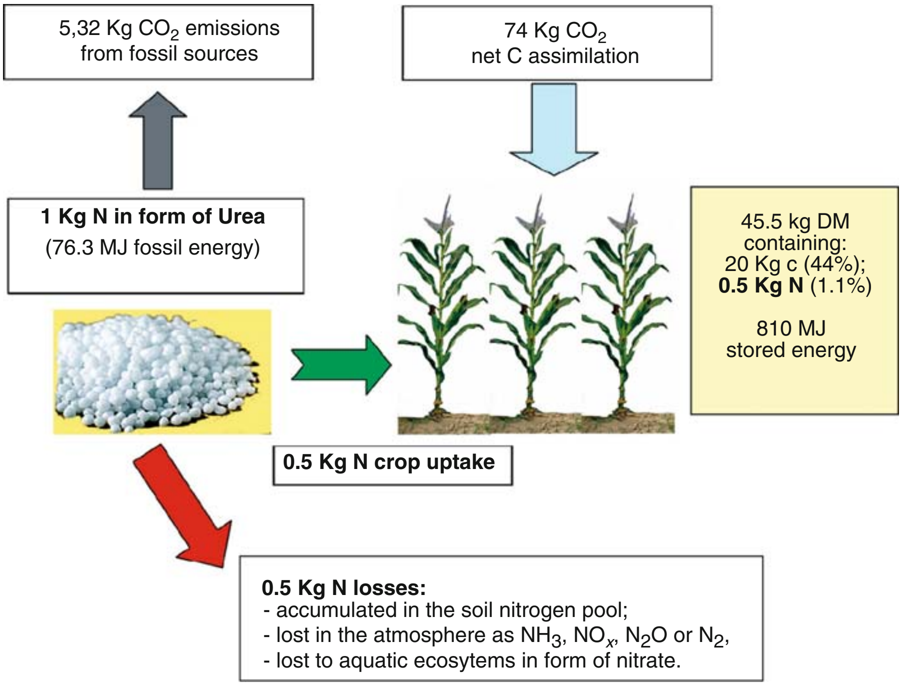

Fig. 5 Outline of the major benefits and detrimental effects of supplementing industrial nitrogen on field crops. The amount of CO_2 assimilated by the crop plant amply makes up the CO_2 emissions deriving from the manufacture of industrial fertilizers. One-half of the nitrogen applied is taken up by the crop and provides valuable proteins to the food chain; the remaining half of the nitrogen supplied is undesirably lost in the environment

milk foods, thereby conflicting with the rising worldwide demand for high-quality food; (2) combustion of N-rich grassland biomass or by-products releases reactive N into the atmosphere and dramatically reduces the residence time of biologically-fixed nitrogen. Since intensively managed crops on fertile soils need to be cultivated anyway to fulfil the dietary needs of populations, the potential role of inedible cereal crop residues in providing bioenergy should be considered. This might spare more marginal land area for forage production or even for full natural use, in order to sustain high levels of biodiversity. Performing a thorough energetic comparison among a few land-use systems is a relatively easy task. In contrast, to identify optimum land-use combinations at higher integration levels is not that simple. Owing to the complexity of terrestrial systems, and the complexity of interactions, a GIS-based modeling effort is needed in order to predict and quantify specific combinations of land use at higher integration levels. This could provide policymakers with the data needed to achieve broad societal goals.

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Plant Drought Stress: Effects, Mechanisms and Management

M. Farooq, A. Wahid, N. Kobayashi, D. Fujita, and S.M.A. Basra

Abstract Scarcity of water is a severe environmental constraint to plant productivity. Drought-induced loss in crop yield probably exceeds losses from all other causes, since both the severity and duration of the stress are critical. Here, we have reviewed the effects of drought stress on the growth, phenology, water and nutrient relations, photosynthesis, assimilate partitioning, and respiration in plants. This article also describes the mechanism of drought resistance in plants on a morphological, physiological and molecular basis. Various management strategies have been proposed to cope with drought stress. Drought stress reduces leaf size, stem extension and root proliferation, disturbs plant water relations and reduces water-use efficiency. Plants display a variety of physiological and biochemical responses at cellular and whole-organism levels towards prevailing drought stress, thus making it a complex phenomenon. CO_2 assimilation by leaves is reduced mainly by stomatal closure, membrane damage and disturbed activity of various enzymes, especially those of CO_2 fixation and adenosine triphosphate synthesis. Enhanced metabolite flux through the photorespiratory pathway increases the oxidative load on the tissues as both processes generate reactive oxygen species. Injury caused by reactive oxygen species to biological macromolecules under drought stress is among the major deterrents to growth. Plants display a range of mechanisms to withstand drought stress. The major mechanisms include curtailed water loss by increased diffusive resistance, enhanced water uptake with prolific and deep root systems and its efficient use, and smaller and succulent leaves to reduce the transpirational loss. Among the nutrients, potassium ions help in osmotic adjustment; silicon increases root endodermal silicification and improves the cell water balance. Low-molecular-weight osmolytes, including glycinebetaine, proline and other amino acids, organic acids, and polyols, are crucial to sustain cellular functions under drought. Plant growth substances such as salicylic acid, auxins, gibberrellins, cytokinin and abscisic acid modulate the plant responses towards drought. Polyamines, citrulline and several enzymes act as antioxidants and reduce the adverse effects of water deficit. At molecular levels several drought-responsive genes and transcription factors have been identified, such as the dehydration-responsive element-binding gene, aquaporin, late embryogenesis abundant proteins and dehydrins. Plant drought tolerance can be managed by adopting strategies such as mass screening and breeding, marker-assisted selection and exogenous application of hormones and osmoprotectants to seed or growing plants, as well as engineering for drought resistance.

Keywords CO_2 • Drought management • Drought response • Hormones • Osmoprotectants • Stomatal oscillation • Stress proteins

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1 Introduction

Faced with scarcity of water resources, drought is the single most critical threat to world food security. It was the catalyst of the great famines of the past. Because the world's water supply is limiting, future food demand for rapidly increasing population pressures is likely to further aggravate the effects of drought (Somerville and Briscoe 2001). The severity of drought is unpredictable as it depends on many factors such as occurrence and distribution of rainfall, evaporative demands and moisture storing capacity of soils (Wery et al. 1994).

Investigations carried out in the past provide considerable insights into the mechanism of drought tolerance in plants at molecular level (Hasegawa et al. 2000). Three main mechanisms reduce crop yield by soil water deficit: (1) reduced canopy absorption of photosynthetically active radiation, (2) decreased radiation-use efficiency and (3) reduced harvest index (Earl and Davis 2003). The reproducibility of drought stress treatments is very cumbersome, which significantly impedes research on plant drought tolerance. A slow pace in revealing drought tolerance mechanisms has hampered both traditional breeding efforts and use of modern genetics approaches in the improvement of drought tolerance of crop plants (Xiong et al. 2006). Although plant responses to drought are relatively well known, plant performance under a more complex environment where multiple stresses co-occur is fragmentary. That is why the plants have to respond simultaneously to multiple stresses, e.g. drought, excessive light and heat, which may coincide in the field. These kinds of investigations are usually not predictable from single factor studies (Zhou et al. 2007).

It is imperative to improve the drought tolerance of crops under the changing circumstances. Currently, there are no economically viable technological means to facilitate crop production under drought. However, development of crop plants tolerant to drought stress might be a promising approach, which helps in meeting the food demands. Development of crops for enhanced drought resistance, among other things, requires the knowledge of physiological mechanisms and genetic control of the contributing traits at different plant developmental stages. Valuable work has been done on drought tolerance in plants. Ingram and Bartels (1996) more than a decade ago elegantly reviewed those appreciable efforts. More recent reviews deal with specific aspects of plant drought tolerance (Penna 2003; Reddy et al. 2004; Agarwal et al. 2006). This review encompasses an overview of the current work reported on some effects and mechanisms of drought tolerance in higher plants and important management strategies to overcome the drought effects, mainly on field crops.

2 Effects of Drought on Plants

The effects of drought range from morphological to molecular levels and are evident at all phenological stages of plant growth at whatever stage the water deficit takes place. An account of various drought stress effects and their extent is elaborated below.

2.1 *Crop Growth and Yield*

The first and foremost effect of drought is impaired germination and poor stand establishment (Harris et al. 2002). Drought stress has been reported to severely reduce germination and seedling stand (Kaya et al. 2006). In a study on pea, drought stress impaired the germination and early seedling growth of five cultivars tested (Okcu et al. 2005). Moreover, in alfalfa (*Medicago sativa*), germination potential, hypocotyl length, and shoot and root fresh and dry weights were reduced by polyethylene glycol-induced water deficit, while the root length was increased (Zeid and Shedeed 2006). However, in rice, drought stress during the vegetative stage greatly reduced the plant growth and development (Fig. 1; Tripathy et al. 2000; Manikavelu et al. 2006).

Growth is accomplished through cell division, cell enlargement and differentiation, and involves genetic, physiological, ecological and morphological events and their complex interactions. The quality and quantity of plant growth depend on these events, which are affected by water deficit (Fig. 2). Cell growth is one of the most drought-sensitive physiological processes due to the reduction in turgor pressure (Taiz and Zeiger 2006). Under severe water deficiency, cell elongation of higher plants can be inhibited by interruption of water flow from the xylem to the surrounding elongating cells (Nonami 1998). Impaired

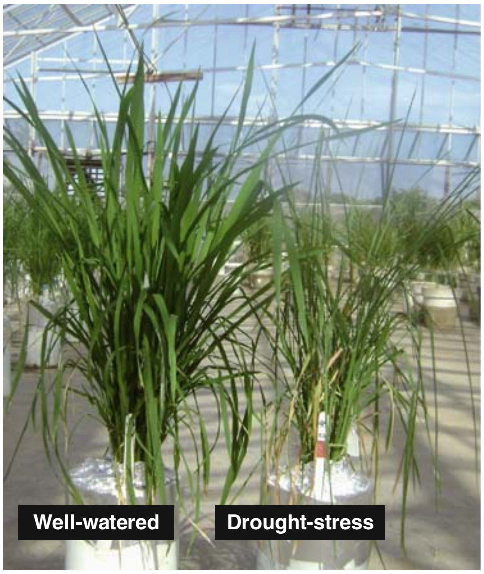

Fig. 1 Effect of drought stress on the vegetative growth of rice cv. IR64. Both the plants were grown under well-watered conditions up to 20 days following emergence. One pot was submitted to progressive soil drying (drought stress). The afternoon before the drought, all pots were fully watered (to saturation). After draining overnight, the pots were enclosed around the stem to prevent direct soil evaporation. A small tube was inserted for re-watering pots. The decrease in soil moisture was controlled by partial re-watering of the stressed pots to avoid a quicker imposition of stress and to homogenize the development of drought stress. A well-watered control pot was maintained at the initial target weight by adding the daily water loss back to the pot. This figure shows the plants 20 days after imposition of drought stress

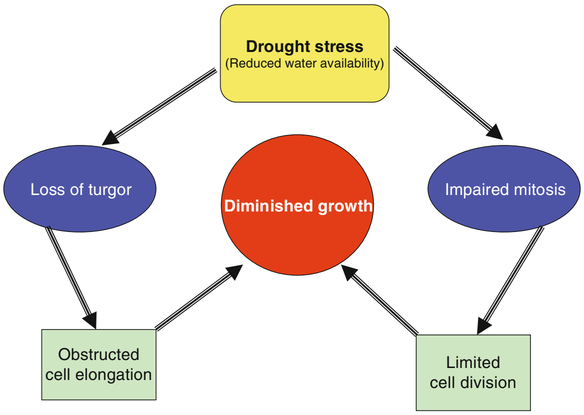

Fig. 2 Description of possible mechanisms of growth reduction under drought stress. Under drought stress conditions, cell elongation in higher plants is inhibited by reduced turgor pressure. Reduced water uptake results in a decrease in tissue water contents. As a result, turgor is lost. Likewise, drought stress also trims down the photo-assimilation and metabolites required for cell division. As a consequence, impaired mitosis, cell elongation and expansion result in reduced growth

mitosis, cell elongation and expansion result in reduced plant height, leaf area and crop growth under drought (Nonami 1998; Kaya et al. 2006; Hussain et al. 2008).

Many yield-determining physiological processes in plants respond to water stress. Yield integrates many of these physiological processes in a complex way. Thus, it is difficult to interpret how plants accumulate, combine and display the ever-changing and indefinite physiological processes over the entire life cycle of crops. For water stress, severity, duration and timing of stress, as well as responses of plants after stress removal, and interaction between stress and other factors are extremely important (Plaut 2003). For instance, water stress applied at pre-anthesis reduced time to anthesis, while at post-anthesis it shortened the grain-filling period in triticale genotypes (Estrada-Campuzano et al. 2008). In barley (*Hordeum vulgare*), drought stress reduced grain yield by reducing the number of tillers, spikes and grains per plant and individual grain weight. Post-anthesis drought stress was detrimental to grain yield regardless of the stress severity (Samarah 2005).

Drought-induced yield reduction has been reported in many crop species, which depends upon the severity and duration of the stress period (Table 1). In maize, water stress reduced yield by delaying silking, thus increasing the anthesis-to-silking interval. This trait was highly correlated with grain yield, specifically ear and kernel number per plant (Cattivelli et al. 2008). Following heading, drought had little effect on the rate of kernel filling in wheat, but its duration (time from fertilization to maturity) was shortened and dry weight reduced at maturity (Wardlaw and Willenbrink 2000). Drought stress in soybean reduced total seed yield and the branch seed yield (Frederick et al. 2001). In pearl millet (*Pennisetum glaucum*), co-mapping of the harvest index and panicle harvest index with grain yield revealed that greater drought tolerance was achieved by greater partitioning of dry matter from stover to grains (Yadav et al. 2004).

Drought at flowering commonly results in barrenness. A major cause of this, though not the only one, was a reduction in assimilate flux to the developing ear below some threshold level necessary to sustain optimal grain growth (Yadav et al. 2004). Moisture

Table 1 Economic yield reduction by drought stress in some representative field crops

Crop	Growth stage	Yield reduction (%)	References
Barley	Seed filling	49–57	Samarah (2005)
Maize	Grain filling	79–81	Monneveux et al. (2006)
Maize	Reproductive	63–87	Kamara et al. (2003)
Maize	Reproductive	70–47	Chapman and Edmeades (1999)
Maize	Vegetative	25–60	Atteya et al. (2003)
Maize	Reproductive	32–92	Atteya et al. (2003)
Rice	Reproductive (mild stress)	53–92	Lafitte et al. (2007)
Rice	Reproductive (severe stress)	48–94	Lafitte et al. (2007)
Rice	Grain filling (mild stress)	30–55	Basnayake et al. (2006)
Rice	Grain filling (severe stress)	60	Basnayake et al. (2006)
Rice	Reproductive	24–84	Venuprasad et al. (2007)
Chickpea	Reproductive	45–69	Nayyar et al. (2006)
Pigeonpea	Reproductive	40–55	Nam et al. (2001)
Common beans	Reproductive	58–87	Martínez et al. (2007)
Soybean	Reproductive	46–71	Samarah et al. (2006)
Cowpea	Reproductive	60–11	Ogbonnaya et al. (2003)
Sunflower	Reproductive	60	Mazahery-Laghab et al. (2003)
Canola	Reproductive	30	Sinaki et al. (2007)
Potato	Flowering	13	Kawakami et al. (2006)

deficit reduced cotton (*Gossypium hirsutum*) lint yield, although the timing, duration, severity and speed of development undoubtedly had pivotal roles in determining how the plant responded to moisture deficit. Lint yield was generally reduced due to reduced boll production because of fewer flowers and greater boll abortions when the stress intensity was greater during reproductive growth (Pettigrew 2004).

Grain filling in cereals is a process of starch biosynthesis from simple carbohydrates. It is believed that four enzymes play key roles in this process: sucrose synthase, adenosine diphosphate–glucose–pyrophosphorylase, starch synthase and starch branching enzyme (Taiz and Zeiger 2006). Decline in the rate of grain growth resulted from reduced sucrose synthase activity, while cessation of growth resulted from inactivation of adenosine diphosphate–glucose–pyrophosphorylase in the water-stressed wheat (Ahmadi and Baker 2001). Water deficit during pollination increased the frequency of kernel abortion in maize (*Zea mays*). Under water stress, diminished grain set and kernel growth in wheat and a decreased rate of endosperm cell division was associated with elevated levels of abscisic acid in maize (Morgan 1990; Ober et al. 1991). In pigeonpea, drought stress coinciding with the flowering stage reduced seed yield by 40–55% (Nam et al. 2001). In rice, on the other hand, water stress imposed during the grain-filling period enhanced remobilization of pre-stored carbon reserves to grains and accelerated grain filling (Yang et al. 2001). In summary, prevailing drought reduces plant growth and development, leading to hampered flower production and grain filling, and thus smaller and fewer grains. A reduction in grain filling occurs due to a reduction in the assimilate partitioning and activities of sucrose and starch synthesis enzymes.

2.2 Water Relations

Relative water content, leaf water potential, stomatal resistance, rate of transpiration, leaf temperature and canopy temperature are important characteristics that influence plant water relations. Relative water content of wheat leaves was higher initially during leaf development and decreased as the dry matter accumulated and leaf matured (Siddique et al. 2001). Obviously, water-stressed wheat and rice plants had lower relative water content than non-stressed ones. Exposure of these plants to drought stress substantially decreased the leaf water potential, relative water content and transpiration rate, with a concomitant increase in leaf temperature (Siddique et al. 2001). A conservative influence of decreased stomatal conductance in non-irrigated plants was negated by a leaf-to-air vapor pressure difference caused by the associated higher leaf temperature. Transpiration rates were similar in both treatments and the lower total water use of the non-irrigated stand resulted entirely from a smaller leaf area index (Craufurad et al. 2000).

Nerd and Nobel (1991) reported that during drought stress, total water contents of *Opuntia ficus-indica* cladode were decreased by 57%. The water-storage parenchyma of the cladodes lost a greater fraction of water than the chlorenchyma, and thus showed a lower turgor potential. In another study on *Hibiscus rosa-sinensis*, relative water content, turgor potential, transpiration, stomatal conductance and water-use efficiency were decreased under drought stress (Egilla et al. 2005).

The ratio between dry matter produced and water consumed is termed as water-use efficiency at the whole-plant level (Monclus et al. 2006). Abbate et al. (2004) concluded that under limited supply, water-use efficiency of wheat was greater than in well-watered conditions. They correlated this higher water-use efficiency with stomatal closure to reduce the transpiration. In another study on clover (*Trifolium alexandrinum*), water-use efficiency was increased due to lowered water loss under drought stress, primarily by decreased transpiration rate and leaf area, and relatively lesser reduction in yield (Lazaridou and Koutroubas 2004). Also, in *Pinus ponderosa* and *Artemisia tridentata,* drought stress did not reduce the water-use efficiency; rather, it was increased, mainly due to a rapid decrease in stomatal conductance with increasing water deficit (DeLucia et al. 1989). (Lazaridou et al. (2003) further reported that leucern (*Medicago sativa*) grown under drought had greater water-use efficiency than that under irrigated conditions, for the same leaf water potential. However, in potato, early season drought stress significantly minimized the water-use efficiency, leading to greatly decreased growth and biomass accumulation (Costa et al. 1997).

In fact, although components of plant water relations are affected by reduced availability of water, stomatal opening and closing is more strongly affected. Moreover, change in leaf temperature may be an important factor in controlling leaf water status under drought stress. Drought-tolerant species maintain water-use efficiency by reducing the water loss. However, in the events where plant growth was hindered to a greater extent, water-use efficiency was also reduced significantly.

2.3 Nutrient Relations

Decreasing water availability under drought generally results in limited total nutrient uptake and their diminished tissue concentrations in crop plants. An important effect of water deficit is on the acquisition of nutrients by the root and their transport to shoots. Lowered absorption of the inorganic nutrients can result from interference in nutrient uptake and the unloading mechanism, and reduced transpirational flow (Garg 2003; McWilliams 2003). However, plant species and genotypes of a species may vary in their response to mineral uptake under water stress. In general, moisture stress induces an increase in N, a definitive decline in P and no definitive effects on K (Garg 2003).

Transpiration is inhibited by drought, as shown for beech (Peuke et al. 2002), but this may not necessarily affect nutrient uptake in a similar manner. Influence of drought on plant nutrition may also be related to limited availability of energy for assimilation of NO_3^-/NH_4^+, PO_4^{3-} and SO_4^{2-}: they must be converted in energy-dependent processes before these ions can be used for growth and development of plants (Grossman and Takahashi 2001).

As nutrient and water requirements are closely related, fertilizer application is likely to increase the efficiency of crops in utilizing available water. This indicates a significant interaction between soil moisture deficits and nutrient acquisition. Studies show a positive response of crops to improved soil fertility under arid and semi-arid conditions. Currently, it is evident that crop yields can be substantially improved by enhancing the plant nutrient efficiency under limited moisture supply (Garg 2003). It was shown that N and K uptake was hampered under drought stress in cotton (McWilliams 2003). Likewise, P and PO_4^{3-} contents in the plant tissues diminished under drought, possibly because of lowered PO_4^{3-} mobility as a result of low moisture availability (Peuke and Rennenberg 2004). In drought-treated sunflower, the degree of stomatal opening of K^+-applied plants initially indicated quicker decline. However, at equally low soil water potential, diffusive resistance in the leaves of K^+-applied plants remained lower than those receiving no K^+ (Lindhauer et al. 2007). In summary, drought stress reduces the availability, uptake, translocation and metabolism of nutrients. A reduced transpiration rate due to water deficit reduces the nutrient absorption and efficiency of their utilization.

2.4 Photosynthesis

A major effect of drought is reduction in photosynthesis, which arises by a decrease in leaf expansion, impaired photosynthetic machinery, premature leaf senescence and associated reduction in food production (Wahid and Rasul 2005). When stomatal and non-stomatal limitations to photosynthesis are compared, the former can be quite small. This implies that other processes besides CO_2 uptake are being damaged. The role of drought-induced stomatal closure, which limits CO_2 uptake by leaves, is very important. In such events, restricted CO_2 availability could possibly lead to increased susceptibility to photo-damage (Cornic and Massacci 1996).

Drought stress produced changes in photosynthetic pigments and components (Anjum et al. 2003), damaged photosynthetic apparatus (Fu J. and Huang 2001) and diminished activities of Calvin cycle enzymes, which are important causes of reduced crop yield (Monakhova and Chernyadèv 2002). Another important effect that inhibits the growth and photosynthetic abilities of plants is the loss of balance between the production of reactive oxygen species and the antioxidant defense (Fu J. and Huang 2001; Reddy et al. 2004), causing accumulation of reactive oxygen species which induces oxidative stress in proteins, membrane lipids and other cellular components (Fig. 3). Some important components of photosynthesis affected by drought are discussed below.

2.4.1 Stomatal Oscillations

The first response of virtually all plants to acute water deficit is the closure of their stomata to prevent the transpirational water loss (Mansfield and Atkinson 1990). This may result in response to either a decrease in leaf turgor and/or water potential (Ludlow and Muchow 1990) or to a low-humidity atmosphere (Maroco et al 1997). The debate as to whether drought mainly limits photosynthesis through stomatal closure or metabolic impairment has continued for a long time (Sharkey 1990; Tezara et al. 1999). During the last decade, stomatal closure was generally accepted to be the main determinant

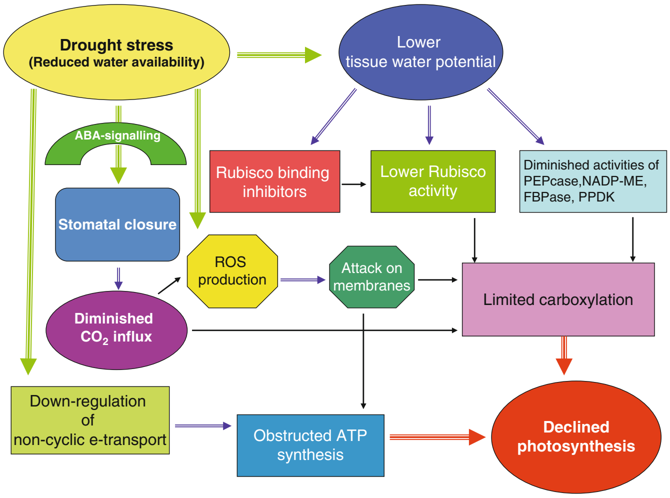

Fig. 3 Photosynthesis under drought stress. Possible mechanisms in which photosynthesis is reduced under stress. Drought stress disturbs the balance between the production of reactive oxygen species and the antioxidant defense, causing accumulation of reactive oxygen species, which induces oxidative stress. Upon reduction in the amount of available water, plants close their stomata (plausibly via ABA signaling), which decreases the CO_2 influx. Reduction in CO_2 not only reduces the carboxylation directly but also directs more electrons to form reactive oxygen species. Severe drought conditions limit photosynthesis due to a decrease in the activities of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), phosphoenolpyruvate carboxylase (PEPCase), NADP-malic enzyme (NADP-ME), fructose-1,6-bisphosphatase (FBPase) and pyruvate orthophosphate dikinase (PPDK). Reduced tissue water contents also increase the activity of Rubisco binding inhibitors. Moreover, non-cyclic electron transport is down-regulated to match the reduced requirements of NADPH production and thus reduces the ATP synthesis. *ROS* reactive oxygen species

for decreased photosynthesis under mild to moderate drought (Cornic and Massacci 1996; Yokota et al. 2002).

When the amount of available soil water is moderately or severely limiting, the first option for plants is to close stomata (Cornic and Massacci 1996). This decreases the inflow of CO_2 into the leaves and spares more electrons for the formation of active oxygen species (Fig. 3). As the rate of transpiration decreases, the amount of heat that can be dissipated increases (Yokota et al. 2002). Various experiments have shown that stomatal responses are often more closely linked to soil moisture content than to leaf water status. This suggested that stomata respond to chemical signals, e.g. abcissic acid, produced by dehydrating roots (Fig. 3), whilst leaf water status is kept constant (Morgan 1990; Taylor 1991; Turner et al. 2001). Environmental conditions that enhance the rate of transpiration also increase the *p*H of leaf sap, which can promote abscisic acid accumulation and concomitantly diminish stomatal conductance. Increased cytokinin concentration in the xylem sap promotes stomatal opening directly and affects the sensitivity of stomata towards abscisic acid (Wilkinson and Davies 2002).

Comparing results from different studies is complex due to interspecific differences in the response of stomatal conductance and photosynthesis to leaf water potential and/or relative water content; the parameters most often used to assess the degree of drought (Cornic and Massacci 1996). It is clear that stomata close progressively as drought progresses, followed by a parallel decline in net photosynthesis. However, stomatal conductance is not controlled by soil water availability alone, but by a complex interaction of intrinsic and extrinsic factors.

2.4.2 Photosynthetic Enzymes

Very severe drought conditions limit photosynthesis due to a decline in Rubisco activity (Bota et al. 2004). The activity of the photosynthetic electron transport chain is finely tuned to the availability of CO_2 in the chloroplast and change in photosystem II under drought conditions (Loreto et al. 1995). Dehydration results in cell shrinkage, and consequently a decline in cellular volume. This makes cellular contents more viscous. Therefore, an increase in the probability of protein–protein interaction leads to their aggregation and denaturation (Hoekstra et al. 2001). Increased concentration of solutes, leading to increased viscosity of the cytoplasm, may become toxic and may be deleterious to the functioning of enzymes, including those of the photosynthetic machinery (Hoekstra et al. 2001).

The level of Rubisco in leaves is controlled by the rate of synthesis and degradation. Even under drought stress the Rubisco holoenzyme is relatively stable with a half-life of several days (Hoekstra et al. 2001). However, drought stress showed a rapid diminution in the abundance of Rubisco small subunit transcripts, which indicated its decreased synthesis (Vu et al. 1999). Rubisco activity is modulated in vivo either by reaction with CO_2 and Mg^{2+} to carbamylate a lysine residue in the catalytic site, or by binding inhibitors within the catalytic site (Fig. 3). Such a binding either blocks activity or the carbamylation of the lysine residue, which is essential for activity. At night, 2-carboxyarabinitol-1-phosphate is formed in many species, which binds tightly to Rubisco, inhibiting catalytic activity. It is reported that tight-binding inhibitors can decrease Rubisco activity in the light. In tobacco (*Nicotiana tabacum*), decrease in Rubisco activity under drought stress was not a primary result of changes in activation by CO_2 and Mg^{2+}, and was rather due to the presence of tight-binding inhibitors (Parry et al. 2002). A rapid decline in photosynthesis under drought was accompanied by decreased maximum velocity of ribulose-1,5-bisphosphate carboxylation by Rubisco, speed of ribulose-1,5-bisphosphate regeneration, Rubisco and stromal fructose bis-phosphatase activities, and the quantum efficiency of photosystem II in higher plants (Reddy et al. 2004; Zhou et al. 2007). Moreover, under severe drought, carboxylation efficiency by Rubisco was greatly declined, and it acted more as oxygenase than carboxylase (Fig. 3).

During water stress, activities of the phosphoenolpyruvate carboxylase, nicotinamide adenine dinucleotide phosphate-malic enzyme, Rubisco, fructose-1,6-bisphosphatase and pyruvate orthophosphate dikinase decreased linearly with lowered leaf water potential (Fig. 3). Pyruvate orthophosphate dikinase activities were decreased 9.1 times during water stress; a much greater reduction than other enzymes, which were from 2 to 4 times, suggesting that pyruvate orthophosphate dikinase is very likely to be the limiting enzyme to photosynthesis under water stress (Du et al. 1996).

2.4.3 Adenosine Triphosphate Synthesis

There is a long-standing controversy as to whether drought mainly limits photosynthesis through stomatal closure (Cornic and Massacci 1996) or by metabolic impairment (Tezara et al. 1999). Evidence that impaired adenosine triphosphate synthesis is the main factor limiting photosynthesis even under mild drought has further stimulated the debate (Lawlor and Cornic 2002). It is reported that impaired photophosphorylation and adenosine triphosphate synthesis are the main factors limiting photosynthesis even under mild drought (Tezara et al. 1999).

Under drought stress, production of limited nicotinamide adenine dinucleotide phosphate maintains the continuation of electron transport, although the status of the reductant may be high even when the fluxes are small, leading to a more increased demand than supply. Under drought stress, non-cyclic electron transport is down-regulated to match the requirements of decreased nicotinamide adenine dinucleotide phosphate production and cyclic electron transport is activated. This generates a proton gradient that induces the protective process of high-energy-state quenching (Golding and Johnson 2003). Support for this model came from the isolation of a mutant deficient in high-energy-state quenching that lacked cyclic electron transport (Munekage et al. 2002). Support for cyclic electron transport under drought also came from non-steady-state measurements (Cornic et al. 2000).

Dissipation mechanisms of excess photon energy under water stress were studied in *ndhB*-inactivated tobacco (cv. Xanthi) mutants, impaired in reduced nicotinamide adenine dinucleotide phosphate dehydrogenase-dependent cyclic electron flow around photosystem I. The relative water content and net CO_2 assimilation was reduced to 30% and almost zero after an 11-day water stress regime in the mutant and wild-type plants, respectively. A decline in photosystem II activity ($\sim$75%), and an increase in malondialdehyde ($\sim$45%), an estimate of lipid peroxidation, were found in both the plant groups when subjected to water stress. Thus, a deficiency in reduced nicotinamide adenine dinucleotide phosphate dehydrogenase-dependent cyclic electron flow around photosystem I did not lead to oxidative damage because the mutant compensated for this deficiency by activating alternative dissipating routes of excess photon energy such as up-regulation of ferredoxin-dependent cyclic electron flow around photosystem I and enhanced accumulation of α-tocopherol (α-toc) quinine (Munné-Bosch et al. 2005).

In fact, the activities of the enzymes of carbon assimilation and those involved in adenosine triphosphate synthesis are retarded and sometimes inhibited depending upon the extent of available moisture. Of these, Rubisco, which shows dual functions, acts as oxygenase under water-limiting conditions; and therefore limited CO_2 fixation is noticed.

2.5 Assimilate Partitioning

Assimilate translocation to reproductive sinks is vital for seed development. Seed set and filling can be limited by availability or utilization, i.e. assimilate source or sink limitation, respectively (Asch et al. 2005). Drought stress frequently enhances allocation of dry matter to the roots, which can enhance water uptake (Leport et al. 2006). De Souza and Da Silv (1987), while analyzing the partitioning and distribution of photo-assimilates in annual and perennial cotton under drought stress, reported that the root-to-shoot dry matter ratio was high in perennial cotton, thereby showing a preferential accumulation of starch and dry matter in roots as an adaptation to drought. Thus, perennial cotton apparently owed its drought resistance to the partitioning of assimilates that favored starch accumulation and growth of the root system. The export rate of sucrose from source to sink organs depends upon the current photosynthetic rate and the concentration of sucrose in the leaves (Komor 2000). Drought stress decreases the photosynthetic rate, and disrupts the carbohydrate metabolism and level of sucrose in leaves that spills over to a decreased export rate. This is presumably due to drought stress-induced increased activity of acid invertase (Kim et al. 2000). Limited photosynthesis and sucrose accumulation in the leaves may hamper the rate of sucrose export to the sink organs and ultimately affect the reproductive development.

Apart from source limitation, the capacity of the reproductive sinks to utilize the incoming assimilates is also affected under drought stress and may also play a role in regulating reproductive abortion (Zinselmeier et al. 1999). Drought-induced carbohydrate deprivation, enhanced endogenous abscisic acid concentration, and an impaired ability to utilize the incoming sucrose by the reproductive sinks are potential factors contributing to seed abortion in grain crops (Setter et al. 2001). A reduced acid invertase activity can arrest the development of reproductive tissues due to improper phloem unloading (Goetz et al. 2001). In addition, drought stress may inhibit important functions of vacuolar invertase-mediated sucrose hydrolysis and osmotic potential modulation. In drought-stressed maize, a low invertase activity in the young ovaries lowers the ratio of hexoses to sucrose. This may inhibit cell division in the developing embryo/endosperm, resulting in weak sink intensity, and may ultimately lead to fruit abortion (Andersen et al. 2002).

In summary, drought stress not only limits the size of the source and sink tissues but the phloem loading, assimilate translocation and dry matter portioning are also impaired. However, the extent of effects varies with the plant species, stage, duration and severity of drought.

2.6 Respiration

Drought tolerance is a cost-intensive phenomenon, as a considerable quantity of energy is spent to cope with it. The fraction of carbohydrate that is lost through respiration determines the overall metabolic efficiency of the plant (Davidson et al. 2000). The root is a major consumer of carbon fixed in photosynthesis and uses it for growth and maintenance, as well as dry matter production (Lambers et al. 1996). Plant growth and developmental processes as well as environmental conditions affect the size of this fraction (i.e. utilized in respiration). However, the rate of photosynthesis often limits plant growth when soil water availability is reduced (Huang and Fu 2000). A negative carbon balance can occur as a result of diminished photosynthetic capacity during drought, unless simultaneous and proportionate reductions in growth and carbon consumption take place.

In wheat, depending on the growth stage, cultivar and nutritional status, more than 50% of the daily accumulated photosynthates were transported to the root, and around 60% of this fraction was respired (Lambers et al. 1996). Drought-sensitive spring wheat (Longchun, 8139-2) used a relatively greater amount of glucose to absorb water, especially in severe drought stress (Liu et al. 2004a,b). Severe drought reduced the shoot and root biomass, photosynthesis and root respiration rate. Limited root respiration and root biomass under severe soil drying can improve growth and physiological activity of drought-tolerant wheat, which is advantageous over a drought-sensitive cultivar in arid regions (Liu and Li 2005).

There are two mitochondrial electron transport pathways from ubiquinone to oxygen in plants. The alternative pathway branches from the cytochrome pathway and donates electrons to oxygen directly by alternative oxidase (Moore and Siedow 1991). The alternative pathway is not coupled with adenosine triphosphate synthesis, but can be induced in response to stress or inhibition of the main electron transfer pathway (Wagner and Moore 1997). When plants are exposed to drought stress, they produce reactive oxygen species, which damage membrane components (Blokhina et al. 2003). In this regard, alternative oxidase activity could be useful in maintaining normal levels of metabolites and reduce reactive oxygen species production during stress. Oxygen uptake by sugar beet was characterized by a high rate, distinct cytochrome oxidase-dependent terminal oxidation and up to 80% inhibition of respiration in the presence of 0.5 mM potassium cyanide. At an early drought stage (10 days), a decrease in the activity of the cytochrome-mediated oxidation pathway was largely counterbalanced by the activation of mitochondrial alternative oxidase, whereas long-term dehydration of plants was accompanied by activation of additional oxidative systems insensitive to both potassium cyanide and salicylhydroxamate (Shugaeva et al. 2007). In summary, water deficit in the rhizosphere leads to an increased rate of root respiration, leading to an imbalance in the utilization of carbon resources, reduced production of adenosine triphosphate and enhanced generation of reactive oxygen species.

2.7 Oxidative Damage

Exposure of plants to certain environmental stresses quite often leads to the generation of reactive oxygen species, including superoxide anion radicals (O_2^-), hydroxyl radicals (OH), hydrogen peroxide (H_2O_2), alkoxy radicals (RO) and singlet oxygen (O_2^1) (Munné-Bosch and Penuelas 2003). Reactive oxygen species may react with proteins, lipids and deoxyribonucleic

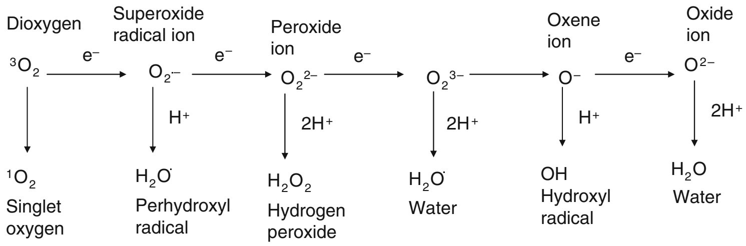

Fig. 4 Generation of reactive oxygen species by energy transfer or sequential univalent reduction of ground state triplet oxygen (Apel and Hirt 2004; reproduced with permission)

acid, causing oxidative damage and impairing the normal functions of cells (Foyer and Fletcher 2001). Many cell compartments produce reactive oxygen species; of these, chloroplasts are a potentially important source because excited pigments in thylakoid membranes may interact with O_2 to form strong oxidants such as O_2^- or O_2^1 (Niyogi 1999; Reddy et al. 2004). Further downstream reactions produce other reactive oxygen species such as H_2O_2 and OH^- (Fig. 4). The interaction of O_2 with reduced components of the electron transport chain in mitochondria can lead to reactive oxygen species formation (Möller 2001), and peroxisomes produce H_2O_2 when glycolate is oxidized into glyoxylic acid during photorespiration (Fazeli et al. 2007).

Mechanisms for the generation of reactive oxygen species in biological systems are represented by both non-enzymatic and enzymatic reactions. The partition between these two pathways under oxygen deprivation stress can be regulated by the oxygen concentration in the system. In non-enzymatic reactions, electron O_2 reduction can occur at higher oxygen concentrations (Apel and Hirt 2004). At very low O_2 concentrations, plant terminal oxidases and the formation of reactive oxygen species via the mitochondrial electron transport chain still remain functional. Among enzymatic sources of reactive oxygen species, xanthine oxidase, an enzyme responsible for the initial activation of O_2, should be mentioned. The electron donor xanthine oxidase can use xanthine, hypoxanthine or acetaldehyde, while the latter has been shown to accumulate under oxygen deprivation (Pfister-Sieber and Braendle 1994; Apel and Hirt 2004). This can represent a possible source for hypoxia-stimulated reactive oxygen species production (Fig. 4). The next enzymatic step is the dismutation of the superoxide anion by superoxide dismutase to yield H_2O_2 (Lamb and Dixon 1997). Peroxidases and catalases also play an important role in the fine regulation of reactive oxygen species in the cell through activation and deactivation of H_2O_2 (Sairam et al. 2005). Several apoplastic enzymes may also generate reactive oxygen species under normal and stressful conditions. Other oxidases, responsible for the two-electron transfer to dioxygen (amino acid oxidases and glucose oxidase) can contribute to H_2O_2 accumulation (Apel and Hirt 2004).

Reactive oxygen species are formed as by-products in the electron transport chains of chloroplasts (Apel and Hirt 2004), mitochondria and the plasma membrane (Sairam et al. 2005). The plant mitochondrial electron transport chain, with its redox-active electron carriers, is considered as the most probable candidate for intracellular reactive oxygen species formation. Mitochondria can produce reactive oxygen species due to the electron leakage at the ubiquinone site – the ubiquinone:cytochrome *b* region (Gille and Nohl 2001) – and at the matrix side of complex I (NADH dehydrogenase) (Möller 2001).

Superoxide radical and its reduction product H_2O_2 are potentially toxic compounds, and can also combine by the Haber-Weiss reaction to form the highly toxic OH^- (Sairam et al. 1998). Many reports show the deleterious effects of reactive oxygen species, whose production is stimulated under water stress (Blokhina et al. 2003). Reactive oxygen species cause lipid peroxidation, and consequently membrane injuries, protein degradation and enzyme inactivation (Sairam et al. 2005). Oxidative stress may also cause protein oxidation, with a loss of enzyme activity and the formation of protease-resistant cross-linked aggregates (Berlett and Stadtman 1997). Oxidatively-damaged proteins accumulate in pea leaves subjected to moderate water stress (Moran et al. 1994).

Overall, the production of reactive oxygen species is linear with the severity of drought stress, which leads to enhanced peroxidation of membrane lipids and degradation of nucleic acids, and both structural and functional proteins. Various organelles including chloroplasts, mitochondria and peroxisomes are the seats as well as first target of reactive oxygen species produced under drought stress.

3 Drought Resistance Mechanisms

Plants respond and adapt to and survive under drought stress by the induction of various morphological, biochemical and physiological responses. Drought tolerance is defined as the ability to grow, flower and display economic yield under suboptimal water supply. Drought stress affects the water relations of plants at cellular, tissue and organ levels, causing specific as well as unspecific reactions, damage and adaptation reactions (Beck et al. 2007). To cope with the drought, tolerant plants initiate defense mechanisms against water deficit (Chaves and Oliveira 2004), which need to be investigated in further detail (Zhou et al. 2007). In the following sections, mechanisms of drought tolerance at different levels are presented.

3.1 Morphological Mechanisms

Plant drought tolerance involves changes at whole-plant, tissue, physiological and molecular levels. Manifestation of a single or a combination of inherent changes determines the ability of the plant to sustain itself under limited moisture supply. An account of various morphological mechanisms operative under drought conditions is given below.

3.1.1 Escape

Escape from drought is attained through a shortened life cycle or growing season, allowing plants to reproduce before the environment becomes dry. Flowering time is an important trait related to drought adaptation, where a short life cycle can lead to drought escape (Araus et al. 2002). Crop duration is interactively determined by genotype and the environment and determines the ability of the crop to escape from climatic stresses including drought (Dingkuhn and Asch Dingkuhn). Matching growth duration of plants to soil moisture availability is critical to realize high seed yield (Siddique et al. 2003). Drought escape occurs when phenological development is successfully matched with periods of soil moisture availability, where the growing season is shorter and terminal drought stress predominates (Araus et al. 2002). In field-grown clones of robusta coffee, leaf shedding in response to drought stress occurred sequentially from older to younger leaves, suggesting that the more drought-sensitive the clone, the greater the extent of leaf shedding (DaMatta 2004).

Time of flowering is a major trait of a crop adaptation to the environment, particularly when the growing season is restricted by terminal drought and high temperatures. Developing short-duration varieties has been an effective strategy for minimizing yield loss from terminal drought, as early maturity helps the crop to avoid the period of stress (Kumar and Abbo 2001). However, yield is generally correlated with the length of crop duration under favorable growing conditions, and any decline in crop duration below the optimum would tax yield (Turner et al. 2001).

3.1.2 Avoidance

Drought avoidance consists of mechanisms that reduce water loss from plants, due to stomatal control of transpiration, and also maintain water uptake through an extensive and prolific root system (Turner et al. 2001; Kavar et al. 2007). The root characters such as biomass, length, density and depth are the main drought avoidance traits that contribute to final yield under terminal drought environments (Subbarao et al. 1995; Turner et al. 2001). A deep and thick root system is helpful for extracting water from considerable depths (Kavar et al. 2007).

Glaucousness or waxy bloom on leaves helps with maintenance of high tissue water potential, and is therefore considered as a desirable trait for drought tolerance (Richards et al. 1986; Ludlow and Muchow 1990). Varying degrees of glaucousness in wheat led to increased water-use efficiency, but did not affect total water use or harvest index. Determination of leaf temperature indicated that, compared with non-glaucous leaves, glaucous leaves were 0.7°C cooler and had a lower rate of leaf senescence (Richards et al. 1986). These authors suggested that a 0.5°C reduction in leaf temperature for 6 h per day was sufficient to extend the grain-filling period by more than 3 days. However, yield advantages are likely to be small as many varieties already show some degree of glaucousness.

3.1.3 Phenotypic Flexibility

Plant growth is greatly affected by water deficit. At a morphological level, the shoot and root are the most affected and both are the key components of plant adaptation to drought. Plants generally limit the number and area of leaves in response to drought stress just to cut down the water budget at the cost of yield loss (Schuppler et al. 1998). Since roots are the only source to acquire water from soil, the root growth, its density, proliferation and size are key responses of plants to drought stress (Kavar et al. 2007).

It has long been established that plants bearing small leaves are typical of xeric environments. Such plants withstand drought very well, albeit their growth rate and biomass are relatively low (Ball et al. 1994). Leaf pubescence is a xeromorphic trait that helps protect the leaves from excessive heat load. Hairy leaves have reduced leaf temperatures and transpiration (Sandquist and Ehleringer 2003) whilst inter- and intra-specific variation exists for the presence of this trait. Under high temperature and radiation stress, hairiness increases the light reflectance and minimizes water loss by increasing the boundary layer resistance to water vapor movement away from the leaf surface. Although drought stress also induces the production of trichomes on both sides of wheat leaves, they had no significant influence on boundary layer resistance.

The water content in drought-treated mature stems declined by 4% and water potential by −0.25 MPa. It is shown that active phloem supply of assimilates and associated water reserves from mature stems was the mechanism that allowed developing stems of *Hylocereus undatus* to maintain growth under drought conditions (Nerd and Neumann 2004). Moreover, girdling the phloem of growing stems rapidly inhibited stem elongation, but secretion of sucrose-containing nectar was maintained during drought. The water potential gradient was in the wrong direction for xylem transport from mature to young growing stems and axial hydraulic conductivity was low to negligible (Nerd and Neumann 2004).

Roots are the key plant organ for adaptation to drought. If tolerance is defined as the ability to maintain leaf area and growth under prolonged vegetative stage stress, the main basis of variation appears to be constitutive root system architecture that allows the maintenance of more favorable plant water status (Nguyen et al. 1997). The possession of a deep and thick root system allowed access to water deep in the soil, which was considered important in determining drought resistance in upland rice (Kavar et al. 2007). Evidence suggests that it is quality, i.e. the distribution and structure, and not quantity of roots that determines the most efficient strategy for extracting water during the crop-growing season (Fig. 5). The drought tolerance of tea, onion and cotton was increased by improved root growth and root functioning. Selection for a deep and extensive root system has been advocated to increase productivity of food legumes under moisture-deficit conditions as it can optimize the capacity to acquire water (Subbarao et al. 1995).

Studies carried out on the effects of alleles of the wheat shoot dwarfing genes on root-shoot dry matter partitioning and drought resistance revealed that cultivars possessing the reduced height gene 1 and reduced height gene 2 gibberellin-insensitive dwarfing genes were more susceptible to drought stress than reduced height gene 1 and reduced height gene 2 tall cultivars (Miralles et al. 1997). The semi-dwarfing genes reduced height gene 1 and reduced height gene 2 resulted in greater root biomass at anthesis due to increased thickening of existing roots using surplus assimilates arising from the restricted stem growth. Thus, the benefit of greater assimilates available for root growth was not expressed as more extensive or deeper root growth. Differences have also been observed in the adaptive response of root distribution to soil drying (Liu et al. 2004a,b).

To summarize, plants may escape drought stress by cutting short their growth duration, and avoid the stress with the maintenance of high tissue water potential either by reducing water loss from plants or improved water uptake, or both. Some plants may reduce their surface area either by leaf shedding or production of smaller leaves.

3.2 Physiological Mechanisms

Osmotic adjustment, osmoprotection, antioxidation and a scavenging defense system have been the most important bases responsible for drought tolerance. The physiological basis of genetic variation in drought response is not clear; in part, because more intricate mechanisms have been suggested. Some of these mechanisms are described below.

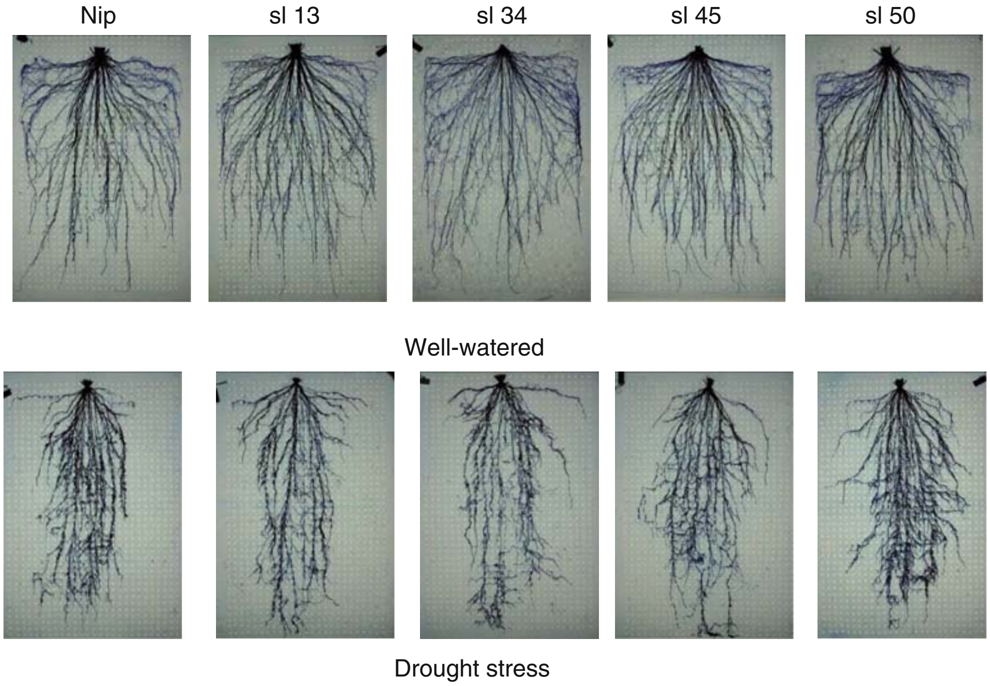

Fig. 5 Root growth and proliferation under well-watered and drought stress conditions in various rice genotypes. Different rice genotypes (Nip, sl 13, sl 34, sl 45, sl 50) were grown under continuous flooded conditions (well-watered) and 15% soil moisture contents (drought stress). The study was conducted in root boxes. The figure shows root proliferation 38 days after seeding (courtesy Ms. Mana Kano)

3.2.1 Cell and Tissue Water Conservation

Under drought stress, sensitive pea genotypes were more affected by a decline in relative water content than tolerant ones (Upreti et al. 2000). In faba bean, determination of leaf water potential was useful for describing the drought effect, but was not suitable for discriminating tolerant from sensitive genotypes. This suggested that water potential was not the defining feature of the tolerance (Riccardi et al. 2001). Nevertheless, other studies opined that determination of leaf water status in the morning and water content in leaves in the afternoon were potentially useful for screening drought tolerance in chickpea (Pannu et al. 1993).

Osmotic adjustment allows the cell to decrease osmotic potential and, as a consequence, increases the gradient for water influx and maintenance of turgor. Improved tissue water status may be achieved through osmotic adjustment and/or changes in cell wall elasticity. This is essential for maintaining physiological activity for extended periods of drought (Kramer and Boyer 1995). Wild melon plant survived drought by maintaining its water content without wilting of leaves even under severe drought. Drought stress in combination with strong light led to an accumulation of high concentrations of citrulline, glutamate and arginine in leaves of wild watermelon. The accumulation of citrulline and arginine may be related to the induction of dopamine receptor interacting protein gene 1, a homologue of the acetylornithine deacetylase gene in *Escherichia coli*, where it functions to incorporate the carbon skeleton of glutamate into the urea cycle (Yokota et al. 2002).

It has been identified that among various mechanisms, osmotic adjustment, abscisic acid and induction of dehydrins may confer tolerance against drought injuries by maintaining high tissue water potential (Turner et al. 2001). With the accumulation of solutes, the osmotic potential of the cell is lowered, which attracts water into the cell and helps with turgor maintenance. The maintenance of turgor despite a decrease in leaf water volume is consistent with other studies of species with elastic cell walls. Osmotic adjustment helps to maintain the cell water balance with the active accumulation of solutes in the cytoplasm, thereby minimizing the harmful effects of drought (Morgan 1990).

Osmotic adjustment is an important trait in delaying dehydrative damage in water-limited environments by continued maintenance of cell turgor and physiological processes (Taiz and Zeiger 2006). The osmotic adjustment also facilitates a better translocation of pre-anthesis carbohydrate partitioning during grain filling (Subbarao et al. 2000), while high turgor maintenance leads to higher photosynthetic rate and growth (Ludlow and Muchow 1990; Subbarao et al. 2000).

3.2.2 Antioxidant Defense

The antioxidant defense system in the plant cell constitutes both enzymatic and non-enzymatic components. Enzymatic components include superoxide dismutase, catalase, peroxidase, ascorbate peroxidase and glutathione reductase. Non-enzymatic components contain cystein, reduced glutathione and ascorbic acid (Gong et al. 2005). In environmental stress tolerance, such as drought, high activities of antioxidant enzymes and high contents of non-enzymatic constituents are important.

The reactive oxygen species in plants are removed by a variety of antioxidant enzymes and/or lipid-soluble and water-soluble scavenging molecules (Hasegawa et al. 2000); the antioxidant enzymes being the most efficient mechanisms against oxidative stress (Farooq et al. 2008). Apart from catalase, various peroxidases and peroxiredoxins, four enzymes are involved in the ascorbate-glutathione cycle, a pathway that allows the scavenging of superoxide radicals and H_2O_2 (Fig. 6). These include ascorbate peroxidase, dehydroascorbate reductase, monodehydroascorbate reductase and glutathione reductase (Fazeli et al. 2007). Most of the ascorbate-glutathione cycle enzymes are located in the cytosol, stroma of chloroplasts, mitochondria and peroxisomes (Jiménez et al. 1998). Ascorbate peroxidase is a key antioxidant enzyme in plants (Orvar and Ellis 1997) whilst glutathione reductase has a central role in maintaining the reduced glutathione pool during stress (Pastori et al. 2000). Two glutathione reductase complementary deoxyribonucleic acids have been isolated; one type encoding the cytosolic isoforms (Stevens et al. 2000) and the other encoding glutathione reductase proteins dual-targeted to both chloroplasts and mitochondria in different plants (Chew et al. 2003).

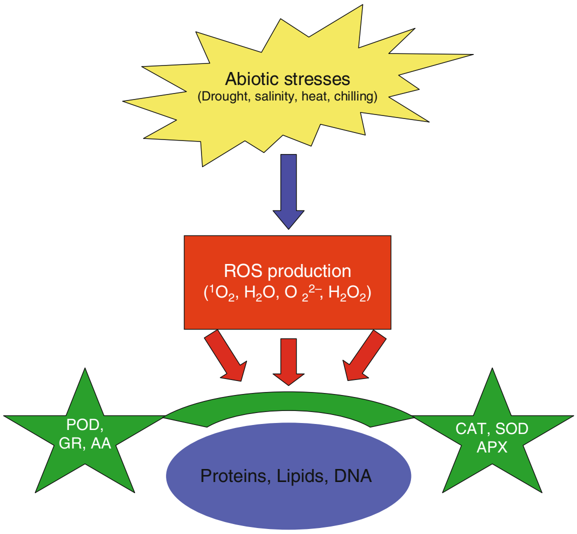

Fig. 6 Role of antioxidant enzymes in the ROS scavenging mechanism. Exposure to abiotic stresses (including drought, chilling, salinity, etc.) leads to the generation of ROS, including singlet oxygen ($^1O_2$), perhydroxyl radical ($H_2O\cdot$), hydroxyl radicals (O_2^{2-}), hydrogen peroxide (H_2O_2) and alkoxy radical (RO). The ROS may react with proteins, lipids and DNA, causing oxidative damage and impairing the normal functions of cells. The antioxidant defense system in the plant cell includes both enzymatic and non-enzymatic constituents. Amongst the enzymatic components are superoxide dismutase, catalase, peroxidase, ascorbate peroxidase and glutathione reductase. Upon exposure to abiotic stresses, tolerant cells activate their enzymatic antioxidant system, which then starts quenching the ROS and protecting the cell. *ROS* reactive oxygen species

Among enzymatic mechanisms, superoxide dismutase plays an important role, and catalyzes the dismutation of two molecules of superoxide into O_2 and H_2O_2; the first step in reactive oxygen species scavenging systems. Lima et al. (2002), from a study utilizing two rapidly drought-stressed clones of *Coffea canephora*, proposed that drought tolerance might, or at least in part, be associated with enhanced activity of antioxidant enzymes. In contrast, Pinheiro et al. (2004) did not find a link between protection against oxidative stress and drought tolerance when four clones of *C. canephora* were subjected to long-term drought.

Carotenoids and other compounds, such as abietane diterpenes, have received little attention despite their capacity to scavenge singlet oxygen and lipid peroxy-radicals, as well as to inhibit lipid peroxidation and superoxide generation under dehydrative forces (Deltoro et al. 1998). The transcript of some of the antioxidant genes such as glutathione reductase or ascorbate peroxidase was higher during recovery from a water deficit period and appeared to play a role in the protection of cellular machinery against damage by reactive oxygen species (Ratnayaka et al. 2003). A superoxide radical has a half-life of less than 1 s and is rapidly dismutated by superoxide dismutase into H_2O_2, a product that is relatively stable and can be detoxified by catalase and peroxidase (Apel and Hirt 2004). These metalloenzymes constitute an important primary line of defense of cells against superoxide free radicals generated under stress conditions. Therefore, increased superoxide dismutase activity is known to confer oxidative stress tolerance (Pan et al. 2006).

Oxidative damage in the plant tissue is alleviated by a concerted action of both enzymatic and non-enzymatic antioxidant systems. These include β-carotenes, ascorbic acid, α-tocopherol, reduced glutathione and enzymes including superoxide dismutase, peroxidase, ascorbate peroxidase, catalase, polyphenol oxidase and glutathione reductase (Hasegawa et al. 2000; Prochazkova et al. 2001). Carotenes form a key part of the plant antioxidant defense system (Havaux 1998; Wahid 2007), but they are very susceptible to oxidative destruction. The β-carotene present in the chloroplasts of all green plants is exclusively bound to the core complexes of photosystem I and photosystem II. Protection against damaging effects of reactive oxygen species at this site is essential for chloroplast functioning. Here, β-carotene, in addition to functioning as an accessory pigment, acts as an effective antioxidant and plays a unique role in protecting photochemical processes and sustaining them (Havaux 1998). A major protective role of β-carotene in photosynthetic tissue may be through direct quenching of triplet chlorophyll, which prevents the generation of singlet oxygen and protects from oxidative damage.

3.2.3 Cell Membrane Stability

Biological membranes are the first target of many abiotic stresses. It is generally accepted that the maintenance of integrity and stability of membranes under water stress is a major component of drought tolerance in plants (Bajji et al. 2002). Cell membrane stability, reciprocal to cell membrane injury, is a physiological index widely used for the evaluation of drought tolerance (Premachandra et al. 1991). Moreover, it is a genetically related phenomenon since quantitative trait loci for this have been mapped in drought-stressed rice at different growth stages (Tripathy et al. 2000). Dhanda et al. (2004) showed that membrane stability of the leaf segment was the most important trait to screen the germplasm for drought tolerance.

Cell membrane stability declined rapidly in Kentucky bluegrass exposed to drought and heat stress simultaneously (Wang and Huang 2004). In a study on maize, K nutrition improved the drought tolerance, mainly due to improved cell membrane stability (Gnanasiri et al. 1991). Tolerance to drought evaluated as increase in cell membrane stability under water deficit conditions was differentiated between cultivars and correlated well with a reduction in relative growth rate under stress (Premachandra et al. 1991). In holm oak (*Quercus ilex*) seedlings, hardening increased drought tolerance primarily by reducing osmotic potential and stomatal regulation, improved new root growth capacity and enhanced cell membrane stability. Among treated seedlings, the greatest response occurred in seedlings subjected to moderate hardening. Variation in cell membrane stability, stomatal regulation and root growth capacity was negatively related to osmotic adjustment (Villar-Salvador et al. 2004).

The causes of membrane disruption are unknown; notwithstanding, a decrease in cellular volume causes crowding and increases the viscosity of cytoplasmic components. This increases the chances of molecular interactions that can cause protein denaturation and membrane fusion. For model membrane and protein systems, a broad range of compounds have been identified that can prevent such adverse molecular interactions. Some of these are proline, glutamate, glycinebetaine, carnitine, mannitol, sorbitol, fructans, polyols, trehalose, sucrose and oligosaccharides (Folkert et al. 2001). Another possibility of ion leakage from the cell may be due to thermal-induced inhibition of membrane-bound enzymes responsible for maintaining chemical gradients in the cell (Reynolds et al. 2001). *Arabidopsis* leaf membranes appeared to be very resistant to water deficit, as shown by their capacity to maintain polar lipid contents and the stability of their composition under severe drought (Gigon et al. 2004).

3.2.4 Plant Growth Regulators

Plant growth regulators, when applied externally, and phytohormones, when produced internally, are substances that influence physiological processes of plants at very low concentrations (Morgan 1990). Both these terms have been used interchangeably, particularly when referring to auxins, gibberellins, cytokinins, ethylene and abscisic acid (Taiz and Zeiger 2006). Under drought, endogenous contents of auxins, gibberellins and cytokinin usually decrease, while those of abscisic acid and ethylene increase (Nilsen and Orcutte 1996). Nevertheless, phytohormones play vital roles in drought tolerance of plants.

Auxins induce new root formation by breaking root apical dominance induced by cytokinins. As a prolific root system is vital for drought tolerance, auxins have an indirect but key role in this regard. Drought stress limits the production of endogenous auxins, usually when contents of abscisic acid and ethylene increase (Nilsen and Orcutte 1996). Nevertheless, exogenous application of indole-3-yl-acetic acid enhanced net photosynthesis and stomatal conductance in cotton (Kumar et al. 2001). Indole-3-butyric acid is a naturally occurring auxin. Drought stress and abscisic acid application enhance indole-3-butyric acid synthesis in maize. Recently, it was revealed that Indole-3-butyric acid synthetase from *Arabidopsis* is also drought-inducible (Ludwig-Müller 2007). Experiments with indole-3-yl-acetic acid and ethylene glycol tetra-acetic acid suggested that calcium and auxin participate in signaling mechanisms of drought-induced proline accumulation (Sadiqov et al. 2002).

Drought rhizogenesis is an adaptive strategy that occurs during progressive drought stress and is reported from Brassicaceae and related families by the formation of short, tuberized, hairless roots. These roots are capable of withstanding a prolonged drought period and give rise to a new functional root system upon rehydration. The drought rhizogenesis was highly increased in the gibberelic acid biosynthetic mutant *ga5*, suggesting that some gibberrelic acids might also participate in this process (Vartanian et al. 1994).

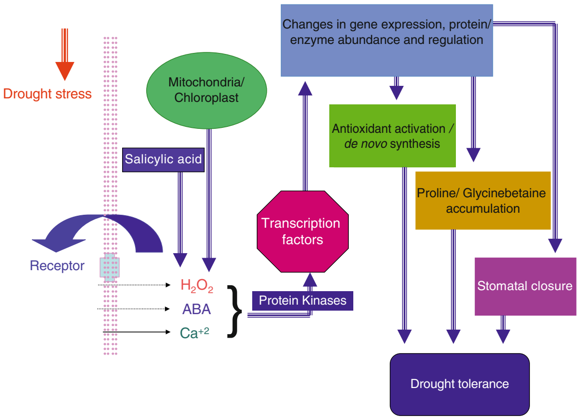

Fig. 7 Proposed cellular events and signaling cascades in a plant cell responding to drought stress. Drought stress is perceived by an unknown mechanism, which then activates the signaling cascades, plausibly by abcissic acid (ABA), hydrogen peroxide (H_2O_2) and calcium (Ca^{2+}). These cascades then activate the synthesis of specific protein kinases which activate more downstream responses such as changes in gene expression. The response to these signaling cascades also results in changes in plant metabolism including activation and synthesis of antioxidants, synthesis and accumulation of osmoprotectants and solutes, and stomatal closure under acute drought stress

Abscisic acid is a growth inhibitor and produced under a wide variety of environmental stresses, including drought. All plants respond to drought and many other stresses by accumulating abscisic acid. Abscisic acid is ubiquitous in all flowering plants and is generally recognized as a stress hormone that regulates gene expression and acts as a signal for the initiation of processes involved in adaptation to drought and other environmental stresses (Fig. 7). It has been proposed that abscisic acid and cytokinin have opposite roles in drought stress. Increase in abscisic acid and decline in cytokinins levels favor stomatal closure and limit water loss through transpiration under water stress (Morgan 1990). When plants wilt, abscisic acid levels typically rise as a result of increased synthesis (Taylor 1991). Increased abscisic acid concentration leads to many changes in development, physiology and growth. Abscisic acid alters the relative growth rates of various plant parts such as increase in the root-to-shoot dry weight ratio, inhibition of leaf area development and production of prolific and deeper roots (Sharp et al. 1994). It triggers the occurrence of a complex series of events leading to stomatal closure, which is an important water-conservation response (Turner et al. 2001). In a study on genetic variation for abscisic acid accumulation in rice, a consistent negative relationship between the ability of detached and partially dehydrated leaves to accumulate abscisic acid and leaf weight was established (Ball et al. 1994). By its effect in closing stomata, abscisic acid can control the rate of transpiration and, to some extent, may be involved in the mechanism conferring drought tolerance in plants.

Abscisic acid induces expression of various water stress-related genes. In a recent study, Zhang et al. (2005) reported a regulatory role of telomeric repeat binding factor gene 1 in abscisic acid sensitivity and drought response during seedling development. Bray (1997) suggested the existence of abscisic acid-dependent and abscisic acid-independent transduction cascades and pathways to act as a signal of drought stress and the expression of specific water stress-induced genes. Abscisic acid produces such changes that confer an ability to maintain cellular turgor to withstand dehydrative forces (Fig. 7).

Ethylene has long been considered a growth inhibitory hormone, although it is involved in environmentally driven growth inhibition and stimulation (Taiz and Zeiger 2006). The response of cereals to drought includes loss of leaf function and premature onset of senescence in older leaves. Ethylene may serve to regulate leaf performance throughout its lifespan as well as to determine the onset of natural senescence and mediate drought-induced senescence (Young et al. 2004). Recent studies suggest that growth promotion is a common feature in ethylene responses. To escape this adversity, plants can optimize growth and tolerate abiotic stresses such as drought, and this response also involves ethylene synthesis (Pierik et al. 2007).

Among the other endogenously produced growth regulating factors, the role of salicylic acid in the induction of tolerance against several abiotic stresses has been emphasized recently. In the case of drought tolerance, the role of endogenously produced salicylic acid is still enigmatic. Salicylic acid potentiates the generation of reactive oxygen species in photosynthetic tissues of *Arabidopsis thaliana* during osmotic stress (Borsani et al. 2001).

Polyamines are known to have profound influence on plant growth and development. Being cationic, polyamines can associate with anionic components of the membrane, such as phospholipids, thereby protecting the lipid bilayer from deteriorating effects of stress (Bouchereau et al. 1999). There has been a growing interest in the study of polyamine participation in the defense reaction of plants against environmental stresses and extensive research efforts have been made in the last two decades (Bouchereau et al. 1999; Kasukabe et al. 2004). Many genes for enzymes involved in polyamine metabolism have been cloned from several species, and their expression under several stress conditions has been analyzed. For example, the apple spermidine synthase gene when overexpressed encodes high levels of spermidine synthase, which substantially improves abiotic stress tolerance including drought (Wen et al. 2007).

Among various polyamines, a rise in the putrescence level is generally due to an enhanced arginine decarboxylase activity (Bouchereau et al. 1999). Compared with sensitive plants, stress-tolerant plants generally have a greater capacity to synthesize polyamines in response to stress, resulting in a two- to three fold rise in endogenous polyamine levels over the unstressed ones (Kasukabe et al. 2004). Recent studies suggested that rice has a great capacity to enhance polyamine biosynthesis, particularly spermidine and spermine in free form and putrescence in insoluble-conjugated form, in leaves earlier in response to drought stress. This was considered as an important physiological trait of drought tolerance in rice (Yang et al. 2007).

3.2.5 Compatible Solutes and Osmotic Adjustment

One of the most common stress tolerance strategies in plants is the overproduction of different types of compatible organic solutes (Serraj and Sinclair 2002). Compatible solutes are low-molecular-weight, highly soluble compounds that are usually nontoxic even at high cytosolic concentrations. Generally they protect plants from stress through different means such as contribution towards osmotic adjustment, detoxification of reactive oxygen species, stabilization of membranes, and native structures of enzymes and proteins (Fig. 8).

Osmotic adjustment is a mechanism to maintain water relations under osmotic stress. It involves the accumulation of a range of osmotically active molecules/ions including soluble sugars, sugar alcohols, proline, glycinebetaine, organic acids, calcium, potassium, chloride ions, etc. Under water deficit and as a result of solute accumulation, the osmotic potential of the cell is lowered, which attracts water into the cell and helps with the maintenance of turgor. By means of osmotic adjustment, the organelles and

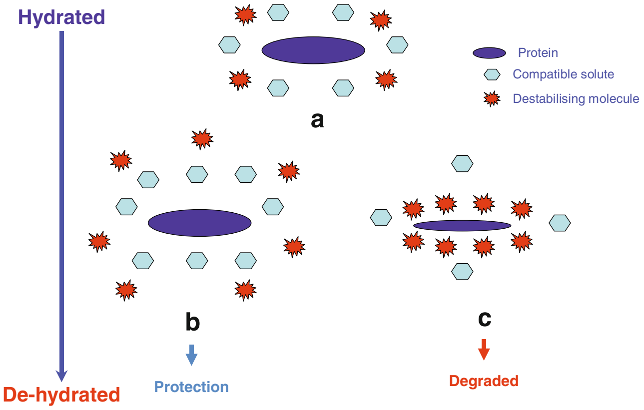

Fig. 8 Role of compatible solutes in drought tolerance. In the hydrated state, the presence of water reduces the interaction of destabilizing molecules (**a**), in tolerant cells the synthesis of compatible solutes preferentially excludes the binding of destabilizing molecules and stabilizes native protein conformation (**b**) and in sensitive cells the lack of compatible solutes results in the preferential binding of destabilizing molecules to the protein surface, leading to degradation (**c**). (Adapted from Hoekstra et al. 2001)

cytoplasmic activities take place at about a normal pace and help plants to perform better in terms of growth, photosynthesis and assimilate partitioning to grain filling (Ludlow and Muchow 1990; Subbarao et al. 2000). As a mechanism, osmotic adjustment has been suggested as an important trait in postponing the dehydration stress in water-scarce environments (Morgan 1990). Variation in osmotic adjustment among chickpea cultivars in response to soil drought has been observed, and seed yield of chickpea was correlated with the degree of osmotic adjustment when grown under a line-source irrigation system in the field (Moinuddin and Khannu-Chopra 2004). Contrarily, Serraj and Sinclair 2002) found no yield advantage from osmotic adjustment in any crop. Nevertheless, further investigations are imperative to establish this controversy.

As mentioned above, osmotic adjustment is accomplished with the accumulation of compatible solutes. Of these, proline is one amongst the most important cytosolutes and its free accumulation is a widespread response of higher plants, algae, animals and bacteria to low water potential (Zhu 2002; Wahid and Close 2007). Its synthesis in leaves at low water potential is caused by a combination of increased biosynthesis and slow oxidation in mitochondria. Despite some controversy, many physiological roles have been assigned to free proline including stabilization of macromolecules, a sink for excess reductant and a store of carbon and nitrogen for use after relief of water deficit (Zhu 2002). Proline contents were increased under drought stress in pea cultivars (Alexieva et al. 2001). Drought-tolerant petunia (*Petunia hybrida*) varieties were reported to accumulate free proline under drought that acted as an osmoprotectant and induced drought tolerance (Yamada et al. 2005).

Glycinebetaine (N,N,N-trimethyl glycine) is one of the most extensively studied quaternary ammonium compounds and compatible solutes in plants, animals and bacteria (Wahid et al. 2007). Many studies demonstrate that glycinebetaine plays an important role in enhancing plant tolerance under a range of abiotic stresses including drought (Quan et al. 2004). The introduction of genes synthesizing glycinebetaine into non-accumulators of glycinebetaine proved to be effective in increasing tolerance to various abiotic stresses (Sakamoto and Murata 2002). Naidu et al. (1998) reported that cotton cultivars adapted to water stress conditions accumulated higher glycinebetaine than the non-adapted ones under drought. In addition to direct protective roles of glycinebetaine either through positive effects on enzyme and membrane integrity or as an osmoprotectant, glycinebetaine may also protect cells from environmental stresses indirectly by participating in signal transduction pathways (Subbarao et al. 2000).

Citrulline, named after *Citrullus*; a Latin name of watermelon, from which it was isolated, is an amino acid. Although not built into proteins during their synthesis, and not encoded by a nuclear gene, several proteins are known to contain citrulline (Kawasaki et al. 2000). Wild watermelon (*Citrullus lanatus*) has the ability to adapt to severe drought stress despite carrying out normal C_3-type photosynthesis, which seem to be correlated with citrulline accumulation (Akashi et al. 2001). Wild watermelon primarily accumulated citrulline followed by glutamate and arginine, in place of proline and glycinebetaine (Kawasaki et al. 2000). Yokota et al. (2002) reported a higher citrulline accumulation in the wild watermelon leaves assuming that citrulline is located only in the cytosol and constitutes 5% of the total volume of the mesophyll cells. Citrulline is a novel and the most effective OH^- scavenger among compatible solutes examined so far. Moreover, it can effectively protect DNA and enzymes from oxidative injuries (Akashi et al. 2001; Bektaşoğlu et al. 2006).

Rapid accumulation of the non-protein amino acid γ-aminobutyric acid was identified in plant tissues upon exposure to stress many years ago. γ-aminobutyric acid acts as a zwitterion, exists in free form, and has a flexible molecule that can assume several conformations in solution, including a cyclic structure that is similar to proline. At physiological *p*H, γ-aminobutyric acid is highly water-soluble (Shelp et al. 1999), and may function as a signaling molecule in higher plants under stress (Serraj et al. 1998). The physiological roles of γ-aminobutyric acid in drought tolerance entail osmotic regulation (Shelp et al. 1999), detoxication of reactive oxygen radicals, conversion of putrescine into proline and intracellular signal transduction (Kinnersley and Turano 2000).

Drought stress initiates a signal transduction pathway, in which increased cytosolic Ca^{2+} activates Ca^{2+}/calmodulin-dependent glutamate decarboxylase activity, leading to γ-aminobutyric acid synthesis (Shelp et al. 1999). Elevated H^+ and substrate levels can also stimulate glutamate decarboxylase activity, leading primarily to γ-aminobutyric acid accumulation. Experimental evidence supports the involvement of γ-aminobutyric acid in *p*H regulation, nitrogen storage, plant development and defense, as well as a compatible osmolyte and an alternative pathway for glutamate utilization (Shelp et al. 1999; Wahid et al. 2007). After drought stress the content of proline was more than 50% and at the end of recovery the γ-aminobutyric acid content reached 27% (Simon-sarkadi et al. 2006).

Trehalose is a non-reducing disaccharide of glucose that functions as a compatible solute in the stabilization of biological structures under abiotic stress (Goddijn et al. 1997). In nature, trehalose is biosynthesized as a stress response by a variety of organisms including bacteria, fungi, algae, insects, invertebrates and lower plants (Wingler 2002). Capacity to produce trehalose, earlier thought to be absent from higher plants, has now been reported to accumulate in high amounts in some drought-tolerant ferns, the resurrection plant *Selaginella lepidophylla* (Penna 2003) and desiccation-tolerant angiosperm *Myrothamnus flabellifolia* (Drennan et al. 1993). The presence of low amounts of trehalose was demonstrated even in tobacco (Goddijn et al. 1997) and many higher plants (Kosmas et al. 2006). Its metabolism may be channelized to enhance drought tolerance in plants (Pilon-Smits et al. 1998; Penna 2003). Physiological roles of trehalose include efficient stabilization of dehydrated enzymes, proteins and lipid membranes, as well as protection of biological structures under desiccation stress (Wingler 2002) rather than regulating water potential (Lee et al. 2004). Karim et al. (2007) reported that enhanced drought tolerance by trehalose depends on improved water status and expression of heterologous trehalose biosynthesis genes during *Arabidopsis* root development.

At a molecular level, exogenously applied trehalose may trigger the abscisic acid-insensitive 4 gene expression but decrease sucrose induction, providing a possible molecular mechanism for the trehalose effect on plant gene expression and growth (Ramon et al. 2007). Trehalose-accumulating organisms produce this sugar in a two-step process by the action of the enzymes trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase when exposed to stress. Improved drought tolerance has been reported in the transgenic plants overproducing trehalose-6-phosphate synthase in spite of minute accumulation of trehalose (Karim et al. 2007).

In fact, plants can withstand drought stress by conserving cell and tissue water principally by osmatic adjustment, maintenance of the antioxidant defense system for the scavenging of reactive oxygen species, and keeping the cell membranes stabilized. Plant growth regulators and polyamines, γ-aminobutyric acid, free amino acids and sugars also play a vital role in drought tolerance by scavenging the reactive oxygen species, stomatal regulation and protection of vital macromolecules, and maintenance of the cell water balance.

3.3 Molecular Mechanisms

Plant cellular water deficit may occur under conditions of reduced soil water content. Under these conditions, changes in gene expression (up- and down-regulation) take place. Various genes are induced in response to drought at the transcriptional level, and these gene products are thought to function in tolerance to drought (Kavar et al. 2007). Gene expression may be triggered directly by the stress conditions or result from secondary stresses and/or injury responses. Nonetheless, it is well established that drought tolerance is a complex phenomenon involving the concerted action of many genes (Agarwal et al. 2006; Cattivelli et al. 2008).

3.3.1 Aquaporins

Aquaporins have the ability to facilitate and regulate passive exchange of water across membranes. They belong to a highly conserved family of major intrinsic membrane proteins (Tyerman et al. 2002). In plants, aquaporins are present abundantly in the plasma membrane and in the vacuolar membrane. The structural analysis of aquaporins has revealed the general mechanism of protein-mediated membrane water transport. Although the discovery of aquaporins in plants has resulted in a prototype shift in the understanding of plant water relations (Maurel and Chrispeels 2001), the relation between aquaporins and plant drought resistance is still elusive (Aharon et al. 2003). Nevertheless, it is believed that they can regulate the hydraulic conductivity of membranes and potentiate a 10- to 20-fold increase in water permeability (Maurel and Chrispeels 2001).

Studies on aquaporins and plant water relations have been carried out for many years. Mercury is a potential inhibitor of aquaporins. This was evident from a number of reports on mercury-induced decline in root hydraulic conductivity, which substantiated that aquaporins play a major role in overall root water uptake (Javot and Maurel 2002), and play a role in cellular osmoregulation of highly compartmented root cells (Maurel et al. 2002; Javot et al. 2003). Reverse genetics provides an elegant approach to explore aquaporin roles in plant water relations (Kaldenhoff et al. 1998). The overexpression of the plasma membrane aquaporin in transgenic tobacco progressively improved plant vigor under favorable growth conditions, but the prolactin-inducible protein 1b gene overexpression had retrogressive influence under salinity, and caused fast wilting under water stress (Aharon et al. 2003). Phosphorylation (Johansson et al. 1998), calcium and *p*H (Tournaire-Roux et al. 2003) are important factors modulating aquaporin activity.

Recently, efforts have been concentrated on investigating the function and regulation of plasma membrane intrinsic protein aquaporins. The aquaporins play a specific role in controlling transcellular water transport. For instance, they are abundantly expressed in roots where they mediate soil water uptake (Javot and Maurel 2002) and transgenic plants down-regulating one or more prolactin-inducible protein genes had lower root water uptake capacity (Javot et al. 2003).

3.3.2 Stress Proteins

Synthesis of stress proteins is a ubiquitous response to cope with prevailing stressful conditions including water deficit. Most of the stress proteins are soluble in water and therefore contribute towards the stress tolerance phenomena by hydration of cellular structures (Wahid et al. 2007). Synthesis of a variety of transcription factors and stress proteins is exclusively implicated in drought tolerance (Taiz and Zeiger 2006).

Dehydration-responsive element-binding genes belong to the v-ets erythroblastosis virus repressor factor gene family of transcription factors consisting of three subclasses, dehydration-responsive element-binding gene1 and dehydration-responsive element-binding gene2, which are induced by cold and dehydration, respectively (Choi et al. 2002). The dehydration-responsive element-binding genes are involved in the abiotic stress signaling pathway. It was possible to engineer stress tolerance in transgenic plants by manipulating the expression of dehydration-responsive element-binding genes (Agarwal et al. 2006). Introduction of a novel dehydration-responsive element-binding gene transcriptional factor effectively improved the drought tolerance ability of groundnut (Mathur et al. 2004) and rice (Yamaguchi-Shinozaki and Shinozaki 2004). After successful cloning of dehydration-responsive element-binding gene1 (Liu et al. 1998), many capsella bursa-pastoris-like genes have been reported to be synthesized in response to drought stress in various plant species including rye and tomato (Jaglo et al. 2001), rice (Dubouzet et al. 2003), wheat (Shen et al. 2003), cotton (Huang and Liu 2006), brassica (Zhao et al. 2006) and soybean (Chen et al. 2007). Introduction of dehydration-responsive element-binding gene1A genes in transgenic tall fescue (*Festuca arundinacea*) showed increased drought resistance with the accumulation of a high level of proline. This indicated the ability of capsella bursa-pastoris 3 to induce drought tolerance (Zhao et al. 2007). Drought stress causes many changes in the expression levels of late embryogenesis abundant/dehydrin-type genes and molecular chaperones that protect the cellular proteins from denaturation (Mahajan and Tuteja 2005).

Heat shock proteins belong to a larger group of molecules called chaperones. They have a role in stabilizing other proteins' structure. Low-molecular-weight heat shock proteins are generally produced only in response to environmental stress, particularly high temperature (Wahid et al. 2007). But many heat shock proteins have been found to be induced by different stresses such as drought, anaerobic conditions and low temperatures (Coca et al. 1994). They are reported to serve as molecular chaperones that participate in adenosine triphosphate-dependent protein unfolding or assembly/disassembly reactions and prevent protein denaturation during stress (Gorantla et al. 2006).

Membrane-stabilizing proteins and late embryogenic abundant proteins are another important protein group responsible for conferring drought tolerance. These increase the water-binding capacity by creating a protective environment for other proteins or structures, referred to as dehydrins. They also play a major role in the sequestration of ions that are concentrated during cellular dehydration (Gorantla et al. 2006). These proteins help to protect the partner protein from degradation and proteinases that function to remove denatured and damaged proteins. Dehydrins, also known as a group of late embryogenesis abundant proteins, accumulate in response to both dehydration and low temperature (Close 1997). In addition to their synthesis at the desiccating stage of seed, they also accumulate during periods of water deficit in vegetative tissues. These proteins are easily identifiable from their particular structural features such as the highly conserved Lysine-rich domain predicted to be involved in hydrophobic interactions, leading to macromolecule stabilization (Svensson et al. 2002).

3.3.3 Signaling and Drought Stress Tolerance

General responses to stress involve signaling stress detection via the redox system, checkpoints arresting the cell cycle and deoxyribonucleic acid repair processes stimulated in response to deoxyribonucleic acid damage. The complexity of signaling events associated with the sensing of stress and the activation of defense and acclimation pathways is believed to involve reactive oxygen species, calcium, calcium-regulated proteins, mitogen-activated protein kinase cascades, and cross-talk between different transcription factors (Kovtun et al. 2000; Chen et al. 2002).

Chemical signals, e.g. reactive oxygen species, calcium and plant hormones are involved in inducing stress tolerance by acting via transduction cascades and activate genomic re-programing (Fig. 7; Joyce et al. 2003). Mitogen-activated protein kinases are important mediators in signal transmission, connecting the perception of external stimuli to cellular responses. Mitogen-activated protein kinase cascades are involved in signaling various stresses, including drought (Wrzaczek and Hirt 2001). Calcium has been established as a ubiquitous intracellular second messenger in plants. Calcium-based signaling systems comprise a receptor, a system for generating the increase in cytosolic calcium, downstream components that are capable of reacting to increased cytosolic calcium, and other cellular systems responsible for returning cytosolic calcium to its pre-stimulus level (Alistair and Brownlee 2004). More recently, it is reported that calcium can improve water stress tolerance in *Catharanthus roseus* by increasing γ-glutamyl kinase and reducing the proline oxidase activities (Abdul Jaleel et al. 2007).

The calcium-dependent protein kinases in higher plant cells are an important group of calcium sensors that decode calcium ion signals in plant cells (Cheng et al. 2002). As a family of unique serine/threonine kinases in higher plants, they perform diverse and important functions in plant signal transduction (Mori et al. 2006). Drought stress increases the cytoplasmic calcium level in living plant cells (Knight 2000). A number of potential calcium sensors, such as salt overly-sensitive 3-like proteins (Zhu 2002) or Casitas B-lineage Lymphoma (Luan et al. 2002) and calcium-dependent protein kinases may further transduce stress-induced calcium signals (Harmon et al. 2000). Mishra et al. (2006) also reported that signaling for cell division and stress responses in plants is mediated through monoammonium phosphate kinases, and even auxins also utilize a monoammonium phosphate kinase pathway for its action.

A number of phospholipid systems are activated by osmotic stress, generating an array of messenger molecules, some of which may function upstream of the osmotic stress-activated protein kinases. Abscisic acid biosynthesis is regulated by osmotic stress at multiple steps. Both abscisic acid-dependent and -independent osmotic stress signaling first modify constitutively expressed transcription factors, leading to the expression of early response transcriptional activators, which then activate downstream stress tolerance effector genes (Zhu 2002). Recently, Wan et al. 2007) reported that amongst the 29 calcium-dependent protein kinase genes identified so far, all contained multiple stress-responsive *cis*-elements upstream in the promoter region (1 kb). Sucrose non-fermenting 1-related protein kinase 2 has also been reported to be capable of mediating signals initiated during drought stress, resulting in appropriate gene expression (Umezawa et al. 2005).

In fact, various chemical signals transduced under drought stress activate an array of genes, leading to the synthesis of proteins and metabolites, conferring drought tolerance in a number of plant species.

4 Managing Drought Stress

Drought stress effects can be managed by production of the most appropriate plant genotypes together with adjustment of agronomic practices (sowing time, plant density and soil management). This is done to ensure that sensitive crop stages occur at the time when likelihood of drought is minimal. Various strategies of paramount importance to accomplish this objective may entail production of appropriate plant varieties and improvement of the existing high-yielding varieties. Efforts have been made to produce drought-tolerant genotypes using the knowledge of responses of plants to drought stress and mechanisms involved as elaborated above. The two most important strategies may include (a) selecting the desired materials as in traditional breeding using molecular and biotechnological means, including production of genetically modified or transgenic plants (Fig. 9) and (b) inducing drought tolerance in otherwise susceptible plants by

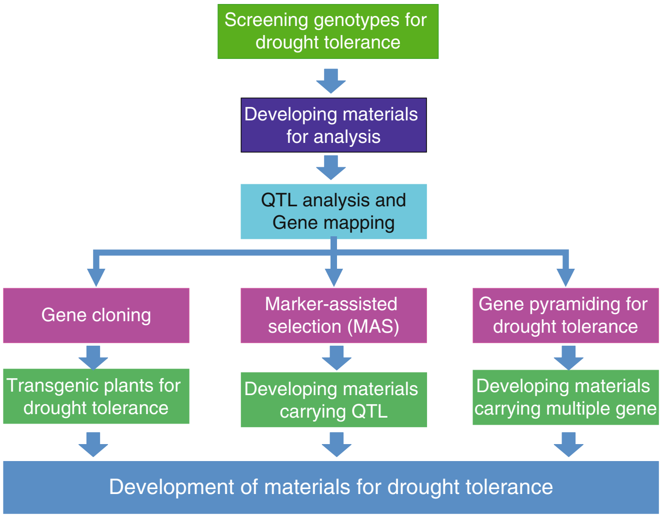

Fig. 9 Developing materials for drought tolerance. Under drought stress conditions, the genotypes showing drought tolerance are selected. To analyze the genotypes for drought tolerance, the materials for analysis are developed. Using developed materials, QTL analysis and gene mapping are conducted. For gene cloning, identified genes or major QTL are analyzed in detail using a large size population. A cloned gene for drought tolerance is transferred into widely adapted varieties. To develop the materials carrying the gene or QTL for drought tolerance, DNA markers which linked to the gene or QTL are used for marker-assisted selection. Similarly, marker-assisted selection is used for developing the materials of gene pyramiding. Gene cloning, marker-assisted selection and gene pyramiding are useful for developing the materials for drought tolerance. *QTL* quantitative trait locus

priming and hormonal application. An account of these efforts is elaborated below.

4.1 Selection and Breeding Strategies

Conventional breeding has been based on empirical selection for yield (Atlin and Lafitte 2002). However, this approach is far from being optimal, since yield is a quantitative trait and characterized by a low heritability and a high genotype × environment interaction (Babu et al. 2003). It is strongly believed that understanding of a physiological and molecular basis may help target the key traits that limit yield. Such an approach may complement conventional breeding programs and hasten yield improvement (Cattivelli et al. 2008). Moreover, even the power of molecular biology for locating important gene sequences and introgressing quantitative trait loci or even for selecting or genetically transforming important quantitative trait loci strongly depends upon our understanding of yield-determining physiological processes (Araus et al. 2002; Kirigwi et al. 2007).

Screening under natural drought stress conditions in the target environments is difficult because of the irregular and erratic drought response. But screening under controlled stress environments and rain-out shelters is more manageable. Selection response in the target population of environments under natural stress can be considered a correlated response to selection in the managed stress environment (Venuprasad et al. 2007). On the other hand, classical breeding is a good approach for developing drought tolerance, which relies upon multi-location testing of progenies in environments representing a random selection of the variation in drought stress in the target environment (Babu et al. 2003). A modification to this strategy involves selection for putative drought-adaptive secondary traits (Ludlow and Muchow 1990), either alone or as part of a selection index. Selection for low-transpiration types, at unchanged water-use efficiency, would result in lower yields under optimum conditions.

In recent studies on unselected populations of doubled-haploid lines, broad-sense heritability of grain yield under reproductive-stage drought stress was comparable with that of grain yield estimated in non-stressed conditions (Atlin and Lafitte 2002; Babu et al. 2003; Venuprasad et al. 2007). Considerable efforts have been targeted at the genetic analysis of secondary traits such as root system architecture, leaf water potential, panicle water potential, osmotic adjustment and relative water content (Jongdee et al. 2002). A suitable secondary trait is (1) genetically associated with grain yield under drought; (2) highly heritable; (3) stable and feasible to measure and (4) not associated with yield loss under ideal growing conditions (Edmeades et al. 2001). However, such traits rarely have high broad-sense heritability like yield under drought stress and are often not highly correlated with it (Atlin and Lafitte 2002).

Hampered water-use efficiency is an initial and the most common plant response to drought stress and plant species/varieties show great variations for this trait. Thus, it is a genetically linked trait. Available reports show that drought-tolerant species reduce the water loss either by reducing the leaf area or restricting stomatal opening or both (Lazaridou et al. 2003; Abbate et al. 2004; Lazaridou and Koutroubas 2004) simultaneously with less effect on the biomass production (Lazaridou et al. 2003; Abbate et al. 2004; Lazaridou and Koutroubas 2004). In the genotypes, which are either unable to adjust their organ size and reduce water loss or sustain the biomass production under water-limited conditions, water-use efficiency is substantially reduced (Costa et al. 1997). Condon et al. (2004) described three key processes in breeding to improve water-use efficiency in crop plants. These include (1) increasing the uptake of available water; (2) improving biomass production per unit transpired water and (3) partitioning of produced biomass towards the harvested product.

4.2 Molecular and Functional Genomics Approaches

For more than two decades, molecular and biochemical studies have identified many of the abscisic acid- and stress-responsive genes and a few of the transcription factors responsible for their induction in crop plants (Buchanan et al. 2005; Poroyko et al. 2005). The products of certain stress-responsive genes could function in alleviating stress damage through still elusive mechanisms (Shinozaki et al. 2003).

Many laboratory and field studies have shown that transgenic expression of some of the stress-regulated genes results in increased tolerance to drought and other stresses. These transgenic approaches are currently the mainstream method to bioengineer drought tolerance in crop plants (Bahieldina et al. 2005). However, enhanced expression of these genes is frequently associated with retarded growth and thus may limit its practical applications. Arising from breeding or bioengineering, the next generation of drought-tolerant crop plants requires better understanding of the molecular and genetic basis of drought resistance (Xiong et al. 2006). In this regard, rice, a submerged plant, offers an excellent model for the precise understanding of drought tolerance phenomena. An increasing number of studies witnesses that rice displays early morphological changes upon exposure to drought at various growth stages (Manikavelu et al. 2006). Since drought tolerance is a genetically controlled phenomenon, many quantitative trait loci for membrane stability and other functionally related phenomena genes have been characterized using bioinformatics tools (Tripathy et al. 2000; Fu et al. 2007).

To identify the less obvious genetic networks that respond to stress, more straightforward and sensitive methods are necessary. The advent of whole genomics and related technologies is providing the necessary tools to identify key genes that respond to drought stress and relating their regulation to adaptive events occurring during stress (Bruce et al. 2002). Differential display was one of the earliest methods of parallel screening for differences in the levels of complementary DNA fragments generated from messenger RNA isolated from samples between experimental treatments (Liang and Pardee 1992).

The progressive cloning of many stress-related genes and responsive elements, and the proof of their association with stress-tolerant quantitative trait loci suggests that these genes may represent the molecular basis of stress tolerance (Cattivelli et al. 2002, 2008). On the other hand, the identification of quantitative trait loci associated with drought tolerance is also an important tool for marker-assisted selection of desirable plants (Fig. 9). In a recent study, mapping of quantitative trait loci for grain yield and its components using a simple sequence repeat/expressed sequence tag marker map explained considerable variation in chromosome 4A of wheat (Kirigwi et al. 2007). It makes clear that the combination of traditional and molecular breeding, marker-assisted selection and genetic engineering may allow a more rapid way to improve abiotic stress tolerance in crops (Chaves and Oliveira 2004).

In summary, to be able to prove that a transgenic plant is more resistant to water stress than the wild type, one would need a rigorous evaluation of the physiological performance as well as water status of transformed plants. This will avoid ambiguous interpretations of the genetic effects on drought resistance of plants (Chaves and Oliveira 2004).

4.3 Induction of Drought Resistance

Drought resistance can be induced by adopting various strategies. Of these, exogenous use of various growth regulating and other chemicals has proven worthwhile in producing drought resistance at various growth stages in a number of plants. An account of these strategies is given below.

4.3.1 Seed Priming

One of the short-term and most pragmatic approaches to overcome the drought stress effects is seed priming. Seed priming is a technique by which seeds are partially hydrated to a point where germination-related metabolic processes begin but radicle emergence does not occur (Farooq et al. 2006). Primed seeds usually exhibit increased germination rate, greater germination uniformity, and sometimes greater total germination percentage (Kaya et al. 2006; Farooq et al. 2007). This approach has been applied to overcome the drought stress effects in a range of crop species.

Improvement of rice and other crops for growing in water-scant areas is of current interest. In the newly introduced aerobic rice culture, the frequency and intensity of drought may increase manifold. Du and Tuong (2002), while testing the effectiveness of different osmotica to improve the performance of direct-seeded rice, noted that osmopriming with 4% KCl solution and saturated $CaHPO_4$ solution was successful in improving the seedling emergence, crop stand establishment and yield under stress. In drought-prone areas primed rice seeds germinated well and seedlings emerged faster and more uniformly, leading to increased yield (Harris et al. 2002). A germination trial of 11 varieties of upland rice under limited soil moisture conditions revealed early and synchronized emergence owing to seed priming (Harris and Jones 1997).

Seed priming improved performance of wheat seeds under drought stress in terms of germination and water-use efficiency of drought-stressed plants by 44% compared with unprimed seeds (Ajouri et al. 2004). The beneficial effects of priming included faster emergence of crop seedlings, early flowering and higher grain yield even under drought stress (Kaur et al. 2005). In sunflower, osmopriming with KNO_3 and hydropriming improved the germination and stand establishment under stress conditions (Kaya et al. 2006).

4.3.2 Use of Plant Growth Regulators

Foliar application of plant growth regulators, both natural and synthetic, has proven worthwhile for improving growth against a variety of abiotic stresses. Drought stress alone inhibited increases in length and fresh weight of the hypocotyl, while applied levels of gibberrelic acid reversed this effect. In this case, gibberrelic acid partially increased the water status of the seedlings and partially sustained protein synthesis (Taiz and Zeiger 2006). Exogenous application of gibberellic acid increased the net photosynthetic rate, stomatal conductance and transpiration rate in cotton (Kumar et al. 2001), and stimulated pollen and seed cone production in Sitka spruce (*Picea sitchensis*) under drought stress (Philipson 2003).

Among other hormones, exogenous application of 1-aminocyclopropane-1-carboxylic acid also improves drought tolerance by delaying senescence (Todd et al. 2004). In another study, exogenously applied uniconazole, brassinolide and abscisic acid increased soybean yields both under well-watered and water deficit conditions. Under water stress conditions, plant growth regulator treatments significantly increased water potential, and improved chlorophyll content (Zhang et al. 2004). Jasmonates, including jasmonic acid and its related compounds, are a group of naturally occurring growth regulators rather recently discovered in higher plants (Creelman and Mullet 1995). Jasmonates play an essential role in the signaling pathway, triggering the expression of plant defense genes in response to various stresses (Koda 1997). Exogenously applied jasmonic acid induced drought tolerance by increasing the betaine level in pear (Gao et al. 2004). Exogenous application of brassinolide, uniconazole and methyl jasmonate in maize improved drought tolerance owing to increased activities of superoxide dismutase, catalase and ascorbate peroxidase, abscisic acid and total carotenoid contents (Li et al. 1998). Benzyladenine is an active cytokinin, which can increase the drought resistance of different plants (Shang 2000).

Salicylic acid can also effectively improve plant growth under drought conditions (Senaratna et al. 2000). In a recent study**,** exogenous application of salicylic acid improved the drought tolerance of winter wheat, which was correlated with an increased catalase activity (Horváth et al. 2007). Both salicylic acid and acetyl-salicylic acid (a derivative of salicylic acid), applied at various concentrations through seed soaking or foliar spray protected muskmelon (*Cucumis melo*) seedlings, subjected to drought stress. However, the best protection was obtained from seedlings pretreated with lower concentrations of salicylic acid (Korkmaz et al. 2007).

The fact that seed imbibition with salicylic acid or acetyl-salicylic acid confers stress tolerance in plants is more consistent with signaling for gene expression rather than their direct effects (Senaratna et al. 2000). The endogenous salicylic acid content was increased in drought-stressed *Phillyrea angustifolia* (Munné-Bosch and Penuelas 2003), suggesting that salicylic acid might have a role in the drought stress response. In wheat, salicylic acid was shown to increase the abscisic acid content, leading to the accumulation of proline (Shakirova et al. 2003). Pretreatment with 0.5 mM salicylic acid for 1 day limited the drought tolerance of 2-week-old maize plants by increasing their polyamine content (Németh et al. 2002). However, soaking grains in acetyl-salicylic acid improved the drought tolerance of wheat.

4.3.3 Use of Osmoprotectants

Osmoprotectants are involved in signaling and regulating plant responses to multiple stresses, including reduced growth that may be part of the plant's adaptation against stress (Fig. 7). In plants, the common osmoprotectants are proline, trehalose, fructan, mannitol, glycinebetaine and others (Zhu 2002). They play adaptive roles in mediating osmotic adjustment and protecting subcellular structures in stressed plants

(Fig. 8). However, not all plants accumulate these compounds in sufficient amounts to avert adverse effects of drought stress (Penna 2003). Ashraf and Foolad (2007) outlined three approaches to increase the concentrations of these compounds in plants grown under stress conditions to increase their stress tolerance: (1) use of traditional protocols of plant genetics and breeding to develop cultivars with natural abilities to produce high levels of these compounds under stress conditions, (2) engineering genetically modified plants capable of producing sufficient amounts of these compounds in response to environmental stresses and (3) as a short-cut method, exogenous use of these osmolytes on plants to enhance their stress tolerance ability.

Exogenously applied glycinebetaine improves the growth and production of some plants under stress (Naidu et al. 1998; Chen et al. 2000; Hussain et al. 2008). In many crop plants the natural accumulation of glycinebetaine is lower than sufficient to ameliorate the adverse effects of dehydration caused by various environmental stresses (Subbarao et al. 2000). Exogenous application of glycinebetaine has been reported to improve drought tolerance in this regard (Hussain et al. 2008). Foliar-applied glycinebetaine improved the growth of plants subjected to water deficit by the maintenance of leaf water status due to improved osmotic adjustment and enhanced photosynthesis, primarily due to a greater stomatal conductance and carboxylation efficiency of Rubisco (Sakamoto and Murata 2002). Exogenous application of glycinebetaine effectively diminished the drought effects in terms of greater number of achenes per capitulum in sunflower (Azam et al. 2005). However, pre-soaking of seeds with glycinebetaine was not effective in preventing the adverse effects of water stress on yield components. Glycinebetaine application at the vegetative stage was more effective in ameliorating the adverse effects of drought (Azam et al. 2005). Glycinebetaine also increased anti-oxidative enzyme activities under water deficit (Ma et al. 2007). Exogenously applied proline enhanced the endogenous accumulation of free proline and improved the drought tolerance in petunia (Yamada et al. 2005).

Inhibitors of polyamine biosynthetic enzymes limit stress tolerance of wheat but the concomitant exogenous application of polyamines restores it (Liu et al. 2004a,b). Exogenous spermidine application before the drought stress significantly improved the stress tolerance in barley (Kubiś 2003). In a recent review, Liu et al. (2007) concluded that though there was variation in effects between polyamines and plant species, exogenous polyamine application to stressed cells or tissues could lead to injury alleviation and growth promotion. Yang et al. (2007) suggested that for rice, to perform well under drought stress, it should have higher levels of free spermidine/free spermine and insoluble-conjugate putrescine.

4.3.4 Silicon

Silicon is the second most abundant element in soils and a mineral substrate for most of the world's plant life. Ample evidence is available indicating that when silicon is readily available to plants, it plays a significant role in their growth, mineral nutrition, mechanical strength and resistance to several stresses (Epstein 1994). It has not been considered an essential element for higher plants yet, partly because its role in plant biology is less well understood (Gong et al. 2003). Nevertheless, numerous studies demonstrate that silicon is an important element, and plays an important role in tolerance of plants to environmental stresses (Savant et al. 1999).

With respect to drought stress, relevant work is limited on silicon. Sorghum (*Sorghum bicolor*) plants grown in pots in the presence of silicon had higher relative water content and dry materials by improving shoot water uptake (Hattori et al. 2001, 2005). Wheat plants applied with silicon could maintain better water status and higher content of dry materials compared with non-silicon treatment under drought (Gong et al. 2003). Exogenously applied silicon lowered the shoot to root ratio, indicating the facilitation of root growth and maintenance of a higher photosynthetic rate and stomatal conductance compared with plants grown without silicon application under drought stress (Hattori et al. 2005). In another study, Gong et al. (2005) opined that the silicon-triggered improvement in drought tolerance of wheat plants was associated with an increase in antioxidant defense, thereby alleviating oxidative stress on functional molecules of cells. Silicification endodermal tissue was found to play an important role in water transport across the root of rice (Lux et al. 1999) and sorghum (Lux et al. 2002). These data, together with the rate of silicon uptake and deposition by sorghum roots (Lux et al. 2003), and the effects of losing root cell walls in sorghum (Hattori et al. 2003), suggested an important role of silicon in water transport and maintenance of root growth under drought stress.

5 Conclusion

Water deficit reduces plant growth and development, leading to the production of smaller organs, and hampered flower production and grain filling. A diminution in grain filling occurs due to a decrease in the accumulation of sucrose and starch synthesis enzymes. Timing, duration, severity and speed of development undoubtedly have pivotal roles in determining how a plant responds to water deficit. Following drought, stomata close progressively with a parallel decline in net photosynthesis and water-use efficiency. Stomatal conductance is not controlled by soil water availability alone, but by a complex interaction of intrinsic and extrinsic factors. Depending upon the availability of moisture, activities of the enzymes of carbon assimilation and those involved in adenosine triphosphate synthesis are decreased and sometimes inhibited. One of the major factors responsible for impaired plant growth and productivity under drought stress is the production of reactive oxygen species in organelles including chloroplasts, mitochondria and peroxisomes. The reactive oxygen species target the peroxidation of cellular membrane lipids and degradation of enzyme proteins and nucleic acids.

Being very complex, the drought tolerance mechanism involves a number of physiological and biochemical processes at cell, tissue, organ and whole-plant levels, when activated at different stages of plant development. Examples of these mechanisms are reduction in water loss by increasing stomatal resistance, increased water uptake by developing large and deep root systems, accumulation of osmolytes and osmoprotectant synthesis. Amongst plant growth substances, salicylic acid, cytokinin and abscisic acid have been reported to play an important role in drought tolerance. Scavenging of reactive oxygen species by enzymatic and non-enzymatic systems, cell membrane stability, expression of aquaporins and stress proteins are also vital mechanisms of drought tolerance. Drought stress effects can be managed by production of most appropriate plant genotypes, seed priming, plant growth regulators, use of osmoprotectants, silicon and some other strategies.

Although physiological mechanisms of drought tolerance are relatively well understood, further studies are essential to determine the physiological basis of assimilate partitioning from source to sink, plant phenotypic flexibility which leads to drought tolerance, and factors that modulate plant drought-stress response. Like most other abiotic stresses, foliar plant parts are more directly impinged upon by drought than roots. However, an understanding of root responses to drought stress, most likely involving root-shoot signaling, is a preferred area of research. Investigations that seek to improve crop performance by increasing osmotic adjustment need to focus on meristematic regions of roots. For effective application and commercial use of exogenous glycinebetaine, proline and other compatible solutes as inducers of drought tolerance, their mechanisms of action, the most optimal concentrations, and appropriate plant developmental stages must be carefully determined. The role of H_2O_2 as a signaling molecule as well as the identification of regulatory components in the pathway that leads to plant responses to drought stress are fundamental clues for future research. Applications of genomics, proteomics and transcriptomic approaches to a better understanding of the molecular basis of plant drought tolerance and improved water-use efficiency under drought are also imperative. Mutants or transgenic plants exhibiting differential capabilities for reactive oxygen species formation and elimination could be useful to elucidate this fundamental point. Molecular knowledge of response and tolerance mechanisms is likely to pave the way for engineering plants that can withstand and give satisfactory economic yield under drought stress.

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Laws of Sustainable Soil Management

Rattan Lal

Abstract The challenge of doubling the world's food grain production by 2030 is even more daunting because of the decrease in per capita arable land area and renewable fresh water resources; increase in risks of soil and environmental degradation; and threat of decrease in use efficiency of inputs because of the projected climate change. Thus, the need for identifying processes, practices and policies that govern sustainable management of soil resources is more critical now than ever before. The goal is to minimize risks of soil degradation by enhancing its resilience and improving ecosystem services of the finite and fragile soil resource. Here, 10 principles are given for sustainable management of soil. This report is an introductory article of the book Sustainable Agriculture, published by Springer, EDP Sciences (Lichtfouse et al. 2009, this book).

1 Introduction

The world population of 1 million about 10,000 years ago increased to merely 1 billion by 1800. The population is projected to be 10 billion by the end of the twenty-first century. Almost the entire increase of 3.3 billion, from 6.7 billion in 2008 to 10 billion by 2100, will occur in developing countries where soil resources are finite and already under great stress. An unprecedented increase in agronomic productivity between the 1960s and 2000, brought about by the Green Revolution technology of growing input-responsive varieties in irrigated soils with high input of chemicals, created a false sense of security and an unnecessary complacency. Consequently, funding support for agricultural research and development has been dwindling (Anonymous 2008). The need for a continued increase in agronomic productivity, from the cropland area and irrigation water resources already committed through increase in use efficiency of inputs with an attendant reduction in losses by erosion and leaching or volatilization, was underscored by the drastic increase in prices of food grains, e.g., wheat, rice and corn, in early 2008. The number of food-insecure people, estimated at 854 million (FAO 2006), increased to 1002 million in 2009 because of the increase in price of the basic food commodities.

The problem of global food insecurity may be exacerbated by the threat of global warming. The projected increase in temperature and decrease in effective precipitation in semi-arid regions may adversely impact agronomic productivity of food staples, e.g., corn, wheat and rice (Lobell et al. 2008; Brown and Funk 2008). Examples of "tipping elements" in these important biomes include the Indian summer monsoon, and Sahel monsoon (Lenton et al. 2008). The adverse impacts of climate change on agronomic productivity may be due to a range of complex but interacting factors. Despite the positive impact of CO_2 fertilization, the net productivity may decrease because of an increase in respiration rate, drought stress and nutrient deficiency. The global energy crisis is also diverting cropland to biofuel plantations, often with positive feedback emissions of CO_2 and N_2O from soils

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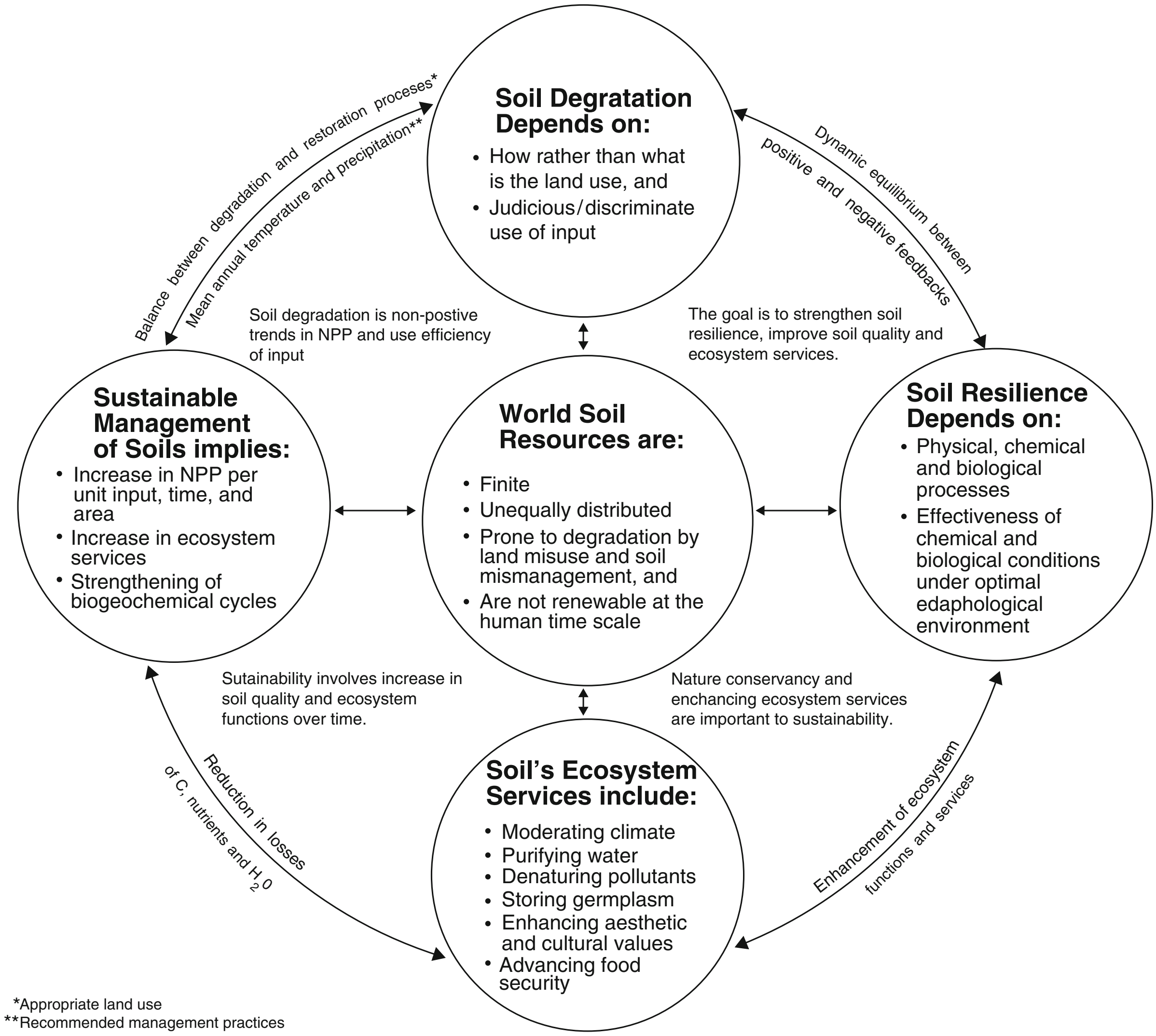

*Appropriate land use
**Recommended management practices

Fig. 1 Properties, processes and practices which govern soil degradation and resilience, and sustainable management

(Searchinger et al. 2008). The competition with biofuel plantations for land is leading to new land being cleared from the tropical rainforest, often with a large carbon debt (Farzione et al. 2008).

While the debate on the magnitude and severity of global warming goes on (Florides and Christodoulides 2008), the problems of soil degradation and desertification are exacerbated by the increasing demand on finite soil resources for the food, feed, fiber and fuel needs of the world's growing population. It is thus important to identify properties, processes and practices that affect sustainable management through setting-in-motion soil restoration trends which have minimal C and water footprints (Fig. 2). Soil degradation and restoration processes are governed by a set of laws as stated below:

2 Basic Principles of Sustainable Soil Management

Law #1

Soil resources are unequally distributed among biomes and geographic regions. Highly productive soils in favorable climates are finite and often located in regions of high population density, and have already been converted to managed ecosystems, e.g., cropland, grazing land and pasture, forest and energy plantations.

Law #2

Most soils are prone to degradation by land misuse and soil mismanagement. Anthropogenic factors leading to soil degradation are driven by desperate situations and helplessness in the case of resource-poor farmers and smaller landholders; and greed, short-sightedness, poor planning and cutting corners for quick economic returns in the case of large-scale and commercial farming enterprises.

Law #3

Accelerated soil erosion and decline in soil quality by other degradation processes depend more on "how" rather than on "what" crops are grown. Productive potential of farming systems can only be realized when implemented in conjunction with restorative and recommended soil and water management practices. Sustainable use of soil depends on the judicious management of both on-site and off-site inputs. Indiscriminate and excessive use of tillage, irrigation and fertilizers can lead to as much as or even more degradation than none or minimal use of these technologies.

Law #4

The rate and susceptibility of soil to degradation increase with increase in mean annual temperature and decrease in mean annual precipitation. All other factors remaining the same, soils in hot and arid climates are more prone to degradation and desertification than those in cool and humid ecoregions. However, mismanagement can lead to desertification even in arctic climates, e.g., Iceland.

Law #5

Soil can be a source or sink of greenhouse gases, e.g., CO_2, CH_4 and N_2O, depending on land use and management. Soil is a sink of atmospheric CO_2 under those land use and management systems which create a positive C budget and gains exceed the losses (Fig. 2a, left). Soil is a source of atmospheric CO_2 when the ecosystem C budget is negative and losses exceed the gains (Fig. 2b, right). Soils are a source of radiatively-active gases with extractive farming which create a negative nutrient budget and degrade soil quality, and a sink with restorative land use and judicious management practices which create positive C and nutrient budgets and conserve soil and water while improving soil structure and tilth.

Law #6

Soils are non-renewable over a human time frame of decadal or generational scales, but are renewable on a geological scale (centennial/millennial). With the increase in the human population, projected to be 10 billion by 2100, restoring degraded and desertified soils over a centennial-millennial scale is not an option.

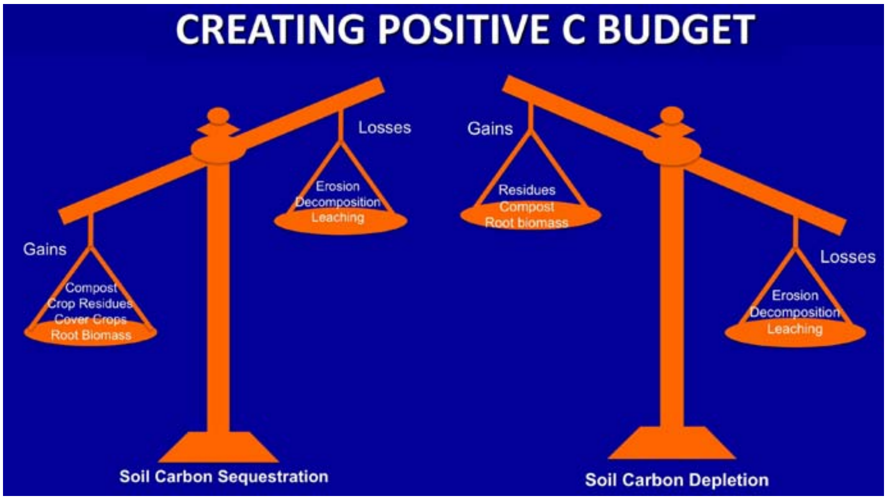

Fig. 2 A positive C (and nutrient) budget is essential to C sequestration

Because of heavy demands on finite resources, soils are essentially a non-renewable resource.

Law #7

Soil's resilience to natural and anthropogenic perturbations depends on its physical, chemical and biological processes. Favorable chemical and biological processes enhance resilience only under optimal soil physical properties, e.g., soil structure and tilth, processes, e.g., aeration, water retention and transmission, and edaphological environments, e.g., soil temperature.

Law #8

The rate of restoration of the soil organic matter pool is extremely slow, while that of its depletion is often very rapid. In general, restoration occurs on a centennial time scale and depletion on a decadal time scale. The rate of restoration and degradation processes may differ by an order of magnitude.

Law #9

Soil structure, similar to an architectural design of a functional building, depends on stability and continuity of macro-, meso- and micropores which are the sites of physical, chemical and biological processes that support soil's life support functions. Sustainable management systems, site-specific as these are, enhance stability and continuity of pores and voids over time and under diverse land uses.

Law #10

Sustainable management of agricultural ecosystems implies an increasing trend in net primary productivity per unit input of off-farm resources along with improvement in soil quality and ancillary ecosystem services such as increase in the ecosystem C pool, improvement in quality and quantity of renewable fresh water resources, and increase in biodiversity.

Soil resources can never be taken for granted. Extinct are the once thriving civilizations, e.g., Mayan, Incas, Indus, Mesopotamia, which chose to ignore their soil resources. Given its importance to human survival and dependence of all terrestrial life, soil quality must be improved, and restored. Soils must be transferred to the next generation in a better state than when received from the previous one.

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Pharmaceutical Crops in California, Benefits and Risks: A Review*

Michelle Marvier

Abstract Crops are being genetically engineered to produce a wide variety of drugs, vaccines and other pharmaceutical proteins. Although these crops may open the door to less expensive and more readily available drugs, there is concern regarding the potential for contamination of human food and livestock feed, as well as environmental harm. The outlook for the production of pharmaceutical crops in California currently appears mixed. To date, 18 federal permits for field trials involving pharmaceutical or industrial proteins have been approved in California. However, the state's farming community and general public have thus far rejected pharmaceutical crop production, and a handful of local governments have recently banned the cultivation of genetically modified crops, including pharmaceutical crops. In light of the many pros and cons, three major approaches – the precautionary approach, risk analysis and cost–benefit analysis – could be used to move the debate about pharmaceutical crops forward.

Keywords Bananas • Blood thinners • Cancer • Carrots • Cholera • Contraceptives • GMO • Growth hormones • Hemoglobin • Hepatitis B • HIV • Influenza • Insulin • Lettuce • Maize • Malaria • Pharmaceutical crops • Protein • Rabies • Risk • Tomatoes • Transgene • Vaccine

1 Introduction

Even science fiction writers did not dream that we would someday use maize fields to produce insulin, or rice paddies to grow anticoagulants. Today, however, crops are being turned into factories producing not just food, but drugs, vaccines, enzymes and antibodies. The first step in using crops to produce pharmaceutically active proteins is the synthesis or isolation of genes that code pharmaceutical proteins, followed by the transfer of those genes into the DNA of crop plants. These transferred genes, or "transgenes", can potentially come from a different plant species, an animal (often a human) or a bacterium. The genetically modified crops are then cultivated and harvested.

In most cases, the crop-produced pharmaceutical protein is extracted, purified and possibly modified further before it is administered to humans or livestock. In some instances, however, crops are being engineered so that a vaccine can be delivered through the direct consumption of leaves, fruits or other plant parts, without the cost and inconvenience of extracting the proteins and delivering them via pills or injections (Sala et al. 2003).

2 Benefits of Pharmaceutical Crops

Scientists are drawn to the genetic engineering of crops as a means of quickly producing large quantities of

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Fig. 1 Proponents of crops genetically engineered to express pharmaceutical proteins believe that these crops could increase the availability of medicines and vaccines, and lower costs. To date, about two-thirds of pharmaceutical field trials in the United States have involved maize, a wind-pollinated species (conventional corn is shown). Credit: USDA-ARS/Doug Wilson

drugs and vaccines, with the hope that this technology can reduce costs and increase the availability of much-needed pharmaceuticals (Fischer et al. 2004; Giddings et al. 2000; Horn et al. 2004; Ma et al. 2003, 2005a). The potential products of transgenic plants include blood thinners, hemoglobin, insulin, growth hormones, cancer treatments and contraceptives. Products already in the pipeline include plant-produced vaccines for hepatitis B, cholera, rabies, HIV, malaria and influenza. One company is developing genetically modified maize (corn) to produce lipase, a digestive enzyme used to treat patients with cystic fibrosis (Fig. 1). Arthritis and other autoimmune diseases are also targets for plant-produced vaccines (Sala et al. 2003). Researchers have focused on maize, bananas, tomatoes, carrots and lettuce as possible oral-delivery mechanisms for such vaccines because these foods can be eaten raw, thereby avoiding the protein denaturing that typically occurs during cooking (Sala et al. 2003). Producing vaccines in food plants would eliminate the need for refrigeration, which limits the usefulness of certain vaccines in many parts of the world (Walmsley and Arntzen 2000).

In some cases, the pharmaceuticals targeted for production in transgenic plants are currently produced by cultures of transgenic animal, bacterial or yeast cells in large vats. Plants are an attractive alternative because they could potentially produce greater yields. This is especially important for monoclonal antibodies (such as etanercept, which is used to treat rheumatoid arthritis) because current production methods cannot keep up with increasing demand (Elbehri 2005). Moreover, faster and less expensive production could reduce prices for consumers. Another major benefit of utilizing plants is the reduced risk of disease transmission; there is concern that producing drugs via mammalian cell cultures or animal milk could facilitate the movement of certain viruses to humans.

Despite these potential advantages, drugs produced by pharmaceutical crops have not yet appeared on the U.S. market. Several are currently making their way through field and clinical trials, and the first drugs derived from pharmaceutical crops could be on the market within a few years (Ma et al. 2005b).

3 Containment Risks

There is a long history of cultivating plants to produce pharmaceutical compounds, and at least one-fourth of modern medicines contain plant-derived ingredients (Raskin et al. 2002). Some plants that are used for pharmaceutical production such as opium poppies are also food crops, such as poppy seeds. Despite such precedents from nature, genetically modifying major commodity grains such as maize and rice to produce pharmaceutical proteins has raised new levels of concern and public anxiety (Stewart and McLean 2004). Although earlier methods of pharmaceutical production often involved cultures of genetically modified cells, these cells were kept under strict confinement in laboratories. The production of pharmaceutical proteins in maize or rice, on the other hand, will typically be done in the field where it will be impossible to completely contain the crops, transgenes and pharmaceutical proteins (Ellstrand 2006) (Fig. 2). Production of these crops in contained greenhouses or underground caves has been proposed as a potential, albeit far less cost-effective, solution.

3.1 Contamination of Food and Feed

In 2002, 130 acres of pharmaceutical maize were cultivated in the United States in field trials. Two-thirds of all pharmaceutical plantings in the United States are maize, but soybeans, rice, potatoes, alfalfa, wheat, tobacco and other crops are also being used.

Fig. 2 Whenever pharmaceutical-producing crops are grown outside, it is virtually inevitable that birds, insects and other wildlife will eat them, resulting in unknown risks to the animals, and that the pollen and seeds will be transported offsite. *Left:* Bees on a corn stalk (credit: Suzanne Paisley/UC Davis). *Right:* A red-winged blackbird (credit: Jack Kelly Clark/UC Davis)

The primary concern is that the public might someday find unwanted medicines in their food or in livestock feed (Elbehri 2005; Kirk et al. 2005; Mascia and Flavell 2004; Peterson and Arntzen 2004).

Food can be contaminated when transgenes are not contained, or if plant products intended only for medicinal uses accidentally comingle with those headed for our dinner tables. Transgenes can escape when pollen from pharmaceutical crops drifts into and fertilizes fields of nonpharmaceutical crops. Due to the energetic costs that producing pharmaceutical proteins likely entails, it is unlikely that transgenes coding for pharmaceutical products would persist for very long within the recipient gene pool. However, even transient transgene flow could cause problems. For example, if transgenic pollen fertilizes seed kernels on a nontransgenic maize plant, the kernels could produce and contain the pharmaceutical protein. Alternatively, if seeds are left behind in the soil following harvest, "volunteer" pharmaceutical plants could establish themselves in subsequent growing seasons, possibly in mixture with nonpharmaceutical crops. Because some pharmaceutical compounds are effective in very low concentrations, even low-level contamination may pose risks.

3.2 *Transgene Escape from Food Crops*

Although pharmaceutical crops are still rarely produced and only under tightly regulated conditions, there already has been one revealing case of transgene escape involving field trials of pharmaceutical maize in Nebraska and Iowa. In November 2002, the U.S. Department of Agriculture (USDA) discovered that ProdiGene had failed to comply with federal regulations in two of its field trials, which involved maize genetically modified to produce a vaccine that prevents diarrhea in pigs. In both locations, ProdiGene failed to destroy volunteer maize plants in the subsequent growing season. In Nebraska, the mistake was not discovered until after the volunteer maize had been shredded and mixed among soybeans that had been subsequently planted at the site (Fig. 3). This meant that 500,000 bushels of soybeans had to be destroyed. In Iowa, there was no mixing with soybeans, but 155 acres of maize surrounding the pharmaceutical-crop test site were destroyed because of possible contamination via pollen from volunteer plants. ProdiGene was fined $300,000 for these violations, and also paid $2.7 million in damages and cleanup costs.

A half-dozen more examples of human error involving other, nonpharmaceutical-producing types of genetically modified crops were reviewed by Marvier and Van Acker (2005). Since the publication of that paper, Syngenta admitted to accidentally distributing the seeds of an unapproved variety of genetically modified insect-resistant Bt10 maize over a 4-year period (Macilwain 2005), and traces of Bayer's Liberty Link 601 herbicide-resistant rice have been detected in both U.S.A. and European long-grain food rice, even though the variety was never approved or marketed (Vogel 2006). The lesson from these events is that human error occurs and, frankly, is unavoidable.

4 Food Vs. Nonfood Crops

The possible escape of pharmaceutical products from engineered crops into the food supply is of concern to the promoters of genetic engineering, as well as environmentalists. For example, an editorial in the journal *Nature Biotechnology* offered two suggestions that could help industry to avoid foreseeable problems (Editors of Nature Biotechnology 2004). First, engineered crops could be geographically isolated to reduce the chances of contamination in the general food supply. For example, pharmaceutical crops might be cultivated on islands where the food crop is otherwise

Fig. 3 In 2002, field trials of pharmaceutical maize went awry when the producer failed to destroy volunteer maize during the subsequent growing season. As a result, 500,000 bushels of harvested soybeans were destroyed in Aurora, Nebraska. Greenpeace activists hung a banner on the silo. Credit: Greenpeace/Laura Lombardi

absent. Second, the editors recommended that food crops should not be used to produce pharmaceutical proteins, and that nonfood crop alternatives such as tobacco might be a wiser choice. The National Research Council (2004) concurred, stating, "Alternative nonfood host organisms should be sought for genes that code for transgenic products that need to be kept out of the food supply" (Fig. 4).

Despite the National Research Council's recommendations, many biotechnology firms are nonetheless using food grains as platforms for pharmaceutical production. As of 2003, over three-quarters of field trials conducted to produce pharmaceutical or industrial proteins (including fibers, plastics and enzymes) had involved maize, a wind-pollinated species (Union of Concerned Scientists 2003). Grain crops are favored because protein yields from the large seeds of maize, rice and barley are typically much higher than those obtained from leaves and other vegetative parts. In addition, pharmaceutical proteins can remain stable in dried grain for several years, compared to the much-reduced stability of these same proteins in leaf tissue. Moreover, maize is generally recognized as safe for ingestion by the U.S. Food and Drug Administration (FDA) and therefore can be used as an inactive carrier, suitable for drug delivery.

Despite these advantages, warnings from critics may be having an effect. A growing number of companies are focusing on tobacco, or even mosses, algae and duckweed, as platforms for pharmaceutical production (Fischer et al. 2004). These plants, however, pose risks of their own that must be considered. Algae and duckweed, if cultivated, would have greater potential than highly domesticated crop species to escape from cultivation.

5 Additional Routes of Exposure

Even if the production of pharmaceutical proteins was limited to nonfood crops, potential risks would remain. Pollen, fine particles of leaves that are crushed during harvest, and possibly even runoff water contaminated with proteins from decomposing plants, could expose people and wildlife that live on or near pharmaceutical-producing fields to the transgenic material. Whenever production occurs outside, birds, insects and other wildlife can consume pharmaceutical crops, regardless of where they are grown or what species they are. Pharmaceutical crops may also affect soils and the community of soil-dwelling organisms.

Such impacts on wildlife and soils have received scarce attention from scientists and regulators, but surely will vary greatly by variety depending on the nature of the protein being produced. One possible strategy to avoid these problems would be to engineer proteins so that they do not become biologically active until after they are extracted and further processed in a laboratory.

Fig. 4 In a 2004 report, an expert panel of the National Research Council recommended that food crops should not be used to produce pharmaceutical crops, suggesting instead that nonfood crops such as tobacco (shown in Virginia) would be a wiser choice. Credit: USDA/Ken Hammond

6 Regulatory Responses

Pharmaceutical crop varieties are not expected to be deregulated; rather, it is likely that they will only be produced in field trials as permitted under USDA regulatory oversight. Initially, field-trial applications for pharmaceutical crops were treated like those for any other regulated, genetically modified crop. However, the USDA recently published stricter requirements specifically designed for plants genetically engineered to produce pharmaceutical and industrial proteins (Federal Register 2003). These new requirements aim to reduce the risk of gene flow and the contamination of food and feed. Confinement measures now required for pharmaceutical crops include greater geographic isolation from other fields of the same species, buffer zones of bare soil around the field edge, scouting for and destroying volunteer plants in subsequent field seasons, and the dedication of equipment for use only on the trial fields.

There is a precedent for the successful segregation of crop varieties intended for use in food from those intended for industry. Rapeseed varieties containing high levels of erucic acid are segregated from those used to produce canola oil, which must contain less than 2% erucic acid (Ma et al. 2005b). Erucic acid is used to create lubricants, coatings and surfactants, but the regular consumption of large amounts of erucic acid has been linked to heart disease in animal studies. Producers of high-erucic-acid rapeseed must maintain a minimum 16.4-foot buffer zone around their fields and clearly label harvested products. In addition, erucic acid levels in canola oil are regularly monitored by various food inspection agencies.

Although this example demonstrates the potential for successful segregation, more stringent protocols would be required to produce pharmaceutical proteins in food crops. In the case of erucic acid, a low level of cross-contamination is acceptable (Bilsborrow et al. 1998), but for pharmaceutical compounds there is generally zero tolerance. Studies examining the potential for the coexistence of other types of genetically modified crops with nongenetically modified varieties demonstrate that contamination can be limited (for example, less than 0.9%) but not entirely prevented (EuropaBio 2006). Moreover, in the rapeseed example, only one or two compounds must be monitored. In contrast, if maize is eventually used to produce some 50 or 100 different pharmaceutical compounds, the costs for systematic monitoring to ensure that none of these compounds contaminates maize intended for food or feed could be prohibitive.

In addition to rules governing how pharmaceutical crops are grown, USDA inspectors have publicly announced that field-test sites of such crops will each be inspected five times during the growing season and twice postharvest (Stewart and Knight 2005). However, based on an audit that included site visits to 91 field-test locations in 22 states, the USDA Office of the Inspector General found that this level of inspection was not consistently maintained. The audit report concluded that weaknesses in the regulatory oversight of genetically modified crop field trials increase the chance that these crops will inadvertently persist in the environment (USDA 2005). Of additional concern, the audit found that:

> At the conclusion of the field test, APHIS does not require permit holders to report on the final disposition of genetically modified pharmaceutical and industrial harvests. As a result, [the inspectors] found two large harvests of genetically modified pharmaceutical crops remaining in storage at the field-test sites for over a year without APHIS's knowledge or approval of the storage facility. (USDA 2005)

Clearly, better adherence to monitoring requirements is needed to minimize the risk of a loss of containment.

Although the 2003 regulations set forth by the USDA are an important step, the proposed rules make no attempt to protect wildlife (fencing or netting are not required), assess how pollen or fine particulate matter from the crop might affect humans, or test soils and groundwater for pharmaceutical residues. Also missing is any requirement that the pharmaceutical variety be readily identifiable. For example, several authors have suggested that pharmaceuticals could be produced in "identity-preserved varieties, such as white tomatoes or maize, which are easily identified by their pigmentation" (Ma et al. 2003).

No specific requirements were proposed for molecular solutions to contamination, presumably because these are not sufficiently developed yet. However, molecular strategies hold great promise for the improved containment of transgenes. Examples include the genetic modification of chloroplast DNA rather than nuclear DNA (for crop species in which pollen does not contain chloroplasts, transgenes would not move with pollen) (Daniell et al. 2002) and the inducible production of pharmaceuticals (for example, the pharmaceutical protein is activated by exposure to ethanol vapor) (Mascia and Flavell 2004). The tissue-specific expression of pharmaceutical proteins may also reduce or eliminate certain avenues of exposure (such as the possibility of exposure via pollen inhalation), and gene deletion technologies could potentially be used to remove transgenes from certain tissues (such as pollen) to reduce the possibility of transgene spread (Keenan and Stemmer 2002).

If transgenes could be contained, then regulations could be much more permissive about which traits are allowed in crop plants. On the other hand, if transgenes will inevitably escape and spread – despite our best intentions for containment – then we must be much more cautious about which traits are allowed to be developed in crop plants. Alternatively, the cultivation of crops engineered to produce particularly hazardous pharmaceutical proteins might be restricted to greenhouses or other enclosed facilities, such as caves (Fig. 5). Although production in such facilities is feasible, it would likely be far more expensive than field production.

Fig. 5 In an abandoned Indiana mine, Controlled Pharming Ventures is working with Purdue University researchers to develop techniques for growing pharmaceutical crops underground, in order to limit risks. Credit: Purdue Agricultural Communications/Tom Campbell

7 Field-Testing in California

The USDA database of field-trial permits for plants expressing pharmaceutical and industrial proteins includes many entries for which the petitioning organization has used a claim of Confidential Business Information to withhold from the public any information regarding the transgene, its source or the traits that have been altered (USDA APHIS 2007). It is therefore difficult to know exactly how many field trials of pharmaceutical crops have been approved in California. However, the Union of Concerned Scientists (2007) estimates that 18 permits for field trials involving pharmaceutical or industrial proteins were approved in California, the earliest in 1996 and one as recently as 2006 (Table 1). According to this analysis,

Table 1 USDA-approved field-trial permits allowing the growth of crops genetically engineered to produce pharmaceutical or industrial proteins in California, 1996–2006

Engineered crop	Applicant	Issued/effective	Source of gene[a]	Pharmaceutical or industrial protein
Maize	Dow	6/2002	CBI[b]	CBI: Unidentified pharmaceutical protein
	Monsanto	3/2001	CBI	CBI: Unidentified transcriptional activator (pharmaceutical)
		3/2001	CBI	CBI: Unidentified transcriptional activator (pharmaceutical)
	Pioneer	3/2000	Unclear[c]	CBI: Unidentified novel protein that may have pharmaceutical or industrial uses
		4/2001	Unclear	CBI: Unidentified novel protein that may have pharmaceutical or industrial uses
		4/2002	Unclear	CBI: Unidentified industrial enzyme and unidentified novel protein that may have pharmaceutical or industrial uses
		4/2004	Unclear	CBI: Unidentified novel protein that may have pharmaceutical or industrial uses
Leaf mustard	USDA Agricultural Research Service	3/2004	Unclear	CBI: Unidentified industrial enzyme
CBI		3/2004	CBI	CBI: Unidentified industrial enzyme
Rapeseed	Pioneer	9/1996	CBI	CBI: Unidentified pharmaceutical protein
Rice	Ventria Bioscience (formerly Applied Phytologics)	3/1997	Humans	Pharmaceutical proteins: Antithrombin and serum albumin
		2/1998	Humans	Pharmaceutical proteins: antitrypsin, antithrombin and serum albumin
		2/1998	CBI	CBI: Unidentified pharmaceutical protein
		5/2000	CBI	CBI: Unidentified pharmaceutical protein and unidentified novel protein that may have pharmaceutical or industrial uses
		4/2001	Humans	Pharmaceutical proteins: antitrypsin, lactoferrin and lysozyme
		4/2003	Humans	Pharmaceutical proteins: lactoferrin and lysozyme
		5/2004	Humans	Pharmaceutical proteins: lactoferrin and lysozyme
Tobacco	Planet Biotechnology	6/2006	Mice, rabbits, CBI	Antibodies to tooth decay and common cold

Source: Union of Concerned Scientists (2007)

[a] Refers specifically to the gene coding for the industrial or pharmaceutical protein

[b] *CBI* Confidential Business Information

[c] Source of gene coding for industrial and/or pharmaceutical protein(s) cannot be determined from publicly available information

California is tied with Kentucky for seventh among U.S. states and territories, after Nebraska with 41 approved permits, Hawaii with 40, Puerto Rico with 39, Wisconsin with 29, Iowa with 27 and Illinois with 19.

7.1 Pharmaceutical Rice

The production of pharmaceutical proteins in transgenic crops is meeting with some resistance in California, as Ventria Bioscience recently discovered (Fig. 6). Ventria had received federal permits to grow approximately 100 acres of pharmaceutical rice in California almost annually since 1997 (see Table 1). However, the company's plans to expand its 2004 field trials to 120 acres of rice engineered with synthetic human genes were met with strong opposition from California rice farmers and environmentalists. Ventria's rice has been genetically engineered to produce lactoferrin and lysozyme, compounds used to treat severe diarrhea in infants. However, farmers were concerned that even low levels of contamination of their rice crops could threaten exports to Asia.

The California Rice Certification Act of 2000 gave the California Rice Commission the authority to devise protocols governing the cultivation of any new rice variety that requires segregation. Despite farmers' concerns, on March 29, 2004, the commission approved planting guidelines for Ventria's expanded plantings in a six to five vote, on the condition that the field trials be conducted in counties such as Orange and San Diego, remote from the state's rice-growing regions. Due to the late timing of the commission's decision and the need to plant immediately, Ventria then asked the California Department of Food and Agriculture (CDFA) to issue an emergency permit for the proposed field trials. On April 9, 2004, CDFA decided not to approve Ventria's proposal because federal regulators at the USDA had not yet completed their review of Ventria's permit application. California regulators essentially deferred to federal regulation, reasoning that federal oversight of the field-trial application is both necessary and sufficient. In 2005, Ventria attempted to move its field trials to Missouri, where it met similar resistance from major rice purchasers.

7.2 Local Bans

Although California regulators may be happy to defer to USDA judgment when it comes to genetically modified crops, the public and local communities are not always so accommodating. Several counties have considered banning genetically modified crops outright, and in some cases bans have indeed been implemented. Bans on all genetically modified plants are in effect in four counties: Mendocino (Measure H, passed by voters in March 2004), Trinity (passed by the county board of supervisors in August 2004), Marin (Measure B, passed by voters in November 2004) and Santa Cruz (unanimously passed by the county board of supervisors in June 2006). In contrast, voters rejected initiatives to ban genetically modified crops in four counties: Humboldt, San Luis Obispo and Butte in 2004, and Sonoma in 2005. Supervisors in several other California counties, including Fresno, Kern and

Fig. 6 In California, rice farmers strongly opposed efforts to grow 120 acres of rice genetically engineered to produce proteins for two pediatric medicines, fearing that their exports to Asia would be jeopardized. *Above*, a California rice farm (not genetically engineered). Credit: ANR Communication Services

Kings, have passed resolutions supporting the use of genetically modified crops.

The political future of local measures, either for or against genetically engineered crops, was recently challenged by Senate Bill 1056, which would have prohibited California counties, towns and cities from passing any local regulation of seeds and nursery plants. However, in September 2006, this bill failed to make it out of committee and died with the close of the legislative year. The failure of this bill leaves open the possibility of additional local restrictions on genetically modified crops in the future.

7.3 Economic Considerations

In the end, economic concerns regarding the containment of food crops may outweigh concerns for the environment or even food safety. The contamination of U.S.-produced rice with the unapproved Liberty Link 601 (herbicide-resistant) variety has had an enormous economic impact on U.S. rice growers. U.S. exporters of long-grain rice lost about $150 million because genetically modified rice is banned throughout most of the European Union, a major importer of U.S. long-grain rice. Even greater economic losses would likely occur if a crop were found to be contaminated with a pharmaceutical protein. Whether pharmaceutical-producing crops will be accepted in California will likely depend on the economic value of other markets that might be placed at risk. A proposal to produce pharmaceutical rice within a major rice-producing area such as the Sacramento Valley is unlikely to be welcomed. However, a proposal to grow that same pharmaceutical rice in an area with very little other rice production may be acceptable.

8 Evaluating Risks and Benefits

All forms of agriculture entail some risks to the environment. Whenever food is grown, some species lose their habitat and some may be poisoned, trapped or shot; species extinctions are also possible. Pharmaceutical crops entail all of these same risks plus additional ones – the contamination of food and feed being the most serious. There are three major approaches to evaluating the potential benefits and risks.

8.1 Precautionary Approach

A precautionary approach typically shifts the burden of proof onto the producer, so that a practice or product is not approved until there is sufficient scientific understanding of the potential risks. This approach has been adopted in many legal and policy arenas, including the transnational movement of living, genetically modified organisms under the Cartagena Protocol on Biosafety. Since all nations with commercial transgenic production must undertake safety testing (of some sort) prior to the commercial production of transgenic crops, a precautionary approach is already being applied to a certain degree (Conko 2003).

However, interpretations of the precautionary approach vary. A strong interpretation mandates that the producer demonstrate the absence of harmful effects prior to the release of the product. Given that harmful effects could be exceedingly rare, this represents an impossible standard from a scientific perspective. In contrast, a weak interpretation mandates that regulators should only consider delaying the approval of a practice or product when sufficient evidence of risk exists (Conko 2003). California counties with moratoria on all transgenic crops are adopting a strong interpretation of the precautionary approach, similar to European countries that require the labeling of any foods with genetically modified plant ingredients.

8.2 Formal Risk-Assessment Framework

The U.S. Environmental Protection Agency embraces a risk-assessment approach in all of its regulatory capacities, including the regulation of chemical pesticides and "biopesticides", such as plants genetically modified to express insecticidal proteins. Risk is defined as a function of both hazard and exposure, such that either a low hazard or low probability of exposure will reduce the assessed level of risk. Hazard is a measure of the harmful effects of the pharmaceutical proteins on people and the environment; as such, not all are equally hazardous. For example, lactoferrin is naturally produced in human tears and breast milk. Assuming that plant-produced lactoferrin is very similar to human-produced lactoferrin, this compound would present little if any hazard to humans.

With regard to exposure, the potential routes and amounts of exposure to pharmaceutical compounds are expected to be highly variable. Exposure will depend upon which crop species is used as the production platform, where it is grown, and where the protein is and is not expressed within the plant (pollen, for example, is highly mobile). The amount of land needed to produce sufficient quantities of particular pharmaceuticals must also be considered; this will depend both upon demand for the product and the protein yields obtained per plant. Incorporating transgenes into chloroplast DNA rather than nuclear DNA could reduce exposure both by limiting the expression of the protein in pollen and by boosting the production of target proteins to a level where sufficient quantities could be produced in very small fields (Daniell et al. 2002).

8.3 Cost–Benefit Analysis

An important component of the cost–benefit analysis approach is "fairness" – who benefits and who pays the costs. Fairness is a core value of many Americans, and environmental policy discussions increasingly focus on equitability and fairness. The precautionary approach and risk-assessment framework do not require the consideration of costs and benefits to stakeholder groups. But one explanation for the public's reluctance regarding the production of pharmaceutical proteins in crop plants could be that the distribution of benefits (primarily to corporations) does not match the distribution of risks (primarily falling upon the general public).

Because biotech and pharmaceutical companies are the primary economic beneficiaries, the key questions for a cost–benefit approach applied to pharmaceutical crop production are whether the economic rewards outweigh the potential risks of unwanted pharmaceutical exposure, and whether the distribution of the costs and benefits is equitable and fair (Elbehri 2005). If economic profits are reinvested into the research and development of new drugs, then additional benefits for human and animal health may be achieved. In addition, drug prices might be reduced if it becomes inexpensive to manufacture drugs in large quantities. However, because most pharmaceutical crops are designed to produce patented pharmaceutical compounds, there would typically be little competition to drive prices lower. Furthermore, the research and development of pharmaceutical crops will likely remain very expensive.

Other potential benefits are possibly increased income for farmers and higher tax revenues (Wisner 2005). There is much hope that pharmaceutical crops will improve farmers' incomes, but these benefits are unlikely in a global market where the production of pharmaceutical proteins in genetically modified crops could be undertaken in whichever nation has the lowest production costs and weakest regulatory restrictions (Wisner 2005). Another important issue for farmers concerns liability for contamination incidents. In the only precedent to date, ProdiGene was held accountable for its mistakes. Communities or regulatory agencies considering allowing the production of pharmaceutical crops will want assurances regarding who pays for any damages.

9 A Promising New Technology?

Like many new technologies, the genetic engineering of crops to produce pharmaceutical products has great promise. Bananas that could cheaply and easily deliver vaccines to children throughout the tropics could be a wonderful invention. But there are downsides; it will be difficult to avoid food contamination and potential harmful effects to wildlife if pharmaceuticals are widely produced in food crops grown out of doors.

Finally, the pros and cons of alternative strategies to achieve the same goals should be assessed (O'Brien 2000). For example, could certain pharmaceutical crops reasonably be confined to greenhouses, caves or other enclosed facilities? Are there other possible routes to the inexpensive and efficient production of drugs that perhaps do not involve the transgenic manipulation of crop plants? The future course of this technology will require thoughtful input from ecologists, public health experts and medical researchers – as well as those who genetically engineer these crops in the first place.

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Coexistence of Genetically Modified and Non-GM Crops in the European Union: A Review

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Abstract The adoption of genetically modified (GM) crops in the European Union (EU) raises questions on the feasibility of coexistence between GM and non-GM crops. Regulations to ensure that different cropping systems can develop side-by-side without excluding any agricultural option are currently implemented or developed by member states. The aim of this review is to explore whether nationally or regionally proposed coexistence strategies comply with the general principles established by the European Commission that ask for science-based and proportionate coexistence measures. In the first part, existing legal requirements and potential sources of adventitious mixing are reviewed. It is discussed what type of coexistence measures might be necessary to keep GM inputs below the legal tolerance threshold of 0.9%. Concentrating on cross-fertilisation as the major biological source of adventitious mixing in maize, it is then assessed to which extent available scientific data on cross-fertilisation can explain the diversity of currently proposed isolation distances by several member states. In the second part, it is analysed whether currently proposed isolation distances reflect contending policy objectives towards GM crops that largely exceed the economic scope of coexistence. It is investigated how coexistence is intersecting with a wider debate about the role of GM crops in agriculture. Based on the analysis of existing cross-fertilisation data, it is concluded that some of the currently proposed isolation distances are not in line with the coexistence principles laid down by the European Commission: they are (1) excessive from a scientific point of view; (2) difficult to implement in practice; (3) rarely proportional to the regional heterogeneity in the agricultural landscape; and (4) not proportional to the farmers' basic economic incentives for coexistence. Hence, the range of proposed isolation distances cannot simply be explained by different interpretations of available scientific data, possible error intervals and remaining uncertainties inherent in the scientific process. It is argued that other than scientific issues must be at play. One might thus claim that coexistence has become an arena of contending values and visions on the future of agriculture and on the role GM crops might play therein.

Keywords Adventitious mixing • Bt-maize • Coexistence • Cross-fertilisation • Fixed measures • Flexible measures• Genetically modified (GM) crops • Isolation distances • Liability • Sustainable development

1 Introduction

The adoption rate of genetically modified (GM) crops shows considerable disparities between different agricultural production regions worldwide. While the global cultivation area of GM soybean, maize, cotton and canola (oilseed rape) reached 114 million hectares in 2007, the total area cropped with GM crops in the European Union (EU) was approximately 110,000 ha (James 2007). Most approved GM crops worldwide are thus currently cultivated outside the EU, but might subsequently be imported and eventually further processed in the EU mostly for feeding purposes. Today,

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Bt-maize expressing the insecticidal protein Cry1Ab from *Bacillus thuringiensis* is the only GM crop to be cultivated in the EU. Bt-maize confers resistance against larvae of certain lepidopteran pests such as the European and Mediterranean corn borer. Following the registration of various Bt-maize varieties derived from the transgenic maize event MON810 in national catalogues and the common catalogue of varieties of agricultural plant species in 2004, the cultivation area of Bt-maize started to gradually increase in the EU, especially in areas where the two lepidopteran pests cause serious infestations. In 2007, the area cropped with Bt-maize for the first time exceeded 100,000 ha with the highest share being grown in Spain (69%), followed by France (19%), the Czech Republic (5%), Portugal (4%) and Germany (3%) (Table 1). Bt-maize plantings in the EU, however, accounted for less than 2% of the total EU maize cultivation area in 2007, compared with 75% in the US (Abbott and Schiermeier 2007).

The disparity in adoption rate of GM crops between the EU and the rest of the world is generally attributed both to societal and political opposition towards agro-food biotechnology and to complex regulatory approval procedures in the EU (Chapotin and Wolt 2007; Herring 2008). In the mid-1990s, the advent of GM crops and their corresponding agro-food products aroused strong societal concerns (Levidow et al. 2005; Devos et al. 2006, 2008d; Levidow and Carr 2007). Fostered by several highly publicised and successive food safety crises, public suspicion towards regulatory authorities, scientists and technocratic decision-making grew (Lofstedt 2006). The media, which were explicitly involved in framing the public perception and societal image-building of agro-food biotechnology (Marks et al. 2007; Maeseele and Schuurman 2008), exacerbated the social amplification of risk (Kasperson and Kasperson 1996). In the late 1990s, the growing societal and political opposition contributed to a de facto moratorium on new market approvals of GM crops. It was adopted at a meeting of the EU Council of environmental ministers in June 1999, where five member states decided not to accept new GM crop market approvals until the existing regulatory frame was revised (Winickoff et al. 2005). Several agro-food biotechnology market applications remained subsequently blocked in the approval pipeline in the EU.

From 1999 onwards, policy-makers started to continuously revise the legal conditions under which GM crops and agro-food products were allowed to be used in the EU to slow down further erosion of public and market confidence (reviewed by Devos et al. 2006).

Table 1 Number of genetically modified (GM) maize varieties registered in national catalogues and/or the common catalogue of varieties of agricultural plant species and the area cropped with GM maize in the European Union (up to December 2007)

		Number of registered (+) or excluded (−) varieties // Area (ha) cropped to GM maize									
	GM maize	2003		2004		2005		2006		2007	
EU country	event	Variety	Area	Variety	Area	Variety	Area	Variety	Area	Variety	Area
Czech Republic	MON810	0	0	0	0	0	270	0	1,290	+11	5,000
	Total	0	0	0	0	0	270	0	1,290	11	5,000
France	MON810	0	17	0	15	0	493	0	5,028	0	21,200
	Total	15	17	15	15	15	493	15	5,028	15	21,200
Germany	MON810	0	<100	0	<100	+3	340	+2	954	0	2,685
	Total	0	<100	0	<100	3	340	5	954	5	2,685
Poland	MON810	0	0	0	0	0	0	0	<30	0	<30
Portugal	MON810	0	0	0	0	0	760	0	1,254	+1	4,500
	Total	0	0	0	0	0	760	0	1,254	1	4,500
Slovakia	MON810	0	0	0	0	0	0	0	<30	0	900
	Bt176	+1	26,090	+2, −1	21,810	−4	0	0	0	w	w
Spain	MON810	+4	6,070	+7	36,410	+14	53,225	+16	53,667	+12	75,148
	Total	7	32,160	15	58,220	25	53,225	41	53,667	53	75,148
The Netherlands	MON810	0	0	0	0	0	0	0	<10	0	<10
	Bt176	0	26,090	0	21,810	0	0	0	0	w	w
EU	MON810	0	6,187	+17	36,425	+14	55,088	+5	62,263	+39	109,473
	Total	0	32,277	17	58,335	31	55,088	36	62,263	75	109,473

w withdrawal from the European market of the transgenic maize event Bt176 and its derived products according to the Commission Decision of 25 April 2007 (2007/304/EC)

The precautionary principle, post-market environmental monitoring and traceability were legally adopted as ways to cope with scientific uncertainties. New institutions such as the European Food Safety Authority (EFSA) were created to provide independent, objective and transparent science-based advice on the safety of agro-food biotechnology applications. Labelling and traceability of GM products became mandatory to ensure consumers' freedom of choice. Because the maintenance of different agricultural production systems is a prerequisite for providing a high degree of consumers' choice, a coexistence policy was adopted in the EU. It specifically aimed at enabling the side-by-side development of different cropping systems without excluding any agricultural option. As such, farmers would maintain their ability to make a practical choice between conventional, organic and GM crops. Since coexistence only applies to approved GM crops that were judged to be safe prior to their commercial release (Sanvido et al. 2007), safety issues fall outside the remit of coexistence (Schiemann 2003; De Schrijver et al. 2007a).

To date there is little experience on how the new legal coexistence requirements could be implemented in the EU. Due to the heterogeneity in farm structures, crop patterns and legal environments between member states, the European Commission follows the subsidiarity principle for the implementation of legal coexistence frames. According to this principle, coexistence should be handled by the lowest authority possible. The European Commission thus limits its influence to gathering and coordinating relevant information based on on-going scientific studies at EU and national level, and to providing guidance to assist member states in establishing best practices for coexistence. These best practices then have to be developed and implemented at national or regional levels.

In the present review, it is explored – after a brief general introduction on coexistence – whether preventive (so-called ex ante) coexistence regulations currently imposed or proposed by member states comply with the general coexistence principles established by the European Commission (European Commission 2003). First, potential sources of adventitious admixtures are considered. Secondly, concentrating on cross-fertilisation as the major biological source of adventitious mixing in maize, preventive coexistence measures are discussed that might be necessary to keep adventitious GM inputs below the legal tolerance threshold of 0.9% in the harvest of neighbouring non-GM maize fields. Given that proposed isolation distances differ considerably among member states, existing scientific cross-fertilisation studies are assessed to define a scientifically appropriate range of isolation distances. Third, it is explored what challenges the implementation of large and fixed isolation distances might entail in practice, and if such isolation distances comply with general coexistence principles laid down by the European Commission. An alternative way of managing coexistence between maize cropping systems through ex ante regulations is discussed. Finally, it is analysed whether the diversity of fixed isolation distances, as imposed or proposed by several member states, reflects contending policy objectives towards GM crops that largely exceed the economic scope of coexistence. Within this context, it is investigated how coexistence is intersecting with a wider debate about sustainable development of agriculture and the role GM crops might play therein.

2 Coexistence of GM and Non-GM Crops

Society typically needs regulation whenever the introduction of a new product or technology leads to an externality or a market failure (Beckmann and Wesseler 2007). A good example is the spray drift of pesticides. Pesticide traces and residues from conventional farming can become a negative production externality if they contaminate neighbouring organic systems, and thereby lower market returns associated with "organic" status. Because organic farming is a production system that avoids or largely excludes synthetic pesticides, plants containing pesticide traces and residues originating in conventional cropping systems are "declassified". If the market does not widely provide formal protection afforded to organic farms from pesticide spray drift, the market fails to serve organic producers. This market failure may justify government intervention, which has to establish clear rules on pesticide use. The cultivation of GM crops is similar, as completely avoiding the unintentional presence of GM material from approved GM crops in non-GM products – the externality – might be impossible in the agricultural context (see Sect. 2.1). Because traces of GM material can occur in non-GM products, a first role for policy-makers is to provide legal standards that ensure the coexistence of GM and non-GM crops (see Sect. 2.3).

However, defining legal standards coping with the potential occurrence of externalities might not be sufficient; once they have been defined, policies need to be designed to avoid market failures. If there is a substantial demand for non-GM crops, this will be reflected by a price difference between GM and non-GM crops. Non-GM crops will yield a price premium on the market, relative to GM crops (see Sect. 4.4). Without government intervention, farmers growing non-GM crops can suffer crop value losses due to externalities caused by adjacent farmers who grow GM crops. If the market itself provides very few incentives for correcting this problem, government intervention may be justified, just like the pesticide use rules introduced by several EU governments. Hence, to correct this market failure and to protect farmers from negative externalities of GM crop cultivation, policy-makers need to define legal coexistence rules which ensure that crop value losses are prevented or minimised (ex ante), or reimbursed (ex post) (see Sect. 2.3).

2.1 *Sources of Adventitious Mixing*

According to Article 43 of Regulation 1829/2003 on GM food and feed that entered into force in April 2004, member states are empowered to take appropriate measures to avoid the unintentional presence of GM material in other products. However, it is recognised that completely avoiding the unintentional presence of GM material in non-GM products is difficult in the agricultural context (Eastham and Sweet 2002; Schiemann 2003; van de Wiel and Lotz 2006; Damgaard et al. 2007). Because agriculture is an open system, a certain extent of adventitious mixing is unavoidable. Various sources have been identified that could contribute to on-farm adventitious mixing between GM and non-GM crops (Fig. 1a): (1) the use of impure seed (Friesen et al. 2003; Jørgensen et al. 2007); (2) cross-fertilisation due to pollen flow between neighbouring fields (Devos et al. 2005; Weekes et al. 2005; Hüsken and Dietz-Pfeilstetter 2007; Sanvido et al. 2008); (3) the occurrence of volunteer plants originating from seeds and/or vegetative plant parts from previous GM crops (Devos et al. 2004; Lutman et al. 2005; Messéan et al. 2007; Gruber et al. 2008); (4) mixing of plant material in machinery during sowing, harvest and/or post-harvest operations (Bullock and Desquilbet 2002; Demeke et al. 2006); and (5) – to a lesser extent – cross-fertilisation from certain sexually compatible wild/weedy relatives and feral plants (Devaux et al. 2007; Jørgensen 2007; Devos et al. 2008c; Knispel et al. 2008; Pivard et al. 2008).

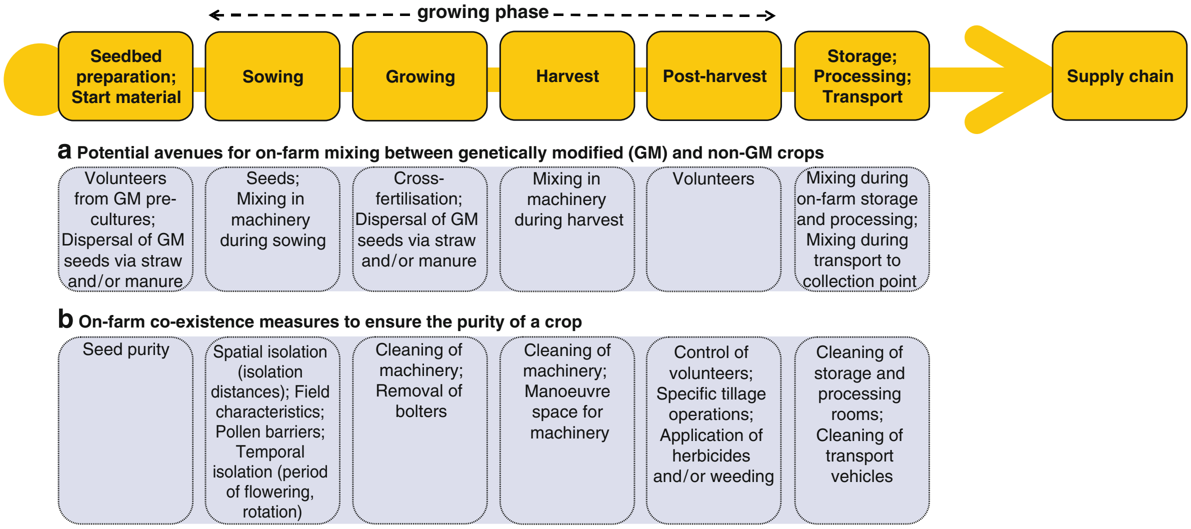

Fig. 1 (**a**) Potential avenues for on-farm adventitious mixing between genetically modified (GM) and non-GM crops, and (**b**) on-farm coexistence measures to ensure the purity of a crop during the production process

2.2 Labelling Thresholds

In response to the difficulty of keeping genes "on a leash", tolerance thresholds were established for the unintentional or technically unavoidable presence of approved GM material in non-GM products. If the content of GM material in a non-GM product exceeds the established tolerance threshold, the product has to be labelled as containing GM material, which may affect its market acceptability (see Sect. 2.3). According to the GM food and feed Regulation, the legal tolerance threshold for conventional food and feed products has been set at 0.9%. Since the scope of coexistence extends from agricultural crop production on the farm up to the first point of sale (e.g., from the seed to the silo), agricultural commodities produced on-farm will have to comply with the labelling requirements at the first point of sale (European Commission 2003).

Organic growers principally aim at keeping their products free from any GM material. Regulation 1804/1999 on organic production of agricultural products states that the use of transgenic organisms and their derivatives is not compatible with the organic production method. The Regulation, however, foresees a de minimis tolerance threshold for the unavoidable presence of GM material in organic products. It was thus anticipated that organic producers would opt for a tolerance threshold ranging between the limit of quantification of a DNA analysis (0.1%) and the tolerance threshold for food and feed products (0.9%). In a press release published on 21 December 2005 (IP/05/1679), the European Commission emphasised that an organic product with an adventitious content of GM material below 0.9% could still be labelled as organic. On 12 June 2007, this point of view was confirmed at a meeting of the EU agriculture ministers where political agreement was reached on a new Regulation on organic production and labelling (IP/07/807). Since the organic sector advocates that GM crops are not compatible with organic farming (Verhoog et al. 2003; Altieri 2005), they are seeking to establish the limit of quantification of a DNA analysis as the basis to determine the tolerance threshold in organic products.

For seeds no tolerance threshold has been defined yet. Considering that seeds are the first step in the production chain and that additional mixing might adventitiously occur at subsequent steps in the production chain, tolerance thresholds for seeds will be lower than 0.9% (Kalaitzandonakes and Magnier 2004). In 2001, the Scientific Committee on Plants proposed tolerance thresholds of 0.3% for cross-pollinating crops, and 0.5% for self-pollinating and vegetatively propagated crops (SCP 2001). As no tolerance thresholds have been established for seeds to date, any seed lot containing approved GM seeds destined for cultivation in the EU has to be labelled as containing GM material.

2.3 Legal Frames on Coexistence

There are principally two strategies member states have established or are developing to warrant coexistence of different cropping systems: ex ante regulations and ex post liability schemes (Beckmann et al. 2006; European Commission 2006; Koch 2007). Regulations are considered ex ante if they have to be followed by GM crop adopters while growing GM crops. Ex ante regulations prescribe preventive on-farm measures that should ensure that tolerance thresholds are not exceeded in neighbouring non-GM agricultural production systems. Contrary to ex ante coexistence regulations, ex post liability schemes are backward-looking: they cover questions of liability and the duty to redress the incurred economic harm once adventitious mixing in a non-GM product has occurred after the cultivation of GM crops.

Preventive coexistence measures: For decades, seed production regulations have specified statutory segregation measures (so-called identity preservation measures) between seed crops and conventional crop production of the same species to maximise varietal seed purity. Apart from seed production, experience with identity preservation systems is also available from the cultivation of different crop types grown for different uses (Sundstrom et al. 2002). Several of the proposed measures to ensure varietal seed and crop purity can be applied within the context of coexistence to limit the adventitious content of GM material in seeds and plant products (Sundstrom et al. 2002; Devos et al. 2004; Kalaitzandonakes and Magnier 2004; Damgaard et al. 2007; Jørgensen et al. 2007; Gruber et al. 2008). These measures include (1) the use of certified seed; (2) spatially isolating fields of the same crop; (3) implementing pollen barriers around fields; (4) scheduling different sowing and flowering periods; (5) limiting carry-over of GM volunteers into the following crop through the extension of cropping intervals; (6) cleaning agricultural machinery and transport vehicles for seed remnants; (7) controlling volunteers and wild/weedy relatives; (8) applying effective post-harvest tillage operations; (9) retaining records of field history; and (10) the voluntary clustering of fields (Fig. 1b). The most drastic preventive coexistence measure is probably banning the cultivation of GM crops in a certain region. The level of containment needed to ensure coexistence is defined by tolerance thresholds: the lower the tolerance threshold, the stricter are the on-farm measures needed to meet labelling requirements.

Liability schemes: Apart from defining the level of containment needed, tolerance thresholds also determine the level of GM material that initiates the need to redress economic harm due to adventitious mixing. Only in the case when the established threshold is exceeded, the product has to be labelled as containing GM material. A lower market price or difficulties in selling products that contain traces of GM material could induce a loss of income. Economic losses are expected to be greater in organic farming than in conventional farming due to the generally higher market value of organic products. Furthermore, organic growers could lose their organic certification, precluding access to markets for organic products for several years. Market attitudes may also impose products to be free of GM material without evidence for actual adventitious mixing, in turn affecting potential markets. Since the late 1990s, major retailers have excluded GM ingredients from their own-brand food products, as a measure to respect consumers' preferences in the EU (Levidow and Bijman 2002; Kalaitzandonakes and Bijman 2003; Knight et al. 2008). A recent qualitative survey of GM food labels in supermarkets in France confirmed that there are almost no "GM" labelled products on supermarkets' shelves, suggesting that food processors still favour non-GM alternatives (Gruère 2006). Moreover, GM foodstuffs reaching retail shelves are targeted by pressure groups opposed to genetic engineering (Carter and Gruère 2003). Due to the possibility of GM admixtures, some food manufacturers are also reluctant to purchase agricultural commodities from regions where GM crops are intensively grown (Smyth et al. 2002). Labelling products as containing GM material does, however, not necessarily lower their market value. In Spain, for instance, GM and non-GM maize are stored and processed together by grain feed manufacturers for sale as animal feed (Messeguer et al. 2006). According to the labelling requirements of the Regulation 1830/2003 on GM food and feed, products such as meat, milk and eggs obtained from animals fed GM feed do not require labelling. Since food companies and retailers only refuse GM maize that enters the food chain, coexistence measures are principally only needed near organic fields and for crops grown for human consumption. However, where the use of non-GM feed is imposed for the production of meat, milk and eggs under specific quality schemes, coexistence measures can be required near non-GM maize fields in which maize is grown for animal feed production. In Germany, for instance, the federal states have recently adopted a new set of rules for the voluntary labelling of "GM crop-free" animal products.

Because GM crop production is the "newcomer" in European agriculture, GM crop adopters are requested by law to take preventive coexistence measures and to bear responsibility for redressing the incurred harm caused by adventitious mixing (European Commission 2003). Provided that the admixture occurred purely accidentally and not due to some misconduct by GM crop adopters, economic losses would in many member states be reimbursed by a compensation fund (Koch 2007). However, if the GM crop adopter causes unlawful damage to a neighbour, he will be required to pay suitable restitution for the full economic loss of the victim. If the farmer suffering the loss deliberately or inadvertently contributed to the damage, his compensation may be reduced or, depending on the circumstances, be forfeited. Considering that various sources can contribute to the adventitious presence of GM material in non-GM products, it can become challenging to establish and prove the causal link between the incurred damage and the farmer or operator responsible for it. In Austria and Germany, for example, all neighbouring GM crop farmers that might have contributed to the admixing are jointly liable for the incurred losses, unless their individual contributions can be clearly determined. In Denmark, causation does not need to be proven strictly: closeness in space and time between a GM crop field and an adjacent non-GM maize field is sufficient to be held liable (Koch 2007). An additional difficulty in defining causation of adventitious mixing is that traces of GM material might only become detected in subsequent steps of production and/or supply chains.

Depending on the member state, the compensation fund will either be provisioned by financial contributions from all growers, only from GM crop adopters, or from GM seed producers, retailers and other actors dealing in the transport and storage of GM crops, and/or from the government (Koch 2007). In Portugal, for example, a flat fee per notification and a tax on GM seeds are demanded as a financial contribution to the compensation fund. Other member states impose or propose fees that vary with the planting area of the GM crop, the dissemination potential of the plant species grown, and/or with the number of neighbouring farmers having at least one non-GM maize field occurring within a specific isolation distance (Beckmann et al. 2006; Koch 2007).

Socio-economic consequences: Coexistence measures imposed by law prior to, during and after cultivation, and laboratory analyses for testing, identifying and quantifying the content of GM material in non-GM products will inevitably entail additional costs to ensure compliance with labelling and traceability requirements (Menrad and Reitmeier 2008). Moreover, farmers may suffer income losses due to restrictions in crop choice and management. Neighbouring farmers could restrict the cultivation possibilities of a farmer who decides to grow a GM crop, if they do not concur with his cropping intention. In the case when a GM crop adopter cannot avoid interference and cannot find mutual agreement with neighbouring farmers, he would have to renounce growing GM crops on his land. Besides spatial restrictions, temporal cultivation limitations may occur due to irreversibility. In a field where a GM crop was grown, it could temporarily be difficult to meet the 0.9% tolerance threshold if a farmer wishes to go back to a non-GM farming system. A conversion time might be required to deplete dormant GM seeds from the seed bank and/or control volunteers and weedy relatives that may contain the transgene (Devos et al. 2004; Lutman et al. 2005; Jørgensen et al. 2007; Messéan et al. 2007; D'Hertefeldt et al. 2008; Gruber et al. 2008).

The cultivation of different crops with GM and non-GM characteristics in the same region can have sociological consequences. GM crop adopters might have to negotiate with neighbouring farmers and landowners, and seek mutual agreement on their respective cropping intentions. Within this context, GM crop growers could be legally obliged to notify in advance their intentions to grow GM crops to neighbours and/or competent authorities. Similarly, contractors intervening in the cultivation or harvest of GM crops might have to be informed about the GM characteristics of the crop. In Belgium, for instance, GM crop adopters are required to dispose of written agreements from neighbours, which subsequently build the basis for an official coexistence approval for the cultivation of GM crops. In other member states, official approval of the government is granted to GM crop adopters before sowing: in Austria, farmers need approval for each single field and crop from local authorities, whilst Hungary, Ireland and the Slovak Republic consider a generic procedure (Beckmann et al. 2006; Koch 2007).

3 Coexistence of Maize Cropping Systems

Since both the cultivation area of Bt-maize and the number of Bt-maize varieties commercially available to European farmers have increased (Table 1), regulating coexistence between maize cropping systems is currently becoming a burning issue in some EU regions. Therefore, sources of adventitious mixing and preventive coexistence measures that might be necessary to keep GM inputs below the legal tolerance threshold of 0.9% are discussed in the following sections.

3.1 Sources of Adventitious Mixing

Various sources can contribute to the adventitious mixing of GM material in non-GM products in maize. Maize is a cross-pollinated crop, relying on wind for the dispersal of its pollen. Most pollen is shed before silks are receptive, although up to 5% self-pollination can occur (Eastham and Sweet 2002). In most EU countries, cross-fertilisation due to pollen flow between neighbouring maize fields represents the major potential biological source of on-farm mixing: there are no cross-compatible wild relatives of maize in the EU, and many shed maize kernels and seedlings do not survive winter cold (Gruber et al. 2008). In Mediterranean regions, however, maize volunteers frequently occur. In Spain, volunteer densities up to 7,000 plants/ha have been observed, which corresponds to approximately 10% of maize planting densities (Melé et al. 2007). If left uncontrolled by weed management practices, shed kernels and – to a lesser extent – kernels on ears remaining on the soil after harvest can germinate and flower under dry and warm conditions. Although these maize volunteers can contribute to the adventitious presence of GM material in the harvest of non-GM maize in the subsequent year, recent field observations demonstrated that their contribution is limited (Melé et al. 2007). Volunteers reaching the flowering stage cross-fertilise neighbouring maize plants only locally. Furthermore, maize is not able to survive as feral populations outside cropped areas in the EU due to its high degree of domestication. Other sources, including the use of impure seed and admixing during sowing, harvest and post-harvest operations, can also contribute to the adventitious GM inputs into non-GM maize. These sources fall outside the scope of this review and will therefore not be addressed.

3.2 Preventive Coexistence Measures

The analysis performed here identified cross-fertilisation as the major potential biological source of on-farm mixing in maize. In the following, preventive coexistence measures are discussed that might be necessary to keep adventitious GM inputs from cross-fertilisation in the harvest of neighbouring maize fields below the legal threshold.

Isolation distances: Given that pollen concentrations and thus cross-fertilisation levels rapidly decrease with increasing distance from the pollen source, spatially isolating GM maize fields from non-GM maize fields is recognised as being an effective on-farm strategy to reduce the extent of cross-fertilisation (Eastham and Sweet 2002; Schiemann 2003). To keep GM inputs from cross-fertilisation in neighbouring non-GM agricultural systems below the legal threshold of 0.9%, member states are currently imposing or proposing largely differing isolation distances, ranging from 15 to 800 m (Table 2).

Various biological, physical, experimental and analytical factors influence cross-fertilisation levels in maize and hence the definition of appropriate isolation distances (reviewed by Devos et al. 2005 and Sanvido et al. 2008). The major influencing factors are the relative sizes of and the distance between donor and receptor fields, and the flowering synchrony between donor and recipient plants, as well as local wind conditions (Debeljak et al. 2007; Hüsken et al. 2007; Messéan and Angevin 2007; Viaud et al. 2007). The available scientific data allows the identification of a number of consistent facts and patterns, which enable making science-based recommendations, for the definition

Table 2 Isolation distances proposed or imposed by different European member states for maize (adapted from European Commission 2006)

Member state	Isolation distance (m) for conventional maize	Isolation distance (m) for organically grown maize	Isolation distance (m) for maize seed production
Czech Republic	70	200	–
Denmark	200	200	200
France	50	–	–
Germany	150	300	–
Hungary	400	800	800
Ireland	50	75	–
Luxembourg	800	800	800
The Netherlands	25	250	250
Poland	200	300	–
Portugal	200	300	–
Slovakia	200	300	–
Spain	50	50	300
Sweden[a]	15[b]/25[c]	15[b]/25[c]	–
United Kingdom	80[b]/110[c]	–	–

[a] Isolation distance doubles if the genetically modified maize variety contains more than one transgene

[b] Fodder maize

[c] Grain maize

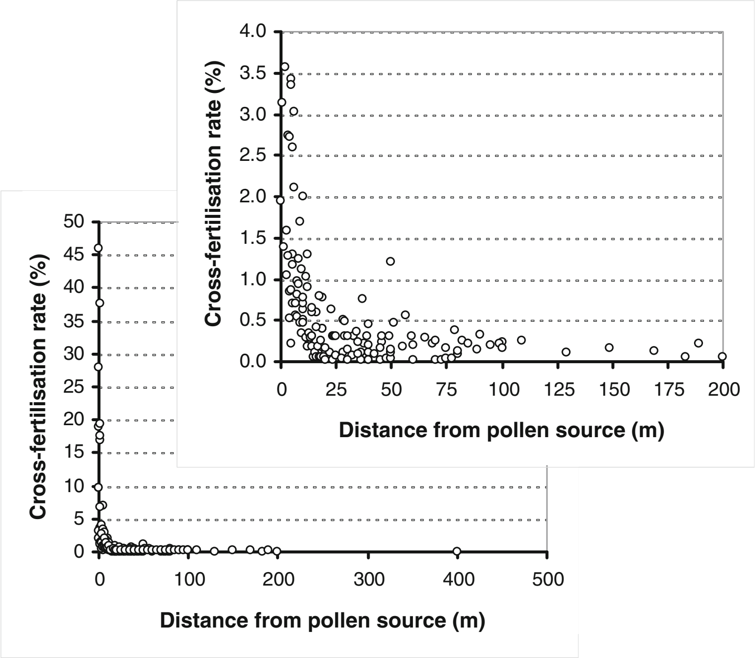

Fig. 2 A meta-analysis of various data of cross-fertilisation between maize fields. Cross-fertilisation levels are represented in relation to the distance from the pollen source. The *upper graph* represents a magnification of the original graph (adapted from Sanvido et al. 2008)

of appropriate and effective isolation distances. Compared with other wind-pollinated species, pollen grains of maize are relatively large and heavy. Due to these characteristics, maize pollen settles to the ground rapidly (Aylor et al. 2003) and has a short flight range (Jarosz et al. 2005). Most cross-fertilisation events occur within 50 m of the pollen source (Fig. 2), while vertical wind movements or gusts during pollen shedding only lead to very low levels of cross-fertilisation over longer distances under suitable meteorological conditions (Bannert and Stamp 2007; Delage et al. 2007; Haegele and Peterson 2007; Viner and Arritt 2007; Lavigne et al. 2008).

Existing scientific literature on pollen dispersal and cross-fertilisation (Devos et al. 2005; van de Wiel and Lotz 2006; Hüsken et al. 2007; Sanvido et al. 2008), and on predictive vertical gene flow modelling at the landscape level (Messéan et al. 2006; Lécroart et al. 2007; Mazzoncini et al. 2007; Beckie and Hall 2008), suggests that isolation distances ranging from 10 to 50 m would be in most cases sufficient to keep GM inputs from cross-fertilisations below the tolerance threshold of 0.9% in the harvest of neighbouring non-GM maize fields. The necessary isolation distance within the range of 10–50 m is influenced by (1) the seed purity of non-GM maize; (2) field characteristics and distribution; (3) (GM) maize share; (4) crop type; (5) differences in sowing and flowering times; and (6) meteorological conditions (Devos et al. 2005; Messéan et al. 2006; Hoyle and Cresswell 2007; Beckie and Hall 2008; Lavigne et al. 2008). An isolation distance of 50 m might in some cases not be sufficient to comply with the current tolerance threshold. This is particularly true for small, long and thin recipient maize fields that are located downwind from a larger GM maize field, where the elongated side is exposed to the GM maize field, and where plants flower synchronously with those of the donor field (Devos et al. 2005; Messéan et al. 2006; Hüsken et al. 2007). Moreover, if local pollen densities of non-GM maize fields are low, as in seed production fields, cross-fertilisation levels increase significantly (Goggi et al. 2007).

Larger isolation distances might also be needed for stacked GM maize varieties to comply with the tolerance threshold. Because a stacked GM maize variety contains more than one transgene (De Schrijver et al. 2007b), a similar cross-fertilisation rate results in a higher content of GM material expressed in percentages of haploid genomes in recipient plants, compared with a single GM maize variety. Moreover, other sources than cross-fertilisation (e.g., seed impurities) could contribute to GM inputs in non-GM products. In this case, GM inputs from cross-fertilisations may thus have to remain substantially below 0.9% in order to allow for a safety margin up to the labelling threshold in agricultural commodities. Because the final GM content in the harvest depends on various factors such as field size and harvesting procedure and because the modelling of this reduction is currently very difficult, the tolerance threshold of 0.9% is taken as an endpoint in the present review. In addition, it is important to bear in mind that no tolerance threshold for the adventitious presence of approved GM material in non-GM seeds has been defined to date. However, based on a meta-analysis of existing cross-fertilisation studies, Sanvido et al. (2008) concluded that an isolation distance of 50 m would be sufficient to keep cross-fertilisation levels below 0.5% at the border of the recipient maize field. Due to mixing of the outer and the inner parts of an entire field at harvest (where the inner parts usually contain lower GM contents than at the field border), the authors assumed the average cross-fertilisation rate would be less than 0.5% in the harvested product.

Pollen barriers: Like isolation distances, pollen barriers consisting of the same crop effectively reduce the extent of cross-fertilisation between neighbouring maize fields. If the outer parts of the maize field function as a pollen barrier, the distance towards the inner field parts increases, in turn increasing the distance GM pollen has to travel for cross-fertilisation (Devos et al. 2005). Moreover, a pollen barrier of maize produces competing pollen and/or may serve as a physical barrier to air, and consequently pollen flow. The extent of cross-fertilisation is reduced much more effectively by a pollen barrier than by an isolation distance of bare ground of the same width (Della Porta et al. 2008). Many research results confirmed that the outer plant rows in a recipient maize field function as a zone that safeguards the centre of recipient fields (Gustafson et al. 2006; Messeguer et al. 2006; Ganz et al. 2007; Sabellek et al. 2007; van de Wiel et al. 2007; Weber et al. 2007; Weekes et al. 2007; Langhof et al. 2008). With a maize barrier of 10–20 m, almost none of the remaining maize harvest in the field contains more than 0.9% GM material. Where isolation distances cannot be implemented, the removal of the first 10–20 m of non-GM maize facing the GM maize field is worth considering.

From an administrative point of view, bordering Bt-maize fields with a pollen barrier of non-GM maize might be favoured since coexistence measures have to be undertaken by the farmer growing GM crops. Not only are Bt-maize growers currently required to undertake coexistence measures, they are also contractually enforced to adopt insect resistance management (IRM) measures. For Bt-maize planting areas larger than 5 ha, a refuge zone of 20% of the transgenic area has to be planted with non-transgenic maize in order to delay the potential resistance development in lepidopteran target pests. The theory underlying the refuge strategy is that most of the resistant pests surviving on Bt-crops will mate with abundant susceptible pests from refuges, and that the hybrid progeny originating from such matings will be killed by Bt-crops, if the inheritance of resistance is recessive (Bates et al. 2005). Both coexistence and IRM measures could thus be combined since the refuge zone could also serve as a pollen barrier. Moreover, by sowing the pollen barrier/refuge zone of non-transgenic maize around GM maize, sowing machinery can be "cleaned" from GM seed remnants. However, from a scientific point of view, it is unclear whether a maize pollen barrier surrounding the donor field reduces the extent of cross-fertilisation as effectively as a pollen barrier of the same depth around the recipient field. Recently, Della Porta et al. (2008) demonstrated that surrounding the recipient field with just two maize rows resulted in the same reduction in cross-fertilisation levels as surrounding the pollen donor with twelve maize rows. Because a maize pollen barrier around the donor is only trapping pollen that flies low and that is not likely to disperse far, the effect of a pollen barrier surrounding the donor field is thought to remain very local and limited (Gustafson et al. 2006; Kuparinen et al. 2007; Langhof et al. 2008). Moreover, in the case of GM herbicide-resistant maize, the cultivation of GM and non-GM maize in the same field might create practical challenges since two different weed management regimes would have to be applied on a single field.

Flowering coincidence: The temporal isolation of GM maize from non-GM maize is another valuable strategy to limit cross-fertilisation between maize cropping systems. This can be achieved by sowing maize at different dates, resulting in a difference in flowering periods (Messeguer et al. 2006; Della Porta et al. 2008). In Spain, for example, non-GM maize sown early in March/April will flower during a short period in June; it will thus mostly fertilise its own silks before GM maize sown in early May starts to flower in July/August. A time lag in flowering synchrony of at least eight days has been proven to reduce the extent of cross-fertilisation between neighbouring maize fields significantly (Messeguer et al. 2006; Palaudelmàs et al. 2007; Della Porta et al. 2008). Sowing non-GM maize early and GM maize late in the season could easily be put into practice in Mediterranean regions. Due to the high infestation of the European and Mediterranean corn borer late in the growing season, there is already a tendency to postpone the sowing of GM maize in irrigated regions in Spain (Messeguer et al. 2007). However, this approach is not feasible in non-Mediterranean regions where the window of suitable weather conditions is too short to postpone sowing, and where this postponement induces yield penalties (Messeguer et al. 2006; Weber et al. 2007; Della Porta et al. 2008).

Crop rotation: Theoretically, farmers might mutually adjust their crop rotations in order to schedule maize crops over different years and to avoid growing GM maize in the proximity of non-GM maize. Such a strategy would demand a very tight discipline and good agreements between neighbouring farmers. In practice, it could be hampered by market-driven production strategies, the share of maize in a specific area, and by growing maize in monoculture, as practised frequently in a number of member states.

GM crop-free regions or GM crop production regions: Although priority is to be given to farm-specific coexistence measures, the European Commission proposes region-wide measures (such as the clustering of GM or non-GM crops) in cases where sufficient levels of purity cannot be achieved by other means (European Commission 2003). An important precondition to installing GM crop-free regions is that farmers jointly decide on a voluntary basis not to grow GM crops in a specific region. If these conditions are met, the competent authority can declare a ban on the cultivation of GM crops for a limited period of time in a specific region. Usually, purely economic considerations (e.g., protection of local traditional agriculture) trigger the decision for the creation of GM crop-free regions. With the installation of a "network of GM crop-free regions", a significant number of such regions has been created all over the EU (Levidow and Boschert 2008). On the other hand, farmers wishing to grow GM crops can demand the creation of GM crop production regions.

Biological confinement: Although most biological confinement tactics are still in their infancy, they could hold great promise to limit the extent of cross-fertilisation between GM and non-GM crops (Chapman and Burke 2006). Instead of inserting transgenes into the nuclear genome, these could be targeted at the organelle genome of plastids and/or mitochondria, generating transplastomic plants (Daniell et al. 2005). Because plastids are absent in pollen of most angiosperm plant species, they are transmitted maternally. Although very low levels of paternal leakage and gene transfer from the chloroplast to the nucleus have been reported in some cases (Ruf et al. 2007; Svab and Maliga 2007), the transmission of cytoplasmic organelles through pollen would greatly reduce the probability of pollen-mediated gene flow. In many plant species such as tobacco, tomato, soybean, cotton and poplar, the usefulness of chloroplast genetic engineering has been confirmed, but it still remains to be achieved in maize (Daniell 2007; Verma and Daniell 2007).

Cytoplasmic male sterility (CMS) is another valuable option to reduce gene flow in maize (Munsch et al. 2007; Weider et al. 2007). CMS plants are characterised by their inability to produce viable pollen. Specific mutations in mitochondrial DNA induce dysfunctions in the respiratory metabolism occurring in anther-tapetum cells during sporogenesis (Budar et al. 2003). Due to this male sterility, CMS plants have been used since the 1950s in maize seed production, as they enable ensuring cross-fertilisations without the need for mechanical or manual emasculation. Within the context of coexistence, the cultivation of CMS GM maize plants might reduce the release of transgenic pollen by up to 80%. To ensure seed set, CMS GM maize plants would have to be interplanted with male fertile maize plants – with either GM or non-GM characteristics – acting as pollen donors. Experimental data show that the use of CMS GM maize hybrid in combination with a second unrelated maize hybrid in the Plus-Hybrid System enables increasing the grain yield in some genetic backgrounds without affecting grain quality, compared with that produced by pure male fertile maize (Stamp et al. 2000; Weingartner et al. 2002, 2004; Feil et al. 2003; Munsch et al. 2007; Weider et al. 2007). However, to make this approach successful, it is important that nuclear fertility restorer genes are absent from the maize breeding pool; otherwise, the mitochondrial CMS trait might be revoked, leading to the restoration of pollen fertility (Pelletier and Budar 2007).

Another currently explored biological confinement system relies on a series of alleles that induces cross-incompatibility between certain maize genotypes. Recipient plants with the homozygous dominant cross-incompatibility allele (GaS) only accept pollen from maize plants with the GaS genotype: non-GaS pollen (ga) from neighbouring hybrids that may or may not contain transgenes will not effect cross-fertilisation. On silks of a heterozygous GaS genotype, pollen with the recessive ga allele competes poorly against GaS pollen. Therefore, ga pollen will only yield partial seed set on styles heterozygous for GaS. However, due to breeding difficulties and genetic side-effects on yield and agronomic performance, the use of the GaS allele as a potential biological confinement system is still in the development pipeline (Hoegemeyer 2005).

4 Challenges Entailed by Large and Fixed Isolation Distances

According to the European Commission guidelines for the development of national strategies and best practices to ensure coexistence, preventive coexistence measures should reflect the best available scientific evidence on the probability and sources of admixture between GM and non-GM crops (European Commission 2003). The selection of appropriate coexistence measures should not only be based on scientific evidence, but measures should also be economically proportionate (= cost-effective) and consider regional and local constraints. Any measures exceeding what is necessary to ensure compliance with the legal tolerance threshold would therefore put an extra burden on farmers wishing to adopt GM crops. This would be in opposition to the EU coexistence objectives aiming at allowing farmers to make a practical choice between conventional, organic and GM crops (European Commission 2003). Several member states are currently imposing or proposing large and fixed isolation distances as the sole means to keep GM inputs from cross-fertilisation below the legal tolerance threshold of 0.9%. In the following sections, it is assessed whether this complies with the science-based, appropriateness, and regional and economic proportionality principles established by the European Commission.

4.1 Science-Based Principle

An analysis of the currently available scientific data on cross-fertilisation shows that in many cases large and fixed isolation distances are excessive from a scientific point of view (reviewed by, e.g., Devos et al. 2005; van de Wiel and Lotz 2006; Hüsken et al. 2007; Beckie and Hall 2008; Sanvido et al. 2008). In practice, shorter isolation distances than those currently proposed by several member states would often be sufficient to ensure compliance with labelling requirements. Cross-fertilisation studies mimicking worst-case commercial on-farm situations demonstrated that isolation distances exceeding 50 m are not always necessary to comply with the labelling threshold of 0.9% in grain maize (Goggi et al. 2006; Gustafson et al. 2006; Pla et al. 2006; Bannert and Stamp 2007; Kraic et al. 2007; van de Wiel et al. 2007; Weber et al. 2007; Weekes et al. 2007; Della Porta et al. 2008). Similar conclusions have been drawn from out-crossing studies performed under real agricultural situations in Spain (Messeguer et al. 2006, 2007) and from predictive vertical gene flow modelling at the landscape level in France (Messéan et al. 2006; Lécroart et al. 2007) and Italy (Mazzoncini et al. 2007). In addition, isolation distances imposed for grain maize might not be appropriate for fodder maize, considering that transgenes present in grains are diluted by vegetative plant parts in fodder maize once harvested (Weber et al. 2007; Hüsken and Schiemann 2007). In many cases, less or no spatial isolation may be required to comply with the tolerance threshold (Devos et al. 2005; Messeguer et al. 2006, 2007; Messéan and Angevin 2007; Sanvido et al. 2008). This may especially be the case with (1) larger and more spatially isolated recipient fields; (2) recipient fields located in an upwind position from the closest pollen source; (3) recipient fields isolated by physical and/or natural barriers (e.g., trees, hedgerows); or (4) non-GM maize plants showing a time lag in flowering period compared with GM maize (Messeguer et al. 2006, 2007; Palaudelmàs et al. 2007; Della Porta et al. 2008).

4.2 Appropriateness Principle

A number of prospective case studies and model simulations have shown that large and fixed isolation distances can be inappropriate in some cases. In areas where maize is grown on a substantial part of the agricultural area and/or where maize fields are small and scattered throughout the cropped area, the implementation of large isolation distances might not be feasible in practice (Perry 2002; Dolezel et al. 2005; Messéan et al. 2007; Devos et al. 2007, 2008a,e; Sanvido et al. 2008). Where maize fields are located in close proximity to each other, it is highly probable that isolation perimeters surrounding GM maize fields would interfere with adjacent non-GM maize fields, in turn affecting the farmers' freedom of choice to grow GM maize. Using geographic information system datasets and Monte Carlo simulations, Devos et al. (2007, 2008a,e) investigated how isolation perimeters around GM maize fields might affect the possibility of farmers to grow GM maize on their fields in Flanders (Belgium) (Fig. 3). With isolation distances larger than 50 m, non-GM maize fields would often be situated within the isolation perimeter imposed for GM maize, especially in areas where (1) a lot of maize is grown; (2) the share of GM maize is high; (3) GM maize is grown on a high number of small maize fields; and/or where (4) GM maize is randomly allocated to maize fields.

Although an isolation distance is generally implemented concentrically around GM maize fields, a GM crop adopter might theoretically also try to achieve the isolation inside his own field if mutual agreement with neighbouring non-GM farmers cannot be found. However, due to the small size of maize fields in certain European regions, this approach may not often be practicable. The area covered by a buffer zone of 25 m is equivalent to approximately 75% of a squared field of 1 ha, 51% of a 3-ha field, 40% of a 5-ha field, and to 24% of a 15-ha field. To cultivate 1 ha of GM maize with a buffer zone of 25, 100 and 200 m imposed by law, fields should have a size of 2, 9 and 25 ha, respectively. Using average Italian farm and field characteristics, Lauria et al. (2005) calculated that less than 4.6% of all Italian farms would have the minimum area necessary to cultivate almost 1 ha of GM maize if buffer zones of 200 m would have to be implemented inside the field of GM maize. However, while the static relationship between the proportion of land available for GM crops and the isolation distance has been recognised in scholarly research on coexistence (e.g., Perry 2002; Beckmann and Wesseler 2007), the dynamic effects have been largely ignored by the scientific community and policy-makers (see Sect. 4.4).

4.3 Regional Proportionality Principle

Considering the existing scientific data, it can be argued that policy-makers enforcing fixed isolation distances do not always take into account a number of factors that largely affect cross-fertilisation in maize. These include regional heterogeneity in (GM) maize share, cropping patterns, field characteristics and distribution, as well as meteorological conditions such as wind direction and speed (Messéan et al. 2006; Lipsius et al. 2007; Devos et al. 2007, 2008e; Ganz et al. 2007; Hoyle and Cresswell 2007; Lécroart et al. 2007; Viaud et al. 2007; Lavigne et al. 2008). Currently imposed or proposed fixed isolation distances mostly ensue from cross-fertilisation studies that were performed under worst-case commercial on-farm situations: the pollen source is grown next to or completely surrounded by a recipient field, and parental plants flower synchronously. As experimental worst-case conditions might not often arise in practice, fixed isolation distances might be too conservative under real agricultural conditions. Under real agricultural conditions, fields may be planted with GM and non-GM maize varieties with different sowing or flowering dates, and maize fields may be mixed with other crops and with physical and/or natural barriers (Devos et al. 2005; Messeguer et al. 2006, 2007; 2007 Sanvido et al. 2008).

4.4 Economic Proportionality Principle

As yet, very few studies have acknowledged that coexistence is only relevant if there are economic incentives for farmers to supply both GM and non-GM maize

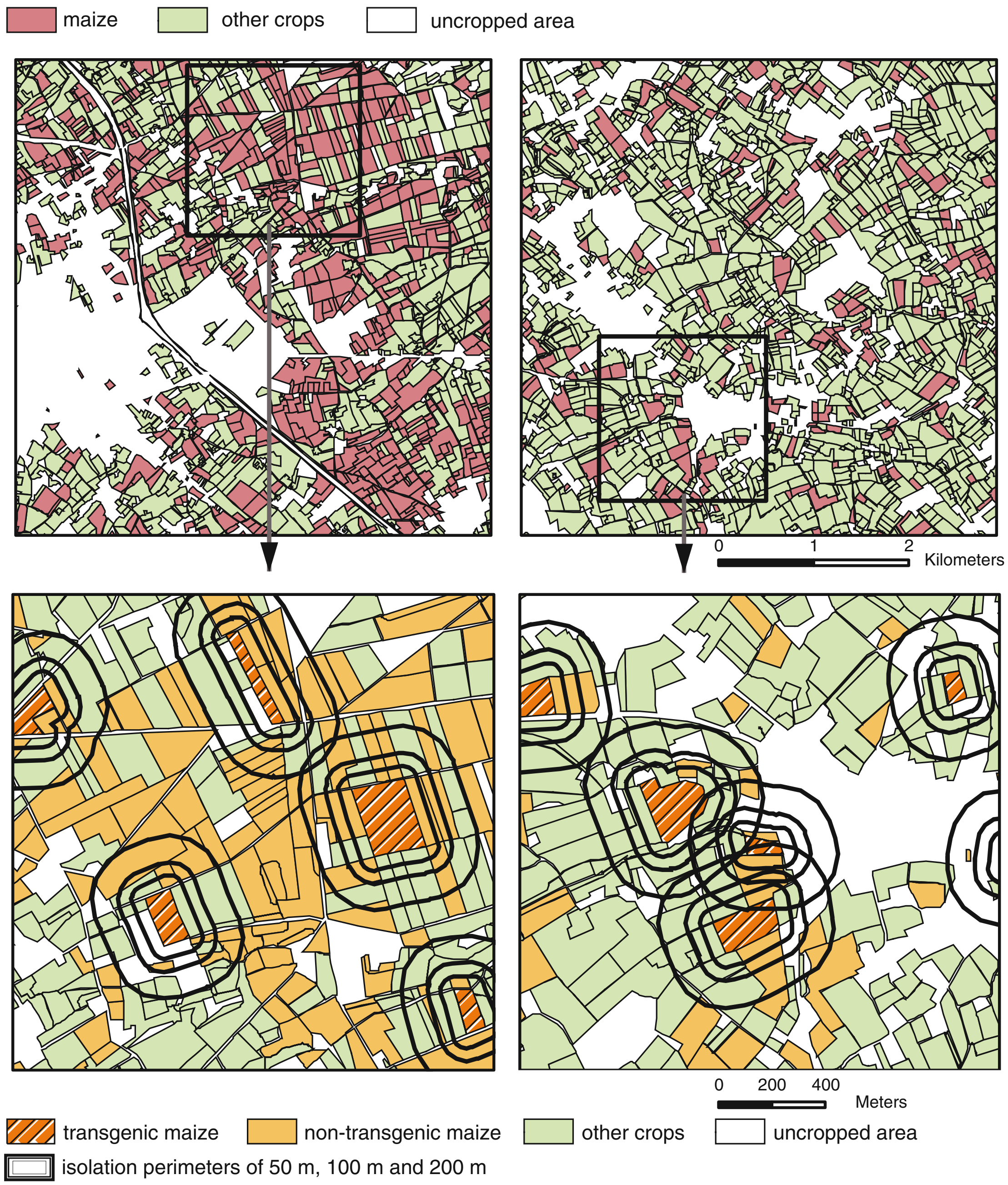

Fig. 3 Maps of selected squares of 25 km^2 in an area with a high share of maize (Bocholt) [*left*] and in an area with a low maize share (Anzegem) [*right*] in Flanders (Belgium). *On top:* share of maize fields. *Below:* concentrically implemented isolation perimeters of 50, 100 and 200 m around some fields planted with genetically modified (GM) maize (Devos et al. 2007, 2008a,e)

(Demont and Devos 2008). Economic incentives for coexistence consist either of (1) the adoption of GM maize as a way to capture "GM gains" or (2) the identity preservation (IP) of non-GM crops as a way to capture "IP gains". GM gains represent economic benefits related to the adoption of GM crops and include productivity and efficacy increases, and production cost reductions, as well as non-pecuniary benefits such as increases in management flexibility (Alston et al. 2002; Demont and Tollens 2004; Demont et al. 2004, 2007, 2008a; Marra and Piggott 2006). IP gains stand for the total additional income generated by price premiums captured for non-GM crops compared with GM crops. If there is a substantial demand for non-GM crops, this will be reflected by a market price premium for IP crops (Bullock and Desquilbet 2002). However, if the content of GM material in IP crops exceeds the tolerance threshold of 0.9%, non-GM crops have to be labelled as "containing GM material" and commercialised at the same price level as GM crops, without yielding any price premium. Even though IP crops do not have to be labelled, it is still the case that costly IP activities are necessary to guarantee the truthfulness of the (implicit) "non-GM" claim. Further down the market chain, incentives to incur the cost of segregating GM and non-GM products naturally reside with suppliers of the "superior" (non-GM) product (Lapan and Moschini 2004).

The balance between GM gains following the adoption of GM maize and price premiums paid for IP maize largely dictates the share of GM and non-GM maize and therefore coexistence (Demont and Devos 2008). Hence, both economic incentives for GM and non-GM maize are vital: if one of them is lacking, coexistence is not a problem of concern because either GM or non-GM maize will not be cultivated. Farmers will only adopt GM maize – and thus invest in imposed coexistence measures – if the benefits of using GM maize exceed the costs of the technology plus the costs of implemented coexistence measures. Other farmer segments might gain more from preserving the "non-GM" status of their production: where price premiums for IP products can be captured due to higher market prices, farmers opting for non-GM maize will have economic incentives to apply coexistence measures. By applying coexistence measures, they avoid adventitious mixing, in turn ensuring a non-GM maize production. As long as benefits exceed costs of growing non-GM maize, these non-GM maize farmers will continue to invest in coexistence measures. Since potential GM gains are lost if a farmer opts for non-GM maize (= opportunity cost) instead of GM maize, IP gains must compensate for lost GM gains (Demont et al. 2008b).

Due to the limited adoption of GM maize in the EU (Table 1), so far no economically important coexistence issues have been reported, even in Spain, the largest GM maize adopter. However, a recent case study focusing on the interplay between incentives and costs of coexistence suggested that imposing large and fixed isolation distances by law is not proportional to the economic incentives of coexistence (Demont et al. 2008b). Under low IP gains (when consumers are not willing to pay significant price premiums for non-GM crops), large and fixed isolation distances generate substantial opportunity costs for GM crop producers as the latter forego GM gains, whilst they are hardly capturing any compensatory IP gains. Under these conditions, if farmers still incur costs due to mere compliance with EU coexistence laws, coexistence costs would not reflect (and hence, would not be proportional to) the economic incentives for coexistence, simply because the incentive – capturing IP gains – is lacking.

On the other hand, under high IP gains (when consumers are willing to pay substantial price premiums for non-GM crops), rational farmers who forego GM gains will attempt to compensate for these opportunity costs by planting non-GM crops and trying to capture IP gains by avoiding any adventitious mixing from GM crops. However, in doing so, they risk triggering a domino-effect at the landscape level that will affect the farmers' freedom of choice to grow GM maize. The domino-effect is a dynamic spill-over effect of farmer decisions induced by enforcing large isolation distances on potential GM crop adopters. It consists of the iterative process of farmers switching their planting intentions from "GM" to "IP" crops to comply with isolation distances and hereby restricting planting options of neighbouring farmers. The domino-effect exacerbates the non-proportionality of large isolation distances by reducing GM crop planting options in the landscape and raising opportunity costs for GM crop adopters (Demont and Devos 2008; Demont et al. 2008b, 2009).

Farmers will only have an incentive to supply IP crops if consumers have (1) strong and sustainable preferences for non-GM crops and (2) are willing to pay significant price premiums for them. If the opposite holds, there is no coexistence issue stricto sensu and coexistence costs will purely reflect the costs of compliance with EU coexistence laws instead of the economic incentives for coexistence. Non-GM crops will not necessarily become more expensive in absolute terms. It may well be that, in equilibrium, average crop prices have decreased as a result of the cost-reducing effect of the GM technology and negative consumer preferences for GM crops, while IP crops are sold at the pre-existing non-GM crop prices. Hence, the IP price premium does not refer to the absolute but to the relative price difference between IP and GM crops.

5 Flexible Coexistence Measures

Based on the presented facts, it can be concluded that large and fixed isolation distances, as currently legally imposed or proposed by several member states, do not comply with the general coexistence principles established by the European Commission: they are (1) excessive from a scientific point of view; (2) difficult to implement in practice; (3) rarely proportional to the regional heterogeneity in the agricultural landscape; and (4) not proportional to the farmers' basic economic incentives for coexistence. To enable appropriate (i.e., a regionally and economically proportionate) coexistence in the long run, it would be necessary to build in a certain degree of flexibility into ex ante coexistence regulations. It may be justified to apply the "newcomer principle" in coexistence regulations with regard to the *financial responsibility* of undertaking coexistence measures and enforce GM crop adopters to reimburse non-GM farmers, provided that the latter agrees to undertaking the measures to ensure coexistence. However, enforcing the *civilian responsibility* of undertaking coexistence measures on GM crop farmers introduces rigidity in regulations, whereas leaving measures open for negotiation between farmers introduces flexibility. Hence, policymakers could support flexibility by allowing plural coexistence measures that are negotiable between farmers on a case-by-case basis, and that are adaptable to different regional and local situations (Furtan et al. 2007; Messéan and Angevin 2007; Demont and Devos 2008; Demont et al. 2008b; Devos et al. 2008e).

In line with the European Commission's guidelines on coexistence, flexibility would enable the development of coexistence arrangements that are adapted to local farming and cropping systems, landscape patterns, farmer strategies and preferences, and to meteorological conditions. Because farmers are heterogeneous with respect to field conditions, managerial expertise, education, market access, pest infestation, and hence the gains they capture from adopting GM crops, flexible measures would be better adapted to the heterogeneity of GM gains (Demont et al. 2008a).

Flexible measures could be designed to be negotiable among GM and non-GM farmers, because both farmer segments have economic incentives to ensure coexistence in the long term. Theoretically, a pollen barrier of non-GM maize – which is a better-suited measure for building flexibility into coexistence regulations than isolation distances – can be planted and cultivated by the GM maize grower, at the expense of an opportunity cost that is equal to the lost GM gain for the area planted with non-GM maize. If the "newcomer principle" is adopted with regard to the financial responsibility of undertaking coexistence measures, a pollen barrier can also be grown by the neighbour, in return for a compensation payment. In the latter case, the area planted with the pollen barrier of non-GM maize is harvested separately, sold as "GM" and, hence, the non-GM farmer does not benefit from any IP gains. The cost of the pollen barrier would, however, be equal (and, hence, proportional) to the lost IP gains. This cost borne by the non-GM farmer could be reimbursed by the GM farmer through a compensatory payment. Demont et al. (2009) illustrated that flexible regulations could be designed in such a way that they encourage farmers to minimise total (opportunity, transaction and operational) coexistence costs, while at the same time satisfying the proportionality condition. If IP gains are negligible compared with GM gains, farmers who grow GM maize will have incentives to persuade neighbouring non-GM farmers to plant a pollen barrier on their field in return for a compensatory payment proportional to their foregone IP gains. They might even persuade the latter to grow GM maize on their fields in order to further minimise costs. If IP gains rise, the opportunity cost of pollen barriers will rise proportionally until it is cheaper for GM farmers to move the pollen barrier to their own field. Further rising IP gains will not affect coexistence costs as all pollen barriers will be planted on GM farmers' fields at an opportunity cost proportional to the GM gain. However, some GM farmers may be attracted by the high IP gains and abandon GM crop production, depending on the magnitude of their GM gains.

It can be observed that national and regional authorities are generally reluctant to adopt flexible coexistence measures due to difficulties in making them operational both from a legal and from an administrative point of view. Some member states have nevertheless already attempted to introduce some flexibility into ex ante coexistence regulations. In the Czech

Republic, for example, farmers can shorten the isolation distance of 70 m towards fields planted with maize provided that every two metres of isolation distance is replaced by one buffer row of non-GM maize around the GM maize field. In Sweden, farmers are able to choose isolation distances from 15 to 50 m depending on the type of maize and on the number of transgenes contained in GM maize hybrids (European Commission 2006).

Computer-based decision support tools may play a crucial role in a future case-by-case-based coexistence approach. They enable the prediction of potential levels of adventitious presence of GM material in the harvest of neighbouring maize fields under various agricultural conditions, and hence the achievable level of coexistence. At the local and regional level, farmers can assess in which maize fields it would not be possible to comply with the established tolerance threshold, and under which conditions both GM and non-GM maize can be grown simultaneously or in close proximity. Outcomes generated by computer-based decision support tools are expected to provide advice to farmers, administrators and policy-makers about the most optimal preventive coexistence measures to be put in place (Beckie and Hall 2008). Examples of such tools, which are currently under validation, include (1) the global index by Messeguer et al. (2006, 2007); (2) the matrix-based approach to a pollen dispersal (MAPOD) model by Angevin et al. (2008); and (3) the SIGMEA maize coexistence (SMAC) Advisor by Bohanec et al. (2007). Although such a case-by-case-based approach will demand much administrative effort, it may be an important step forward in making coexistence workable in practice, and in reaching appropriate and regionally and economically proportionate coexistence at the regional and landscape levels.

6 The Coexistence Paradox

Focusing on the broad range of isolation distances proposed by several member states to ensure the spatial coexistence between maize cropping systems, one might presume that the coexistence policy objectives of some member states do not solely aim at keeping the adventitious presence of GM material in non-GM maize products below the tolerance threshold of 0.9%, but at totally avoiding any adventitious presence of GM material. The broad range of isolation distances proposed by member states cannot simply be explained by different interpretations of available cross-fertilisation data, possible error intervals and uncertainties inherent in the scientific process. Moreover, some member states (e.g., Austria) prescribe isolation distances towards ecologically sensitive areas such as nature conservation areas (Dolezel et al. 2007; Levidow and Boschert 2008). This illustrates that more than economic issues, as defined in the European Commission's coexistence guidelines, are at play in the coexistence debate since isolation towards nature conservation areas represents a safeguard measure related to the environmental safety of an approved product. Although it is often mixed into the coexistence debate, safety issues fall outside the remit of coexistence since these crops were judged to be safe prior to their commercial release (Schiemann 2003; De Schrijver et al. 2007a; Sanvido et al. 2007).

Viewed in a broader societal context, the diversity of proposed isolation distances reveals conflicting rationales on coexistence. One group of actors attaches itself to the European Commission's definition, which states that coexistence purely refers to the potential economic loss and impact of the admixture of GM and non-GM crops. Another group, in contrast, extends the economic issue, mentioning different additional concerns related to genetic engineering. By broadening the debate, they consequently fuel the confusion about the wider discussion on the acceptability of genetic engineering and that on coexistence of different cropping systems. The techno-scientific discussion about isolation distances is in fact hiding an underlying discussion about the type of agriculture wanted in the EU. Thereby, it is debated whether GM crops might play a role in the type of agriculture wanted and whether they might contribute to the construction of a sustainable system of crop production. On an even more fundamental level, one can detect a conflict of values pertaining to the importance of individual freedom of choice and to the trust in markets as regulators of consumer preferences. It may be argued that the broad range of isolation distances, which is supposed to satisfy standards of EU legislation, is in fact reflecting a coexistence paradox that effectively accommodates an irreconcilable divergence of positions towards GM crops.

6.1 Opponents' Rationale on Coexistence

Several lines of argumentation can be identified when looking at the reasoning put forward by opponents to explain their aversion towards agro-food biotechnology applications. Opponents perceive GM crops as being a further step in the industrialisation of agriculture. With the adoption of GM crops and their associated management practices, agricultural developments follow an agro-industrial path, which is associated with high productivity and efficiency (e.g., monocultures, genetic uniformity) in order to compete with standardised agricultural commodities on a global market (Hubbell and Welsh 1998; Marsden 2008; Russell 2008). Opponents expect that GM crops will undermine agricultural developments focusing on added value of agricultural commodities (e.g., local speciality "niche" products) and environmentally friendly production systems such as organic agriculture (Verhoog et al. 2003; Altieri 2005; Levidow and Carr 2007; Binimelis 2008). Moreover, opponents claim that the dependence of farmers on the biotechnology industry would increase due to the need to rely upon specific chemicals for pest control, and that technology fees related to the adopted GM crops would increase input costs and create a culture of surveillance (Beckie et al. 2006).

GM crops and their associated management practices are further thought to reinforce adverse environmental effects and the negative impact of farming on biodiversity due to intensive agriculture. The vicious "agro-chemical treadmill" would be perpetuated and even aggravated without marking a substantial break with the environmentally harmful past of intensive agriculture. Through the reliance on a component-based chemically intensive production system, symptoms of agricultural problems would be treated rather than causes. Opponents claim that the reliance on good agricultural practices (e.g., sound crop rotation) would take away many causes of agricultural problems, in turn making some current chemical-based therapies redundant (Hubbell and Welsh 1998; Graef et al. 2007; Malézieux et al. 2008; Powles 2008). Instead of being a remedy to current agricultural problems, as claimed by proponents, GM crops are therefore perceived by opponents as a new source of problems that are even worse than those GM crops were meant to solve. Opponents point to a number of different environmental and agricultural drawbacks associated with the cultivation of GM crops such as (1) the development of noxious, invasive weeds and the loss of the genetic identity of native species due to vertical gene flow to cross-compatible wild/weedy relatives; (2) the invasion of GM crops into natural habitats; (3) adverse impacts on non-targeted species; (4) the disruption of biotic communities, including agro-ecosystems; (5) the development of resistance in the targeted pest/pathogen population; (6) the reduction or loss of farmland biodiversity; and (7) negative changes in physical, chemical and biological soil characteristics, resulting in decreased soil quality. Finally, appealing to the unnaturalness and irreversibility of genetic modification, various actors describe the technology as involving a high level of scientific uncertainty, thus necessitating strong precautionary measures in order to avoid a technology out of control (Brom 2000; Verhoog et al. 2003; Streiffer and Rubel 2004; Madsen and Sandøe 2005; Lassen and Jamison 2006).

Various actors oppose to the possible integration of GM crops into existing agricultural systems and regions through the installation of GM crop-free regions and through the promotion of large and fixed isolation distances in ex ante coexistence regulations. Opponents thereby often refer to a number of consumer surveys (such as the Eurobarometer) indicating that large parts of the European public seem to share scepticism towards GM crops (Gaskell et al. 2006). The argument is therefore that GM crop-free regions, ensuring more "natural" food and feed production, are in line with consumer preferences. According to Jank et al. (2006), GM crop-free regions create a specific image for marketing regional products and services such as tourism. By preventively banning GM crops in certain areas, opponents are not only defending alternative "less industrialised" cropping systems (Marsden 2008), but they are also protecting the "perceived" value of potentially affected agricultural regions (Kaiser 2007). Within this context, pressure groups, regional/local governments, municipalities and farmers forged coalitions and succeeded in putting their prerogatives (including their sovereignty) on the agenda. Through the creation of an impressive number of GM crop-free regions across the EU, these coalitions imposed their democratic right to decide whether GM crops can be cultivated in their region. As such, coexistence is no longer a matter of private choice of farmers, who should have the freedom to choose between conventional, organic and GM crops. In effect, they claim the right to locally decide and interpret questions of safety and ensuing precaution relating to GM crops independent from the European level. From an ethical point of view, it is interesting to note that the stress on regional/local sovereignty is bought at the price of individual freedom of choice. It may thus not be too far fetched to maintain that an underlying conflict of values is one of the driving forces behind this opposition.

Finally, because the interests and preferences of non-GM crop adopters are perceived to be not fairly balanced against those of GM crop adopters and due to the individualisation of liability and redress schemes, opponents claim that coexistence will promote conflicts and ruin personal relationships between neighbouring farmers. Interests and preferences of a small group of early GM crop adopters are anticipated to outbalance those of an agricultural minority system (e.g., organic farming) (Bello et al. 2007).

6.2 Proponents' Rationale on Coexistence

In contrast to the opponents' view, proponents attach themselves to the European Commission's definition on coexistence, exposing a different view on the role of agricultural biotechnology. In their opinion, coexistence is feasible, provided that (1) techno-scientifically-based coexistence measures are implemented proportional to economic incentives; (2) good agricultural practices are followed; and (3) good agreements are made between farmers. Because agro-food biotechnology applications undergo a thorough risk assessment prior to commercialisation, proponents argue that GM crops have been proven to be safe and even safer than their conventional counterparts. Moreover, they see GM crops as a more sustainable alternative to current crop production systems that would help to minimise or even remedy adverse effects of intensive agriculture. This includes the substitution of environmentally harmful input factors by less harmful ones and improved eco-efficiency through the reduction of external chemical inputs (such as pesticides and fertilisers).

In areas with high infestation of the European and Mediterranean corn borer, claimed benefits of Bt-maize are (1) higher yield levels compared with non-GM maize varieties; (2) less pesticide treatments; (3) lower pest damage, resulting in decreased levels of mycotoxins (e.g., fumonisin); and therefore (4) enhanced safety and quality for animal and human consumption (Demont and Tollens 2004; Wu 2006 2007; Gómez-Barbero et al. 2008). Proponents even argue that greater efficiency, productivity and management flexibility would enhance economic competitiveness. In the case of GM herbicide-resistant crops, the biotechnology-based weed management strategy is thought to replace a set of currently used herbicides by broad-spectrum, non-selective herbicides with better environmental profiles, and to reduce the amount of active ingredients applied and herbicide doses used (Nelson and Bullock 2003; Brimner et al. 2005; Cerdeira and Duke 2006; Graef et al. 2007; Kleter et al. 2007, 2008; Bonny 2008; Devos et al. 2008b; Duke and Powles 2008; Gardner and Nelson 2008; Shipitalo et al. 2008). The adoption of GM herbicide-resistant crops and their associated management practices might (1) increase the flexibility in timing of weed management; (2) simplify weed management; (3) reduce management time; (4) lower the risk for crop injury; (5) facilitate the adoption of no-till or reduced-till planting procedures; and (6) generate less concern with carry-over damage to rotational crops (Marra and Piggott 2006; Sanvido et al. 2007; Devos et al. 2008b; Duke and Powles 2008; Gianessi 2008). Where higher-than-average herbicide rates and numbers of active substances are needed for weed control, improved control of troublesome weeds combined with a reduction in overall herbicide-use rates and number of used active ingredients might translate into economic benefits for farmers.

Furthermore, proponents would insist that only the market can in the long run provide reliable indications of true consumer preferences, and should thus be allowed to regulate balanced proportions of available GM and non-GM products. However, this statement only holds if actors in the market have access to perfect information. Since the presence of traces of GM material in food is a credence attribute, the problem of asymmetric information arises. The seller of GM products has access to information that cannot be verified by the buyer through searching or experience. If consumers perceive GM products to be different from their traditional counterparts, then demands for the banning of GM products and labelling requirements are rational (Giannakas and Fulton 2002).

The mandatory labelling system was set in place in the EU to reduce resulting welfare losses (Philips 1988). If this labelling system reflects the necessary information for the consumer to satisfy his perception regarding safety and environmental concerns, the welfare losses will be reduced. However, if labelling is not considered reliable or the threshold does not fulfil consumers' needs, welfare losses are created and long-term market indications do not reflect true preferences of consumers. Furthermore, forcing suppliers of GM products to incur labelling costs (such as in the EU) may be counterproductive from a welfare perspective (Lapan and Moschini 2004). Therefore, to achieve a socially desirable outcome, the cost of a trustworthy mandatory labelling regime has to be proportional to the consumers' willingness to pay for IP crops.

In the opinion of proponents, GM crops and their associated management practices enable sustaining intensive agriculture more safely through reduced environmental damage. Therefore, they argue that GM opponents currently misuse coexistence as a pretext to place a new barrier in the path of GM crops. They invert the aforesaid reasoning put forward by GM opponents and question whether an existing agricultural cropping system has the right to take hostage of a new cropping system. The complaint appeals to considerations of fairness towards innovators to prove the viability of their product on the market as long as these are found to be safe (Kaiser 2007).

7 Conclusion

The controversy about and stigma of transgenic agro-food products still hold in the EU (Herring 2008). Although regulations should ensure that different cropping systems can develop side-by-side, coexistence has become another arena of contending values and visions on future agriculture and on the role agro-food biotechnology might play therein (Devos et al. 2008d; Levidow and Boschert 2008). The economic scope of coexistence, as defined in the European Commission's guidelines on coexistence, has been widened with issues of environmental safety, sustainable development of agriculture, globalisation, dependence and protection of local producers. Unsolved debates about the safety of GM crops held at EU or national levels have moved to regional/local levels, where the debate continues in the context of coexistence, displaying at least some features of so-called "not-in-my-backyard" (NIMBY) arguments (Kaiser 2007). Thereby, any distinction between environmental, agricultural, economic and socio-ethical issues proved to be blurred, fuelling the confusion about the wider debate about the acceptability of genetic engineering and the coexistence of GM and non-GM crops in the EU (Devos et al. 2008d; Levidow and Boschert 2008). The main conflict line is between those that promote agro-food biotechnology applications as a safe and sustainable alternative to current crops and agricultural management practices, and those that defend less-industrialised cropping systems – as a future "alternative" agricultural path – by preventively banning this novel agricultural technology.

In principle, the maintenance of different cropping systems should be ensured in European agriculture by tolerating a certain level of adventitious mixing between cropping systems. In practice, however, there seems to be low or no political willingness to tolerate any adventitious mixing from GM crops in some EU regions. To comply with the zero tolerance policy in these regions, large and fixed isolation distances are imposed by law in ex ante coexistence regulations. However, legally imposing large and fixed isolation distances entails various challenges. Based on the performed review, it is concluded that large and fixed isolation distances do not comply with the general coexistence principles set by the European Commission: they are (1) excessive from a scientific point of view; (2) difficult to implement in practice; (3) rarely proportional to the regional heterogeneity in the agricultural landscape; and (4) not proportional to the farmers' basic economic incentives for coexistence. Therefore, one could interpret the deliberate use of large and fixed isolation distances as the sole preventive coexistence measure in ex ante coexistence regulations as a new local substitute for the lifted de facto moratorium. One could even go a step further by arguing that the use of large and fixed isolation distances is complementing similar political attempts intending to place a barrier on the path of GM crops. These include invoked safeguard clauses, which provisionally restrict or prohibit the use and/or sale of approved GM agro-food products on national territories or the proclamation of GM crop-free areas, which are currently emerging all over the EU. The irony is that it was the adoption of the EU coexistence policy – as the final building stone of the restyled regulatory frame on GM agro-food products – that contributed to the lifting of the de facto moratorium on new GM crop market approvals in 2004.

To move towards appropriate (i.e., regionally and economically proportionate) coexistence, there is an urgent need to build in a certain degree of flexibility into ex ante coexistence regulations. As such, it remains to be seen whether the coexistence policy will ever succeed in appeasing the contending normative positions raised on agricultural futures and the role agro-food biotechnology might play therein, not to mention letting different cropping systems exist "peacefully" side-by-side in practice.

Disclaimer

Opinions and views expressed in the present article are strictly those of the authors, and do not represent those of the organisations where the authors are currently employed.

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Agro-Environmental Effects Due to Altered Cultivation Practices with Genetically Modified Herbicide-Tolerant Oilseed Rape and Implications for Monitoring: A Review

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Abstract Genetically modified herbicide-tolerant oilseed rape or canola (*Brassica napus* L.) is at the forefront of being introduced into European agriculture. Concerns have been raised about how genetically modified oilseed rape cultivation and the modified cropping practices might impair the agro-environment. The present review compiles and categorises evidenced and potential agro-environmental effects of cultivating genetically modified oilseed rape and assesses the data quality of published references. Cropping practice changes were identified for (a) the introduction of genetically modified oilseed rape cultivation per se, (b) time, mode and rate of herbicide application, and spraying frequencies, (c) soil tillage and cover crops, (d) crop rotations and (e) coexistence measures to avoid mixing of genetically modified and non-genetically modified cultivation systems. Agro-environmental effects identified are directly linked to the herbicide tolerance technology and may impact ecological processes on various scales. The herbicide-tolerant oilseed rape biology, genotype and co-existence constraints with neighbouring agricultural systems also entail various agro-environmental effects. The potential and especially the well-evidenced adverse effects on the agro-environment, according to European legislation, require a systematic monitoring of genetically modified oilseed rape. The most evidenced adverse effects to be monitored are persistence and/or spread of feral herbicide-tolerant oilseed rape and volunteers, transfer of herbicide tolerance to wild relatives and decline in agrobiodiversity, and development of herbicide tolerance in weeds, as well as adverse effects on field organisms and/or soil bio-geochemical cycles. Other well-evidenced potential adverse effects include reduced crop rotation options, increased late-season herbicide drift and pollution, and implications for microbial and faunal activities due to altered agrochemical profiles, as well as implications of feral herbicide-tolerant oilseed rape on neighbouring habitats.

Keywords Agricultural practice • Agro-environmental effects • Canola • Genetically Modified herbicide tolerance • Monitoring • Oilseed rape

1 Introduction

Introducing genetically modified (GM) crops with new traits implies changing farming practices. Among the commercially cultivated GM crops, herbicide tolerance is the dominant trait (68% area), followed by insect resistance (19% area) (James 2006). Of the GM herbicide-tolerant (GMHT) plant species with tolerance to either glufosinate or glyphosate, oilseed rape and its canola cultivars cover 5% of the global biotech area of 102 million hectares. 18% of the 27 million hectares of cultivated oilseed rape is genetically modified. However, growing interest in biofuels is expected to boost oilseed rape acreage and the proportion of biotechnology involved.

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Changes in land use and farming practices (Boutin and Jobin 1998) over the past few decades have been shown to affect farmland biodiversity (Benton et al. 2002; Krebs et al. 1999; McLaughlin and Mineau 1995). The new cropping techniques introduced with both GM and also non-GM (conventionally bred) HT plants (Champion et al. 2003; Hayes et al. 2004; Johnson 2004) may impact farmland biodiversity (Firbank and Forcella 2000). Companies develop and offer new HT varieties that enable farmers to optimise their practice in weed suppression; for instance, using only one complementary herbicide application, reduction to one application only, thus reducing the active ingredient (ai) rate. Some of their direct effects on the agro-ecosystem such as enhanced weed suppression and consequences thereof on fauna were focused on in the Farm Scale Evaluations (FSE) (Firbank et al. 2003a,b). Others (Devos et al. 2004; Hayes et al. 2004; Légère, 2005) discuss a number of indirect agro-environmental effects of their cultivation; for instance, transfer of HT to volunteers or wild relatives by vertical gene flow through pollination and the subsequent formation of interspecific hybrids. Interpreting those effects as solely due to HT plant cultivation is challenging due to the ongoing change of production factors in agricultural practice (Robinson and Sutherland 2002), inherently involving many degrees of freedom.

In accordance with the precautionary principle, the Directive 2001/18/EC regulates the release of GM crops into the agro-environment, applying a step-by-step approval process (European Commission, 2001). Environmental GM crop releases thus need to be accompanied by environmental monitoring to detect potential adverse effects, either direct or indirect, immediate or delayed, on human health and the environment. These adverse effects, for instance, are unacceptable levels of gene flow from GM crops to wild relatives, their spread in the environment or adverse effects on single species or species groups, thus reducing biodiversity. With respect to potential adverse environmental effects of GMHT crops at present, there is an overlapping of competencies between the pesticide Directive 91/414/EEC (European Commission 1991) and the Directive 2001/18/EC on the deliberate release of GMOs. Some of the agro-environmental effects discussed in the following will thus fall into the remit of the pesticide directive.

This review paper systematises and categorises pathways of both direct and indirect potential and evidenced agro-environmental effects of practice changes when introducing GMHT oilseed rape in European agriculture. It does not cite literature where no agro-environmental effects have been recorded, and thus targets issues relevant for GM crop monitoring (European Commission 2001). The sampled indicators, sampling methods and data quality of the literature are evaluated to indicate the evidence for identified effects, which subsequently can be selected for the obligatory monitoring of GM crops.

The term "agro-environment" for this review is defined as the area cultivated with GMHT oilseed rape along with neighbouring fields and biotopes. The term "oilseed rape" in the following includes the canola cultivars. The term "direct effects" is defined as intended results from practice changes, whereas the term "indirect effects" is defined as unintended consequences of either practice changes or preceding intended direct agroecosystem effects.

2 Methodology of Categorising Changes and Agro-Environmental Effects

Practice changes may induce different pathways of agro-environmental effects (Senior and Dale 2002; Squire et al. 2003). It is therefore necessary to categorise practice changes and respective effects on the agro-environment and indicate their pathways using hierarchies (Hayes et al. 2004) and schemes of influencing factors (Graef et al. 2007). However, there is an inherent abundance and diversity of direct and indirect pathways that may be triggered by a single practice change (Fig. 1). Direct effects are directly connected to a practice change, whereas indirect effects occur as a result of preceding direct effects. For instance, the HT technology enhances weed suppression (Owen 1999) and may reduce erosion due to denser plant cover (Agronomy guide 1999/2000), but may increase herbicide drift because spraying height is increased due to later-season spraying along with further developed, higher crops (Johnson 2001). On the other hand, many different changes in agricultural practice may lead to a single agro-environmental effect only; for instance,

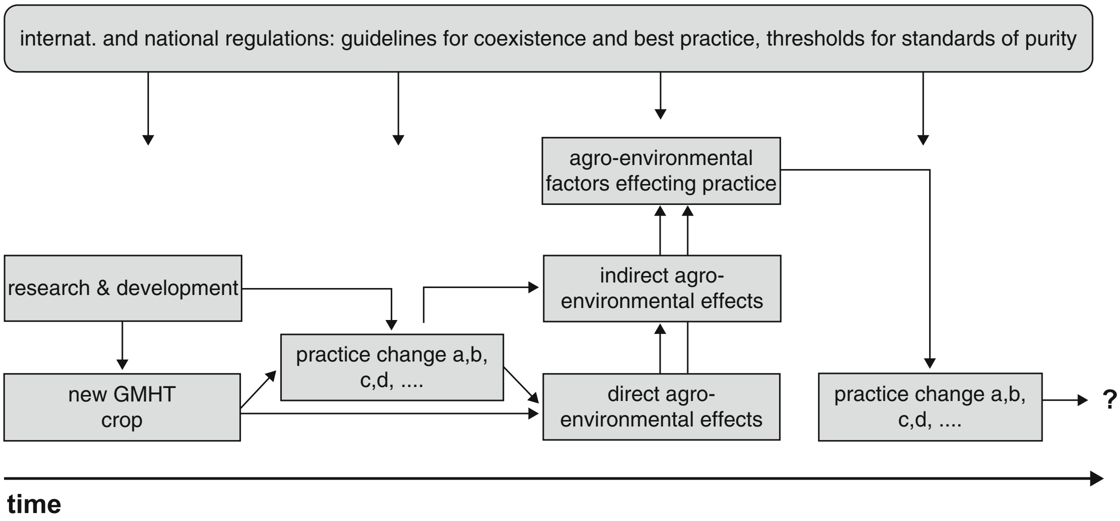

Fig. 1 Conceptual scheme of factors influencing practice changes and subsequent agro-environmental effects when introducing a new GMHT crop (adapted from Graef et al. 2007)

a decline in agrobiodiversity (Benton et al. 2002; Firbank and Forcella 2000).

The present review therefore hierarchically categorises practice changes and agro-environmental effects as strictly as possible. Practice changes and agro-environmental effects may entail indirect changes and indirect agro-environmental effects, respectively, because many pathways have multiple implications and vice versa. This leads to redundant information. Thus, to avoid inflating information this categorisation was not followed exclusively and fully consistently (Table 1). Evidence of observed practice changes and agro-environmental effects was approximated from the referenced sources using evaluation scores for different quality aspects (Krayer von Krauss et al. 2004).

3 Practice Changes with GMHT Oilseed Rape Cultivation

Cultivating GMHT rape instead of conventional rape may lead to direct, indirect, immediate, delayed and/or cumulative practice changes. They can be categorised into the following groups: (a) the introduction of GMHT oilseed rape cultivation, (b) time, mode and rate of herbicide application, and spraying frequencies, (c) soil tillage and cover crops, (d) crop rotations and (e) coexistence measures to avoid mixing of GM and non-GM cultivation systems. Table 1 presents an overview of practice changes with the implementation of GMHT oilseed rape cultivation and their agro-environmental effects.

3.1 *Introduction of GMHT Oilseed Rape Cultivation*

GMHT oilseed rape is not yet authorised for commercial cultivation in Europe but has been cropped for 10 years in the USA and Canada (Benbrook 2004; James 2006). In European agriculture it will introduce a new weed control technology, altering existing cropping systems (Canola Council of Canada 2001; van Acker et al. 2003). Together with the increased acreage of HT oilseed rape, the overall cropping frequency of oilseed rape over the years may increase; at the expense of conventional oilseed rape varieties, the summer fallow acreage may also be reduced (Schütte et al. 2004). Due to increased weed suppression, GMHT oilseed rape cultivation can be expanded to areas which were not suitable for oilseed cultivation before (Beckie et al. 2006).

Table 1 Evidence of agro-environmental effects resulting from practice changes with increased GMHT oilseed rape cultivation and evaluation of data quality (adapted from Graef et al., 2007; Krayer von Krauss et al., 2004; Squire et al., 2003)

Practice changes	Chain of potential agro-environmental effects	References[1]	Evaluation of data quality[2]			
			Proxy[3]	Method[4]	Data validation[5]	Overall evidence[6]
introduction of GMHT oilseed rape and/or increased acreage	increased weed suppression → less biomass, food and flowers for field organisms after spraying → lower abundance of various herbivores, pollinators and beneficial species (pest antagonists) → decrease in agrobiodiversity	1, 3, 4, 5, 6, 7, 9, 10, 25, 37, 38, 39, 40,46, 49	3,3,3,3,3,3,3,3, 2,2,3,3, 3,2,3	2,2,2,3,2,2,2,2, 3,2,3,3, 3,2,2	1,1,1,3,1,1,1, 1,3,2,3,3, 3,2,2	9
	development of herbicide tolerance in weeds	1, 2, 3, 9, 16, 18, 35,54,55	3,3,3,3,3,3, 3,3,3	2,2,2,2,2,2, 3,2,2	1,1,1,1,2,2, 2,2,2	8
	persistence of HT volunteers and/or transfer of HT to neighbouring oilseed rape fields	20, 21, 26, 27, 36, 51, 54, 55, 56	3,3,3,3,3, 3,3,2,3	2,3,3,3,2, 3,2,2,3	3,3,3,3,2, 3,2,2,3	9
	persistence of feral HT oilseed rape (no spread yet documented)	1, 2, 4, 15, 19, 53, 57	2,2,2,3,3,3, 3	2,2,2,3,2,2 3	1,1,1,3,2,2, 3	9
	transfer of one or multiple HT to weedy or wild relatives (no spread yet documented)	1, 2, 4, 17, 18, 19, 33, 57	1,1,1,2,3,3, 3,3	2,2,2,2,2,3, 3,3	1,1,1,1,2,3, 3,2	9
	decrease in crop species diversity → subsequent decrease in agrobiodiversity	1, 3, 7, 9, 12, 30, 31, 32	1,1,1,2,1,3, 3,2	2,2,1,2,2,2, 3,1	1,1,1,2,2,2, 3,1	9
	reduced crop rotation options	1, 3, 9, 20, 23	1,1,1,2,2	2,2,1,1,2	1,1,1,1,2	6
	decrease and/or shift of weedy species and weed seed bank	5, 35, 36, 37, 38, 39, 40, 54, 55	3,3,3,3, 3,3,3,2	3,2,2,3, 3,3,2,2	3,2,2,3, 3,3,2,2	9
	little or no evidence: impact on flora, sedentary invertebrate species, impact on migratory and wide-ranging species, changed quality of leaf litter, changed crop competitiveness, changed insect resistance, pleiotropic and epigenetic genome effects, horizontal gene transfer of HT to microorganisms, impact on soil functions	41, 42, 43, 46, 47, 48, 49, 52, 55	2,2,2,2,2, 2,2,1,2	1,1,1,2,2, 2,2,2,2	1,1,1,1,1, 1,1,1,1	5
→ reduced herbicide ai amount, reduced no. of spray rounds, use of one broadband herbicide only	less negative impacts on field organisms and/or soil compaction	3, 4, 5, 10, 11, 12, 13, 25, 46, 52, 58	3,3,3,2,3,1, 2,2,3,2,2	2,2,3,2,3,2, 2,2,2,2,2	1,1,2,2,2,2, 2,2,1,2,2	8
→ higher herbicide & insecticide applications in formerly uncultivated areas	various adverse effects on field organisms and/or soil bio-geochemical cycles	1, 3, 29, 31, 46, 47	1,1,2,2,3,3	2,2,2,3,2,2	1,1,2,3,2,2	8
→ enhanced precision farming techniques with less pollution and drift	less negative effects on field organisms, neighbouring fields and habitats	1, 4	2,2	2,2	1,1	5
→ glyphosate and glufosinate use instead of other mre persistent or toxic herbicides	less residual activity to followcrops, less adverse effects on field organisms	4, 8, 46, 55, 58	2,3,3,3,2	2,2,2,2,2	1,1,2,2,2	7
→ glyphosate and/or glufosinate use instead of other less toxic herbicides	adverse effects on field organisms and/or aquatic communities in neighbouring habitats	45, 47, 55	3,3,3	3,2,2	3,1,2	9
→ post-emergent spraying	more biomass for feeding organisms until spraying	5, 9, 25, 39, 40	3,3,2,3,3	3,2,3,3,3	3,2,3,3,3	9
	less erosion due to more weed biomass and residues	8, 55	3,3	2,2	2,2	7
	increased drift and pollution due to higher late-season wind speeds and/or increased spraying height	6, 44, 47	3,3,3	2,2,2	1,2,1	7

→ change in spray schedules of insecticides and fungicides due to modified herbicide spraying	positive or negative implications for microbial and fauna activities	1, 4, 9, 13, 37, 47	2,2,3,2,2,2	2,2,2,2,2,2	1,1,1,2,1,1	6
→ minimum till associated with HT oilseed rape cultivation	increased competitiveness of perennial weeds	1, 14, 32	3,2,2	2,3,1	1,3,1	8
	less soil compaction, higher soil biodiversity	1, 8, 9, 24, 34, 55	1,1,2,3,3,3	2,2,2,3,3,2	1,1,1,2,3,2	8
control of HT oilseed rape volunteers in followcrops	reduced crop rotation options (e.g. wider rotations or crops with other HT traits)	20, 21, 22, 23	3,3,3,3	3,3,3,2	3,3,3,3	9
	→ various positive or negative implications for field organisms and soil bio-geochemical cycles	1, 2, 3, 16, 32	1,1,1,2,2	1,1,1,2,1	1,1,1,1,1	5
	changes in tillage system → positive or negative implications for soil degradation and erosion	1, 3, 17, 20, 21, 22, 23, 32	1,1,2,2,2,1, 1,2	1,1,2,2,2,2, 2,2	1,1,1,2,2,2, 1,2	6
control of increased HT in weeds	increased ai amount, different types of herbicides, higher spraying frequency	1, 2, 3, 12, 13,18, 47	3,2,3,3,2,2, 3	2,2,2,3,3,2, 2	1,1,1,2,3,2, 2	8
	→ various adverse effects on field organisms and/or soil bio-geochemical cycles	1, 2, 3, 16, 17, 25, 29, 32	3,1,3,2,1,2, 3,2	2,2,1,2,2,2, 3,1	1,2,2,2,1,3, 3,1	9
in case of survival or spread of feral HT oilseed rape or wild relatives	various adverse effects on neighbouring habitats	15, 19, 47	2,3,3	2,2,2	1,1,1	6
in case of decreased summer fallow acreage	less food and flowers for various herbivores, pollinators and for beneficial species (pest antagonists)	3, 28, 29, 37	2,2,2,2	1,3,2,2	1,3,3,2	8
in case of increased yield potential → increased fertiliser use	increased nutrient leaching	1, 8, 30	1,1,3	2,3,2	1,1,2	7
coexistence measures to reduce vertical gene flow	reduced crop rotation options, isolating fields of GM oilseed rape, modified tillage, and sowing and harvesting at a modified crop development stage	2, 13, 21, 23, 50	3,3,3,3,2	2,2,2,2,2	2,2,2,2,1	7
	→ various positive or negative implications for field organisms and/or soil bio-geochemical cycles	32, 45, 47	2,2,2	2,2,2	2,2,2	6

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[1] References legend (E: Expert opinions; M: Models; R: Review; O: Original data): 1 (Hayes et al., 2004/E, M); 2 (Devos et al., 2004/R); 3 (Schütte et al., 2004/E, R); 4 (Senior and Dale, 2002/R); 5 (Bohan et al., 2005/O); 6 (Owen, 1999/E); 7 (Krebs et al., 1999/E, R); 8 (Agronomy Guide, 1999/2000/E, O); 9 (Werner et al., 2000/E, M, R); 10 (Madsen et al., 1999/E, M); 11 (Canola Council of Canada, 2001/E, O); 12 (Benbrook, 2004/R, O); 13 (Champion et al., 2003/O); 14 (Frick and Thomas, 1992/O); 15 (Crawley and Brown, 2004/O); 16 (Orson, 2002/E, O); 17 (van Acker et al., 2003/E, R); 18 (Légère, 2005/E, R); 19 (Wilkinson et al., 2000); 20 (Colbach et al., 2005/M); 21 (Gruber et al., 2004/O); 22 (Lutman et al., 2005/O); 23 (Pekrun et al., 2005/M, O); 24 (Jordan et al., 2004/O); 25 (Strandberg et al., 2005/O); 26 (Funk et al., 2006/O); 27 (Damgaard and Kjellsson, 2005/M); 28 (Denys and Tscharntke, 2002/O); 29 (Robinson and Sutherland, 2002/R, O); 30 (Pacini et al., 2003/O); 31 (Benton et al., 2002/R, O); 32 (McLaughlin and Mineau, 1995); 33 (Halfhill et al., 2004/O); 34 (Thorbek and Bilde, 2004/O); 35 (Owen and Zelaya, 2005/O); 36 (Begg et al., 2006/E, R); 37 (Hole et al., 2005/R); 38 (Firbank et al., 2005/O); 39 (Heard et al., 2003b/O); 40 (Heard et al., 2003a/O); 41 (Heinemann and Traavik, 2004/O); 42 (Nielsen and Townsend, 2004/O); 43 (Rensing et al., 2002/O); 44 (Johnson, 2001/E, O); 45 (Relyea, 2005/O); 45 (Squire et al., 2003/O); 46 (Watkinson et al., 2000/M, E); 47 (Züghart and Breckling, 2003/R); 48 (Regal, 1994/E, R); 49 (Firbank and Forcella, 2000/E, R); 50 (Schiemann, 2003/E); 51 (Rieger et al., 2002/O); 52 (Brimner et al., 2005/R); 53 (Garnier and Lecomte, 2006/M); 54 (Beckie et al., 2006/R, O); 55 (Cerdeira and Duke, 2006/R); 56 (Gruber and Claupein, 2007/M, O); 57 (Yoshimura et al., 2006/O); 58 (Kleter et al., 2007/R).

[2] Evaluation of data quality (adapted from Krayer von Krauss et al. (2004): evaluation scores for different quality aspects of sources.

[3] "Proxy" refers to how closely the measured or observed effects and indicators resemble the actual effects and indicators about which information is desired (1: weakly correlated, 2: well correlated, 3: direct measurement.

[4] "Method" refers to the quality, mode and accuracy of the methodological design and to the degree to which empirical or expert observations have been used to produce the data (3: well-established method and precise experiments, 2: acceptable method, indirect measurements, modelled or expert-based data, 1: preliminary methods, thumb estimates).

[5] "Data validation" describes the statistical design, number of replications, spatio-temporal representativeness (3: well-established statistical design, cross-checks against independent sources, long period, 2: existent statistical approach, few replications, not independent measurements, shorter period, review of case studies, 1: weak and indirect validation).

[6] "Overall evidence" is calculated from the sum of maximum scores per single source.

3.2 Time, Mode and Rate of Herbicide Application, and Spraying Frequencies

In HT oilseed rape cultivation, herbicides with a wide spectrum of activity – glyphosate or glufosinate – are often applied at the post-emergence stage until early bolting. Timing is more flexible and the application of only one herbicide simplifies weed control (Champion et al. 2003; Hin et al. 2001). In a few cases, due to the low residual activity of the active ingredients two applications may become necessary (Pallutt and Hommel 1998; Beckie et al. 2006). In conventional agriculture either glyphosate or glufosinate are applied at the preseeding or preemergent stage to clear fields before crop emergence, preharvest as desiccants and postharvest for volunteer control.

With HT oilseed rape, the intention is to reduce the number of spraying rounds (Madsen et al. 1999) and the active ingredient (ai) amount and to rely preferably on one broadband herbicide only, which reduces work and costs to farmers (Beckie et al. 2006; Canola Council of Canada 2001). During the first years of cultivating GMHT oilseed rape, most farmers reduce ai rates and application frequencies (Brimner et al. 2005; Champion et al. 2003; Benbrook 2004). After years of continued cultivation in some areas (a) weeds may become herbicide-tolerant through natural adaptation and selection pressure, especially if different HT crops resistant to the same herbicide are cultivated in the same rotation (Beckie et al. 2006; Devos et al. 2004; Hayes et al. 2004; Service 2007), (b) HT oilseed rape volunteers may occur in subsequent rotations due to harvest seeds falling to the ground (Beckie et al. 2006; Légère 2005) (Fig. 2), (c) HT volunteers may evolve in non-HT oilseed rape fields due to pollen-mediated gene flow from neighbouring HT oilseed rape fields and due to neighbouring volunteers resulting from HT oilseed rape seed banks and seed impurities (Damgaard and Kjellsson 2005; Gruber and Claupein 2007; Pekrun et al. 2005), (d) HT weedy relatives (Daniels et al. 2005) or interspecific hybrids (Devos et al. 2004) may evolve due to pollen-mediated gene flow, (e) the composition of weed communities can change (Cerdeira and Duke 2006; Beckie et al. 2006) and (f) multiple HT oilseed rape may develop due to pollen-mediated gene flow from neighbouring HT oilseed rape fields (Hall et al. 2000). Consequently, ai rates, application frequencies and numbers of ai may increase again, particularly in low-disturbance seeding systems (Senior and Dale 2002).

Fig. 2 Oilseed rape volunteers in winter wheat (source: Dr. Sabine Gruber, Universität Hohenheim, http://www.biosicherheit.de)

3.3 Tillage and Cover Crops

Conservation tillage, no-tillage and cover crops help to prevent soil erosion and generate a higher soil bioactivity (Cerdeira and Duke 2006; Duke 1999). HT crops facilitate the use of enhanced crop cover and no-tillage or reduced-tillage, minimising weed pressure (Légère 2005; Pekrun et al. 2005). Because it requires less tractor use, the practice reduces soil compaction. The use of no-tillage or reduced-tillage systems has greatly increased since the introduction of HT crops (Service 2007). In present European agriculture, glyphosate is sprayed pre-seeding in reduced-till systems and on fallow land; with HT oilseed rape the herbicide is applied after crop emergence. In Canada the recommended integrated weed management is not largely practised (Beckie et al. 2006), but HT oilseed rape is often grown in weedy fields to reduce the weed seed bank in subsequent years. If HT weeds and HT oilseed rape volunteers in the followcrops develop, the necessary control is likely to trigger more intensive tillage (Gruber et al. 2004).

3.4 Crop Rotations

Crop rotations help control pests, diseases and weeds and can save pesticides and fertilisers. With GMHT oilseed rape, both additional and less crop species can be expected for future rotations (Schütte et al. 2004). Seed dispersal of oilseed rape may lead to HT volunteers in subsequent crops of the rotation (Colbach et al. 2005; Gruber et al. 2004, Sweet et al. 2004), which may require specific measures such as wider rotations or crops with other HT traits. In general, most dispersed oilseed rape seeds germinate rapidly, either during the late season or in the following year. However, in case seeds get into deeper soil layers through deep ploughing secondary dormancy can be induced (Devos et al. 2004; Pekrun et al. 2005). As a result, seeds can persist for years in the soil. After growing GMHT oilseed rape, returning to a conventional oilseed rape in the crop rotation may become difficult due to HT volunteers and their seed admixture in the harvest (Messéan et al. 2007).

3.5 Coexistence Requirements

A number of practice changes may also become necessary due to coexistence requirements with GMHT oilseed rape cultivation to avoid GM material presence in non-GM crop production (Devos et al. 2005; European Commission 2003b; Schiemann 2003). They generally aim at reducing vertical gene flow to avoid contamination of non-GMHT oilseed rape; for instance, by increasing the time span between successive rape varieties, isolating fields of GM oilseed rape and introducing isolation distances, modified tillage, and sowing and harvesting at a modified time schedule using other varieties (Colbach et al. 2005; Gruber et al. 2004; Lutman et al. 2005; Pekrun et al. 2005).

4 Effects of Practice Changes on the Agro-Environment and Implications for Coexistence

Analogously to the practice changes with the introduction of GMHT oilseed rape, potential direct, indirect, immediate, delayed and/or cumulative agro-environmental effects may occur. We followed a hierarchical categorisation (Table 1), although in some cases it is difficult to distinguish between practice changes and agro-environmental effects because the latter may also be perceived as indirect practice changes (Graef et al. 2007) triggered by GMHT oilseed rape cultivation. One example is potential changes in tillage systems and crop rotations to control volunteers. Some effects on the agro-environment may be induced by several different direct or indirect mechanisms that may work singly or cumulatively. For instance, the potential decline in agrobiodiversity inter alia may result from increased weed suppression, decreased crop species diversity or change in the agrochemical profile (Champion et al. 2003; Owen and Zelaya 2005; Squire et al. 2003). A number of agro-environmental effects are restricted to the cultivated fields and field margins (Denys and Tscharntke 2002). Others may extend to neighbouring fields and/or habitats (Colbach et al. 2005) and to the wider environment (Züghart and Breckling 2003; Crawley and Brown 2004). Whether effects of GMHT oilseed rape cultivation are considered as being positive, not relevant, relevant for monitoring or even meriting withdrawal of further approval is discussed further below. We grouped the potential agro-environmental effects into three categories.

4.1 Introduction of GMHT Oilseed Rape to the Farming System and Agro-Environmental Effects Directly Linked to the HT Technology

A number of agro-environmental effects of the new technology have a predominantly agronomic relevance. More efficient weed suppression, for instance, is the most prominent aim and effect of the HT technology (Beckie et al. 2006; Bohan et al. 2005; Owen 1999). However, after applying this technology for several years, the potential challenges include, for instance, development of HT in weeds (Benbrook 2004; Owen and Zelaya 2005; Service 2007), a shift of weedy species and the weed seed bank (Heard et al. 2003a; Firbank et al. 2005; Cerdeira and Duke 2006), and the persistence of HT oilseed rape volunteers in subsequent rotations (Sweet et al. 2004; Gruber et al. 2004; Légère 2005). To control HT oilseed rape volunteers in followcrops, crop rotations have to avoid oilseed rape and the HT traits for longer periods and may have to change the tillage system (Colbach et al. 2005; Gruber et al. 2004). This may affect field organisms and soil bio-geochemical cycles (McLaughlin and Mineau 1995; Orson 2002). Increased ai amount, different types of herbicides or higher spraying frequency to control HT in weeds (Hayes et al. 2004; Schütte et al. 2004) may have various adverse side effects on agrobiodiversity. Post-emergent spraying enables the buildup of more biomass for feeding organisms until spraying (Werner et al. 2000; Strandberg et al. 2005) and reduces erosion due to more weed biomass and residues (Agronomy guide 1999/2000). However, post-emergent spraying may also increase herbicide drift into the agro-environment, for instance, due to increased spraying height (Johnson 2001). Post-emergent spraying also often entails a change in spray schedules of insecticides and fungicides, with potential implications for microbial and faunal activity (Champion et al. 2003; Thorbek and Bilde 2004). The HT technology supports minimum till, which reduces soil erosion and compaction, and enhances soil biodiversity, but may increase the competitiveness of perennial weeds (Frick and Thomas 1992; McLaughlin and Mineau 1995).

If the HT technology is widely adopted, herbicide and other pesticide applications in formerly uncultivated areas can be expected, for instance, where weed pressure has not yet allowed cultivation. This may have various potential effects on field organisms and soil bio-geochemical cycles even on a large scale (Benton et al. 2002; Cerdeira and Duke 2006; Robinson and Sutherland 2002).

4.2 Impact on Ecological Processes on Different Scales

Direct agro-environmental effects of applying glyphosate and/or gyphosinate compared with other herbicides have been controversially discussed. Increased mortality of amphibians has been observed by Relyea (2005) and may be possible for other non-target organisms too (Richard et al. 2005; Züghart and Breckling 2003). Some studies, however, indicate less herbicide toxicity and persistency than other herbicides (Agronomy guide 1999/2000; Squire et al. 2003).

While the persistence of non-HT or HT oilseed rape has been evidenced in several habitats (Crawley and Brown 2004), its invasiveness has not yet been proved. Populations that have established outside the agricultural fields often become extinct after 2–4 years (Crawley and Brown 2004). Other studies suggest that feral oilseed rape populations can persist far longer (8–10 years) (Pessel et al. 2001). Unless the habitats are disturbed on a regular basis (e.g. herbicide application, soil disturbance) or replenished with seed from seed spillage from passing traffic, feral oilseed rape populations will eventually be displaced. Feral oilseed rape populations thus have been reported along transport routes due to seed spillage (Garnier and Lecomte 2006; Yoshimura et al. 2006) (Fig. 3). Depending on the

Fig. 3 Ruderal oilseed rape on a grass verge next to a country road (source: Barbara Elling, Universität Osnabrück, http://www.biosicherheit.de)

road management practices and herbicides used, the HT populations may persist longer than their non-GM counterparts may.

Oilseed rape has many cross-compatible wild relatives (Daniels et al. 2005). However, viable hybrids that germinate, flower and develop viable seeds are only formed in a few cases; for instance, with Brassica rapa. Nonetheless, cross-compatibility remains a major concern (Hayes et al. 2004). Furthermore, HT oilseed rape, if it becomes invasive, may have various adverse effects on neighbouring habitats (Légère 2005; Züghart and Breckling 2003).

A number of studies have detected changes in the agrobiodiversity as one of the most prominent effects with GMHT oilseed rape cropping (Bohan et al. 2005; Heard et al. 2003a,b; Watkinson et al. 2000): HT allows more efficient weed control, leading to fewer surviving flowering plants to provide food for various feeding organisms. Overall, countryside biodiversity may also be affected over the long term, for instance, due to altering current herbicide management regimes or decreasing the number of cultivated crop species (Werner et al. 2000; Hails 2002).

Some effects, which are less specific for the HT trait but more of general ecological concern, were identified. But their long-term negative impact remains to be definitively proved; for instance, the pleiotropic and epigenetic genome effects of the GM plant (Regal 1994) or the horizontal gene transfer of HT to microorganisms (Heinemann and Traavik 2004). Adverse effects may occur on decomposers and soil organisms (Heuer et al. 2002), thus hampering soil functions or bio-geochemical cycles (Züghart and Breckling 2003). Squire et al. (2003) mention potential effects on sedentary invertebrate species, migratory and wide-ranging species, changed quality of leaf litter, altered crop competitiveness, and changed insect resistance.

4.3 HT Oilseed Rape Biology, Genotype and Effects on Co-existence with Neighbouring Agricultural Systems

The specific oilseed rape biology, i.e. volunteer growth, the high rate of pollen spread and cross-pollination (Begg et al. 2006; Damgaard and Kjellsson 2005) combined with the modified HT genotype, is likely to impair co-existence with neighbouring non-GM agricultural systems (European Commission 2003b; Schiemann 2003). The transfer of HT to neighbouring oilseed rape fields is well evidenced (Colbach et al. 2005; Daniels et al. 2005; Rieger et al. 2002). Genes can be transferred by outcrossing and hybridisation with non-GM oilseed rape crop and wild relatives, and might increase crop and weed management efforts (Crawley and Brown 2004; Wolfenbarger and Phifer 2000). Fitness parameters of GMHT oilseed rape and relative hybrids may be enhanced, especially if selection pressure through herbicide applications is applied, leading to invasiveness into both neighbouring fields and natural habitats (Wilkinson et al. 2000; Snow 2003). HT can also be transferred to volunteers and feral oilseed rape, which can backcross into non-GM oilseed rape (Züghart and Breckling 2003). Different HT cultivars grown nearby can develop stacked genes and lead to volunteer hybrids with multiple tolerances (Hall et al. 2000; Simard et al. 2005). To avoid contamination of non-GMHT oilseed rape or to reduce it below a specified level of purity, a number of practice measures are being discussed (Devos et al. 2004; Gruber et al. 2004).

These practice measures – for instance, increasing the time span within a crop rotation between successive rape varieties, isolation distances between GM and non-GM fields, pollen barriers, modified tillage, and sowing and harvesting at a modified crop development stage – can also trigger various agro-environmental effects as mentioned above, depending on the type of measure (Werner et al. 2000; Champion et al. 2003; Squire et al. 2003).

5 Monitoring Requirements and Reference Basis

According to the Directive 2001/18/EC on the deliberate release of GMOs, monitoring of adverse effects of GMO cultivation must be based on good scientific practice (European Commission 2001). Whether adverse effects are considered relevant to be monitored is determined by an environmental risk assessment (e.r.a.) and by a decision-making process based on scientific evidence (Andow and Hilbeck 2004; Damgaard and Lükke 2001) and/or expert judgements

(Krayer von Krauss et al. 2004; Hayes et al. 2004). As experienced in the GMO debate so far, decisions will also largely depend on political and societal assessments such as defined standards for purity of seed (Devos et al. 2007; European Commission 2003a). Such decisions should also be made based on predefined thresholds of observed agro-environmental effects. In some cases, the e.r.a. may also identify a need and methods for risk mitigation. Potential effects of large-scale cultivation to a small extent can be drawn from the limited-scale studies required prior to market releases (Mellon and Rissler 1995; Lang 2004; Faivre et al. 2004; Prasifka et al. 2005).

It is important to note that some agro-environmental effects identified fall beyond the monitoring remit of the Directive 2001/18/EC of the deliberate release of GMOs. For instance, coexistence restraints such as outcrossing to neighbouring non-GM fields is considered as a socio-economic issue, and monitoring herbicide resistance in weeds should be monitored under the pesticide Directive 91/414/EEC.

Agro-environmental effects of GMHT oilseed rape cultivation compared with conventional oilseed rape or other crops are diverse and manifold. They are difficult to monitor because of constantly changing land use and cultivation systems and production factors (Gafsi 1999; Senior and Dale 2002) and shifting political frameworks (European Commission 2003b; Gaskell and Tanner 1991). Coming new trait generations of GM crops will also have implications for crop management changes (Hails 2002; Lheureux and Menrad 2004) and subsequent monitoring activities. Cultivation systems may also differ depending on the ecoregion (Kropff et al. 2001), requiring the inclusion of different types of spatial agro-ecological reference data (Graef et al. 2005a). Changing crop management factors must be included as covariables in an adaptive GMO monitoring design (Stein and Ettema 2003) using different spatial and temporal scales. Structured information from different agricultural systems is required as a covariable too, if possible using a typology (Orians and Lack 1992; Landais 1998). The monitoring design also implies selecting different indicators depending on the respective scale level, for instance, the enterprise, the landscape or the state level (Osinski et al. 2003). For the biometric analysis of monitoring data, this implies predefining a sufficient number of sites and replications, their spatial distribution, the indicator parameters measured, and their standard deviation. Here, power analysis can be used beforehand to achieve monitoring results with a tolerable error probability (Perry et al. 2003; McDonald 2003; Lang 2004).

6 Assessment on Effect of Practice Changes and Implications for Monitoring

As shown above, both adverse and positive agro-environmental effects can be triggered by practice changes due to HT oilseed rape cultivation (Table 1), whereby GMO monitoring will focus on detecting the adverse effects. Not every potential adverse effect related to GM cropping can be monitored.

Therefore, prioritising and selecting potential adverse effects and related responsive indicators thereof are required. The prioritisation in this review is done by applying the criteria (a) agro-environmental damage (type of effect, magnitude of negative consequences) (European Commission 2001) and (b) scientific evidence of an adverse effect. The prioritisation of scientific evidence was carried out based on an expert assessment of the data quality of available literature (Table 1). Applying the evaluation methodology and criteria of Krayer von Krauss et al. (2004) scores of three (very good quality), two (acceptable quality) and one (weak quality) were given for three quality aspects of sources: (a) proximity of the measured effects and indicators to real effects and indicators about which information is desired; (b) methodological design, mode, accuracy and degree to which empirical or expert observations were used to produce the data; and (c) data validation, statistical design, number of replications and spatio-temporal representativeness. The maximum scores per single source and quality aspects were summed up to achieve an overall rating that shows the present evidence of an observed agro-environmental effect.

Hence, based on the overall evidence of agro-environmental effects resulting from practice changes (Table 1), among all identified effects the most-evidenced adverse agro-environmental effects that need to be monitored (evaluation scores 8–9) might be persistence and/or spread of feral HT oilseed rape and volunteers, transfer of HT to wild relatives and decline in agrobiodiversity, and development of herbicide tolerance in weeds, as well as adverse effects on field organisms and/or soil bio-geochemical cycles. Other well-evidenced adverse agro-environmental effects (evaluation scores 6–7) include increased late-season herbicide drift and pollution, and implications for microbial and faunal activities due to altered agrochemical profiles, as well as implications of feral HT oilseed rape on neighbouring habitats. For a number of adverse agro-environmental effects, only little evidence is available (evaluation scores 3–5). These include impact on migratory species, changed quality of leaf litter, pleiotropic and epigenetic genome effects, horizontal gene transfer of HT to microorganisms, and implications for soil bio-geochemical cycles.

Priorities for monitoring adverse effects may vary regionally due to differing ecoregions in the EC (Kropff et al. 2001) and they will also depend on regionally differing value judgements of environmental damage (Devos et al. 2006). Other criteria, all of which may differ regionally, could also be applied to prioritise monitoring requirements. These are, for instance, (a) the probability and/or uncertainty of the occurrence of an adverse effect (Krayer von Kraus et al. 2004), (b) the convenience of including monitoring networks already established (Graef et al. 2005b), and (c) the practicability of monitoring and measuring specified indicators (McDonald 2003; Stein and Ettema 2003). These, however, have not been included due to the aggregated level of this review. Note that, legally, poorly evidenced or uncertain adverse effects must also be included in the GMO monitoring (European Commission 2001). Examples include those adverse effects which (a) occur in a delayed fashion, for instance, transgene spread into the wild (Crawley and Brown 2004, Wilkinson et al. 2000); (b) happen rarely, for instance, horizontal gene transfer to soil organisms (Nielsen and Townsend 2004); (c) occur indirectly, for instance, tri-trophic interactions (Schuler 2004) and reduction of farmland birds (Benton et al. 2002), and (d) have not yet been foreseen (Hails 2002; Wolfenbarger and Phifer 2000).

Conservational aspects and obligations may also drive monitoring priorities. For instance, adverse GMO effects on the European Natura 2000 network areas protected under the Council Directive 92/43/EEC (European Commission 1992) or on other ecologically sensitive regions may be judged as more important than those on intensively cultivated land.

Knowledge about adverse effects of HT oilseed rape cultivation can be conferred to other HT crops if practice changes coincide. The monitoring requirements, however, by all means must be determined on a case-by-case basis (European Commission 2001).

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Bacillus thuringiensis: Applications in Agriculture and Insect Resistance Management – A Review

Vincent Sanchis and Denis Bourguet

Abstract *Bacillus thuringiensis* (*Bt*) is a sporulating, Gram-positive facultative-aerobic soil bacterium. Its principal characteristic is the synthesis, during sporulation, of a crystalline inclusion containing proteins known as δ-endotoxins or Cry proteins. These proteins have insecticidal properties. The considerable diversity of these toxins, their efficacy and their relatively cheap production have made *Bt* the most widely used biopesticide in the world. It is used in the fight against many agricultural crop pests – mostly lepidopteran and coleopteran larvae – notably in the creation of new plant varieties expressing *Bt cry* genes. For human health, *Bt* can be used for the effective control of populations of several dipteran disease vectors. The aim of this review is to provide an overview of the use of *Bt* for crop protection and to deal with the problem of the emergence of insects resistant to this biopesticide. We will begin by presenting various aspects of the biology of this entomopathogenic micro-organism, focusing on the diversity and mode of action of the insecticidal toxins it produces. We will then present several examples of utilization of commercially available *Bt* products used as sprays or as transgenic crops. Finally, we will describe the principal strategy for the use of *Bt* transgenic plants, developed so as to prevent or delay the emergence of resistance in target insect populations.

Keywords *Bacillus thuringiensis* • Biopesticide • Cry Protein • δ-Endotoxin • Resistance management • Transgenic plants

1 Introduction

The use of entomopathogenic micro-organisms for regulating the populations of insect pests was first proposed at the end of the nineteenth century by several pioneering scientists, including Louis Pasteur. A large range of micro-organisms such as bacteria, viruses, fungi and protozoans have since been identified as potential candidates for use in biocontrol strategies against insect pests (Riba and Silvy 1989). Given the undesirable effects of chemical insecticides and public health problems in tropical countries, these biopesticides – which also present the advantage of having only a minor impact on the environment – have come to occupy a stable, although modest position in the insecticide market. The biopesticide market currently accounts for about 600 million US dollars, or 2% of the worldwide crop protection market, with about 90% of all biopesticide sales involving products based on *Bacillus thuringiensis* (*Bt*). There are many reasons for this success: the larvicidal activity of *Bt* is rapid but sustained, *Bt* can be applied with standard equipment and its effects on beneficial insects and non-target organisms are negligible. The advantages of *Bt* have not escaped biotech companies, which began introducing *Bt* genes into many crop plants, including cotton and maize, at the end of the 1980s. The insertion of these genes leads to the production of *Bt* toxins in various tissues, protecting the plant against attacks by several highly damaging pests. However, the use of these transgenic crops remains highly controversial in Europe, but is increasing year after year over the world. Hence, the cultivation of transgenic plants expressing genetically modified *Bt* genes has increased considerably in recent years, reaching

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more than 32 million hectares worldwide in 2006 (James 2006). This expansion of the area under *Bt* crops has greatly increased the selection pressure exerted on the pests targeted by the toxins, increasing the risk that mutations conferring greater tolerance to *Bt* toxins will be selected. An increase in the frequency of these mutations in pest populations would decrease the efficacy of these genetically modified plants, perhaps even rendering them ineffective. In the face of this risk of resistance, a resistance management strategy specifically adapted to transgenic plants was proposed and has been operational in the US since 2000. It is difficult to estimate the true efficacy of this strategy but, in 2006, six years after its introduction, no increase in resistance level to *Bt* crops has yet been recorded.

2 The Bacterium

Bacillus thuringiensis comprises bacteria from the *Bacillus cereus sensu lato* group capable of synthesizing during sporulation a protein crystal consisting of δ-endotoxins with insecticidal activity. This crystalline inclusion may make up about 25% of the dry weight of the bacterium (Fig. 1). *Bt* was first isolated in 1901, from infected silk worms, *Bombyx mori* (L.), by the Japanese bacteriologist S. Ishiwata (Ishiwata 1901). It was subsequently rediscovered in 1911 by the German biologist Berliner, who isolated it from infected chrysalids of the Mediterranean flour moth, *Ephestia kuehniella* (Zell.), collected from a mill in the province of Thuringe (Berliner 1915). He called this bacterium *Bacillus thuringiensis*. Agronomists soon became interested in the entomopathogenic properties of *Bt*, because small amounts of preparations of this bacterium were sufficient to kill insect larvae rapidly. The first formulation based on *Bt* was developed in France in 1938, under the name "Sporéine", but the first well-documented industrial procedure for producing a *Bt*-based product dates from 1959, with the manufacture of "Bactospéine" under the first French patent for a biopesticide formulation. Commercial formulations of *Bt* consist of spore/crystal preparations obtained from cultures in fermentors; the preparations are dried and used in a granulated or wettable powder formulation for use as a spray. δ-endotoxins are highly diverse, resulting in a generally restricted activity spectrum for each individual toxin, and are innocuous to plants, animals and almost all non-target insects (bees, ladybirds and other auxiliary biological control agents) (Marvier et al. 2007). The industrial-scale production of *Bt* is now well controlled and relatively simple, and is competitive in terms of cost, and this obviously contributes to its success.

Fig. 1 Transmission electron micrograph of a longitudinal section of *Bacillus thuringiensis* towards the end of sporulation, showing the spore (*black ovoid structure*) and the protein crystal with insecticidal properties (*bipyramidal inclusion*). Photo: from Institut Pasteur, Station Centrale de Microscopie Électronique

3 Diversity of the δ-Endotoxins (Cry Proteins) of *Bacillus thuringiensis*

The first gene encoding a δ-endotoxin was entirely sequenced in 1985 (Schnepf et al. 1985). Around 400 *cry* genes encoding δ-endotoxins have now been sequenced (Crickmore et al.,2005). The various δ- endotoxins have been classified into classes (Cry 1, 2, 3, 4, etc.) on the basis of amino acid sequence similarities. These classes are composed of several subclasses (Cry1A, Cry1B, Cry1C, etc.), which are themselves subdivided into subfamilies or variants (Cry1Aa, Cry1Ab, Cry1Ac, etc.). Current nomenclature for δ-endotoxins includes 51 classes (Cry 1 to Cry 51) and a current list of δ-endotoxins genes can be found on the Internet at http://www.lifesci.sussex.ac.uk/home/Neil_Crickmore/Bt/ holo2.html. The genes of each class are more than 45% identical to each other. The product of each individual *cry* gene generally has a restricted spectrum of activity, limited to the larval stages of a small number of species. However, it has not been possible to establish a correlation between the degree of identity of Cry proteins and their spectrum of activity. The Cry1Aa and Cry1Ac proteins are 84% identical, but only Cry1Aa is toxic to *Bombyx mori* (L.). Conversely, Cry3Aa and Cry7Aa, which are only 33% identical, are both active against the Colorado potato beetle, *Leptinotarsa decemlineata* (Say). Other Cry toxins are not active against insects at all, but are active against other invertebrates. For example, the Cry5 and Cry6 protein classes are active against nematodes. More recently, binary toxins from *Bt* designated as Cry34Ab1/Cry35Ab1, active against various Coleopteran insect pests of the Chrysomelidae family have also been characterized. They have been assigned a Cry designation, although they have little homology to the other members of the Cry toxin family. The Cry34A and Cry35A are 14-kDa and 44-kDa proteins, respectively, that function as binary toxins showing activity on the western corn rootworm, *Diabrotica virgifera virgifera* (LeConte) (Ellis et al. 2002). Dow AgroSciences and Pioneer Hi-Bred have constructed transgenic corn expressing this binary toxin (see Table 1).

In addition to the δ-endotoxins, other toxins may be produced by various isolates of *B. thuringiensis*. One such toxin class is the Vegetative insecticidal protein (Vip) 3A (Estruch et al. 1996) which has broad toxicity against lepidopteran species. Genetically engineered products expressing Vip3A are also being evaluated in cotton and maize plants. Although it has similar properties to the δ-endotoxins, the Vip3A toxin has not been classified as a δ-endotoxin.

4 Specificity, Structure and Mode of Action of δ-Endotoxins

δ-endotoxins act on the cells of the intestinal epithelium of susceptible insects. Following ingestion, the crystals first dissolve in the intestinal tract, facilitated by the reducing conditions and high pH typical of the insect gut. δ-endotoxins are in fact protoxins of around 135 kDa. They are cleaved in vivo by the digestive proteases of the host to generate mature toxins

Table 1 Examples of genetically engineered Bt plants approved for sale

Crops	Target insects	Genes	Event	Trade name	Company
Potato	Colorado potato beetle	*cry3A*	Various	New Leaf	Monsanto
Cotton	Bollworms and budworms	*cry1Ac* and *cry2Ab*	15985	Bollgard II	Monsanto
		cry1Ac and *cry1F*	281-24-236 + 3006-21-23	WideStrike	Dow Agrosciences and Pioneer Hi-Bred
Corn	European corn borer	*cry1Ab*	MON810	YieldGard	Monsanto
		cry1Ab	Bt11	Agrisure CB	Syngenta
Corn	Western bean cutworm European corn borer Black cutworm Fall armyworm	*cry1F*	TC1507	Herculex I	Dow Agrosciences and Pioneer Hi-Bred
Corn	Western corn rootworm	*cry3Bb1*	MON863	YieldGard Corn Rootworm	Monsanto
Corn	Western corn rootworm Northern corn rootworm Mexican corn rootworm	*cry34Ab1/35Ab1*	DAS-59122-7	Herculex RW	Dow Agrosciences and Pioneer Hi-Bred
Corn	European corn borer Corn rootworm	*cry1Ab* + *cry3Bb1*	MON810 + MON863	YieldGard Plus	Monsanto
Corn	Western bean cutworm European corn borer Black cutworm Fall armyworm Western corn rootworm Northern corn rootworm Mexican corn rootworm	*cry1F* + *cry34Ab1/35Ab1*	TC1507 + DAS-59122-7	Herculex Xtra	Dow Agrosciences and Pioneer Hi-Bred

of about 65 kDa (the amino-terminal part of the protoxin). The peptide sequence of the carboxy-terminal part of the molecule that is dispensable for toxicity contains almost all the cysteine residues of the protein and is believed to play a role in the formation of disulfide bridges linking δ-endotoxins in the crystal. The high pH and reducing conditions prevailing in the guts of most susceptible insects therefore seem to be necessary for the destabilization of ionic bonding and the disruption of intermolecular disulfide bridges. Some 65–75 kDa Cry proteins lacking the carboxy-terminal extension found in the long protoxins, and that is eliminated by proteolysis, have also been identified, e.g. the Cry2A and Cry3A proteins. Following their solubilization and activation, δ-endotoxins bind to receptors on the surface of intestinal epithelial cells in susceptible insects (Van Rie et al. 1990). The first three-dimensional structure of an activated δ-endotoxin, the Cry3Aa toxin, was determined in 1991 (Li et al. 1991); the toxin is composed of three distinct domains (Fig. 2) and its structure suggests that it is able to create pores in epithelial membranes.

Fig. 2 Three-dimensional structure of activated Cry3Aa toxin. Schematic diagram showing the three domains of the protein. (Image courtesy of D.J. Ellar, University of Cambridge, United Kingdom)

The first domain consists of seven hydrophobic alpha helices, at least five of which have structural characteristics (length, distribution of polar residues) enabling them to insert into the cytoplasmic membrane. The second domain consists of three groups of anti-parallel beta-strands, terminating in loops at the apex of this domain (Fig. 2). Various studies in which one or several of the amino acids present in these loops were modified have shown that these amino acids are involved in the interaction between the toxin and its receptor in insects (Smedley and Ellar 1996). The third domain has a beta-sandwich structure and may be responsible for the stability of δ-endotoxins in the insect gut after activation. However, several studies have suggested that domain 3 may also be involved in the specific binding of the toxin to its receptors (de Maagd et al. 2000, 2003). The specific receptors of some of the proteins of the δ-endotoxin family have been identified and shown to be membrane aminopeptidases (Knight et al. 1994) or proteins of the cadherin family (Vadlamudi et al. 1995). Currently, 38 different aminopeptidases have been reported for 12 different lepidopterans (for a review, see Pigott and Ellar 2007).Bravo et al. (2004) elucidated the stages involved in the binding of Cry1Ab to its receptors and the ensuing interactions between toxin and receptor. Two cadherin-like receptors and an aminopeptidase N act sequentially. The monomeric form of Cry1Ab seems to bind preferentially to cadherin-like receptors and this binding is followed by proteolysis, resulting in a conformational change facilitating toxin oligomerization. The resulting oligomers have a higher affinity for the aminopeptidase N-type receptor, and probably for other glycolipid or sugar molecules of the *N*-acetylgalactosamine type. The phase following the binding of the toxin to its receptor, and the possible contribution of the receptor to toxicity, have not been completely elucidated. However, it is widely believed that the toxin acts by osmocolloidal cytolysis, following the formation of pores in intestinal cells (Knowles 1994). In other words, pore formation may disturb ion exchange, leading to cell lysis. A mechanism involving an intracellular signaling phenomenon following the binding of the toxin to a cadherin-like receptor, leading to apoptosis, has also been proposed (Zhang et al. 2006). However, these two mechanisms (osmotic shock due to the formation of pores and/or apoptosis) are not necessarily exclusive. Intoxication manifests itself physiologically as almost immediate paralysis of the digestive tract, preventing food intake (Angus 1954). This paralysis is followed by destruction of the intestinal epithelium. This results in communication between the haemolymph and the intestinal cavity, leading to a decrease in intestinal pH, in turn allowing the spores ingested with the crystal to germinate and the resulting vegetative cells to multiply in the insect cadaver.

5 *B. thuringiensis* and Its Uses in Crop Protection and Disease Vector Control

Bt is remarkably non-toxic to humans and to a large extent non-target fauna and is easy to use, making it a popular alternative to chemical treatments for crop protection. Most of the *Bt* formulations are used to control many common leaf-feeding caterpillars, including caterpillar pests on vegetables, the larvae of the gypsy moth, *Lymantria dispar* (L.), in forests, and European corn borer (ECB) larvae, *Ostrinia nubilalis* (Hbn.), in corn fields. Despite the immense diversity of the strains containing different *cry* toxin genes only two subspecies of *Bt* have been developed into sprayable products (*kurstaki* and *aizawaï*) to control lepidopteran pests. The most common trade names for these commercial products include Dipel®, Javelin®, Thuricide®, Worm Attack®, Caterpillar Killer® and Bactospeine®, but many small companies sell similar products under a variety of trade names. Similarly, one strain belonging to the subspecies *morrisoni* (known as *tenebrionis*) was developed as a commercially successful product against *L. decemlineata*. The discovery in 1977 of the *Bt* H-14 strain – known as *Bacillus thuringiensis* var. *israelensis* (*Bti*) – which is highly toxic to mosquito and blackfly larvae (both vectors of tropical diseases, such as malaria, onchocercosis and dengue fever) has led to *Bt* being also widely used in the urban control of mosquitoes and the peridomestic and rural control of blackfly. Many commercial *Bt* products that utilize *Bti* are also available; among them Vectobac®, Teknar®, Bactimos® and Skeetal®. The World Health Organization (WHO), through the Onchocercosis Control Program (OCP), has been an important promoter of the use of *Bti* against dipteran larvae. *Bt*-based formulations have been used intensively, since the 1980s, on the rivers of West Africa, with the aim of combating the blackfly species complex, which is responsible for transmitting *Onchocerca volvulus* (Leuck.), a microfilarial parasite causing river blindness. In this vector control strategy, the cycle of transmission is broken by eliminating blackfly larvae by the aerial coverage of fast-flowing rivers with insecticides in 11 countries in West Africa; this required the weekly treatment of up to 50,000 km of river irrigating 1.3 million km^2. In order to assess the environmental impact of such treatments a network of sampling stations was established. Despite the increase in *Bt* use, the ecological assessment by hydrobiologists is reassuring, in that no irreversible effect of the insecticides used on aquatic ecosystems has been detected (Levêque et al. 1988; Calamari et al. 1998). In France, *l'Entente Interdépartementale pour la Démoustication* (EID) – an agreement between administrative districts concerning mosquito control for both economic reasons and to encourage tourism – has led to a pest control program covering several *départements* (French administrative units) and regions (the Atlantic coast, Rhône, Isère, Savoie, the Western Pyrenees, the Mediterranean, etc.). For example, a LIFE-Environment project based on the use of *Bt* was adopted by the Mediterranean EID in 1999 (http://www.eid-med.org). This organization is responsible for mosquito control measures along the whole of the Languedoc-Roussillon coast. Similarly, in Germany, a hundred towns and villages along the Rhine Valley have united to form an organization responsible for mosquito eradication: the Kommunale Aktionsgemeinshaft zur Bekämpfung der Schnakenplage Ludwigshafen (KABS). The KABS covers more than 300 km of river and about 600 km^2 of flood-prone land. Between 1988 and 1999, about 90% of the 170,000 hectares of potential larval breeding sites were treated with preparations based on *Bt* (Becker 2000).

Nevertheless, despite the increasing use of biological insecticides for the control of dipteran vectors of tropical diseases, *Bt* products remain most widely used in agriculture, which still accounts for more than 60% of the market for these bioinsecticides. The distribution of sales in this market remains highly uneven: geographically, 55% of all sales are in North America and only 8% in Europe. In terms of production systems, forests and fruit and vegetable crops account for 80% of *Bt* bioinsecticide use. In the future, the increased competition from transgenic plants and new chemistries may have an impact on sprayable *Bt* products in vegetable, forest and tree fruit markets. However, new market opportunities for *Bt* may arise as consumers seek alternatives to products that have been sprayed with conventional chemical insecticides. Indeed, given the regulations concerning the use of insecticides innocuous to mammals and non-target animals in force for certain types of crops, these products are, in some cases, the only available option in cases of infestation. In 2001, in the US, more than 20,000 ha of brassica and tomato crops (corresponding to 60% of the total area under brassicas and 40% of the area under tomato), together with 40,000 ha of vines (10% of the entire area under vines), 35,000 ha of almond orchards and 23,000 ha of apple orchards (18% and 13% of the area under these trees) were treated with *Bt* (Walker et al. 2003). In forests, almost 3.5 million hectares of forest were treated with various *Bt* formulations between 1980 and 1998, to combat the spruce budworm, *Choristoneura fumiferana* (Clem.) (521,000 ha), the western spruce budworm, *Choristoneura occidentalis* (Free.) (547,000 ha) and *L. dispar* (2,435,000 ha). In Canada, between 1980 and 1999, almost 6 million hectares of forest were treated by aerial spraying with products based on *Bt*. It is also estimated that 1.8 million hectares of forest in Europe (corresponding to about 26% of the area treated) were treated with *Bt*-based products between 1990 and 1998 (Van Frankenhuyzen 2000).

6 The Expression of *Cry* Genes in Plants

Several teams working in the domain of plant transgenesis decided to make use of the insecticidal potential of *Bt* to generate genetically modified plants expressing δ-endotoxin genes (Fig. 3). A first decisive step in this direction was taken in 1987, with the production of tobacco plants transformed with the *Bt cry1Ab* gene (Vaeck et al. 1987) a gene whose product is active against the European corn borer, one of the main pest attacking maize in the US and Europe. This insect, due to the way it attacks plants, is particularly difficult to control with a standard insecticide treatment. The young ECB caterpillars burrow into the apical bud and then penetrate into the interior of the stem, creating a network of holes in the soft tissue. Thus, the insect rapidly finds shelter from classical insecticides and the damage it causes is not immediately apparent. A promising approach to control this type of pests was to create genetically engineered maize plants, expressing a *cry Bt* transgene in the tissues that are prone to the insect attack, in order to neutralize it before causing major damage.

Fig. 3 Transgenic tobacco transformed with the cry1C gene. On the *left*, an untransformed control plant. On the *right*, tobacco transformed with the *cry1C* gene, modified for expression in plants. In both cases, 40 *Spodoptera littoralis* second instars were placed on the leaves. The photograph shows the damage after 72 hours (photograph courtesy of J. Tourneur, INRA)

The development of new methods of plant transformation, based on electroporation or particle bombardment, subsequently made it possible to transfer *Bt cry* genes into most plants, including monocots such as maize. However, despite the use of strong promoters, toxin production in plants was initially too weak for effective agricultural use (Koziel et al. 1993). Unlike plant genes, *Bt* genes have a high A + T content (66%), which is a suboptimal codon usage for plants, and potentially leads to missplicing or premature termination of transcription (De la Riva and Adang 1996). The coding sequence of *cry* genes has been modified (without modifying the encoded peptide sequence) to ensure optimal codon usage for plants, and this allowed toxin production in plants to be increased by two orders of magnitude (Perlak et al. 1991). This strategy has been successfully used in many plants: cotton, rice and maize have been transformed with modified *cry1* genes and potato has been transformed with a modified *cry3A* gene (Table 1).

Bt potatoes were first developed and sold by Monsanto in the United States in 1994 under the NewLeaf trademark for control of the Colorado potato beetle (Perlak et al. 1993). The use of NewLeaf potatoes led to a significant reduction in pesticide use and cost savings for growers. However, they were only considered as a marginal niche market by Monsanto and sales were discontinued in 2001. In 1996, authorization was obtained for the cultivation and sale in the US of transgenic plants expressing certain lepidopteran active *cry* genes (reviewed in Sanchis 2000). Today, *Bt* maize and *Bt* cotton are cultivated on a large scale, throughout the world. In 2006, these transgenic crops covered an area of 32.1 million ha (James 2006). Insect-resistant crops covered 19 million ha (19% of the area under genetically modified organisms (GMOs)) and crops with a combination of transgenic traits (insect resistance and herbicide tolerance) covered 13.1 million ha (13% of the area under GMOs) (James 2006). *Bt* cotton adoption has resulted in a significant decrease in the use of insecticides in all cases studied (25% of all insecticide used in agriculture worldwide is for cotton cultivation). By contrast, *Bt* maize adoption has induced only a little decrease in insecticide use, since the pests *Bt* maize is designed to resist were not usually controlled by insecticide applications (James 2006).

In 2006 *Bt* cotton was grown in nine countries: Australia, Argentina, China, Colombia, India, Indonesia, Mexico, South Africa and the United States. *Bt* maize was grown in fourteen countries: Argentina, Canada, Colombia, the Czech Republic, France, Germany, Honduras, the Philippines, Portugal, Slovakia, South Africa, Spain, Uruguay and the United States (James 2006).

Bt cotton was first adopted in India as hybrids in 2002. In this year India grew approximately 50,000 hectares of officially approved *Bt* cotton hybrids. Three years later, in 2005, the area planted to *Bt* cotton in India reached 1.3 million hectares, and in 2006, 3.8 million hectares (60%) of the 6.3 million hectares of hybrid cotton in India (which represents 70% of all the cotton area in India) was *Bt* cotton. However, in 2007, it has been reported that in southern Punjab farmers had to spray pesticides worth over $120 millions to save their cotton crop from the mealy bug (a new insect pest on cotton considered deadlier than the American bollworm) that is now threatening the cotton crop in Punjab and elsewhere in the region (Singh-Ashk 2007). At least 25% of the crop has already been destroyed. This points out that we should never forget that introducing new technologies can also give rise to a new set of problems, including pest shifts.

In China, 64 improved cotton varieties have been approved for environmental release. Of these, many varieties have been examined by the national government and confirmed as pest-resistant and high-yielding. These have been put into production in 12 provinces and *Bt* cotton is the most extensively grown transgenic crop in China today. In 2005, China grew 3.3 million hectares of *Bt* cotton, occupying about 66% of the national cotton area (Huang et al. 2007). However, like in India, *Bt* cotton is clearly not as profitable as it is in the US. The problem in China is not due to the bollworm developing resistance to *Bt* cotton but, as happened in India, because of secondary pests that are not targeted by the *Bt* cotton and which previously were controlled by the broad-spectrum pesticides used to control bollworms (Wang et al. 2006). This problem could be circumvented, since a particularity of China is the availability of new *Bt* cotton varieties developed by the public research group. A series of transgenic *Bt* rice lines transformed with modified *cry1A*, *cry1Ab* or *cry1Ac* genes have also been approved for large-scale pre-productive trials and are now in the process of rigorous biosafety assessment which is the last step before commercialization (Huang et al. 2007).

7 The Regulations Concerning GMOs in Europe and in France

In Europe, the authorization procedure for the voluntary dissemination and sale of genetically modified organisms (GMOs) is fixed by European directive 2001/18/CE, dating from March 12, 2001. A moratorium was nonetheless imposed, at the instigation of five Member States, including France, at the Council of Environment Ministers in June 1999, in the absence of precise and reliable mechanisms for tracing and labeling GMOs. This moratorium was lifted in 2003, following the adoption of community-wide regulations for GMO traceability and labeling. However, only six countries within the European Union currently authorize the sale of *Bt* maize (Spain, Germany, Portugal, France, the Czech Republic and Slovakia). Portugal and France lifted moratoria of four and five years, respectively, on the cultivation of *Bt* maize in 2005, whereas the Czech Republic authorized the planting of *Bt* maize for the first time in 2005. In 2007, according to GMO Compass (http://www.gmo-compass.org/eng/agri_biotechnology / gmo_planting / 191.eu_growing_area.html), 75,000, 20,000, 5,000, 3,000 and 2,500 ha of *Bt* maize were sown and harvested in Spain, France, the Czech Republic, Portugal and Germany. In France, a draft law relating to GMOs was approved during its first reading at the Senate on March 23, 2006. This law transposes the European directives 90/219/CEE (relating to the restricted use of GMOs) and 2001/18/CE into French law. Nonetheless, the French government delayed the presentation of this law on GMOs to its parliament until after the presidential elections held in May 2007. Actually, this law will be discussed early 2008 after the French "Grenelle de l'Environnement" ecological talks. Unveiling the country's new environmental policy. French president Sarkozy proposed a temporary freeze on the planting of genetically modified crops in France after the government received the results of an evaluation by a new authority on GMOs early in 2008. Nevertheless, GMOs with EU-wide authorization obtained before 1999 can be sold in France without additional national authorization.

8 Status of GMO Maize in France in November 2006

The European catalog of varieties currently includes 35 GMO varieties, including:

- 1 *Bt*11 maize variety, highly tolerant to the ECB and glufosinate, from Syngenta.
- 20 Mon810 maize lines, protected against the ECB, from Monsanto.

However, in France, the authorization to cultivate and to disseminate seeds of GMO varieties authorized for sale by the EU requires their listing in the national catalog of species and varieties. This listing depends on a decision by the Minister of Agriculture, based on the advice of a consultative committee, the Permanent Technical Selection Committee (CTPS, *Comité Technique Permanent de la Sélection*). Varieties are listed in the catalog if they pass DUS tests (distinctness, uniformity and stability tests) and VCU (value for cultivation and use) tests. In France, GEVES (*le Groupe d'Étude et de Contrôle des Variétés et des Semences*; the Variety and Seed Study and Control Group) is responsible for carrying out these tests for the CTPS, making it possible to determine whether the proposed varieties merit listing in the official catalog. Varieties derived from the Mon810 lineage have been authorized in France and are listed in the national catalog. By contrast, authorization has not yet been sought for the *Bt*11 variety, which is therefore not listed in the national catalog.

The sale of genetically modified maize seeds may be authorized for a period of up to 10 years. This authorization is accompanied by monitoring (biovigilance), the use of seeds for the evaluation of possible effects of the transgenic crop on the environment (emergence of moths resistant to *Bt* toxin, effects on populations of non-target insects and on soil bacteria) and monitoring of animal consumption of the maize produced (changes in the digestive flora). A biovigilance committee was set up for this purpose in March 1998. This committee includes scientific experts and lay representatives.

9 Resistance to the δ-Endotoxins of *Bt*

The scientific community considers the emergence of populations of pests, resistant to the toxins produced by different varieties of *Bt* crops, to be probable. The resulting resistant larvae would be able to eat the foliage of these transgenic plants, which would therefore become ineffective at controlling the damage caused by this pest. This concern is based on past experience in the domain of crop protection. We have long been aware of the development of resistance to chemical pesticides in insect pests. Fifty years of theoretical and practical studies have shown that an increase in the number of resistant individuals over time is almost inevitable in populations exposed to chemical treatments. Certain populations of aphids, such as the cotton aphid, *Aphis gossypii* (Glov.), have become resistant to almost all the major classes of insecticide known. Thus, all insecticides have a high probability of becoming ineffective. There is no theoretical reason why this should not also be true for *Bt* toxins. As for antibiotics, herbicides and fungicides, the generalization of resistance to *Bt* toxins is likely to be a particularly severe problem whilst the number of toxins potentially useful against crop pests remains small.

10 The High Dose-Refuge Strategy

The "high dose-refuge" (HDR) strategy for managing resistance to *Bt* plants has been implemented in several countries after it was set, for the first time, in the United

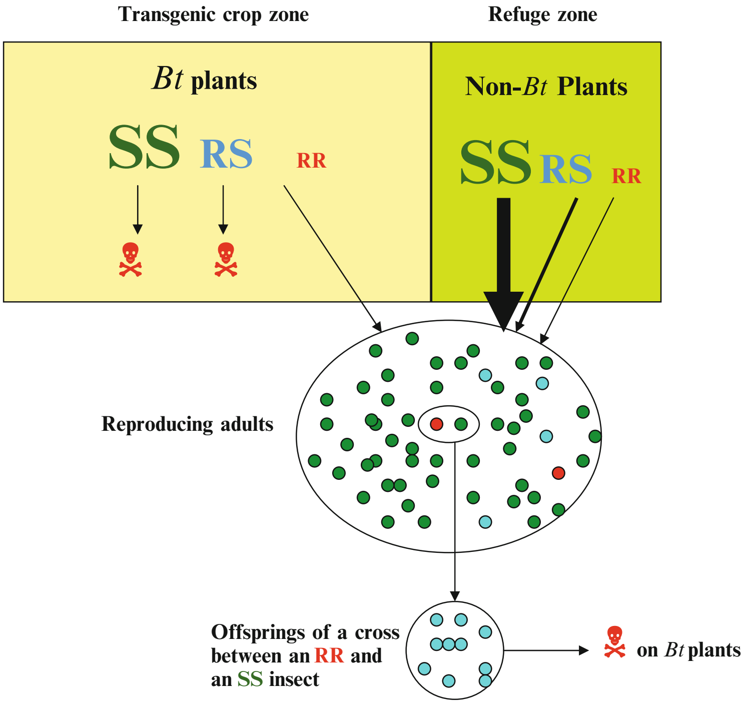

Fig. 4 Schematic representation of the "high dose-refuge" (HDR) strategy. The success of the HDR strategy depends on resistance being a rare and recessive trait and the genetically modified plants producing a dose of toxin sufficient to kill all homozygous susceptible individuals (SS-green) and all heterozygous individuals with both resistance and susceptibility alleles (RS-blue)

States. This method is one of the best-known strategies for slowing the development of resistance in pest populations. It involves growing plots of *Bt* crops producing large amounts of toxin alongside non-*Bt* crops' plots (referred to as refuge zones), in which the larvae of target insects are not exposed to the toxin; these larvae therefore constitute a reservoir of susceptible individuals (Alstad and Andow 1995). An understanding of genetics is required to comprehend the functioning of the HDR strategy. Resistance is a consequence of genetic mutations. We can therefore distinguish between the wild-type form of the gene, known as the susceptibility (S) allele, and the mutated form, known as the resistance (R) allele. Insects with two susceptibility alleles are SS homozygotes and are susceptible. Insects with one copy of each allele are RS heterozygotes and insects with two copies of the resistance allele are RR homozygotes (and are consequently resistant). The HDR strategy is based on the observation that Bt resistance is rarely dominant (Bourguet et al. 2000; Tabashnik et al. 2003) and, in some pests like the ECB, initially at low frequency (Bourguet et al. 2003). If the *Bt* plants produce sufficiently large amounts of toxin one might therefore expect these plants to kill all SS homozygotes and all RS heterozygotes. If this is the case, only a few RR homozygotes can develop and emerge from the *Bt* crop plots. Provided the high-dose and refuge plots are appropriately spaced, RR individuals are likely to mate with SS individuals from the refuge zones (but see Dalecky et al. 2006). The offspring of these crosses will consist mostly of susceptible RS heterozygotes unable to develop on the *Bt* plants in the next generation, thereby decreasing the frequency of the resistance alleles (Fig. 4). Note that these alleles might eventually remain at low frequency if they are associated with a fitness cost – i.e. if the resistant RR and/or RS individuals have a lower fitness than the SS in the absence of selective pressure; in this case, the *Bt* toxin (e.g. Higginson et al. 2005).

The amount, and location size of refuges that are necessary will differ depending on the mobility and ecology of the insect and whether or not the refuge is sprayed with any chemical control. This approach also assumes that mating will be random between insects living in the refuges and those in the crop being sprayed or the genetically modified crop. This strategy is currently used in commercial production in several countries.

11 Evolution of Resistance in Natural Populations

The first case of resistance to *Bt* toxins selected in the laboratory was in a population of Indian meal moths, *Plodia interpunctella* (Hbn.) (McGaughey 1985). Strains resistant to one or several *Bt* toxins have since been selected in about 10 insect species (Tabashnik 1994, 2003). However, the situation in the field remains very different. To date, the only natural populations that have really developed resistance following *Bt*-based treatments have been populations of diamondback month, *Plutella xylostella* (L.). The first resistant lines of this lepidopteran were detected in populations sampled on watercress in Hawaii. One of these populations had been subjected to 15 treatments with *Bt*-based biopesticides over the course of 18 months and the other had been subjected to between 50 and 400 such treatments between 1982 and 1989.

The use of transgenic plants has greatly increased the selection pressure on target pest populations. However, the several thousand ha of *Bt* maize planted in France during the first field trials and the agricultural production of such crops by a few producers until the years 2006–2007 have had much less impact than the 3 million ha of conventional maize planted each year, to the extent that the selection pressure exerted on ECB populations may be considered negligible. As expected, susceptibility monitoring over the last five years, carried out by the Plant Protection Service (SPV; *Services de la Protection des Végétaux*) at the request of the Biovigilance Committee, has found no evidence for the evolution of resistance in French ECB populations. More surprisingly, no resistance problem has been detected among ECB populations in North America (Alves et al. 2006; Stodola et al. 2006), where *Bt* maize crops have been grown since 1996. Similarly, the monitoring of ECB populations in Spain – the only European country in which *Bt* crops have been planted over large areas – has revealed no change in susceptibility more than five years after these crops were first introduced (Farinos et al. 2004). Nevertheless, several ECB strains with a lower susceptibility to the principal *Bt* toxins produced by *Bt* maize varieties have been selected in the laboratory (Chaufaux et al. 2001; Farinos et al. 2004; Alves et al. 2006). The level of resistance of these strains – resistance factor of 10 to 1,000 – is, however, too low to ensure the survival of larvae on the foliage of *Bt* maize plants, particularly on the foliage of varieties generated by transformation events *Bt*11 and Mon810, in which large amounts of toxin are produced. Finally, estimations made jointly in France and the USA suggest that the frequency of resistance alleles in natural ECB populations is sufficiently low to allow a sustainable management of resistance (Bourguet et al. 2003; Stodola et al. 2006).

Without going into detail, there is limited evolution of resistance to *Bt* cotton in populations of target pest insects feeding on this crop (Tabashnik et al. 2005).

There are several possible reasons for the lack of emergence of resistance to *Bt* plants in target pest populations. The first is that the alleles conferring such resistance were initially – before the introduction of *Bt* plants – present at such a low frequency that, despite possible increases over the last decade, these alleles remain too rare for detection in the field or selection in the laboratory of resistant individuals. Alternatively, the principal areas in which *Bt* cotton and *Bt* maize crops have been planted on a large scale over the last few years – the US and Canada, in particular – have been managed using the HDR system. The presence of plots of conventional crops not producing toxins, acting as a refuge, may have significantly maintained resistance at a low level. A third possible reason is that the use of *Bt* crops remains limited. For example, over the entire American Corn Belt, *Bt* maize has never covered more than 30% of the area under maize. Even in the regions most affected by ECB damage, *Bt* crops rarely cover more than 70–30% of the area under maize. The cultivation of conventional maize varieties by many producers decreases the selection pressure on ECB populations, by providing large natural refuge zones free of *Bt* toxins. It is also possible that the cost of resistance – the decrease in the fitness of resistant individuals compared to susceptible individuals in the absence of *Bt* toxin – is sufficiently high for there to be selection against these alleles in the absence of the toxin. These four possible explanations are, of course, not exclusive. There is reason to believe that all four of these factors have contributed to the non-emergence (for ECB) or limited evolution (for pests targeted by *Bt* cotton) of resistance during the first 10 years of *Bt* plants' cultivation.

12 Conclusion

One of the main advantages of microbial control agents is that they can replace, at least in part, some of the most dangerous chemical insecticides. The use of these safer and biodegradable biological control agents also has a number of ecological advantages. One of these advantages results from their high level of selectivity, their infectious or lethal action being limited to a few species. They are therefore often used in organic agriculture, which is becoming increasingly popular with consumers. Many studies have also highlighted the benefits of exploiting *Bt* for the protection of crops and forests. Progress in molecular genetics has also made it possible to use *Bt cry* genes as a genetic resource for transgenesis and for the construction of transgenic plants resistant to insects. *Bt* maize and *Bt* cotton, which constitutively produce δ-endotoxins, are an effective means of controlling their pests – especially the "borers" due to their "endophytic" habits – greatly increasing productivity. However, in some cases, secondary pests that are not killed by the *Bt* toxins produced by the current transgenic varieties of these two crops might significantly decrease the value of this technology, as recently shown in China and India. The extension of pesticide formulations containing *Bt* will depend essentially on our capacity to improve the performance of the products used: activity levels, activity spectrum, quality and stability of formulations, and persistence in the field. The emergence of resistant insects is a problem that both *Bt* sprays and plant products are likely to face in the future. This phenomenon has already been observed in the laboratory, and is likely to become much more acute in natural conditions if *Bt* use in agriculture and for human health applications spreads, or in cases of the non-rational use of large-scale transgenic crops expressing *cry* genes. For this reason, many research programs have been launched to anticipate the risk of resistant populations emerging and to design or refine strategies – such as the HDR strategy – for slowing and/or preventing the emergence of resistance. Second-generation transgenic plants are currently being developed. In particular, it is planned to generate plants expressing at least two *cry* genes encoding toxins recognizing different receptors. Approaches of this type should help to slow the emergence of resistance in insects. However, more detailed studies of the mode of action of δ-endotoxins and of the mechanisms inducing resistance to biological insecticides are also required. An understanding of the mechanisms and genes associated with resistance, and thus of ways to control them, is essential for the future rational use of bioinsecticides and transgenic plants based on *Bt*.

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Genetically Modified Glyphosate-Tolerant Soybean in the USA: Adoption Factors, Impacts and Prospects – A Review

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Abstract Transgenic crops are the subject of lively debate and controversy. Despite such controversy, transgenic soybean has undergone a rapid expansion. Among various types of transgenic crops, herbicide–tolerant crops appear to many to be of limited interest, especially in Europe. Nonetheless, herbicide-tolerant crops are the most widely spread in the world. Indeed, glyphosate-tolerant soybean was notably the most cultivated transgenic plant in the world in 2006. In the USA 91% of soybean was transgenic in 2007. How can this particularly significant diffusion in the USA be explained, and what are its impacts? Such issues are addressed in this article, using surveys, studies of numerous statistical data and literature analysis. A first section underlines the importance of soybean in the current development of transgenic crops in the world, and the favourable context for their expansion in the USA. Then follows an analysis of the advantages and drawbacks of transgenic soybean for American farmers. Factors explaining the rapid diffusion of transgenic soybean are also analysed. A comparison of transgenic vs. conventional soybean reveals that transgenic glyphosate-tolerant soybean allows both the simplification of weed control and greater work flexibility. Cropping transgenic soybean also fits well with conservation tillage. Transgenic soybean has an economic margin similar to conventional soybean, despite a higher seed cost. The next section describes the evolution of the use of herbicides with transgenic soybean, and some issues linked to the rapid increase in the use of glyphosate. At the beginning a smaller amount of herbicides was used, but this amount increased from 2002, though not steadily. Nonetheless, the environmental and toxicological impacts of pesticides do not only depend on the amounts applied. They also depend on the conditions of use and the levels of toxicity and ecotoxicity. The levels of ecotoxicity seem to have somewhat decreased. The success of transgenic soybeans for farmers has led to a higher use of glyphosate as a replacement for other herbicides, which has in turn led to a decline in its effectiveness. However, the issue here is not only genetic engineering in itself, but rather the management and governance of this innovation. Finally, the prospects of transgenic soybean are addressed. Transgenic soybean with new traits should be placed on the market. The conclusion describes economic context of the development of the first transgenic crops.

Keywords Agricultural economics • Genetically modified crop • Glyphosate • Herbicide • Herbicide tolerance • Impact • Soybean • Transgenic crop • United States

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1 Introduction[1]

Transgenic crops are the subject of lively controversy due to the hopes raised by the new traits that can be introduced into plants and the diverse fears they provoke concerning their effects on the environment, health and the economy. The most widespread transgenic crops during the first 12 years of their diffusion since 1996 have been tolerant to herbicides, particularly glyphosate. In 2006, this trait was present in 81% of the surface area of transgenic crops, which represented a total of 102 million hectares. This expansion of herbicide-tolerant (HT) crops seems somewhat surprising as it goes against one of the expectations concerning the applications of biotechnologies. Indeed, it was hoped that the latter would lead to a form of agriculture that enhanced life processes and thus required fewer chemical products. How can this high diffusion be explained, and what are its effects, particularly in terms of the evolution of herbicide use? Among the transgenic crops, one of them, soybean tolerant to glyphosate herbicide, or Roundup Ready® (RR) soybean, stands out due to its particularly high expansion level and the extent of the area it covers. Indeed, it is the most widespread transgenic crop on the planet, representing 57% of the entire area under transgenic cultivation in 2006. Furthermore, it is the only plant for which a majority (64% in 2006) of the area cultivated in the world is transgenic; whereas for other crops this proportion is far lower, often non-existent. Finally, in the USA it has been massively adopted.

It therefore seems useful to seek a better understanding of the adoption factors and impacts of glyphosate-tolerant soybean, especially since in Europe, transgenic crops are often presented as holding little interest for farmers. In terms of impacts, one question is often asked: how has herbicide use evolved for this transgenic herbicide-tolerant crop? This is one of the points that will be addressed more particularly. Indeed, Western agriculture is often criticised for using too many pesticides, a factor leading to the weak sustainability of its practices. Thus, it is useful to understand better the herbicide consumption of transgenic crops, particularly HT ones. Part of this text will focus on this issue without addressing the other economic or environmental aspects dealt with in other papers (Nelson 2001; Kalaitzandonakes 2003; Wesseler 2005; Duke and Ragsdale 2005; Gomez-Barbero and Rodriguez-Cerezo 2006; Sanvido et al. 2007).

An inventory of transgenic crops around the world and in the USA is presented first (Sect. 2). Then diverse factors at the origin of HT soybean spread in the USA, particularly at an agro-economic level, are analysed (Sect. 3). Evolutions in the use of herbicides, and agro-economic and environmental impacts are then studied in more detail (Sect. 4). Finally, some prospects of transgenic soybean in the USA are tackled (Sect. 5).

This paper is based on multiple sources: on the one hand, interviews with American scientists and actors in the agricultural and para-agricultural sector on explicative factors and the impacts of the adoption of HT soybean; on the other hand, scientific articles, symposium papers and agronomic extension newsletters, and finally, the collection, analysis and processing of the different statistical data available. In particular, USDA (US Department of Agriculture) statistical data on the use of different herbicides on soybean-cultivated land from 1990 to 2006 have been analysed in order to pinpoint trends in this matter.

2 An Uneven Expansion of Transgenic Crops Around the World and in the USA: The Importance of Herbicide-Tolerant Soybean

In mid-2007, 19 transgenic species had each been authorised in at least one (sometimes a single) country for cultivation, human consumption or animal consumption. In total, thirteen types of traits have been introduced into these species by transgenesis: herbicide tolerance, resistance to certain insects or viruses,

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[1] Acronyms used in this article are given below. The terms "transgenic crop" and "genetically modified (GM) crop" are used interchangeably. The current term of "genetically modified organism" (GMO) is also used for transgenics in general.

Acronyms

Bt: *Bacillus thuringiensis*
EIQ: Environmental Impact Quotient
GM: genetically modified
GMO: genetically modified organism
RR: Roundup Ready®
CT: conservation tillage
HT: herbicide-tolerant
EU: European Union
USDA: United States Department of Agriculture
USDA-NASS: USDA National Agricultural Statistics Service
USDA-ERS: USDA Economic Research Service

Table 1 Distribution of transgenic crop acreage in the world in 2006 (in million hectares) (from James 2007)

By country	10^6 ha	Percent	By crop	10^6 ha	Percent	By transgenic trait	10^6 ha	Percent
USA	54.6	53.5	Soybean	58.6	57	Herbicide tolerance (HT)	69.9	68
Argentina	18.0	17.7	Corn	25.2	25			
Brazil	11.5	11.3	Cotton	13.4	13	Insect resistance (Bt)	19	19
Canada	6.1	6.0	Canola	4.8	5			
India	3.8	3.7	Other (squash, papaya)	<0.5	<0.5	Insect resistance and herbicide tolerance	13.1	13
China	3.5	3.4						
Paraguay	2.0	2.0				Virus resistance or other	<0.1	<1
South Africa	1.4	1.4						
Total	102	100	Total	102	100	Total	102	100

HT herbicide-tolerant; through herbicide tolerance, plants have been genetically modified to tolerate the effects of a broad-spectrum herbicide, such as glyphosate. *Bt* variety resistant to some pests through *Bacillus thuringiensis* toxin (Bt); it is achieved by inserting a gene from the bacteria *Bacillus thuringiensis*, which creates a toxin that affects some insects

etc. (Agbios 2007). However, the number of transgenic species cultivated today on a large scale is much lower than the 19 authorised and their diffusion remains very condensed (Table 1). Thus, in 2006, three crops (soybean, corn and cotton) represented by themselves 95% of the world's GM acreage. Moreover, four countries (the USA, Argentina, Brazil and Canada) totalled 88% of the world's transgenic crop acreage. One particular trait, tolerance to a herbicide (sometimes associated with another) was present in 81% of transgenic crops (James 2007). Meanwhile, for the main plants cultivated throughout the world, the share of transgenic varieties is very low, often non-existent, except for soybean, cotton, canola and corn. Thus, in 2006 on a world scale, the total acreage of transgenic crops (102 million hectare) added up to little more than 7% of all the planet's crops (approximately 1.4 billion hectare, permanent crops excluded).

In the USA, which accounted for 54% of the transgenic crops cultivated worldwide in 2006, one crop, HT soybean, has progressed significantly more quickly than the others (Table 2). In 2007, it represented 91% of the surface area dedicated to soybean cultivation in the USA, and even 97% in South Dakota and 96% in Mississippi and Nebraska (USDA NASS 2000–2007).

Table 2 Proportion of the main transgenic crops in the USA, 1996–2007 (in percentage of the total surface of each crop planted). (Source: USDA ERS 2007a; USDA NASS 2000–2007)

Year	HT soybean	Bt and/or HT Corn	Bt and/or HT cotton
1996	7	4	17
1997	17	12	25
1998	37	25	45
1999	47	37	48
2000	54	25	61
2001	68	26	69
2002	75	34	71
2003	81	40	73
2004	85	45	76
2005	87	52	79
2006	89	61	83
2007	91	73	87

HT herbicide-tolerant, *Bt* variety resistant to some insects through Bt toxin

There are many factors behind the success of transgenic crops in the USA, and in particular of HT soybean. The development of any innovation in agriculture can generally be explained by a combination of institutional, economic, agronomic, social and cultural factors which it is not possible to analyse in detail here. Very briefly, the rapid development of biotechnology in the USA was favoured by the contextual framework of the country: undeniably, there exists in the USA a firm faith in progress, business and innovation (Bonny 2005a). Moreover, the legislative process and government policy in the USA are more strongly influenced by lobby groups and less by public opinion than in the EU. In the American approach to regulation, decision-making rests on the one hand on scientific considerations, and on the other on the legal responsibility of the private sector and manufacturers: any problems which might arise will be settled through the courts. Because of this, the expectations placed on state regulation are fewer than in some EU countries. The American situation is also characterised by a rather high level of confidence in the agencies responsible for food safety. Moreover, there is a highly developed level of cooperation between private companies and public research bodies, notably the universities and the USDA Agricultural Research Service; this cooperation involves private companies, farmers and producer associations. Finally, Monsanto, which is behind most of the transgenic crops currently in use, has had effective strategies for rapid market penetration through their thorough knowledge and experience of regulatory approval procedures, through licensing policies *(branded seed 'storefront' and broad licensing accelerates market access and trait penetration)* and through gene stacking (Monsanto 2003); the latter, for example, increases the value of seeds as it includes two or three technology fees rather than just one.

Furthermore, at least until recently, biotechnology was generally regarded favourably in the USA, unlike in the EU (Bonny 2003). There are few inquiries into identical questions that allow a comparison of opinions in the USA and the EU; the surveys that do show a more favourable opinion of GMOs in the USA (Bonny 2007). This is the case, for example, with the Environics poll in 2000 (FAO 2004), the Worldviews survey (2002), of certain questions in the Canada–USA polls and the Eurobarometer in 2005 (Canadian Biotechnology Secretariat 2005; Eurobarometer 2006). Indeed, in the USA the relationship with food is, in general, often different from that in the EU, with a stronger sensitivity to its practicality and less attachment to local produce. Furthermore, American agriculture is largely orientated towards export and biotechnology is considered to be a competitive factor.

Thus, HT soybean has a particular position as it is the most widespread transgenic crop. Furthermore, a very high proportion of soybean cultivated is transgenic. How can this great expansion be explained, particularly in the USA at farm level, beyond the general context that has been rapidly presented?

3 Agro-Economic Advantages of Herbicide-Tolerant Soybean for US Farmers

3.1 Agro-Economic Advantages that Compensate for the Drawbacks

At the farming level, there are many factors behind the rapid development of HT soybean (Alexander 2006). Table 3 gives an overview of its advantages and disadvantages, the relative importance of which will differ in each particular situation. One of the principal advantages of HT soybean for farmers comes from the fact that weeding is simplified, at least in the short term. Previously, farmers used several herbicides and some weeds were still difficult to control. Transgenic cultivation allows for easier weed management because only a single product is required. Moreover, the period when weed treatments can be applied is slightly longer, offering greater flexibility of work and diminishing the risk of intervening too late if weather conditions prevent treatment at the appropriate time. Furthermore, the herbicides used previously were in certain cases fairly persistent and could affect subsequent crops and even the soybean itself (UIUC 1999; Carpenter and Gianessi 1999, 2000, 2001, 2002; Bullock and Nitsi 2001; Nelson 2001; Gianessi et al. 2002).

For farmers, the economic advantage of HT soybean in relation to conventional soybean depends among other things on the difference in margin. The higher cost of transgenic seed – the "technology fee" – is generally balanced out by the reduced cost of herbicides. A comparison of conventional and transgenic soybean shows that they have broadly similar margins, sometimes slightly higher for transgenic soybean. However, various other aspects reinforce the agro-economic advantages of HT soybean for the farmer. These various other agro-economic effects are significant:

– *Relatively easier weed management and simplified herbicide applications* free up time for other activities. This aspect, although hard to quantify, is significant, as the work of a farmer consists of multiple tasks which are sometimes in competition with each other at busy times, particularly in cases of multifunctionality or multiactivity. In any case, the time freed is often of important value to farmers (Fernandez-Cornejo 2005; Gardner and Nelson 2007a).
– *Reduced risk of failed weed control:* with HT soybean the period when herbicides can be applied is slightly longer, which is an advantage when the weather is bad or where there are large areas to be treated. However, treatments which are applied too late will have an adverse effect on yield (Knezevic et al. 2003; Owen 2007).
– *HT soybean cultivation often goes hand in hand with other techniques* such as cultivation in rows

Table 3 Assessment elements of the advantages and drawbacks of glyphosate-tolerant soybean

Advantages	Drawbacks
1. Agro-economic advantages – Easier weed management in general because only one herbicide is needed – Greater work flexibility (due to a slightly longer period to treat against weeds) which makes other activities more possible – Rather similar or slightly higher margins than those with conventional soybean because of a reduced cost of herbicide treatments – Lower economic risk of bad weeding – Easier crop rotation: non-residual glyphosate does not harm the following crop in contrast to some other herbicides used previously – Quite frequently, fewer herbicide treatments – Fewer working hours and a lowered use of equipment for treatments in general – HT soybean is well suited to conservation tillage	1. Agro-economic drawbacks – Because of technology fees in addition to seed costs, seeds are more expensive, and must not be saved – For soybean, very low risk of pollinating neighbouring soybean crops, but increased need to keep the various harvests well separated to avoid mixing of grains – Greater care necessary in the seed-processing industry to avoid the accidental presence of GM seeds in a bag certified “GM-free” – Greater dependence on the input-supplier firms because of the contract stipulating not to save seeds, and sometimes to use a branded glyphosate rather than a generic one – Potential difficulties in controlling volunteers of the previous crop if it was also tolerant to the same herbicide – Potential risk of difficulty in selling or exporting to some markets which want GM-free products
2. Environmental advantages – Low toxicity of glyphosate leads to a decrease in the environmental impact of herbicide treatments – (Variably) reduced number of tractor or spraying machine trips – Often associated with conservation tillage which reduces ground erosion and some environmental damage	2. Environmental drawbacks – The growth of glyphosate use has led to the development of weeds resistant to this herbicide. Therefore, other herbicides probably more toxic than glyphosate will be needed
3. Food safety – Glyphosate replaces other often more toxic weedkillers, therefore potential reduction of toxicological and ecotoxicological risks	3. Food safety – Potential risk of accumulating metabolites resulting from the degradation of glyphosate and its adjuvants in the plant or in the soils

sown closer together and the techniques of “conservation tillage” (CT) (Barnes 2000; Marra et al. 2004; Cerdeira and Duke 2006). These techniques are being developed because of various programmes to limit erosion and conserve soil: in 1995, 48.6% of the soybean was cultivated in this way and 61.3% in 2004 (CTIC 2004). Several studies underline the good association between conservation tillage and HT crops which allows weed problems previously met with these techniques to be resolved (ASA 2001). The USDA surveys indeed showed that in 2002, the proportion of CT was higher (67%) with GM varieties than with non-GM varieties of soybean (51%).

– *The contractual agreement not to save seeds for the following year’s sowing increases* the cost of HT soybean seed. The importance of saving seed varies according to the country and the crop. In the USA, in 1998 15–20% of soybean cultivation land was sown with seeds saved from the farmer’s previous season’s harvest and not purchased on the market. In other countries, such as Argentina (a major soybean producer), this figure was as high as 25–35% and was more than doubled by black market purchases from other farmers outside official commercial channels (US GAO 2000). But the companies took this into account in their pricing policies for GM seeds: thus, in 1998 HT soybean seeds were on sale at much lower prices in Argentina than in the USA. This brought protests from American producers, who felt they were being penalised. For farmers, one of the main questions on the subject is the evolution in price of the technology fee. The evidence of a farmer questioned on the contracts issue sheds some light on the point of view of American farmers.

> Farmers for the most part did not have a problem with the contracts required from Monsanto. They understood the benefits of the program and that Monsanto needed a return on their investment. They did not like the ability not to save seed, but farmers were increasingly buying new seed every year before RR technology became available. One of the main issues for not saving seed was the rapid improvement in RR varieties each year. Not only was saving the seed illegal, but you needed to get the best performing genetics on the farm as early as possible. A bushel per acre of increased yield would pay for the new

> seed. There might actually be more resistance by farmers today to Monsanto's polices because of the increase in the technology fees. (I think they started at $4.00–5.00 per bag, and are at $7.00–8.00 today.) The other issue unpopular with US farmers is the lack of a technology fee charge to farmers in South America. As we see Brazil and Argentina taking a larger share of the global soybean market, farmers are upset that this competition does not have to pay the fees we do. Monsanto has to figure this one out. (Illinois farmer, personal communication, 2003)

Gene flow between neighbouring crops of conventional soybean and GM soybean does not present any problems. Soybean, which is 99% autogamous, poses few risks in terms of cross-pollination with neighbouring non-GM crops of the same species, unlike canola and corn. But vigilance is required in a number of different areas, in particular in the seed-processing industry in order to avoid GM seeds being accidentally mixed with seeds certified as "GM-free", which some farmers choose in order to sell them at a premium in specific markets. Otherwise, there is a risk of tricky questions of liability arising if farmers targeting specific niches in the market were to find that their produce lost its certification as a result of GM seeds being accidentally present in their seed. Downstream, separated channels to preserve identity also exist, where a rigorous separation of batches is necessary (Bullock and Desquilbet 2002).

3.2 Transgenic Soybean Is of Variable, Quite Often Positive, Economic Interest

The difference in margin between HT and conventional soybean is difficult to quantify as there are wide variations in the cost of soybean production between farms (Foreman and Livezey 2002) and as there were more than 317,600 farms growing soybean in 2002. Moreover, seed, herbicide and soybean prices have varied over the past few years (Bullock and Nitsi 2001; Ash 2001). A lower expenditure on herbicides due to the lower price of glyphosate and less treatments compensates approximately for the extra cost of transgenic seeds (Sankula et al. 2005). Therefore, the cost of GM soybean production is generally similar to or slightly lower and the margin quite often similar to or slightly higher than for conventional soybean. However, the difference between HT and conventional soybean depends on the weeds present and the herbicides (or other means of control) used: for conventional, there is a wide range of possibilities; for transgenic, a certain range also exists – Monsanto proposes several formulations according to the additive type and concentration. In any case, the cost of herbicides was reduced for many farmers whether they used HT varieties or not, because of a drop in all herbicide prices (see below) (Bullock and Nitsi 2001).

In order to compare the results of crops cultivated with different production techniques, there is often an attempt to consider the costs of production or the margin in each case. However, this has its limits as the comparison is closely linked to price ratios which can vary quite markedly. It is therefore helpful to complement it with a quantitative analysis of the production factors used. Furthermore, it is important to remember an important point which is often forgotten: the farm must be considered as a system and the analysis of one production in isolation should be avoided. In particular, establishing the production costs of one crop independently of other possible productions and its interaction with the functioning of the whole farm can give a distorted picture as it ignores various opportunity costs. So, we have seen that HT soybean may have other advantages for the producer: simplification of weed control freeing up time for other activities or areas of production, a fair correlation with conservation tillage and hence development of this (synergy effect), non-persistence of herbicides, etc. Finally, micro-economic profitability calculations often ignore longer-term, economic or environmental external costs.

4 Impacts of the Expansion of Soybean on the Use of Herbicides

4.1 Questions on Sources and Methods

A controversial point often brought up in Europe concerning GMOs is the evolution in the quantity of pesticides used. Thus, this section focuses on this issue without tackling other economic or environmental aspects. The trends in the use of pesticides with transgenic crops are to be looked at case by case as they vary according to the new trait type introduced, the plant considered, the pedoclimatic conditions and the socio-economic context. With HT soybean, the usual conventional herbicides are for the most part removed and substituted with glyphosate. However, conventional herbicides are used in very variable doses per hectare; the recommendations can vary from $10\,\mathrm{g\,ha^{-1}}$ to $1.3\,\mathrm{kg\,ha^{-1}}$ according to the molecule, whereas glyphosate is often spread at a dose of approximately $0.75\,\mathrm{kg\,ha^{-1}}$. So, if, for example, 1.5 glyphosate treatments replace 3 conventional treatments, the assessment of quantity in $\mathrm{kg\,ha^{-1}}$ would be highly variable according to the weedkillers used previously, but it would not have a significant meaning. A simple evaluation of the quantity of herbicide used before and after the development of transgenic soybean is insufficient. To appreciate their environmental and toxicological impact, it is necessary to balance the level of weedkiller use by taking into account the conditions of application and by using toxicity and ecotoxicity indicators.

To assess the impacts of HT soybean, diverse methodological questions arise. Indeed, a comparison of weedkiller use on GM and non-GM soybean in the same year is not enough because the two cultivated areas can have different characteristics; farmers could use, for example, HT soybean where the infestation with weeds is greatest. Ideally, the different heterogeneity factors need to be separated before establishing the effects of using HT varieties (Heimlich et al. 2000; Fernandez-Cornejo and McBride 2002; Bonny and Sausse 2004). It is also necessary to have access to detailed data on the herbicides applied. In the USA, different statistical sources exist in this area, but they rarely allow a comparison of the use of herbicides on transgenic and conventional soybean. Admittedly, sample surveys are carried out each year with farmers on the main crops in order to evaluate the use of fertilisers and pesticides (USDA NASS 1991–2007). But these surveys establish this use globally per crop without separating their use on GM and non-GM soybean. Nevertheless, the use on the two types could be evaluated for the rare years where more detailed surveys were carried out by the USDA, the ARMS (Agricultural Resources Management Surveys). However, it would necessary to have access to the survey's detailed individual documents, which was not possible. The only access to differentiated results for GM and non-GM soybean was for 1997–1998, where a detailed analysis was carried out by the USDA services; but this concerns only the very first years of HT soybean. Thus, the trends in the use of herbicides were studied globally for the soybean acreage by using the annual USDA survey on this topic (USDA NASS 1991–2007).

These USDA surveys on the herbicides used are sample surveys concerning most of the soybean-producing states, but with a variable number of states, depending on the year. The surveys always include the major soybean-producing states, but the number included of states producing low quantities varies depending on the year. To eliminate these variations, we have brought the herbicides used back to the total surface of soybean included in the survey each year, thus establishing the mean doses of herbicides per hectare. The values can be compared from one year to the next as the states that are not surveyed grow low quantities and so have rather little influence on the average. However, given the sampling variation from one year to the next, these doses of herbicide per global hectare of GM and non-GM soybean must be considered cautiously: these are approximate evaluations.

4.2 Rapid Growth in the Use of Glyphosate Progressively Replacing a Large Majority of Former Herbicides

The trends in soybean treatments from 1990 to 2006 show that the progression in HT varieties leads to a progressive substitution of many herbicides formerly used with glyphosate (Fig. 1). In particular, imazethapyr, trifluralin, imazaquin and pendimethalin were widely used in 1995, and much less in 2006. Thus, from 1995 to 2006 the percentage of soybean acreage treated with imazethapyr decreased from 44% to 3%, and the percentage treated with pendimethalin decreased from 26% to 3%.

What has the evolution in the number of herbicide treatments been? Before HT varieties, in 1995, soybean received approximately 2.7 herbicide treatments. The use of transgenic soybean has allowed the number of treatments to be reduced (Heimlich et al. 2000; Benbrook 2004; Brookes and Barfoot 2005; Fernandez-Cornejo and Caswell 2006). This reduction is difficult to evaluate considering the diversity in weeding practices as well as the fact that glyphosate is (and

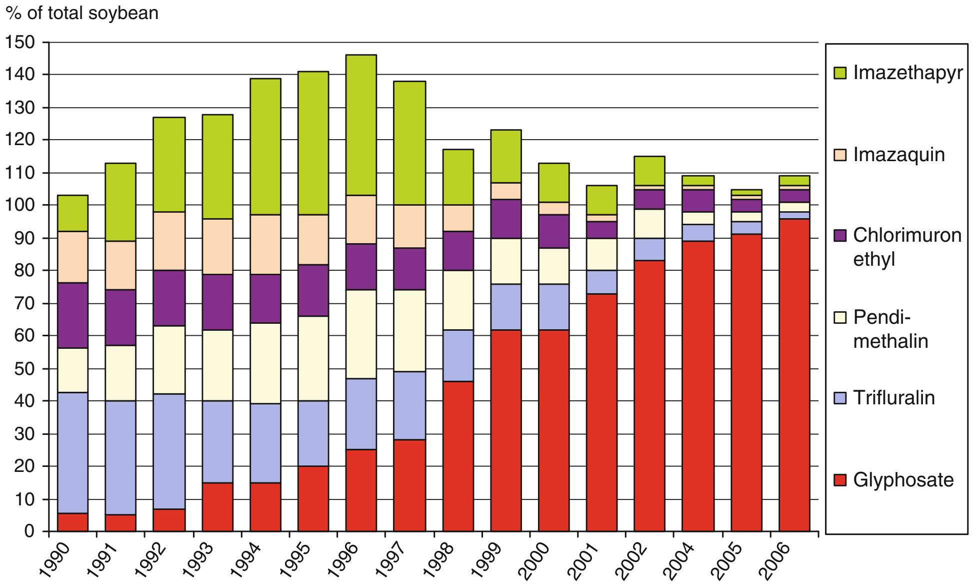

Fig. 1 Main herbicides used on total soybean acreage, 1990–2006 (as percentage of soybean surface treated by each herbicide) (from USDA NASS 1991–2007). With the development of glyphosate-tolerant soybean, this herbicide is used far more extensively. Indeed, it replaces the herbicides used previously; the figure shows only a few of the latter

was already in 1996) also used with non-transgenic varieties, notably in the case of no till: the available statistics do not allow distinction between the different types of use. USDA surveys show a decrease in the number of treatments from 1996 to 2001; thereafter, a near stagnation at approximately 1.9 treatments, then a slight increase in 2006 (2.1 treatments).

In terms of the quantity of herbicides used over a given surface area of soybean (Fig. 2), that of glyphosate has of course increased due to the rapid expansion of the transgenic varieties that represented 89% of all soybean in 2006. There also seems to have been a slight increase in the number of glyphosate treatments per hectare of soybean treated over the last few years. The total quantity of herbicides spread over soybean initially decreased from 1996 to 2001, but seemed to undergo two quite marked increases in 2002 and 2006. In this way, globally, on a given surface area of soybean, the total level of herbicide use in 1996 seems to have been reached again in 2005 and overtaken in 2006 (Fig. 2). However, we cannot deduce from these observations that compared with conventional soybean, HT soybean requires less herbicide in the first years, but then more, since other factors intervene in the evolutions of herbicides used. Other than the possible effects of weather variations, these particularly include the development of conservation tillage (CT) and the drop in herbicide prices.

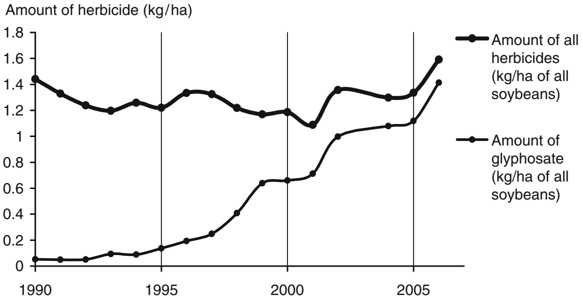

Fig. 2 Quantity of herbicides, and in particular of glyphosate, on total US soybean acreage, 1990–2006. With the rapid expansion of transgenic soybean from 1996, the quantity of glyphosate used increases as it replaces the other herbicides. The total quantity of herbicide spread decreases between 1996 and 2001, but then increases in a non-continuous manner. This can be explained by different factors. NB. The quantities of herbicides used have been brought back to the total acreage of soybean to eliminate the effect of variations in the overall soybean surface area, but the values are approximate due to sampling error. Source: calculations of the author based on USDA NASS (1991–2007)

Indeed, with CT, as weeds can no longer be controlled by being buried during ploughing, an increase

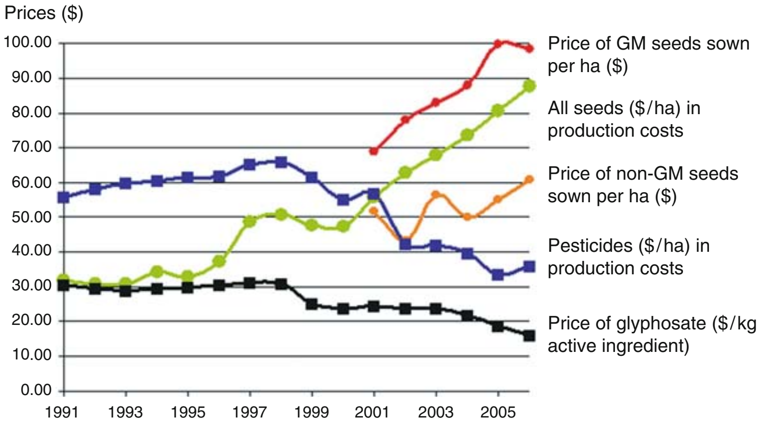

Fig. 3 Price of glyphosate ($ per kilogram of active ingredient), price of GM seeds and non-GM seeds sown per hectare ($) and costs of pesticides and seeds in soybean production costs per hectare, 1991–2006 (the seed price is the price for the mean seed dose used for soybean). Source: author's calculations from USDA NASS (1992–2007) and from USDA ERS (2007b)

in the use of herbicides is quite often observed. As for price, the diffusion of HT soybean having brought about the replacement of certain formerly used weedkillers by glyphosate, the agro-chemical firms that produced them have markedly decreased their prices since 1996 to limit market losses and stay competitive (Fig. 3). This has induced a global reduction in herbicide treatment costs for all soybean producers whether they use transgenic varieties or not (Lemarié 2000; Bullock and Nitsi 2001). This drop in herbicide prices may have contributed to a certain increase in the quantities used. As for seeds, their price has increased over the years, meaning that in soybean production costs, the seed cost has increased while that of herbicides has dropped (Fig. 3). However, overall, between 1995 and 2006, the share of the seeds + herbicides cost has varied relatively little in the total production costs of soybean.

4.3 Environmental Impacts

As indicated previously, the quantity of weedkiller alone would not be a valid indicator of its effect on the environment. It is necessary to balance each herbicide with indicators that take into account its environmental and toxicological impacts. Numerous parameters and indicators exist on the matter, assessing herbicide impacts on human health, animal health, various organisms (bees, birds, mammals, etc.) and several environments (soil, water, etc.). The use of composite indicators elaborated using combinations of basic indicators is necessary in order to carry out global evaluations: through different methods they aggregate the various data on the toxicity and ecotoxicity of each pesticide (Devillers et al. 2005). However, these composite indicators are numerous: more than 42 indicators have been listed by Devillers et al. (2005). Amongst them, the EIQ, Environmental Impact Quotient, perfected by Kovach (1992), was used here. It simultaneously takes into account three important aspects: effects on workers, effects on consumers and water, and ecological effects, and could be applied to the majority of herbicides spread on soybean. For its calculation, the different effects of herbicides are established on the basis of toxicity parameters related to the applicators and agricultural workers on the one hand, to consumers and leaching on the other, and finally to fish, birds, bees, beneficial insects and soil organisms. Regarding its calculation method, the higher the EIQ, the higher the environmental impact, i.e. the more toxic the herbicide is considered to be.

The EIQ was here established for each herbicide used on soybean, then overall for all herbicides used annually by multiplying the amount of each herbicide used per hectare by its EIQ, and by then adding the values. So, for each year we assess the field EIQ value of all soybean herbicides, a kind of environmental footprint of these herbicides. This impact indicator decreased from 1994–1996 (29.15) to 2001 (20.4), but tends to slightly increase in 2002 (23.8) and 2006 (25.7). The toxicity of the herbicides used, considered overall, seems therefore to have decreased with the adoption of GM crops. But this diminution tends to subside after several years, and particularly in 2006 as the quantities spread increase. Other work using another indicator or analysing different HT crops over less than 10 years also obtained a decrease in the level of toxicity of the herbicides applied (Nelson and Bullock 2003; Gardner and Nelson 2007b; Brookes and Barfoot 2005).

4.4 Appearance of Glyphosate-Resistant Weeds

The significant increase in the use of glyphosate has diverse causes in addition to the rapid progression of herbicide-tolerant crops (Woodburn 2000). The glyphosate patent expired in September 2000 in the USA (in 1991 in some other countries), generics developed and competition between firms was fierce, especially as it concerned the most popular herbicide. Furthermore, Monsanto sought to increase its sales as they provided it with liquid assets while it was investing heavily in research and the acquisition of seed companies (Bonny 2005b). High glyphosate gross profit was essential for Monsanto so long as that of its other sector (seeds and genomics) was still in the early stages of development. The increased use of glyphosate, whether Monsanto's Roundup or generic versions, notably took place through HT plants, non-agricultural consumption, or conservation tillage. The statistics of the Environmental Protection Agency (EPA) show that in the USA, the annual use of glyphosate in thousands of tonnes of active ingredient increased from 3.2 in 1987, to 16.3 in 1997, to 32 in 1999, and nearly 50 in 2001, taking into account all uses, including agricultural and others. Glyphosate for agricultural use increased from 3,000 tonnes in 1987 to 40,000 tonnes in 2001, a 13-fold multiplication in 14 years (Aspelin and Grube 1999; Donaldson et al. 2002; Kiely et al. 2004).

This high increase in the use of glyphosate – formerly spread on much smaller areas – has led to the appearance of weeds resistant to this herbicide (Heap 2007; Owen and Zelaya 2005; Cerdeira and Duke 2006). Glyphosate-resistant weeds have already appeared in the USA in different states (eight weeds at mid-2007), as well as elsewhere in the world (13 weeds in total at mid-2007). This emergence was very predictable because of the high selective pressure for weeds, even if certain properties of glyphosate have slowed this in comparison with other herbicides that have known a similar phenomenon (Service 2007). This partial loss in glyphosate's efficiency is considered prejudicial, as it will have to be supplemented or replaced by other herbicides that are generally more noxious and difficult to use compared with glyphosate: hence, there is a risk of loss on a global environmental level (Service 2007; Marsch et al. 2006). In this way, the present substantial expansion in the use of glyphosate may prove to be disadvantageous in the medium term, not so much for Monsanto, whose main sales are now transgenics and genomics, but above all globally.

Thus, the total quantity of herbicide used on soybean initially decreased, but then seems to rise in 2002, and especially in 2006, overtaking the previous levels. Nonetheless, the environmental assessment of HT soybean development using a composite indicator improves somewhat. But what will its evolution be in the years to come? It is necessary to continue the analysis to examine how the total quantities of herbicide and the environmental impact indicator evolve, especially since glyphosate-resistant weeds have appeared and other types of HT crops are likely to be placed on the market.

5 Some Technological Prospects of Transgenic Soybean Over the Next Few Years

For 12 years, one trait introduced by transgenesis was dominant in GM soybean, and among all transgenics: Monsanto's herbicide tolerance. Will new traits be diffused over the years to come? This seems probable. Indeed, the big companies, Monsanto, Syngenta, Dupont/Pioneer, Bayer, BASF and Dow, that have actively invested in transgenics, continue their research while being engaged in fierce competition. On the one hand, other glyphosate-tolerant crops, in addition to soybean, corn, cotton and canola, will most certainly be marketed in the USA, even though this is sometimes the subject of heated debate due to fears of losing a share of the export market. Indeed, HT wheat, which was on the point of being commercialised in 2004, was not in the end, to avoid a decrease in purchases by different countries. On the other hand, concerning soybean, Monsanto is preparing a new generation of HT soybean: the "Roundup RReady2Yield" soybean, which should have a better yield as well as being glyphosate-tolerant; and also a new type of soybean tolerant to another herbicide, Dicamba (Monsanto 2006; Service 2007; Hinsch 2006; Steiner 2006).

Firms other than Monsanto envisage commercialising other glyphosate tolerance traits, notably the GAT system, Glyphosate ALS (acetolactate synthase) Tolerance, by Pioneer/DuPont, and for corn, Agrisure Glyphosate Tolerance by Syngenta. Tolerance to another herbicide, imidazolinone, has also been developed in soybean by BASF and EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária, Brazilian Agricultural Research Corporation). However, in the USA, the proportion of HT soybean should hardly increase in the years to come as it is close to the maximum.

In addition, for soybean, the companies involved are working on different composition modifications that may concern human or animal foodstuffs or processing, and on perfecting varieties tolerant to soybean cyst nematode or rust. A new soybean has been commercialised from 2006. As well as being glyphosate-tolerant, it has a slightly modified composition, with a lower concentration of linolenic acid in order to reduce trans-fatty acid formation during industrial processing. Although this trait was introduced using conventional genetic methods, the fact that it was inserted into HT soybean means that this new soybean (called Vistive) is transgenic. Other new traits in the soybean pipeline include a higher betaconglycinin content to improve taste and texture in products such as soy milk and meat alternatives as well as a higher stearidonic acid content to increase the quantity of functional omega-3 fatty acids (Hinsch 2006; Steiner 2006). Work is also in progress on soybean for energy usage and its transformation into biodiesel. It is also probable that transgenics with two or three traits introduced simultaneously for different objectives ("stacked genes") will be diffused.

If the present and future developments envisaged for transgenic crops are compared with what was hoped for – or at least presented – more than a decade ago, there appears to be a gap in the realisation times. In 1994, Robert Fraley, currently chief technology officer at Monsanto, hoped for the development of "food processing" traits from the end of the 1990s, followed by plants with modified composition for nutrition or health purposes as of the year 2000 (Fig. 4). These prospects are indeed still present, but have a more distant commercialisation date. The GMOs marketed since 1996 have sometimes been criticised for not responding to consumers' needs, but only to those of certain farmers. Some companies, aware of this, now present their future developments in terms of their potential interests for farmers, consumers and processors (Tinland 2007). And traits such as drought resistance, with a much more obvious potential interest than herbicide tolerance, are clearly in the pipeline for firms.

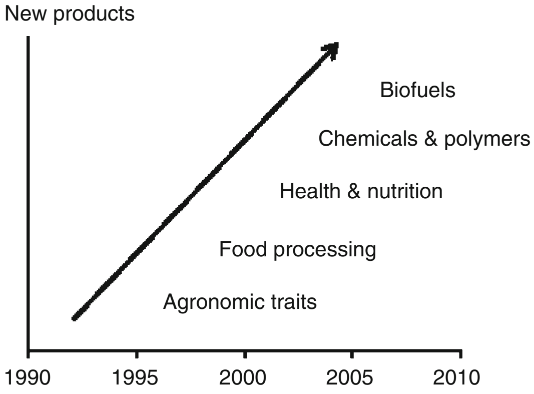

Fig. 4 Development prospects for new products using agbiotechnology in the next 15 years, as anticipated in 1994 by Fraley (from Fraley 1994, modified). These prospects are still present, but have a more distant commercialisation date

Finally, it must be noted that the transgenic character of American soybean has not brought about any lasting serious difficulties for exports, contrary to what is sometimes thought. Indeed, world soybean imports have increased and are projected to rise. However, imports of north-American origin that were formerly predominant have been and should be increasingly surpassed, notably by imports from Brazil where GM soybean is also expanding (Fig. 5). This is particularly the result of the production increase in South America, especially as production costs are lower than in the USA, notably because of lower land prices. As the Asiatic market grows, outlets are opening for GM soybean, as well as for non-GM soybean which has a preserved identity on specific markets, such as human foodstuffs in certain countries.

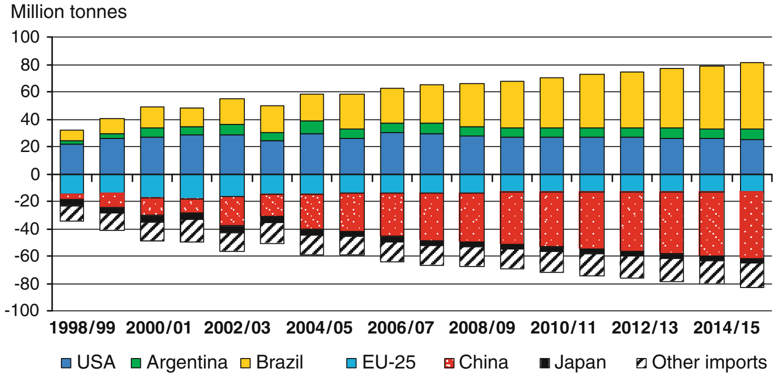

Fig. 5 Evolution and forecast of world trade in soybean grain, 1998/1999–2015/2016. Exports are represented above the abscissa, imports below; trade in soybean meal is not taken into account here. The increase in the proportion of transgenic soybean (30% in 1999, 64% in 2006 at world level) does not affect the demand. The leadership of the USA in export diminishes faced with the expected growth of Brazilian exportations, also largely transgenic. Source: FAPRI (2007)

6 Conclusion

6.1 Herbicide-Tolerant Soybean: Adoption Factors and Impacts on Herbicide Use

In the USA, HT soybean spread rapidly and had a high adoption rate. There are different reasons behind this, including an institutional, economic and cultural context favourable to this innovation, its interest for farmers, support from numerous actors, and the vigorous Monsanto strategy. For farmers, HT soybean has agro-economic advantages compared with conventional soybeans: easier weed control management, greater application flexibility, no herbicide persistence, etc. HT and conventional soybean gross margins are frequently similar. The extra price of transgenic seed (technology fee) is normally offset by the reduced herbicide cost, even though there has been a trend towards a rise in transgenic seed prices over the years. In the short term at least, the advantages of HT soybean seem to override its disadvantages, such as more expensive seed, risk of difficulties in controlling volunteers if two HT crops tolerant to the same herbicide are planted in succession, etc. The economic appeal of HT soybean for farmers seemingly comes from the effects it brings, chiefly the simplified weedkilling process making more time available for other activities, and the good combination with conservation tillage that can thus be developed. This last point is also important from an environmental point of view as conservation tillage leads to significant reduction in soil erosion, to better carbon sequestration, to an increase in organic matter in the soil, etc.

As far as concerns the changing amounts of herbicides used, it is difficult to analyse changes linked to the development of HT soybean using the currently available statistics. Surveys carried out by the USDA on agro-chemicals applied every year establish the usage of various herbicides globally for each crop, without differentiating between HT or conventional varieties. Only global data are therefore available on changes in herbicide applications for all soybeans, with no possibility of comparing HT and conventional soybeans, or differentiating between the various uses of glyphosate (except for two years when there was an additional survey). In addition, the USDA surveys always include the major soybean-growing states, but not all the soybean-growing states, the number depending on the year. Thus, the herbicide amount used per hectare of soybean must be considered cautiously. Few, if any, standard, conventional herbicides are used on transgenic soybean, almost all having been replaced by glyphosate. But dose rates per hectare for conventional herbicides can vary widely, depending on the molecule. The change in herbicide quantities used in kilogram per hectare therefore varies tremendously depending on the herbicides applied previously. The assessment for the majority of US soybean suggests that the total quantity of herbicides applied per unit surface area decreased initially between

1996 and 2001, but tended to rise afterwards, although not steadily. A weight assessment of this type has little significance, however. To assess the environmental and toxicological impacts of herbicides, their quantity must be weighted by taking into account their conditions of application and their toxicity and ecotoxicity, using appropriate indicators. The calculation of such a composite indicator for herbicides used on all soybean shows that their environmental impact improved when the growth rate of the proportion of HT soybean was high. However, more recently there was a stagnation or a slight deterioration; nevertheless, the present environmental impact remains better than it was before 1996. But how will this trend evolve if more herbicides are used over the next few years? Herbicide consumption and its impacts must be monitored, especially as other HT plants exist and are likely to be placed on the market in the years to come.

Between 1996 and 2006, with the development of glyphosate-tolerant crops, this herbicide was used far more extensively as it gradually replaced the weed-killers used previously. This increase in the use of glyphosate is also based on its frequent association with conservation tillage and the drop in its price. But one knock-on effect of its extensive use has been the appearance and development of some weeds resistant to it. Nevertheless, the issue here is not really genetic engineering in itself, but rather the management and governance of this innovation.

6.2 Assessment of the Impacts of Transgenic Crops: Methods and Issues

The impacts of GMOs having been the subject of many critical and controversial commentaries, it seems necessary to recall certain points. Firstly, the impacts of GMOs cannot be addressed globally and generally. Indeed, to evaluate transgenic crops, a case-by-case approach is necessary according to the transgene type and conditions of use, the possible alternatives for each situation, and the people concerned; it is also necessary to carry out multi-criteria assessments, integrating the context and evolution dynamics. Moreover, history has shown that innovations always evolve considerably between the first products and those developed afterwards, because of technical and scientific advances, general socio-economic evolutions and changes in context, and finally, the reactions of all those involved. So, we cannot judge GMOs in general solely on the basis of the GMOs widely diffused to date and the trees of the first GMOs must not hide the wood of biotechnology. Finally, the "technical impacts" are not determined a priori, they depend on how the innovation is directed, implemented, regulated and used in practice, and therefore on the economic, social, institutional and cultural context in which it is inserted. Therefore, the management and governance of the innovation and techniques are major factors; the expression "technical impacts" is thus hardly adequate.

At the beginning of the 1980s, biotechnology was presented as a new wave of innovations, a new technological paradigm, based on the better use and enhancement of life processes. It seemed likely to surpass some of the limits of the previous wave of innovations, relying namely on chemistry and fossil fuels. However, its birth and first years were difficult. The first widespread transgenic crops, those that are herbicide-tolerant, have, through this characteristic, often disappointed. In the 1980s or the 1990s, the potential of biotechnology to allow plants to be more "self-sufficient", not reliant, for example, on different pesticides, was often evoked. Yet HT plants, the most widespread at present, go hand in hand with the use of a herbicide, even if it is considered less noxious than others. The gap compared with the announcements made twenty years ago results from different economic or technical factors explaining the development of this type of GMO in the first place.

6.3 The First Decade of Transgenic Crops and Its Assessment

The extension of transgenic crops is highly uneven, depending on the country. In France, GMOs have crystallised numerous oppositions, hence there is a strong blockage towards them. However, in addition to some French companies, it is above all public research that has in fact been slowed, which has indirectly increased what was feared: the monopolistic position and the domination of major international firms. This has led to a lack of investment in biotechnology applications that could be more geared towards the public good or beneficial for a greater number and for the environment, which in turn strengthens opposition.

The first transgenic crops developed in an economic context marked by the financialisation of the economy, leading the major companies to seek rapid profitability which was not always in line with certain sustainable development objectives that had been laid out. The major firms initially worked on traits such as herbicide tolerance as they were technically faster to identify and to transfer into quite a high number of species through genetic engineering, which enabled a relatively fast return on investment. In addition, the substantial investments made by certain chemical and agro-chemical companies which bought seed firms, and the context of heightened competition with the domination of extremely demanding financial markets, weakened certain firms and/or led to aggressive behaviour. This is perceived by part of the population as unethical and in contradiction with some of the objectives announced. More generally, are intense competition and the quest for fast, high profit dictated by the financial markets compatible with sustainable development objectives? Sustainable development does not solely rely on reducing pollution, but also on more harmonious economic and social relations, and on greater cooperation between the actors involved. Wouldn't biotechnology have more chance of developing and also being accepted if competition gave way to more cooperative behaviour, which does not depend on the biotechnology sector alone.

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Small Eats Big: Ecology and Diversity of Bdellovibrio and Like Organisms, and their Dynamics in Predator-Prey Interactions

Shemesh Yair, Davidov Yaacov, Koval Susan, and Edouard Jurkevitch

Abstract The ecological role of predation is well established in the animal world. Not so in the bacterial realm where the number of known bacterial predators is small and their phylogenetic affiliations largely unknown. The best-characterized bacterial predators belong to the *Bdellovibrio-Bacteriovorax* group (*Bdellovibrio* and like organisms, the BLOs). As predation at this trophic level may be of ecological significance, there is a need to better understand the diversity and the phylogeny of bacterial predators as well as the kinetics of their interactions with their prey. Such studies could also help to develop new approaches for the control of plant and animal Gram negative pathogenic bacteria. Here, we present a short review on the ecology, diversity and the taxonomy of predatory bacteria, with an emphasis on BLOs as well as on the dynamics of the interaction between a selected strain of *Bdellovibrio bacteriovorus* and its *Erwinia carotovora* subsp. *carotovora* prey under high and low predator:prey ratios.

Keywords Bacterial predation • Bdellovibrio • BLO • Predatory bacteria

Résumé Quand le petit mange le grand: Ecologie et diversité de *Bdellovibrio* et organismes appérentés, et leurs dynamiques dans les interactions prédateur-proie Le rôle écologique de la prédation est bien établi dans le monde animal. Ce n'est pas le cas des bactéries où le nombre de prédateurs bactériens connus est faible et leurs affiliations phylogénétique largement inconnues. Les prédateurs bactériens les mieux caractérisés appartiennent au groupe des *Bdellovibrio-Bacteriovorax* (*Bdellovibrio* et organismes apparentés, les BLOs). Comme la prédation à ce niveau trophique peut avoir une incidence écologique, nous avons besoin de mieux comprendre la diversité et la phylogénie des prédateurs bactériens tout comme les cinétiques de leurs interactions avec leur proie. De telles études pourraient aussi aider à développer de nouvelles approches pour le contrôle des bactéries Gram négatif pathogènes pour les plantes et les animaux. Ici, nous présentons une brève synthèse sur l'écologie, la diversité et la taxonomie des bactéries prédatrices, avec une attention particulière portée aux BLOs tout comme sur la dynamique de l'interaction entre une souche sélectionnée de *Bdellovibrio bacteriovorus* et sa proie *Erwinia carotovora* subsp. *carotovora* avec des rapports prédateur/proie élevé et faible.

Mots clés Bactérie • Bdellovibrio • BLO • Prédation

1 The Wonders of Bacterial Predation

Predation is of utmost importance for ecological balance, nutrient acquisition and energy flow, as it is present at every trophic level. It is well studied in the animal kingdom but much less researched at the microbial level, with most of the research on bacterial predation having been performed with

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phages, protozoan and metazoan bacterial predators. Bacterial predation of bacteria is even much less understood although it may play an important role in bacterial ecology.

Bdellovibrio and like organisms (BLO) as they are now denominated, are Gram negative cells, possessing one sheathed polar flagellum, enabling very rapid swimming at up to 100 body-length s^{-1} (Stolp, 1967). This motility confers these organisms the title of the fastest motile bacteria (Young, 2002). However, the most striking characteristic of most BLOs is their unique predatory behavior: BLOs are obligate predators of Gram negative cells. Most only grow and replicate within the periplasmic compartment of their hosts. Attachment and penetration of the substrate cell by a BLO, free-swimming attack cell is quickly followed by the inactivation of the substrate cell's metabolism and by a loss of prey viability and the formation of a bdelloplast, as the BLO-invaded cell-BLO is called. The bdelloplast offers BLO protection against photooxidation damage (Friedberg, 1977), phage attack (Varon and Seijffers, 1975) and increased resistance to pollutants (Markelova, 2002; Varon and Shilo, 1978). Filamentous growth and DNA replication of the invading BLO occurs within the bdelloplast. Exhaustion of the cytoplasmic content of the prey leads the long, intraperiplasmic BLO cell to division by multiple fission into progeny attack cells which grow a flagellum, lyse the ghost remnant, and burst outside (Ruby, 1991; Stolp and Starr, 1963).

The overall volume of research on these fascinating organisms is rather small and has little expanded since the 1990s. The reasons for that may essentially be technical: Isolation of BLOs is not always successful, and demands a dedicated isolation procedure, including differential centrifugation, filtrations and a double layered growth medium in Petri dishes. This is often an addition too cumbersome and heavy to the design of experiments not necessarily focused on bacterial predation and results in the fact that BLOs are seldom looked for. Moreover, as these are not dominant populations, they are almost never detected in rDNA clone libraries obtained from the environment (about 25 environmental clones clustering with BLOs can be found in the Gene Bank). Therefore, because they do not form colonies on standard growth media, and because they are not represented in clone libraries, they have been neglected by microbiologists.

1.1 Survival of Bacterial Predators

The two-membered BLO-substrate cell system can be described in terms of a parasite-host as well as in terms of a predator-prey relationship, as it exhibits features relevant to both definitions: the substrate cell is invaded, a prerequisite for replication, (parasite) but its cell machinery is not used by the BLO while its contents constitutes its food base and the substrate is killed in the process (predation). Moreover, this model, as well as other microbial models, has been used for modeling predator-prey interactions, as it is convenient and accurate to measure. The BLO-prey interaction has been described as an oscillating system with inconsistent periodicity (Afigenova et al., 1978; Varon, 1979). Although the Lotke-Volterra model has been applied to describe the oscillations of the system and its maintenance (Varon and Zeigler, 1978), in the natural world a "decoy" effect can be expected to occur as most of the cells surrounding the predator may not be potential prey, leading to ineffective predator-prey encounters. Such a decoy effect would damp the oscillations and would likely reduce the probability of prey extinction (Wilkinson, 2001).

Under laboratory conditions, a high density of prey is necessary for BLO survival. Various authors have reported that minimal prey concentrations of 10^5 to 10^6 CFU g^{-1} soil or mL^{-1} are required (Keya and Alexander, 1975; Uematsu, 1980). Using the Lotka-Volterra model, Varon and Zeigler (1978) calculated that in order to give BLOs a 50% chance of survival, at least 3×10^6 prey cells were needed. Therefore, it was generally concluded that BLOs only survive in special ecological niches. However, these calculations were performed based on two-membered cultures serving as models. Since BLOs are usually not stringently specific in their host range, the concentration of substrate cells in natural settings may well be high enough to sustain predatory populations. It is now accepted that only a fraction (ranging from less than 1 to a few percent) of the bacterial cells contained in environmental samples is amenable to cultivation (Amann et al., 1995). Rice et al. (1998), who quantified the number of BLO-susceptible bacteria in an estuarine environment found that 70–85% of the recovered bacteria were preyed upon by BLOs isolated from the same sampling sites. Assuming 10% cultivability for the bacteria retrieved in the samples, it was calculated that the level of susceptible populations was sufficient to ensure survival of the predators.

As explained below, biofilms can potentially provide a habitat fit for predation by BLOs in low microbial density biotas, the predator expending beyond that realm during bacterial population surges.

The cell composition of BLOs is rapidly altered and viability quickly reduced in starved BLO bacterial suspensions kept without a prey (Hespell et al., 1974; Marbach et al., 1976). However, BLOs were shown to survive long periods in nutrient-poor environments (Daniel, 1969; Fry and Staples, 1976), and have been retrieved from long-term stored dry soils (Germida, 1987). It was suggested that population heterogeneity (Varon and Shilo, 1978), higher resilience of bdelloplasts (Sanchez Amat and Torrella, 1990) and the formation of bdellocysts (Varon and Shilo, 1978), although the number of strains able to develop this morphology seems to be rather limited, could explain survival. No knowledge on molecular responses to starvation is available.

1.2 Environmental Niches

BLOs are quite ubiquitous in natural and manmade habitats. They are commonly retrieved from soil, are associated with the rhizosphere of plant roots, are found in water of various qualities – in rivers, in the brackish environment of estuaries, in the open sea, at the various stages of treatment in water treatment plants – and associated with biotic and abiotic surfaces (Kelley et al., 1997). BLOs have been retrieved from the gills of crabs (Kelley and Williams, 1992), from oyster shells (Kelley et al., 1997), and more recently from hen and mammals feces (Schwudke et al., 2001). The number of BLOs detected in environmental samples using the double-layer isolation procedure – as for the isolation of phages, a suspension of potential prey cells is poured as a soft agar layer on top of bottom agar, to form a layer of cells in which plaques will develop – is usually low, ranging from tens to tens of thousands of plaque forming units per gram or milliliter of sample. Also, BLO strains exhibit different prey ranges. Although most are able to use a number of prey, BLOs have been isolated that can only utilize one type of substrate cell (see below). In other words, BLOs do not represent dominant populations, a fact that is not unexpected, as predators generally do not numerically dominate ecosystems.

Although BLOs are aerobic, and oxic conditions appear to best sustain their multiplication, it was shown that halotolerant strains are able to grown under microaerobic conditions, and that – at least – marine BLOs are also able to survive anoxic periods as attack phase cells or as bdelloplasts (Schoeffield et al., 1996). Spells of low oxygen tension occur in soils and in water and the BLOs seem to be adapted to these conditions. Morever, BLOs have been isolated from the feces of humans, horses and hens (Schwudke et al., 2001). Stable colonization or transient passage through the gut implies that at the very least, these BLOs are able to cope with anaerobic conditions. The range of possible niches that can support growth and survival of BLOs may therefore be larger than solely permanent aerobic biotas.

BLOs are also found associated with surfaces and biofilms. In the continuous space between the solid phases of biofilms, dissolved chemicals, suspended particles and cells move freely (Wanner, 1989). Biofilms may provide a sustainable habitat for BLO multiplication and survival: Whereas planktonic BLO cells were not systematically recovered from tested seawater samples, biofilms-associated BLOs were detected at a much higher rate (Kelley et al., 1997; Williams et al., 1995). Biofilms may offer BLOs improved conditions for growth and survival, especially in oligotrophic habitats as the gel-matrix can sustain a higher concentration of potential prey along with physical protection: surface-associated BLOs were shown to survive various environmental insults whereas free-living cells died rapidly (Markelova, 2002). It is hypothesized that the small size of BLOs, their high motility and their mode of multiplication can play a role in shaping the structure of biofilms, which are naturally composed of consortia of microorganisms. Fratamico and Cooke (1996) reported that a BLO isolate effectively reduced the level of biofilm *E. coli* cells on stainless steel.

1.3 BLO Diversity

BLOs are part of the class delta-*Proteobacteria*. Only recently, have the natural diversity of BLOs been addressed systematically and their phylogeny revisited. Phylogeny based on the analysis of the 16S rRNA gene lead to the definition of two genera, *Bdellovibrio* and *Bacteriovorax* (Baer et al., 2000). To date, *Bdellovibrio* comprises only one species, *B. bacteriovorus*, while the *Bacteriovorax* genus is composed of two species, *B. starrii* and *B. stolpii*. Marine BLOs exhibit a different GC content than the terrestrial strains (Taylor et al., 1974) and require sodium, potassium and calcium for growth. They recently were shown to form a separate cluster in *Bacteriovorax* (Snyder et al., 2002). Other studies reveal that new species of both *Bdellovibrio* and *Bacteriovorax* should be defined (unpublished data). A study using a combined analysis of the 16S rRNA gene and prey range of soil and rhizosphere isolates showed that BLOs belonging to both these genera include various heterogeneous sub-groups that can be found co-existing in the same environment (Jurkevitch et al., 2000). Moreover, prey range and phylogenetic affiliation appear not to be linked. Culture-independent analysis of BLOs can now be envisaged, as BLO-targeted oligonucleotides have been designed (Jurkevitch and Ramati, 2000).

A variation on the theme of "classical" intracellular predation has been reported by Koval and Hynes (1991), with the isolation of a BLO that has no periplasmic stage in its life cycle. This predator was isolated from raw sewage on lawns of *Caulobacter crescentus* cells that do not form an S-layer. This strain (named JSS) did not enter the periplasmic space of the prey cell, but it remained attached at its surface and utilized the cytoplasmic contents of the prey. No bdelloplast was formed, and the empty prey cell retained its original shape. Growth was by binary fission at the prey cell surface. Interestingly, of the potential prey cells tested, *C. crescentus* was the only prey organism suitable for predation by this strain (unpublished data). Recently, more BLOs were isolated on lawns of *C. crescentus* from garden soil, compost and again from raw sewage. These BLOs resembled strain JSS in that they remained extracellular during predation on caulobacters, and could not use *E. coli* as a prey cell (Fig. 1e). Thus, other predatory bacteria resembling JSS may be found in other ecological niches.

Also, a number of other bacteria have been described as "micropredators". Most are extracellular (*Ensifer*, *Vampirovibrio*, *Vampirococcus* [Esteve and Gaju, 1999]). Interestingly, a Gram negative bacterium invading and dividing within the cytoplasm of its prey – *Daptobacter* – has been described (Guerrero et al., 1986). Moreover, gliding bacteria such as *Myxobacteria*, *Cytophaga* or *Herpetsiphon* are endowed with the capacity to lyse and utilize living bacterial cells as food substrate. The different strategies exhibited by these predators were recently summarized by Martin (2002): wolfpack, or group

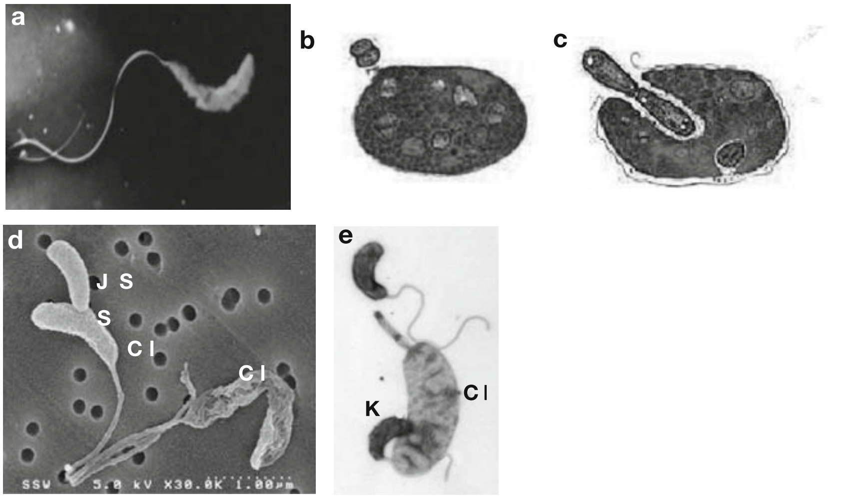

Fig. 1 (**a**) *Bdellovibrio bacteriovorus* strain SNE. (**b**) *Vampirovibrio*. (**c**) *Daptobacter*. (**d**) A *Bdellovibrio* strain JSS cell (JSS) attached to a *Caulobacter cresentus* prey (Cl). To the right of the attacked cell an emptied Cl cell. (**e**) Isolate KL8 (K), isolated from compost and preying on *C. crescentus* Similarly to strain JSS, the predator does not penetrate the substrate cell (Cl). (**a, b**) Electron microscopy. (**b, c**, drawings; Guerrero et al., 1986)

predation, describes predation by a number of predatory cells excreting hydrolytic enzymes, e.g. predation by *Myxococcus*; Epiobiotic, fits predation by *Vampirococcus* and by *Bdellovibrio* strains JSS and KL8 (Fig. 1e), when a predatory cell attaches to the prey, degrades and assimilates prey components; direct invasion, occurs when a predator invades the prey's cytoplasm (*Daptobacter*), and; Periplasmic describes predation for almost all BLOs. Only BLOs appear to be obligate predators, the other bacteria being able to grow heterotrophically and multiply in the absence of prey. The phylogenetic affiliations of most of these bacteria – and therefore their evolutionary relationships – are unknown.

Electron micrographs of various predatory bacteria are shown in Fig. 1. A phylogenetic tree based on 16S rDNA analysis of the BLOs is presented in Fig. 2 (Snyder et al., 2002).

2 Dancing with the Wolves: Dynamics of Prey-Predator Interactions

2.1 *BLOs as Biocontrol Agents of Phytopathogens*

As seen above, the dynamics of predation by BLOs has been the subject of a number of studies (Afigenova et al., 1978; Varon, 1979; Varon and Zeigler, 1978; Wilkinson, 2001). Few of the studies performed have compared the dynamic behavior of different BLO strains. Also, there is a lack of knowledge on the kinetics of the various changes occurring in a developing lysate, i.e. release of attack cells, bdelloplast formation and prey reduction.

A limited number of studies have been published on the potential of BLOs as biocontrol agents. The most comprehensive work on using BLOs to control phytopathogenic bacteria was performed by Uematsu (1980) who showed that BLOs efficiently reduced *Xanthomonas oryzae* populations from rice paddy field water but obtained mixed results against *E. carotovora* spp.*carotovora* in soil. Soybean rhizophere BLO isolates were used to control bacterial blight caused by *Pseudomonas glycinea* (Scherff, 1973), and a significant reduction in disease severity and in systemic symptoms were observed. The possibility of a deleterious impact of BLOs on plant growth-promoting rhizobacteria was brought forward by Germida (1987), who isolated BLOs parasitic to *Azospirillum brasilense* from soils. Another study showed an increase in rhizosphere BLOs preying upon fluorescent pseudomonads in Chinese cabbage inoculated with a beneficial strain of *Pseudomonas fluorescens* (Elsherif and Grossmann, 1996).

Erwinia carotovora subsp.*carotovora* (Ecc) is the cause of soft rot diseases in many crops, including vegetables, flowers and tubers, resulting in large scale losses. BLOs are potential biocontrol agents to control these diseases, but knowledge on predator-prey interactions is needed for a judicious application of such systems.

2.2 *Preying Behavior at High and Low Predator Prey Ratios*

A number of BLO strains able to prey on Ecc were isolated from soil and water and their ability to utilize various preys was analyzed on double agar plates (Table 1). Although most isolates behaved similarly, preying on all proposed prey, the efficiency of plaque formation differed. For example, strain CHI -isolated from a river in Spain- and strain SJE, originating from a soil in Israel, preyed most efficiently on *E. coli*, while strain FCE, isolated from the rhizosphere of strawberry, could only use *E. coli* and Ecc. As expected, none of the isolates could prey upon *Bacillus megaterium*, a Gram positive bacterium. The kinetics of plaque formation and growth also differed between the strains, using Ecc as a prey. Plaques became visible after two (strains SNE, CHI) or three (strains PRE, DPE, FCE and SJE) days. The final sizes of the plaques varied by up to 130%, with strain SNE forming very large (>7 mm in diameter) plaques while plaques of strains FCE and DPE only reached two to three mm in diameter (Fig. 3). It was also observed that after 8 days, plaques from certain strains were still expanding (strains SNE, SJE) while others seemed to have reached their maximal size (the remaining strains). This shows that remarkable differences occur between BLO isolates in their abilities to use similar prey. Efficiency of plaque formation may be linked to the ability of the predator to irreversibly attach to prey cells, as

Fig. 2 Neighbour-joining tree of BLO isolates. A neighbour-joining tree was constructed for the 17 salt-water and nine freshwater isolates by aligning these sequences with other selected members from the prokaryotic domain. Listed beside each organism or strain name is the GenBank accession number (in parentheses). Numbers at branch-points represent confidence values obtained after bootstrap analysis of the neighbour-joining tree using 1,000 replicates (Snyder et al., 2002., with permission)

this capacity appears to be the first step for successful predation (Gray and Ruby, 1991).

Strain SNE, which exhibited a more rapid and sustained growth as plaques than the other isolates, was identified as *B. bacteriovorus*, based on amplified ribosomal DNA restriction analysis and 16S rDNA sequencing (not shown). This strain was grown in liquid culture and formed lysates with Ecc. As BLOs grow in liquid culture with concomitant exploitation of the prey population, the cell suspension clears. Therefore, the development of a lysate in liquid culture can be tracked by simple spectrophotometric readings. However, this type of measurement, as well as the plating of prey cells and BLOs in double agar

Table 1 Efficiency of plaque formation of soil and river water BLOs on different prey cells. Efficiency relates to the relative number of plaque formed in comparison to the number of plaques formed on *Erwinia carotovora* subsp. *carotovora*. X = no plaque growth

Original prey	Origin	*E. carotovora*	*E. coli*	*P. syringae*	*A. tumefaciens*	*A. brasilense*	*B. megaterium*	Strains
E. carotovora	Soil, Israel	1	1	1	1	1	X	SNE
E. coli	River, Spain	1	10	1	1	1	X	CHI
E. coli	River, Spain	1	1	X	X	X	X	FCE
E. carotovora	Soil, Israel	1	1	1	1	1	X	SRE 11
E. carotovora	Soil, Israel	1	1	1	1	1	X	SRE13
E. carotovora	Soil, Israel	1	1	1	1	1	X	DPE
E. carotovora	Soil, Israel	1	1	1	1	1	X	PRE
E. carotovora	Soil, Israel	1	10	1	1	1	X	SJE

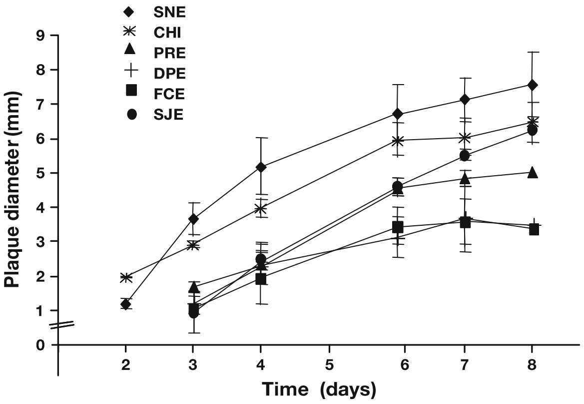

Fig. 3 Kinetics of plaque formation of BLO isolates growing on *Erwinia carotovora* subsp. *carotovora*. A single plaque of the tested predator was suspended in diluted nutrient broth with about 10^8 CFU ml^{-1} Ecc prey. A lysate was obtained (usually overnight) yielding 2–5 × 10^8 cells. ml^{-1} of predators. Lysates were filtered (0.45 μm) to remove remaining prey cells, mixed with the tested prey in soft agar, and then poured on a diluted nutrient agar Petri dish. Plaque growth was examined daily for eight days. Bars represent standard error when larger than the signs

standard growth media can only yield the concentrations of the remaining prey population and of the plaque-forming attack cells, respectively, while bdelloplast formation and the dynamics of progeny cell release remain undetected. Round bdelloplasts, larger prey cells and small predatory attack cells can be differentiated after DAPI staining and counted under epifluorescence microscopy (Fig. 4a), enabling the tracking of each of these populations. This was used to follow the dynamics of two-membered cultures in suspensions containing predator and prey at different ratios. At a 10:1 predator to prey ratio, the viable prey population decreased by three to four orders of magnitude within less than one hour, followed by a much slower decline. At a 1:1 ratio, no change in prey concentration could be detected during the first two hours, which was followed by a second phase of rapid decline (Fig. 4b).

In the former case, the rapid loss in prey viability was probably due to rapid infection of the substrate cells by attack cells, while in the latter case, infection was not as efficient. This is clearly seen in Fig. 4c, which depicts the kinetics of bdelloplast formation while also tracking the attack cells' population. At a 10:1 predator:prey ratio, the almost entire prey population was transformed into bdelloplasts in a quasi-synchronous manner (McCann et al., 1998) with progeny bursting from these bdelloplasts after about two and a half hours. Under these conditions, multiple infections occur (as seen by video microscopy, not shown), with more than one predator penetrating the substrate cell, resulting in an undetectable increase in total attack cell population after progeny release (about five progeny cells are made per infected prey). At the start of the experiment with the lower ratio, the concentration of attack cells decreased by about 50% within 30 minutes, while a similar corresponding level

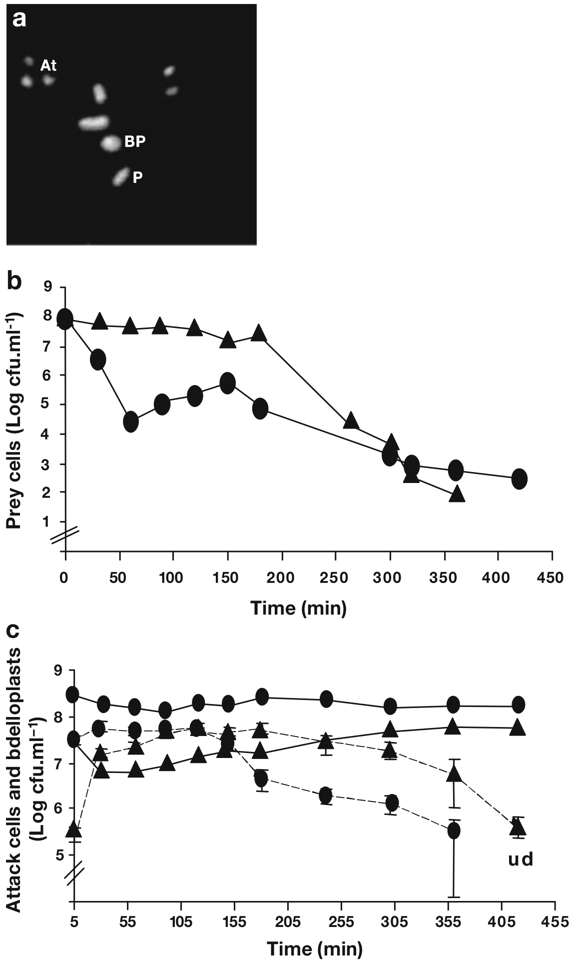

Fig. 4 Dynamics of prey consumption, bdelloplast formation and *Bdellovibrio* attack cell release at two different predator: prey concentrations as measured by DAPI staining, cell tracking by epifluorescence microscopy and colony counting. Predator and prey were mixed at 1:1 and 10:1 ratios and samples taken during the following seven hours. One µl per sample was spotted in a well on a gelatin-treated Teflon-covered glass slide, dried, dehydrated and stained with 0.7 mg ml^{-1} of 4,6-diamidino –2-phenylindole (DAPI) for 10 minutes, followed by washing with ice-cool water. After air-drying, cells were counted under an epifluorescence microscope. Twenty fields were counted or at least 400 of each bdelloplasts and attack phase cells. The concentration of prey was measured by dilution plating on nutrient agar. In all cases, triplicate samples were used. (**a**) DAPI staining of *Erwinia carotovora* subsp. *carotovora* prey (P), bdelloplast (BP) and *Bdellovibrio bacteriovorus* SNE attack cells (At). (**b**) Survival of *Erwinia carotovora* subsp. *carotovora* prey cells (2×10^8 CFU ml^{-1} at the start of the experiment) after exposure to 2×10^8 (▲, 1:1 ratio) or 2×10^9 (●, 10:1 ratio) pfu ml^{-1} of *B. bacteriovorus* strain SNE. (**c**) Dynamics of bdelloplast formation (—) and attack cells (- - -). Ratio key is as in (**b**). u.d. under minimal detection level. Experiments were performed three times, and one representative experiment is shown. Bars represent standard error when larger than the signs

of bdelloplasts was formed. The remaining prey cells kept dividing, with few of these prey showing attack cells attached onto them. Progeny were released gradually, starting two and a half hours after mixing the prey and the predator, leading to an increase in the concentrations of attack-phase predators. Under these conditions, the level of bdelloplasts remained constant for a longer period than at the high predator: prey ratio, due to continuous bdelloplast formation, and the release of new, attack phase cells was more gradual than at the high predator:prey ratio.

At a 10:1 predator:prey ratio, attack cells are always more numerous than at a 1:1 ratio. However, and after one round of cell replication (in the present case about 150 min), bdelloplasts and "freshly released" attack cells are more numerous at a 1:1 ratio. It appears that predation is more efficient at the 1:1 ratio, requiring 200 min to bring prey population to a level that took 350 min to reach at a 10:1 ratio.

Although no such regulation can be seen in this type of experiments, some kind of bdelloplast population density control mechanism may be at play, as was reported with a strain of marine BLO that exhibited growth arrest upon rapid dilution of bdelloplasts (Varon et al., 1983). Growth was rescued by the addition of polyamines, which have been shown to increase growth in prey-independent BLOs (Gray and Ruby, 1991). Another factor that may influence the dynamics of predator:prey interactions is the recent finding that the mutation of methyl-accepting chemoreceptors in *B. bacteriovorus* leads to a reduction of predation, suggesting that chemotaxis is involved in finding the prey (Lambert et al., 2003).

Newly formed BLOs appear to be more active than older cells (Hespell et al., 1974) and these experiments show that slow release of attack cells may be a better strategy for the control of target populations than massive input of predators during a short period. But, if one is to apply BLOs in the real world, we think that the cause of the non-eradication of prey cells in lysate cultures should be studied. Also, the influence of predator:prey ratio on the survival of the a targeted prey (such as a pathogen) should be studied in more natural settings in which other prey and non-prey species are present.

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Identification of Traits Implicated in the Rhizosphere Competence of Fluorescent Pseudomonads: Description of a Strategy Based on Population and Model Strain Studies

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Abstract The lack of consistency of the beneficial effects of inoculated fluorescent pseudomonads has often been related to their bad survival in the rhizosphere. In this review, we describe the strategy followed over the last decade to study traits involved in the rhizosphere competence of these bacteria. The diversity of indigenous populations associated with plant roots was first compared to that of populations associated with uncultivated soils in order to identify traits that discriminate these populations. The involvement of these bacterial traits in the rhizosphere competence was then assessed by comparing the competitiveness of a wild-type strain to that of mutants affected in the corresponding phenotypes. Finally, traits shared by populations adapted to the rhizosphere were identified by comparing both the competitiveness in the rhizosphere and the metabolism of a collection of bacterial strains. The data yielded indicated that rhizosphere competent pseudomonads show a specific metabolism especially characterized by the efficiency of the pyoverdine-mediated iron uptake and by the ability to reduce nitrogen oxides.

Keywords Diversity • Metabolism • Model strain • Mutant • Population

Résumé – Identification de caractères impliqués dans la compétence rhizosphérique des *Pseudomonas* spp. fluorescents: description d'une stratégie basée sur des études de populations et de souche modèle. Le manque de fiabilité des effets bénéfiques déterminés par les *Pseudomonas* spp. fluorescents inoculés a souvent été attribué à leur mauvaise survie dans la rhizosphère. Au cours de cette synthèse, nous décrivons la stratégie suivie lors des dix dernières années pour étudier les caractères impliqués dans la compétence rhizosphérique de ces bactéries. La diversité des populations indigènes associées aux racines a d'abord été comparée à celle des populations associées à des sols nus. L'implication de ces caractères bactériens dans la compétence rhizosphérique a ensuite été évaluée en comparant la compétitivité d'une souche modèle à celle de mutants affectés dans les phénotypes correspondants. Finalement, des caractères partagés par les populations adaptées à la rhizosphère ont été identifiés en comparant la compétitivité rhizosphérique et le métabolisme d'une collection de souches. Les données recueillies indiquent que les *Pseudomonas* spp. fluorescents adaptés à la rhizosphère présentent un métabolisme spécifique caractérisé, en particulier, par l'efficacité de leur système d'acquisition du fer basé sur les pyoverdines et par leur aptitude à réduire les oxydes d'azote.

Mots clés Population • Diversité • Souche modèle • Mutant • Métabolisme

1 Introduction

Saprophytic fluorescent *Pseudomonas* spp. constitute an oxidase-positive group, including various species *Pseudomonas fluorescens*, *P. putida*, *P. chlororaphis*

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(Bossis et al., 2000; Palleroni, 1984) and more recently described species, as for example, *P. jessenii* (Verhille et al., 1999) or *P. lini* (Delorme et al., 2002). All bacteria belonging to this group share the ability to produce soluble yellow-green pigments, pyoverdines, which act as siderophores for these bacteria (Meyer and Abdallah, 1978). These microorganisms are considered to be rhizobacteria, since their densities and activities are stimulated in the rhizosphere (Bowen and Rovira, 1976; Höfte et al., 1992; Kluepfel, 1993; Schroth et al., 1992). They are known to synthesize a variety of secondary metabolites (Budzikiezwicz, 1993; Dowling and O'Gara, 1994; Leisinger and Margraff, 1979). Some of them exert a toxic activity against various pathogenic and deleterious microorganisms, contributing to a reduction of their saprophytic growth (microbial antagonism) (Cook et al., 1995; Gutterson, 1990). Microbial antagonism against pathogenic agents decreases the frequency of root infections and of diseased plants, whereas antagonism against deleterious microorganisms enhances plant growth (Schippers et al., 1987). Plant growth promotion can also be ascribed to metabolites affecting the plant physiology such as growth substances (Glick, 1995). Specific bacterial metabolites may elicitate defense reactions of the host plant (induced systemic resistance) (Van Loon et al., 1998).

Consequently, some fluorescent pseudomonads are known to improve plant health and/or growth (Gamalero et al., 2002; Lemanceau and Alabouvette, 1993; Lifshitz et al., 1987; Weller, 1988). They have been shown to play a role in the natural suppressiveness of soils to fusarium-wilts (Alabouvette and Lemanceau, 1996; Baker et al., 1986) and to take-all (Cook and Rovira, 1976; Lucas et al., 1989; Raaijmakers and Weller, 1998). Indeed, fluorescent pseudomonads are considered as potential biocontrol agents of soilborne diseases and several studies have demonstrated their efficacy as microbial inoculants (Cook et al., 1995; Haas et al., 1991; Lemanceau and Alabouvette, 1991; Moulin et al., 1996; Schippers et al., 1995; Thomashow and Weller, 1988). Despite the positive effects reported in these studies, overall biological control of soilborne diseases achieved by fluorescent pseudomonads is often inconsistent (Lemanceau and Alabouvette, 1993; Weller, 1988). This inconsistency has been partially associated with inefficient root colonization by the introduced bacteria (Schippers et al., 1987). Indeed, a clear relationship has been established between suppression of the wheat root disease take-all and that of fusarium-wilts by different strains of fluorescent pseudomonads and the densities of these bacteria in the rhizosphere of the corresponding host plant (Bull et al., 1991; Raaijmakers et al., 1995). In order to improve the efficacy and the consistency of the biological control, the use of rhizosphere-competent strains is required. To fulfill this requirement, progresses must be made in our knowledge of bacterial traits promoting rhizosphere-competence of fluorescent pseudomonads.

When initiating the strategy described in the present review, most of the studies consisted in evaluating the impact of traits, expected to be important for root colonization, such as presence of flagella (De Weger et al., 1987; Howie et al., 1987; Scher et al., 1988), pili (Vesper, 1987), agglutinin (Anderson et al., 1988), membrane lipopolysaccharides (De Weger et al., 1989), porin OprF (De Mot and Vanderleyden, 1991), protease (O'Sullivan et al., 1991) or phenazines (Mazzola et al., 1992). Evaluation of the involvement of these traits consisted in comparing the survival of a model strain and of mutant of that strain affected in the phenotype studied. Even if this strategy contributed to progress in the knowledge of the traits promoting rhizosphere competence of the strains studied (Lugtenberg and Dekkers, 1999; Lugtenberg et al., 2001), it also had limitations. Indeed, results obtained with specific strains and experimental conditions could not always be extended to other strains (De Weger et al., 1987; Howie et al., 1987; Scher et al., 1988). The differences that were recorded between strains are not surprising considering the high diversity of fluorescent pseudomonads which have been described largely after these differences were first noted (Clays-Josserand et al., 1995; Delorme et al., 2002; Keel et al., 1996; Laguerre et al., 1994; Latour et al., 1996; Lemanceau et al., 1995; Mac Spadden Gardener et al., 2000; Mavrodi et al., 2001; Meyer et al., 2002; Raaijmakers and Weller, 2001). Furthermore, the strategy described above did not reveal the importance of traits others than those expected at first to be involved in rhizosphere competence which are not necessarily the most relevant. Thus, the knowledge of the bacterial traits involved in rhizosphere competence was necessarily partial. This was already clearly stressed by De Weger et al. (1995) when comparing the phenotype of the strain *P. fluorescens* WCS365 to those of the 20 mutants, obtained by random mutagenesis, showing an impaired root colonization compared to the wild-type strain. Among these 20 mutants, 3 did not present any difference compared to the wild-type strain in the traits expected to be involved in the rhizosphere competence, clearly indicating that other unknown traits were involved in the rhizosphere competence of *P. fluorescens* WCS365. Surprisingly, at that time little attention was given to the possible involvement of carbon and energy metabolism of the bacteria in their rhizosphere competence despite the fact that the characteristics of the rhizosphere are mostly influenced by the release of photosynthetates by the host-plant (Lynch and Whipps, 1990; Whipps, 1987).

Altogether these observations led us to propose in the early 1990s a strategy based both on population and on model strain studies. We first compared the diversity of populations associated with roots and with uncultivated soils in order to discriminate traits expected to be involved in the rhizosphere competence in a non targeted way. The hypotheses, raised from the population studies, were then evaluated by comparing the competitiveness of a wild-type strain and of isogenic mutants affected in the phenotypes discriminating the rhizospheric and soil populations. Besides the emergence of hypotheses, the initial population studies enabled us to select strains representative of the diversity of populations from rhizospheric and bulk soils. The reduced number of strains considered allowed us to compare their metabolism and their competitiveness in the rhizosphere and then to identify traits shared by the rhizosphere competent populations.

In this review, we describe this strategy developed over the last years in our group to identify traits involved in the rhizosphere competence of fluorescent pseudomonads.

2 Comparison of the Diversity of Populations from Rhizospheric and Bulk Soils

The first step of our strategy consisted in raising hypotheses on traits possibly involved in the rhizosphere competence of fluorescent pseudomonads in a non-targeted way. For that purpose, the diversity of indigenous populations of fluorescent pseudomonads associated with roots and with uncultivated soil was compared in order to possibly identify discriminating phenotypes assumed to be involved in the rhizosphere competence. Since soils are oligotrophic environments whereas the great majority of soilborne microorganisms, including the fluorescent pseudomonads, are heterotrophic, we hypothesized that nutrient competition and consequently carbon and energy metabolism of bacteria would play a major role in their adaptation to the rhizosphere. This environment is indeed characterized by the release of a significant part of the photosynthetates that would be more readily used by the microorganism having the most adapted catabolism. This catabolism is based on electron transfers between donors (organic compounds) and acceptors (coenzymes, Fe–S proteins, cytochromes, oxygen, nitrogen oxides). Rhizospheric and soil populations were then characterized for their ability to use a wide range of organic compounds and their ability to mobilize iron and to respire nitrogen oxides. More specifically, the ability of the bacterial strains to grow in the presence of 1 of the 49 sugars, 49 organic acids or 49 amino acids some of which having been described as component of the root exudates (Vancura, 1980, 1988), has been tested using API strips (BioMerieux). Ferric iron is a cofactor of proteins indispensable to the oxido-reduction processes of respiratory chain and is then essential for microbial metabolism. However, the concentration of Fe^{3+} available to the soilborne microflora is very low (Lindsay, 1979), in such way that this ion is usually a limiting factor for microbial growth and activity in soil habitats (Loper and Buyer, 1991). The ability to mobilize ferric iron of the bacteria was assessed by measuring their Minimal Inhibitory Concentration (MIC) of 8-hydroxiquinolin, a strong iron chelator (Geels and Schippers, 1983); (Lemanceau et al., 1988). Bacteria able to reduce NO_3^- (nitrate reducers) and among them those able to produce N_2O and/or N_2 (denitrifiers) were determined by biochemical methods (Clays-Josserand et al., 1995).

In order to enhance the possible rhizosphere effect, plants were continuously cultivated for 5 cycles of 8 weeks each in a given soil (Dijon, France) and the bacterial isolations were made on the last culture. The Dijon soil was kept uncultivated and was maintained in the same environmental conditions as the cultivated soil. Furthermore, in order to stress more clearly the plant effect on the soilborne populations, they were isolated along a horizontal gradient (rhizospheric soil, rhizoplane, and root tissues). All these experiments were performed with two plant species (*Linum usitatissinum* L. and *Lycopersicon esculentum* Mill.). Numerical analysis of the results enabled us to cluster isolates showing a high level of similarity (Latour, 1996).

The data yielded indicate that the plant affects both the structure and diversity of the indigenous soilborne populations of fluorescent pseudomonads. Indeed, some of the phenotypic clusters only included rhizospheric isolates whereas another only included soil isolates, clearly indicating that the rhizospheric isolates show differences in their abilities to use organic compounds compared to those of soil. More precisely, it was shown that the ability to use specific sugars (saccharose, trehalose, xylose), polyols (inositol, sorbitol) and amino acids (citrulline, trigonelline) is more frequent among rhizospheric populations than soil populations (Latour and Lemanceau, 1997); (Lemanceau et al., 1995). Overall, rhizospheric strains showed a higher similarity than those isolated from bulk soil, indicating that the diversity of the rhizospheric populations was reduced compared to that of the soil populations. This reduced diversity was even more strongly expressed for the endophytic populations. The ability to mobilize iron of the rhizospheric and soil populations was also shown to differ significantly. Indeed, the frequency distribution of the strains in the different MIC differed significantly according to their origin. The rhizospheric populations were mostly distributed in the classes corresponding to the highest concentrations of 8-hydoxiquinolin, indicating that they were more able to mobilize ferric iron in deficiency conditions than soil populations (Lemanceau et al., 1988). Finally, the proportion of strains able to respire nitrates was significantly higher in the populations associated with the roots (90 and 82% in the root tissues of flax and tomato, respectively), than in the uncultivated soil (55%). Among these strains, the proportion of denitrifiers gradually and significantly increased in the vicinity of tomato roots (44, 68, 75 and 94% in uncultivated soil, rhizosphere, rhizoplane and root tissues, respectively) and was higher in the flax rhizoplane (66%) than in the uncultivated soil.

Altogether, these data indicate that plants select specific populations of fluorescent pseudomonads and that compared to soil populations they are (i) more able to mobilize ferric iron, (ii) more frequently nitrate reducers and denitrifiers, (iii) able to use specific organic compounds as carbon and energy sources.

Since microbial inoculations would be performed on plants growing in various soils, the impact of the soil type on the plant selection towards the indigenous soilborne populations was further assessed. The two plant species were grown in two different soils and the populations were analyzed as described above. The influence of the soil type on the rhizosphere effect was then considered by comparing data collected in two different soils (Dijon and Châteaurenard, France). Although the selection recorded previously in the Dijon soil was checked in the Châteaurenard soil, this selection appeared to differ in the two soils (Latour et al., 1996). The populations associated with the roots of a given plant species cultivated in the two soils differed significantly in their ability to use the organic compounds tested. Differences in the indigenous soilborne populations only partly accounted for the variations of the populations associated with a given plant species cultivated in these two soils. Indeed the variation of the rhizosphere effect determined by a same plant species was shown to differ in the two soils even when their indigenous microflora was destroyed by γ-irradiation and replaced by the same calibrated community of fluorescent pseudomonads. The rhizosphere effect was more strongly expressed in the Châteaurenard soil and the structure of the populations re-isolated differed significantly in the rhizosphere of the plants cultivated in the Châteaurenard and in the Dijon soil (Latour et al., 1999). These data clearly indicate that it was not possible to identify common organic compounds specifically used by fluorescent pseudomonads associated with the roots of plants cultivated in different soils.

In contrast, indigenous populations of fluorescent pseudomonads selected by the two different plant species cultivated in the two soils have in common the ability to efficiently take up iron (Lemanceau et al., 1988) and to respire nitrogen oxides (Clays-Josserand et al., 1999). From these observations, we then hypothesized that these two traits might be involved in the rhizosphere competence of fluorescent pseudomonads.

3 Evaluation of the Involvement of Nitrate Reductase and Pyoverdine in the Rhizosphere Competence of a Model Strain

The second step of our strategy consisted then in assessing the above referred hypotheses, using a model strain and isogenic mutants of this strain. The *P. fluorescens* strain C7R12 is a rifampicin-resistant mutant of the strain C7 (Eparvier et al., 1991), previously isolated from the rhizosphere of flax cultivated in Châteaurenard soil (Lemanceau et al., 1988). This strain was chosen as a model strain since it was shown (i) to be a good denitrifying strain (Clays-Josserand et al., 1995), (ii) to efficiently mobilize the iron (Lemanceau et al., 1988), (iii) to be rhizosphere-competent (Eparvier et al., 1991) and (iv) to be a biocontrol agent (Lemanceau and Alabouvette, 1991). Fluorescent pseudomonads have evolved an efficient strategy of iron uptake based on the synthesis of the siderophore pyoverdine and its relevant outer membrane receptor (Meyer et al., 1987). The possible implication of pyoverdine, in the ecological competence of *P. fluorescens* C7R12 in soil and rhizosphere, was evaluated using a pyoverdine minus mutant (Pvd$^-$) obtained by random insertion of the transposon Tn*5* (Mirleau, 2000). The Tn*5* flanking DNA was amplified by inverse PCR and sequenced. The nucleotide sequence was found to show a high level of identity with *pvsB*, a pyoverdine synthetase. As expected, the mutant Pvd$^-$ was significantly more susceptible to iron starvation than the wild-type strain despite its ability to produce another unknown siderophore. As with the wild-type strain, the mutant Pvd$^-$ was able to incorporate the wild-type pyoverdine and 5 pyoverdines of foreign origin, but at a significantly lower rate despite the similarity of the outer membrane protein patterns of the two strains (Mirleau et al., 2000). The survival kinetics of the wild-type strain and of the mutant Pvd$^-$ in bulk and rhizospheric soil were compared in gnotobiotic and non-gnotobiotic conditions. In gnotobiotic conditions, when inoculated separately both strains showed a similar survival in soil and rhizosphere suggesting that iron was not a limiting factor. In contrast, when inoculated together, the bacterial competition was favorable to the pyoverdine producer C7R12. The fitness of the mutant Pvd$^-$ in the presence of the indigenous microflora, even when coinoculated with C7R12, was assumed to be related to its ability to uptake heterologous pyoverdines. Altogether, these results suggest that pyoverdine-mediated iron uptake is involved in the ecological competence of the strain *P. fluorescens* C7R12 (Mirleau et al., 2000).

The involvement of nitrogen oxide respiration in the rhizosphere competence of *P. fluorescens* C7R12 was also assessed by comparing the competitiveness of the wild-type strain to that of a mutant affected in nitrate reductase synthesis (Nar$^-$). Nitrate reductase catalyses the first step of denitrification. The corresponding experiments were performed under gnotobiotic conditions. The Nar$^-$ mutant was obtained by site-directed mutagenesis (Mirleau et al., 2001). The selective advantage given by nitrate reductase over the wild-type strain was assessed by measuring the dynamic of the mutant-to-total-inoculant (wild-type strain plus mutant) ratio. The Nar$^-$ mutant clearly showed a lower competitiveness than the wild-type strain, indicating that nitrate reductase is important. However, the selective advantage given by nitrate reductase was more strongly expressed under conditions of lower aeration (Mirleau et al., 2001). Comparison of the competitiveness of the Pvd$^-$ and Nar$^-$ mutants indicated that the competitive advantages given to C7R12 by nitrate reductase and pyoverdine were similar. A double mutant (Pvd$^-$Nar$^-$), obtained by site-directed mutagenesis of the Pvd$^-$ mutant, presented the lowest competitiveness (Mirleau et al., 2001).

Altogether, these data indicate the importance of pyoverdine-mediated iron uptake and nitrate respiration on the fitness of the biocontrol agent *P. fluorescens* C7R12. The competitive advantage given to the wild-type strain by pyoverdine and nitrate reductase over the defective mutants was expressed not only in the rhizosphere but also in bulk soil, indicating that these two bacterial traits are implicated in the bacterial saprophytic competence in soil environments.

4 Identification of Bacterial Traits Shared by Rhizosphere Competent Populations

The conclusions made following the model strain approach described above are obviously only valid for the model strain studied and only concerned two traits.

In order to extent our conclusions to populations of fluorescent pseudomonads and to assess the possible involvement of other metabolic activities, we came back to a population approach for the next step of our strategy. In contrast with the first step of the strategy aiming at the identification of traits shared by the populations selected by the plant, we now assessed metabolic characteristics shared by populations adapted to the rhizosphere.

The adaptation to the rhizosphere was evaluated by measuring the competitiveness of the studied populations in the rhizosphere of tomato cultivated in soil, in the presence of the indigenous microflora and microfauna (non-gnotobiotic conditions). The corresponding experiments are very labour intensive and could then only be performed on a limited number of strains (23 strains). Twenty-one strains came from the selection of one strain from each of the clusters previously defined (Latour et al., 1996; Lemanceau et al., 1995) with the 340 strains tested. These 21 strains are then expected to be representative of the diversity of the larger bacterial collection from which they are issued (Delorme, 2001). Two reference strains were added: (a) *P. fluorescens* C7R12 studied in the model strain approach (see Section "Evaluation of the Involvement of Nitrate Reductase and Pyoverdine in the Rhizosphere Competence of a Model Strain") and (b) *P. fluorescens* A6 known for its ability to promote plant growth (Gamalero et al., 2002) and to suppress soilborne diseases (Lemanceau and Samson, 1983; Berta et al., unpublished data). The results obtained with these 23 strains clearly indicate the high diversity of the strains for their competitiveness in the tomato rhizosphere. Indeed, the survival rate of the strains varied from 0.11% for the less competitive strain to 61.4% for the most competitive (Delorme et al., unpublished data).

Relations between these observations and metabolic characteristics already described above – such as the ability to assimilate a wide range of organic compounds and the ability to reduce NO_3^- and/or N_2O, and additional traits such as the ability to synthesize extracellular enzymes (gelatin liquefaction, levan production) (Lelliot et al., 1966), to synthesize *N*-acyl-homoserine-lactones (NAHL) involved in quorum sensing (Elasri et al., 2001), to synthesize phenazines (antibiotic compounds) (Raaijmakers et al., 1997), the characterization of the pyoverdine-mediated iron uptake (siderotype, ability to incorporate heterologous pyoverdines) (Meyer et al., 1997) – were evaluated by appropriated statistical methods. Multiple correspondence analyses were first applied to identify possible traits explaining the bacterial competitivity, and were then followed by mean multiple comparisons and variance analyses to determine if these traits were indeed involved in the rhizosphere competence of the fluorescent pseudomonads (Delorme, 2001).

These statistical analyses indicated that the populations selected by the plant were more competitive than the populations isolated from bulk soils. However, some strains isolated from the rhizosphere were not necessarily competitive in this environment, confirming then the relevance of the competitivity experiments developed by Delorme et al. (unpublished data).

Excepted one strain, the most competitive strains all belonged to the same siderotype, which was different from that of the less competitive strains showing various other siderotypes. The only strain differing by its siderotype from the other competitive strains also differed from these strains by its ability to synthesize NAHL and phenazine. The most competitive strains were all able to reduce nitrogen oxides to dinitrogen gas. In contrast, the most competitive strains did not belong to the same phenotypic clusters indicating that these strains could not be differentiated from the less competitive on the basis on their auxanogram. The only electron donors used in common by the most competitive strains was trehalose. Altogether, these data clearly show the importance of the carbon and energy metabolism and more especially of specific electron acceptors (ferric iron, nitrogen oxides) in the rhizosphere competence of the fluorescent pseudomonads.

All the most competitive strains had the gelatinase. Half of them produced levansucrase whereas none of the less competitive showed this ability. These enzymes contribute to modifications of the root environment which may be favorable for bacterial survival in the rhizosphere. Indeed, the activity of the gelatinase, a protease with a broad spectrum, leads to the release of essential amino acids and then contribute to their increased availability for the bacteria (Curl and Truelove, 1986; Simons et al., 1997). In the same way, the synthesis of the levan, a polymer of the fructose known to be present in the rhizosphere, contribute to the aggregation of soil adhering to roots and then favor a more porous structure in rhizosphere soil (Bezzate et al., 2000).

Concerning bacterial secondary metabolism, statistical analyses of the data indicated that the ability to produce NAHL explains rhizosphere competence of the fluorescent pseudomonads tested. The only strain producing phenazine, showing also the ability to synthesize NAHL, appeared to be competitive in the rhizosphere, however this was not significant since there was only that strain having this ability among those tested.

A major conclusion of this study is that the rhizosphere competence has a multifactorial determinism that confirms the limitations of the research made only on specific traits. As an example, it appeared that (a) the most competitive strains have the ability to use trehalose, (b) however some non-competitive strains show also this ability, (c) but these non-competitive strains do not have the ability to denitrify and then (d) the competitive strains present both the ability to use trehalose and to denitrify (Delorme, 2001).

Another major conclusion is that depending on the strains, the strategy of adaptation to the rhizosphere differs. The data yielded allowed us to stress two types of behavior. In the first one, the strains show a specific carbon and energy metabolism. They share the same siderotype, they are able to fully reduce nitrogen oxides and they have the ability to assimilate trehalose and to produce gelatinase. The competitivity of the second type would rather be ascribed to its ability to antagonize the indigenous microflora through the synthesis of antibiotic (phenazine) and its probable regulation through NAHL production as described in the strain *P. aureofaciens* 30–84 (Wood et al., 1997).

5 Discussion

This review describes the strategy that we developed over the last decade in order to identify bacterial traits involved in the rhizosphere competence of fluorescent pseudomonads. The originality of this strategy is to have associated both population and model strain approaches (Fig. 1).

Our study strategy first consisted in comparing indigenous populations associated with roots and bulk soils in order to identify traits allowing the discrimination of these two types of populations, these traits being then expected to be involved in the rhizosphere competence. During these diversity studies, our attention was focused on the bacterial carbon and energy metabolism because of the importance of trophic relations among microorganisms, mostly heterotrophic, in soils (Lemanceau et al., 1988; Lockwood, 1964) and rhizospheres (Curl and Truelove, 1986; Duijff et al., 1999). The diversity studies were conducted in the rhizosphere of two plant species cultivated in two different soils, and in these same soils kept uncultivated. Comparison of the diversity of bacterial populations associated with a given soil and with roots of a given plant species was also performed by Mavingui et al. (1992) and Frey et al. (1997). And the possible crop specificity towards fluorescent pseudomonads was previously showed by Glandorf et al. (1993). However, to our knowledge, our studies were among the very first to include both the effects of the plant species and of the soil-type on the selection achieved by the host-plant towards bacterial soil populations. These studies allowed us to identify traits shared by pseudomonads populations selected by two different plant species cultivated in two different soils.

The implication of these traits in the rhizosphere adaptation was then evaluated by a model strain approach that consisted in comparing the rhizospheric competitiveness of a wild-type strain to that of mutants. This approach based on the comparison of the survival kinetics of wild-type strains to that of mutants, affected in specific phenotypes, has been commonly used to assess the involvement of these phenotypes in the rhizosphere competence of various bacterial strains (Carroll et al., 1995; De Weger et al., 1995; Duijff et al., 1997; Höfte et al., 1992; Mazzola et al., 1992). However, an originality of our work was to evaluate the relative importance of two bacterial traits thanks to the use of single and double mutants. Another originality was to assess the impact of a specific environmental parameter (soil aeration) on the involvement of the studied traits in the rhizosphere competence of the wild-type strain. The choice of this parameter was made since aeration is known (a) to vary a lot in the rhizosphere and (b) to affect bacterial fitness in the rhizosphere (Höjberg and Sörensen, 1993; Höjberg et al., 1999; Meikle et al., 1995).

Besides the identification of traits expected to be involved in rhizosphere competence, our early diversity studies enabled us to select strains representative of the populations associated with soils and roots. This reduced number of strains made possible the evaluation of their competitiveness in the rhizosphere.

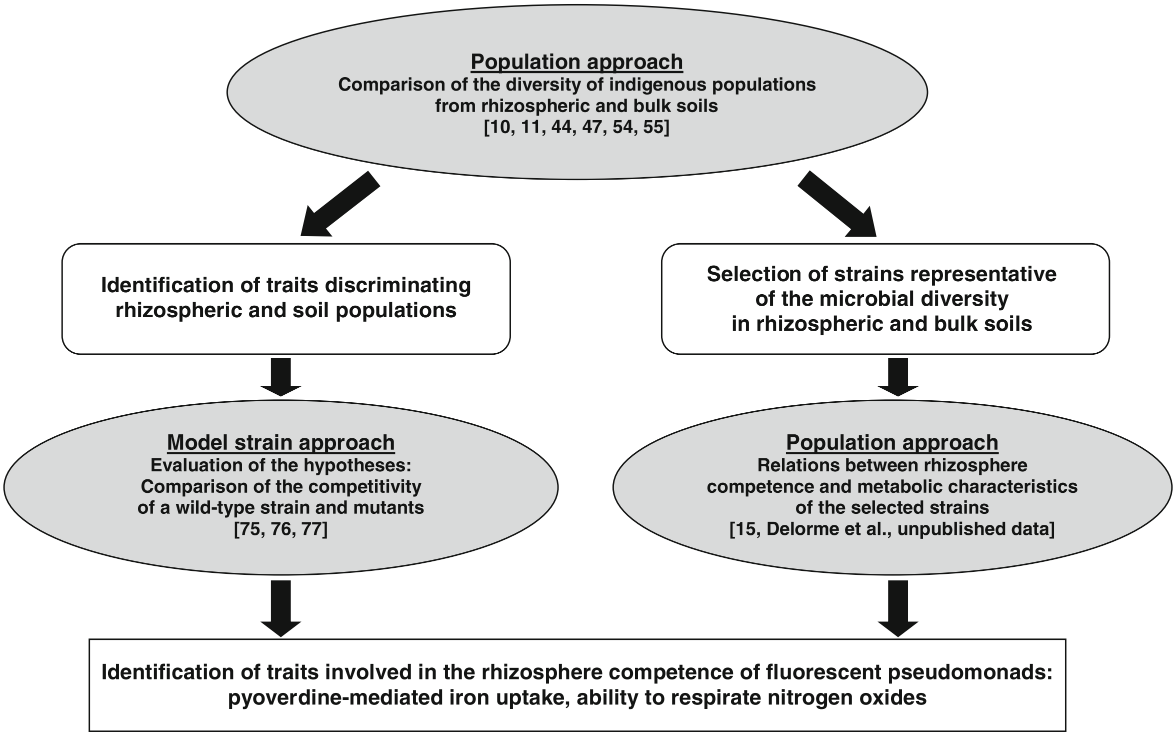

Fig. 1 Schematic representation of the approaches followed to identify bacterial traits involved in the rhizosphere competence of fluorescent pseudomonads

A similar approach based on the selection of strains representative of the diversity, assessed by PCR-RAPD, of the 2,4-diacetylphloroglucinol-producing *Pseudomonas* spp. enabled Raaijmakers and Weller (2001) to select the superior root-colonizing *P. fluorescens* Q8r1-96. In our case, the level of competitiveness in the rhizosphere of the selected strains was compared to several characteristics of their metabolism in order to identify traits shared by populations adapted to the rhizosphere. This type of relation was applied by Ellis et al. (2000) to identify conserved traits in fluorescent pseudomonads with antifungal activity.

The main conclusion obtained following the strategy developed is that the carbon and energy metabolism of fluorescent pseudomonads plays a major role in their rhizosphere competence.

More specifically our data show the importance of the ability to use ferric iron and nitrogen oxides as electron acceptors in the rhizosphere competence of fluorescent pseudomonads. This conclusion agrees with the fact that (a) fluorescent pseudomonads require oxygen or nitrogen oxides for oxidization of all the substrates, excepted arginine (Latour and Lemanceau, 1997), and that (b) the ferric iron and oxygen content is usually low in the rhizosphere (Höjberg and Sörensen, 1993; Höjberg et al., 1999; Loper and Henkels, 1997). The ability to use efficiently both types of electron acceptors is favorable to the respiratory potential of fluorescent pseudomonads. This potential directly affects the assimilation of nutrients by *Pseudomonas* and consecutively their multiplication. Indeed, aerobic respiration and denitrification allow both the production of ATP by oxidative phosphorylation and the reoxidation of NADH and $FADH_2$ coenzymes. This reoxidation is required for the cycle of Krebs that appears to play a major role for pseudomonads metabolism and more specifically for the nutrient assimilation by these bacteria (Latour and Lemanceau, 1997). Ecological observations made by the group of Lugtenberg on the rhizosphere competence of the model strain *P. fluorescens* WCS365 are in agreement with these metabolic characteristics. Indeed, the ability to use organic acids in the rhizosphere, oxidized by the cycle of Krebs, are involved in the rhizosphere competence of this model strain (Lugtenberg et al., 2001), whereas the ability to use sugars by Entner-Doudoroff pathway does not confer a competitive advantage to this bacterial strain (Lugtenberg et al., 1999).

In fact, from our data it was not possible to identify a common auxanogram shared by the populations being the most competitive in the rhizosphere. This observation could be related to the ability of the pseudomonads to use a wide range of organic compounds which would then allow them to adapt their metabolism to various environments and more specifically to the evolution of composition of the root exudates according to the plant species, the plant development and the soil type.

Despite the progresses made in the knowledge of the bacterial traits involved in the rhizosphere competence of fluorescent pseudomonads, our study strategy presents limitations. The first one is that only metabolic traits, mostly related to carbon and energy metabolism of bacteria, were taken in account. Other relations than trophic between the microflora and the host-plant may probably also play a role in the rhizosphere competence of the fluorescent pseudomonads: molecular recognition through signal molecules and/or membrane characteristics (Dénarié et al., 1996; Duijff et al., 1999), ability to stand or detoxify toxic compounds, etc... A second limitation is that even if the number of traits tested were important during the population studies, they still remain quite low and moreover were chosen a priori. Other strategies than the one described in the present review were aimed at searching untargeted traits involved in the rhizosphere competence of model strain, following an approach based on random mutagenesis to identify genes targeted by the insertion sequence in mutants showing an impaired root colonization (Lam et al., 1990; Lugtenberg and Dekkers, 1999) or to identify genes preferentially expressed in the rhizosphere (Rainey, 1999).

Development of methods of functional genomic and proteomic should allow to provide new insights on the microbial determinism of the rhizosphere competence. Further research should also take more in consideration the effect of the host-plant in the interaction plants – microorganisms, and plant traits affecting the rhizosphere microflora should also be determined. The final goal would be to identify plant/microbe couples the most favorable for the survival and activity of the beneficial introduced organisms.

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Progress in Mechanisms of Mutual Effect between Plants and the Environment

Hong-Bo Shao, Li-Ye Chu, and Biao Li

Abstract Higher plants play a major role in keeping a stable environment on the globe. They regulate global climate and surroundings in many ways at different levels such as molecular, cellular, organ, individual, community, regional, ecosystem and global ecosystem levels. This article will focus on the abiotic aspect of the environment. Readers interested in the biotic aspect can read recent publications by Garcia-Brugger et al. [Early signalling events induced by eliators of plant defenses. Mol. Plant Microbe In. 19 (2006) 711–724], Lecourieux et al. [Calcium in plant defence-signalling pathways, New Phytol. 171 (2006) 249–269], and Conrath et al. [Priming: Getting ready for battle, Mol. Plant Microbe In. 19 (2006) 1062–1071], for related progress. Plant behavior and character expression are controlled at the molecular level by gene expression and environmental cues. In a persistently changing environment there are many abiotic adverse stress conditions such as cold, drought, salinity and UV-B, which influence plant growth and crop production. Unlike animals, higher plants, which are sessile, cannot escape from their surroundings, but adapt themselves to changing environments by inducing a series of molecular responses to cope with these problems. The physiological processing basis for these molecular responses is the integration of many transduced events into a comprehensive network of signaling pathways. Here, higher plant hormones occupy a central place in this transduction network, frequently acting in conjunction with other signals, to regulate cellular processes such as division, elongation and differentiation, which are the fundamental basis for higher plant development and related character expression. Stress factors are also major ecological factors influencing the environment, which are general environmental stimuli and cues to higher plants. Molecular responses to environmental stresses have been studied intensively over the last few years. The findings show an intricate network of signaling pathways controlling perception of environmental signals, the generation of second messengers and signal transduction. In this review, up-to–date progresses are introduced in terms of functional analysis of signaling components and issues with respect to the agricultural environment and sustainable development. These advances mainly include identification of the abiotic stress-responsive genes, extensive realization of the mutual concerted relationship between plants and the environment on different scales, molecular mechanisms of stress signal transduction and pathways, and so on. Here, a general network of stress-responsive gene expression-control model is proposed, with an emphasis on the integration between stress signal transduction pathways and the agricultural environment.

Keywords Agricultural sustainable development • Biological measures • Eco-environment • Environmental stresses • Global climate change • Higher plants • Network of signaling transduction • Plant biology

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1 Introduction

Human beings have stepped into the twenty-first century, during which sustainable and healthy utilization of the environment and resources and their own health concerns are the most important issues. Those issues are tightly linked with agriculture and the environment, in which biology, in particular plant biology, plays a major role because plants offer the globe a renewable resource of food, building material and energy (Abbott 2003; Ballare 2003; Asada 2006; Bergantion et al. 2002; Becker et al. 2002; Bao and Li 2002; Breusegem and Dat 2006; Brodribb et al. 2005; Cook et al. 2005; Darnell 2002; Deng et al. 2002, 2003; Garcia-Mata and Lamattina 2002; Finkestein et al. 2002; Gao and Li 2002; Dharmasiri and Estelle 2002; DeLong et al. 2002; Dodds and Schwechheimer 2002; Eckardt 2002a–c; Flexas and Medrano 2002; Friml and Palme 2002). The biological environment is a crucial part of nature, on which human beings depend for sustainable development. The character expressions of higher plants are controlled by developmental cues and environmental stimuli, most of which are factors such as low temperature, drought, salinity and UV-B radiation (He et al. 2002; Helliwell et al. 2002; Hagen and Guilfoyle 2002; Ha et al. 2005; Hui and Jackson 2006; Liscum and Reed 2002; Leon et al. 2001; Liu et al. 2000, 2003a–c; Liu and Zhu 1998; Jia et al. 2003; Jordan 2003; Kolbe et al. 2006; Milborrow 2001; Medina et al. 1999; Marfinez-Mardrid et al. 2002; Moller et al. 2002; Chow and McCourt 2003; Mouradov et al. 2002; Mlotshwa et al. 2002; Mao et al. 2002; Shao 1993; Shao et al. 2004, 2005a–c, 2006a–d, 2007). Understanding the mechanisms by which higher plants perceive environmental stimuli and transmit the signals to cellular machinery to activate adaptive responses is of vital importance to biology (Mark and Antony 2005; Munns 2005; Napier et al. 2002; O'Connell and Panstruga 2006; Sudha and Ravishankar 2002; Sa et al. 2003; Shen et al. 2003; Swarup et al. 2002; Shao 2001a–c; Shao 2003, 2005). Knowledge about stress signal transduction is also the basis for continued development of crop breeding and transgenic strategies to improve stress tolerance in forest, grass, crops and, especially, decomposing poisonous substances of circumstance, and vegetation succession (Shao et al. 2006; Sessitsch et al. 2006; Somerville and Dangl 2000; Sakuma et al. 2006; Rabbani et al. 2003; Rensink and Buell 2004; Roberts et al. 2002; Tardieu 2003; Tarcgnski et al. 1993; Torres et al. 2006; Rossel et al. 2002; Rizhsky et al. 2002; Trewavas 2002; Xue et al. 2002; Xiong et al. 2002; Yu et al. 2002; Yang et al. 2006; Vasil 2002, 2003; Voloudadis et al. 2002; Vardy et al. 2002; Wang and Peng 2003; Wang et al. 2003; Nguyen 2003; Sakuma et al. 2006; Schumpp et al. 2003; Chinnusamy et al. 2006; Malamy 2005; Zhang et al. 2002, 2003; Bauer and Bereczky 2003; Conrath et al. 2006; Garcia-Brugger et al. 2006; Lecourieux et al. 2006). Here, we integrate up-dated information and put forward a general stress signal transduction pathway model from the angle of the agricultural environment, the purpose of which is to establish a connecting bridge between molecular biology and ecology and to instruct environmental construction.

2 A General Model for the Stress Signal Transduction Pathway in Higher Plants

Animals perceive their local environments by complex signal transduction processes. Intelligent responses are computed, and fitness is increased by behavioral changes that commonly involve movement. Movement is a fundamental part of the animal lifestyle that arises in evolution from the requirements to find food and to mate. The same happens in higher plants and sessile higher plants must also change behavior to increase fitness as the local environment fluctuates (Boniotti and Griffith 2002; Zhu 2003; Zhang et al. 2002, 2003; Liu and Zhu 1998; Liu et al. 2000; Swarup et al. 2002; Wu and Tang 2004; Wright et al. 2006; Zhou et al. 2003). The ubiquitous distribution of light has never provided evolutionary pressure to develop movement; instead, behavioral changes are exemplified by phenotypic plasticity (Jordan 2003; Moller et al. 2002; Munns 2005). However, the need for detailed environmental stimuli, accurate sensing, assessment and intelligent computation is just as strong (Trewavas 2002). A stronger spatial dimension network underlies signal transduction, for instance, and higher plants must be able to detect gradients in signals such as light and resources such as nitrate and water (Shao 2003a, b, 2005a–c, 2006a–d; DeLong et al. 2002; Wright et al. 2006). Higher plant development itself is also polar (Zaninotto et al. 2006;

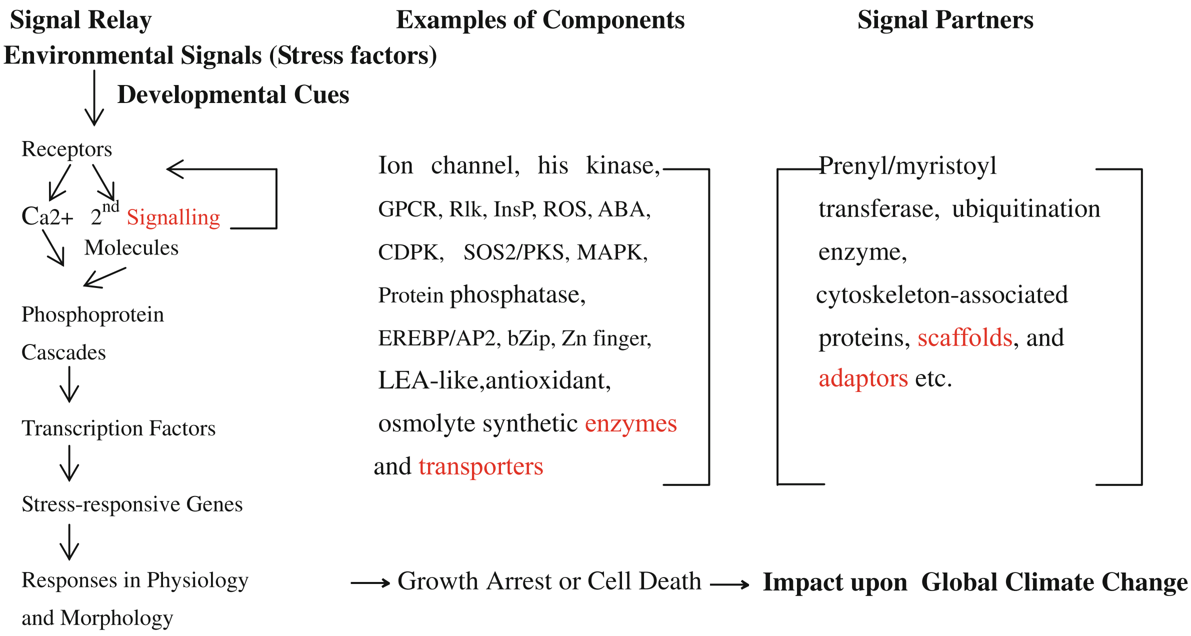

Fig. 1 A common framework model for the signal transduction of abiotic stress in higher plants (Shao et al., 2005, 2006; Trewavas 2002; Rizhsky et al. 2002; Wang et al. 2003; Takemoto and Hardham 2004; Millar et al. 2003; Boudsocq and Lauriere 2005)

Zhu 2000, 2001; Zhu et al. 1997, 1998). The spatial dimension is satisfied in many ways. Higher plant cells place receptors, channels, G proteins and kinases in specific membranes. Some signaling protein complexes are permanent, such as relatively stable and perhaps hardwired COP9 signalosome. Other signaling protein complexes are likely to be ephemeral and formed immediately as a result of signaling(Boniotti and Griffith 2002; Bergantion 2002; Becker et al. 2002; Bao and Li 2002; Dodds and Schwechheimer 2002; Liscum and Reed 2002; Trewavas 2002; Sudha and Ravishankar 2002; Zhu 2003). There are at least 300 receptor kinases in *Arabidopsis*, and most of them are membrane-bound. Incompatibility and disease defense signal transduction use receptor kinases. After ligand binding and autophosphorylation, such kinases may act as nucleation sites for the construction of ephemeral signaling complexes that contain many proteins (Leon et al. 2001; Foyer and Noctor 2005; Boudsocq and Lauriere 2005; Takemoto and Hardham 2004; Arholdt-Schmitt 2004; Luan and Gupta 2004; Desikan et al. 2003; Millar et al. 2003). Although there are some differences in different higher plants, a common signal model for stress transduction pathways exists in higher plants (Boniotti and Griffith 2002; Darnell 2002; He et al. 2002; Liu et al. 2000, 2003; Jia et al., 2003; Munns 2005; Shao et al. 2003a, b, 2006a–d; Sessitsch et al. 2006; Rossel et al. 2002; Xiong et al. 2002; Yu et al. 2002) (Fig. 1). This model begins with the perception of signals from environments, followed by the generation of second messengers such as inositol phosphates and reactive oxygen species.

Second messengers can modulate intracellular Ca^{2+} levels, often initiating a protein phosphorylation cascade that finally targets proteins directly involved in cellular protection or transcription factors controlling specific sets of stress-regulated genes. The products of these genes may participate in the production of regulatory molecules such as the plant hormones abscisic acid, ethylene and salicylic acid (Marfinez-Madrid et al. 2002; Mouradov et al. 2002; Mark and Antony 2005; Sakuma et al. 2006; Roberts et al. 2002; Chu et al. 2005; Bauer and Bereczky 2003). Some of these regulatory molecules can, in turn, initiate a second round of circulation. More and more facts from different disciplines of natural sciences and social sciences have clearly shown that molecular biology is the leading discipline during the twenty-first century, through which many issues may obtain an eventual resolution. It is time to take much more care with our environment, on the basis of considering a vast amount of data involved in biology, physiology, pedology, environmental stress and molecular biology. How to integrate this information available, how to analyze the data completely, how to establish a tied relationship among different data obtained at different levels and accuracy are the main challenges confronting us, which are the key points for us to improve our environment and conduct sustainable development (Hui and Jackson 2006; Shao 2001; Sessitsch et al. 2006; Bauer and Bereczky 2003; Malamy 2005; Schumpp et al. 2003; Desikan et al. 2005).

We are facing a fluctuating world, in which the surroundings are worse and worse and the resources are more and more limited: our final goal is just to adapt ourselves to such circumstances and utilize them in the best way, and to have the optimum survival space through our knowledge. A recent Floral Genome Project, an ambitious undertaking linking phylogenetic, genomic and developmental perspectives on plant reproduction, funded by the National Science Foundation of the USA from October 2001 through 2006 will provide us with more comprehensive knowledge about the origin, conservation and diversification of the genetic architecture of flowers, which will also give us insights into global changes (Eckardt 2002; Somerville and Dangl 2000; Rossel et al. 2002; Chinnusamy et al. 2006; Nguyen 2003). Rossel et al. (2002) used microarray techniques and Arabidopsis as experimental materials to explore the relationship between global changes and gene expression, enforcing and further bearing out the multiplicity and universality that higher plants are adapted to changing environments from the starting point of gene expression. Much research is needed on this frontier and overlapping field.

The past years have seen great strides in dissecting the molecular basis of environmental stress signal transduction in higher plants. Advances in our understanding of the integration of higher plant signaling processes at the transcription level have relied, rely and will continue to rely heavily on the application of genetic approaches in the model plant *Arabidopsis thaliana*. Such studies have helped, in the first instance, to identify important components of hormone and other stress signaling pathways. An integrative signaling function has often been elucidated through the pleiotropic hormone response phenotype of the null mutation or by subsequent second-sited mutation screens (Zaninotto et al. 2006; Jia et al. 2003; Xiong et al. 2002). At the protein level, novel interactions between newly discovered components from nominally discrete signaling pathways will be detected through the application of two-hybrid proteomic-based approaches or the use of high-throughput protein chip-based technologies. Microarray-based expression analysis represents the genomic technology most likely to have an immediate impact on this area of research. The ability to profile the entire *Arabidopsis* genome opens up unprecedented opportunities to study different environmental stress signals at the level of gene expression. However, great care must be taken in experimental design to ensure that meaningful results are obtained. For instance, the researcher must ensure that comparisons are made between materials at equivalent developmental stages when profiling a hormone mutant vs. wild type. Equally importantly, validation of initial expression profiling results must be obtained with either independent alleles or related hormone mutants. Remember, these results should be compared with those obtained from other higher plants as much as possible (O'Connell and Panstruga 2006; Hui and Jackson 2006; Sessitsch et al. 2006; Shao et al. 2005a–c, 2006a–d; Tardieu 2003; Arnholdt-Schmitt 2004; Foyer and Noctor 2005).

In summary, given the rich molecular biology and other branch information resources available, *Arabidopsis* will continue to represent the model experimental system to study environmental stress signal transduction and cross-talk in higher plants. Nevertheless, we must not overlook the rich diversity of signaling mechanisms that has evolved in other higher plant species and endeavor to adopt a comparative and integrative research approach on a global scale. Signaling may follow the above model, although some different components are often involved (Bergantion et al. 2002; Breusegem and Dat 2006; Cook et al. 2005; Dharmasiri and Estelle 2002; Eckardt 2002; Mark and Antony 2005; Moller et al. 2002; Rabbani et al. 2003; Mlotshwa et al. 2002).

Signal transduction processes are very complicated, requiring the suitable spatial and temporal coordination of all signaling molecules involved in the transduction process. Therefore, there are some molecules that take part in the modification, delivery or assembly of signaling components, but do not directly relay the signal. They are very critical for the precise transmission of stress signals. These proteins include protein modifiers (e.g., enzymes for protein lipidation, methylation, glycosylation and ubiquitination), scaffolds and adaptors (Finkelstein et al. 2002; Friml and Palme 2002; Hagen and Guilfoyle 2002; Milborrow 2001; Medina et al. 1999; Liu et al. 2000; Jordan 2003; Mao et al. 2002; Pinto et al. 2002; Shao et al. 2005a–c, 2006a–d; Chinnusamy et al. 2006).

3 Multiplicity of Higher Plant Stress Signals

Low temperature, drought, high salinity and UV-B radiation are common complex abiotic stresses that possess many different related attributes, each of which may provide the higher plant cell with quite different information. This results in the multiplicity and complexity of higher plant adaptation to fluctuating environments for the sake of tuning well and succeeding, which involves cell-to-cell communication and coordination among different organelleles – such as chloroplast and nucleus and mitochondrion, respectively and integratedly (Jordan 2003; Mao et al. 2002; Chow and McCourt 2003; Malamy 2005). For instance, water stress may immediately bring about mechanical constraints, changes in activities of macromolecules, and decreased osmotic potential in the cellular milieu, and ion concentration change (Deng et al. 2002, 2003; Rizhsky et al. 2002; Shen et al. 2003; Sakuma et al. 2006; Zhu et al. 1997). We indicated that different pretreatments of barley mature embryos heavily influenced the hormone (abscisic acid, gibberellic acid and others) and ion (Na, K, Ca, Mg and Fe) changes in barley embryos and endosperms, further affecting the morphological processes of the subsequent callus due to abnormal signal transduction of environmental stress (Sudha and Ravishankar 2002; Shao et al. 2006; Sessitsch et al. 2006). High salt stress includes both an ionic and an osmotic component, each of which is chemical and physical stress, respectively. The multiplicity of the corresponding information embedded in such stress signals underlies one aspect of the complexity of stress signaling.

On the basis of this multiplicity and complexity, it is impossible that there is only one sensor that perceives the stress condition and controls a subsequent signaling. Rather, a single sensor might only regulate branches of the signaling cascades that are initially one aspect of the stress condition. For example, cold is known to change membrane fluidity (Mark and Antony 2005; Munns 2005; Shao and Chu 2005; Xiong et al. 2002; Chu et al. 2005; Wu and Tang 2004). A sensor measuring this change could initiate a signaling cascade responsive to membrane fluidity but would not necessarily control signaling initiated by an intracellular protein whose conformation/activity is directly altered by cold. Therefore, there may be multiple primary sensors that sense the initial stress signal.

Secondary messengers such as higher plant hormones and other signals can trigger another cascade of signaling events, which can differ from the primary signaling in time, i.e., lag behind, and in space, e.g., the signals may diffuse within or among cells, and their receptors may be in different subcellular locations from the primary sensors (Bao and Li 2002; Liscum and Reed 2002; Swarup et al. 2002; Shao et al. 2005). These secondary signals may also differ in specificity from primary stimuli, may be shared by different stress pathways, and may underlie the interaction among signaling pathways for different stresses and stress cross-protection. Therefore, one primary stress condition may activate multiple signaling pathways differing in time, space and outputs. These pathways may connect or interact with one another using shared components generating an intertwined network (Mark and Antony 2005; Shao 2001a–c; Shao 2003a, b, 2005a–c, 2006a–d; Zhu 2000, 2001; Bauer and Bereczky 2003; Zhang et al. 2002).

4 Functional Analysis of Stress Signal Transducti and Related Stress-responsive Genes

Much functionally genetic analysis of stress signal transduction has been carried out by applying a wide range of *Arabidopsis thaliana* mutants. Much research has implied that the process of signal transduction is quite complicated and includes a series of biochemical reactions, in which there is the stage of perception of the primary signal sensor, the generation of secondary signal molecules through the connection of repetitive Ca^{2+} transients, resulting in different outputs with different biological significance (Eckardt 2002; Mouradov et al. 2002). Abscisic acid is a main environmental stress-responsive plant hormone

(Flexas and Medrano 2002; Finkelstein et al. 2002; Marfinez-Madrid et al. 2002; Pinto et al. 2002; Shao et al. 2003a, b, 2006a–d; Trewavas 2002; Sakuma et al. 2006; Yang et al. 2006; Chinnusamy et al. 2006). Many studies of the connection between abiscisic acid and different stress-signaling pathways have been limited by the paucity of signaling mutants. To facilitate genetic screens for stress-signaling mutants, transgenic Arabidopsis were engineered that express the firefly luciferase reporter gene (LUC) under control of the RD29A promoter, which contains both the abiscisic acid-responsive element and dehydration-responsive element (Eckardt 2002). Seeds from the RD29A LUC transgenic plants were mutagenized with ethyl methane sulfonate or T-DNA, and seedlings from mutagenized populations were screened for altered RD29A-LUC responses in response to stress and abscisic acid treatments. The occurrence of mutations with different responses to stress or abscisic acid or combinations of the stimuli revealed a complex signal transduction network in three-dimensioned directions and suggested that there should be extensive connections among cold, drought, salinity, UV-B and the abscisic acid signal transduction pathway (Eckardt 2002). The identification and cloning of some of the mutations have been able to provide new insights into the mechanisms of stress and abscisic acid signal transduction.

The effect of UV-B on gene expression has been extensively reviewed (Jordan 2003; Wright et al. 2006; Boudsocq and Lauriere 2005). It is very important to repeat a number of key points. The potential of UV-B to directly damage DNA suggests this could be a means to influence gene expression. The overwhelming evidence, however, suggests that modification of gene expression is far more complex and specific. For example, gene expression is simultaneously up- and down-regulated by exposure to UV-B. The effect of UV-B on gene expression is strongly influenced by developmental stage and DNA damage levels do not correlate with changes in gene activity. UV-B also affects the gene expression at different levels from transcription, translation and post-translational modification (Eckardt 2002; Wright et al. 2006). The general belief is that most nuclear-encoded genes seem to be influenced at the level of transcription by light, whereas chloroplast-coded genes seem to be largely affected at translation. Many authors suggest that gene expression can be modified by changes in light, perception/signal transduction, metabolic feedback, changes in photosynthetically active radiation, or changes in the balance between photosystems (Bergantion et al. 2002; Flexas and Medrano 2002; Munns 2005; Shao et al. 2005a–c; Nguyen 2003; Liang et al. in press). For instance, changes in carbohydrate metabolism affect gene expression and UV-B is known to modify carbohydrate levels in higher plants. Therefore, UV-B-induced changes in gene expression could be modified through carbohydrate feedback. High light has been demonstrated on many occasions to ameliorate UV-B-induced responses, including gene expression.

Salt, drought, and to some extent, old stress cause an increased biosynthesis and accumulation of abscisic acid, which can be rapidly catabolized following the relief of stress. Many stress-responsive genes are up-regulated by abscisic acid. The role of abscisic acid in osmotic stress signal transduction was previously addressed by studying the stress induction of several of these genes in the *Arabidopsis* abscisic acid-deficient mutant, abscisic acid1-1 and dominant abscisic acid-insensitive mutants abi 1-1 and abi 2-1. A general conclusion from these studies was that whereas low temperature-regulated gene expression is relatively independent of abscisic acid, osmotic stress-regulated genes can be activated through both abscisic acid-dependent and abscisic acid-independent pathways (Eckardt 2002; Xiong et al. 2002; Chinnusamy et al. 2006). Increased abscisic acid levels under drought and salt stress are mainly achieved by the induction of genes coding for enzymes that catalyze abscisic acid biosynthetic reaction. The abscisic acid biosynthetic pathway in higher plants is understood to a great extent. Most recent studies imply that all of these genes (i.e., ZEP, NCED, AAO3, and MCSU) are likely regulated through a common cascade that is dependent on Ca^{2+} (Zhu et al. 1998; Sakuma et al. 2006; Zaninotto et al. 2006).

Molecular studies have identified many genes that are induced or unregulated by osmotic stress (Zhu et al. 1997; Zhu 2003). Gene expression profiling using cDNA microarrays or gene chips has identified many more genes that are regulated by cold, drought or salt stress. Although the signaling pathways responsible for the activation of these genes are largely unknown, transcriptional activation of some of the stress-responsive genes is understood to a great extent, owing to studies on a group of such genes represented by COR 78 /L7178 (Chinnusamy et al. 2006; Zhu et al. 1997). The promoters of this group of genes contain both the abscisic acid-responsive element and the dehydration-responsive element. Transcription factors belonging to the EREBP/AP2 family that bind to the above transcription factors were isolated and termed as CBF1/DREB1B, CBF2/DREBC and CBF3/DREB IA (Liu and Zhu 1998; Liu et al. 2000; Medina et al. 1999; Marfinez-Madrid et al. 2002; Chinnusamy et al. 2006). These transcription factor genes are induced early and transiently by cold stress, and they, in turn, activate the expression of target genes. The similar transcription factors DREB2A and DREB2B are activated by osmotic stress and may confer osmotic stress induction of target stress-responsive genes (Liu and Zhu 1998). Several basic leucine zipper transcription factors (named ABF/AREB) that can bind to ABRE and activate the expression of ABRE-driven reporter genes have also been isolated. AREB1 and AREB2 genes need abscisic acid for full activation, since the activities of these transcription factors were reduced in the abscisic acid-deficient mutants, abscisic acid 2 and abscisic acid-insensitive mutant, abi1-1, but were enhanced in the abscisic acid-hypersensitive era1 mutant, probably due to abscisic acid-dependent phosphorylation of the proteins.

5 Environmental Stress-responsive Transcriptional Elements

Plants can sense, process, respond to environmental stress and activate related gene expression to increase their resistance to abiotic stress (Shao and Chu 2005; Chu et al. 2005; Shao et al. 2005a–c, 2006a–d; Rabbani et al. 2003; Vasil 2002, 2003; Voloudadis et al. 2002; Xue et al. 2002; Vardy et al. 2002; Wang et al. 2003). Environmental stress-inducible genes can be mainly divided into two types in terms of their protein products: one type of genes, whose coding products directly confer the function of plant cells to resist environmental stress such as late-embryogenesis abundant protein, anti-freezing protein, osmotic regulatory protein, enzymes for synthesizing betaine, proline and other osmoregulators; the other type of genes, whose coding products play an important role in regulating gene expression and signal transduction, such as the transcriptional elements for sensing and transducing the protein kinases of mitogen-activated protein and basic leucine-zip factors and others (Eckardt 2002; Milborrow 2001; Medina et al. 1999; Tardieu 2003; Shao et al. 2006; Wu and Tang 2004; Foyer and Noctor 2005). Transcriptional elements are defined as the protein combining with the specialized DNA sequence of eukaryotic promoters or the protein with structural characteristics of a known DNA-combining region, whose main function is to activate or suppress the transcriptional effect of corresponding genes. Up to now, hundreds of transcriptional elements of environmental stress-responsive genes in higher plants have been isolated, which regulate and control the stress reaction related to drought, salinity, cold, pathogens and heat. In the genome of *Arabidopsis* and rice, they have about 1,300–1,500 genes for coding transcriptional elements, most of which have not been identified functionally. Recent study has shown that the transcriptional elements involved in plant stress responses mainly include four kinds: APETALA2/EREBP, bZIP, WRKY and MYB. Typical transcriptional elements in relation to environmental stress have been summarized in Table 1 for reference.

6 Conclusion

Globally, food production will have to be tripled to meet the demand for food of the 12 billion inhabitants of the world by the year 2050 (Vasil 2002, 2003); meanwhile, including most of the people in our country, India, Northern Korea and Thailand, dietary requirements are changing as a result of their improving buying power. It is evident that food supply is the first challenge. The second challenge is eco-environmental degradation, mainly including deficits of water resources and pollution, soil erosion and desertification, decrease in biodiversity owing to widespread use of agro-chemicals, and increase in natural disasters resulting from different forms of biotic/abiotic stress factors (Ballare 2003; Liu and Zhu 1998; Shao et al. 2005a–c, 2006a–d; Yang et al. 2006; Zhu 2000, 2001, 2003; Zhu et al. 1997, 1998). These factors lead to great losses in food production annually. Plants are evolving in a concerted manner (Abbott 2003), and declining resources essentially enhance a rapid decrease in species, resulting in a vicious circle. Facing such severe global change and considering all of the technologies developed, it is firmly believed that biological measures (mainly plant methods) are the best solution not only for meeting the food needs of the ever-growing population, but also for protecting

Table 1 Typical transcriptional elements in higher plants

Plant materials	Factors	Binding sites/Factor Types
Arabidopsis thaliana	ABI5/AtDPBF	ABA response elements (ABREs)/bZIP
A. thaliana	AtDPBF2	ABA response elements (ABREs)/bZIP
A. thaliana	AtDPBF3/AREB3	ABA response elements (ABREs)/bZIP
A. thaliana	AtDPBF4	ABA response elements (ABREs)/bZIP
A. thaliana	AtDPBF5/ABF3	ABA response elements (ABREs)/bZIP
A. thaliana	ABF1	ABA response elements (ABREs)/bZIP
A. thaliana	ABF2/AREB5	ABA response elements (ABREs)/bZIP
A. thaliana	ABF4/AREB2	ABA response elements (ABREs)/bZIP
A. thaliana	GBF3	ABA response elements (ABREs)/bZIP
A. thaliana	AB53	RY/sph elements/B3 domain proteins
A. thaliana	ATMTB2	MTC
A. thaliana	ATHB6	HD-Zip
A. thaliana	ATHB7	HD-Zip
A. thaliana	ATHB12	HD-Zip
A. thaliana	ABI4	AP2
Oryza	TRAB1	ABA response elements (ABREs)/bZIP
Oryza	OsVPI	RY/sph elements/B3 domain proteins
Zea mays	VP1	MYB
Triticum	EmBP-1	ABA response elements (ABREs)/bZIP
Avena	AtVPI	RY/sph elements/B3 domain proteins
Helianthus	DPBF5,-2,-3	ABA response elements (ABREs)/Bzip
Phaseolus	ROM2 (repressor)	ABA response elements (ABREs)/Bzip
Phaseolus	PIARF	RY/sph elements/B3 domain proteins
Craterestinma	Cpvp1	RY/sph elements/B3 domain proteins
Daucus	C-ABI3	RY/sph elements/B3 domain proteins
Populus PtABI3 RY/sph elements/B3 domain proteins		

and improving our eco-environment (resources) in a sustainable development way (Vasil 2002, 2003). Plant molecular biology is the basis of plant biotechnology, which is the best way to solve the problem above, at least to increase productivity on limited land under cultivation, with less water and under worsening eco-environmental conditions (Shao 2003a, b, 2005a–c, 2006a–d; Shao and Chu 2005; Chu et al. 2005; Vasil 2002, 2003).

The elaboration of higher plant form and function depends on the ability of a plant cell to divide and differentiate and the information-communicating status between higher plants and circumstances, e.g., soil compaction, water situation and climate parameters. The decisions of individual cells to enter the cell cycle, maintain proliferation competence, become quiescent, expand, differentiate or die depend on the perception of various signals. These signals can include hormones, nutrients, light, temperature and internal positional and developmental cues, which also have an influence upon the function of stress signals displaying the condition of the plant. In fact, higher plant development is the basis for higher plants to be adapted to the environment; otherwise, the environment brings about diversity of higher plant development (Asada 2006; Darnell 2002; Dodds and Schwechheimer 2002; Gao and Li 2002; Mao et al. 2002; Liu et al. 2003; Mark and Antony 2005; Munns 2005; Shao et al. 2005a–c, 2006a–d; Boudsocq and Lauriere 2005). From this point, there is a closed relationship among higher plants at different levels, the environment and their development, whose regulating mechanism is gene expression and control in time and space (Munns 2005; Eckardt 2002; Sa et al. 2003; O'Connell and Panstruga 2006; Shao et al. 2005, 2006). Of course, it is possible to increase the water-use efficiency (WUE) of main crops through biotechnology after clear mastering of the mechanism (Deng et al. 2002, 2003; Marfinez-Madrid et al. 2002; Napier et al. 2002; Sudha and Ravishankar 2002; Shao 2001; Shao and Chu 2005; Shao et al. 2005; Sakuma et al. 2006; Tarcgnski et al. 1993; Wang et al. 2003). Genetic approaches are important tools for analyzing complex processes such as stress signal transduction. Conventional genetic screens based on stress injury of tolerance phenotypes have been applied with success. However, such screens may not be able to identify all components in the signaling cascades due to functional redundancy of the pathways in the control of plant stress tolerance. The accessibility of the Arabidopsis genome and rice genome framework and various reverse genetics strategies for generating knockout mutants should lead to the identification of many more signaling components and a clear picture of abiotic stress signaling networks (Rensink and Buell 2004; Xiong et al. 2002; Shao et al. 2005, 2006; Arnholdt-Schmitt 2004; Chinnusamy et al. 2006). Molecular screens such as the one using the RD29A-LUC transegene as a reporter are beginning to reveal novel signaling determinants. Similar methods may prove useful for the study of their pathways, such as osmolarity sensing. Adoption of forward and reverse genetic approaches will improve our understanding of signaling mechanisms in higher plants.

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Biodiversity: Function and Assessment in Agricultural Areas: A Review

Boris Clergue, Bernard Amiaud, Frank Pervanchon, Françoise Lasserre-Joulin, and Sylvain Plantureux

Abstract Biodiversity has become a central concept in agronomical research since the Rio de Janeiro summit in 1992. Agricultural areas include a unique biological diversity which is the basis of human activities. Conservation of this biodiversity in agricultural and protected areas is therefore fundamental and requires an operational approach. Biodiversity is a complex entity which can be spread over several levels (genes, species, ecosystems and ecological processes) and can be related to three main functions: (a) patrimonial functions, (b) agronomical functions and (c) ecological functions. The patrimonial function concerns conservation of aesthetic of landscape and threatened species. Biodiversity function according to relationships with agricultural activities describes the biotic and abiotic stress resistance, and the production of cultivated ecosystems. Biodiversity is also involved in ecological functioning by the existence of typical habitats with particular species. The relevance of assessment tools is required in order to understand and evaluate the impact of farm practices on the different compartments of biodiversity at the patch scale to the landscape scale. Different methods, like direct measurements with biodiversity indexes, biotic indicators and models are described and their suitability and limits are discussed.

Keywords Agricultural area • Assessment • Biodiversity • Indicators • Sustainable agriculture

Résumé – Biodiversité: fonction et évaluation dans l'espace agricole Depuis le sommet de Rio de Janeiro en 1992, la biodiversité est devenue un concept incontournable pour la recherche agronomique. Les espaces agricoles contiennent une diversité biologique particulière qui est à la base de nombreuses activités humaines. La conservation de cette biodiversité dans les espaces agricoles et les surfaces protégées est donc fondamentale et nécessite une approche opérationnelle. La biodiversité est une entité complexe qui peut être abordée à différents niveaux hiérarchiques (gènes, espèces, écosystème et processus écologiques) et être reliée à trois fonctions d'intérêt majeur: (i) fonctions patrimoniales, (ii) fonctions agronomiques et (iii) fonctions écologiques. Les fonctions patrimoniales incluent le rôle du paysage et des espèces possédant un statut de protection. La fonction agronomique concerne les résistances aux stress biotiques et abiotiques, et les capacités de production des surfaces cultivées. La dimension écologique se traduit par l'existence d'habitats et d'espèces typiques impliqués dans le fonctionnement des écosystèmes. Des outils d'évaluation pertinents sont indispensables pour comprendre et évaluer l'impact des pratiques agricoles sur la biodiversité. Différentes méthodes de caractérisation de la biodiversité, comme les mesures directes par le calcul d'indices de diversité, l'utilisation de bioindicateurs et la construction de modèles sont décrites et leurs avantages et leurs limites sont discutées.

Mots clés Biodiversité • Évaluation • Espace agricole • Indicateurs • Agriculture durable

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1 Introduction

Biodiversity has become a central concept in agronomical research since the Rio de Janeiro summit in 1992 (CBD, 1992). This event indicated a world consciousness of the importance of biodiversity protection for sustainable development (Brundtland, 1987). Biodiversity protection can be motivated by pragmatic reasons. For example, biodiversity represents a potential reserve of new compounds for medicine, interesting genes for plant breeding and services for agriculture (Altieri, 1999; Duelli and Obrist, 2003; Paoletti et al., 1992; Peeters and Janssens, 1995). Biodiversity is also considered as a mankind heritage and human being cannot decides on the existence or not of a species (Cairns, 1997).

Considering the role of agriculture in the preservation of biodiversity appears to be a key issue. For a better biodiversity conservation at large scale of territories, knowledge and creation of conservation tools are necessary not only in protected and restricted areas but also in agricultural areas. At the European Community scale, agricultural areas are more important (44%) than protected areas which represent less than 5% (Piorr, 2003). In addition, mosaic landscape based on a melting of agricultural and semi-natural areas represent a particular reserve of biodiversity. Finally, biodiversity preservation in agricultural lands produces new challenges: to conciliate production necessities with respect for the environment (Altieri, 1999; Buchs, 2003; Peeters and Janssens, 1995; Vereijken et al., 1997). Additional studies have been conducted in urban landscapes (Breuste, 2004; Lofvenhaft et al., 2004; White et al., in press), and in natural areas (Bootsma et al., 1999; Chiarucci et al., 2001; Lomolino, 1994; Oldfield et al., 2004; Partel et al., 2004), but these specific cases will not be developed in this paper.

Protection of biodiversity requires assessment methods in order to understand disturbance effects on biodiversity, monitoring its state and the relevance of agri-environmental measures. However, biodiversity is a very complex entity with the interaction of different scales (species, community, ecosystem, landscape) in interaction. Biodiversity is not only a concept which expresses the "variety of life" but is also a socio-political construction and an ecological measurable entity (Gaston, 1996). Thus, operational definitions of biodiversity are necessary to determine research directives, biological conservation measures and make environmental policies.

For instance, Noss (1990) has described biodiversity by a hierarchic approach based on the distinction between "composition", "structure" and "function" applied at different scales (Fig. 1). The work of Noss has been a key-reference in ecological studies for monitoring biodiversity. Biodiversity "composition" is an inventory of characteristics, such as biomass production, species abundance, presence of threatened species or habitat proportions. Biodiversity "structure" is the organization of biodiversity components and the relations between them. These components take into account structural data about population (sex, ratio, morphological variability, . . .), habitat (slope, foliage density, . . .) and landscape (connectivity, fragmentation, patch size, . . .).

The third level, biodiversity "function", is the whole of particular ecological processes, such as demographic processes or population dynamic and genetic. The functional groups theory is another operational approach which links biodiversity to ecosystem processes. Each functional group is related to an ecosystem process such as organic matter decomposition or nitrogen mineralization (Lehman and Tilman, 2000; Loreau, 2000, 2001; Loreau et al., 2002; Tilman, 1999; Walker et al., 1999). An ecosystem process becomes an ecosystem service according to a human point of view. For example, biomass production of grassland ecosystem represents forage production for cattle. Ecosystem services form therefore a basis for human life (Schläpfer, 1999).

Agricultural areas contain a unique and useful biodiversity which results from farm management. In order to promote sustainable agriculture, knowledge and conservation of biodiversity need clarifications on two points: (a) the biodiversity concept, especially the integration of the benefits of biodiversity, and (b) assessment methods used to evaluate and monitor biodiversity.

2 Biodiversity as a Multi-Function

Biodiversity is a complex entity which can be spread over several levels. Authors have given, therefore, different ways to define biodiversity as a sum of several functions.

Noss (1990) proposed a hierarchic approach involving the concept of the term "function" of biodiversity. He used it to define all the processes which

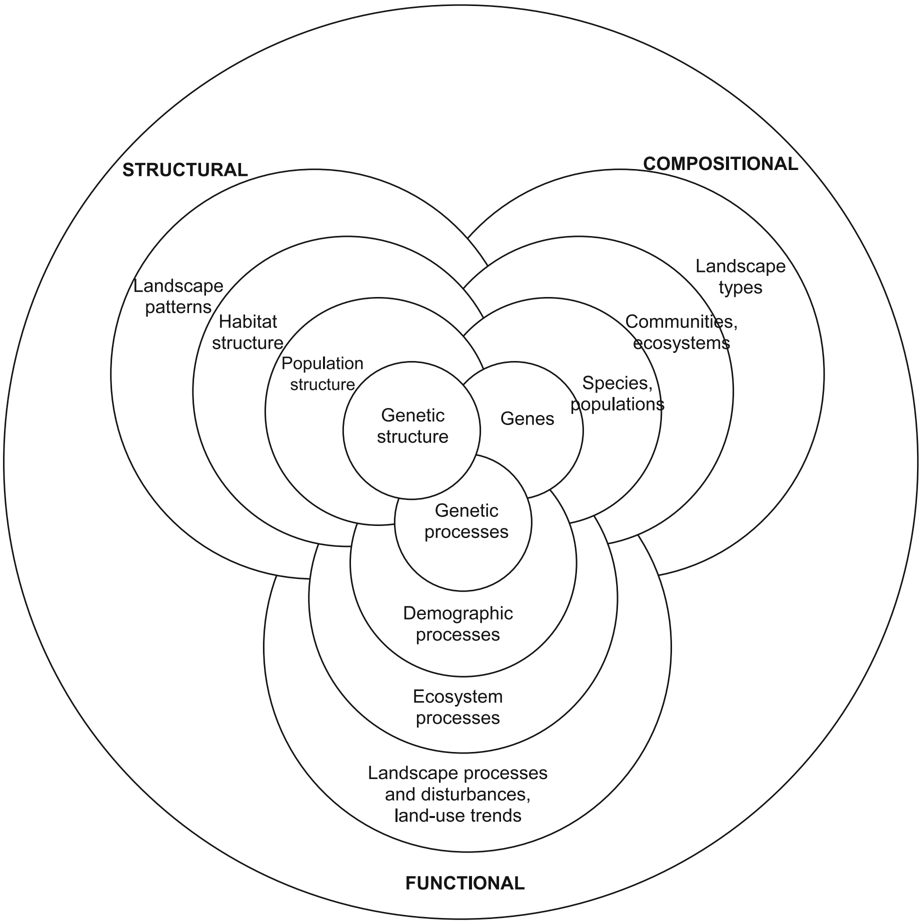

Fig. 1 Compositional, structural and functional biodiversity, shown as interconnected spheres (modified from Noss, 1990)

occurs at the different scales: gene, species-population, community-ecosystem and regional landscape (Fig. 1). Nutrient cycling and energy flow are especially taken into account. But Noss has focused on the ecological functions of biodiversity.

On the contrary, Peeters et al. (2004) have expressed biodiversity functions essentially according to relationship with agricultural activities. Biodiversity is split in three parts: (a) agricultural biodiversity, (b) para-agricultural biodiversity, (c) extra-agricultural biodiversity. “Agricultural biodiversity” represents the variety of life directly used for the farming production. It involves animal and plant species, races and varieties. “Para-agricultural biodiversity” (also called “functional biodiversity”) is the variety of life indirectly used for farming production such as soil fauna, auxiliary fauna, pollinators, grassland plant diversity and more generally ecosystem services. “Extra-agricultural biodiversity” represents biodiversity in production areas which does not contribute to production. These are mainly particular species especially endangered species (orchids, butterflies, great mammals, . . .).

Gurr et al. (2003) also reviewed benefits of biodiversity for agricultural production such as pest management which favoured enhancement of natural enemies. They also proposed a hierarchy of biodiversity benefits based on the different scales of biodiversity. For instance, pest management is obtained at the patch scale by changing practices, and at the landscape scale by the integration of non-crop vegetation which increases diversity (Fig. 2).

The definitions of Peeters et al. (2004) and Gurr et al. (2003) showed that agricultural activities are

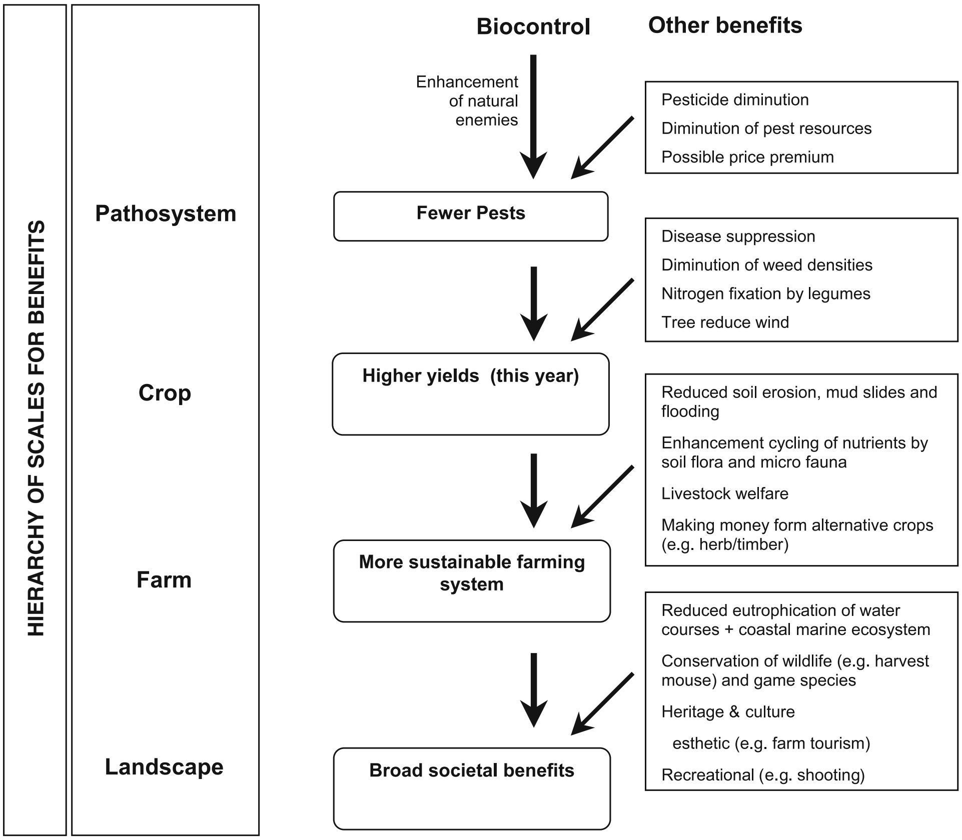

Fig. 2 The hierarchy of scale for potential benefits of multi-function agricultural biodiversity (Modified from Gurr et al., 2003)

strongly linked to biodiversity components. Paoletti (1995) and Paoletti et al. (1992) previously highlighted, by an inventory of biodiversity components, that agricultural production is based on biodiversity.

Duelli and Obrist (2003) have reviewed the different aspects of biodiversity with both an ecological approach and an agronomical approach. They separated these aspects into three parts which motivated preservation and studies on biodiversity: (a) conservation (threatened species protection), (b) biological control (antagonist species diversity), and (c) resilience (ecosystems processes). The Duelli and Obrist (2003) approach presents a biodiversity concept which manages several functions or ecological services. The three parts may be respectively extended to three main functions: patrimonial functions, agronomical functions and ecological functions. The approach of Gurr et al. (2003) highlighted the necessity of taking in account the action of these functions at several scales.

2.1 Patrimonial Functions

The biodiversity of a site is related to history, and thus constitutes a patrimony. This patrimony is a common heritage with both a natural or biological and a cultural patrimony. More often than not, these two patrimonies are inter-related. Patrimonial functions are present at different scales: at landscape scale, biodiversity contributes to aesthetic and at smaller scale, to particular habitat, species and a genetic patrimony.

2.1.1 Aesthetic Function

Biodiversity contributes to the aesthetic value of the landscape, this is also called visual or scenic quality. At the landscape scale, patrimony has therefore an aesthetic function. The aesthetic function creates an identity feeling for residents, and a recreation object for tourists.

For the European Landscape Convention (Europe Co, 2000), "landscape means an area as perceived by people, whose character is the result of the action and interaction of natural and/or human factors". Thus, the aesthetic value includes natural and cultural elements of the landscape. Steiner (1991) stated that "usually, a landscape is that portion of land or territory which the eye can comprehend in a single view, including all its natural characteristics."

Aesthetic values result from the relationship between landscape and an observer. Observation provokes in the observer a visual perception that is associated with thoughts and feelings. The NGO European Academy for the Culture of Landscape (Petrarca) attributed the first landscape description in Europe to the Italian poet Francesco Petrarca (1304–1374). At the "Mont Ventoux" summit (Vaucluse, France) Petrarca related an observation experiment. He observed a panorama, the nature which he perceived as a totality: a landscape. This observation is considered as an aesthetic perception. Nohl (2001) explained precisely the aesthetic perception process (Fig. 3). He differentiated several levels of perception especially between, on the one hand, results of observation and interpretation, and on the other hand, objective (narrative aspect) and subjective approaches (poetic aspect). But Weinstoerffer and Girardin (2000) underlined that the first landscape studies which began in the 1970s had used only an "objective pole" with a descriptive science. This point of view includes naturalistic approaches and agro-ecological approaches: the first approach study landscape structure by inventories of the characteristics (vegetation, relief, soil, geology, climate), the second approach as is a taking into account of agricultural and semi-natural elements (Weinstoerffer and Girardin, 2000). Since earlier work of Shafer et al. (1969), more recent landscape studies have included both objective and subjective approaches (see for example: Arthur et al., 1977; Bosshard, 1997; Briggs and France, 1980; Palmer, 2004, 1997; Vereijken et al., 1997). Colquhoun (1997) and Bosshard (1997) pointed out that subjective approaches have the same scientific rigour as the objective approaches. This conviction is based on the works of the German poet and scientist Goethe (1749–1832) in botany (*Plant metamorphosis*, 1789) and optics (*Theory of colours*, 1810). The American philosopher Emerson (1803–1882) also sustained this point of view especially in his essay *Nature* (1836).

In addition, Schüpbach (2003) underlined the fact that the tourist industry and landscape protection organizations (see for example, SOS-Arvel) use aesthetic perceptions in order to raise the public conscience of the landscape.

Analyses of these perceptions showed that humans have "a natural attraction for diversity which is source of pleasure, satisfaction, or happiness" (Weinstoerffer and Girardin, 2000). A preserved natural landscape provoked the same feelings (Arriaza et al., 2004; Palmer, 2004, 1997).

Biodiversity gives origin to an aesthetic function at the landscape scale but Nohl (2001) showed another complexity level: "If one compares the appearance of

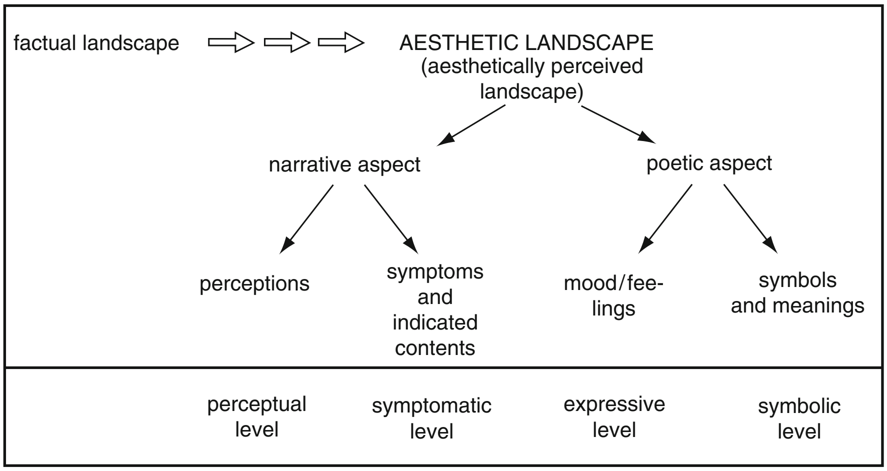

Fig. 3 Aesthetic perception of landscape and levels of aesthetic cognition (modified from Nohl, 2001)

today's landscape with that of premodern and early modern time, one recognizes that the landscape did not only lose its wealth of elements but also its **sense of unity** which gave form to that variety". Landscape aesthetic is thus a result of the diversity of elements and their cohesion or organization.

An agricultural landscape is a complex assemblage of agricultural, semi-natural and rural areas (Piorr, 2003) and constitutes a mosaic of many elements. Heterogeneity is a parameter that helps to understand the organization of mosaic landscapes. Heterogeneity is the diversity of landscape elements (patches) and the complexity of their spatial relationships. Fragmentation and connectivity are measures that characterize landscape heterogeneity. Fragmentation gives information on the spatial organization of an habitat by patch size, while connectivity describes the spatial relationships between patches (Burel and Baudry, 1999). These spatial parameters permit to understand preferences of the observers.

A landscape can offer some aesthetic qualities to the inhabitant or tourist but landscapes contain other elements which the public prefer and ecological characteristics. These elements also have a patrimonial value.

2.1.2 Patrimonial Function at Other Scales

Biodiversity can also have a patrimonial interest that is more due to its historical and socio-cultural context than its visual quality. At small scales, patrimony includes habitats, species, and genetic patrimony.

At the European scale, the Directive 92/43/EEC (Habitats Directive) on the conservation of natural habitats, wild fauna and flora has established the European ecological network Natura 2000. Natura 2000 aims to maintain vital elements of the natural patrimony. These natural area are also related to economic activities (agro-forest production, rural tourism), hobbies (hunting, fishing, outdoor hobbies, ...), and contribute to maintain the quality of rural life.

Following the Convention on Biological Diversity the signatory states must contribute to species conservation, this is especially the case for threatened species (CBD, 1992) that belong to natural patrimony. Based on the Red list concept of the World Conservation Union (IUCN), threatened species are registered for particular area.

So-called flagship species are used to increase public interest and attract funding for ecological matters (Caro, 2000). These species often are threatened species. Flagship species can be a plant (orchids, ...) or an animal (butterflies, eagle, bear, wolf, ...) with sometimes a cynegetic value (partridge, hare, ...). Flagship species belong, therefore, to cultural and natural patrimony.

Pervanchon (2004) owing to a request from French Regional Natural Park managers found that rarity characterises patrimonial value in permanent meadows. The rarity criteria of a species is based on the rarity index of Janssens (1998). Pervanchon (2004) proposed a definition of a patrimonial species which cover the concepts of both flagship and threatened species. A patrimonial species is "a rare or threatened species which needs local management and which may be a flagship species and may have cultural importance." (Pervanchon, 2004). The Patrimoniality concept is used in ecological studies in this sense (see for example Fustec, 2000; Lefeuvre et al., 2003; Pasche et al., 2004).

At the genetic scale, natural and agronomic species have a genetic patrimonial value. Genetic diversity allows species perenniality and species adaptation to environment changes. In addition, knowledge of genetic diversity gives measures for the breeding and conservation of plants (Bataillon et al., 2003) and animals (De Rochambeau et al., 2003). This may also help in conservation of wild species and forest management (Gerber et al., 2003). Conservation of genetic resources has been committed internationally especially via the Global Programme for the Management of Farm Animal Genetic Resources (FAO) and the Global Plan of Action for the Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture (FAO, Leipzig, June 1996).

2.2 Agronomical Functions

Agricultural production can be considered as linked to different biodiversity functions. This biodiversity may control crop and meadow stresses (pests, diseases, dryness, deficiencies, ...) and support essential plant functions such as reproduction via pollinators. Biodiversity acts on agronomic parameters at different scales: at the patch scale, at the matrix scale which includes semi-natural boundaries (bark, ditches, hedgerow), and at the landscape scale with hedgerow webs (connectivity and fragmentation) or forest areas.

2.2.1 Biotic Stress Resistance

Pest Control

Biodiversity can control pest population by two mechanisms: on the one hand, floristic diversity implies a decrease in host-species (bottom-up effect), while on the other hand, an increased diversity of predators control pest populations (top-down effect) (Gurr et al., 2003).

Arthropods and birds are the main auxiliaries. Presence of these useful fauna is strong correlated with semi-natural areas (Jeanneret et al., 2003a, c).

In the case of the vole, their outbreaks are strongly correlated with land cover. High values of the meadow/crop area ratio indicate a high risk of outbreaks (Giraudoux et al., 1997). Millán de la Peña et al. (2003) showed that habitat diversity (connectivity vs openness) allowed a diversity of rodent and thus decreased the generalist species.

A high species diversity within a community enhances its resistance to invasion of alien species. The works of Levine et al. (2002) and Shea and Chesson (2002) reviewed the different studies examining this theory. The majority of studies were carried out on plants in grasslands. In addition, they indicated that the most diverse natural communities were the most frequently invaded.

Disease and Nematode Control

Crop protection against diseases is an important part of farm budget. The diversity of plant and soil organisms may help to control pathogenic microorganisms, especially fungi (Alabouvette et al., 2004; Reeleder, 2003) and plant-parasitic nematodes (Widmer and Abawi, 2002). In addition, disease control by biodiversity helps to reduce pesticide inputs. Crop rotation (diversity in time) and the diversity of organisms in organic amendment are management practices which increase soil biological activity. For example, wheat diseases can be reduced by cultivar blending (Jackson and Wennig, 1997), while compost amendment increases soil biological activity and controls turfgrass diseases (Nelson, 2003).

The presence of hedgerows limits propagation of some diseases (e.g. Oidium) by reducing wind, but can induces other disease in shaded and wet area.

2.2.2 Abiotic Stress Resistance

Biodiversity Benefits on Soil Properties

Soil biota regulates many ecological processes: litter decomposition, nutrient cycling, pathogen control, mineral weathering, . . . From an agronomical point of view, the processes of decomposition, immobilization and mineralization liberate nutrient elements according to plant growth (Paoletti et al., 1992). Thus, losses by leaching are limited as plants absorb necessary elements. Moreover, symbiotic associations with mycorhizal fungi increase nutrient availability e.g. of phosphorus, and increase plant water uptake. Mycorhizal symbiosis are therefore important for plant growth. There are present in all plant species except in Brassicaceae family (Strullu, 1985). Soil biota can also weather minerals by production of chelating agents and catalyse redox reactions (Altieri, 1999).

The diversity of soil organisms and their abundance are involved with processes that affect soil structure. Crossley et al. (1989) defined the influence of each organism category (microflora, microfauna, mesofauna, macrofauna) on each soil structure. These organisms act as much on particle aggregation and humification as porosity creation and organic-mineral phase melting. Soil structuring increase growth of plant roots, anchorage and fluid circulation (air and soil solution). Soil structuring also increase penetration of rain water.

At the landscape scale, wind erosion is a soil quality matter often neglected. In openfield landscape without plant cover, a low speed wind ($4\,\mathrm{m\,s^{-1}}$) may provoke soil erosion of small particles. Humus is mainly present in the soil upper layers and can be taken away by wind. The presence of hedgerow limits wind speed and thus soil erosion. The carbon value of hedgerow board soils is the highest (CNRS, 1976). The whole hedgerow/bank/ditch creates lateral discontinuity which limits water and particle lateral transfers. This process reduces soil erosion by hydric transfer due to for example superficial runoff and hypodermic flow. In the soils affected by this erosion, especially for sloped patches, soil is always more deep in bank uphill slope than in down slope (Ruellan, 1976). Erosion modifies the quantity of the different soil elements (sand, silt, clay) which have consequences both on soil structure and fertility. Moreover, presence of the whole hedgerow/bank/ditch allows a better infiltration and thus a greater water stock than in openfield landscape (Ruellan, 1976). Diversity and organization of landscape elements influence soil water availability, and thus plant growth and yield.

Microclimate

Microclimates are strongly connected to regional climate but they are also linked to local geomorphology (slope, aspect, relief which reduces winds) and human activities. The diversity of landscape elements, such as hedgerows, acts on climatic parameters (wind speed, Potential Evapo-Transpiration).

At the patch and regional scales, bocage structure decreases wind speed about 30–50% (CNRS, 1976). The effect of bocage on patch microclimate is due to landscape structure both at the patch and regional scales (Fig. 4) This wind speed diminution decreases Potential Evapo-Transpiration by 4–6%. Although several effects of bocage are known, effects on farm production are difficult to measure. In a bocage, the day air temperatures are higher and the night temperatures are lower in comparison with open areas.

In addition, pluviometry is higher in the presence of a hedgerow web than in forest area (Soltner, 1973).

2.2.3 Pollination

In addition to domestic honeybee (*Apis mellifera*), pollination is done by a diversity of insect (bumblebees, wild bees, . . .). A high diversity of habitat increase the occupancy rate of bumblebees (Barron and Wratten, 2000). These pollinators are more efficient than the honeybee in unfavourable climatic conditions (cold, overcast sky). Pollination allows fecundation of entomophile plants, especially dicotyledonous plants (Mineau and McLachlin, 1996). For crop production such as rape or sunflower, pollination directly affects a yield component, the seed number. In grasslands, pollination allows reproduction of entomophile species. The consequences of pollination are that sexual reproduction maintain genetic diversity that vegetative reproduction cannot. This genetic diversity increases adaptation to environmental stress.

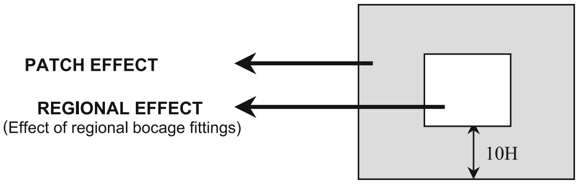

Fig. 4 Duality effect in a bocage patch (modified from CNRS, 1976). With H: total height of bank and vegetation, about 10 m in the Western France bocage

2.2.4 Crop and Animal Production

Regulation of biotic and abiotic stress and pollination are one of several agronomical functions of biodiversity. Aggregation of these functions gives biodiversity effects on crop production. However, other factors are linked to crop production. For example, species forage value and species richness of a grassland is correlated to forage production. In addition, species diversity influences crop and forage production and also quality of dairy products (milk and cheese).

In the case of hedgerow effects on production components, effects must be seen at patch and landscape scale. In a bocage grid, the culture response of hedgerow effects shows a spatial heterogeneity. For the climatic effect, the center of the patch is controlled by regional context and the boundaries by local context (Fig. 2).

The presence of hedgerow influences also growth rhythms and yield. A spatial heterogeneity is observed for precocity and yield in strips which are perpendicular to dominant winds. Favourable areas spread on 2–6 times hedgerow height.

At the landscape scale, bocage increases crop precocity in comparison with open areas. However, effects on yield are often contradictory because there is also an interaction between plant cultivar and its area.

Protected Designation of Origin cheeses are characterized by typical sensory properties (taste, odor, texture). In order to understand links between cheese properties and a geographical area, a "Terroir", several chemical studies were carried out. These studies have demonstrated clearly that some odor-active compounds (aldehyde, ester, and terpenoid compounds) found in grassland species can be transferred to the milk and cheese (Buchin et al., 1999; Carpino et al., 2004; Cornu et al., 2001; Jeangros et al., 1997; Vaillon et al., 2000). Dairy product quality is therefore related to floristic diversity. A diversity of these compounds are produced by plant species adapted to particular habitat (high mountain pasture, extensive practices), especially dicotyledonous species such as *Achillea* sp., *Meum* sp., *Thymus* sp. or *Geranium* sp. (Dorioz et al., 2000; Mariaca et al., 1997). Odor-active compounds form a fingerprint of dairy product and may be use for traceability (Vaillon et al., 2000).

2.3 Ecological Functions

According to Duelli and Obrist (2003) biodiversity implies in ecological functioning is involved in some services for agricultural activities but diversity is also related to some ecological aspects. Biodiversity creates (a) typical habitats, (b) includes particular species, and (c) is related to ecosystem functioning.

2.3.1 Habitats

Habitat is the place where an organism or population occurs naturally. The habitat of a (plant or animal) species is its "place of residence" (Odum, 1971), that means the area to which it is adapted and which it is able to occupy. A habitat type includes specific factors (ecological conditions) which allow the species to survive and to reproduce successfully. If the habitat quality changes (e.g. due to anthropogenic impact) or the ecological requirements of the species change, it is forced to retreat from its place of residence (Buchs, 2003).

In addition, in agricultural areas, the presence of extensive practices allows formation of habitats with a specific biodiversity (Burel and Baudry, 1995).

Abandonment of these practices causes species impoverishment. In order to prevent this phenomenon, Environmental Sensitive Areas (ESAs) have been established by the CAP (CE 797/85). European Directives Habitats (92/43/EEC) and Birds (79/409/EEC) allowed establishment of the Natura 2000 network. Natura 2000 areas are specific habitats or landscapes that are selected for their biological diversity, presence of specific or threatened habitats and species. According to a plant diversity point of view, these habitats are also characterized by a particular phyto-sociologic community (Muller, 2002).

2.3.2 Specific Species

Biodiversity includes particular species in relationships with ecosystem processes. The literature has given several names to groups of species that are related to certain ecological functions. These different names may be cross-checked.

Indicator species are species which are used for many reasons such as an indirect measure of the health of ecosystem (*condition indicator*), identification of an area of high species richness (*biodiversity indicator*) or as markers of population size for other species (*population/guild indicator*) (Landres and Verner, 1988).

Keystone species have an important ecological function either in sustaining ecosystem functions or in sustaining populations of other species. For example, barrage building by beavers creates a wetland, while a cavity dug by a woodpecker may be used for nesting of other bird species. Keystone species are therefore precious tools for ecosystem conservation (Simberloff, 1998). However, they are not the panacea. Not all ecosystems have keystone species. According to Bengtsson (1998), ecosystem engineer (earthworm in soil, *Daphnia* in aquatic foodweb) are like keystone species.

Umbrella species are used to locate the edges of a conservation area. These are species that need a large area to survive. Conservation of this area provides protection to co-existing species.

Using terms like **focal species** and**surrogate species** provoked a semantic and scientific polemic (Armstrong, 2002; Caro, 2000). But focal species and surrogate species are sometimes used to design indicator species or other particular species.

2.3.3 Ecosystem Processes and Nutrient Cycling

Many studies (de Ruiter et al., 1994, 2002; Hinsley and Bellamy, 2000; Kromp, 1999; Smeding and de

Snoo, 2003; Smeding and Joenje, 1999) have shown clearly that farming practices (fertilisation, pesticides, tillage) affect population size and the dynamics of several groups (microbes, protozoa, vascular plants, nematodes, arthropods, annelids, vertebrates). In the face of biodiversity losses, ecologists have begun investigating these damages to ecosystem functioning. The relationship between diversity and ecosystem stability has been the most studied and debated since the 1950s (Loreau et al., 2002).

According to several authors (de Ruiter et al., 1994, 2002; Loreau et al., 2002), biodiversity is linked to ecosystem processes: matter, energy and nutrient cycles. Although these relationships are known, especially by the food web concept, their understanding is limited. The majority of studies and models are based on the relationship between diversity and stability (community and ecosystem process stability) (Lehman and Tilman, 2000; Loreau, 2000, 2001; Tilman, 1999), but are often led on single trophic-level and at small scales.

Loreau et al. (2002) supposed that the first theoretical studies applied the conventional wisdom (don't put all your eggs in the one basket). In this vision, diversity of pathway provides stability. Below a threshold of biodiversity loss, stability is therefore broken and involves a cascade reaction of species loss and the ecosystem is endangered. But many results showed that diversity is related to different stability properties. A greater diversity is not always favourable to community stability and process stability. One of the hypothesis about ecosystem functioning involves idiosyncratic process. Species make different contributions to ecosystems depending on certain conditions (e.g. community composition, . . .) (Naeem et al., 2002).

Griffiths (2000, 2001) illustrated another complexity of ecosystem functioning: the redundancy of functional groups by a stress-on-stress experiment. The first stress is a disturbance applied on soil samples. It decreases the biodiversity but not all ecosystem processes. For example, organic matter decomposition may be greater than before disturbance. At the second stress, ecosystem processes decrease strongly. These experiments showed that the first stress has affected biodiversity stability while the second stress has affected process stability. Hence, ecosystem processes are not linked directly to biodiversity. As there is a functional redundancy in the soil community, a distinction must be observed between community and processes.

Loreau et al. (2002) reviewed the different stability properties such as resilience or resistance. Resilience is "a measure of speed at which a system returns to [a stable] state after a perturbation". While resistance is "a measure of ability of a system to maintain its original state in the face of an external disruptive force". Resistance is the stability propriety of the ecosystem, for example, against invasion by non-native species.

Raffaelli et al. (2002) suggested there was an urgent need to orient modelling on biogeochemical cycling and therefore research at larger-scale. "There have been very few attempts to explore the effect of biodiversity on the functioning of full ecosystems comprising higher trophic levels, decomposers and nutrient cycling and none as yet have considered stability explicitly" (Loreau et al., 2002). Ecological studies consider two ecosystem divisions: above-ground/below-ground, either plant-herbivore-predator or soil community. But studies are often limited to small scale of soil and plant associated (see for example de Ruiter et al., 1994; Smeding and de Snoo, 2003) and are not included mammals or birds. A multitrophic approach are argued to examine ecosystem process holistically.

Many authors (Lehman and Tilman, 2000; Spehn et al., 2000; Tilman, 1999) have given information on the relationship between plant diversity and above-ground biomass of grassland. Biomass production is greater with species rich communities than the most productive monoculture.

According to de Ruiter et al. (2002) future research must focus on these links between biodiversity stability and process stability. Knowledge of these key-properties will allow an understanding of the risks and effects of human disturbances.

3 Biodiversity Assessment

Assessment tools are required to quantify and evaluate the impact of agricultural activities on biodiversity. Many methods have been proposed either by direct measures on the site, or by indirect measures. Biodiversity studies are generally focused on one scale: either at habitat, patch scale, or at landscape scale.

3.1 Direct Measures of Biodiversity

3.1.1 Simple Indexes

The biodiversity definition provided by the Convention on Biological Diversity takes composition (species, ecosystem, . . .) and structure (ecological process) into account. The taxonomic richness is the first biodiversity measure which gives the number of taxa (family, genus, species, variety, ecotype) per unit area. This method is the most commonly used and represent the simplest expression of the diversity. Nevertheless the value of this criterion used alone is limited as species number must be compare to a reference number for a particular habitat.

Diversity indices are another method that uses the number of taxa and their abundance (Table 1). For example, communities which have the same number of species may differ in the abundance of each species.

The Shannon–Weaver index (Shannon and Weaver, 1949) (H) is the most used index. It gives information on community complexity and can vary from 0 (one species alone) to $\mathrm{Log}_2 S$ (where all species have the same abundance). But this index is not sensitive to strength variation. In the case of two ecosystems which have the same number of species but one has twice as many individuals as the other, the Shannon–Weaver Index gives the same value (Pitkänen, 1998). The Shannon–Weaver index is also used at landscape scale to evaluate diversity of landscape elements (Forman, 1995; Nagendra, 2002). The Shannon–Weaver index is used as an alpha-diversity index, because it gives information at species level.

The beta-diversity could be defined as the difference in species composition between different communities. Beta-diversity is larger when there are fewer common species between different communities (Fang and Peng, 1997). The Whittaker index (Whittaker, 1972) could be the most suitable among the beta diversity indices available. This is partly because they are easy to calculate and interpret (Wilson and Shmida, 1984). It can vary from 0 to 2.

Gamma or regional diversity is the total number of species occurring in a system (Maguran, 1988; Whittaker, 1975).

The evenness (Pielou, 1966) (J) is a measure of abundance heterogeneity between species in a community. This parameter can vary from 0 to 1. The maximum is obtained when all species have the same abundance in the study site. Evenness is calculated from Shannon–Weaver Index H.

Touzard and Clément (2001) used another parameters to describe diversity of plant community: the dominance. The dominance (D) is measured from the inverse of the Simpson diversity index (Simpson, 1949). When the dominance value is high, the study site contains species with high abundance.

Janssens (1998) used another parameter: the rarity index which is an important parameter for biodiversity conservation. The rarity index may be used to give the patrimonial value of a study site (Pervanchon, 2004). Peeters et al. (2004) proposed vulnerability as a parameter which gives sensitivity of a taxon to extinction.

These different methods show that are many diversity measures but their suitability for use in different domain (soil microflora, arthropods, plants, landscape elements) is not always clear (Fang and Peng, 1997; Hill et al., 2003; Wilson and Shmida, 1984).

3.1.2 Biotic Indicators

Direct measurement of biological diversity is frequently used for biodiversity studies. But this measurement is inconvenient due to high cost in time and money, and the necessity for competence in species determination of very diverse organisms (soil arthropods, plants, birds, . . .). In addition, a sample represents a

Table 1 Indices used for biodiversity description

Indices	Formula	Abbreviations
Species richness (S)	$S = \Sigma n_i$	n_i = species i
Alpha diversity ($H_\alpha{}'$) (Shannon and Weaver, 1949)	$H_\alpha{}' = p_i \log_2 p_i$	p_i = frequency of the species i
Beta diversity ($H_\beta{}'$) (Whittaker, 1972)	$H_\beta{}' = S/m - 1$	S = species number (all samples) m = average number of species per sample
Evenness (J) (Pielou, 1966)	$E = H_\alpha{}'/\log_2 S$	
Dominance (D) (Simpson, 1949)	$D = \Sigma p_i{}^2$	
Rarity index (I_R) (Janssens, 1998; Pervanchon, 2004)	$I_R = \Sigma C_i/S$	C_i = rarity coefficient of the species i (from 1 to 13)

picture of biodiversity which changes all the time (day/night, weather, season, years). Thus, scientists have tried to find indirect or surrogate measurements to determine biodiversity. Instead of measure all the biodiversity, many scientists hold the view that the dynamics of taxa gives a picture of the dynamics of biodiversity (Doring et al., 2003; Duelli and Obrist, 1998, 2003; Kati et al., 2002, 2004; Landres and Verner, 1988; Mac Nally and Fleishman, 2004; Perner, 2003; Perner and Malt, 2003). An important contribution on using of biotic indicators was given in the special issue of Buchs (2003). Works of Duelli and Obrist (1998) suggested arthropod higher taxa were better biotic indicators in terms of their ease of sampling and relationship with biodiversity. Assessment tools must be easily usable in order to be generalized for other cases study and to help decision-makers involved with land use management.

3.2 Evaluation of Biodiversity Functions by Models

3.2.1 Modelling Approaches Considering Life Beings as Dynamic Systems

Most of the models in ecology are based on a physical approach of individual organisms, populations or ecosystems. Life beings are not considered in their all complexity, but as dynamic systems which are determined by their state, as stated by physics (Stewart, 2002). For instance, it is the case of plant species competition models (Gounot, 1960; Schippers and Joenje, 2002). Gounot's model is one of the first theoretical ecosystem model. It is based on compartments which correspond to elements of the grassland such as the cattle, the soil nutrients, the micro-organisms, the plant biomass. Matter and energy flows circulate between these compartments. Independent variables of the model are climate and grassland management. VEGPOP 2 is a recent model based on compartments, but it is operational thanks the high improvement of scientific knowledge since the 1970s (Schippers and Joenje, 2002). This model needs field experiments for plant species parameters concerning physiology, resources allocation, nitrogen flow, flowering or population dynamics (see Table 2 for details). VEGPOP 2 predicts the Shannon index, the plant biomass and the vegetation spatial dynamics (Schippers and Joenje, 2002). Numerous other models are based on statistical analyses to quantify flows and compartments (for instance, see Bai et al., 2001). Three kind of analyses can be distinguished: the classical linear regression models, the linear generalised relations among which the Gaussian, the binomial and the Poisson's distributions (Yee and Mitchell, 1991) and the generalised additive models. These models are up-to-now largely used in ecology and they were well described elsewhere (Guisan et al., 2002).

Beside these models, several models were inspired by the application of physics concepts. For instance, thermodynamics (Zhang and Wu, 2002) or automatics (Matsinos and Troumbis, 2002) can help to predict structure, dynamics and functioning of ecosystems.

From the 1990s, the concomitance of the chaos theory, the account of interactions between ecosystems and the improvement of computer performance was at the origin of numerous individual-based models in ecology (Judson, 1994). Now, numerous different models were available to explain or predict vegetation structure and dynamics of ecosystems (Ejrnaes and Bruun, 2000; Koleff and Gaston, 2001; Laterra and Solbrig, 2001; Loreau, 1998; Pacala and Crawley, 1992; Peters, 2002; Wilson et al., 2002). None of these models evaluate the impact of farming practices on biodiversity, they only explain or predict vegetation structure or dynamics.

3.2.2 Models Predicting the Threatening Level of Natural Resource

Potential impact models are issued from German works of the 1970s on ecological risks (Freyer et al., 2000). Impact means the level from which resources and/or ecological functions are threatened by a harmful use to ecosystems health. Potential means that not only impact models are in part based on field measurements, but they are limited by available data and approximation inherent in modelling (Freyer et al., 2000). The model of Freyer et al. (2000) predict the level of natural resource threatening due to human activities such as pesticides and nitrogen inputs or mechanical action (e.g. ploughing). This model can be applied at various scales (see Table 2 for details).

Table 2 Comparison between several models and indicators which evaluate the impact of agricultural practices on biodiversity and agronomic value of grasslands

Characteristics of the models		Model of Freyer et al. (2000)	VEGPOP2 (Schippers and Joenje, 2002)	Model of Pervanchon (2004)
Model type		Potential impact mode	Vegetation dynamic model	Expert based model associated with fuzzy logic
Model objectives		Evaluation of human activities impacts on ecological functions	Evaluation of human activities impacts on plant diversity (biomass, Shannon Index)	Evaluation of human activities impacts on ecological and agronomic function of any kind of herbaceous surfaces
Targeted users		Unknown	Unknown	Agricultural development managers
Model structure Parameters	Human activities	Pesticides inputs, ploughing, drainage, proportion of the different activities at landscape scale	Hay cutting, N fertilisation, herbicides	Hay cutting, grazing, water management (drainage, irrigation), N and P fertilisation, calcareous inputs
	Environment characteristics	Groundwater, soil, climate, species, biotopes, landscape and amenities	Field perimeter	Corine habitat, N and P soil fertility, soil depth, pH, soil moisture, temperature
	Studied species	Unknown: species and biotopes are together in the frame of "environment protection"	Parametrisation for four plant species (*Poa annua*, *Holcus lanatus, Anthoxantum odoratum*, *Festuca ovina tenuifolia*)	2912 European plant species
Model outputs		Potential impact of human activities on resources	Shannon index, plant biomass	Plant species lists (names) according to agricultural practices
Input data		Measured data, maps, statistical data	Fertilisation and disturbance levels	Data given by farmers and maps
Scale		Landscape, field, biotope or species	Field boundary	Herbaceous surface (e.g., grassland)
Calculation methodology				
Statistical analysis		None	Yes: relations for spatial representation of plant competition	None
Fuzzy logic		Yes: exponential, logarithmic, multilinear and linear functions	None	Equivalent of sigmoid functions
Other methods	Type of equation	Ratio and average of weighted parameters	None	Choice of the minimum: plant species presence probability is the minimum of the all probabilities
	Justification	None	—	Precaution principle and limiting factor theory
Application area		Various biotopes or landscapes	Areas where the four species are parameterised	Four French Regional Natural Parks
Model generalisation		Unknown	Unknown	Any herbaceous surfaces where some of the 2,912 species are potentially present
Validation		Unknown	Satisfying results for some parts of the model	Satisfying results for French permanent grasslands; not tested for other surfaces
Computerised version		Yes	Yes	Yes

3.2.3 Models Based on Life Traits

Expert models are a novel modelling approach: they are based only on the knowledge of some traits or biological characteristics of animal or plant species. There is no need of statistical analyses or empirical relations to elaborate such models, but only field observation and biometric measurements to build a database. Once the database is built, expert models can predict very efficiently the species presence in any ecosystems. These modelling approaches are the first concrete applications of functional groups theory based on life traits of plant species to predict animal or plant presence according to human activities and environmental factors (Pervanchon, 2004).

For instance, Pervanchon (2004) developed an expert model which predicts plant species presence in any herbaceous ecosystems. This model is based on a database of 17 life traits or biological characteristics already identified in literature for 2,912 plant species. In order to predict the presence probability of grassland plant species, the information of the traits and characteristics are aggregated with data on farming practices and environment factors by fuzzy logic associated with expert system. With such a model, it is possible to predict a list of plant species with their patrimonial value, without realizing floristic relevés. The validation results of this model highlighted that it is only necessary to improve the knowledge on life plant traits to improve the expert models (Pervanchon, 2004).

The use of species traits to predict the presence of species according to human activities and environment factors was also developed successfully to predict the presence of *Syrphidae* in any ecosystems (Speight and Castella, 2001). If for plants, the scientific knowledge on biological traits still has big gaps, for *Syrphidae*, the traits are well detailed and the lists of predicted species by the model and observed species in ecosystems are very similar.

3.3 Surrogate Measures of Biodiversity: Landscape Metrics

Ecologists have suspected for a long time that landscape composition and landscape pattern are highly significant for species diversity. However, the way in which species diversity behaves in landscapes with different spatially arrangements is largely unexplained (Steiner and Kohler, 2003).

One solution is to measure the elements that are related to biodiversity. Landscape parameters may be correlated with species diversity of many groups (Jeanneret et al., 2003a–c). As a first step, a biodiversity parameter is studied in relation to spatial information. For example, data are searched on the presence of a target species in different habitats. After determining the link between the abundance of the species and spatial structure, this link is modelled and then validated. At the end, landscape data are only necessary for monitoring the target species. Actually, a higher diversity level at the landscape scale is used to predict a lower diversity level (species richness, . . .) (& * & Jeanneret et al., 2003a), and even if biodiversity is linked to landscape parameters, there are no general models.

For this reason, very many indicators based on spatial information have been built. Piorr (2003) reviewed agri-environmental indicators and landscape indicators used in the European Union.

The OECD had produced agri-environmental indicators which were adjusted to the driving force-state-response (DSR) framework (OECD, 1997, 1999, 2001). DSR indicators focus on the causes of change in environmental conditions in an agriculture area, the effects of agriculture on the environment and the efficiency of any actions taken.

The OECD Expert Meeting, May 1999, in Paris suggested more concrete indicators (Morard et al., 1999). One goals was to select relevant landscape indicators for that data are available. An example for the EU territory monitoring is the Corine-Land-Cover (CLC, 2000). This monitoring at the EU level allows determination of anthropogenic impacts on landscapes. Initiatives aiming to preserve the quality of landscapes can be designated. But at the EU level monitoring is limited. A specific level must be chosen and the data are of limited significance to specific analysis.

The European Community initiated a project proposal on agri-environmental indicators called the PAIS project. This project contained indicators within the domain of landscapes, rural development and agricultural practices which were applicable at EU level. Thirty six landscape indicators had chosen as relevant. At the moment these landscape indicators cannot give answers regarding biodiversity. The future working steps will be to determine the relevant landscape indicators that are related to biodiversity and nature protection purposes (Piorr, 2003).

In order to follow landscape development, several European countries have produced landscape conservation schemes and landscape indicators. These monitoring programmes have helped in the planning and delimitation of nature conservation areas.

Peeters et al. (2004) reviewed a list of direct indicators (specific biotic indicators, natural area rate, . . .) and indirect indicators (rate of area with a high slope, fertiliser quantity, . . .) to promote sustainable management of grasslands. However, these indicators can give answers which are contradictory and frequently do not facilitate decision-making.

3.4 Agro-Ecological Indicators

Girardin et al. (2000) adopted the interaction matrix (Leopold et al., 1971) for an environmental impact assessment methodology. This method evaluates the effects of farm production practices on different components of the agroecosystem. Evaluation modules, which characterise the impact of a production practice on an environmental component, can be aggregated to yield two types of indicators. Agro-ecological indicators reflect the impact of one production practice on all environmental components concerned, while *indicators of environmental impact* reflect the impact of all production practices concerned on one environmental component (van der Werf and Petit, 2002).

Pervanchon et al. (2002) proposed a methodology to evaluate the impact of agricultural practices on grassland biodiversity. Agro-ecological indicators are predictive tools and help in decision-making. These indicators use easily accessible data that can be collected by non-specialists However, the building of indicators is dependent on scientific knowledge and indicators are only suitable if they are validated for sensitivity and usability value (Girardin et al., 1999).

4 Conclusion

Functional vision is an operational approach which permit clarification of the complex concept of biodiversity. Biodiversity is too large to be entirely assessed by a single criterion. Biological diversity must therefore be evaluated according to precise objectives: ecological, agronomical or patrimonial approaches. Many tools have been built to assess biodiversity but they measure only some parts of biodiversity. For example, models are often limited to simple systems, while validation of indicators shows the complexity of these systems. Future studies ought to examine understanding of the relationships between biodiversity and agro-ecosystems with complementary approaches (agronomy and ecology) and produce suitable tools that permit decision-making. Studies frequently examine only one scale whereas these relationships are relevant at different scales and are interconnected.

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Mixing Plant Species in Cropping Systems: Concepts, Tools and Models: A Review

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Abstract The evolution of natural ecosystems is controled by a high level of biodiversity, in sharp contrast, intensive agricultural systems involve monocultures associated with high input of chemical fertilisers and pesticides. Intensive agricultural systems have clearly negative impacts on soil and water quality and on biodiversity conservation. Alternatively, cropping systems based on carefully designed species mixtures reveal many potential advantages under various conditions, both in temperate and tropical agriculture. This article reviews those potential advantages by addressing the reasons for mixing plant species; the concepts and tools required for understanding and designing cropping systems with mixed species; and the ways of simulating multispecies cropping systems with models. Multispecies systems are diverse and may include annual and perennial crops on a gradient of complexity from 2 to *n* species. A literature survey shows potential advantages such as (1) higher overall productivity, (2) better control of pests and diseases, (3) enhanced ecological services and (4) greater economic profitability. Agronomic and ecological conceptual frameworks are examined for a clearer understanding of cropping systems, including the concepts of competition and facilitation, above- and belowground interactions and the types of biological interactions between species that enable better pest management in the system. After a review of existing models, future directions in modelling plant mixtures are proposed. We conclude on the need to enhance agricultural research on these multispecies systems, combining both agronomic and ecological concepts and tools.

Keywords Agrobiodiversity • Agroforestry system • Competition • Crop model • Cropping system • Facilitation • Plant mixture • Resource sharing • Species mixture

1 Introduction

1.1 Intensive Monocultures vs. Multispecies Systems

Intensive agricultural systems are often based on optimising the productivity of monocultures. In those systems, crop diversity is reduced to one or very few species that are generally genetically homogeneous, the planting layout is uniform and symmetrical, and external inputs are often supplied in large quantities. Such systems are widely criticised today for their negative environmental impacts, such as soil erosion and degradation, chemical contamination, loss of biodiversity and fossil fuel use (Giller et al. 1997; Griffon 1999; Tilman et al. 2002). Conversely, multispecies cropping systems may often be considered as a practical application of ecological principles based on biodiversity, plant interactions and other natural regulation mechanisms. They are assumed to have potential advantages in productivity, stability of outputs, resilience to disruption and ecological sustainability, although they are sometimes considered harder to manage (Vandermeer 1989). A majority

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of the world's farmers, particularly those located in tropical regions, still depend for their food and income on multispecies agricultural systems, i.e. the cultivation of a variety of crops on a single piece of land (Vandermeer et al. 1998). Those systems, which are often without synthetic inputs and based on integrated management of local natural resources and, in many cases, on rational management of biodiversity, theoretically offer numerous ecological advantages.

1.2 New Issues

Faced with the critical situation of intensive monocultures, new conceptual ways of constructing sustainable agroecosystems are being sought (Malézieux and Moustier 2005a, b). Several agronomists recently proposed that traditional multispecies systems could be used as models for designing sustainable cropping systems (Gliesmann 2001; Altieri 2002). Jackson (2002) proposed imitating the structure of the prairie ecosystem, composed of a number of species of different functional groups, to achieve resilience to changes in climate and water supplies, and to pests and other natural disturbances. Ewel (1999) enhanced the role of woody perennial species in the sustainability of ecosystem functioning in the humid tropics and proposed forest-like agroecosystems. Such systems are usually complex, as they are based on several species, and may involve combinations of perennial and annual, woody and non-woody plants.

Agricultural research now has an adequate tool-box of methods and models for technology development in monospecific cropping systems, but its suitability for more complex systems is unsure. Methods for designing multispecies systems barely exist. Systemic agronomy concepts (crop management sequences, cropping system), and especially the tools derived from that discipline, scarcely deal with the complexity of multispecies systems. In particular, the modelling tools widely used today in agronomy are not well adapted to simulating them. New models are required to represent, assess and design sustainable multispecies cropping systems.

This article addresses those questions, reviews concepts suitable for use in dealing with multispecies systems and attempts to identify shortcomings in terms of tools, thereby proposing new avenues of research. It is based on a wide range of systems, such as simple or complex, uniform or heterogeneous and intercropped species, such as annual and perennial, herbaceous and woody, etc. The article is structured in three parts, focusing successively on the following issues: (1) the reasons for mixing species, i.e. benefits and drawbacks, (2) the concepts and tools used for understanding and designing cropping systems with mixed species and (3) the models existing and needed for simulating multispecies cropping systems.

2 Benefits and Drawbacks of Mixing Plant Species

2.1 The Role of Biodiversity in Ecosystems

The relationship between biological diversity and ecosystem functions has been and continues to be the focus of much work in the ecology field (Loreau et al. 2001). In contrast with most agricultural systems, biomass productivity in natural ecosystems is achieved through a high genetic diversity of plants involving different complementary functional groups. Although numerous studies report that plant communities with some degree of genetic heterogeneity have advantages over pure stands, debates and controversies remain on the exact role of biodiversity in ecosystem functioning and productivity (Loreau et al. 2001). Recent work by various authors has thus shown positive correlations between the richness of species and different ecological processes such as primary productivity, nutrient retention and resilience after stress. However, studies have particularly focused on natural prairie ecosystems (Hector et al. 1999; Loreau et al. 2001; Tilman et al. 1996, 1997) or natural forest ecosystems (Vila et al. 2003; Kelty 2006; Erskine et al. 2006). Very few studies have concentrated on cultivated ecosystems (Altieri 1999). In agroecosystems, biodiversity may (1) contribute to constant biomass production and reduce the risk of crop failure in unpredictable environments, (2) restore disturbed ecosystem services, such as water and nutrient cycling and (3) reduce risks of invasion, pests and diseases through enhanced biological control or direct control of pests (Gurr et al. 2003). Some features of biodiversity in natural systems may offer a basis for designing multispecies systems (Ewel 1986). For instance, persistent ground cover and minimum soil disturbance, which minimises erosion, is the basis for the development of 'conservation agriculture', involving both minimum tillage and cover crop use in annual cropping systems. The frequent presence of deep-rooted perennials in natural ecosystems, one advantage of which is to enable more complementary water and nutrient use by plants, has led to the numerous agroforestry systems that exist in the world. More generally, biodiversity remains the basis for traditional farming in the tropics and multispecies systems still provide food for a majority of poor farmers in developing countries.

2.2 The Different Ways to Mix Plant Species in Cropping Systems

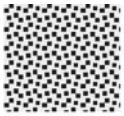
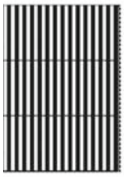
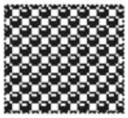
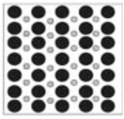
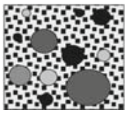
In world agriculture, a multitude of different cropping systems can be identified on the basis of their composition, design and management. The agroecological areas involved in species mixing extends, in theory, to all cultivated zones, be they temperate or tropical, dry or humid. In what follows, the multispecies systems described consist of growing several crops simultaneously in the same field, or more generally, of mixing several plant species within the same field: field crop species, pasture species, trees, or combinations of these. Multispecies systems have been the subject of numerous typologies and classifications that may include various criteria such as (1) the permanence of a specific crop assemblage or, conversely, the frequency of land-use rotation, (2) the intensity of intercropping, i.e. the number, type and level of spatio-temporal occurrence of crops within the field and (3) the percentage of tree canopy cover in the field (Garcia-Barrios 2003). The existence of trees with crops (agroforestry) is an important feature and, within that category, the specific arrangement of species, which refers to the simultaneous or sequential arrangement of trees and crops, or the spatial structure, which refers to the mixed or zonal arrangement, are discriminating factors (Huxley 1983; Nair 1993; Torquebiau 2000). Our aim here is not to give an account of that abundant variability, but rather to highlight the major types existing, bringing out the existence of a multivariate gradient ranging from pure stands (cultivation of a single species, or a single genotype or even a single clone) to cropping systems that function along the same lines as a natural ecosystem. Table 1 illustrates that variability, with certain properties that might be exerted as soon as genotypes are mixed within the same species. The degree of complexity increases, which might be expressed by the number of intercropped species, the nature of those species (existence to varying degrees of wild species 'managed' by the farmer), the number of strata making up the vertical profile and the simultaneous existence of several groups of species (annual/perennial, woody/non-woody). Pictures 1–3 show examples of row intercropping, row agroforestry in Europe and complex agroforestry in the humid tropics, respectively.

2.3 Advantages of Mixing Species

2.3.1 Effects on Stability

The idea that the species diversity of ecological communities contributes to stability is among ecology's most venerable hypotheses (Frank and McNaughton 1991), but there are few data on how those properties are associated in agroecological systems. Biodiversity is the most obvious feature in multispecies systems, but its real function often remains vague. Some authors (Altieri 1999; Swift et al. 2004) make a distinction between *planned biodiversity*, principally the crops and plant species included intentionally in the system by the farmer, and *associated diversity*, i.e. soil flora and fauna, herbivores and carnivores, decomposers, etc., that colonise the system. Swift and Anderson (1993) proposed a comparable classification of biodiversity in agroecosystems that distinguished productive biota from resource biota (organisms that contribute to pollination, decomposition, etc.) and destructive biota (weeds, insect pests, microbial pathogens, etc.). It has been reported for numerous taxa in various conditions that associated diversity is positively correlated to planned biodiversity (Vandermeer et al. 1998). A schematic representation of this relation is given in Fig. 1. Although the form of the relation between planned and associated biodiversity, and the exact processes involved, remain open questions (Perfecto et al. 1996, Swift et al. 1996), it is certainly a key for understanding the ecological functions offered by multispecies systems

Table 1 Different forms of species mixtures in agricultural systems. Systems are classified according to a gradient of complexity, including the number and type of plant species (annual vs. perennial), the horizontal and vertical structure of the mixture, and the life cycle duration of the species (*S* short cycle, *L* long cycle)

Type of System	Number of species	Horizontal heterogeneity	Number of strata	Duration	Example/location
Annual crops					
Combination (intraspecific mixture)	1		1	S	Cereals
Relay cropping (time overlap only during one part of the life cycle of each species) - Crops or crop and service plant	2		1 or 2	S	Maize/beans, groundnut/cotton (Africa)
Row intercropping (growing two or more species in rows) - Crops with crops or crops with service plant	2		1 or 2	S	Cereals/herbaceous legumes and grasses. Ex : Rice/arachis pintoï (Europe, South America)
Mixed intercropping (no distinct row management)	2-n		1	S	2 species (maize-sorghum, maize-cassava, etc.) to n species (tropical garden e.g. rice, maize, tomato, cassava, etc.) (humid tropics), annual grassland (Europe)
Perennial crops					
Perennial grasses	2-n		1	L	Grassland (North America, Europe, Australia, etc.)
Agroforestry (crop with trees)					
Sequential agroforestry (crop temporarily mixed with trees)	2		2	S-L	Pineapple/rubber tree (Humid Asia)
Row agroforestry.					
Crop under service trees	2		2	S-L	maize/ green manure legume tree (Tropics)
Herbaceous Crop under tree crop	2		2	S-L	Cereal/wood tree (Europe, N.America) Pineapple/coconut tree, pasture/coconut tree (Humid Asia)
Service plants under tree crop	2-n		2	S-L	Pueraria/oil palm tree (Asia, Africa), Grass/vineyard (Europe)
Tree crops	2		1to 3	L	Cocoa/coconut tree (Oceania) Coffee/wood tree (Central America)
Complex agroforestry (trees, shrubs and crops)	3-n		2 to 5	S, L	Tropical homegardens; cocoa, coffee and rubber agroforests (humid tropics)

Picture 1 Wheat and mint intercropping in furrow irrigated raised bed systems. Indo-gangetic plain, India (*Courtesy of G. Gupta, with permission of KASSA http://kassa.cirad.fr.*)

Picture 2 Harvest of a wheat crop in an 8-year-old poplar–cereal intercropping system in the South of France. The poplar density is 75% of the density of a forestry plantation, and wheat is grown on 85% of the plot area, resulting in a high relative density, indicating a design that is closer to an additive than a substitutive design. The land equivalent ratio (LER) is over 1.3. (*Photo C. Dupraz.*)

Picture 3 Cocoa agroforestry system in Costa Rica (Talamanca region). Cocoa agroforests include numerous cultivated and sub-spontaneous plant species, i.e. timber trees, fruit trees, palms, banana trees in complex and heterogeneous mixtures. Comparable multistrata systems are found in the humid tropics with coffee, rubber, or coconut palms as major cultivated crops (*Photo E. Malézieux.*)

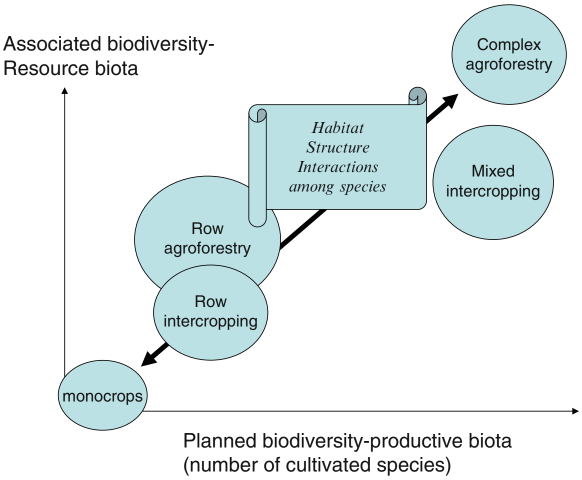


Fig. 1 Relationship between planned biodiversity (plant species introduced and cultivated intentionally by the farmer) and associated biodiversity (species that colonise the agroecosystem) (From Altieri 1999; Vandermeer et al. 1998)

(Altieri 1999). Intentionally mixing plant species will create new habitats for associated species, mainly when the structure of the system is modified (introduction of trees, for instance). In sole crop systems, the mutually beneficial functions and natural subsidies that lend stability and sustainability to natural systems are usually destroyed and require energy subsidies. According to Hobbs and Morton (1999), the stability and sustainability of managed systems could be increased by replacing external energy subsidies with the mutually beneficial functions found in nature through biodiversity. Hence, multispecies systems might or might not improve productivity, but might improve sustainability by improving the ability to resist or rebound in the face of disruptive effects, i.e. resilience.

2.3.2 Effects on Yield and Quality

The advantage of a mixture has often been assimilated to a higher yield of the mixture when compared with an equal area divided between monocultures of the components in the same proportion as they occur in the mixture. Advantage may also be considered when the yield of the mixture is higher than the yield of its best components grown in a monoculture over the whole of the same area, a less frequent situation called transgressive deviation. In a study based on published data on 344 binary mixtures, Trenbath (1974) reported that most mixtures were recorded as yielding at a level between the yields of the components' monocultures. A minority of mixtures were recorded as yielding outside the range defined by the yields of the components grown in a monoculture.

Mixing species can also influence product quality, although different processes may interfere. In coffee (*Coffea arabica* L.) agroforestry systems in Central America, shade due to timber or shade trees promotes slower and more balanced filling and uniform ripening of berries, thus yielding a better-quality product than a monoculture of unshaded plants (Muscler 2001). Fodder quality may be improved when forage is grown under trees, but that is probably due to an improvement in the nutrient balance (Lin et al. 2001). Intercropping legumes and cereals may result in a higher nitrogen content in the cereal grains, hence improving that quality criterion (Bulson et al. 1997). In field experiments in Europe in the 2002/2003, 2003/2004 and 2004/2005 growing seasons, intercropping wheat with faba bean (Denmark, Germany, Italy and UK) and wheat with pea (France) regularly increased the nitrogen and sulphur concentration in cereal grains, hence increasing the wheat quality for breadmaking (Gooding et al. 2007).

2.3.3 Effects on Pests and Diseases

Some crop combinations offer advantages in terms of reducing pests and diseases (Trenbath 1993; Hauggaard-Nielsen et al. 2001). The effect of mixing crops on weed suppression is also well documented (Liebman and Altieri 1986; Bulson et al. 1997; Welsh et al. 1999; Hauggaard-Nielsen and Jensen 2005) although studies on the mechanisms governing those effects are rare.

Numerous studies have shown a significant reduction in harmful insects in mixed cropping systems compared with monocultures of the same species (Nickel 1973; Perrin 1977; Vandermeer 1989). Andow (1991) analysed 209 studies on crop mixtures involving 287 different species of parasitic insects. The insects were significantly fewer in 52% of cases (149 species) compared with monocultures, and greater in 15% of cases (44 species). In conservation tillage agriculture, Dempster and Coaker (1974) found that the use of clover as a cover between rows of brassica crops reduced populations of three insect pests (*Brevicorne brassicae* L., *Artogeia rapae* L. and *Erioischia brassicae*). Andow et al. (1986) showed similar results on insect pests with living mulches interseeded in cabbage.

A particular type of mixed crop is called trap cropping. Trap crops are 'plant stands that are, per se or via manipulation, deployed to attract, divert, intercept and/or retain targeted insects or the pathogen they vector, in order to reduce damage to the main crop' (Shelton and Badenes-Perez 2006).

The reducing effect of crop mixes on diseases (Deadman et al. 1996; Jing Quan Yu 1999; Kumar et al. 2000; Kinane and Lyngkjær 2002) or nematode harmfulness (Egunjobi 1984; Rajvanshi et al. 2002) has been shown in numerous studies.

However, the balance of effects can be complex: for instance, heavy shading in cocoa agroforests may increase pod rot (*Phytophthora megakarya*), but may at the same time reduce insect (*Sahlbergella singularis*) attacks and impacts. Reducing or increasing shade intensity by controlling associated forest trees is therefore an important component of integrated pest and disease management in cocoa agroforests (Berry 2001). The great variability of responses to pests and diseases in multispecies systems therefore requires a clearer understanding of the mechanisms involved in those biological interactions.

2.3.4 Environmental Impacts

In addition to agricultural products, multispecies systems may provide environmental services that have impacts beyond the field scale, either spatially, e.g. services to the local or the global community, or temporally, e.g. modifications of the environment for future generations. Factors that interact in multispecies

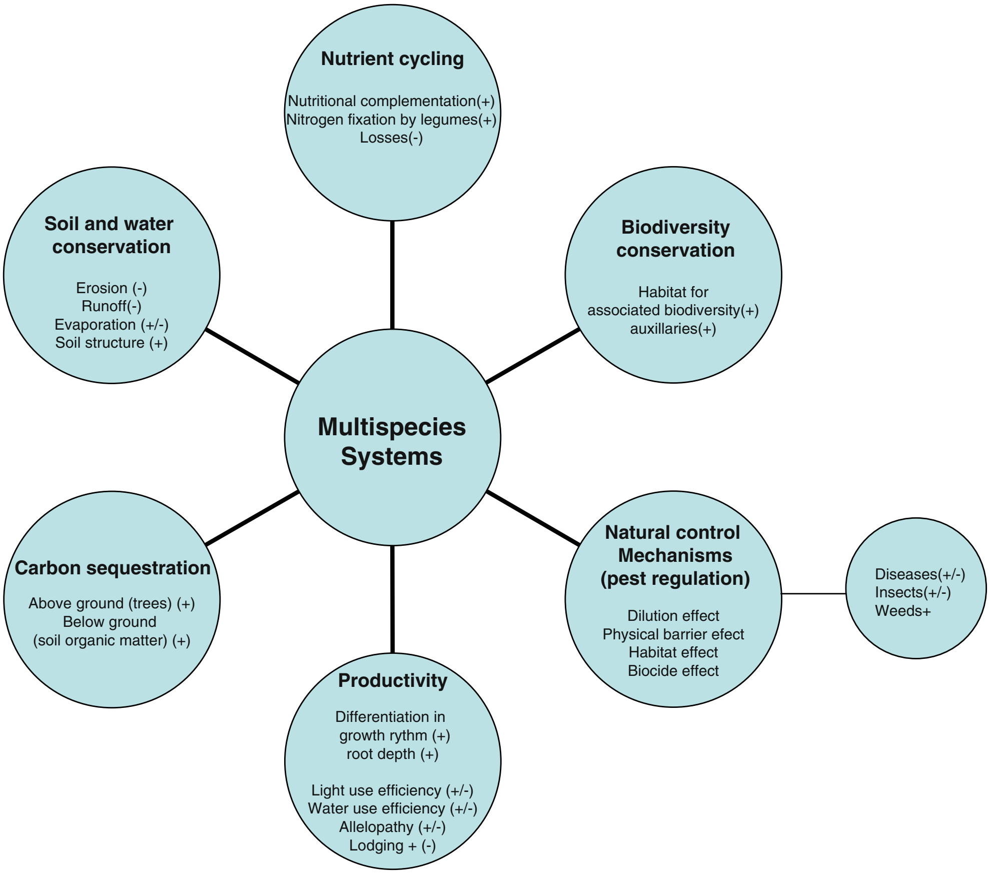


Fig. 2 Processes and induced properties in multispecies systems

systems and may impact on both production and protection functions are summarised in Fig. 2. The most documented environmental services are related to the following areas:

1. Biodiversity conservation: the enhanced diversity of plants in a field may host a larger range of species, from plants to insects, birds to mammals, above- or belowground (Brussaard et al. 2007; Perfecto et al. 2003) (Fig. 3). For crops such as coffee and cocoa, biodiversity often differs less between natural habitats and low-intensity multispecies systems than it does between low-intensity and high-intensity systems (Donald 2004). Beyond conservation issues, higher biodiversity can have local effects, such as greater resilience to abiotic or biotic disruptions, particularly through greater microbial diversity in the soil (Giller et al. 1997; Altieri 1999; Swift et al. 2004).
2. Nutrient recycling by coexisting species exploring different soil depths: this has been particularly documented in agroforestry systems where the deeper rooting system of trees brings up nutrients from deeper soil layers, increasing nutrient-use efficiency and reducing nutrient leaching from the soil layers explored by the crops (Van Noordwijk et al. 1996).
3. Soil conservation and water quality: multispecies systems may increase soil cover, root presence in the topsoil and obstacles to run-off on the soil surface, hence decreasing soil erosion, having a positive impact, on a watershed scale, on the water quality of rivers and on the intensity of floods (Swift et al. 2004).
4. Multispecies systems can sequester carbon over pure crop stands. Trees and/or cover crops (Vandermeer et al. 1998; Scopel et al. 2005) may also enhance the soil carbon content, thus participating in climate change mitigation.

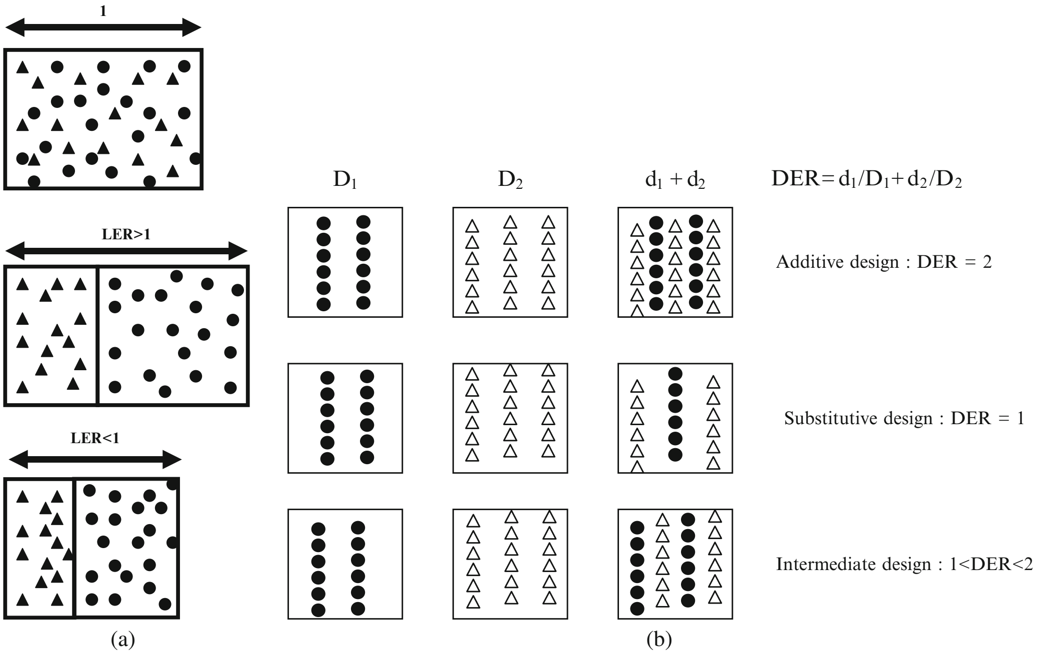


Fig. 3 Land and density equivalent ratios. (**a**) The land equivalent ratio (LER) of a multispecies system is the area needed to produce the same outputs as one unit of land with a pattern of sole cropping; (**b**) the density equivalent ratio (DER) indicates the crowding of the mixture. The symbols represent the plant population density

Multispecies systems can also provide other services, linked to the quality of the environment: trees over crops can provide shade and shelter for animals and humans, and, on a landscape scale, enhance the aesthetical value of land. However, such services are difficult to assess.

2.3.5 Economic Profitability

The economic profitability of multispecies systems is firstly related to their productivity. Despite difficulties due to the number of products involved, specific tools have been developed to assess that productivity. The LER concept (Land Equivalent Ratio, developed hereafter) has been extended to take into account the duration of land occupancy by crops (Area × Time Equivalence Ratio, ATER (Hiebsch and McCollum 1987)) or to incorporate monetary returns (Monetary equivalent ratio, MER (Adetiloye et al. 1989)). However, other issues have to be considered when assessing economic profitability (Follis 1993):

1. *Product time range*. When setting up perennial plantations such as oil palms or rubber trees, crop mixing is widely used to generate income in the first years of the plantation, when the palms or trees are still unproductive, do not produce any economic returns and also occupy a confined field space (Eichhorn et al. 2006). As investments and products are needed or delivered at different times, a financial appraisal of these agroforestry systems calls for tools developed to assess forestry projects, such as Discounted Cash Flow, and the results of the comparisons depend on the discount rate chosen. Nevertheless, most studies show an economic advantage for multispecies systems used for that purpose, when compared with pure forestry projects.
2. *Income stability*. Stability has often been presented as the main reason for adopting multispecies systems in situations exposed to risk, such as poor tropical agriculture. Multispecies systems that provide several products can maintain a more stable income, particularly if price variations for those products are not correlated (Ramirez et al. 2001). Multispecies systems also often require lower external fertiliser and pesticide inputs. That lower dependence on external inputs makes multispecies systems more resilient to external changes, such as product prices.

Intercropping a new crop with a traditional crop is also a way of cautiously entering a new market, without much knowledge, as shown by fruit production in cocoa agroforestry systems in Cameroon (Dury and Temple 1999).

3. *Evaluation of ecological services*. Multispecies systems may also offer ecological services, either for the internal benefit of the fields in which they are cultivated (pollination or soil conservation) or for external benefits, such as water quality, biodiversity or the beauty of the landscape. Valuing those services is becoming an important issue, to incorporate them into the economic appraisal of cropping systems. Several examples exist of payment for such services, particularly in Latin America (Sherr et al. 2007), but accurate assessment is still needed.
4. *Labour productivity*. Mixing is also assumed to be conducive to an adequate distribution of labour over the year. Unfortunately, very little on-farm research has been published on labour distribution and labour productivity in multispecies systems compared with pure crops. Some data are available on annual intercrops, such as the 'abonera' system in Honduras, where velvet bean (*Mucuna deeringianum*) is sown in the rows of corn. That system shows higher labour productivity, even after the second year of establishment, whatever the discount rate chosen (Sain et al. 1994). In Brazil, the direct sowing mulchbased cropping system, combining cover crops before, after or during the main crop, is widely adopted on mechanised farms (Scopel et al. 2004). The adoption of these systems by smallholders is mainly motivated by labour savings (Bolliger et al. 2006), but precise labour data are not available. Multispecies systems may also favour the equity of income distribution within the family, as in West Africa, where, for instance, nuts of the shea tree (*Vitellaria paradoxa*) scattered in the fields are for the exclusive benefit of women.

Although frequent, the advantages and benefits of multispecies systems must not be overgeneralised: not all crops are beneficial in mixtures, since they do not systematically generate ecological and/or economic benefits, and may involve more complex or higher inputs of labour. Even when advantages are recognised, multispecies systems are sometimes more difficult to manage and require substantial farmer skills and specific research efforts.

3 Concepts and Tools Needed for Understanding and Designing Multispecies Systems

3.1 The Conceptual Frameworks of Agronomy and Ecology

3.1.1 The Framework Provided by Agronomists

Agronomy is a discipline that concentrates on both the biophysical functioning of the cultivated field, and on the reasoning of actions taken in plant production (Sébillotte 1978; Doré et al. 2006). Progress in understanding plant-soil interactions on a field scale has been widely based on a simplification of reality, the cultivated plant stand being assimilated to a single homogeneous crop. A widely-used approach in agronomy is big leaf representation based on an energy balance where the plant stand is assimilated to an area of thermodynamic exchange with its surrounding environment. That approach, which is very robust for describing biomass growth in pure crops (Monteith 1977; Gosse et al. 1986), has also been used on sparse crops and to some extent on multispecies systems, by cutting the plant cover into horizontal sections to take into account the vertical heterogeneity of the stand (Wallace et al. 1991). Whilst it has given some interesting results, particularly for characterising competition for light and its impact on biomass (Wallace et al. 1991; Keating and Carberry 1993; Cruz and Sinoquet 1994), a functional approach to the plant stand becomes necessary to account for competition and facilitation processes in the canopy (see below). A similar approach was used for soil and root colonisation in the different soil layers, with similar limits. Using indicators established on monospecies stands can raise problems for multispecies stands; that is notably the case with N because the nitrogen nutrition index is difficult to interpret for intercrops (Corre-Hellou 2005). One of the essential limits for applying the usual agronomic approaches to multispecies systems is therefore to consider the plant stand as a collection of identical individuals. Some attempts to consider canopy heterogeneity and its dynamics have appeared recently for pure stands, allowing for the consideration of emerging properties of the system due to canopy heterogeneity and its evolution: for instance, cohort models have been used to represent and simulate intraspecific heterogeneity in pure stands of bananas due to phenology lags (Tixier et al. 2004). Architectural representations that consider the plant stand as a sum of differentiated individuals are doubtless another efficient way of representing multispecies system functioning, since they can integrate environmental heterogeneity and the impact of architectural organisation on the functional activity and phenotypical plasticity of plants (Soussana and Lafarge 1998; Prusinkiewicz 2004).

As regards the reasoning of actions, agronomy has produced a theoretical corpus based on decision rules for crop management, incorporated into cropping system (Sébillotte 1974, 1978, 1990), technical system (Osty et al. 1998) or action model concepts (Aubry et al. 1998). Agronomists can call upon methods developed for evaluation and design: multicriteria evaluation (Rossing et al. 1997; Loyce et al. 2002), agronomic diagnosis (Doré et al. 2008), designing based on models or expert evaluations. All these concepts and tools should be applicable to multispecies systems as they account for interactions between techniques, long-term cumulative effects and multi-criteria objectives for a crop. However, whilst not ruling them out, they do not facilitate the consideration of characteristics such as heterogeneity and the numerous interactions between individual plants specific to multispecies systems. Hence, multispecies systems require the development of new knowledge, as intercrops involve more complex functions when compared with the respective sole crops. It also calls for the designing of decision rules enabling coordinated management of several cultivated species and even, in some cases, sub-spontaneous species that may have different functions. The complexity of multispecies systems and the specific properties that emerge from them often make it difficult to accept the hypothesis of homogeneity that lies at the basis of many agronomy tools. It may therefore be necessary to revise the concepts used and develop specific, new models and tools.

3.1.2 The Framework Provided by Ecologists

The relations between plant interactions and plant community structures have long received the attention of ecological research (Clements et al. 1926). The question of how biotic diversity and ecosystem functions are related is now considered one of the fundamental questions in ecology (Hobbs and Morton 1999). In natural systems, the composition of plant species can change in line with a productivity (resource) gradient (Tilman 1984). For instance, species richness may decline as soil fertility increases (Abrams 1995). The research conducted by ecologists therefore provides a rich theoretical framework for approaching the role of biological diversity in ecosystem functioning. However, attempts to apply that theoretical framework to cultivated ecosystems are few and far between. Main (1999) addressed the important question of how much biodiversity is enough in an agricultural context. There is certainly no absolute answer to that question, because all systems are dynamic and solutions may depend on place and time, and also because criteria need to be specified to address the sustainability of cropping systems or agriculture. The answer should be more qualitative than quantitative: the ecologists Ewel and Bigelow (1996) emphasised the fact that the mix of life-forms, not the mix of species, exerts control on ecosystem functioning.

That framework provided by ecology primarily relies on three principles based on the hypotheses of complementarity, facilitation and selection of species possessing particular traits (Erskine et al. 2006). The principle of complementarity considers that the diversity of ecological attributes arising from a large number of species provides easier access to limited resources. The principle of facilitation suggests that overall productivity can be increased when some species, e.g. nitrogen-fixing species, can enhance the growth of other species. The principle of selection or sampling assumes that systems containing a large number of species have a greater probability of containing species that are highly adapted to the limiting conditions faced by the system. The diversity of species may also reduce instability in the ecosystem processes through asynchronous responses of the different species to environmental fluctuations. Those different aspects enable ecologists to interpret the effects of biological diversity in ecosystems based on two major variables of the ecosystem: its productivity, often measured by the biomass present, and its stability. Despite the wide range of applications, few studies have been conducted to analyse the relevance and applicability of those three principles for cropping systems. However, whilst the principles of complementarity and facilitation could be tested in an agricultural context, the theoretical principle of the effect of selection or sampling cannot be easily studied because mixtures of species in cropping systems do not give rise to random distribution but to a deliberate choice, reasoned and guided by the farmer.

Taking into account competition between species is a preferential point of entry for both agronomy and ecology, which has strongly influenced the concepts and tools developed by the two disciplines. Competition has received close attention in ecological research (Keddy 1989; Goldberg and Barton 1992) but the explicit consideration of the facilitation principle might renew the concepts and tools, as shown by Bruno et al. (2003). By shifting the balance between competition and facilitation towards facilitation processes (Callaway and Walker 1997; Anil et al. 1998), multispecies systems form a new element at the interface of the two disciplines.

3.2 Measuring Multispecies System Productivity

Simple methods can be used to assess the benefits of multispecies systems by estimating their productivity using the Land Equivalent Ratio (LER, Mead and Willey 1980). LER compares the yields obtained by growing two or more species together with yields obtained by growing the same crops as pure stands. For two mixed species, the LER equation is as follows:

$$\begin{aligned}\text{LER} &= \text{mixed yield1/pure yield1}\\ &\quad + \text{mixed yield2/pure yield2}\end{aligned}$$

The resulting LER indicates the amount of land needed to grow both species together compared with the amount of land needed to grow pure stands of each (Fig. 3a). A LER greater than 1.0 indicates mixed systems are advantageous, whereas a LER less than 1.0 shows a yield disadvantage.

A Density Equivalent Ratio (DER) can be defined as a measure of the crowding of the mixture:

$$\begin{aligned}\text{DER} &= \text{mixed density1/pure density1}\\ &\quad + \text{mixed density2/pure density2}\end{aligned}$$

Additive, substitutive and intermediate designs may be used to combine species in mixed cropping systems (Fig. 3b). The key assumption in the use of the LER is that the densities of plants in the sole cropping controls are close to the optimum. The null hypothesis (LER = 1) means that inter- and intraspecific interactions are equivalent.

The properties of multispecies systems are not always derivable from the properties of individual species. Collective dynamics may lead to emergent properties that cannot be deduced from species properties alone, i.e. redistribution of the soil-water resource by shrubs in agroforestry systems. This makes it more complicated to define a proper methodology for studying multispecies systems compared with studies involving one species.

Loreau and Hector (2001) developed an approach to separate the ‘selection effect’ from the ‘complementary effect’ in ecological systems. According to these authors, a selection effect occurs when changes in the relative performances of species in a mixture are non-randomly related to their performances in a monoculture. In their approach, the authors proposed measuring the selection effect in a mixture of N species by a covariance function derived from the Price equation in evolutionary genetics (Price 1970, 1995a). Here, we propose an adaptation of that function, which could be used by agronomists to estimate the selection and complementary effects resulting from a mixture of several cultivated species.

Let us consider N species cultivated in a monoculture in N fields with areas equal to $s_1, \ldots, s_i, \ldots, s_N$, respectively. Note $z_i = s_i \times y_i$ the crop product obtained with a monoculture of the ith species in the ith field, where y_i is the crop product per unit area. Crop products can be expressed as yields or as gross margins. It is more worthwhile expressing y and z as gross margins when the monetary prices of the crops are very different.

Now, let us consider a mixture of the N species. Note $z_i' = s_T \times y_i'$ the crop product obtained for the ith species when the N species are cultivated as a mixture on a total area defined by $s_T = s_l + \ldots + s_N$. Then, the covariance function defined by Loreau and Hector (2001) can be expressed as:

$$\operatorname{cov}(R_{z_i}, z_i) = \frac{1}{N}\sum_{i=1}^{N}\left(R_{z_i} - \bar{R}_z\right)(z_i - \bar{z}) \qquad (1)$$

where $R_{z_i} = \left(\frac{z'_i}{z_i} - 1\right)$ is the relative gain for the ith species which results from the mixture of the N species.

According to Loreau and Hector (2001), the covariance (1) can be used to measure the 'selection effect'. A positive covariance indicates that the highest relative gains $R_{z_i}, i = 1, \ldots, N$, are obtained for the species giving the best results when cultivated in a monoculture. On the other hand, a covariance near zero indicates that those relative gains are not linked to the performance of the N species in the monoculture.

A small covariance does not necessarily indicate that the overall gain resulting from the mixture of the N species is small. Indeed, it is easily shown that the overall gain is a sum of two terms:

$$\Delta = \sum_{i=1}^{N} z'_i - \sum_{i=1}^{N} z_i = N \times \operatorname{cov}(R_{z_i}, z_i) + N \times \bar{R}_z \bar{z} \tag{2}$$

where Δ is the overall gain resulting from the mixture of the N species, $\bar{z} = \frac{1}{N}\sum_{i=1}^{N} z_i$, and $\bar{R}_z = \frac{1}{N}\sum_{i=1}^{N} R_{z_i}$. The first term is proportional to the covariance $\operatorname{cov}(R_{z_i}, z_i)$ and the second term is proportional to the average value $\bar{R}_z$ of the relative gains obtained for the N species. This second term can be used to measure the complementary effect of the species in the mixture. A positive value of $\bar{R}_z$ indicates that, on average, the relative gains obtained for the different species are positive.

3.3 Resource Sharing in Multispecies Systems

3.3.1 The Principle of Competition vs. Facilitation

Referring to Vandermeer (1989), '*competition* is the process in which two individual plants or two populations of plants interact such that at least one exerts a negative effect on the other, while *facilitation* is the process in which two individual plants or two populations of plants interact in such a way that at least one exerts a positive effect on the other; double facilitation is equivalent to mutualism'. Multispecies systems may maximise beneficial interactions while minimising competition. In comparison with homogeneous pure cropping systems, different species that are sharing a common space interact together and with the environment in an information feedback loop, where the environment affects the plants and the plants reciprocally affect the environment (Fig. 4). These types of interactions give them a set of properties including competition for space, competition for light between canopies and competition for water and nutrients between root systems.

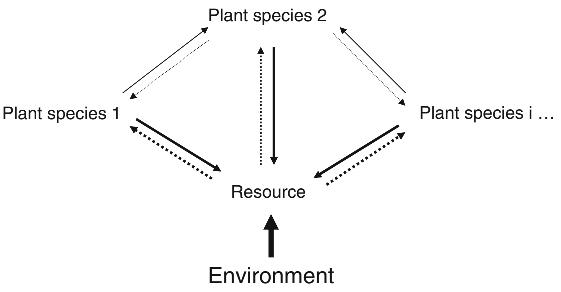


Fig. 4 Interaction between plants for resources (from Grace and Tilman 1990). In this context, plants have an effect on the abundance of a resource and other plants respond to the change. Both the effect (*line*) and the response (*dashed line*) must be of appropriate sign for competition to occur

The agronomic advantages of multispecies systems are the result of differences in the competitive ability for growth factors between plant components. In terms of competition, this means that the components are not competing for the same ecological niches and that interspecific competition is weaker than intraspecific competition for a given factor (Wit de and Van Den Berg 1965). The ecological niche concept (Vandermeer 1989) underlies the fact that the different species involved may have different resource requirements at different times, as well as different sources of nutrition, e.g. root exploitation of superficial soil layers by one species vs. deeper exploitation by the other, different growth patterns, or different affinities for the same nutrient, e.g. nitrogen in NO_3^- form vs. NH_4^+ available form.

3.3.2 Aboveground Competition for Light

Of all the major environmental factors that contribute to reported multispecies system merits, the capture and use of solar radiation is the one that has received the most attention (Keating and Carberry 1993). Overyielding by mixtures has often been attributed to a more efficient use of light by their canopies. Trenbath (1974) reported that an 'ideal' leaf arrangement could be approached by a mixture of a tall erect-leaved genotype and a short, prostrate-leaved genotype. Among aboveground factors, the factors that affect the light regime of plant canopies are the amount of light and quality of incident radiation, the canopy architecture and the optical properties of the leaves and the soil (Sinoquet and Caldwell 1995). In comparison with pure, uniform stands, light capture depends on (1) the fraction of incident photosynthetically active radiation (PAR) that is partitioned by heterogeneous canopies and intercepted by each species, and (2) the efficiency with which intercepted radiation is converted by photosynthesis. While direct measurement techniques have been slow to develop, light modelling within multispecies systems has quickly matured (Sinoquet and Caldwell 1995). A number of models are now available with different levels of complexity for multispecies systems, but field applications face some practical limitations. Compared with pure stands, multispecies systems contain significant spatial variations in leaf area density and leaf angle distribution that are difficult to simulate.

3.3.3 Belowground Competition for Water and Nutrients

Belowground competition occurs when plants decrease the growth, survival or fecundity of neighbours by reducing available soil resources. Contrary to aboveground competition, which primarily involves a single resource, light, plants compete for a broad range of soil resources including water and at least 20 mineral nutrients that differ in molecular size, valence, oxidation state and mobility within the soil (Casper and Jackson 1997). The components of the mixture may be complementary in a spatial sense by exploiting different layers of the soil with their root systems. Components of a mixture may complement each other nutritionally (different needs in quantities, preferential use of different chemical forms). Mixtures of leguminous and non-leguminous species are well known in that regard, and provide repeatable examples of overyielding due to nutritional complementation. To study those interactions, three aspects have to be taken into account that address the resource supply to the roots, the characteristics of the root system and the demand for water and carbon allocation, respectively (Fig. 5):

1. Resource supply to the roots involves four main processes: the distribution of resources in the soil and their availability, which depends on soil biophysical and chemical properties, interception by the roots ($<10\%$), mass flow, which affects water and mobile nutrients such as NO_3^-, and diffusion, which affects nutrients such as P and K.
2. Root system characteristics include morphological plasticity – root location in time (Caldwell and Richards 1986) and space (Willigen and Van Noordwijk 1987), investment in root biomass, root length or surface – and physiological plasticity – rate of resource uptake in relation to enzyme functioning.
3. The demand for water. Water distribution depends on the partitioning of evaporative demand between the species' components, and on soil evaporation (Ozier-Lafontaine et al. 1997, 1998).

An analysis of the belowground processes and resource use by plants presents tremendous challenges as there are still general methodological difficulties despite the advances made in techniques and equipment design. For example, roots of the component plants can intermingle (Gregory and Reddy 1982), making the task of separating the respective root systems very cumbersome. Staining techniques generally fail to distinguish one root system from another. Other possibilities, such as isotopic discrimination of 13C between C3 legumes and C4 cereals are efficient but require special equipment (Wong and Osmond 1991; Lichtfouse 1997).

3.3.4 Intercrop and Resources

For a long time, plant ecologists have intensively studied competition and facilitation along resource gradients (Donald 1958; Grime 1977; Tilman 1988; Wilson and Tilman 1988). However, empirical results and theories remain controversial (Garcia-Barrios 2003). Schematically, competition might be high in a high resource environment, while facilitation might be greater under harsh conditions (Bertness and Callaway 1994). More generally, the interpretation of interactive effects

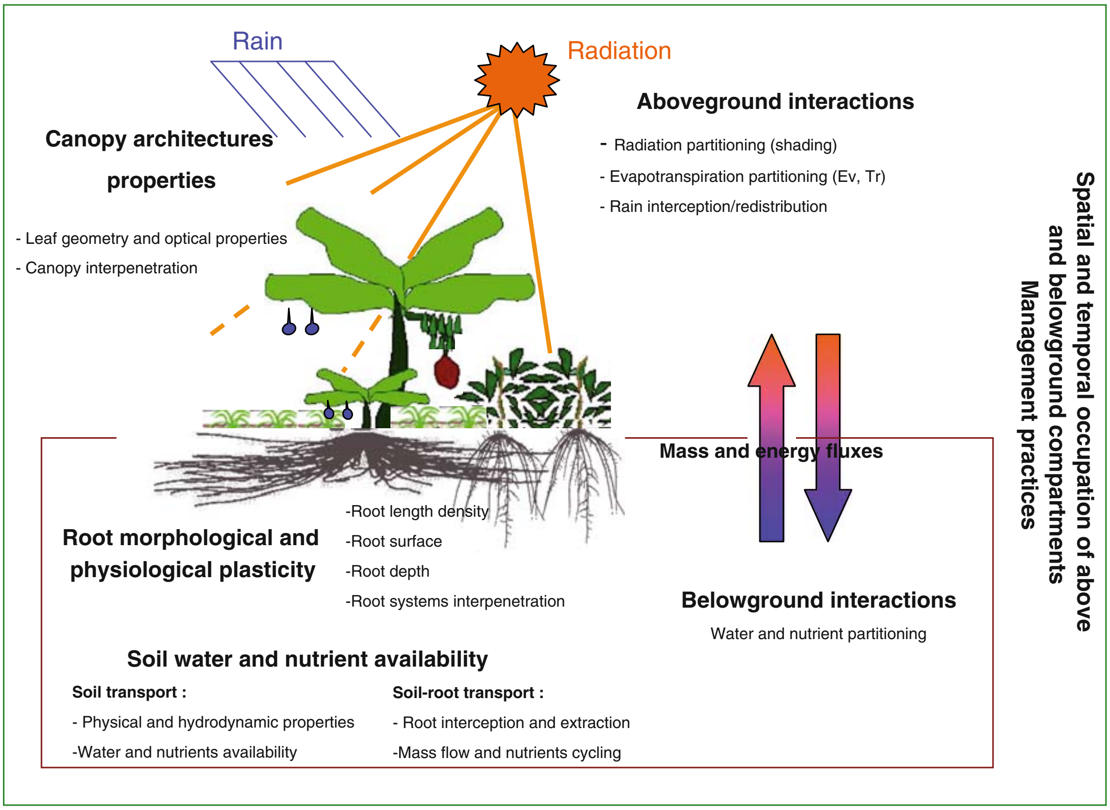


Fig. 5 Above and belowground competition for resources in multispecies systems. The functionning of such systems is not only conditioned by the availability of environment resources but also by the ability of the component species to share them

between intercrop component activities and resources in the environment is extremely complex. For example, specific crop growth affects soil shading and light interception and therefore also temperature; plant water uptake changes soil water content in the rhizosphere, which affects microbial decomposition rates; decomposition rates affect soil texture, water-holding characteristics, rooting profiles and nutrient availability for crops. It remains very difficult to disentangle those processes experimentally. Thus, dynamic simulation models of those systems are valuable for interpreting all these processes and interacting conditions (see Sect. 4).

3.4 Biological Interactions

These refer to a variety of processes that include allelopathy and competition with weeds, along with pest and disease interactions with plants.

3.4.1 Interactions with Weeds

Two types of action are identified to explain the reduction in weed biomass frequently observed: (1) competition for resources such as light, water, nitrogen or other nutrients, and (2) allelopathy (Liebman and Dick 1993), though the distinction between those effects sometimes remains difficult.

Allelopathy refers to inhibition of the growth of one plant by chemical compounds released into the soil from neighbouring plants. It may inhibit a mixture: tree species such as *Gliricidia sepium or Leucaena leucocephala* used in agroforestry are reported to have allelopathic effects on maize and rice seedlings (Nair 1993). Conversely, the use of specific species may enable better control of weeds and thereby be a benefit of mixing. However, little is known about allelopathic mechanisms for weed control in a mixture. Beyond those allelopathic mechanisms, the suppressive effect on weeds is observed through competition when the cultivated species are complementary in resource uptake: nitrogen requirements (legumes vs. other plants), photosynthesis metabolism (C3 vs. C4 plants) and different soil exploration by roots depending on the species. The complementarity between cultivated species often makes it possible to capture a greater quantity of resources in the case of intercrops vs. pure stands, thereby reducing the resources available for weed growth (Liebman and Dick 1993; Bulson et al. 1997; Hauggaard-Nielsen et al. 2001; Hauggaard-Nielsen and Jensen 2005). In mixtures combining a cereal and a legume, the greater competitiveness of the mixture compared with monocultures is due to the fact that cereals are more competitive than legumes in taking up nitrogen from the soil due to faster root development and demand (Corre-Hellou et al. 2006). Mixing species may also reduce the specific diversity of the weed stand and lead to a change in biomass distribution between weed species (Poggio 2005).

3.4.2 Interactions Between Crop Mixtures and Diseases and Pests

In order to explain interactions between mixtures and diseases and pests, a distinction is made between different processes:

1. *The dilution effect*. The hypothesis of resource concentration put forward by Root Root (1973) reflects the fact that the mixture gives rise to a “dilution” of the host plant in the plant cover, making the parasite or pest less efficient at locating and colonising its host plants. An increase in the proportion of non-host plants in a mixture enhances that effect (Sibma et al. 1964; Trenbath 1993).
2. *The physical barrier effect*. The previous theory of Root (1973) is completed by the disruptive crop hypothesis (Vandermeer 1989). By modifying the structure of the stand and the architecture and microclimate of the cover, the mixture modifies the location of the host plant, thereby affecting disease spread or disrupting the parasitic insect’s search for feeding or mating sites (Francis 1990). Conventional cereal crops can disrupt insects in their visual search for smaller crops (Ogenga-Latigo et al. 1992), and the existence of a lower crop storey may, likewise, affect the visual search for a potential host.
3. *The habitat effect*. Introducing species with a contrasting plant architecture creates a new habitat which in turn modifies populations of predators. For instance, Jones and Sieving Jones and Sieving (2006) reported a change in the behaviour of insectivorous birds with the introduction of a single row of sunflowers in organically grown vegetables.
4. *The chemical effect*. A mixture may contain species that produce substances that have negative effects on diseases and pests, such as nematodes, that are parasites on another component in the mixture. That is the case for certain intercropping systems based on cover crops dedicated to controlling nematodes (Yeates 1987; Rodriguez-Cabana and Kloepper 1998). Those different effects can be combined in different ways: for example, cover crops used in mixtures may affect plant parasitic nematodes (a) as non-host plants affecting nematode reproduction, (b) by producing root exudates stimulating nematode reproduction in the absence of hosts and causing nematode death, (c) by producing root exudates with nematicide properties and (d) by producing compounds in the foliage which, once incorporated into the soil, have nematicide properties. Functions (a) and (d) can be utilised in crop rotations, where they ensure a preventive function (cleansing), whilst functions (b) and (c) can be taken advantage of in mixtures, as those two control methods can be utilised in overlapping cycles.

4 Modelling Plant Mixtures

4.1 The State of the Art

Models of plant mixtures apply to various systems: crops and weeds, mixtures of crop varieties, intercropping of different crop species, tree and crop mixtures (agroforestry, tree-shelters) and tree species mixtures (mixed forests). The non-linear behaviour of multispecies systems cannot be accounted for by simply studying or modelling plant components independently (House et al. 2003).

4.1.1 Modelling is the Only Way to go with Multispecies Systems

Design decisions made from the small amount of empirical evidence from the few available trials on multispecies systems are inherently weak, as plant development and productivity in mixtures are site- or weather-specific (Vandermeer 1989). Land Equivalent Ratio assessments based on a few years of measurements are highly questionable (Dupraz 1998; Vandermeer 1989). In mixture studies, innovative planting designs have been developed to reduce the land area needed for mixed-species plantation experiments, by focusing on individual plant analysis rather than plot-level analysis (Kelty 2006). However, the numerous combinations between species, environments and practices are not within reach of traditional factorial experimental approaches. In dynamic systems with heterogeneous structures, a system approach is required to improve understanding of the processes involved, and to evaluate adequate management schemes. There is a need for dynamic modelling tools to evaluate how wide ranges of soil conditions, various weather sequences and different management schemes modify the yield and environmental impact of multispecies systems.

4.1.2 Modelling Interspecific Relationships

All models of multispecies systems simulate interspecific interactions which are key determinants of the structure, the dynamics and the productivity of mixed plant communities (Caldwell 1995; Aerts 1999). As seen previously, in contrast with sole cropping, multispecies systems have parallels with basic ecological principles (Lefroy et al. 1999; Ranganathan 1992). Plant interaction models are categorised as being either empirical, providing only a description of the outcome of competition, or process-based, offering a representation of the physiological processes underlying plant growth. Empirical models are useful for making predictions within the range of data used to parameterise them but are not suitable for extrapolation. Mechanistic models that are based on the behaviour of individual plants are based on 'focal plant–neighbour plant' interactions (Liu and Ashton 1995). In contrast to empirical models, process-based models have the ability to make predictions outside the range of data used in their parameterisation, making them the models of choice for designing plant species mixtures. In order to simulate both competition and facilitation, it is necessary to achieve a balance in aboveground and belowground interactions in resource capture by the component species (Lose et al. 2003; Ong and Huxley 1996). Moreover, in modelling approaches, a balance needs to be maintained between process and pattern, between temporal and spatial aspects. Multispecies dynamic models for resource partition must include appropriate plasticity mechanisms in the plant models, solve the resource sharing for multiple resources simultaneously, and couple plant and crop models often built with different concepts (Fig. 6).

4.1.3 A Review of Current Multispecies System Models

A comparison of some representative multispecies system models is presented (Table 2). Relatively few models have been developed for mixtures of tree species (Bartelink 2000; Coates et al. 2003) and mixtures of herbaceous species (Brisson et al. 2004; Caldwell and Hansen 1993; Carberry et al. 1996; Tsubo et al. 2005), including crop-weed models (Deen et al. 2003; Park et al. 2002, 2003), but even fewer for mixtures of trees and crops (Garcia-Barrios and Ong 2004; Mobbs et al. 1998). Competition models for trees only usually run on a yearly time step, while competition models for crops only, or crops and trees, run on a daily time step.

Multispecies system models can be divided into three groups depending on spatial discretisation of the simulated scene. Most models ignore the spatial heterogeneity of plant mixtures, and simplify the system to a one-dimensional representation. They include CROPSYS (Caldwell and Hansen 1993), APSIM (Carberry et al. 1996), Yield-sAFe (Van der Werf et al. 2007) and GEMINI (Soussana and Lafarge 1998). Those models mimic sole crop modelling, considering the system to be composed of two species instead of one, and assume that both aboveground and belowground stand components are horizontally homogeneous. In the second group of models, a first level of spatial heterogeneity is introduced through discretisation of the system into some linear or circular areas between which flows of mass or energy occur. Some intercropping models such as STICS-CA (Brisson et al. 2004) and most tree belt-crop models (Huth et al. 2003) follow that approach. The WaNuLCas model (Van Noordwijk and Lusiana 1998)

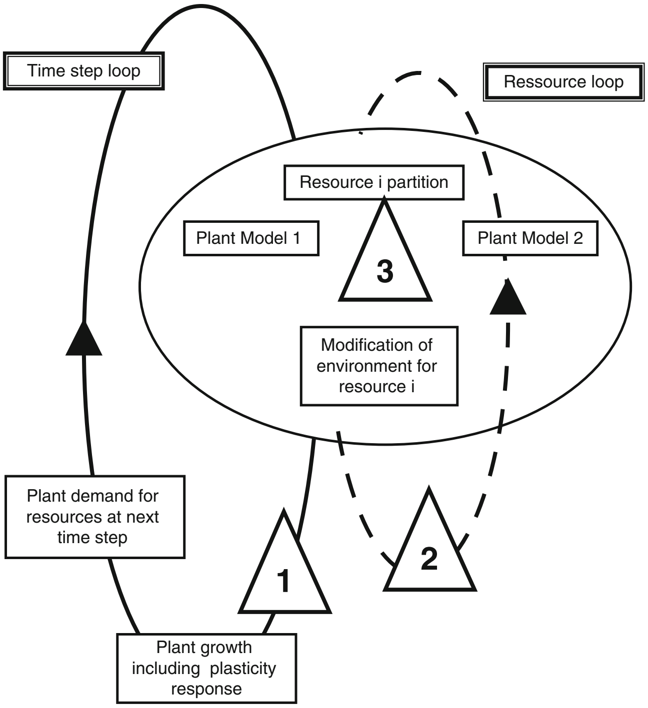
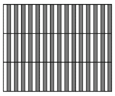
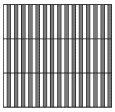
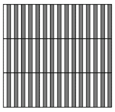
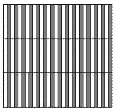
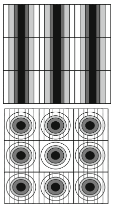
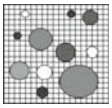
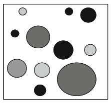
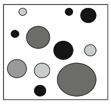
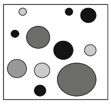


Fig. 6 The three crux of multispecies dynamic models for resource partition: including appropriate plasticity mechanisms in the plant models (1), solving the resource sharing for multiple resources simultaneously (2), and coupling plant models often built with different concepts (3)

includes four zones of tree-crop interactions with decreasing intensity. The possible schedule of a sequence of crops to be grown over time in each zone makes it possible to encompass a broader range of systems in terms of species diversity and spatial structure. The third group includes spatially explicit models, based on modelling individual plants that interact together. This is most common for trees in mixed stands (Bartelink 2000; Coates et al. 2003) but those models often ignore belowground interactions and focus on light partitioning between trees. Very few spatially-explicit models have been developed for annual plants; some models deal with grassland mixtures (Soussana and Loiseau 2002), but most are neighbourhood population dynamic models that often ignore competition for belowground resources (Stephen et al. 1990). SeXI-FS (Manson et al. 2006; Vincent and Harja 2002) and Hi-sAFe (Dupraz et al. in preparation) are models that explicitly integrate both above- and belowground competition for resources on a plant scale, using a distance-dependent and an individual-based modelling approach. By including demographic processes (mortality, recruitment), SeXI-FS simulates the long-term dynamics of the spatial structure. For belowground interactions, progress has been achieved with the development of 2D mechanistic models that include distributed source-sink functions (Ozier-Lafontaine et al. 1998; Lafolie et al. 1999), and in some cases algorithms to account for minimum energy resolution (Adiku et al. 2000). By coupling structure and function at different levels of complexity, these biophysical models provide a clearer understanding of the importance of the different components involved in water competition, i.e. demand partitioning, soil hydrodynamic properties, root distribution and priority in root water extraction.

Time steps in all these models vary from 1 year to one day or less, with possible integration over the course of one or more growing seasons, up to a century for forest models. The models have not been designed for the same purpose, or for the same users, and a comparison is therefore tricky. They often combine simplicity and complexity: one model might be very simple regarding one mechanism, while being more realistic and close to mechanistic models for simulating other processes. Most of the models are used as

Table 2 Comparison of some multispecies models designed for intercropping, agroforestry and forestry

	Specific diversity (number of species)	Spatial pattern heterogeneity (horizontal)	Time step and scale	Aboveground interactions	Belowground interactions	Type of system	Objectives	General comments
CROPSYS (Caldwell and Hansen 1993)	2 [crop/crop]		Day time step n cycles	Canopy : 1D, 2 layers Light balance	Soil : 1D, n layers Water and N competition	Tropical linear intercropping systems	Growth and yields of annual species Risk evaluation/Decision making	Dedicated to crop/crop interactions
STICS-CA (Brisson et al. 2004)	2 [crop/crop] or [tree/crop]		Day time step n cycles	Canopy : 1D, geometric shape Light balance Microclimate feed-back implemented	Soil : 1D, n layers Water and N competition Plasticity of root system implemented, but no C allocation between shoots and roots	Temperate and tropical linear intercropping systems	Growth and yields of annual and perennial species	Suitable for tree/crop or crop/crop interactions No tree management (pruning)
GEMINI (Soussana et al., 2000)	2 [crop/crop]		Day time step n cycles	Canopy: 1D, n vertical layers Light balance	Soil : 1D, n vertical layers N uptake	Temperate grasslands	Grassland species coexistence, dynamic and productivity.	Simulation of phenotypic plasticity in response to shade and nitrogen deprivation Explicit description of the plant shoot and root morphogenesis and architecture Crop management (animal module)
Yield-SAFE (Van der Werf et al., 2007)	2 [tree/crop]		Annual time step Long term production dynamic	Canopy: : 1D, n vertical layers Light balance	Soil : 1D, n vertical layers Water and N uptake	Temperate linear intercropping systems	Growth and yields of annual and perennial species. Evaluation of profitability of agroforestry systems	
WaNuLCAS (van Noordwijk and Lusiana, 2000)	> 2 [tree/crop]		Day time step n cycles Long term production dynamic	Canopy : 1D n layers Light balance Microclimate feed-back not implemented	Soil : 2D, 16 compartments Water, N and P uptake Tree roots may interact with crop roots in the 16 compartments N tree fixation Crop roots do not interact each other and are limited in vertical compartments Plasticity of tree root system	Tropical linear ou circular intercropping systems; sequential agroforestry	Growth and yields of annual and perennial species. Evaluation of sustainability and profitability of agroforestry systems	Tree management Sustainabilty : SOM (Century model) Limits of the STELLA programming platform
Hi-sAFe (Dupraz et al., 2007)	> 2 [tree/crop] and [tree-tree]		Day time step n cycles Medium term production dynamic	Canopy : 3D Light balance	Slope included Soil : 3D Water and Nitrogen balance	Temperate agroforestry systems with any design for tree and crop position	Growth and yields of annual and perennial species. Carbon, nitrogen and water budgets for environmental appraisements	Crop and Tree management Unsuitable for long term prediction Plasticity of tree root systems Water table
COMMIX (COMpetition in MIXtures). (Bartelink, 2000)	> 2 [tree-tree]		Year time step Long term production dynamic	Canopy : 3D Light balance Space occupation	Ignored	Temperate mixed forests	Forest productivity Exploration of thinning scenarii	Phenological differences between species not included
SORTIE/BC (Coates et al, 2003)	> 2 [tree-tree]		Year time step Long term production dynamic	Canopy : 3D Light balance Space occupation	Ignored	Temperate and boreal mixed forests	Forest dynamic and productivity Exploration of thinning scenarii	Demographic processes included (recruitment and mortality)
SexI-FS (Vincent and Harga, 2002)	> 2 [tree-tree]		Year time step Long term production dynamic	Canopy : 3D, continu Light balance Space occupation	Soil : 1D horizontally discrete	Tropical agroforests	Forest dynamic and productivity Exploration of prospective management scenarios	Demographic processes Module of crown shape flexibility in response to light and space limitations

research tools rather than management tools. Knowledge gaps have been identified and are discussed in the following section.

4.2 Future Directions in Modelling Plant Mixtures

4.2.1 Designing an Appropriate Working Environment to Deal with Spatial and Temporal Patterns

One of the main characteristics of multispecies systems is the wide range of spatial arrangements (strip or mixed systems, alley crops with various plant-plant distances, mixtures of annuals and perennials, vertical discontinuities in agroforests, windbreaks, etc.) and temporal arrangements (simultaneous vs. sequential) that farmers opt for depending on their agroecological purposes. Such a platform is only partially designed in WaNulCAS and Hi-sAFe for agroforestry systems. A real breakthrough is needed in the design of a flexible platform that involves both multispatial and temporal management, including annual (intercrops, cover crops) and perennial (agroforests, forests) arrangements. The CAPSIS platform (Coligny et al. 2003) provides an attractive example of such operational platforms designed for the simulation of forest dynamics and productivity.

Dynamic simulation models need to integrate competition for different resources in time and space, so as to predict highly non-linear response patterns. A key point in future modelling challenges remains the need for a link between different models geared towards process levels and, above all, between different spatial and temporal scales. That objective means creating bridges between ecophysiology, population biology and functional ecology.

Table 2 focuses on the modelling of multispecies systems as a whole, but other modelling tools are available and relevant to the analysis of some multispecies system processes or components. For example, radiation models based on 3D architectural mock-ups and ray-tracing (Dauzat and Eroy 1997) can be used to derive parameters of the turbid medium analogy widely applied in multispecies system models (Lamanda et al. 2007). In addition, conceptual population biology models may be helpful in exploring the coexistence of species in mixtures. To that end, use of functional traits and groups used in ecology to characterise and simulate natural ecosystems such as rainforests (Gourlet-Fleury et al. 2005) may be of great interest for simulating complex multispecies systems, such as agroforestry systems in the humid tropics (Malézieux et al. 2007).

4.2.2 Challenges Related to the Level of Process Description in Mechanistic Models

The large number of possible species combinations, management practices and site-dependent interactions in multispecies systems indicates that a pure empirical approach would be unsuitable for most problems to be solved. Although intensive work has been devoted to simulating abiotic interactions (light, water) in multispecies systems, efforts need to be focused on (1) better knowledge of interactions for resources, which requires both suitable discretisation of the above- and belowground environment (voxellisation) and realistic simulation of the physical properties of mass transport laws – simulation of changes in soil physical properties, due to tillage and biological activities, must also receive greater attention (Stockle 1999) – and (2) an appropriate understanding and multi-scale representation of the plasticity of roots, shoots and crowns involved in the process of adaptation to heterogeneous and competitive environments. Through its decisive role in resource acquisition and use, physiological and morphological plasticity may alter the sign and magnitude of interactions among plants and, as a consequence, the performance and dynamics of the system (Callaway et al. 2003).

Biotic investigations still remain the poor relation of modelling research on multispecies systems, although many models have been developed to simulate the growth and activity of weed, pest and disease populations (Doyle 1997; Van Oijen 1995). As pointed out by Stockle (1999), weed, pest and disease effects are ignored in most comprehensive models, as a result of the complexity of dealing with a potentially large number of species for each plant of interest. This field of research is particularly dependent on population ecology concepts – population dynamics, epidemiology – and the functional ecology of the soil (role of micro- and macroorganisms).

In other respects, assessing the impact of climate change and CO_2 elevation scenarios on multispecies system productivity and the environment will continue to be an important field of research, particularly for simulating the ability of species to adapt to changing temperatures, moisture availability, atmospheric CO_2 concentration and other aspects of climate change, i.e. pest pressure.

5 Conclusion

Despite its potential advantages and the huge diversity of multispecies systems existing in world agriculture, mainstream agronomic research has largely focused on monocrop systems, with very little interest in ecological interactions between species in mixed systems. Today, it is barely feasible to simulate multispecies systems and, due to the absence of efficient models, it is difficult to understand the effects of the different factors that interact within those systems. The relevance, but relative limitation of the concepts and existing tools of systemic agronomy in alone representing and simulating multispecies systems and their properties certainly reveal the need to find new representations to account for the particular processes brought into play. As shown in this article, the numerous mechanisms involved in species mixing highlight the need to deal with their complexity by combining concepts from diverse disciplines (agronomy, ecology, epidemiology, etc.), although the necessary link with ecology largely remains to be constructed.

As emphasised by Gurr et al. (2003), mixing species in cropping systems may lead to a range of benefits that are expressed on various space and time scales, from a short-term increase in crop yield and quality, to longer-term agroecosystem sustainability, up to societal and ecological benefits that include recreation, aesthetics, water and soil quality and flora and fauna conservation, including endangered species. Understanding such interactions between cropping systems and the environment means working on a broader spatial scale than the farmer's field and means considering the mosaic of fields that structure the landscape, and its evolution over a longer time scale.

For all these issues, multispecies systems are today a real challenge for agricultural research, and more specifically for systemic agronomy. It is time to understand and integrate their complex functioning and develop an adequate tool-box for checking and ensuring their technological development.

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Saffron, An Alternative Crop for Sustainable Agricultural Systems: A Review

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Abstract Saffron (*Crocus sativus* L.) is an autumnal flowering geophite whose dried stigmas, well known for their aromatic and colouring power, have been used since immemorial time as a spice in human nutrition, for medicinal purposes and as a dye. Many doubts remain on its origin; it was probably selected and domesticated in Crete during the Late Bronze Age. Saffron is a triploid geophyte species, self- and out-sterile and mostly male-sterile and therefore unable to produce seed, that reproduces by means of corms. Furthermore, it has a reverse biological cycle compared with the majority of cultivated and spontaneous plants: flowering first in October–November, then vegetative development until May, which means that the vegetative development is not directly important for production of stigmas, but for the production of new corms. Due to its unique biological, physiological and agronomic traits, saffron is able to exploit marginal land and to be included in low-input cropping systems, representing an alternative viable crop for sustainable agriculture. Notwithstanding this great potential and the considerable increase in new generation consumer demand for saffron, the future of the plant is still uncertain. Indeed, the main obstacles to saffron production are: (1) the limited areas of cultivation in countries where it is traditionally grown, (2) the great amount of sophisticated spice, (3) management techniques executed by hand, and (4) the very high price of the spice. Here we review the main biological, genetic and ecological traits associated with agronomic management techniques of saffron in relation to environmental conditions. Colour, taste and aroma are the essential features on which the quality of saffron stigmas is founded. In turn, these aspects are strictly connected with the biomolecular composition of the stigmas, namely, the carotenoids and their derivatives. With this in mind, the biosynthetic pathway that leads to the formation of saffron secondary metabolites and their abundance in the spice is presented, together with the biomedical properties commonly associated with saffron. Furthermore, a detailed overview of the more recent instrumental methods to assess the quality of saffron, strictly from a chemical point of view, will be discussed.

Keywords Carotenoids • *Crocus sativus* • Quality • Saffron • Sustainability • Yields

1 Introduction

Saffron (*Crocus sativus* L.) belongs to the large family of Iridacee and to the genus *Crocus*, which includes about 80 species distributed primarily in the Mediterranean and south-western Asia (Fig. 1). Among these, saffron, recognised as the most expensive spice in the world (Winterhalter and Straubinger 2000; Fernandez 2004), certainly represents the most interesting and attractive species, for the colouring, bitterness and aromatic power of its dried stigmas.

Saffron is a geophite herbaceous plant, whose stigmas have been used from ancient times as a spice in

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Fig. 1 Saffron (*Crocus sativus* L.)

food, as a dye, in perfumes and cosmetics preparation and for medicinal purposes (Basker and Negbi 1983). Nowadays, it is almost exclusively used for food colouring and flavouring, even though recent studies are boosting interest in its medical properties. Saffron is known only as a cultivated species; it propagates solely vegetatively by means of corms, underground stems acting as storage and reproduction structures, and does not produce seeds or exist as a spontaneous plant.

Saffron is cultivated in a wide range of environments with mild to dry climates. For a long time, saffron has been neglected by researchers and farmers since it was considered a minor crop used only for agricultural diversification. However, in the last few years it is gaining a more interesting role in low-input agricultural systems and as an alternative crop. Moreover, saffron is a very attractive crop for organic and low imput agriculture considering that no irrigation, chemical fertilisation or chemical weed treatments are applied in some environments in which it is cultivated. The main management techniques such as corm planting, flower harvest, stigma separation and corm lifting are carried out manually and this contributes to its high price. Commercial saffron is defined as "*the stigmas of C. sativus L. dried, dark red in colour and trumpet shaped, serrated or indented at the distal end. The length is between 20 and 40 mm. The stigmas may be isolated or joined in pairs or threes at the end of the portion of the style, which is white/yellow in colour*" (ISO 2003). Saffron's colour, bitter taste and aroma are its three main and particular characteristics, which are associated with three different molecular features: crocins, picrocrocins and safranal, respectively.

These and other characteristics make saffron one of the most interesting alternative rediscovered crops, especially for the Mediterranean environment, where the hot, dry summer climate inhibits the spread of pathogenic disease. The relaunching of saffron production requires research studies, improvement in knowledge and a synergic action between scientific and empiric information.

2 Origin and Distribution

The word saffron probably comes from the Persian sahafaran, that derives from asfar, meaning yellow. Its centre of origin is not entirely certain. Some information is given on saffron origin and domestication: Vavilov indicates the Middle East (1951), while other authors suggest Asia Minor or the south-west Greek islands as its probable area of origin (Tammaro 1990). Results from recent research (Negbi 1999) show that *Crocus sativus* was probably selected and domesticated in Crete during the Late Bronze Age. From here, it spread to India, China and the Middle Eastern countries. From these latter, the Arabs brought saffron to all Mediterranean Europe (Ingram 1969). Anyway, according to recent archaeological studies on ancient coins and inscriptions (Manganaro 2001), saffron was already cultivated in the Greek–Roman period in Sicily.

Even if many legends surround the origin of saffron, the first detailed historic information dates back to Papyrus Ebers (1550 years B.C.) that documents the use of saffron for medical purposes and frescoes in the Minoic Palace of Knossos (about 1500 years B.C.), depicting young girls gathering crocus flowers in baskets. Many other historic references are reported in the Old Testament and in the texts of many Greek (such as Theophrastus, Aeschylus, Pindarus, Hesiod, Hippocrates and others) and Roman (such as Plinus, Largus and Celsus) writers, documenting its use for the preparation of perfume, food and as a dye.

Due to the small area of cultivation and the minor importance given to this crop, information on its diffusion throughout the world is uncertain. Total world saffron production is estimated at about 205 tons per year

Table 1 Estimate of saffron world production (modified from Negbi 1999)

Country	Area (ha)	Production (kg)	
Iran	47,000	160,000	Ehsanzadeh et al. 2004
India	–	8,000–10,000	Fernandez 2004
Greece	860	4,000–6,000	Fernandez 2004
Azerbaijan	675	–	Azizbekova and Milyaeva 1999
Morocco	500	1,000	Ait-Oubahou and El-Otmani, 1999
Spain	200	300–500	Fernandez 2004
Italy	35	120	(personal communications)
France	1	4	Girard and Navarrete 2005
Turkey	–	10	Thiercelin 2004
Switzerland	–	0.4	Negbi 1999

(Fernandez 2004). In the last century, saffron cultivation areas changed completely: in European countries, despite an increase in the price of saffron, traditional cultivated areas (Spain, Italy and Greece) underwent a severe reduction. In Spain, saffron dropped from 6,000 ha in 1971 to 200 ha today (Fernandez 2004), in Greece from 1,600 in 1982 to 860 according to the most recent information (Skubris 1990; Fernandez 2004) and in central Italy (Abruzzo) it fell from 300 ha in 1910 to 6 ha some years ago. On the contrary, an enormous increase has been registered in Iran in the last 30 years. The main reason for this change is certainly due to the high requirement of manual labour, concentrated into a few days and into a few hours a day, and to the increase in labour costs. Today, the main producer countries are Iran, India and Greece (Table 1). Iran has the widest area cultivated with saffron: Ehsanzadeh et al. (2004) report an area of 47,000 ha, most of which is grown in the Khorasan province. In India, saffron is widely cultivated in Kashmir, while there are notably less areas, even if considerable given the typology of the crop, in Greece (Kozani, western Macedonia), Azerbaijan (Aspheron peninsula) and Morocco (Taliouine area). There are also small cultivation areas in Italy, about 35 ha, for the most part concentrated in Sardinia (about 25 ha in S. Gavino, Cagliari province) and Abruzzo (about 6 ha in Altopiano di Navelli, L'Aquila), France, Turkey, Switzerland, Israel, Pakistan, China, Egypt, United Arab Emirates, Japan and Australia (Fernandez 2004).

The major saffron-importing countries are Germany, Italy, the USA, Switzerland, the United Kingdom and France (International Trade Centre 2006). Spain imports large quantities of saffron as well, especially from Iran, Greece and Morocco for re-export and for its internal market needs.

3 Genetic Traits

Saffron is a triploid geophyte species ($x = 8$; $2n = 3x = 24$) (Mathew 1977; Ghaffari 1986), self- and out-sterile and mostly male-sterile (Grilli Caiola 2005) and therefore unable to produce seed. Its sterility depends on an irregular triploid meiosis, resulting in many anomalies in sporogenesis and gametophyte development (Chichiriccò 1999; Grilli Caiola 2004) and then in a production of abnormal pollen. In fact, at maturity, about 70% of the ovules of *C. sativus* contain a normal Polygonum-type sac (Battaglia 1963; Chichiricco 1984; Grilli Caiola and Chichiriccò 1991), while a very high incidence of low pollen viability and germination due to meiotic abnormalities was detected (Chichiriccò and Grilli Caiola 1984; Grilli Caiola 2004). For these reasons saffron presents self-sterile pollination. In the past, seeds in the field have been reported only once (Piccioli 1932), while in vitro cross-pollination (fertilisation) of the ovary of *C. sativus* with pollen of *C. cartwrightianus* (Grilli Caiola 1999, 2005) and *C. tomasii* Ten. (a self-incompatible, but cross-fertile species) (Chichiriccò 1999) resulted in the production of capsules and viable seeds. *C. hadriaticus* is able to fertilise *C. sativus* as well (Grilli Caiola et al. 2001). On the contrary, pollination of other *Crocus species* with pollen of *C. sativus* did not result in production of any seeds (Grilli Caiola 2005). Even if Angiosperms can also produce apomictic embryos, this was never detected in saffron (Chichiriccò 1996; Grilli Caiola 2005).

The genetic origin of *C. sativus* is not clear: it may have occurred by autotriploidy from a wild *Crocus*, probably by fertilisation of a diploid unreduced egg cell by a haploid sperm cell or a haploid egg cell by two haploid sperms (Chichiricco 1984; Grilli Caiola 2004, 2005), or by allopolyploid through the hybridisation of *C. cartwrightianus* and *C. hadriaticus* (Castillo et al. 2005). Information on saffron ancestors is not univocal: Brighton (1977) in a kariological study suggested that possible ancestors of *C. sativus* are *C. cartwrightianus* or *C. thomasii*. Recent AFLP analysis (Amplified Fragment Length Polymorphisms) confirmed that the quantitative and qualitative traits of their DNA are compatible with *C. sativus* (Zubor et al. 2004). Between these, some authors indicate *C. cartwrightianus* as the most probable ancestor (Mathew 1999; Brandizzi and Grilli Caiola 1998; Grilli Caiola et al. 2004). Moreover, flowering in *C. cartwrightianus* has close similarities to *C. sativus*.

Brighton (1977) affirms that saffron exhibits fairly homogenous and stable biological traits all over the world and differs only in minor morphological and biochemical characteristics such as some morphometric features (Tammaro 1990). This observation was in part confirmed by a recent investigation into the DNA of saffron from five different locations (Europe and Israel) with RAPD methodology (Random Amplified Polymorphic DNA) that did not identify any genomic differences (Grilli Caiola et al. 2004). However, the samples from different countries showed clear morphological differences, so we may assume that the screening method used was unable to detect genetic differences.

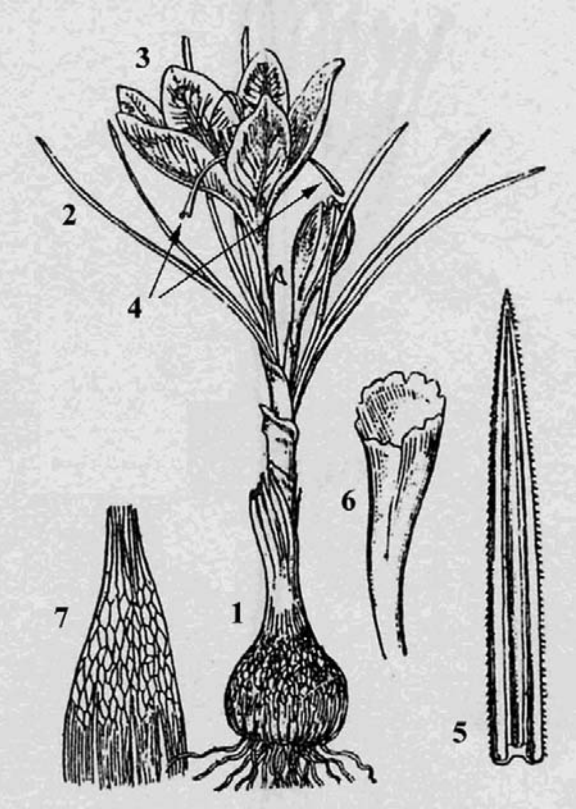


Fig. 2 Main morphological characteristics of saffron. (1) Corm; (2) Leaves; (3) Tepals; (4) Stigmas; (5) Close-up of the leaf; (6) Close-up of the terminal trumpet-like part of the stigmas; (7) Reticulated tunic of the corm (modified from Pignatti 1982)

4 Description

4.1 Morphology

Morphologically saffron, being a clone, has great uniformity over a wide cultivated area (Brighton 1977; Mathew 1977). The corms, a tuberous-bulb formation, are squashed, flattened at the base, to about 4.5–5.5 cm diameter, and covered by several reticulated fibrous tunics (Fig. 2). Corms have one or two main buds in the apex position and about (depending on the dimension) 4–5 or more secondary buds, arranged irregularly in spiral form. Corms derived from secondary buds are smaller than corms produced by apical buds. Each mother corm produces 1–3 medium-big daughter corms from apical buds and several small corms from lateral buds, depending on the size of the mother corm. Leaves (from 6 to 9) are erect, narrow, grass-like and dark green coloured. The flower, usually one or several, but even as many as 12, is composed of a perianth of six violet tepals (perigon) connate at the base in a long and narrow tube. The pistil is composed of an inferior ovary from which a slender style, 9–10 cm long, arises. The style is divided into three dark red branches, each one up to 30–40 mm long, named stigmas, which droop over the perianth segments. Three stamens with two lobed anthers each are also present.

Some variants of saffron with a higher number of stigmas have been reported by Estilai (1978), Dhar et al. (1988), Piccioli (1932) and Gresta et al. (2008). However, they do not reappear the following year and so should be considered somoclonal variations that do not pass on to the next generations (Grilli Caiola, personal communication). Saffron has two types of roots: fibrous, thin roots at the base of the mother corm, and contractile roots formed at the base of lateral buds

(Kalesi et al. 2004), thicker than the former with a tuber organ aspect that give the corm the ability to maintain depth in the soil (Chio-Sang 1996). *C. sativus* may be distinguished from other spontaneous *Crocus* species lacking in aromatic power and that are sometimes used in the sophistication of the spice, essentially for the greater dimension (gigantism) of vegetative (leaves, corms and floral axes) and flowering parts (stigmas, stamens and tepals).

4.2 Biology and Physiology

Saffron is an autumnal flowering geophite characterised by a long summer rest in which the plant survives periods of drought by means of corms (Fig. 3). Its biological cycle starts with its above-ground vegetative growth at the first autumn rains with the emission of leaves and flowers almost immediately and ends with the production of replacement corms in about 220 days. Unlike many other species of the *Crocus* genus that flower in the winter-spring period, in saffron flowering can occur from mid-October to the end of November, essentially depending on the climatic conditions. It generally starts from 60 to 90 days after planting, mainly depending on sowing time, but snowy and cold periods may retard flowering. Flower induction is a very complicated mechanism in saffron. Little information is available on flower induction in saffron; nevertheless, flowering seems to be mainly influenced by environmental factor such as Molina et al. (2004a) ascribe the ability to influence the beginning and duration of flowering to temperature, while an inferior or negligible role is ascribed to soil water content. Certainly, as in most geophyte plants, both seasonal and daily thermoperiodism are involved as the main environmental factors inducing flowering (Halevy 1990).

Saffron is considered a subhysteranthous plant (Mathew 1977, 1982). Indeed, the phenological sequence of the different phases is not predetermined: flowers can appear before, at the same time or after leaf appearance. Hysteranthy is a strange phenomenon present in many geophytes such as *Scilla autumnalis*, *Uriginea maritima Amaryllis belladonna*, *Pancratium sickenbergeri*, *Colchicum alpinum*, *Sternbergia colchiciflora*, *Ornithogalum pyrenaicum*, etc., that flower before leaf emergence, supporting flowers only with the storage nutrients concentrated in the corm (Dafni et al. 1981). In Mediterranean geophytes, this response can be explained as a strategic adaptation to the temporal unpredictability of the onset of rain after the summer drought (Debussche et al. 2004). Hysteranthy may be of great importance in saffron with respect to mechanisation of the flower harvest. Flower-cutting machines could be used to harvest flowers and avoid damaging the leaves. Hysteranthy can be induced by controlled temperature during corm storage: a dry storage of corms at 15°C for 35 days resulted in flowering prior to leaf appearance (hysteranthy) and in a more synchronous flowering (Plessner et al. 1989). Flower and corm production was also improved by the 15°C pre-treatment.

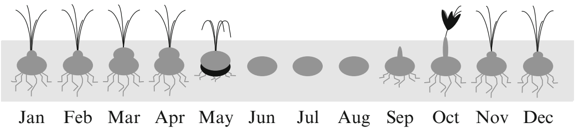


Fig. 3 Biological cycle of saffron (from Alvarez et al. 2004)

A short growth phase of the vegetative part is also detectable in early spring, in which the photosynthetic activity of leaves allows the formation of replacement corms. Afterwards, when the temperature rises, leaves wither, and the plant remains only belowground by means of the corm. Root growth occurs from autumn to the spring period, in which the mother corm is completely empty and daughter corms are produced to propagate the plant. The average size of replacement corms is inversely related to their number (Negbi 1990).

5 Adaptation

5.1 Climate

Saffron is cultivated in very different environmental conditions with good results: in Italy, saffron is cultivated in Navelli, from 650 to 1,100 m above sea level (a.s.l.) with an average annual rainfall of about 700 mm, and in Sardinia in S. Gavino Monreale, from 50 to 140 m a.s.l. with 300–600 mm rainfall. In Greece, cultivation areas are located in Kozani Macedonia, about 650–700 m above sea level, and precipitations are around 560 mm per year, while in Spain, La Mancha and Castille, it is cultivated under 250–500 mm rainfall, usually in irrigated conditions (Tammaro 1990).

Average annual temperatures are around 11.3°C in Navelli with 20–22°C in summer and 2–3°C in winter, when snow can cover the land for up to one month; in Sardinia 16–20°C with 25°C in summer and 10°C in winter; in Kozani 12.5°C with 23°C in summer and 2–5°C in winter, and in La Mancha and Castille 16–20°C with 25°C in summer and 5–7°C in winter. In India, saffron is cultivated in areas with a sub-tropical climate (Sampathu et al. 1984). Mollafilabi (2004) reports that saffron is able to tolerate substantial cold temperatures of −18°C, and occasional winter snow, even if with reduced productivity. Significant snow damage in saffron was ascertained only during flowering because of flower freezing and decomposing (Tammaro 1990), while minor problems were observed in leaves even with short-lasting snow (Ait-Oubahou and El-Otmani 1999). Autumn temperatures in Azerbaijan saffron-cultivated areas fall to −5.9°C (Azizbekova and Milyaeva 1999). During the summer rest period, maximum temperatures of 30–40°C can be tolerated as well.

Saffron prefers direct sun exposure, even though in India it is cultivated together with almond trees. According to Fernandez (2004) and Mollafilabi (2004), the best climatic conditions for high yields are rainfall in the autumn, warm summers and mild winters.

5.2 Soil

Saffron grows on a wide range of soils. Skrubis (1990) indicates that the best performances are achieved on well-drained clay-calcareous and deep soil. Fernandez (2004) suggests that clay is a good soil for saffron, while Sampathu et al. (1984) report that saffron requires a well-ploughed sandy-loamy soil or a well-drained clay soil. Saffron is also cultivated on sandy soil in Azerbaijan (Azizbekova and Milyaeva 1999). Tammaro (1999) suggests that the humus-clay soil of Navelli guarantees good water storage for saffron. Saffron grows well in salty soil, while a limiting factor could be calcium carbonate deficiency (Mollafilabi 2004). Good soil pH ranges from neutral to slightly alkaline. Conflicting information is reported on nutrient needs among different authors (Goliaris 1999; Tammaro 1999; Skrubis 1990).

6 Management Techniques

6.1 General

Most crop management techniques, above all planting, weeding, flower picking and separating, are performed by hand all over the world (Bali and Sagwal 1987; Ingram 1969; Tammaro and Di Francesco 1978). For this reason, saffron cultivation is painstaking and expensive. Saffron cultivation is generally carried out as a perennial cycle, but an annual crop system is adopted in Navelli, Italy. Perennial crop techniques have highly variable durations from place to place: from 3–4 years in Spain, 6–8 years in India and Greece and up to 12 in Morocco (Ait-Oubahou and El-Otmani 1999). With the aging of the saffron field, generally after 4–5 years, spice production declines because of increasing competition for water and nutrients, fungal infection due to overcrowding (Sampathu et al. 1984) and the reduced size and reproduction capability of corms. In a 10-year experiment, Grilli Caiola (2005) observed that corms left in the soil without management techniques continue producing daughter corms for up to 3–5 years and afterwards they degenerate and are no longer able to reproduce vegetatively. Di Crecchio (1960) and Tammaro (1990) reported similar conclusions. Every year in perennial crop techniques, daughter corms creep upwards by about 2 cm from the mother corm, and when they reach the soil surface they must be lifted and replanted.

In Navelli, where annual cultivation represents a strategy to avoid parasite infection, the corms are lifted up annually at the beginning of the summer, selected for size (diameter greater than 2.5 cm), and checked for possible defects, such as rot, parasites, viruses, etc., before replanting (Tammaro 1999). This continual selection of the best plants, even in the absence of sexual reproduction, may lead to the conservation of the highest morphological and productive characteristics. In annual cultivation used in Navelli after ploughing, a ridging hoe is used to prepare the “raised beds” about 30 cm from soil level, where 3–4 rows of corms, 20–30 cm apart, are placed with the apex in the upward direction. Raised beds allow better drainage of the soil and less effort in management techniques. In the highly humid environment of Navelli, perennial cultivation has been very unsuccessful due to the severe attack of root rot (Degli Espinosa 1904).

6.2 Corm Planting (Methods, Rate and Time) and Harvesting

Before planting, the soil should be completely cleared of weeds, ploughed at a depth of 25–30 cm, and left to rest from a few weeks to the entire winter (Navelli). To avoid fungal infection, corms can be disinfected before planting by immersion for around 5 min in a benomil, captan or copper-based solution.

Sowing depth and spacing differ in annual and perennial crops. Sowing in perennial crops is deeper (10–20 cm) and wider spaced (10–15 cm between corms and 20–25 between rows) compared with annual crops (8–10 cm depth, 3–8 cm between corms and about 15 between rows), but shorter spacing is also used in intensive crop systems. Negbi et al. (1989) have shown that rooting and flowering are not affected by planting depth.

Sowing is commonly carried out by hand. Larger corm size has a well-documented positive effect on spice production in the same and following years (De Mastro and Ruta 1993; De Juan et al. 2003; Negbi et al. 1989; Gresta et al. 2008), namely, increasing corm size leads to a greater number and higher weight of replacement corms (Negbi et al. 1989; Lombardo et al. 2005). In unfavourable experimental site conditions in New Zealand, significant effects of corm size on flowering were detected only in the second crop season (McGimpsey et al. 1997). Generally, 2.5 cm is considered the smallest size below which corms need to be placed in a nursery for vegetative reproduction.

No univocal information is available for the most appropriate sowing time. In fact, like many other traditional crops, adopted agricultural techniques depend more on local practices passed on from generation to generation rather than scientific knowledge. Corms are sown in the second half of August in central Italy (Navelli), from the second half of June in Spain, between the middle of July and end of August in India, between May and September in Greece and between the end of August and the beginning of September in Morocco (Negbi 1999). Recently, Gresta et al. (2008) showed that an earlier sowing time (end of July) results in a higher and better quality of saffron stigmas compared with a later one (end of August). In any case, corm dimensions and sowing time may affect daily flower production but not its duration (Fig. 4).

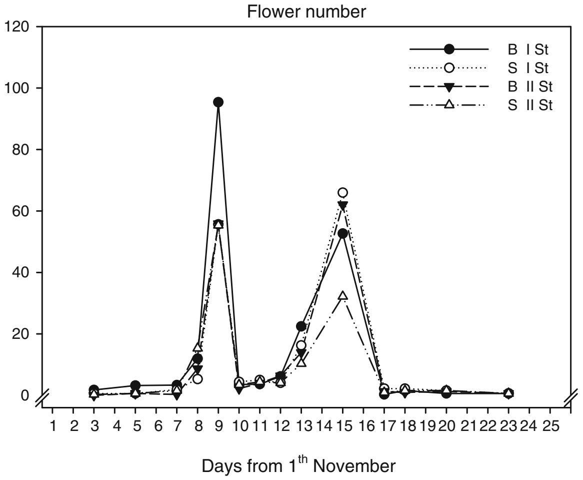


Fig. 4 Daily flower production N m^{-2} d^{-1} in relation to corm dimensions (Big: B and Small: S) and sowing time (I: early, II: late) (from Gresta et al. 2008)

After leaf withering, corms are lifted up, generally from May to July, but also in September in Morocco, and kept in jute or hemp bags until planting. The external tunic can be removed, leaving only the interior one. In spring, when corms pass to the natural summer rest, leaves can be cut and therefore this is a good moment for weed control.

6.3 Crop Rotation

Saffron cultivation needs to be included in a crop rotation. No definitive information is available on the effect of the previous crop on saffron yield and quality, but traditionally between 3 and 8 years should pass before it is cultivated on the same soil. Tammaro (1999) reports that a decrease in stigma production and an increase in weed number have been observed when saffron was cultivated on the same soil. In central Italy, saffron is profitably rotated with legumes and wheat (Tammaro 1999).

6.4 Fertilising

The application of about 20–30 tons per ha of organic manure is the most common fertilisation practice all over the world (Koocheki 2004; Tammaro 1990), while 40 units of N, 30 units of P_2O_5 and 40 Units of K_2O are applied in Greece (Goliaris 1999). Mature horse and cow manure of about 25–30 t ha^{-1}, without any chemical fertilisation, determined good results in Navelli (Tammaro 1999). On the contrary, incorporation of organic matter before planting did not improve saffron yields in New Zealand (McGimpsey et al. 1997). Behzad et al. (1992) found that 25 t ha^{-1} of cow manure significantly increased the dried stigma production in a soil with low organic content (0.3% in Organic Carbon), but had no effect in a soil with 1.0% O.C. They also observed that annual distribution of 50 kg ha^{-1} of nitrogen increased saffron yields, and that phosphorus and potassium seems unnecessary. Sadeghi (1980) reports promising results of applying chemical fertiliser, while, in a three-year experiment in two sites in Iran, Behnia et al. (1999) found contrasting results on nitrogen fertiliser, and no effect was shown by the application of phosphorus. Urea foliar fertilisation applied on saffron in winter (from January to March) resulted in a significant increase in flower number in a 2-year experiment carried out in Iran (Hosseini et al. 2004).

6.5 Irrigation

Irrigation is not a necessary practice. Water requirements of saffron are low and can be satisfied by the scarce rainfall when cultivated in semi-arid conditions. Even in the Mediterranean environment, saffron is not watered in many cultivated areas (Sardinia, Abruzzo, Greece, etc.) (Tammaro 1999; Skubris 1990). Some authors (Koocheki 2004; Mosaferi 2001) report up to 3,000 m^3 flood irrigation per year in Iran and up to 500 $m^3\,ha^{-1}$ in Morocco (Ait-Oubahou and El-Otmani 1999). Experiments carried out in Greece (Skrubis 1990) demonstrate that irrigation at the beginning of September resulted in an earlier onset of flowering, while irrigation at the end of September and during October determined an increase in production. Late irrigation could result in a worsening of the quality traits of saffron, especially if watered just before flowering. Certainly, the most crucial moment for irrigation is after summer to awaken the corms, but this coincides with autumn rains so, excepting a severe drought season, this may be considered unnecessary.

6.6 Weed Control

Weeds are probably the main problem for saffron, since it is unable to compete, above all because of its very low height. Nevertheless, due to the short time between the corm planting and flowering, severe weed problems start the following spring in perennial cultivation. From flowering, weeds can be left to grow until May and then cut without damaging the crop when the saffron leaves wither.

Weeds are managed by hand in annual crops in Italy, while in perennial crops a good chemical control is generally achieved with 10 kg ha^{-1} of Simazine (Gesatop 50%) or Atrazina (Gesaprim 50%) (Goliaris 1999). In Iran, broad leaves are controlled with pre-emergence and post-emergence treatments of Sencor (Metribuzin) and narrow leaves with Gallant (Haloxy fopetoxy-ethyl) treatments after flower harvest. Pre-emergence treatments with Sonalan (Ethyl fluralin) are also used (Mollafilabi 2004). During the summer rest, general herbicides such as Roundup (Glyphosate) or Buster (2,4-D, 2,4-DP) are applied.

Little research has been carried out on indirect weed control methods. Interesting results in reducing weeds with agronomic methods have been obtained with wood chips and sawdust mulch (Galigani and Garbati Pegna 1999; McGimpsey et al. 1997; Zanzucchi 1987). Experimental trials to control weeds with plastic films as dead mulch are on-going in southern Sicily (Sortino O., personal communication).

6.7 Harvesting and Separating

Gathering saffron flowers requires care and intensive manual labour: the flowers only grow a few centimetres above-ground and, depending on vegetative activity, might be surrounded by several leaves which must not be damaged otherwise daughter corms will not be produced. The flowers are harvested manually, generally by family members, by cutting the base of the flower stem with the fingernail. About 350–450 man hours are needed to harvest 1 kg of the spice, corresponding to between 200,000 and 400,000 stigmas, depending on the unitary weight. The saffron flower is highly ephemeral; given its very short life, it should be picked the same day of flowering and placed in baskets. The best practice is to pick the flower early in the morning each day, when the corolla is still closed, thereby preventing the stigmas from losing colour and quality, avoiding any sudden deterioration by wind or rain (Zanzucchi 1987; Tammaro 1990) and allowing a ready separation into their constituent parts. After harvest, stigmas must be separated from the tepals and stamen as soon as possible by opening the corolla and cutting the stigmas with the fingers below the branching where the style changes colour (from red to yellow).

6.8 Mechanisation

Tentative mechanisation procedures of some crop techniques in saffron have been carried out (Galigani 1982, 1987; Galigani and Garbati Pegna 1999), but it is a rather difficult crop. Lack of mechanisation in saffron is certainly due to the delicacy of corms and flowers, which require handling with care, but also to the considerable variation in size of corms. Other reasons are the cultivation of saffron in countries with very low manual labour costs and, on the contrary, the limited areas of land to which this crop is devoted in high labour-cost countries. Planting requires regular and correctly oriented placement of the corms. A modified onion planter has been used to plant saffron, but the impossibility of placing the corms with the apex in the upward direction led to a delay in emergence and a decrease in production. In fact, the corm reduces emergence when the apex is not pointing upward. A potato planter was also tested, enabling more control for corm orientation, but resulting in lower production compared with the onion planter. A normal hoeing machine can be used to mechanise weed control, by adapting row distance, especially in the first year. Lower efficacy was obtained the following year with the increase in weeds and the rising of the daughter corms. During the rest period of the crop, flaming was also used with good results against young weeds (Galigani 1987). If hysteranthy is manifested in saffron, flower harvesting can easily be mechanised with calibrated and adapted mowing or grass-cutting machines, without cutting the leaves. No suitable results have been obtained for flower separation, while adapted bulb- and tuber-picking (such as a potato digger) can replace human labour successfully for corn lifting.

6.9 Drying and Storage of Stigmas

Drying and storage methods are very important because a poor undertaking of this procedure can completely compromise qualitative features of saffron (Carmona et al. 2005). According to the ISO norm, the moisture content may range between 10 and 12% (ISO-3632 2003). Many methods are used for the dehydration of saffron. Concerning Italian production, the stigmas are normally spread over a large area and dried at room temperature in the sunlight or with forced air. In Navelli, dehydration is traditionally carried out by placing the stigmas on a sieve 20 cm above a charcoal fire (Tammaro 1999), while in Sardinia it is performed by drying stigmas in the sun or at room temperature (for several days) or in the oven at low temperature (35–40°C) in less time until moisture is reduced to 5–15%. Saffron is sun-dried in India and Iran, and toasted over hot ashes in Spain, while it is dried slowly at 30–35°C in dark rooms in Greece; therefore, many procedures are applied and, as is usual in such cases, there are still substantial disagreements over the best drying conditions (Raina et al. 1996; Carmona et al. 2005; Gregory et al. 2005).

Storage of saffron must be done in the dark and possibly in a modified atmosphere, since saffron pigments are light-, oxygen- and temperature-sensitive. The best way to store saffron spice is to keep it hermetically closed in darkened glass containers, and possibly at low temperature (5–10°C). (Mannino and Amelotti 1977; Alonso et al. 1990).

6.10 Flower Yield

Yield is quite a difficult parameter to forecast in saffron: saffron yield is in fact a function of many agronomic, biological and environmental factors able to exert a great influence on production. As far as is known, production is strictly influenced by dimension (De Mastro and Ruta 1993; Lombardo et al. 2005; Gresta et al. 2008) and storage conditions of corms (Molina et al. 2004b), climatic conditions (Tammaro 1990, 1999), sowing time (Gresta et al. 2008), cultural techniques (annual or perennial), crop management (irrigation, fertilisation and weed control) and disease. Moreover, saffron production increases from the first to the third–fourth years of cultivation (McGimpsey et al. 1997). Generally, one hectare of saffron may produce 10–15 kg of dried stigmas, but it can range widely, depending on the abovementioned factors, from 2 to 30 kg.

Yields of 2.5 kg ha^{-1} are reported in Kashmir, India and Morocco (Bali and Sagwal 1987; Sampathu et al. 1984) in rain-fed conditions, while it can reach 15 kg ha^{-1} in Spain under irrigation and fertilisation (Sampathu et al. 1984; Dhar et al. 1988). In irrigated Moroccan areas, yields of about 2.5–6 kg are obtained (Ait-Oubahou and El-Otmani 1999). In Iran, the average yield of saffron is around 5.4 kg ha^{-1} (Behzad et al. 1992). In New Zealand, McGimpsey et al. (1997) reached yields of 24 kg ha^{-1} of dried stigmas. A production of 29 kg ha^{-1} was recorded in Navelli (Tammaro 1999), but this is not comparable with the other yields because an annual cropping system is used and only the biggest corms are replanted every year.

6.11 Pests and Disease

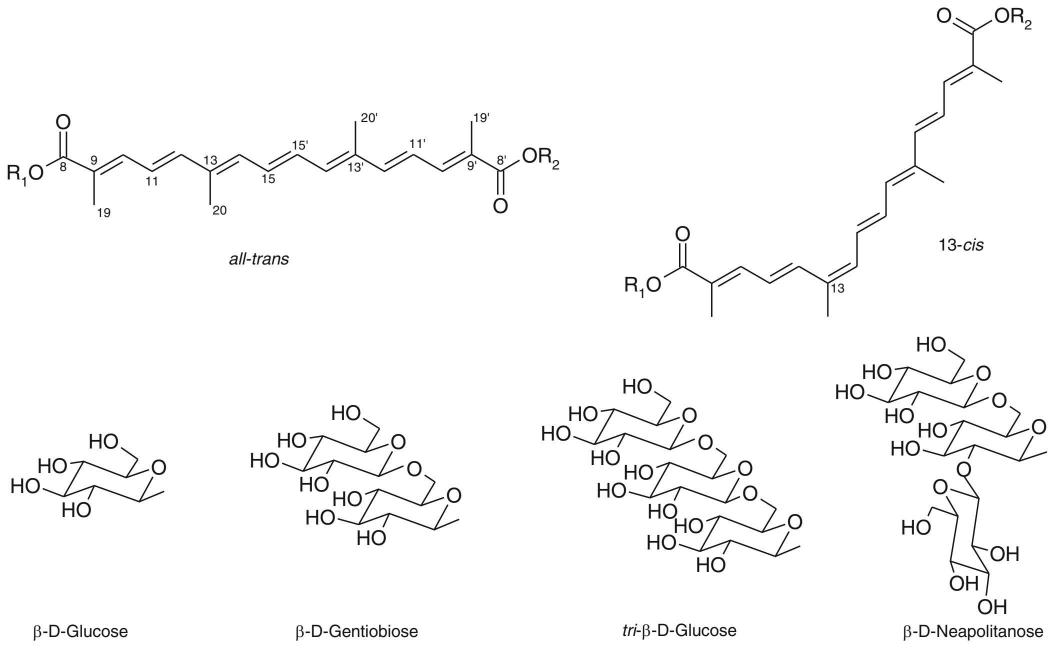
The worst enemies of saffron are rodents and fungi (Tammaro 1999; Goliaris 1999). Moles, rats and rabbits can easily damage corms or eat leaves. Fungal attacks are mostly promoted by humid conditions. High moisture percentage together with high temperatures create ideal conditions for the rapid development and spread of nematodes and fungi (*Fusarium*, *Penicillium*, *Rhizoctonia*, etc.) and consequently corm rot. These conditions generally occur in the hot and rainy spring. Tammaro (1999) indicates that temperatures above 10–12°C with rainy weather are a favourable climatic combination for the establishment of fungal disease on saffron. On the contrary, the hot and dry Mediterranean summer inhibits the spread of parasites. To avoid fungal infection, the best practices are crop rotation, the removal and burning of infected plants and corm treatments with anti-fungal products before planting, such as benomil or copper-based solution.

7 Qualitative Characteristics of Stigmas

7.1 Chemistry of Saffron: Secondary Metabolites

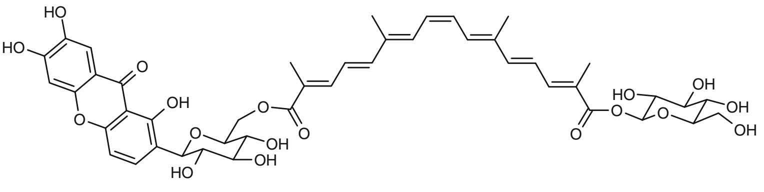
Apart from the ubiquitous primary metabolites such as carbohydrates, minerals, fats and vitamins (Sampathu et al. 1984), the *Crocus sativus* L. plant contains a large number of components belonging to different classes of secondary metabolites, namely, carotenoids, monoterpenoids, flavonoids and anthocyanins. Carotenoids are the most characteristic and important components of saffron stigmas, responsible for the particular colorant features of this spice. They include both fat-soluble carotenoids such as lycopene, α- and β-carotene and zeaxanthin, and, mainly, the water-soluble C_{20} apocarotenoid, crocetin (8,8'-diapo-8,8'-carotenedioic acid), and its ester derivatives, with one or more molecules of sugar, the *trans* crocetin (β-D-digentibiosyl) ester being the most important and abundant component of this class (Lozano et al. 1999; Carmona et al. 2006c; Rychener et al. 1984) (Fig. 5). Amongst the other minor components belonging to this class, β-crocetin and γ-crocetin (Fig. 5), the mono- and dimethyl ester of crocetin, respectively, and mangicrocin (Fig. 6), an unusual xanthone-carotenoid glycosidic coniugate, have also been identified (Ghosal et al. 1989; Fernandez 2004).

The other two typical features of saffron spice, namely the bitter taste and flavour, again derive from the carotenoid oxidation products: the bitter glucoside picrocrocin and safranal, respectively (Pfander and Schurteberger 1982) (Fig. 7). The first, picrocrocin, is a colourless glycoside, and is considered the main bitter principle of saffron, even though other components, such as flavonoids (*vide infra*), concur to give saffron's bitterness (Carmona and Alonzo 2004). Picrocrocin, according to the accepted biogenesis,



a) R_1=R_2= β-D-neapolitanoside (only *all-trans*)
b) R_1=R_2= β-D-gentiobioside
c) R_1=R_2= β-D-glucoside
d) R_1=R_2= CH_3 (γ-crocetin)
e) R_1=R_2= H (crocetin)
f) R_1= *tri*-β-D-glucoside, R_2= β-D-gentiobioside
g) R_1= β-D-neapolitanoside, R_2= β-D-gentiobioside
h) R_1= β-D-neapolitanoside, R_2= β-D-glucoside
i) R_1= β-D-gentiobioside, R_2= β-D-glucoside
l) R_1= β-D-gentiobioside, R_2=H
m) R_1= β-D-glucoside, R_2=H (only *all-trans*)
n) R_1= CH_3, R_2=H (β-crocetin)

Fig. 5 Crocetins and their glucoside ester (crocins) in saffron



Mangicrocin

Fig. 6 Mangicrocin, an unusual xanthone-carotenoid glycosidic conjugate

should derive, like the members of the crocin family, from the enzymatic degradation of zeaxanthin; in turn, the transformation of picrocrocin gives the volatile safranal (Sampathu et al. 1984; Pfander and Schurteberger 1982). The latter is the main volatile component of saffron, responsible for the particular aroma of this spice. Other typical volatile components of saffron are listed in Fig. 8; all possess the same skeleton of safranal, and like this latter, are considered to derive from picrocrocin, even though the recent discovery of several new glycosides suggests that picrocrocin is not the sole glycosidic aroma precursor in saffron (Straubinger et al. 1997, 1998; Carmona et al. 2006c). However, it is worth underlining that the saffron essential oil is very prone to absorbing oxygen and becoming thick and brown, and because of this high instability, this volatile oil is not commercially available (Sampathu et al. 1984).

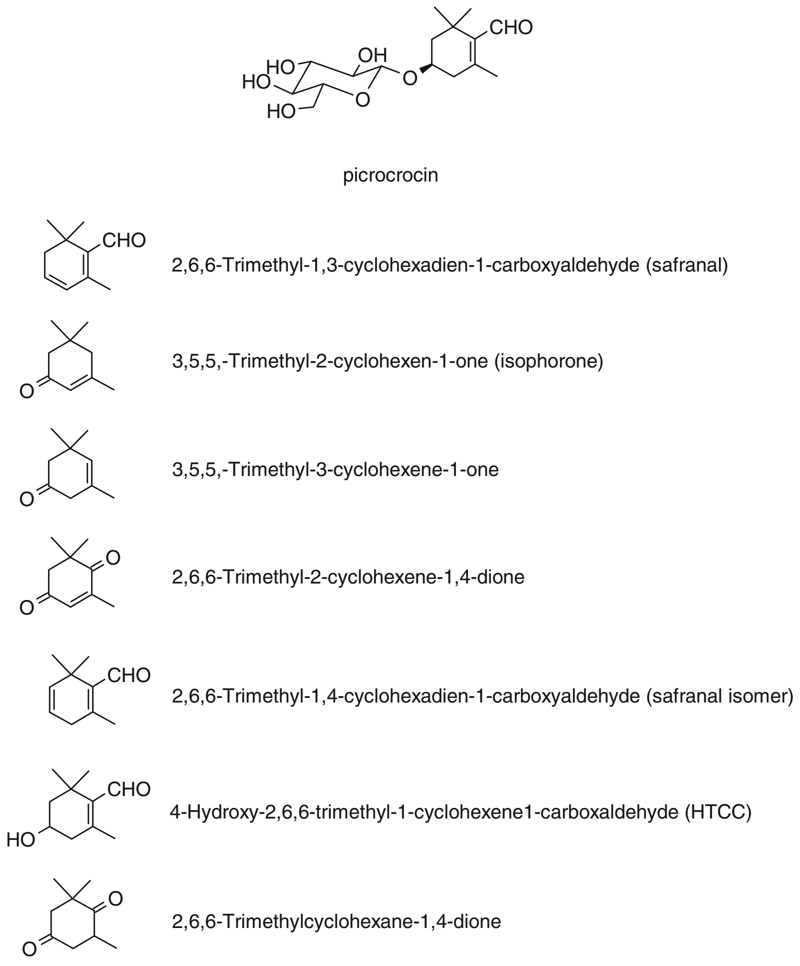


Fig. 7 Picrocrocin and the main constituents of saffron aroma

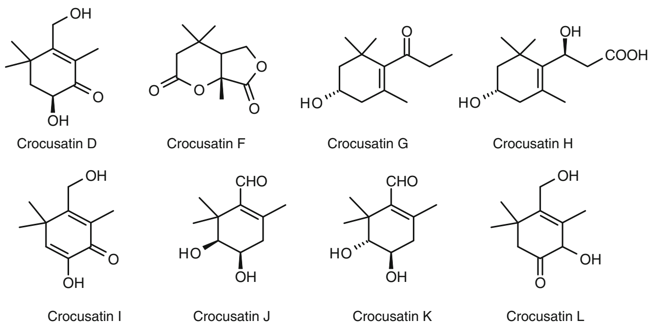
7.2 Minor Components of Saffron

Several minor components belonging to different classes of natural substances have been isolated from stigmas and other plant parts, mainly petals and corms. Terpenoids are the most frequently recovered components; the crocusatins (Fig. 8), present in stigmas and petals and showing a significant antityrosinase activity, represent a substantial group (Li and Wu 2002, 2004). To the same class of substances, namely, terpenoids, belong several glycosidic derivatives (Fig. 8) which, as previously mentioned, are considered precursors of volatile saffron components alternative to picrocrocin (Straubinger et al. 1997, 1998). Moreover, a series of flavonoids, all glycosidic derivatives of kaempferol, have recently been characterised in the stigmas of saffron; these polyphenols probably concur together with picrocrocin to produce the bitter taste of saffron (Carmona et al. 2007). The scenario of the secondary metabolites from *C. sativus* is completed by some anthraquinones (Gao et al. 1999) and an anthocyanin (Maroto 1950; Saito et al. 1960), isolated from corms and petals, respectively, and reported in Fig. 9.

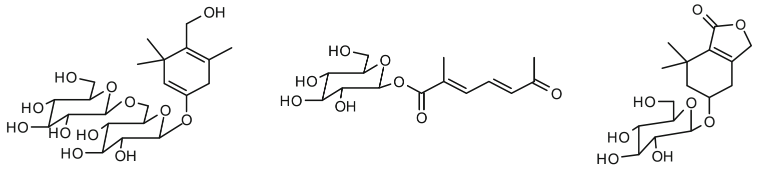
7.3 Biosynthesis: Argumentation on the Synthetic Pathways

In 1982, it was suggested that the biogenesis of the colour, bitter taste and aroma principles of saffron

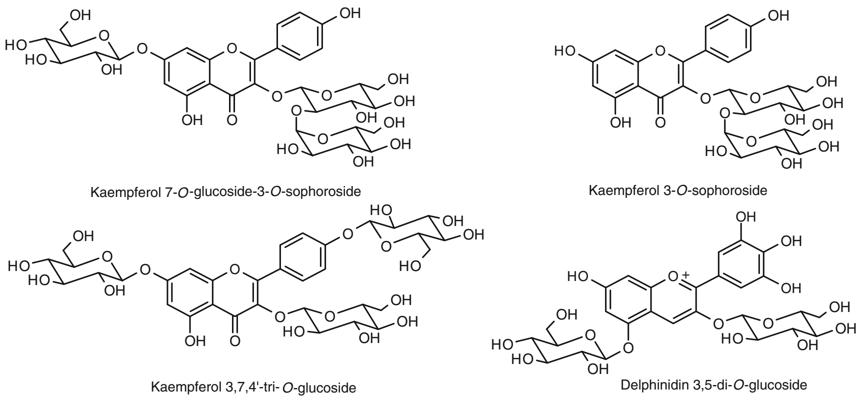
Selected monoterpenoidic compounds, crocusatins:



Selected glycosidic aroma precursors different from picrocrocin:



Selected polyphenols:



Selected antraquinones:

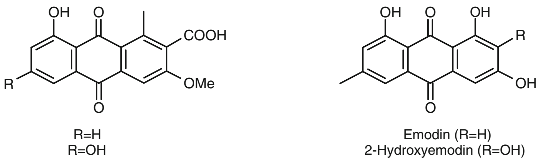


Fig. 8 Minor secondary metabolites in saffron

derives from the bio-oxidative cleavage of the C_{40} oxygenated carotenoid zeaxanthin (Pfander and Schurteberger 1982), that leads to the formations, on one hand, of the C_{20} apocarotenoid pigments, and on the other, of picrocrocin and safranal (Fig. 9).

Crocetin and its related compounds have so far been detected only in some Crocus species, *Crocus sativus* L. being the most important one, and in the fruit of *Gardenia jasminoides* Ellis. This limited occurrence in nature prompted hypothesising that their biosynthetic pathway could be promoted by a specific enzymatic oxidation mechanism not involving well-known and ubiquitous lipoxygenases (Wu et al. 1999) or xanthine oxidase systems (Bosser and Belin 1994). This enzyme, called CsZCD (zeaxanthin cleavage dioxygenase), in fact, specifically catalyses the synthesis

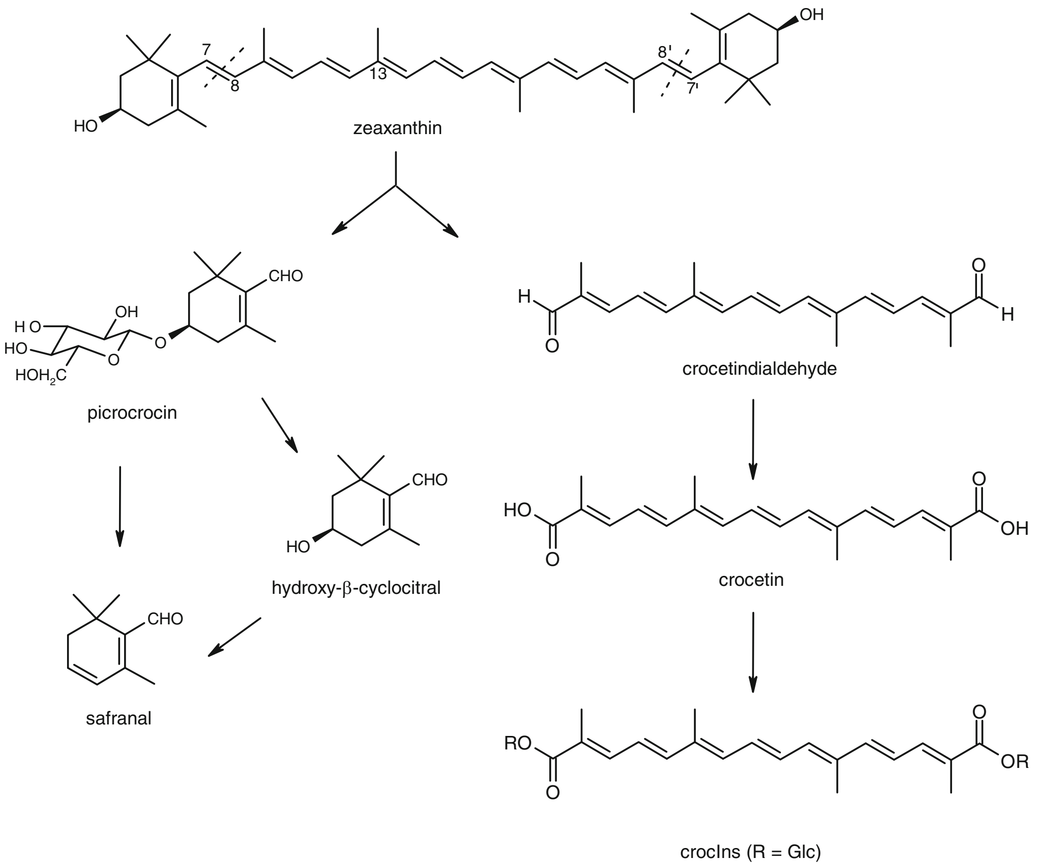


Fig. 9 Generally accepted hypothesis for the generation of the secondary metabolites in saffron from a common precursor (zeaxanthin)

of crocetin dialdehyde and hydroxy-β-cyclocitral (the latter further bio-modified into safranal) from zeaxanthin, and unlike other dioxygenases, CsZCD is expressed specifically in *Crocus* chromoplasts (Bouvier et al. 2003; Rubio Moraga et al. 2004; Castillo et al. 2005).

However, some controversies have recently been reported about the possible pathways proposed for crocetindialdehyde formation (see Fig. 9). An in-depth study of this topic, beyond the scope of this paper, has recently been tackled by Carmona and Alonzo (2004), who conclude that the biosynthetic pathway of apocarotenoids in saffron still remains unknown.

Moreover, very recently (Carmona et al. 2006b), starting from observations on some anomalies evidenced on the quantitative relationships between picrocrocin and safranal, have proposed an alternative pathway for the biosynthesis of saffron volatiles; according to their experimental results, safranal could be generated not only from picrocrocin but also from the crocetin chain by thermal degradation.

7.4 Evaluation of Quality: Aroma, Bitter Taste and Colouring Power According to ISO Norm

Saffron quality is chemically defined as the sum of the colouring power of its pigments, the bitterness of picrocrocin, and the intensity of aroma due to the composition of its volatile oil. Chemical requirements for saffron filaments or powder are given by the ISO norm No. 3632-2 (2003), and presented in Table 2.

This norm is based on a spectrophotometric test at three different reading wavelengths, namely, 440, 330 and 257 nm, corresponding to the maximum absorbance of crocetin esters, safranal and picrocrocin,

Table 2 ISO norm for the quality of saffron

Test method	Characteristic	Requirements	
		Filaments	Powder
ISO/TS 3632-2:2003, art. 7	*Moisture and volatile matter, % (m/m), max*	12	10
ISO 928:1997, art. 8 & ISO/TS 3632-2:2003, art. 12	*Total ash, % (m/m), max*	8	8
ISO 930:1997, art. 7 & ISO/TS 3632-2:2003, art. 13	*Acid-insoluble ash, % (m/m), on dry basis, max:*		
	Categories I and II	1	1
	Categories III and IV	1.5	1.5
ISO 941:1980, art. 7	*Solubility in cold water, % (m/m), on dry basis, max*	65	65
	Bitterness, expressed as direct reading of the absorbance of picrocrocine, on dry basis, min:		
ISO/TS 3632-2:2003, art. 14	Category I	70	70
	Category II	55	55
	Category III	40	40
ISO/TS 3632-2:2003, art. 14	*Safranal, expressed as direct reading of the absorbance at about 330 nm, on dry basis:*		
	Min	20	20
	Max	50	50
ISO/TS 3632-2:2003, art. 14	*Colouring strength, expressed as direct reading of the absorbance of crocine at about 440 nm, on dry basis, min:*		
	Category I	190	190
	Category II	150	150
	Category III	100	100
ISO/TS 3532-2:2003, art. 14	*Synthetic hydrosoluble acid dyes*	0	0

respectively. The absorbance can be directly related to the concentration of the molecules present in solution, provided the latter is exactly the same for all samples, as must also be the other experimental conditions (temperature, path length, solvent used, physical form, etc.); the values obtained are an estimation of the quality of the sample submitted to the analysis. Different categories have been established (I–III), and are reported in Table 2. Although the ISO norm for saffron quality is based on a test that is rapid, cheap and easily performed (only a spectrophotometer is needed for an accurate measurement), there are definitely several disadvantages: it is difficult to distinguish between authentic saffron and contaminants (the most recent adulterants have similar-shaped absorbance spectra to authentic natural pigments), and therefore to reliably assign a quality category on the international market (Lozano et al. 1999; Zalacain et al. 2005b; Zougagh et al. 2005ba).

7.5 Evaluation of Quality. Analytical Methods in the Analysis of Saffron: Chromatographic Methods, UV–Vis (Ultaviolet Visible) and Fluorescence Detection

Chromatography allows the separation of the pigments based on their different affinity to a stationary phase, packed on a column or present as a layer on an inert support, and a mobile phase.

In the last two decades, the most common chromatographic method used for the qualitative and even semi-preparative separation and analysis of saffron constituents was thin-layer chromatography (TLC), with silica gel used as the stationary phase and a mixture of highly polar solvents, namely, butanol, acetic acid and water as the mobile phase (Sampathu et al. 1984). More recently, reverse-phase high-performance liquid chromatography (RP-HPLC), coupled with a UV-Vis detector or, more often, a UV-Vis-DAD (Diode Array Detector) for non-volatile constituents (Caballero-Ortega et al. 2007), and Gas Chromatography (GC), with a mass spectrometer (MS) detector for the volatiles (Roedel and Petrzika 1991; Narasimhan et al. 1992; Tarantilis and Polissiou 1997) are the methods of choice, allowing the separation on an analytical level and the identification and quantification of the metabolites of interest. Due to the peculiar characteristics of the molecules belonging to the crocetin esters (high degree of conjugation and a certain rigidity of the terpenoid scaffold) fluorescence can also be used. The use of fluorimetry offers a better selectivity and sensitivity, though more precautions must be taken in sample handling (Trigoso and Stockert 1995; Vickackaite et al. 2004).

7.6 Other Methods of Analysis

The development of new analytical techniques has prompted researchers to explore new methods to evaluate saffron quality and chemical composition, mainly in order to characterise chemotaxonomic connections between, for instance, the composition and geographical origin of the sample and/or composition and storage conditions. For example, two volatile components, namely, 3,5,5-trimethyl-2-cyclohexenone (isophorone) and 2,6,6-trimethylcyclohexane-1,4-dione (Fig. 7), together with the flavonoidic fraction and in particular kaempferol 3-sophoroside (Fig. 8), have been demonstrated to be useful in determining saffron's geographical origin (Carmona et al. 2006a, 2007). Furthermore, with the advent of the holistic approach and metabolomics, there is the need for reliable, reproducible high-throughput devices to analyse large amounts of samples in order to build up libraries of spectra available for comparison.

Although RP-HPLC/UV-Vis-DAD (Reverse-Phase High-Perfomance Liquid Chromatography Ultraviolet Visible Diode Array Detector) is still the most widely used method, near-infrared spectroscopy (NIR) (Zalacain et al. 2005b), non-aqueous CE (Capillary Electrophoresis) (Zougagh et al. 2005b) and proton nuclear magnetic resonance (^{1}H-NMR) (Assimiadis et al. 1998; Tarantilis and Polissiou 2004) have recently been developed with some success so far. Therefore, the exploitation of even more sophisticated analytical techniques with highly standardised procedures appears essential to guarantee the quality of the spice, to determine its geographical origin and to counter adulterations in order to evaluate and certify the best productions.

7.7 Adulterations

Adulteration of saffron dates back to the Middle Ages in Europe, and given its high value, the penalty for those adulterating this spice could be death (*Safranshou Code*). One of the first systematic collections of these fraudulent practices, most of them still in use, has been documented by Maish (1885). Adulteration is normally carried out with vegetable or synthetic substances, as well as with inorganic and organic matter. The most common adulteration is with different parts of the flower itself: styles, stamen, strips of the corolla; other vegetable adulterants often commonly used are: safflower, calendula, poppy, arnica, onion skins, turmeric, annatto, capsicum and stigmas of maize (Maish 1885). Amongst the synthetic substances tartrazine, ponceau 2R, methyl orange, eosin and erythrosine are the synthetic dyes most frequently reported (Carmona and Alonzo 2004; Sampathu et al. 1984; Zalacain et al. 2005a). Saffron is also sometimes adulterated by the addition of oil, honey, glycerine, solutions of potassium or ammonium nitrate, and dry meat fibres (Sampathu et al. 1984).

7.8 Biological Properties: Use in Folk Medicine and in Modern Clinical Trials

The use of saffron goes back to ancient Mediterranean civilisations (Greece, Egypt and Rome), as well as many areas of the Middle and Far East. Its main and common use is to give colour, flavour and aroma to food, and to a lesser extent it is reported to be used as a dye and ingredient of perfumes. The use of saffron as a food additive is so widespread in the world that almost every national cuisine comprises a dish strongly characterised by saffron, namely, Spanish paella, Italian risotto, French bouillabaisse, Iranian plov, Azerbaijani paklova, etc. In central Sicily, for example, saffron is used to produce 'Piacentinu Ennese', an old and traditional cheese, whose taste, colour and flavour is strongly influenced by this spice (Horne et al. 2005).

As a medicinal plant saffron is still used in traditional medicine in several countries (Russia, India and Iran) (Mathew 1982; Tamarro 1990), and from a toxicological point of view it can be considered safe since its $LD_{50} = 20\,g\,kg^{-1}$ (Bisset 1994). It has traditionally been considered as an anodyne, antidepressant, respiratory decongestant, antispasmodic, aphrodisiac, diaphoretic, emmenagogue, expectorant and sedative. It was used in folk medicine as a remedy against scarlet fever, smallpox, colds, asthma, eye and heart disease, and cancer (Abdullaev 2002). Saffron can also be used topically to help clear up sores and to reduce the discomfort of teething infants (Abdullaev and Espinosa-Aguirre 2004).

Among the secondary metabolites present in saffron the ester derivatives of crocetin, together with safranal, are nowadays the most studied to evaluate their biological activity. Recent data show that saffron possesses tyrosinase inhibitory (Li and Wu 2002, 2004), anticonvulsant (Hosseinzadeh and Younesi Hani 2002), antinflammatory (Hosseinzadeh and Younesi Hani 2002) and mutagenic activities (Abdullaev and Espinosa-Aguirre 2004), and cytotoxic and antigenotoxic effects (Abdullaev et al. 2003), as well as antiamyloidogenic activity in Alzheimer's disease (Papandreou et al. 2006). Cancer chemopreventive and tumoricidal properties of saffron extracts are worth special mention, as scientific research in the last 5 years (2001–2006) has focused on this specific topic, with numerous encouraging results supported by in vitro and in vivo assays (Abdullaev 2002; Ahmad et al. 2005; Hosseinzadeh et al. 2005; Konoshima and Takasaki 2003; Magesh et al. 2006; Ochiai et al. 2004; Soeda et al. 2005).

8 Conclusions and Prospects of Saffron

From an agronomic point of view, saffron is a very unusual plant for its agrological and ecophysiological characteristics. It is unable to produce seeds and multiplies by means of a subterranean stem. It does not exist in the wild state and only very recent studies have been addressed to the individuation of ancestors so that we have not yet had the possibility of acquiring information from these for improving the crop. Saffron has a reverse biological cycle compared with the majority of cultivated and spontaneous plants: flowering first in October-November, then vegetative development until May, which means that the vegetative development is not directly important for production of stigmas, but for the production of new corms. The plant itself has an annual cycle, but the crop is perennial, precisely owing to its vegetative multiplication. Saffron has a low water use and a very low harvest index (Fernandez 2004). Above all, the parts harvested for production are the stigmas, from which a very expensive spice is obtained, probably a unique case in an agronomic context. Last but not least, saffron cultivation has been neglected for many decades by farmers, who have relegated it to adverse soil and climate conditions, and by research, which has led to a lack of innovation. All these reasons should induce revision of the most common agronomic knowledge for an effective revaluation of the crop. Synergy between the empirical knowledge of producers and scientific knowledge is able to generate new agronomic knowledge, especially in poorly-known crops, such as saffron, in which technical management represents a major hindrance to development (Girard and Navarrete 2005).

At the moment, saffron's future is rather uncertain: major productions come from the Middle East and South Asia with a low price, but often without quality control. This may mean that without changes in crop techniques and without the introduction of quality identification methods, many areas of saffron cultivation in Europe will decrease rapidly in the next few years.

A significant input to high quality saffron production could come from the wide sector of functional foods, nutraceuticals and dietary supplements. The growing interest of consumers and industry in the development of new products endowed with functional features, or health benefits, is testified by market expenditure analyses, which report that $20.5 billion were spent on dietary supplements during 2004 in the USA (Burdock et al., 2006). Saffron has many qualities to find a suitable place within this field. Consumer interest in saffron is growing, for example, especially in the USA and UK, though largely attracted by the low price and not by the quality.

According to Grilli Caiola (2004) and Fernandez (2004), increasing saffron production and quality can be achieved by means of plants with more flowers per plant, flowers with a higher number of stigmas, increased stigma size or stigmas with a greater amount of dye and aroma. The sterility of saffron limits the application of conventional breeding approaches for its further improvement. Vegetative multiplication, in fact, offers the major advantage of maintaining the plant's genetic traits, but does not allow any genetic improvement. In fact, corm multiplication does not induce genome variations. Traditional plant breeding techniques, based on a massive selection of the best samples among natural or cultivated populations, is restricted to searching for a quote of variability deriving from some rare mutations. Even though no genetic differences were detected with RAPD methodology, morphological difference was observed (Grilli Caiola 2004). At the moment, we cannot exclude the possibility that differences may still exist among saffron from different places; maybe a deeper genomic analytical determination will soon be able to reveal variability. Genetic improvement in saffron should mainly be addressed to breeding with wild ancestral species and to the selection of spontaneous or induced mutations. The characteristic infertility of *C. sativus* intraspecific pollination and its fertility with pollen from other *Crocus* species suggests a possible breeding programme of hybridisation with *C. thomasii,* a closely related, highly fertile and vital wild species common in southern Italy (Chichiriccò 1999). Integration of saffron with wild *Crocus* species can lead to fertility, resistance to pathogenic fungi and improvement in saffron spice quality (Negbi et al. 1989). Naturally, pollination of saffron with a different pollen donor species (interspecific pollination) will generate a seed with different genetic traits from the original triploid saffron. Moreover, in Navelli, the selection of corms carried out each year has produced a significant "genetic pool", which must be studied above all with respect to corm dimensions and disease resistance (traits for which the selection is made).

Multistigma flowers have been found by many authors (Estilai 1978; Dhar et al. 1988; Gresta et al. unpublished data), but unfortunately they are not stable and prove useless for cultivation. An artificial source of variability in order to obtain some improvements in saffron yield, even if no interesting results have been obtained, can be considered with colchicine (Zaffar et al. 2004) or physical irradiation (Khan 2004).

More promising results may derive from biotechnological tools, the improvement of agronomic management and individuation of areas in which saffron is able to achieve the highest results (Fernandez and Escribano 2000). Promoting cultivation in areas with dry Mediterranean summers, in which fungal infections are less widespread and where perennial cultivation is realisable, avoiding parasite attacks and weed overcrowding, may be a possible strategy.

The spread of saffron cultivation should also undergo mechanisation. Achieving a total mechanisation in saffron is almost impossible, but suitable tools and existing machinery already available for other crops can certainly reduce manual labour in saffron and accelerate some operations, thereby reducing production costs. Improvement in research on mechanisation of saffron crop techniques can lead to interesting results, but the more delicate operations are very far from being mechanised and should be done by hand. Hard labour requirements are partially recovered by the high price of the spice, with the drawback of a very small market. Pure mechanisation studies have obtained the first results on sowing and lifting of corms, but not on flower picking. Instead, what might make these studies more applicable would be the adaptation of the crop to mechanisation. In this context, a central role in any developmental programme could be played by the typical feature of hysteranthy of the saffron corm. Genetic (selection) and biological (storing conditions) studies devoted to hysteranthy able to prolong the leaf appearance at the complete end of flowering, could prove an important step towards mechanisation. Achievement of hysteranthy in saffron cultivation would mean the possibility of mechanising flower harvest, thus avoiding cutting leaves, notably reducing production costs and the price of the spice, giving saffron cultivation a major boost. Moreover, the use of appropriate crop techniques such as plant density, sowing time, use of living or dead mulch, etc., can lead to successful results, the spreading of cultivation and reduced price.

High quality saffron production from selected areas with appropriate and safe management techniques cannot compete in the world market with the saffron from low-cost manual labour-intensive countries, but must be addressed towards a potential niche market of high-level quality. To reach it, more attention should be focused on using modern techniques and the evaluation and promotion of saffron quality. The process must be accompanied by traceability, quality marking in order to attract more consumer interest, the adoption of organic agriculture management techniques (no pesticide and chemical fertilisation) and the reduction in manual labour. In saffron, the commercial products (stigmas) are not storage structures as in most cultivated plants, so an increase in nutrients in the soil is not directly linked to an increase in stigma weight. Certainly, a fertile soil is the basis for good saffron production, but organic manure represents the best support for saffron, especially under non-irrigated conditions, supplying nutrients, but above all, improving soil moisture and soil structure. In very nutrient-poor soil, limited chemical fertilising can be adopted.

The biological and agronomical traits of saffron (autumn flowering, overcoming adverse season by corms, very low fertiliser requirements and good adaptation to poor soil) make it an alternative plant for low-input agriculture, able to offer good production in sustainable agricultural systems. It may be considered a viable alternative crop for marginal lands, especially where low water availability severely limits the cultivation of many crops. Certainly, the improvement and, above all, the diffusion of knowledge on this species will encourage farmers in low-fertility areas to increase their income with saffron cultivation.

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Digital Imaging Information Technology Applied to Seed Germination Testing: A Review

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Abstract The application of digital imaging information technology to seed germination testing is discussed. This technology is reviewed in light of recent interest on the development and adoption of sustainable agrosystems joined with a modern strategy of "precision agriculture", which provides new complex information tools for better crop production. Basic concepts on the patterns of image analysis descriptors of imbibing seed performance are described with the objective of demonstrating the potential of this technique to be adequate for overcoming problems encountered with a standard seed germination test. The application of different image analysis system prototypes in monitoring seed germination of *Brassica*, as well as several other crop species, has provided encouraging results, highlighting the reliability of this technique to quickly acquire digital images and to extract numeric descriptors of germination and radicle growth events. Another aspect of digital imaging is the possibility to determine the colour space of a two-dimensional seed surface. Experiments carried out on lentil seed germination have shown that quantitative changes in Red–Green–Blue (RGB) colour component density may be considered as markers of the start of germination. In addition, the extracted RGB data may be used to trace a virtual three-dimensional surface plot allowing a better analysis of colour distribution on the lentil's surface. RGB colour density can also be used to determine any variation in colour due to the 'browning effect' as a result of advancing seed deterioration. The potential of RGB markers in classifying sub-samples and maintaining high germination quality in aged seed samples represents a non-destructive method in seed testing and sorting. As a conclusion, the information flow deriving from digital image processing should be integrated with other bio-morphological, taxonomic and 'omic-system' databases. The final target should be an interrelated and complex database for a deeper functional and structural knowledge of plant species, which can respond to the needs of farmers, seed industries, biodiversity conservation and seed basic research.

Keywords Computerised image analysis • Seed colour components • Seed shape and size descriptors • Seed testing and sorting

1 Introduction

Modern farming reflects a highly complex activity, where gains in crop yield depend directly on the continuous supply of energy and resources, such as intensive mechanisation and the development of agrochemicals to fertilise crops and control both weeds and pests, and crop selection versus monocultures that substitutes traditional varieties of agro- and eco-types. Therefore, when examining these problems it is impossible to separate the development of a more self-sustained agriculture by agroecosystems, which practise "ecological agriculture", from the modern strategy of "precision agriculture" which provides tools for reducing input costs, increasing yields and reducing environmental impacts in order to make decisions associated with

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crop production (Altieri et al. 1983; Cox 2002). In agroecology control, the remote-sensing techniques applied to classifying different cultivation area boundaries use digital imaging and the colour space value ratio (Peña-Barragán et al. 2008). Information technologies also include the development of digital image processing, focused mainly on cultivar identification (Keefe and Draper 1986) and crop grading (Anquar et al. 2001), weed detection in seed samples (Granitto et al. 2002) or in open fields (Pérez et al. 1997), and on seed quality testing and sorting (Dell'Aquila 2007). In this last case, the needs for development and improving seed quality tests are various, as reviewed by Hampton (1995). Farmers require information to expect rapidity and uniformity of seedling emergence, and seed industries need improved specific tests to guarantee the best levels of seed quality for production and trade purposes. Seed banks, devoted to ex situ conservation of biodiversity, require routine germination testing procedures before rejuvenating high quality accessions conserved in cold storage rooms, with the main target of avoiding plant genetic erosion and contributing to agroecosystem maintenance (Clergue et al. 2005). Furthermore, seed analysts and researchers are interested in developing new automated and non-destructive techniques to characterise seeds with well-defined genetic and physiological quality traits and improve knowledge in seed biology research.

Automated computer methods which utilise high-speed image capturing and data processing are the most advanced methods providing a high degree of accuracy in seed quality testing and sorting. Interest is increasing in the development of machine vision systems to replace human visual inspection, usually employed in germination tests under the rules of the ISTA (2005) and AOSA (2000). In analysing growth, the seed analyst studies rates of biological change with subjective methods. The germination test results essentially in a yes or no question, and the inspection of normal or abnormal seedlings at the end of the test depends on seed analysts' expertise. In addition, timing of the start of germination for individual seeds within a seed population is not accurately recorded because of the limited working time of the operator. To overcome these problems, many researchers have designed a number of machine vision system prototypes for the inspection of germinating seeds through the introduction of new image analysis parameters which can correlate biological changes more accurately and objectively. The understanding of growth rate patterns by image analysis parameters could represent a new method to investigate germination performance of many crop seed species in an automated way. As a result, new algorithms and hardware architectures have to be developed for high-speed extraction of raw data, such as seed digital images, and numeric data, such as dimensional measurements, shape factors and colour space density. Integration of image analysis data of seed germination performance with genomic and proteomic data could contribute to a better definition of the 'information technology' which accounts for acquisition, recording and elaboration, and communication of information.

This review reports features of image analysis system prototypes specifically designed for and widely applied in seed germination and vigour testing. The development of these technologies shows their potential in seed science study with the perspective of integrating traditional methods in seed quality assessment with those that provide more accurate and informative biomorphological data.

2 Image Processing in Seed Germination Testing

2.1 Seed Dimension and Shape Changes During Imbibition: Searching for a Pattern

Seeds have a three-dimensional (3D) shape, while captured images displayed on the monitor or on a printed page are in two-dimensional (2D) format (Loomis et al. 1999). In this case, the digital seed images can be assumed as a 2D object having both dimensions placed along the orthogonal axes of a Cartesian plane. One way to describe the growth of a biological structure is by the velocity at which its points move, with respect to a fixed system of coordinates (Coen et al. 2004). These ideal patterns can be represented by vectors, which form a mathematical vector field for an object in movement. In the case of the seed imbibition process (Bewley 1997), the first phase of rapid water uptake can be described geometrically as an enlargement of a disk which reflects radially an isogonic growth, where each region grows at the same rate in all directions (Fig. 1).

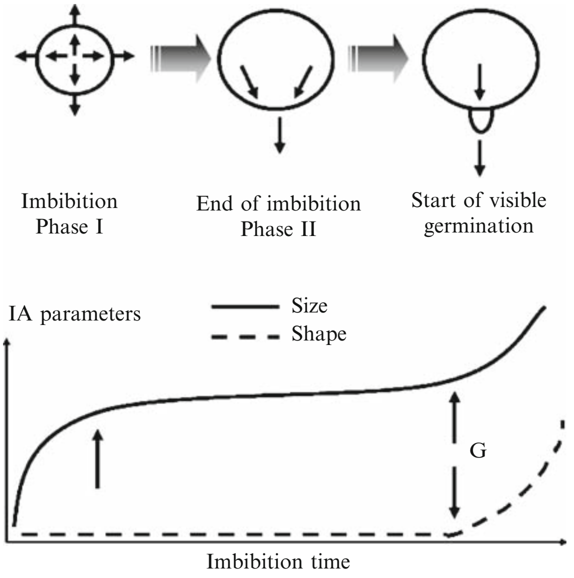

Fig. 1 Note the changes in velocity fields (*on the top*) for an imbibing object-seed during the imbibition Phase I, at the end of imbibition Phase II, and at the start of radicle emergence from the seed coat. The related plotting of size or shape image analysis parameters vs. imbibition time (*on the bottom*) shows curvature and inflection points (*arrows*) which describe the seed imbibition process up to a visible germination (G)

Consequently, velocity fields change this type of direction and depend on a single direction corresponding to the reference point. The second phase of seed imbibition represents the stage at which growth is preparing to change direction and finalises in the emergence of the radicle tip, that is the signal of the so-called 'visible germination'.

These geometric changes result in a dynamic model which is inclusive of physiological, biochemical and molecular processes (Prusinkiewicz 2004). Developmental models are commonly represented by growth pattern plotting, in which metric measurements of a 2D object size, e.g. area, perimeter, length and width, or shape numeric factors, e.g. roundness, calculated with the formula: perimeter$^2/4\pi$ Area, and aspect, calculated with the ratio between the longer axis and the shorter axis of the ellipse equivalent to the seed area, are plotted against time units to give a polynomial curve and equation (Silk 1984). In the case of a seed, the completion of the first and the second phases of imbibition is marked by two inflection points, the latter marking the change in dimensions of the seed-object due to radicle protrusion from the seed coat, and coinciding with the time of germination completion or start of visible germination, in physiological and agronomic terms, respectively (Bewley 1997). When the shape change descriptors are used, during the two early phases of water uptake no apparent change in shape occurs, while curvature starts when the radicle tip protrudes from the seed coat with the related change in the shape of the seed-object. In fact, assuming that a seed with a circular shape has a roundness factor of 1 at the start of imbibition, a different shape of the seed, due to changes occurring with radicle protrusion from the seed coat, should produce a roundness factor higher than 1 (Dell'Aquila 2004a). The inflection point of the related curve marks the start of visible germination.

2.2 The Case of Brassica Seed Germination: A Model for Image Analysis Application

The most studied seeds are those of the *Brassica* genera because their morphology and shape are suitable for image analysis measurements. Assuming that a seed of cauliflower (*Brassica oleracea* L.) or broccoli (*Brassica oleracea* L.) approximates a sphere and that linear expansion during the first phase of water uptake is similar along both Cartesian coordinates, measurements of size and shape changes can be closely correlated with the increase in seed fresh weight, as previously reported (McCormac and Keefe 1990; Dell'Aquila et al. 2000). Similar results were also obtained with seeds of winter wheat (*Triticum aestivum* L.) and oat (*Avena sativa* L.), with a shape differing from a sphere. Cereal seeds with 9.5–10.5% moisture content were subjected to a 'moistening' procedure to gradually reach 21–22% moisture content or to a 'drying' procedure to reach the original hydration state (Kruse 2000). Using a machine vision system, the results showed that seed length, width and thickness increased linearly with moisture content with approximately the same proportionality. These basic findings allowed the design of a computer-aided image analysis system (Fig. 2a), devoted essentially to monitoring seed image features during swelling and subsequent germination, operating in the laboratory of image analysis of the Institute of Plant Genetics

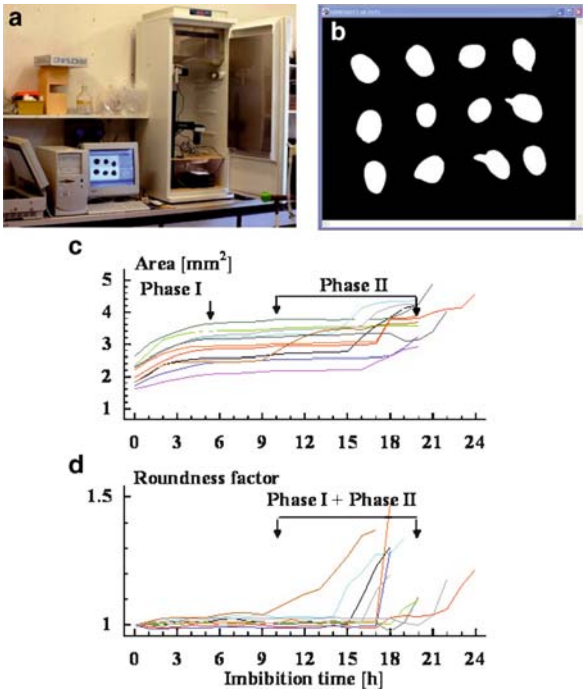

Fig. 2 The machine vision prototype (**a**) operating in the IGV-CNR (Bari, Italy) has been designed to capture digital images of *Brassica* seeds and to produce seed silhouettes (**b**) by image segmentation processing. Image analysis software can measure seed size and shape parameters, and data can be plotted to obtain, e.g., time courses of area (**c**) and roundness factor (**d**) increase. Arrows indicate, in (**c**), the end of Phase I (6 h) and that of Phase II of area increase coinciding with the start of germination (11–21 h), respectively, and in (**d**), the end of Phase I + Phase II coinciding with the start of germination (11–21 h) as detected by the increase in the roundness factor (adapted from Dell'Aquila 2004a)

of the National Research Council (IGV-CNR, Bari, Italy). A thermostatic chamber at 25°C was designed to include a colour Charged-Coupled Device (CCD) camera (Micropublisher 3.3 M pixels, Qimaging, Canada), a timer-dependent lighting system, and a holder for a Petri dish containing polymerised agarose where a sample of a maximum of twelve *Brassica* seeds could be placed. Alternatively, agarose was substituted with highly concentrated NaCl for salt stress imbibition trials (Dell'Aquila 2003), or different temperature regimes were used to evaluate their effects on germination performance (Dell'Aquila 2005). The computer unit was standardised using a CCD camera with a 55-mm telecentric lens or a flat-bed scanner (Sharp mod. JX-330, Japan) for image capturing, a commercial imaging board, and a high-power personal computer running MS® Windows XP. The most recent version of the commercial software package ImagePro-Plus™ (IPP; Media Cybernetics, USA), or alternatively the open source freeware software ImageJ (http://rsb.info.nih.gov/ij/) were used for digital image processing. Time-lapse seed images were captured every hour in an automated way and saved on the hard disk in the most common graphic formats with a full colour option. Image segmentation was carried out to streamline the process of object contour identification and to define the silhouette of the seeds (Fig. 2b). Image segmentation is the process of grouping pixels of a given image into homogeneous regions with respect to the contour of the object and the surrounding background, i.e. the inert support where seeds were placed. We used the multi-thresholding algorithm, which is able to differentiate the 2D colour pair histograms (i.e., RG, RB, GB segmentation maps), giving a comprehensive segmentation map (Kurugollu et al. 2001). A macro was created with Image-pro's macro language (IPBasic, Media Cybernetics, USA) for the conversion of pixels into millimetres, object number counting, image analysis parameter measuring and data transfer to a MS® Excel worksheet.

Image analysis systems have been applied by several researchers to automatically test germination percentage in a large population of seeds. (Van der Heijden et al. 1999) used a system controlled by a single computer program to study the germination time course in tomato (*Lycopersicon esculentum* L.), lettuce (*Lactuca sativa* L.), *Arabidopsis* and *Brassica* seeds under different temperature and water stress conditions. A more sophisticated image acquisition system was developed to capture images of different trays, containing plugs in which lettuce, cauliflower and tomato seeds were grown for subsequent transplanting (Ureña et al. 2001). Once the CCD camera was positioned over a given tray, a label placed on the tray surface containing the serial number in bar code form was read. Then, an image of the tray was obtained, allowing the examination of the cells to search for germinated seeds; the degree of seedling development was classified using fuzzy logic, and processed data on germination percentage and length of each seedling represented indices of speed of germination. More recently, Ducournau et al. (2004, 2005) elaborated new algorithms based on the idea that the emergence of a radicle tip at a defined time results in a modification of the binary images. The system was tested to study germination of sunflower (*Helianthus annuus* L.) seeds, and detailed germination curves were obtained, allowing a perfect fit in a probit model (Ellis and Roberts 1981).

2.3 Information Flow Generated by the Computer Imaging Process

The image analysis system designed at IGV-CNR can be applied to study the imbibition process in several seed species and to set up new markers of seed germination and radicle growth rate. In this context, the generated information flow can be summarised as follows:

(1) Time-lapse seed images in the most common graphic file formats [usually Joint Photographic Experts Group (JPEG)] or Tagged Image File (TIF)] can be easily stored in the hardware to implement bio-morphological databases of plant species. The image sequencing can be assembled in a digital animation file format [usually Audio Live Interleave (AVI)] which can simulate in a short time the germination behaviour of a seed species. Digital seed catalogues or image libraries, used to study botanical structures for educational training as well as to share images via the Internet, have been provided by the Ohio State University, USA (Loomis et al. 1999; McDonald et al. 2001; http://www.cse.ohio-state.edu/~fujimura/seed/). A database was also developed at IGV-CNR to store information including images of several crop seeds, their germination simulation and elaboration of image analysis features collected from a 2D imaging system. These data have been published on the following website: http://germimaging.ba.cnr.it for free download and educational purposes.

(2) A number of image analysis parameters for a single seed within a seed population may be extracted, including: metric size change descriptors (area, perimeter, length and width), shape change numeric factors (roundness and aspect), and surface colour space values (Red, Green, Blue colour primaries, and medium grey level density). Commercial or open source image analysis software packages can offer a number of algorithms to measure directly dimensions, shape and colour density of the desired object, and to combine these to calculate more complex parameters (Sundblad et al. 1998).

(3) Recent computer technology has developed powerful hardware and software suitable for elaborating in real time a large amount of data for statistical and graphical processing. As an example, data elaboration allows one to plot image analysis parameters (e.g., seed area and roundness factor) versus imbibition time, generating time-course patterns with curvature and inflection points for each individual seed (Fig. 2c, d). In highly viable cauliflower seeds a large variation in the time of the second phase of area increase contributed to different timing of the start of germination. When the imbibition process was monitored by the roundness factor, a first phase of no apparent shape change from the start of imbibition to radicle emergence was followed by a second phase of rapid increase with distinct curves. Image analysis was also applied to seed samples with different viability. The sensitivity of the technique in discerning the occurrence of the third phase of seed area increase or the second phase of roundness factor increase in deteriorated seeds, as well as in seeds subject to salt or temperature stress, provided a further test of the technique's flexibility (Dell'Aquila et al. 2000; Dell'Aquila 2003, 2005). Seed area and roundness factor have been tested as effective markers of seed swelling and start of germination, with the advantage of being measured on each individual seed within a seed population (Dell'Aquila 2004b). When immaturity, storage conditions or pathogen contamination affect the quality of a seed lot, individual seeds are not damaged to the same extent, resulting in the occurrence of an ample range of quality, possibly with distinct sub-populations. Computerised image analysis is a promising technique to investigate and detect any seed-to-seed variation within an apparently homogeneous population.

(4) The rate of rapid area or roundness factor increase in the last phase of imbibition, mostly due to protrusion of the radicle tip and its growth, may be correlated with the corresponding metric measurement of radicle length of a single germinated seed, when 'visible germination' can be assessed in a germination test. Highly significant correlation coefficients have been obtained in *Brassica*, radish (*Raphanus sativus* L.), lentil

(*Lens culinaris* Medik.), lettuce, pepper (*Capsicum annum* L.), tomato and carrot (*Daucus carota* subsp. *sativa* L.) seeds (Dell'Aquila 2004a, b). In this way, the radicle elongation rate may be assessed indirectly by the corresponding seed area or roundness factor increase rates, confirming the usefulness of image analysis parameters in seed vigour assessment.

A promising field of application of image analysis is seed vigour testing, using a scanner image capturing technique. Sako et al. (2001) obtained digital images of lettuce seedlings, and the extracted image measurements were used to generate a vigour index based on morphological features. A similar method was also developed to assess vigour of different lots of small-sized horticultural seeds (Geneve and Kester 2001; Oakley et al. 2004). Moreover, in cotton (*Gossypium hirsutum* L.), the problem of overlapping seedlings that can interfere with the measurements of elongation rate has been overcome with the design of a new algorithm which measures each seedling independently (Xu et al. 2007). Readers can find more information on the application of computer image analysis in seed vigour testing in the recent review of Dell'Aquila (2007).

3 Red–Green–Blue Colour Space Evaluation in Seed Digital Images

3.1 The Case of Lentil Seed: From Two-Dimensional Digital Imaging to Three-Dimensional Surface Simulation

Physiological studies have demonstrated that there are only three colour sensors utilised by human visual recognition, which are associated with long (Red, R), medium (Green, G) and short (Blue, B) wavelengths of light (Fairchild 1998). All visible colours, as displayed on most computer monitors, can be represented by varying combinations of these primaries. Even if colour mapping is defined by a numeric range of RGB values (from 255-255-255 for the so-called 'white' colour to 0-0-0 for the so-called 'black' colour), many colour models are used to represent colour space with other primaries, such as hue, saturation, intensity value (HSV), or hue, saturation and lightness (HSL), or cyan, magenta and yellow (CMY). To study colour features of seed digital images we used the RGB colour order system by extraction of any colour information whose appearance is generally altered by surface texture, lighting, shading effects and viewing conditions. By automated image analysis all these factors must be considered in the characterisation of a coloured image of a seed through a series of colour histograms. One of these may be graphically displayed on a Cartesian plane by extracting the number of pixels of each primary colour along a virtual line connecting the seed grain and emerging radicle tip. In the visualisation scheme of Fig. 3 the implemented algorithms follow a similar progression: (1), selection of RGB colour space; (2), quantification of RGB pixel values; and (3), computation of histograms by plotting RGB data

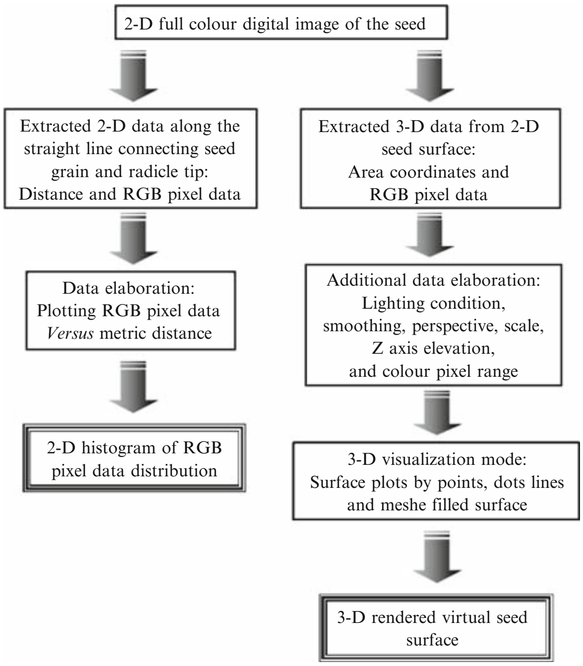

Fig. 3 Digital seed image processing scheme by Red–Green–Blue (RGB) value computation. On the left, the image processing flow shows that extracted two-dimensional (2D) data from the digital image of a seed surface can be computed to generate RGB histograms by plotting colour components (Y-axis) vs. distance (X-axis). On the right, the data elaboration flow is reported, which takes into account an unlimited number of RGB histograms distributed on a plane (X- and Y-axes) with combinations of RGB primary pixel values (Z-axis). The extracted 2D data from the seed surface can be further processed using optional tools useful for enhancing the rendering performance of a three-dimensional (3D) seed surface simulation

(Y-axis) versus distance (X-axis) reported in metric units. Otherwise, we can consider an unlimited number of colour histograms placed on a plane (X-and Y-axes) with combinations of RGB primary pixel values (Z-axis). The extracted data of coordinates and primary colour density can be integrated with optional data, useful for enhancing the rendering performance of a 3D seed surface simulation. In addition, the choice of visualisation mode by surface dots, lines, meshes and filled surface may help a better plotting of the 3D surface of the studied seed.

As an example, RGB data, extracted along the virtual straight line connecting the radicle tip and the corresponding free border of a lentil seed coat, may be used to plot the related histogram (Fig. 4). Before radicle emergence (14 h of imbibition, Fig. 4a, b) RGB density distribution is representative of the seed coat colour space which is limited by 0-0-0 pixel values corresponding to the black background holder where seeds are placed. At 15 h of imbibition (Fig. 4c, d), the start of radicle protrusion from the seed coat can be evidenced by a dropping point between the RGB

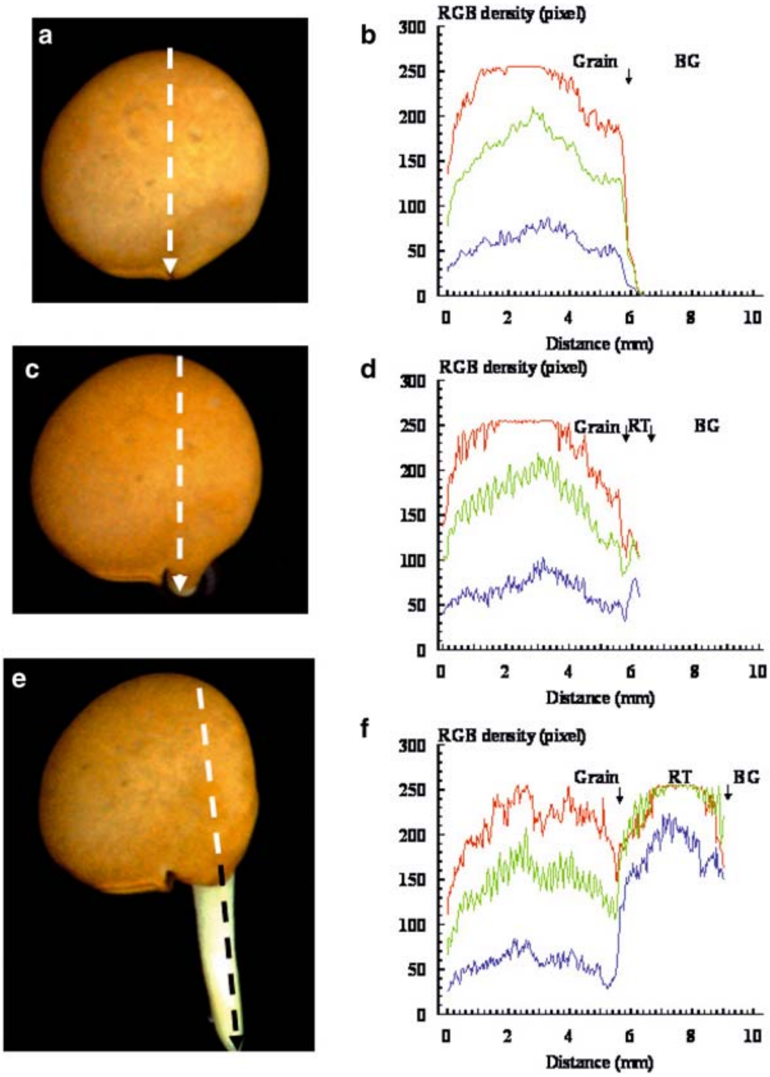

Fig. 4 Seed images, captured by a charged-coupled device – camera at 14 (**a**), 15 (**c**) and 21 h (**e**), can be a source of two-dimensional imaging data along the straight *dashed line* connecting the seed grain and radicle tip (RT). Related Red–Green–Blue (RGB) density values are plotted as histograms (**b**, **d** and **f**, respectively) in a Cartesian plane having the metric distance as X-axis and RGB density as Y-axis (adapted from Dell'Aquila 2007). *BG* background

distribution of the seed coat and that corresponding to the emerging radicle tip. Timing of the occurrence of this inflection point marks the start of ‘visible germination’. At 21 h of imbibition (Fig. 4e, f), when radicle length reaches 2.5–3 mm, the RGB component histogram clearly shows two sequentially different distributions of RGB colour primaries due to the seed grain and developed radicle with a brown or white–yellow colour, respectively. The 3D rendering option lets us obtain a 3D surface plot of a lentil seed at 14, 15 and 21 h in mesh mode using the plug-in ‘Interactive 3D surface plot’ of the image analysis software ImageJ (Fig. 5). By changing the modality of smoothing and Z-axis elevation modality, surface features can be enhanced and certain quality factors such as seed coat rugosity, disease and defects can be determined quantitatively using the facilities of image analysis processing.

More recently, a new technology has been developed known as dynamic speckle, or biospeckle, an optical phenomenon produced when living materials, such as biological tissues, are illuminated by laser light. The term speckle refers to a random granular pattern which can be observed when a highly coherent light beam is diffusely reflected on a surface with a complicated structure. Features of seed tissue images, acquired by a CCD camera, can be amplified and assessed by their speckle activity by appropriate algorithms (Braga et al. 2003). The biospeckle technique has proved to be a potential non-destructive methodology to assess bean (*Phaseolus vulgaris* L.) seed viability, even if water content in the seeds can affect measurements. The technique has also been applied to detect the presence of fungi colonies on bean seed coats (Braga et al. 2005).

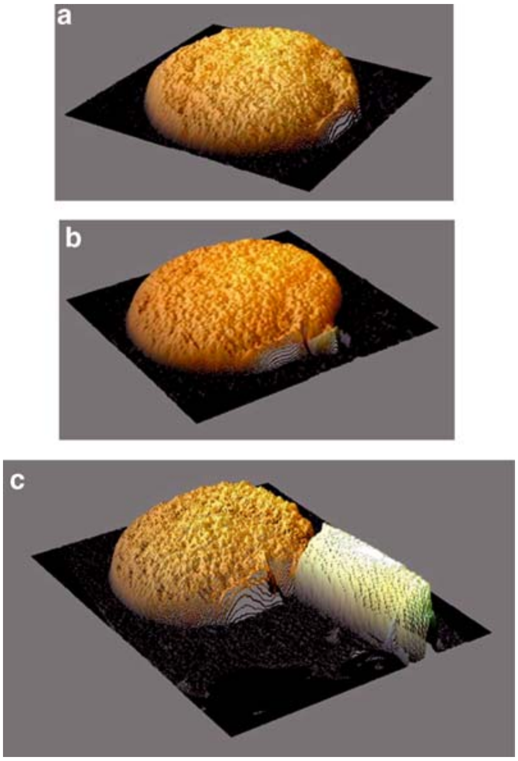

Fig. 5 Three-dimensional (3D) rendering option is used to obtain a virtual 3D surface plot of lentil seeds at 14 (**a**), 15 (**b**) and 21 h (**c**). The elaboration is made in mesh mode by the interactive 3D surface plot plug-in of the ImageJ software package

3.2 Red–Green–Blue Data as Markers of Seed Viability

Object surface colour space is a physical feature which can be used for effective quantitative recognition during image analysis processing. The experimental approach extracts automatically the colour content of a restricted or entire space of the seed surface over a large collection and sequence of images. The Red, Green and Blue colour components alone can be quantified as medium density for a normalised seed area, and so data can be easily compared and used for cluster or discriminating analysis. We analysed RGB colour components on lentil seed samples stored under deteriorating conditions to investigate the potential of this image analysis parameter to be a valid seed viability marker (Dell’Aquila 2006). Images of differently deteriorated lentil seed samples were captured by a flat-bed scanner with full colour option after a calibration with Kodak Q-60 target to ensure an accurate transposition of dye densities to RGB pixel values. The density values of Red, Green and Blue colour primaries, or alternatively a medium RGB value corresponding to the density colour of a grey image, were determined using the software package IPP v. 6.01. RGB density distribution for each seed sample was graphically displayed by defining equal classes of pixel unity on the X-axis and by plotting the number of seeds in each class on the Y-axis. Three fractions can be extracted, whose borders were chosen so that two fractions (with high and medium colour density) contained seeds with high viability and the third fraction, with low colour density, the rest of seeds, according to the method used in sorting cabbage seeds with a chlorophyll fluorescence marker (Dell'Aquila et al. 2002).

A sequence of seed images may be acquired during different periods of ageing under different environmental storage conditions. Colour differences between deteriorated seeds can be due to accumulation of Amadori and Maillard products, obtained by reduction of sugars or protein aminogroups to form fructosyl derivates or glycate proteins, whose interaction produces polymeric brown products (Wettlauer and Leopold 1991; Sun and Leopold 1995). The effect has been described in legumes, where colour change can be quite heterogeneous within a seed sample and seeds which maintain their original colour at full maturity tend to preserve high vigour (Priestley 1986). As a result, the visible physical change is the discoloration or browning of the seed coat, as shown for lentil seeds stored over 51 days at 14.4% moisture content and 40°C (Fig. 6a, b). Based on the symmetric distribution of density values for each colour component in lentil seed samples aged for both 0 and 51 days, the three seed fractions can be defined with different RGB value borders (Fig. 6c). Alternatively, it is possible to use an overall medium RGB index (255 grey levels) in both seed samples with different value borders (Dell'Aquila 2006). At 0 d deterioration Fraction I contained few seeds (1.33%) with 78% final germination (G), while at 51 d deterioration a large amount (44.16%) of seeds was found with the lowest germination percentage (25% G). Conversely, in Fraction III at 0 d deterioration more seeds (61%) had 98% G compared with 51 d deteriorated ones (10.6% in number and 75% G). The largest amount of seeds was found in Fraction II from both 0 and 51 d deteriorated seed samples (76.5 and 54.16%, respectively, with 39 and 55% G, respectively). The RGB marker was also used in sorting deteriorated seeds of cucumber (*Cucumis sativus* L.), lettuce and tomato (Dell'Aquila 2007). These findings confirmed that a RGB marker may identify seed sub-groups with different germination quality and variable seed distribution using a non-destructive technique. Collected deteriorated seed images from different species together with RGB threshold values can be stored in an electronic archive and constitute a database of deterioration patterns useful for elaborating a strategy of highly viable seed sorting and survival prediction.

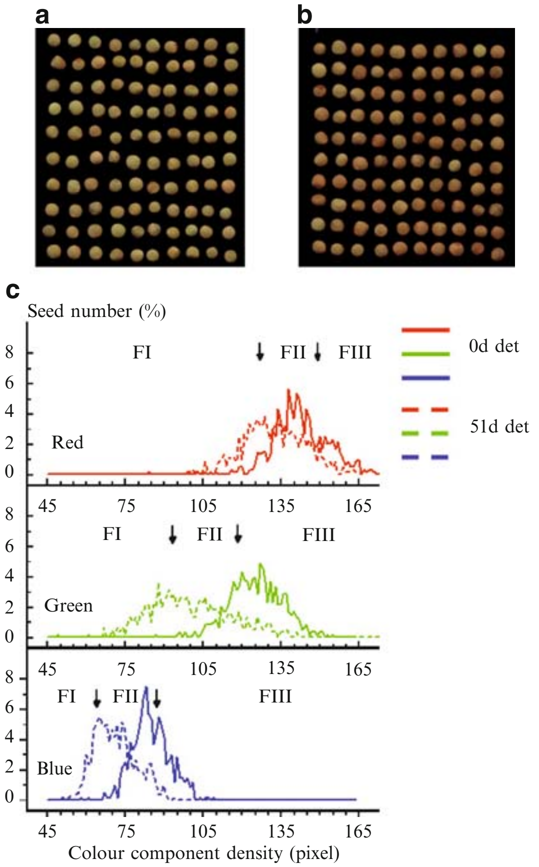

Fig. 6 The 'browning effect' due to deterioration (det) is shown in the captured digital images of lentil seeds stored under 14.4% mc and 40°C conditions for 0 (**a**) and 51 d (**b**; adapted from Dell'Aquila 2006). The extracted Red–Green–Blue (RGB) colour space data from two-dimensional imaging are used to plot seed number percentage vs. the changes in colour primary component density at 0 and 51 d of deterioration (**c**). The *arrows* indicate the selected border of each fraction (FI, FII and FIII, respectively) for each colour component, having different viability patterns during advancing ageing

4 Perspective of Imaging Information Technology as a Tool in Seed Science and Technology

In the twenty-first century, plant researchers have a range of opportunities provided by technological and knowledge advances. Many of these tools can be considered under a unique subject, 'information technology', which by definition is concerned with the management of biological system databases (Cox 2002). More recently, inspection of plants utilises modern image acquisition aided by computer technology, providing a highly adaptable tool to produce digital images suitable for subsequent processing. Automated image analysis systems applied to the study of seed quality provide a semi/non-destructive evaluation tool of seed performance leading to germination, or in the dry state an estimate of the morphological features useful for seed testing and sorting. As a result, time-course patterns can give information on hydration status or colour space quality. In addition, medium density of RGB colour primaries can be used as a non-destructive marker in classifying seeds with different viability levels within a seed population. These findings are in agreement with the recent success of optical methods which incorporate high-speed optical sensing and data processing techniques to facilitate quality evaluation and sorting of many agricultural products with a high degree of accuracy (Chen and Sun 1991). The success of this new technology has also been supported by the declining costs of computer hardware and the opportunities to acquire image analysis and graphical software packages with new algorithms designed to implement the capability of data processing and plotting.

Both digital imaging and numeric data acquisition and elaboration, as well as their utilisation, should be aimed at integration with more complex databases. The final target of information technology is that any database is transferred in the right way to implement other databases, e.g., those generated by so-called ‘omic systems’, for an up-to-date knowledge of plant functional and structural changes and modelling. As an example, we have calculated that in the case of a sample of twelve broccoli seeds’ imbibition at 25°C for 24 h, the extracted data could be quantified in 288 single seed images and 2.592 image analysis numeric data (Dell’Aquila 2007). This amount of data, the minimum needed to describe broccoli seed imbibition and germination, may be further processed statistically and displayed graphically. The creation of an extensive database could allow matching of different bio-morphological characteristics of a large number of seed species to improve genetic purity analysis, taxonomy screening, germination and vigour prediction. Keys (1982) first developed a computerised automated seed analysis system in order to enable analysts to estimate physiological parameters of seeds and compare the values obtained with the standards for seeds of known quality. Many years later, an image-based database was designed at the National Seed Storage laboratory of USDA-ARS (Fort Collins, USA), with the aim of concentrating data of seed physical characteristics and, so, to enhance conservation and utilisation of seed germplasm (Howarth and Stanwood 1993). A ‘Seed Identification Key’ using a computerised database, also available commercially in a CD format, has been developed to identify prohibited and restricted seeds and to reduce the impact of noxious weeds on the environment and agricultural production (Gupta et al. 2005). Daoust et al. (2005) described a machine learning technique that employed the use of an inexpensive commercial scanner and a modern PC. The image analysis software used allowed the definition of 21 seed species with the digitised images and the measurement of size parameters and average colour on each seed. The classification determined the closest matching species for each seed using a global database highly configurable to specific seed types.

5 Conclusion

An information system consists of two components: a computer (images, data, information processing engine) and human-computer interaction (Lew et al. 2007). In the case of seed inspection by a vision machine system, the overall goal is to extract from a two-dimensional digital image a considerable amount of data in order to describe germination and radicle growth, and the colour space density of the seed surface. The sophistication of non-destructive methods has evolved rapidly, and the availability of high-speed data acquisition and processing technology has encountered a renewed interest in seed researchers. The up-to-date designed machine vision systems are prototypes which need to be highly automated with the implementation of new algorithms, so that valid and flexible image analysis parameters can integrate or substitute the visual inspection of a large seed sample for germination and vigour testing. Efforts should be made in the future to utilise this kind of information technology, which can be included in the global key to precision agriculture and sustainability, in transferring standardised data to a seed analyst for decision-making or recording purposes.

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Managing Weeds with a Dualistic Approach of Prevention and Control: A Review

Randy L. Anderson

Abstract Scientists have theorized that weed management would be more efficient if prevention tactics were integrated with control tactics. The goals of prevention are to reduce weed community density and improve crop tolerance to weeds. Here we describe the impact of this approach in the semiarid steppe of the United States. As a result, producers have reduced herbicide inputs and costs by 50% compared to conventional practices. Critical factors for success with this approach are rotation design and no-till practices. Rotations comprised of two cool-season crops followed by two warm-season crops are the most disruptive of weed population growth. The impact of rotation design on weed community density is enhanced by no-till. Crop tolerance to weeds is improved by systems of cultural tactics. The tolerance is greatest when three tactics are combined together. This dualistic approach of prevention and control effectively controls weeds with four-crop rotations such that herbicides are not needed in some crops of the rotation. Weed density is so low that crop yield is not affected by weed interference. With this approach, herbicides are a choice rather than a requirement for cropping success in the semiarid steppe of the United States.

Keywords Crop diversity • No-till • Rotation design • United States

1 Introduction

Producers in the United States are fortunate to have a vast arsenal of herbicides to control weeds. Because herbicides were initially so effective, producers and scientists perceived herbicides as the "*silver bullet*," controlling weeds with one management tactic. Herbicide-based control, however, has failed to achieve long-term weed management (Mortensen et al. 2000; Weber and Gut 2005). Even with herbicides, weeds remain prominent in croplands and producers still lose considerable crop yield due to weeds (Bridges 1994). Furthermore, herbicide resistance is forcing producers to use more expensive management tactics, thereby increasing production costs. Thus, scientists and producers in the United States are seeking a broader perspective to weed management than relying primarily on herbicides (Lewis et al. 1997). One possible approach is expanding management tactics to include a prevention component (Pedigo 1995; Ferron and Deguine 2005). Prevention tactics are planned to disrupt weed population growth, with one effective tactic being rotations comprised of crops with different life cycles (Streibig 1979).

No-till practices have begun about 30 years ago in Brazil primarily as a means of reducing soil erosion (Bernoux et al. 2006). At present, about 63 million ha are under no-till systems worldwide, with the USA having the largest area of about 21 million ha, following by Brazil with about 20 million ha (Bernoux et al. 2006). In the semiarid steppe of the United States, crop rotations are changing because of no-till practices. Previously, producers followed a winter wheat (*Triticum aestivum* L.)-fallow rotation. During fallow,

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neither crops nor weeds are allowed to grow. Therefore, precipitation during the fallow interval is stored in soil for future crop use. Soil water gained during fallow reduces yield variability and crop loss due to drought stress. However, preserving crop residues on the soil surface with no-till has increased precipitation storage in soil such that more crops can be grown before fallow is needed again (Farahani et al. 1998). Producers now grow warm-season crops such as corn (*Zea mays* L.), proso millet (*Panicum miliaceum* L.), sorghum [*Sorghum bicolor* (L.) Moench] and sunflower (*Helianthus annuus* L.) along with cool-season crops such as winter wheat and dry pea (*Pisum sativum* L.) (Anderson et al. 1999).

This diversity in crops with different life cycles provided an opportunity for producers to develop weed management systems that integrate prevention with control tactics (Fig. 1). For prevention, cultural tactics are used to reduce weed community density and improve crop tolerance to weed interference. Another suite of tactics controls weeds in crops. This approach enables producers to effectively control weeds with 50% less cost compared with producers using less diverse rotations or the conventional system of winter wheat-fallow (Anderson 2005a).

With this paper, we explain the cultural tactics and ecological reasoning that led to this dualistic approach with weed management in the semiarid steppe of the United States. Our example may provide insight and ideas for producers and scientists elsewhere to develop similar programs. Even though crop choices and cultural tactics can vary among regions, integrating prevention tactics with control tactics may lead to successful weed management less dependent on herbicides.

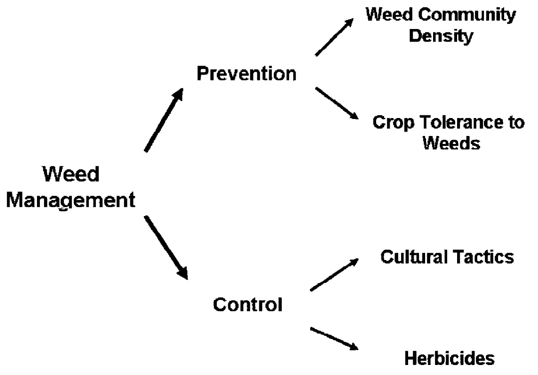

Fig. 1 A conceptual framework for developing a broader perspective with weed management that integrates prevention with control tactics (Adapted from concepts described in Pedigo (1995))

1.1 *Weed Community in the Semiarid Steppe*

In the United States steppe, annual weeds are the prevalent species infesting grain crops. The weed community includes cool-season weeds such as downy brome (*Bromus tectorum* L.), jointed goatgrass (*Aegilops cylindrica* Host), kochia [*Kochia scoparia* (L.) Schrad.], and Russian thistle (*Salsola iberica* Sennen & Pau). These weeds emerge during the cooler months of the growing season, either in September and October or late March through early May. Prominent warm-season weeds are green foxtail [*Setaria viridis* (L.) Beauv.], field sandbur [*Cenchrus longispinus* (Hack.) Fern.], stinkgrass [*Eragrostis cilianensis* (All.) E. Mosher] and redroot pigweed (*Amaranthus retroflexus* L.), which emerge during mid-May through July. Less than 1% of the land area is infested with perennial weeds.

2 Prevention: Reducing Weed Community Density

With annual weeds, the seed is the key component of population dynamics. Strategies for prevention emphasize cultural tactics that decrease the number of weed seeds in soil, reduce weed seedling establishment, and minimize seed production by plants that escape control tactics (Fig. 2). Cultural tactics can be grouped into five categories: rotation design, crop sequencing, no-till, crop residue management, and competitive crop canopies. In the following text, we describe tactics used for each category and their impact on weed dynamics.

2.1 *Arranging Cool-season and Warm-season Crops in Rotation*

Both cool- and warm-season crops are commonly grown in the region. Different planting and harvest

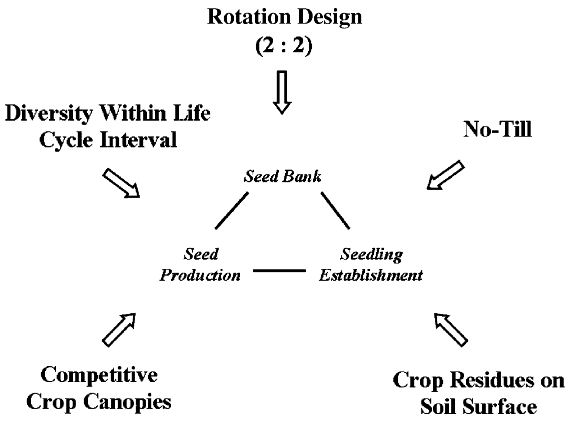

Fig. 2 Five components of a prevention approach to reduce weed community density in the semiarid steppe of the United States. The 2:2 designation refers to rotations comprised of two cool-season crops followed by two warm-season crops. Cultural tactics in each component disrupt weed population dynamics by minimizing weed seed survival in soil (seed bank), seedling establishment, or seed production (Adapted from Anderson (2005a))

dates among these crops provide more opportunities for producers to prevent either plant establishment or seed production by weeds. For example, green foxtail emerges between mid-May and early July, then begins flowering in early August. Winter wheat is harvested in early July, thus producers can control green foxtail before it flowers and produces seeds. A similar opportunity occurs with cool-season weeds; they are easily controlled before planting warm-season crops such as corn or sunflower to prevent seed production.

The impact of rotations on weed community density is related to seed survival in soil. With downy brome and green foxtail, approximately 20% of seeds are alive 1 year after seed shed, whereas less than 5% of their seeds are alive after 2 years (Anderson 2003). Rotating crops with different life cycles favors the natural loss of weed seeds across time because producers can prevent new seeds being added to the soil. With less seeds in the soil, fewer seedlings emerge in following crops (Sagar and Mortimer 1976; Roberts 1981).

However, long-term rotation studies using conventional herbicide programs show a striking trend; weed density increases if rotations consist of one cool-season crop followed by one warm-season crop, such as winter wheat–proso millet (Anderson 2005a). In contrast, if rotations are arranged in a cycle of four, with two cool-season crops followed by two warm-season crops, weed density declines with time. Comparing trends across three rotation studies, weed seedling density was eightfold higher in two-crop rotations compared with four-crop rotations comprised of cool- and warm-season crops.

Another trend noted with long-term rotation studies is that with four-crop rotations, weed density increases if the same crop is grown 2 years in a row (Anderson 2003). When winter wheat is grown 2 years in a row, density of the winter annual grasses, downy brome and jointed goatgrass, escalate rapidly. Seed decay of weeds in the soil is minimal during the 3-month interval between winter wheat harvest and planting, thus seedling emergence is high in the second winter wheat crop. In contrast, replacing one crop of winter wheat with another cool-season crop, such as dry pea, provides an opportunity to reduce seedlings of winter annual weeds that emerge over winter because dry pea is planted in late March or early April.

A similar benefit occurs when a warm-season sequence of corn and sunflower is used rather than 2 years of corn; corn is planted in early May whereas sunflower is planted 3–4 weeks later. Diversifying crops with different planting dates within a life-cycle category, i.e., warm-season crops, accentuates the benefit gained with rotations comprised of 2-year intervals of cool- and warm-season crops. Examples of 4-year rotations used in the region are winter wheat–corn–sunflower-fallow or dry pea-winter wheat–corn–proso millet; like sunflower, proso millet is planted 3–4 weeks later than corn.

2.2 No-till Interacts with Rotation Design to Affect Weed Density

No-till systems help weed management by keeping weed seeds near the soil surface and exposing seeds to environmental extremes and predation (Sagar and Mortimer 1976). For example, a study in the Canadian prairies showed that more than 50% of green foxtail seeds were alive after 2 years when buried 10 cm in soil, contrasting with less than 10% of seeds surviving when they remained on the soil surface (Fig. 3). Even when seeds were buried only 1 cm in soil, survival was still twofold greater after 2 years compared with seeds on the soil surface.

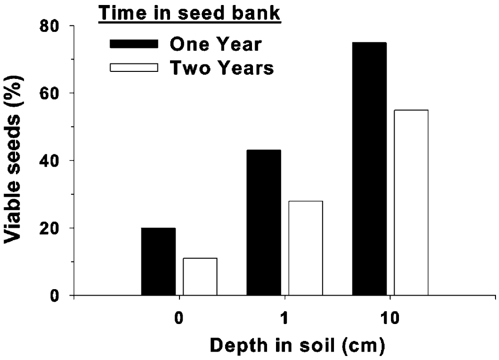

Fig. 3 Impact of depth in soil on seed survival of green foxtail. Means for each depth within each year differ as determined by 95% confidence intervals. Study conducted in the semiarid prairies of Canada (Adapted from Banting et al. (1973); Thomas et al. (1986))

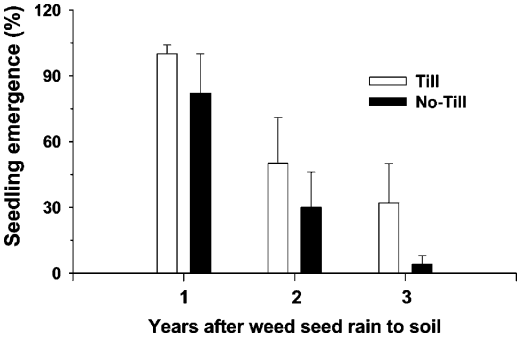

Fig. 4 Effect of tillage on weed seedling emergence across time. Weed seeds were not added to the soil after initiation of studies; tillage occurred in the tilled treatment each year. Data expressed as a percentage of the treatment with highest number of weed seedlings in each study. Standard error bars were derived from yearly means among studies (Adapted from Egley and Williams (1990); Popay et al. (1994); Anderson (1998))

The impact of no-till on weed seed survival in soil, however, is related to rotation design. This relationship was demonstrated with a series of studies that recorded weed seedling emergence in no-till and tilled treatments for 3 years (Anderson 2005a). The sites were naturally infested with weeds, but after initiation of each study, further weed seed rain was prevented. Averaged across all studies, seedling emergence was similar between tilled and no-till in the first year, whereas in the second year, the difference between tillage treatments was about twofold (Fig. 4). In contrast, seedling emergence was eightfold greater with the tilled system in the third year; the benefit of no-till in reducing seedling emergence increased across time.

This interaction among seedling emergence, tillage, and time is one reason why no-till rotations with 2-year intervals of cool- and warm-season crops are effective in reducing weed density. By preventing weed seed production across 2 years, such as eliminating seed rain of cool-season weeds during the warm-season crop interval, weed seedling density is drastically reduced when a cool-season crop is grown in the third year with no-till.

Rotation design also helps weed management in tilled systems, but the impact is less compared with no-till systems (Anderson 2004). The reason tillage reduces the rotation effect on weed density is that weed seeds survive longer when buried in soil, thus leading to more weed seedlings in following years (Sagar and Mortimer 1976).

A further benefit of no-till is that crop residues lying on the soil surface reduce establishment of weed seedlings; in the semiarid steppe, each 1,000 kg/ha of winter wheat residues on the soil surface reduces weed seedling establishment 14% (Wicks et al. 1994). To enhance crop residue suppression of weed emergence, producers grow taller cultivars of winter wheat at seeding rates 50% higher than normal, with low rates of N and P banded with the seed at planting. These tactics increase residue production 2,000–2,500 kg/ha (Anderson 2003). Similar suppression of weed establishment occurs with residues of other crops.

2.3 Competitive Crop Canopies Reduce Weed Growth and Seed Production

To minimize seed production of weeds that escape control tactics in crops, crop competitiveness to weeds is increased with cultural tactics such as increased seeding rate or fertilizer placement. A key to effectiveness, however, is that several tactics need to be combined (Anderson 2003). With sunflower, a single cultural tactic, such as narrower row spacing, higher plant population, or delayed planting, reduced weed biomass 5–10% compared to conventional practices used by producers (Fig. 5). When two practices were combined, biomass suppression approached 20–25%. However, weed biomass was reduced almost 90%

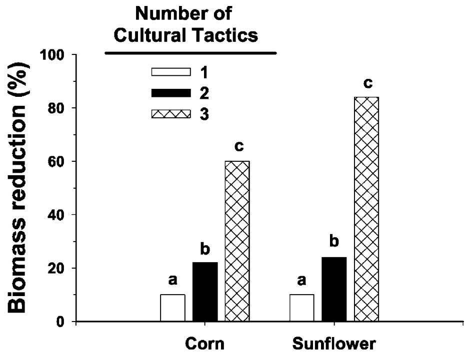

Fig. 5 Synergism of cultural tactics with suppression of weed biomass in corn and sunflower. Tactics include increased seeding rates, narrower row spacing, fertilizer placement, and delayed planting, with treatments compared to the conventional system used by producers. Bars with an identical letter within a crop are not significantly different based on Fisher's Protected LSD (0.05). Means for single tactic treatments did not differ from the conventional system. Study conducted at Akron, Colorado, United States (Adapted from Anderson (2003))

when three tactics were integrated together. Combining tactics synergistically enhanced sunflower suppression of weed growth. A similar trend occurred with corn and higher plant population, narrow row spacing, and fertilizer placement; again, a system of three cultural tactics greatly reduced weed growth (Fig. 5).

This synergistic trend with cultural tactics in suppressing weed growth also occurs with proso millet and winter wheat (Anderson 2003). With proso millet, a cultural system comprised of a tall cultivar, higher seeding rate, and N banding by the seed, reduced seed production of redroot pigweed 90% compared with conventional practices (Anderson 2000a).

3 Prevention: Improving Crop Tolerance to Weed Interference

Along with reducing weed density, a second component of prevention is improving crop tolerance to weeds (Fig. 1). Earlier, we discussed cultural systems in corn that reduced weed growth (Fig. 5); this approach also improves corn tolerance to weeds (Anderson 2000b). Common practices for growing corn in the semiarid steppe include a target population of 37,000 plants/ha, row spacing of 76 cm, and N fertilizer applied broadcast. To assess corn tolerance to weeds, we evaluated three cultural tactics: (1) banding N near the seed; (2) higher corn density (47,000 plants/ha); and (3) narrow row spacing (38 cm). All possible combinations of these tactics were evaluated, with the study established in no-till. The conventional system comprised of common practices was included for comparison, and treatments were split into weed-free and weed-infested subplots.

Yield loss due to weed interference was only 13% when three cultural tactics were combined (Fig. 6). In contrast, yield loss was 43% with the conventional system, a threefold increase compared to the production system with three cultural tactics. If only one or two cultural tactics were used, yield loss due to weed interference was still reduced, but not to the extent of combining three cultural tactics.

We achieved similar results with improving proso millet tolerance to weeds (Anderson 2000a). A cultural system comprised of a tall cultivar, seeding rate increased 50% above normal, and banding N fertilizer near the seed, was established with no-till. The conventional system used by producers was established with tillage and N fertilizer applied broadcast. Each treatment was split into weed-free and weed-infested subplots.

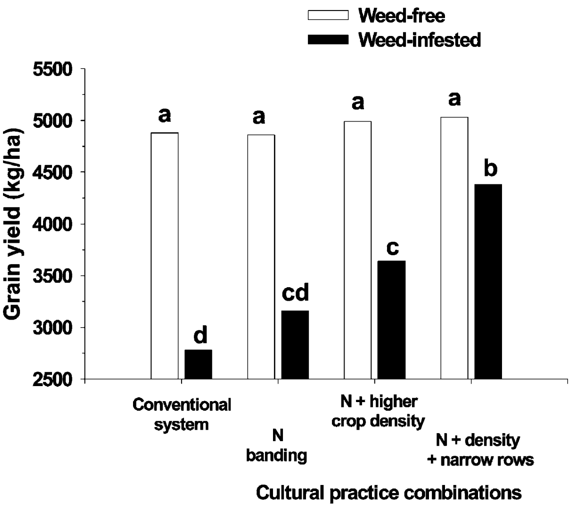

Fig. 6 Corn grain yield in weed-free and weed-infested conditions as affected by cultural tactic combinations. Conventional system was 37,000 plants/ha at a row spacing of 76 cm, with N fertilizer broadcast at planting. Cultural tactics were banding N near the seed, increasing crop density to 47,000 plants/ha, and reducing row spacing to 38 cm. Data averaged across 3 years; bars with the same letter are not significantly different based on Fisher's Protected LSD (0.05). Study conducted at Akron, Colorado, United States (Adapted from Anderson (2000b))

The cultural system eliminated yield loss due to weed interference whereas weeds reduced grain yield 29% in the conventional system. Yield differences reflected system impact on weed biomass, which was ninefold greater with the conventional system. Cultural systems also improve tolerance of winter wheat and sunflower to weeds (Anderson 2003).

4 Control: Benefits Gained with Prevention Tactics

Producers gain a multitude of benefits with lower weed community density and more crop tolerance to weed interference. Herbicide efficacy is improved whereas cultural tactics become a more viable alternative to herbicides. Lower weed density reduces the need for herbicides to manage weeds.

4.1 Improved Herbicide Performance

Both soil- and foliar-applied herbicides are more effective at lower weed density (Winkle et al. 1981). For example, Dieleman et al. (1999) found that number of broadleaf weeds surviving treatment of foliar-applied herbicides was related to initial density of plants. Hoffman and Lavy (1978) reported a similar trend with weed density and atrazine activity in soil; more plants escaped control at higher densities.

Increasing crop competitiveness with cultural tactics also improves herbicide efficacy (Derksen et al. 2002; O'Donovan et al. 2006). Scientists in the Netherlands are defining the minimum lethal herbicide dose needed for efficient weed control in various crops (Mortensen et al. 2000); lower weed density and improved crop competitiveness should enhance success of this approach also.

4.2 Reduced Input Costs

The economic impact of the dualistic approach to weed management in the semiarid steppe was determined by comparing eight producers who followed this approach with eight conventional producers (Anderson 2005a). Producers who included prevention tactics in weed management reduced herbicide inputs and cost 50% compared with producers following conventional practices. Cost of weed management was less because lower weed community density reduces the need for herbicides. With winter wheat–corn–proso millet-fallow, producers grow winter wheat and proso millet without in-crop herbicides; weed density is so low that crop yield is not affected by weeds.

A second trend noted with this economic survey is that net returns were fourfold greater for producers with diverse rotations compared with conventional producers. The dualistic approach for weed management was a key factor with improved economics; savings in weed management costs comprised one half of the increase with net returns by producers using crop diversity and no-till rotations.

4.3 Cultural Tactics as Alternatives to Herbicides

Cultural tactics can effectively substitute for herbicides if weed density is low (Forcella et al. 1993). As noted earlier, a system comprised of three cultural tactics to improve competitiveness of proso millet eliminated yield loss due to weeds; in contrast, weed interference reduced yield 29% with the conventional system used by producers (Anderson 2000a). A similar benefit was achieved with sunflower; yield loss was eliminated with a system of cultural tactics whereas weed interference reduced yield 24% with the conventional system (Anderson 2003).

Weed management in crops grown in wide rows (76 cm or more), such as corn, often require high herbicide or tillage inputs to control weeds. Recent advances with weed control equipment may provide an alternative to herbicides or tillage. An in-row cultivator has been developed that effectively removes weeds in the corn row, especially in fields with low weed seedling density (Schweizer et al. 1992). In addition, a mower has been developed that controls weeds between rows of soybean (*Glycine max* L.) and corn (Donald et al. 2001). With lower weed community densities due to prevention tactics, a system comprised of these two tactics may control weeds in wide-row crops without herbicides.

4.4 *Ancillary Benefits for Managing Other Pests*

Prevention tactics for weed management, such as crop diversity, also helps manage other pests. A prominent example occurs with sunflower. The sunflower stem weevil (*Cylindrocopturus adspersus* Leconte), a native insect of the steppe, burrows into the stem during the growing season (Knodel and Charlet 2002). Sunflower tolerates normal infestation levels without yield loss. However, phoma (*Phoma macdonaldii* Boerma), a native soil-borne fungus, proliferates when sunflower is grown more frequently than once every 4 years (Anderson 2005b). Phoma weakens the sunflower stem, thus accentuating the impact of stem weevil injury and leading to extensive lodging before harvest. If phoma is present, producers need to spray insecticides for the stem weevil to prevent severe yield loss. Similar results have been achieved by scientists in the Netherlands, where insects, diseases, and weeds are being managed with less pesticides in cropping systems that include diverse rotations and prevention planning (Lewis et al. 1997).

5 The Dualistic Approach to Weed Management

A key aspect of the dualistic approach is that multiple tactics need to be integrated into the production system, as shown with tactics used to reduce weed community density (Fig. 2). Density of weed communities often escalates if management includes only a few tactics, especially when herbicide input is reduced. For example, no-till rotations comprised of only one or two crops (Anderson 2005a) or cropping systems that include extensive tillage (Anderson 1999) lead to more weeds. In addition, seed production of weeds escaping control tactics in the crop can minimize the benefits of crop diversity and no-till if cultural tactics are not used to improve crop competitiveness. The dualistic approach to weed management requires more planning and management than the herbicide-based approach. However, the dualistic approach has transformed weed management in the semiarid steppe. Producers have found that herbicides are a choice rather than a requirement for cropping success with this approach (Anderson 2005a). Additionally, eliminating herbicide use in some years is reducing selection pressure for resistant weeds.

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Mechanical Destruction of Weeds: A Review

D. Chicouene

Abstract Methods of mechanical destruction of weeds in relation to their biology are reviewed. Depending on the stage of growth and type of weed, i.e. the depth at which new shoots can be formed and the ability to withstand burial, implements inflict damage on weeds in different ways: cutting, burial, uprooting. The various types of damage are shown. The three main methods of destruction are analysed and compared. These are: lethal damage, particularly effective in the case of plantlets; the exhaustion of reserves, effective where either deep or surface organs are targeted; withering, of special interest where sub-surface parts should be involved. Stored reserves can be depleted by either cutting the foliage, burying foliar parts or severely cutting up surface organs of propagation. Withering is accomplished by either cutting the roots, by exposing the roots to the air or by shaking out rootlets. It is shown that the kind of damage is strongly dependent upon the type of organ involved. Advances in our understanding of the way in which mortality occurs are discussed in the light of research.

Keywords Creeping roots • Exhaustion • Morphology • Physiology • Plant • Plantlets • Rhizomes • Reserves • Weed control • Withering

1 Introduction

The mechanical control of weeds is one of the main traditional methods used in plant production. The expansion of sustainable agriculture in developed countries has brought mechanical destruction methods to the forefront again, as they avoid the use of herbicides. An analysis of cultural methods, as related to the biology of weed plants, forms the basis of research into better weed control. This is an aspect already emphasised by Chancellor (1968). Following intervention in a crop, an understanding of the mechanisms causing weed death or weed survival will be a determining factor in optimizing control methods. Depending on the kind of weed involved, the various types of destruction methods should also be reviewed. More often than not, however, where mechanical methods of control of vascular green plants are used, only the final result at the end of the season is taken as a measure when comparing different protocols (*vide* Rasmussen, 1992: Harrowing of plantlet weeds; Palis, 1996: The control of *Elymus repens*). For many perennial species the mechanisms by which regeneration occurs are discussed in terms of the relationship between the organs and the damage inflicted. This is particularly so in the case of organs which regenerate after intervention (e.g. Irmisch, 1857, who showed that the organogenetic capacity of each organ depends on the plant concerned; Korsmo, 1930; Salisbury, 1962; Leakey, 1981).

Besides, the mechanisms by which death occurs following the use of mechanical control methods are

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the subject of only sparsely scattered communications and tend to be limited to a restricted range of perennial weeds:

- The role of the actual level of reserves in *Cirsium arvense* needed for regeneration to occur was suggested by Prentiss (1899) and added to by Welton et al. (1929) regarding the way in which the level of reserves in the same plant is tied to its annual cycle, by Barr (1940) regarding the use of existing reserves in *Convolvulus arvensis* and by Timmons (1941) regarding the optimum number of interventions on *Convolvulus arvensis* aimed at exhausting its reserves.
- In the control of perennials, Hitchcock and Clothier (1898) suggested preventing the build up of reserves, plus the straightforward pulling up of annuals and biennials. Both these aspects were taken up by Brenchley et al. (1920), but only in the case of plantlets deprived of their capacity to regenerate.
- In their vast review of the subject Robbins et al. (1942) tackle the subject of the effect of untargeted weed control (i.e. before emergence) on plantlets, pointing out the difference between the importance of the reserves of deeply rooted perennials and that of dehydration for surface rooting species.
- With plants possessing only subsurface underground parts (particularly *Elymus repens* and *Agrostis* sp. pl.), Fail (1956) proposed exhausting reserves by programming at least two successive interventions: the first enabling the production of new shoots with the second killing them off.
- In addition to annuals and biennials at the plantlet stage which had been totally deprived of their capacity to regenerate, Muzic (1970) made a distinction between preventing the build up and using up of reserves – these being influenced by meteorological conditions.
- With damaged perennial grasses (*Elymus repens*, *Holcus mollis* and *Agrostis gigantea*) Hakansson and Wallgren (1975) observed drying up and exhaustion of reserves in rhizomes only – particularly those near the surface.
- For some plantlets arising from seed, Jones et al. (1996) showed that they could be controlled by simply cutting back to soil level or by burial.

The three main methods of control are exhaustion of reserves, provoking withering and depriving the plant of any capacity to regenerate. In addition, several authors suggest exposure to cold (e.g. Muenscher, 1955). With such varied points of view an overall historical review is needed.

The factors influencing the ploughing up of stubble in an area in western France possessing a hyperoceanic temperate climate were analysed by Chicouene (1999). Underground organs are often sectioned horizontally and, depending on the particular weed involved and the depth in the soil, either both upper and lower parts, or just one or neither is capable of regeneration. Thus, the reasoning and methods involved in ploughing up stubble, etc., aimed principally at causing withering or exhaustion of reserves, differ according to the type of weed. The weed list for the area studied showed that each species was characterised by: a calendar governing the capacity for regeneration (Chicouene, 1996), a vegetative period and, for those with underground propagation, the way in which the organs of propagation and regeneration were arranged. Plants dormant in winter, theoretically sensitive to cold, tended to have the deepest organs, often below the ploughed layer. Such comparative lists are, however, available for only a very few areas.

Reviewing historically the diverse mechanisms leading to the weed's death is useful when trying to judge whether trial protocols have, a priori, been optimised and whether the strategy used in mechanical control is best adapted to the particular weed involved. Such mechanical control covers stubble ploughing, tillage, harrowing, furrowing, ground preparation for sowing, etc. This is a conceptual framework that deals with factors influencing the plants' sensitivity to being damaged, the damage types induced by various implements, and the discovery in a systematic way. Justifying the effort involved in using a particular method of mechanical control for a chosen weed assumes choosing the right implement, adjusting it properly, and the criteria for deciding on the correct timing for intervention. The types of damage inflicted on each plant type should be analysed when working out a strategy. This implies that weeds should be classified by different forms in which they occur and the various ways in which implements inflict damage on.

2 Ways in Which Implements Work in Relation to the Characteristics of Regenerating Organs

Before studying the way different implements act against weeds it is worth analysing each of the above two aspects separately in order to show how implements vary in their effect.

2.1 *The Weed Plants and the Organs Responsible for Regeneration*

A description of the factors governing what happens to the weed plant centres on the position of the underground organs and the plant's state of growth. According to the type of organ involved in regeneration (particularly those buried deep) weed plants are classified according to Tables 1 and 2. Such plants are either stationary species or those which propagate vegetatively.

2.1.1 Surface Regenerating Organs

Organs situated on the soil surface and give rise to new shoots correspond to plants for which all stems are aerial, and are the only parts capable of regeneration. The plants involved include those producing stolons, stationary species, fully grown perennials and annuals (there was no evidence of underground propagation in the latter in the flora studied) and those at the plantlet stage.

2.1.2 Underground Regenerating Organs

The underground organs capable of forming new shoots (i.e. tap roots, rhizomes and creeping root systems) form at depths which vary from species to species (Hakansson, 1982; Chicouene, 1992, 1999). They can be classified as follows:

(a) Superficial organs situated in the first few centimetres of the soil and so affected by top soil work as *Hypericum perforatum*. These belong to rhizomatous plants and some stationary species, plus those with creeping root systems.

(b) Those extending into the remaining workable layer and are unaffected by top soil work as *Mentha arvensis*.

(c) Those extending below the ploughable layer (in plants propagating vegetatively the orthotropic part

Table 1 Relationship between depth of regenerating organs and effectiveness of implement's mode of action

	Type of implement				
Regenerating organs	Horizontal blade	Horizontal blade	Discs, moleboard, plough	Tines (vibratory curved)	Subsoiler tines
(non dormant)	Deep cut (≈1 dm)	Sub-surface cut (2 cm deep)	Green parts buried (overturned or not)	Uprooting (to 1 dm) green parts and roots left exposed	Rootlets shaken leafy parts left untouched
Plantlet (without reserves)	(0 to +) W	+(+)[a] W	+ E (& W)	+ W	(+) W
Surface organs (stolons or rosettes)	0 to (+) W	+ W	+ E (& W)	+ W	(+) W
Sub-surface organs (in first 1 dm)	(0) W	(+) W & E	+(+) E (& W)	+ W	(+) W
Surface and underground organs	(+ to 0) W & E	(+ to 0) W & E	+(+) E (& W)	+ to 0 W & E	+(+) W (& E)
Underground organs	+ E	(+) E	+ to (+) E (& W)	(+) to − E	(+) W (& E)
Deeply buried organs	+ E	(+) E	+ to (+) E	(+) to − E	(+) W (& E)

[a] Dependent on depth of emergence

+ = effective; 0 = ineffective; − = negative effect and to be avoided; () = marginal; W = withering; E = exhaustion of reserves

Table 2 Relationship between regenerating organs and method of destruction and effectiveness of damage inflicted on weed organ

Withering					Exhaustion of reserves	
Regenerating organs	Rootlets shaken out	Underground organs exposed to air	Roots cut (near surface)	Leaves cut	Regenerating organs also cut up	Green parts buried
Organs on soil surface	+(+)	+(+)	+	(0)[a]	(+)	+
Sub-surface	+(+)	+	(+)[b]	(+)[c]	+	(+)
Sub-surface and underground	If tap roots: + If not: (+)	0	(+)	(+)	(+)	(+)
Underground	+(+)	0[d]	−	+	0	(+)[e]
Deep down	0	−	−	+	0	(+)[e]
Type of implement	Long prongs ripper tines	Vibratory curved tines	Horizontal sectioning	Horizontal sectioning	Horizontal rotary blades	Discs furrow plough

+ = effective; 0 = difficult; − = not possible; () = marginal

[a] Moving partly eliminates leafy parts

[b] Cutting roots which arise at different depths can be difficult – one should pass just below the deepest plagiotropic organs

[c] Necessitates being able to pass at approximately 1-cm depth

[d] An intervention, involving a sorting out, will be needed

[e] Because of the usually low depth of burial, results will not tend to be significant

of the underground stem is quite long) such organs being, at best, hardly affected by sub-soiling, as *Equisetum telmateia*.

(d) Hybrid types of organ: these being a combination of "surface" organs and those descending to varying soil depths – or simply being a combination restricted to the latter. A combination of type "a" and "b" would, for example, correspond to the whole of the workable soil layer. A plant such as *Taraxacum officinale* with a tap root possessing a pronounced capacity for regenerating (Mann and Cavers, 1979) falls into a combination of "a" + "b" + "c".

2.1.3 Dormant Regenerating Organs

Dormant organs and seeds are capable of sprouting and germinating provided that dormancy is not pronounced, surface moisture is sufficient, and burial is not deep as far as reserves are concerned. For example, fine seed will not germinate if covered by more than 1 cm of soil, whilst large dormant organs sprout even if buried at the bottom of the plough furrow.

2.1.4 Special Case of Plantlets

Weed plantlets are fragile prior to and just after emergence; plant reserves, having been used up, are usually incapable of regenerating. Plant architecture closely resembles that of surface organs (i.e. less than 2 cm from the soil surface, above or below).

2.2 Ways in Which Implements Work and Type of Damage Inflicted

The types of damage inflicted on weed plants (including those Chicouene, 1999, described for stubble ploughing) can be classified as follows:

- Horizontal sectioning of generally deeply buried underground organs, e.g. by using a stubble-harrow with horizontal blades resulting in an upper and lower plant part.
- Straightforward burial (e.g. when ploughing furrows) results in green parts being covered with soil.
- Burial resulting from overturning (e.g. mould-board or disc plough) which involves a degree of uprooting due to horizontal sectioning.
- Vertical sectioning cuts up those organs which are generally growing horizontally (particularly those laying perpendicular to the path of implements with moving parts).
- The crushing and shredding of organs caused by disturbance (particularly that caused by tine harrows).

Movement of underground organs to the surface, or towards it. Implements such as a flex-tine cultivator result in a certain degree of separation of the weeds from their soil (i.e. uprooting).

Certain implements cause more than one type of damage, for example:

- Cultivators and vibro-cultivators cause the above last three types
- Ploughs are noted for sectioning deep down and burial due to over-turning
- Rotary cultivators result mainly in horizontal superficial sectioning plus partial burial and overturning
- After ploughing, rotary cultivators and ordinary cultivators reduce the amount of burial caused by the plough by, for example, bringing stolons closer to the surface
- Cover crop implements section organs in various directions (mainly horizontal, but also vertical) plus a degree of partial burying
- Potato harvesters result in a sorting out, along with important horizontal sectioning

The way in which each implement works can depend on soil texture and structure. In loose ground for example, sorting out is better whilst vertical sectioning is poorer.

2.3 Impact on the Plant

The way in which an implement damages the weed plant is determined by the regenerating organ's own particular architecture (Table 1). The depth at which the regenerating organs are found, combined with the particular way in which the implement works, results in one of three different situations:

- Organs formed on the surface can either be deprived of their roots (cut near the surface), have their roots exposed to the air or be simply buried.
- Organs situated in worked soil layers may suffer any one of the types of damage mentioned above (i.e. uprooted, buried, etc.). However deep roots and stem parts can pass between machine prongs and are largely unaffected by vertical sectioning.
- Those regenerating organs situated below the worked soil layer can finish up deprived of green parts irrespective of the depth various implements section them. Nonetheless vertical sectioning has little or no effect and, as in the previous situation, the end result is a proliferation of this particular type of weed.

Such results can sometimes be dependent on weed plant phenology. For example Magnuson et al. (1987) considered the production of new shoots in *Cirsium arvense* from stem sections to be no more than accessory.

After intervention, seed may finish up being covered, and cracked ground can also be responsible for seed being buried. Depending upon size such burial has differing results: germination of larger seed being favoured, whilst that of fine seed being less likely. However, ploughing may bring each seed to the surface where it may still germinate.

3 Destruction and Organs Likely to Regenerate

The degree to which weed plants withstand exposure to mechanical destruction varies according to their reserves. The tendency for resistance to being damaged or for survival appears to be as follows:

1. The survival rate for plantlets is less than that for fully grown plants; a phenomenon already remarked upon by Klingman (1961), Arny (1927) and Anonymous (1968)
2. The survival rate for fully grown annuals is less than that for fully grown perennials (as Hitchcock and Clothier [1898] and Anonymous [1968] have already pointed out).

Fully grown perennials are therefore generally the most difficult of all to combat, whilst plantlet and annual weeds are easier to eradicate. However, exceptions probably occur in the latter case. The actual mechanisms by which death occurs in annual and perennial weeds are not usually mentioned in the literature, even though exhaustion of reserves and withering are often cited as the cause in fully grown perennials.

In practice, one of two situations is possible after inflicting damage: either some plants (or plant fragments) retain the capacity to regenerate if left lying on the surface with sufficient moisture; or others never regenerate, even when conditions are favourable.

What follows below is an analysis of the various types of destruction in which plant organs retain the capacity for regeneration. This is followed up by a review of the different types of damage that are fatal. An intermediate type of damage involving the slowing down and restriction of plant development, due to amputation of plant organs (e.g. an annual's roots), is not dealt with.

3.1 Eradication of Organs Capable of Regenerating

Included under this heading are those plants, or at least their detached parts, remaining capable of regeneration under favourable conditions. Actual regeneration depends on numerous parameters which are compared below and considered from the stand point of the two main limiting factors i.e. water loss and exhaustion of reserves (Tables 1–3).

3.1.1 Principles

Withering

Numerous authors write about causing withering in weeds; for example Robbins et al. (1942) (for weeds with surface rooting systems), FAO (1958) (where the action of cultivators on *Elymus repens* rhizomes is cited), Evans (1962) (in the case of ground disturbance of put-aside land aimed at limiting perennials), Anonymous (1968) (judged worthwhile for surface roots in soil with a poor water retention capacity), Hakansson (1982) (writes of the value, where warm climates operate, of leaving the rhizomes and roots to dry out), FAO (1988) (suggests drying out by leaving exposed to the air), Jones et al. (1996) (who write about annual weeds) and Zimdahl (1993).

Although withering is valid for organs found at various depths, it is probably more effective for those near or actually on the surface. In practice, it consists of separating the plant of its roots in one of three ways (Table 2):

- Amputating the roots by cutting them near their point of insertion (in dry weather stems dessicate rapidly).
- Exposing the roots to the air (for example, where surface rooting weeds are involved, after the passage of a potato harvester).
- Shaking out the rootlets on regenerating organs (main roots and root stock) by passing deep down below them; for example, in the control of *Rumex*

Table 3 Importance of factors involved in exhaustion of reserves and withering

Factor	Exhaustion of reserves	Withering
Meteorology	No apparent effect	Determinant (dry spell needed afterwards)
Organ reserves	Determinant	Theoretically no, or little, effect
Physical parameters of regenerating organs	Length × diameter × Amount of reserves (× Depth of soil to be traversed)	Impermeability (× diameter) (× Length of remaining roots) (× Number of leaves left)
If fragile (e.g. plantlet): "duration" and depth to which worked	Single intervention at surface level (with death occurring after several days)	A single intervention at surface level followed by a day without rain
If resistant (e.g. adult perennial): type of organ involved	Large organs	Impermeable organs (epidermis, suber) (large diameter also slows drying out)
—	—	—
Depth of soil to be worked	–As deep as possible (if upper part regenerates) –Just above regenerating organs (where lower fragment sprouts)	Depends on depth of sprouting organs (pass just below them)
Duration and number of interventions (depends on aim, i.e. slowing down of spread/eradication)	For complete eradication: at least 3–4 times at intervals of 2–3 weeks (slow action)	Possibly one month of sunny dry weather (one period sufficient if good climate)
Type of plants destroyed (regenerating organs)	All types (but difficult to obtain for organs close to surface)	Organs on surface, organs near to surface, (indeed deep tap roots)
Implements and interventions	Plough, furrowing, discs, cutting, finger harrows used on cereal crops, hoes, stubble cultivators, potato harvesters, flexityned hoes	Finger harrows used on cereal crops, hoes, stubble cultivators, potato harvesters, flexityned hoes

crispus with sub-soiler. It should be noted that where foliage remains exposed to the air and attached to regenerating parts, transpiration will contribute to the loss of water in underground organs.

Exhausting Reserves

Exhausting the stored reserves of either the whole plant or detached parts is dealt with in a number of reviews and treatises on weed science (e.g., Buckman, 1855; Hitchcock and Clothier, 1898; Brenchley, 1920; Muenscher, 1955; FAO, 1958, 1988; Klingman, 1951; Evans, 1962; Anonymous, 1968; Muzic, 1970; Fryer and Evans, 1970; Hakansson, 1982; Zimdahl, 1993). Certain authors advise acting before the accumulation of reserves, others exhausting stored reserves and yet others both (*vide* Muzic, 1970). Where storage organs last for more than one season and function more than once, both methods are often used in tandem.

Stored reserves can be exhausted in one of three ways:

- Where weeds possess only very deep regenerating organs they are finally worn down after repeatedly destroying green parts. Such a solution is advocated by Robbins et al. (1952), Muenscher (1955), FAO (1958), and Anonymous (1968), and in particular, has been tried out by Welton et al. on *Cirsium arvense* (1929), by Barr on *Convolvulus arvensis* (1940) and by Pavlychenko, especially on *Cirsium arvense* and *Sonchus arvensis* (1940). Cutting most of the underground orthotropic stem, plus green parts, should help the depletion process when such parts have a poor capacity for regenerating.
- By severely cutting up surface storage organs and then allowing them to sprout, and then killing them off by various methods, as preconized by Fail (1956).
- By burying the green parts to force the plant to produce new growth to reach the surface. This may be repeated if burial is not deep enough to exhaust the plant in a single pass which is the case with certain large rosettes which produce new growth after being well ploughed up.

In theory reserve depletion is independent of meteorological conditions. However Muzic (1970) suggested that conditions favouring growth accelerate the process.

Plant organs are considered depleted when, following intervention, they no longer produce new aerial shoots at a time when the species would normally be growing (assuming growth to be seasonal). However actual mortality of these organs remains to be verified.

If a plant has both regenerating surface and underground organs, a campaign against those on the surface helps to exhaust deeper organs at the same time, provided that:

- All orthotropic stems originating deep down are decapitated, and (in the case of rhizomatous weeds) the upper part extracted.
- All green parts are ploughed under.

3.1.2 Tolerance

The plant variables effecting the degree to which it withstands mechanical intervention differ according to the particular physiological processes leading to its death.

Withering

For death due to withering, which may take anything from less than a day to a month or longer, the rate of drying is dependent on the impermeability and thickness of the organ, plus the degree to which leaves persist. If it should rain before complete drying out and the plant re-develop roots, the operation should be repeated in order to avoid simply slowing down development (i.e. causing no more than a temporary perturbation). Regenerating organs that are deeply buried cannot be properly brought to the surface and are, therefore, poorly accessible to this kind of destruction – except perhaps sub-soiling which may affect tap rooted weeds. Grummer (1963) shows that short pieces were more susceptible than longer fragments of rhizomes of *Elymus repens*.

Exhausting Reserves

Concerning eradication of perennials, optimising the number and the length of time between interventions is dealt with by various authors, in particular by: Timmons on *Convolvulus arvensis* (1941), Seely on

Cirsium arvense (1952) and Derscheid et al. (1961) on *Cirsium arvense* and *Sonchus arvensis*. They reveal that a delay of more than 10 days will suffice. The precaution of preventing leaf growth, mentioned by Hitchcock and Clothier (1898) and Pavlychenko et al. (1940), does not seem to be of use. Timmons (1941), in fact, showed that the amount of reserves held in the roots of *Convolvulus arvensis* continued to decrease up to 15 days after emergence, whilst Muzic (1970) thought that young aerial shoots used up more reserves than they produce.

To obtain complete exhaustion of reserves in perennials one will, generally, need to intervene several times at intervals of a few weeks (i.e. during the growth season). For a weed propagating vegetatively the actual number of interventions will vary according to whether the aim is to slow down its progression, to stabilise its extent at its current limits, to obtain a decrease in its abundance or to eliminate vegetative organs. It is possible that, with certain species, a single intervention each year will not be enough to obtain a decrease in population size. Moreover, Hodgson (1972) showed that tolerance to intensive perturbation of *Cirsium arvense* populations varied according to the ecotype involved. As a consequence a control programme should not be based on information from a single population.

Numerous workers have demonstrated, with weeds which have rhizomes or creeping roots, the relationship between the size and the depth of buried organ fragments, and their capacity to regenerate different studies have been undertaken for species normally possessing (according to Chicouene, 1992) underground propagation at different depths. For example, Prentiss (1889) investigated the length and diameter of creeping roots of *Cirsium arvense*, Hakansson (1967) different stages of development of *Elymus repens* rhizomes and (1969) the creeping roots of *Sonchus arvensis*, Hakansson and Wallgren (1975) the rhizomes of *Elymus repens*, *Holcus mollis* and *Agrostis gigantea*, and Bourdot (1984) the probable perennial rhizomes of a winter-green dicotyledon: *Achillea millefolium*. All of these studies showed that the more reserves an organ contained, the more it was capable of regenerating; i.e. the more interventions it was able to withstand. It may therefore be worthwhile looking at using a sub-soiler to cut up deep organs down in order to reduce the number of interventions needed to exhaust reserves, so reducing the overall time for this to occur. The commonly used types of implement are ineffective in cutting up underground organs as rhizomes of *Equisetum telmateia*.

With an intact root system the greater the depth of soil the plant has to penetrate to reach the surface the more, in theory, it uses up its reserves. Fewer interventions will, therefore, be needed. Such circumstances can be created by either burying green parts and regenerating organs deep down, or by cutting off a large section of the underground orthotropic stem. Nevertheless, with *Convolvulus arvensis*, Barr (1940) was unable to show such an effect in a trial which compared results from one depth and double that depth. In a trial run by Timmons (1941), and which covered depths varying by three times, depth had little effect on the number of interventions needed. The performance of different morphological types would therefore be worth investigating during their period of underground development. Certain plants produce only a single finely drawn out leaf, whilst others produce a stem with burrowing leaves straight away (Chicouene, 1991).

If organs for vegetative propagation are only distributed throughout the worked soil layer then any shoots of those tips exposed to the air will have no soil to traverse to find daylight. One would therefore expect a weak effect on the wearing down of reserves caused by a single intervention – which is probably why Pavlychenko et al. (1940) failed in their efforts to control *Elymus repens* in rainy years.

Certain authors have based themselves on the level of reserves (e.g. Welton et al. (1929) writing about *Cirsium arvense*; Arny (1932) writing about five different species one of which was the winter-green rhizomatous *Elymus repens* and Barr (1940) writing about *Convolvulus arvensis*), and they have all sought to intervene during flowering when reserves were at their lowest. With *Elymus repens*, however, there is little variation in the level of reserves. Observations were primarily done on summer dicotyledons possessing creeping roots. Kingman (1961) probably falls into such a case when he based his programme for exhaustion of reserves on theory. The difficulty in such trials is knowing the level of reserves for organ death to occur. Another gap in our present knowledge is that we do not know whether it is better to wait until the plant reserves reach their lowest annual level naturally, or to intervene as soon as possible. The latter could possibly lead to lower levels than those attained naturally despite the plant's normal cycle being upset.

The reasoning regarding the level of reserves needs to take into account their total overall amount – in which the actual size of the plant organs plays a role. Thus, where tubercles are annual, e.g. particularly with rhizomatous summer dicotyledons, intervention can be planned for when they are absent. This presupposes a knowledge of the phenology of the renewal of organs and the formation of tubers, as well as the degree to which the crop itself prevents weed development. By anticipating the accumulation of reserves, and the consequent formation of organs for vegetative propagation, it is possible to limit the spread of weed patches.

3.1.3 Combinations of Different Methods of Eradication

Exhausting reserves and causing withering are tactics that can be used simultaneously or separately. A combination of both methods – possible during a dry spell – involves simultaneously subjecting the same plant organ to both such tactics. Successive work during a dry spell can correspond to such a situation (Hakansson and Wallgren [1975] and Palis [1996] writing about *Elymus repens*) for organs found throughout the worked soil layer in which the work mixes and fragments them and amputates the roots of their rhizomes.

In the case of plants sectioned horizontally, where both parts can regenerate, elimination is obtained by allowing the upper part to wither, or in combination withering with exhaustion of reserves, and exhausting the reserves of the lower half (Table 2). Should rain occur after such an operation then only the exhaustion of reserves will have some effect (weak). If dryness is not pronounced it may be possible to calculate the depth at which sectioning should occur to ensure that the upper part withers. However the shallow depth at which sectioning must be done will dictate a greater number of interventions if the lower part's reserves are to be exhausted.

Logically withering does not cut off food reserves nor does exhaustion reduce water reserves. Also, where a succession of interventions is involved, particularly those aimed at surface organs, changing the method would probably translate to going back to square one. With superficial plagiotropique organs, starting by allowing plant fragments to sprout (which exhausts their reserves) under moist conditions, and then allowing the new sprouts to dry out after a second intervention is, theoretically, the only exception to this rule. Experimental evidence concerning all such aspects is indispensable in planning a practical strategy for the species, conditions and development stages. This is also needed for estimating the effectiveness and sensitivity to failure.

3.1.4 Dormant Vegetative Organs

All weed species are not growing at the same time, nor do all tubercles of a species with a seasonal growth cycle enter this cycle simultaneously. Consequently the weed flora of a given field will not be controlled simultaneously. Exhaustion of food reserves will obviously be ineffective if plant organs are not growing. For at least some weeds appearing in winter causing them to wither in summer will also produce equally poor results (those species with dormant tubercles above surface show high resistance in summer), It sometimes happens that disturbance sparks off unseasonal growth (Chicouene, 1991) and depending on the size of plant fragments, sprouting may be possible even after burial.

Exposing underground organs (normally those of weeds appearing in the summer) to the cold (as suggested by Muenscher [1955] and King [1966]) or to winter pests can cause them to die. However this may not be enough to make the practice a viable one – particularly because many summer weeds possess organs below the worked soil layer (Chicouene, 1992).

3.2 Lethal Damage

3.2.1 Principles

In contrast to the previous group, no part manages to regenerate a new plant, even when situated on the surface or even when not exposed to water loss. The literature provides little information on the subject. In general what is called lethal damage here is more or less implicitly assessed as such, since the weed's disappearance is considered to be simply due to intervention. According to the type of weed control used, the approach to the subject falls into one of two categories:

- The destruction of plantlets, particularly those on prepared ground left lying before sowing and those hit by untargeted harrowing, where no type in particular is concerned (certain authors put the accent on plantlets of perennials) is cited by Brenchley (1920), Arny (1927), Drottij (1929), Robbins et al. (1942), FAO (1958, 1988), Klingman (1951), Evans (1962), Anonymous (1968), Muzic (1970), Jones et al. (1995) and Rasmussen (1996). Ilnicki and Fertig (1962) and Boyd and Murray (1982) show seedlings clipped of perennial *Solanum* (*Solanum carolinense* or *S. eleagnifolium*) were died until the age of 10 days.

– The destruction of fully grown, or nearly fully grown plants during hoeing, harrowing and other mechanised work is broached by the following: Hitchcock and Clothier (1898); Robbins et al. (1942) who stipulate that top growth should be destroyed; Evans (1962) who limits himself to entertaining the idea of upsetting growth; King (1966); Anonymous (1968); Muzic (1970) who puts the accent on removing the rooting system; FAO (1988) and Jones et al. (1966) who compare different types of damage in annuals.

Closely cropped cuts should also be mentioned (i.e. cuts at the level of the aerial part of the hypocotyls – when it exists – which probably correspond to certain trial procedures used by Jones et al., 1995) as well as the chopping up of aerial organs in many young and fully grown erect annuals, particularly those with a long drawn out stem.

Lethal damage particularly concerns those plantlets left lying on prepared ground before sowing, and also those damaged during the harrowing of germinating autumn sown cereals, in damp periods. Damage involves plant organs harmed by crushing, being torn apart or being sectioned. The organs concerned form part of the plant axis (i.e. stem, hypocotyle, mesocotyle or even the root) or possibly just the leaves (cotyledons and coleoptyle). Both aerial as well as underground parts may be involved and this leads to a single main part being left (in the case of amputation of the cotyledons) or two or more, in the case of fragmentation. No part succeeds in regenerating, possibly for more than one reason: the organogenetic capacity is not yet properly developed (arguably, a problem involving growth substances), insufficient reserves for forming new buds and roots; and severe weakening caused by lack of sap, or due to pathogenic organisms and pests. Such hypotheses largely remain to be tested to enable improvement in mechanical techniques.

3.2.2 Tolerance

Mechanical damage from withering, exhaustion of reserves, or lethal damage to the plant, generally lead to plant death. The period during which plants are most exposed is when they are at the plantlet stage, when shoots begin to appear. The damage is probably lethal for all plant parts, although this remains to be verified. Proof should take into account the stage of development. The species involved is probably important here. The hypocotyl, or other plant part, incurs damage, particularly with respect to underground parts and the depth from which shoots are produced.

With fully grown plants, particularly annuals, cutting the main stem is more difficult than with young plants. Decapitating aerial parts is not necessarily enough to kill the plant.

Those traits describing the plant's sensitivity to lethal damage are useful when discussing tolerance. Sclerification, the size of main axes, the relationship between size of plantlet and seed, the depth from which shoots appear are all worth taking into account. Due to the mechanical vibration that accompanies the use of tine harrows such implements are probably more effective in inflicting damage. The mechanisms leading to the weed's death following damage are, from an organographic standpoint, probably similar to those following either weeding using thermal techniques or contact weed killers.

Different degrees of control are possible, with lethal damage being the most extreme. At the other end of the scale is the slowing down of development. The differences, as far as wearing the plant down is concerned, might simply be no more than quantitative.

4 Conclusion

On analysing the mechanisms by which growing weed plants are mechanically destroyed, three main processes appear to be involved: direct lethal injury, exhaustion of reserves and withering. Observation points to each process depending on the type of injury inflicted, along with the actual biology of the plant itself and, in the case of withering, the lack of water to which the plant is exposed.

Where plantlets are involved, all three types of injury appear easily attainable. On the other hand perennial weeds possessing tougher organs involve a more difficult type of destruction to attain:

- Withering (always dependent on the lack of water) is more of a candidate with species with sprouting organs near the surface – though tap rooted plants constitute an exception where sub-soilers are used.
- Exhausting the weed plant, a slow but sure process, appears more suitable against plants with deep regenerating organs, but not exclusively so.

In the use of mechanised control methods the most important aspects of the biology of the weed plant are the depth at which those organs likely to regenerate occur, their possible dormant season and, possibly, their life span. Such an approach is valid where intervention involves the whole plot area, or simply just a part (e.g. between crop rows). But where a succession of interventions is involved, changing the method probably translates into going back to square one.

The optimisation of mechanised methods of control should take into account: the biology of the weed plants involved, the pedoclimate within the soil, crop rotation (choice of crops and length of time between each). and, where applicable (i.e. in the case of plants exhibiting seasonal growth), the time of year. Working out the mechanisms by which weeds die after damage would enable the effect of the weather to be evaluated, and would also influence the choice of implement used. Information relating to the comparative biology of the weed plants involved ought to take into account the above aspects as well as a statistical evaluation of the general principles outlined above i.e.:

- The number of interventions needed, plus the time between each one and the degree of dehydration to which the weed plant is exposed – all calculated according to the object in view (which may be anything from slowing down the weed's progression to its complete eradication).
- The probability of the damage to the weed plant actually having an effect
- The difficulty in uprooting, with regard to both the desired end result and the stage of development at the time of intervening.

In evaluating how a particular technique has performed, the time before it is possible to record results and the relevant information to be recorded will depend on the biology of the particular weed involved. The time of year in which growth and propagation occur and the time before new growth is produced after intervention are all important here. When evaluating trials, particularly with perennial weeds propagating vegetatively, it would be better to think first of recording the reduction in plagiotropic growth (as measured against a control) than the number of aerial shoots produced (e.g. per unit surface area).

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Sustainable Pest Management for Cotton Production: A Review

Jean-Philippe Deguine, Pierre Ferron, and Derek Russell

Abstract Cotton cultivation, often highlighted for its excessive consumption of plant protection products, is taken as a model to illustrate the development of the ideas and practices of crop protection over the last 50 years. Cotton is grown in 69 countries on 30–35 million hectares and the production exceeded 20 million tones of lint in recent years. Despite the continual improvement in the performance of chemical control strategies, harvest losses remain very high, of about 30%. The largest consumer of pesticides in the world, the cotton production system has the advantage of having been an experimental model for many crop protection programmes under various agronomic conditions and in the presence of diverse pest complexes. Without attempting an exhaustive bibliography, this review explores how and why the ideas underlying crop protection have significantly evolved since the advent of synthetic pesticides. After a spectacular demonstration of yield growth through the application of chemical control, cotton production was rapidly confronted by the secondary effects of this control. These included the appearance of evolved insecticide resistance and the appearance of new damage caused by pests considered up to then as of only secondary importance. In extreme cases, the economic viability of the production systems themselves have been compromised following increases in the application rate and frequency of insecticidal treatments. In general, harvest losses have remained high despite the constantly improving technical performance of pest control chemicals. Two models of the future of crop protection can be drawn: total pest management which involves the eradication of pests, and integrated pest management (IPM), which aims at the management of pest populations below economic thresholds by a mixture of chemical control and a suite of alternative control measures. The first method, total pest management is limited in agricultural systems to particular cases in which the pest in question has no significant alternate hosts in the vicinity of the crop system. On the other hand, the application of IPM is constrained both by the difficulties in exploiting the concept of an 'intervention threshold' and by the limitations of many of the specific non-chemical techniques proposed, but does have the advantage of taking into consideration the full pest complex in a cropping system. In practice, it has been a calendar schedule, largely of insecticidal treatments, established on the basis of earlier local observations which has been most widely adopted by growers. This strategy has produced significant improvements in production in the cotton producing countries of francophone Africa and elsewhere. This has led to area-wide integrated pest management which takes into account the potential for natural factors to regulate populations in a specific region. In cotton production, biological control by introduction and acclimation of beneficial arthropods has not been notably successful because of the difficulty of developing a suite of beneficial organisms capable of responding effectively to the diversity of pests in the system, the annual nature of the crop, and the disrupting effects of chemical control measures directed against the remaining pests. Only inundative biological control has had significant success and then in particular cases where the pressure

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of chemical insecticides has been reduced. More benefit is to be obtained from the active conservation of the indigenous fauna of beneficial organisms. In spite of an increased general environmental awareness, in practice it has been the growth of evolved resistance to pesticides which has had the dominant role in constraining the growers to a more rational use of control strategies. These can be illustrated by the development of window strategies for control measures across the growing season, initially in Australia. The reduction in chemical control treatments made possible by the efficacy of genetically modified cotton has shown the positive role that indigenous natural enemies can play. At the same time, however, there has been a growth in the importance of pest species which are unaffected by Bt toxins. For example, the sucking pests are progressively coming to displace the vegetative and fruit feeding caterpillars as key pests of Bt cotton. Taking into account the spatio-temporal dimension of natural population regulatory factors has led to changes in agricultural practices and production systems. In cotton, for example, production systems maintaining permanent ground cover, are having increasing success. Intercropping and trap cropping have been favourable to the maintenance of beneficial arthropod complexes and unfavourable to the growth of pest populations. This new design context for crop protection in general and for cotton in particular, in applying the principles of agroecology, moves towards the concept of a truly sustainable agriculture. This implies a change of strategy towards a total systems approach to sustainable pest management, characterised by a movement from a paradigm of pest control field-by-field, through farm-by-farm and agroecosystem-by-agroecosystem, to a landscape by landscape approach.

Keywords Agroecology • Area-wide pest management • Biotech cotton • Chemical control • Conservation biological control • Crop losses • Eradication • Farmscaping • Integrated pest management • Pesticide resistance • Total pest management

1 Introduction

Since the widespread use of synthetic pesticides against plant pests from the middle of last century, the crop protection community has been searching for guiding principles, capable of responding both to the needs of agricultural production and the constraints imposed by a sustainable development of the planet (Lewis et al. 1997). Chemical control rapidly revealed its limitations, as well as its possibilities, and alternative solutions to pest management problems have been recommended since at least the 1960s. A new strategy was developed under the rubric 'integrated control', envisaging the employment of a range of different control measures, constrained by their compatibility and the requirement for minimising noxious effects on the wider environment.

Experience has shown that putting in place effective biological control procedures has required a significant reduction in chemical treatments, a condition which producers have found difficult to accept. In their defense, it must be said that the alternative solutions proposed have often been difficult to put into practice and frequently insufficiently or unreliably effective. These problems arise in large part from our still inadequate understanding of the mechanisms which determine the dynamics of pest populations in their agro-ecosystems (Geier 1966). Since that time, a number of significant stages in the thinking on crop protection have been passed through, of which the first, under the term 'Integrated Pest Management' or IPM, abandoned the idea of comprehensive pest control and replaced it with the concept of the management of pest populations. In retrospect, this realisation of the importance of the interactions between populations within agro-ecosystems came late. It is now considered as a necessary precursor to the true management of pest populations within the global functioning of ecosystems (Altieri and Nicholls 1999).

Despite these difficulties, a biological, then ecological, orientation has underlain the development of crop protection over the last 50 years (Geier and Clark 1978, 1979; Perkins 1982; Pimentel 1995; Walter 2003). This process has been marked by multiple and diverse interpretations of the concept of IPM (Kogan 1998). In total, numerous technical innovations have been proposed, without, however, bringing any really significant change in the management of pests in major crops (Lewis et al. 1997), due no doubt to an unrealistic approach to the complexities of the phenomena concerned. The debate has been re-animated recently, both by the spectacular success of the recent advances in biotechnology and by genuinely taking into account the need to preserve biological diversity.

As much for socio-economic as for ecological reasons, from here comes a re-examination of farming systems traditionally practiced, via an innovative agroecological approach (Dalgaard et al. 2003).

In this context, cotton production offers the potential to analyse the fruits of a phytosanitary experience, both rich and frequently controversial, in a range of agro-ecological situations, ranging from subsistence farming to industrial production systems (Ferron et al. 2006). Cotton trading is today the object of a socio-economic investigation by the World Trade Organisation, whose scope and accuracy is likely to have a significant impact on the economics of cotton pest management in the future. For these various reasons, cotton production is taken here as a case study illustrating the development of the concepts of crop protection and their strengths and weaknesses.

We have not attempted here an exhaustive coverage of the vast cotton literature, but rather have identified the most significant papers, which illustrate the development of thinking in cotton pest management. Emphasis is given to the entomological literature because of the importance of yield losses caused by insect pests of cotton. We have grouped theoretical and applied papers to produce a synthesis illustrated by concrete examples and have then attempted to draw lessons from this experience, with a view to supporting the adoption of a new strategy for cotton crop protection.

Following this introduction, there are five chapters and a conclusion. The first is a reminder on one hand of the importance of yield losses caused by arthropods, microorganisms and weeds, and on the other hand an examination of the particularities of cotton cultivation, to provide a foundation for an understanding of the case studies to follow. The second section is devoted the paradigm of chemical insecticide use. The third section examines two parallel, but eventually convergent ideas in the management of pest populations. The fourth chapter is dedicated to biological and biotechnological alternatives to chemical control. The most recent agro-ecological approaches are the object of the final section. The particular richness of the literature on the two final themes reflects the importance that they are given today. In the conclusions, we weigh up the significance of these shifts in thinking.

2 Cotton Crop Losses and Key Pests

Despite the continual improvement in the performance of chemical control strategies, harvest losses remain very high and certainly are not declining. Recent data shows, for example, that losses average 26–30% for sugar beet, barley, soyabean, wheat and cotton, 35% for maize, 39% for potato and 40% for rice. The relative importance of the different types of biotic stresses responsible for yield losses varies with the plant cultivated, the system of cultivation, the indigenous flora and fauna and local climatic and soil factors. Globally however, the potential yield losses may be partitioned to insects (37%), weeds (34%) and then to viruses and microorganisms (11%) (Oerke and Dehne 2004).

2.1 Cotton Production: Cultivation Systems and Harvest Losses

Grown in 69 countries, in five continents, on 30–35 million hectares (Berti et al. 2006; ICAC 2006), the cultivated cotton species, *Gossypium hirsutum, G. barbadense, G. arboreum and G. herbaceum* are a primary source of the world's industrial textiles and stock feed and are a major source of cooking oil for much of the world. Production is limited to tropic and subtropical areas of high temperature. It is essentially a small-farmer activity providing the principal cash income for poor families in numerous developing countries, although in a few places it has been adapted to large scale, high-input industrial agricultural systems (Australia and USA in particular).

Since the second world war, global production has been growing. Production exceeded 20 million tones of lint in recent years, from an essentially stable production area: 60% of this production is from Asiatic countries (particularly China, India, Pakistan, Uzbekistan and Turkey), 25% is produced by countries in the Americas (particularly USA and Brazil), and around 10% by African countries including Egypt, Mali, Côte d'Ivoire, Benin, Burkina Faso, Zimbabwe. The remaining 5% is divided between Australia, Greece and Spain. Some characteristics of the world cotton production are given in Table 1. Average yields have

Table 1 Some general characteristics of the world cotton production

- More than 80% of the global production is in the hands of seven countries: China (25%), USA (20%), India (16%), Pakistan (9%), Brazil (5%), Turkey (4%), Uzbekistan (4%)
- 80% of global production is obtained from small farmer systems in developing countries, with an average farm area usually less than 1 ha (0.3 ha for China for example)
- 80% of the global production area is concentrated in 10 countries: China, USA, India, Pakistan, Uzbekistan, Turkey, Brazil, Turkmenistan, Mali, Benin

been over 600 kg/ha of lint since the early 1990s, but this hides an astonishing diversity of national average yields: 1,667 kg/ha in Australia, around 1,000 kg/ha in Brazil, in China, Greece, Mexico, Spain, Syria and Turkey, 730 kg/ha in USA and only 300–350 kg/ha in India and most of the African producer countries, with Egypt, growing *G. barbadense* on irrigated land, as an exception (ICAC 2005, 2006).

To this diversity of agrarian structures and yields, must be added those of growing systems, ranging from subsistence peasant agriculture to large scale industrial systems. Eight different production systems are generally recognised, as a function on one hand of climatic conditions (temperate or tropical, either arid, semi-arid or humid) and on the other hand by the level of input use (minimal, moderate or intensive) (Hearn and Fitt 1992). Cotton plants are demanding of water in the early vegetative growth stages and 55% of the global cotton area is irrigated (ICAC 1996). Irrigated systems find their place in agro-ecosystems as diverse as those of the humid Matto Grosso of Brazil and the Uzbekistan desert. This extreme diversity of production systems allows us to illustrate the different plant protection strategies currently in place.

Today, in spite of the potent chemical means of control, principally relying on synthetic insecticides, the harvest losses are in the order of 30% (animal pests 12%, microorganisms and viruses 10% and weeds 7%), with significant differences between countries. For example, the harvest loss occasioned by insect pests alone are estimated at 24% in sub-sahelien Africa, at 13% in South America but only 7% in Australia where insect control has been more effective and expensive (Oerke and Dehne 2004; Oerke et al. 1994). To these harvest losses, it is necessary to add the lost market value due to contamination of the lint by the exudates of certain sucking pests (cotton stickiness), which complicates the industrial treatment of the cotton. The manual harvesting of cotton, as practiced by the enormous number of small-scale producers does at least, by allowing segregation of stained cotton and reduction in accidental trash contamination, enhance the marketable value of the harvest.

2.2 *Diversity and Development of the Pest Complex in Cotton*

The insect fauna associated with cotton is rich and diverse. However, of the more than one thousand species found on cotton, only 10 or a dozen are significant potential pests. They are either pests of the fruiting parts (flower buds or squares, flowers, and the developing seed capsules or bolls) – causing excision of these parts from the plant, consuming the seeds and destroying or staining the fiber – or they are leaf feeders, root feeders or sucking pests, attacking particularly young shoots and developing leaves. There are monophagous species, almost restricted to the genus *Gossypium* (*Anthonomus*, *Diaparopsis*), oligophagous feeding on plants in the family Malvaceae and closely related families (*Pectinophora*, *Dysdercus*, *Earias*) or polyphagous (*Helicoverpa*, *Heliothis*, *Cryptophlebia*, *Spodoptera*, *Helopeltis*) (Matthews and Tunstall 1994). The heliothine lepidopteran species complex (*Heliothis virescens*, *Helicoverpa armigera*, *Helicoverpa zea*) is considered as the most dangerous, attacking numerous other cultivated plants which are often associated with cotton in a range of cropping systems (Vaissayre 1995 and Table 2).

The relative economic importance of these different pests varies, depending on the agro-ecosystem considered and changes in response to selection pressure to which they are subject (Kabissa 2004a; King et al. 1996). These changes are particularly notable in low spray environments and where modifications to the growing systems are made possible by the advancement and extension of new agronomic techniques. It is remarkable that sucking pests (*Miridae* and *Pentatomidae*) are today considered as key pests in the mid-south and southeast states of the US cotton belt, even though traditionally it was the progressive

Table 2 Geographic distribution of the *Heliothine Lepidopteran* species complex (Singh and Sohi 2004)

Species	Geographic distribution	Main host plants
Helicoverpa armigera	Africa, central and south eastern Asia, Australia, southern Europe, India, New Zealand and many eastern Pacific Islands	**Cotton**, groundnut, maize, pulses, rapeseed, safflower, sorghum, soybean, sunflower, tobacco, tomato, etc.
Helicoverpa zea	North and South America	**Cotton,** maize, sorghum, soybean, sunflower, tomato
Helicoverpa punctigera	Australia	Chickpea, **cotton**, lucerne, safflower, soybean, sunflower
Heliothis virescens	North and South America	**Cotton**, soybean, sunflower, tobacco, sunflower

migration of the boll weevil from equatorial humid regions which was the main determinant of phytosanitary interventions. This development of the pest complex of cotton adds to the diversity of these systems of culture across the globe and cautions prudence when attempting generalisations about these systems.

Cotton is a weak competitor with weeds, particularly during emergence and the early vegetative stages, as a result of its C_3 metabolism. Weeds can thus cause severe losses to the quality and quantity of the harvest (Bryson et al. 1999). It is for this reason that manual weeding is one the major constraints of the small-scale cotton farmer, while large scale operations have recourse to chemical herbicides. One hundred weed species are recorded as associated with cotton, but only a dozen of these are responsible for significant yield losses. Weeds of foreign origin are the most common and dangerous, as they are frequently more competitive in the absence of their natural control factors. As a result of its great adaptability, this weed flora requires constant attention from the grower, with quantitative and qualitative modifications to the flora impacting rapidly under the effects of environmental and agrochemical selection (Charles and Taylor 2004). Additionally, certain weeds are hosts of cryptogamic or viral diseases and others provide refuge for insect pests, though they may also play an important role in the production of natural enemies of pests (El-Zik and Frisbie 1985; Showler and Greenberg 2003). The management of weed populations can therefore not be undertaken independently of the phytosanitary context of the cropping system as a whole.

Cotton is susceptible to diverse plant diseases. The most significant and the most common of these are cryptogams, frequently associated with the presence of nematodes, particularly *Meloidogyne incognita*. The cotton seedling disease complex comprises principally species of *Pythium*, *Fusarium*, *Rhizoctonia solani* Keuhn and *Thielaviopsis basicola* (Berk. and Br.), but also by *Glomerella gossypii* Edgerton and *Ascochyta gossypii* Woron. The manifestation of these diseases is tightly linked to environmental conditions and is therefore very variable, from one year to another and from one field to the next. Cultural practices have an important preventative role as has the choice of disease resistant varieties. The development of cultural practices and the deployment of resistant varieties have resulted in changes in the importance of different diseases. For example, the systematic treating of seeds, in the USA, has resulted in a very significant reduction in the threat of seedling diseases. On the other hand, the spread of cultivars derived from *G. barbadense* in the USA, India and Israel, has favoured foliar attack by *Alternaria macrospora*.

2.3 Cotton Phenology, Compensatory Growth and Risk Analysis

Three major phenological stages are usually distinguished in cotton grown as an annual plant: (a) plant establishment and vegetative growth; (b) fruit formation; (c) boll growth and maturation. The duration of each of these is variable, depending on the variety, the climate and the agronomic practices adopted, but sufficiently predetermined to enable the establishment of a predictive calendar on the bases of average values. Some varieties are more determinate in their growth patterns than others. More determinate varieties show shorter growing seasons but also less

Table 3 Cropping systems and main pests in the Cotton Belt according to the climatic conditions

Cropping systems and pests	Climate irrigated desert	Climate semi-arid	Climate humid
Cotton production Areas	• *Far West*: Arizona and California	• *Southwest*: New Mexico, Centre of Texas	• *Southeast*: Alabama, Florida, North Carolina • *Midsouth Delta*: Arkansas, Louisiana, Mississippi, Tenessee • *Costal areas* of Texas
Main crops	**Fruits and vegetables** cotton, maize, maize, sorghum, lucerne, thistle, wheat	**Cotton** maize, sunflower, sorghum, soybean, lucerne, wheat	**Soybean and rice** cotton, maize, sorghum, sunflower, wheat, orchards
Main insects	*Pectinophora gossypiella*, *Lygus hesperus*	*Pseudatomoscelis seriatus*	*Anthonomus grandis*, *Helicoverpa zea*, *Heliothis virescens*
Main weeds	*Cyperus* spp., *Ipomea* spp.	*Ipomea* spp., *Amaranthus* spp.	*Ipomea* spp., *Amaranthus* spp., *Senna obtusifolia*

growth compensatory ability in the case of stresses, the risk of which is greater in unirrigated and high temperature conditions (Russell and Hillocks 1996). Taking the earlier example of the American cotton belt and merging the interaction of these factors, significant differences can be seen in the different regions (Table 3). The total period of growth, from planting to harvest, varies from 140 days in the High Plains, to 155 days in the Southeast (including the Mississippi Delta and the Mid South), up to 195 days in the West (El-Zik and Frisbie 1985).

It has been shown that the initial period of plant growth, the 30–40 days after planting, determines the maximum yield which can be expected. The management of later events, including pest attack, will, at best, only allow the development of that yield potential. The often spectacular impact of the range of early season pests like thrips, cutworms, leafminers and aphids, is frequently without significant effect on the eventual yield, because of the strong capacity for growth compensation of cotton during its vegetative growth stage, provided agronomic conditions are optimal (Wilson et al. 2003; Rosenheim et al. 1997). The first fruiting buds appear 5–8 weeks after sowing, later in the Western USA (60 days) then in the Delta (39 days) and in the High Plains (45 days), and the first white flowers open 3 weeks later, between 60 and 80 days after sowing. The majority of bolls are formed during the first three weeks of flowering (85% in the Southeast and the High Plains, 64% in California). Boll maturation therefore commences 65–90 days after sowing and lasts until the last boll opens, which can range from 140 to 200 days from sowing depending on the region. The first bolls to be formed have the shortest maturation period. When taken with the other characters promoting earliness mentioned above, this has militated in favour of early maturing/short season varieties. In addition to minimising the costs associated with having the crop in the ground, there are benefits in reducing the period for build up of multiple generations of some pests, particularly the bollworms, which can have a long period of activity between the 50th and 110th day after sowing (El-Zik and Frisbie 1985; King et al. 1996), but not all. For example sucking pest attack on desiccating short season cottons simply occurs earlier in earlier maturing varieties (Russell and Hillocks 1996).

In conclusion, cotton does not escape the rules of good agricultural practices, which have been summed up in the following way: ‘Early planting, the use of rapid fruiting and early maturing varieties, optimum fertilisation and irrigation, plant spacing, trap crops, early harvest and crop residue disposal have long been recognised and adopted as excellent measures for reducing potential mite and pest damage in cotton production’ (King et al. 1996).

3 The Chemical Cotton Pest Control Paradigm

The commercialisation of synthetic pesticides, in the middle of last century, fundamentally transformed traditional strategies of crop protection. Their remarkable immediate efficacy, their ease of use and their relatively low cost when compared with the benefits obtained, ensured their rapid and widespread adoption. This yield protection, for the first time, allowed growers to capitalise fully on modern techniques of varietal selection, fertilisation and irrigation and to come close to capturing the full genetic yield potential of the selected varieties (Bottrell and Adkisson 1977).

The ongoing improvement in the performance of these insecticides further explains the continuing success of this technical solution, which largely remains the dominant pest control strategy today. However, serious undesirable secondary effects rapidly began to manifest themselves. The major risks, in addition to those arising from the manipulation of highly toxic materials, lay in the lack of specificity of action of the molecules, the persistence in the environment of certain degradation products and the capacity of the pests to evolve resistance to the compounds. These factors not only risked aggravating the pest management situation they were supposed to assist with, but also led to an economic impasse by virtue of their insupportable augmentation of the costs of production. Cotton today takes over 18% of world insecticide use and significant proportions of other pesticides globally (Table 4).

Bearing in mind the yield gains initially obtained with these chemical materials, the temptation for growers was to intensify their use, both to overcome their limitations and as a form of insurance against yield loss, but usually without verifying whether this risk was sufficiently real to justify the costs involved. These practices frequently resulted in unnecessary increases in the pressure of both key and secondary pests, due to the reduction of the beneficial arthropod fauna which is unintentionally decimated by these non-selective treatments. To an extent then, increasing pesticide applications against an unquantified risk became a self-fulfilling prophesy, generating pest populations requiring control. The spontaneous reaction of most growers was to increase the dose and frequency of insecticide applications, which additionally selected strongly for evolved resistance. The phenomenon of cross-resistance between diverse families of active ingredients frequently rendered inoperable any recourse to other insecticide classes. This situation of increasing use of ineffective materials gave rise to the expressions ‘the pesticide treadmill’ (van den Bosch and Aeschlimann 1986) and ‘the chemical paradigm’ (Perkins 1982; Walter 2003).

3.1 *The Pesticide Treadmill*

Since the invasion of the US cotton belt by the boll weevil at the end of the nineteenth century, American cotton producers have oscillated, several times,

Table 4 World market of the different families of pesticides in 2002 (in million US $) (Bocquet et al. 2005, modified)

Crops	Herbicides	Insecticides	Fungicides	Other*	*Total*
Straw cereals	17.3	3.6	21.7	18.3	14.9
Maize	18.1	8.8	0.1	0.9	11.3
Rice	7.4	11.7	10.2	6.5	9.1
Soybean	14.6	1.9	1.7	2.1	8.2
Colza	3.0	0.9	1.6	1.2	2.1
Sunflower	1.4	0.3	0.1	0.1	0.9
Cotton	**3.7**	**18.3**	**0.7**	**23.6**	**7.6**
Sugar beet	3.5	0.7	0.8	0.4	2.2
Sugar cane	2.1	1.2	0.0	1.0	1.4
Potatoes	1.5	3.7	8.6	3.8	3.7
Vineyard	1.1	2.3	11.1	2.7	3.6
Pip fruits	0.8	4.1	6.2	2.6	2.9
Other fruit and vegetables	8.5	28.3	24.1	19.4	17.3
Other crops	16.4	13.3	12.3	16.8	14.8
Total (%)	100	100	100	100	100
Total ($ mill)	**12,490**	**6,363**	**5,425**	**872**	**25,150**

*Growth regulators, anti-slug specialities, nematicides from optimism to doubt with regard to the efficacy of chemical control. We can establish the accelerating succession of active materials used. For example, in the Mid South and Southwest: (a) calcium arsenate, from the 1920s to the mid 1940s, applied for the first time by airplane. The secondary effect of these applications was to make aphids and the bollworms/budworms into pests of major importance, (b) DDT and other organochlorines, from the mid to late 1940s until 1972, with resistance to DDT from 1961. This favoured infestations of spider mites by elimination of their natural enemies, (c) organophosphates and carbamates, in the late 1950s until the present, with resistance manifesting itself from the early 1970s, inducing the use of mixtures of different organophosphates, (d) pyrethroids alone or in mixtures, from the early 1980s until today (King et al. 1996; Reynolds et al. 1975).

Doubt has frequently been transformed into despair, when the accelerating frequency of treatments and the simultaneous increase in doses, without increases in efficacy of control, has led the producers to an economic impasse (NAS 1976). Many of the examples of these catastrophic situations relate to the USA (Smith and Reynolds 1972), but the most spectacular concern the countries of Latin America (El Salvador, Guatemala, Nicaragua, Peru). In these regions, it was not unusual to apply 20 sprays of DDT, if not more, in the late 1940s (Wille 1951). In the particular case of the Canete valley in Peru, by 1956 it had become practically impossible to control pest outbreaks despite repeated applications (15–25 per season). In Central America (El Salvador, Nicaragua) the frequency of the interventions reached 30 times, even 50 times, in a period of 90 days, (Boza Barducci 1972; Smith and Reynolds 1972). In states like Louisiana (USA), where more moderate schedules were implemented, their ecological consequences were nonetheless disastrous. When unfavourable climatic conditions, in particular in years of drought, were added to the critical phytosanitary position, it was common for producers to abandon the cultivation of cotton (Newsom 1972; Smith and Reynolds 1972).

It is in this context that a diagnosis was made of the situation (Doutt and Smith 1971; Smith and Reynolds 1972). This provided the rationale for the development of pest scouting to which most authors still subscribe today. The embracing of pesticides in cotton production followed five successive stages in a repetitive cycle:

(a) In an initial phase, the growing of cotton is one of the elements of subsistence agriculture, with very low yields and no system of phytosanitary protection;
(b) Whenever irrigation is possible, cotton becomes one of the most profitable crops and becomes a major resource, justifying protection measures; this is the exploitation phase, in which growers have recourse to chemicals applied on a calendar basis;
(c) After some years of production of this blind and often intense chemical application, its efficacy declines; it becomes necessary to commence applications earlier in the season and to prolong them up to harvest; often pest populations reappear at higher levels than originally after these applications; the substitution of one active ingredient for another does not improve the situation; occasional secondary pests become permanent, major pests. This is the crisis phase, which is generally marked by an intensification of chemical treatments and a marked increase in the costs of production;
(d) The profitability of production is then brought into question, first in production systems on poorer soils, then over whole production areas; this is the disaster phase;
(e) There may then be a following recovery phase, provided the phytosanitary strategy is changed and is more objective, following the concepts of integrated production and pest control described below.

3.2 The Staggered Targeted Control System, A Step Towards Integrated Control of Cotton Pests

Full IPM systems, where interventions are made only on the basis of pest forecasts made from scouting, require a level of sophistication on the part of the users, which is difficult to obtain and maintain, especially in small-farmer systems where a weak extension service is likely to be added to the farmers own lack of knowledge and input resources. For that reason, calendar treatments, with pre-determined insecticides at pre-determined rates, were the mainstay of most national recommendations in most developing countries for many years. Appreciating that this frequently resulted in unnecessary applications and unnecessarily high doses, but at the same time appreciating that subsistence farmers cannot afford to take risks with the yields of what is often their only cash crop, scientists in West Africa have developed techniques of calendar treatments, determined locally and recommended by the local scientists and extension agents in response to the development of the cotton crop and the local pest population dynamics. These have had a lasting success in francophone Africa, providing a limited form of rational crop protection. These decisive technical advances have permitted local variation, as a function of infestation levels and risk, of the quantities of active ingredient required by a range of different intervention programmes (Cauquil and Vaissayre 1995; Silvie et al. 2001). In different areas and in response to different farmer constraints, extension capacity and insecticide availability situations, the following programmes have been used, forming a sort of progression towards a full IPM programme:

(a) The conventional ultra low volume programme (ULV): 4–6 treatments on a calendar basis, made every 14 days starting on the 45th day after plant emergence, using ultra low volumes of 1 L/ha, using pre-prepared pyrethroid/organophosphate mixes in ultra low volume formulations.
(b) The dose-frequency control programme: 8–12 calendar treatments made at 7 days intervals, starting at the 45th day after plant emergence, in very low volume formulations of 10 L/ha, using one third of the doses utilised in the preceding programme.
(c) The staggered control programme: 4–6 treatments on a calendar basis every 14 days, each one followed 7 days later by scouting the field for pests, allowing variation in the doses of insecticide used in subsequent applications, using very low volume formulations. In this programme, the types of active ingredient remain determined in advance of the season. At least a reduced dose of insecticide is systematically applied, as a security measure, at each of the 4–6 calendar application dates. Higher doses may be used if the pest situation warrants it.
(d) The staggered-targeted control programme: a calendar spray programme, where the choice of insecticides and of doses applied are a function of observations of the pests in the field: (1) where the observations are made on the eve of calendar treatments timed every 14 days from the 45th day after plant emergence. This allows the user to define the type and dose of active ingredient for each application, without modifying their number, which remains the classic 4–6 per season; (2) where observations are made in the field 6 days after the calendar treatment (timed every 14 days), this allows a supplementary application to be made where necessary on the day following the scouting and therefore 7 days after the preceding calendar treatment. In principle the number of treatments varies from a fixed 4–6, where intermediate applications are found to be unnecessary, to 8–12 in the extreme case where such applications were always necessary (Vaissayre and Deguine 1996). Whatever the results of these applications, at least a reduced does of insecticide is always applied at the calendar dates in both these versions of the staggered-targeted control programme (STC).

The ease of utilising the low, or very low, volume equipment and the economies generated by a reduction in the quantity of active ingredient applied, which often reaches 40–50%, has assured the success of these strategies – particularly in Mali and Cameroon (Fig. 1). However, there are inevitable difficulties produced by the necessity for the small-scale farmers (frequently semi-literate) to undertake the necessary scouting of the fields and to make the choice of the insecticides and the appropriate dose to use. It is for this reason that the promoters of these schemes have recommended limiting the field observations to estimates of the levels of the major bollworm, *H. armigera* (Silvie et al. 2000, 2001).

The utilisation of pyrethroid/organophosphate mixtures in these strategies of rational control, specific to the countries of francophone Africa, is likely to have been a major reason why the phenomenon of evolved resistance to the pyrethroids, so common elsewhere in the world, did not manifest itself in W. Africa until 1996. It is now known that the organophosphate component of the mixtures undermines certain mechanisms of metabolic resistance to the pyrethroid component, thus restoring its efficacy. From the end of the 1990s, it has been recommended that the first two calendar applications be made with endosulfan or

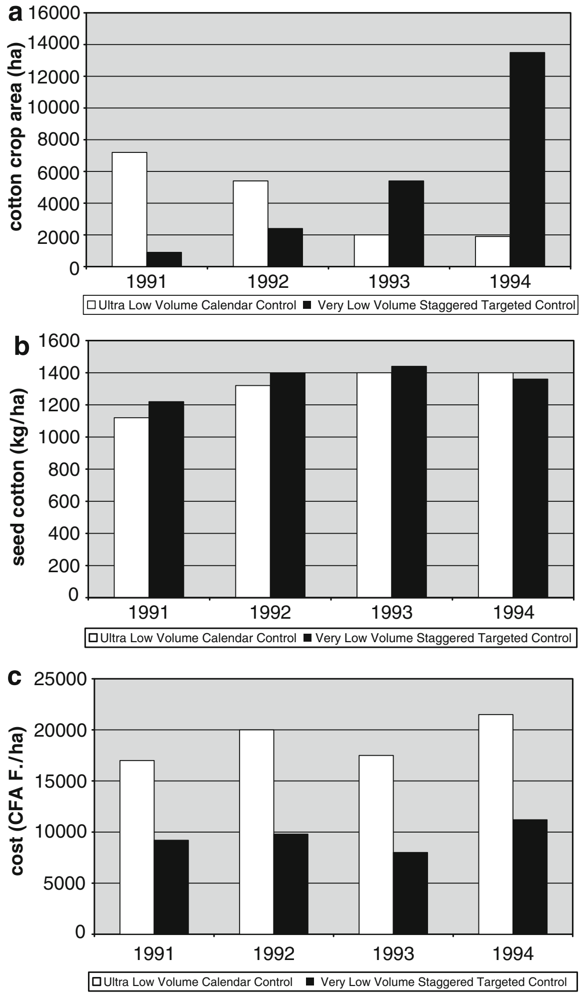

Fig. 1 (**a**) Changes in cotton crop area according to the type of the protection programme in Cameroon (Touboro region); (**b**) Yield over time according to the type of the protection programme in Cameroon (Touboro region); (**c**) Changes in the cost of protection according to the type of the protection programme in Cameroon (Touboro region)

profenophos, which do not show cross-resistance with the pyrethroids (Martin et al. 2004). Alternatives to the employment of endosulfan have been proposed, using the new and non cross-resisted active ingredients of spinosad and indoxacarb. If these are widely adopted then the staggered, targeted control systems will come closer to the Australian 'window' strategy described below, but with the addition of the compulsory, calendar aspect of applications for yield insurance (Nibouche et al. 2004; Ochou and Martin 2003).

3.3 Conventional Cotton Crop Protection at the Crossroads

There is only a very limited range of novel active ingredients for cotton insecticides able to replace those materials which are severely compromised by evolved resistance. It is eminently sensible to pay particular attention to alternative strategies, including those offered by biotechnology or by techniques of cultural control of pests (Carter 2005). In addition to indoxacarb (oxadiazine) already mentioned, the main newer materials are methoxyfenozide (diacylhydrazine), various insect growth regulators and imidacloprid (neonicotinoid), frequently utilised as a seed treatment for preventative management of aphids, leafhoppers and whiteflies in the early part of the growing season. Amongst the newer active ingredients of biological origin, in addition to spinosad, the avermectins (emmamectin benzoate) and clorfenapyr (pyrrole) offer useful control materials (Russell 2004a). This modest list of newer (and non-cross resisted) active ingredients is added to reducing number of 'conventional' insecticides (essentially organophosphates, carbamates, pyrethroids and endosulfan), as these are withdrawn from many markets in the name of preservation of the environment (Casida and Quistad 1998; Horowitz and Ishaaya 2004).

Amongst the new techniques under development, those relevant to site-specific, or precision farming, are often presented as an appropriate response to current economic and environmental constraints in cotton production. They currently reside in the domain of research, envisaging an improved control of the use of phytosanitary products based on the growing characteristics of the crop and the level of infestation. The optimisation of classic techniques of spray applications have already allowed important progress to be made. A new stage has been reached recently with the ability to record intra-field variability in yield through the use of remote sensing and geopositioning systems with computer-aided decision tools. At present this is limited to the application of fertilisers, plant growth regulators and defoliants (Bagwell et al. 2005; Gurr et al. 2004; Hanks et al. 2005). Experiments using pest management products under complex protocols have been undertaken in the USA, where they are proving to be of great interest.

4 Integrated Cotton Pest Management

In reaction to the pressing phytosanitary situation, two apparently divergent concepts will be elaborated here in parallel, as being particularly well illustrated in cotton culture: the eradication and the management of pest populations (Myers et al. 1998; Perkins 1982). These two concepts have today converged in the idea of area-wide pest management (Hardee and Henneberry 2004). Of particular importance in gaining producer acceptance of integrated pest management since the 1980s has been the increase in costs of production caused by the progressive development of insecticide resistance.

4.1 Eradication–Suppression Strategy, or Total Cotton Pest Management

The spectacular success of eradication operations, by release of sterile males against the screw worm fly, *Cochliomyia hominovorax*, and the immediate and remarkable success of synthetic insecticides in the absence of resistance, encouraged the development of the combined use of these two techniques against plant pests in the 1960s in the USA. Two preliminary experiments (the Pilot Boll Weevil Experimentation Trials) took place in Louisiana/Alabama (1971–1973) and in North Carolina and Southern Virginia (1980–1982). At the same time the Optimum Pest Management Trial, over an enormous area in Mississippi, enabled the protocols to be validated in cotton. Despite the sometimes hotly debated results, the strategy was progressively applied with success, from 1993, to the majority of states in the US cotton belt, allowing a reduction of approximately 50% in the quantity of pesticides used, while augmenting the yield by around 10% (King et al. 1996).

The programme of eradication/suppression took place in three successive stages of 3–4 years each: (1) mapping to identify the exact location of each cotton field using Global Positioning Systems (GPS), (2) monitoring with boll weevil pheromone traps using a standardised trapping density and placement appropriate to active-eradication and post-eradication zones, (3) the application of cultural, mechanical and chemical control measures. In most of the area, this began in the autumn with seven aerially applied chemical treatments in infested fields for the control of diapause individuals. The following year, applications were made from bud formation to harvest at a level depending on the results of earlier scouting. Over the years, the number of fields being treated fell considerably. A system of surveillance for possible re-infestations was put in place, in particular through the use of pheromone traps (Grefenstette and El-Lissy 2003). In South America especially, traps luring boll weevils with pheromones and host plant volatiles and then killing them with malathion, have played a major part in the control of weevils and the prevention of their spread, often supported by national cotton re-invigoration programmes (Plato et al. 2007). The key to success of these operations lies in the adhesion of the producers to an internally agreed programme of collaboration and of intensification of insecticide treatments, and to a good level of co-operation between federal and state agencies and the other players in the cotton production system. The quality of the technicians assisting locally in the eradication strategy has been of central importance. However, finding finance to continue the eradication–suppression programme, remains a major headache for the USA, despite the reduction in costs which continues to benefit producers (Smith 1998).

At the end of the 1960s, an eradication programme for the pink bollworm (*Pectinophora gossypiella*) using the male sterile technique was also in place in California, as part of community-wide and season-long pest management programme (EL-Lissy et al. 2003; Henneberry and Naranjo 1998; King et al. 1996). In 2001 a bilateral programme of action between Mexico and the United States was adopted on the basis of four intervention strategies: (1) extensive pest surveys; (2) transgenic, lepidopteran caterpillar resistant, cotton; (3) pheromone applications for mating disruption; and (4) releases of sterile pink bollworm moths.

4.2 Cotton Pest Integrated Control, An Unaccomplished Concept

Having brought together a panel of experts, in 1967, to define the concept of integrated control, the FAO attempted to draw up a compendium of practices for the main cropping systems, amongst which was cotton (Frisbie 1984). In the meantime, the strategy had evolved, moving from a harmonious combination of chemical control measures to a management of populations in the same agro-ecosystem, under the title 'Integrated Pest Management' (IPM). The ecological bases of this new concept, with its three levels of complexity – population, community and ecosystem – were eventually validated in the conclusions of the UN Conference on Environment and Development (Rio de Janeiro, Brazil 1992) which assigned a central role for IPM in agriculture as part of its 'Agenda 21' (Kogan 1998).

Bearing in mind the concern to support the use of natural factors in the regulation of pest populations and to apply alternative biological control techniques, the initial preoccupation was to limit the role of chemical control. The innovative programmes then being practiced in California made use of the following major methods: '(1) pest sampling and population prediction methods; (2) pest economic thresholds; (3) naturally occurring biotic mortality agents and their role in restraining or suppressing pests and potential pest species; (4) the impact of artificial control practices on them; (5) pest phenologies as they relate to injury potentials and the timing of artificial control measures; (6) cultural and agronomic practices and their possible employment in insect population management; (7) development of alternative or supplementative ecologically selective chemical and microbial controls' (Flint and van den Bosch 1981). In the majority of cases, however, only the relevant, simple, criteria plus the principles of rational chemical control, rather than a true IPM, were used. So for example (1) scouting and economic injury levels for spray decision making, and (2) the use of more effective pesticides or application of lower doses of broad spectrum insecticides, were being used in the USA in the mid 1980s to evaluate the impact of these technical innovations on four major crops (apple, cotton, lucerne and soya bean). The evolution of true IPM programmes was slower than might have been wished (Ehler and Bottrell 2000; Kogan 1998).

A major role was given to pest scouting as a technique for the prediction of risks. This had been undertaken for a long time under a series of empirical rules, but became the key to all economic and management decisions with more sophisticated protocols and risk calculation methods. The growers, used to 'insurance' insecticide applications on a calendar basis, were soon confronted with the difficulties of putting into practice sampling and diagnostic techniques regarded as necessary for decision making. In the countries where cotton is both intensively and extensively grown, particularly USA and Australia, it is not uncommon for these new activities to be undertaken by professionals – crop consultants or pest managers (King et al. 1996). The capture of computerised data in the field made it possible to design dynamic injury threshold levels, adjusted to the stage of plant development (King et al. 1996). In the developing countries, where the majority of the producers are often semi-literate and reliant on their own knowledge in the absence of an adequate extension system, the FAO organised, in collaboration with local institutions, programmes of farmer education (Farmer Field Schools or FFS) in cotton, on the system initially developed for the promotion of IPM in rice (Ooi 2004; Russell 2004b). These season-long processes of education of groups of farmers in the principles and practices of IPM through a discovery learning process are undoubtedly effective in locally raising the understanding of the cotton agro-ecosystem and in raising yields while reducing pest management costs (Prudent et al. 2006, 2007). Ooi et al. (2005) report on the results of the largest of these cotton FFS, in seven Asian countries from 2000 to 2005. However, FFS suffer from their relatively high costs of implementation (especially in the training of trainers) which has made it difficult for them to have any major impact over significant areas (there are for example 2,360 cotton farmers per 1,000 ha in Bangladesh as opposed to about 10 in the same area in the USA) (Russell 2004b). Simpler, less knowledge intensive but cheaper, programmes have been put in place in Uganda (with USAID support) using simple pest scouting pegboards to assist in decision making for the key pests (Matthews 1996; Sekamatte et al. 2004a, b) and have been demonstrated nationally in India in the context of insecticide resistance management (see below).

4.3 Cotton Pest Insecticide Resistance Management and the 'Window Strategy'

Faced with the importance of harvest losses occasioned by pest populations resistant to insecticides, insecticide resistance management (IRM) strategies have been developed, sometimes with great urgency. This new pressure has frequently distracted the attention of growers from other aspects of IPM. A number of strategies were developed in the 1970s, with examples in Zimbabwe, Egypt and Australia (Sawicki et al. 1989). The Australian example grew to provide a global model for sustainable management in cotton systems (Forrester et al. 1993; King et al. 1996; Kranthi et al. 2004a, b; Russell 2004b; Russell et al. 2000). In 1983, pyrethroid treatments for control of caterpillars of *H. armigera* failed in central Queensland. The problem concerned not only cotton producers but equally the majority of farmers in the region, bearing in mind the polyphagous nature of the pest (chickpea, lupin, wheat, rapeseed, safflower, sunflower, maize, sorghum, pigeon pea etc.). The goal therefore was to develop and implement a strategy appropriate to all the farmers in the region. The upshot was a new strategy, the 'window strategy' building on earlier understanding of the management of populations (Geier 1966).

Applying the idea of economic damage thresholds, at most three successive applications of pyrethroids were allowed during an interval of no more than 35 days (Window Stage II), in the middle of the vegetative growth period (September to the end of April). This period corresponds to the minimum development period for a generation of *H. armigera* in the field, out of the 4–5 annual generations (not all in cotton). The growing season was divided into three windows:

(a) Window Stage I – from September to January (with sowing of cotton in mid-November), where only applications of endosulfan, thiodicarb, products from *Bacillus thuringiensis* and later methomyl or chlordimeform as ovicides could be used, in order to preserve the beneficial arthropod fauna and avoid infestations of mites, whiteflies and aphids.
(b) Window Stage II – from the beginning of January to the beginning of February, allowed the use of endosulfan or pyrethroids (with a maximum of three applications).
(c) Window Stage III – from February to the end of April, excluded the use of endosulfan and permitted the use of organophosphates (Forrester et al. 1993).

These arrangements eventually became part of an enlarged programme of integrated resistance management, involving spatial considerations (mosaic and refuge strategies) and temporal considerations (alternations, rotations and window strategies). Today the enlarged programme comprises five successive Windows, thanks to new understanding of the biology of populations in relation to insect resistant GM cotton (Holloway 2005).

A modified, and of necessity simplified, version of this window strategy was developed for the millions of Indian cotton growers (Aggarwal et al. 2006; Kranthi et al. 2004a, b; Russell et al. 2004). With donor and then national support, this has become the recommended cotton pest management system on India's 8 million ha of cotton, approximately halving insecticide use, increasing yields and doubling cotton profitability for adoptees in all 11 cotton states (Russell and Kranthi 2006).

The manifestation of resistance to herbicides by weeds did not really begin to pose a problem for intensive agriculture until the mid 1970s, with the use of triazine, but has become a significant issue subsequently. After a phase of passing from one active herbicide ingredient to another, this phenomenon gave rise to a movement for integrated weed management (IWM). Its promoters emphasise the importance of taking into account the whole agricultural system within which the weed is present (Buhler et al. 2000). In Australia, the objective is the development of a system which progressively reduces the weed seed bank in the soil while continuing to ensure the sustainability of the on-going crop production (Charles and Taylor 2004). The recommendations, again essentially limited to the localised actions at the level of the single producer, are intended to assist the growers to reduce their herbicide use and slow the development of herbicide resistance (Roberts 2000).

5 Biologically Based Integrated Cotton Pest Management

In the 1990s the phytosanitary industry launched a new generation of active ingredients under the name of biorational, or low-risk pesticides. These had lower toxicity to non-target organisms and the environment. Expressions such as 'biologically intensive IPM' held appeal as a return to more natural control systems and in many cases did indeed prove effective (Frisbie and Smith 1991). Bajwa and Kogan (2004) survey these new initiatives and assess their contribution to the management of pest populations, when deployed in conjunction with cultural control practices, selection of IPM compatible varieties and the deployment of genetically transformed plants. Spurgeon (2007) reviews ecologically based IPM in cotton to 2003 for the US situation.

5.1 Biotech Cotton: Springboard to IPM or Another 'Technological Fix'?

From 1995, the commercialisation of Bt-cotton has permitted a reduction of 50–80% in the number of insecticide treatments against the most dangerous lepidopteran caterpillars, with variable gains in yields depending on the efficacy of the preceding chemical control practices (Shelton 2002). Rushed, and sometimes illegal, adoption of agronomically inappropriate varieties containing the Bt genes has from time to time resulted in yield losses in certain areas, particularly parts of India in the early years of Bt cotton commercialisation. The first Bt cotton generation was developed primarily with *Heliothis viriscens* and *Pectinophora gossypiella* in mind. However, other species, particularly *Helicoverpa armigera,* are primary targets in the Old world, despite its lower sensitivity to Cry1Ac toxin, which is of increasing importance as the bioavailability of the toxin in the plant declines later in the season (Dong and Li 2007; Kranthi et al. 2005; Olsen et al. 2005; Rochester 2006; Wan et al. 2005). Infestations of less susceptible pest groups such as the hemiptera, is favoured by the reduction of chemical applications against the key lepidopterous pests and this sometimes necessitates additional insecticidal interventions (Fitt 2004; Men et al. 2005; Wu et al. 2002). The additional seed price and/or 'technology fee' charged by the biotechnology provider can greatly affect the economics of the deployment of Bt cotton. An economic study undertaken across diverse ecological regions of Argentine during the 1999/2000 season showed that the growing of Bt cotton did indeed result in a reduction of insecticide applications, however the saving was insufficient on its own to offset the increased price of the transgenic seeds. A yield advantage of c. 460 kg/ha of seed cotton would be necessary to achieve additional profitability (de Bianconi 2002). In the small-scale farming systems of South Africa, it is the absence of a realistic assessment of risk which has led growers to continue their programmes of frequently unnecessary insecticide applications, reducing the profit potential of Bt cotton and this phenomenon is increasingly seen in China and India, emphasising the need for farmer training in the utilisation of transgenic cotton (Hofs et al. 2006a, b; Vaissayre et al. 2005).

As the commercialisation of Bt cotton increases rapidly, the risks of evolved resistance come increasingly to the fore. National systems generally oblige growers to set aside non-Bt cotton areas of host plants for the key pests (refuge zones), to allow the dilution of any genes for resistance through genetic mixing with populations of susceptible insects emerging from the refugia. In the USA, for example, a high dose/refugia strategy has been adopted, where the toxin level in the plant is calculated as being much more than is necessary to kill any heterozygous resistant insects which might emerge from the mating of extremely rare homozygous resistance insects from the Bt crop with the much more common homozygous susceptible insects from the refugia. Three different options are available to producers today (5% external unsprayed refuge; 20% external sprayed refuge and 5% embedded refuge), depending on the local cropping patterns and the understanding of the movement of insects between crops and areas (Tabashnik et al. 2005). A collective arrangement which could be put in place by groups of farmers is under study. In 2007, the United States Environmental Protection Agency (EPA) has approved a natural refuge option for Bollgard II cotton planted from Texas, excluding some counties where pink bollworm is a significant pest. In these eligible regions cotton producers can take advantage of non-cotton crops, where cotton bollworms and tobacco budworms are present, as a refuge. These recommendations may require alteration in the light of new understanding of the mode of action of these toxins and the modalities of resistance development in the various pest species concerned (Andow and Zwahlen 2006; Carrière et al. 2004; Vacher et al. 2003). In particular the high dose/refuge strategy assumes that the evolved resistance will be recessive with respect to the toxin levels in the plants currently deployed. This proves not to be the case for *H. armigera* in China or India (Russell 2004b).

In Australia, under comparable growing conditions with large areas of cotton monocrop, but with a pest complex dominated by *H. armigera*, the emphasis has been on good agricultural practices, with the most important of these being to limit the area of cotton sown with single gene Cry1Ac Bt varieties to a maximum of 30% of the cotton grown per farm unit (Fitt 1997). By contrast, in China and India, where cotton is cultivated by a large numbers of growers practicing a polyculture, many of which crops are alternate hosts for *H. armigera*, the need for planted refugia has not been so pressing. China has no policy for planted refugia. India has an one, modeled quite closely on the US system, but encouraging the planting of refuges as border rows to Bt cotton fields. However, this policy is widely disregarded by growers (Ravi et al. 2005; Russell and Deguine 2006; Sharma 2005; Wu et al. 2004). In the case of *P. gossypiella*, which is restricted to feeding on the Malvaceae which, with the exception of okra, are uncommon in cropping systems, this attitude to refugia may prove unwise.

The second generation transformed Bt varieties express simultaneously more than one entomotoxin (gene pyramiding), allowing an enlargement of the spectrum of activity of the Bt plants to include activity against other species (Table 5). The widespread Cry1Ac/Cry2Ab plants are effective against *H. armigera*, *H. zea*, *Heliothis virescens*, *Spodoptera frugiperda*, *S. exigua* and *Pseudoplusia includens* (Sankula and Blumenthal 2004). The Chinese inclusion of cowpea trypsin inhibitor with Cry1Ac in some varieties may add a level of anti-feedant activity for certain sucking pests. Stacking genes for non-cross resisted toxins which have efficacy against key pests provides a further bulwark against resistance development (Andow and Zwahlen 2006). In Australia, production has moved entirely to dual-gene Bt cottons with the permitted area of Bt cultivation rising from 30% to 70% of the total (Holloway 2005).

The genetic determination of resistance to these entomotoxins seems likely to be less simple than originally expected and the efficacy of the measures in place to prevent resistance may be significantly less than hoped (Ferré and van Rie 2002). The emerging complex pattern of pyramided genes may also exacerbate the risks of resistance development (Gahan et al. 2005; Gurr et al. 2004). Xu et al. (2005) found a truncated cadherin (cell adhesion molecule), which is a binding site for Cry1Ac in the insect gut, to be a mechanism of resistance in Chinese *H. armigera*, but the pattern of sites of action and consequently cross-resistances seems likely to be as complex with Bt toxins as it has been with conventional insecticides. Similar observations were made in India and on pink bollworm strains from Arizona (USA) (Gujar et al. 2007; Tabashnik et al. 2005).

Genetically modified cottons expressing genes conferring herbicide tolerance are also experiencing a growing commercial success. These two properties, herbicide tolerance and insect resistance, sometimes in tandem, were present in 28% of global cotton production in 2005 (Bt cotton 4.9 mha; Bt/herbicide tolerant cotton 3.6 mha; herbicide tolerant cotton 1.3 mha (James 2005)). Globally, plants on 82% of

Table 5 Characteristics of the commercial varieties of Bt Cotton

Commercial name	Company	Expressed Bt genes	Spectrum of efficacy
First generation			
Bollgard	Monsanto (U.S.A.)	Cry1Ac	*Heliothis virescens*, *Helicoverpa armigera*, *Pectinophora gossypiella*, *Earias spp.*
Guokang	Academy of Sciences of China	Cry1Ac	*Helicoverpa armigera*, *Pectinophora gossypiella*, *Earias spp.*
Second generation			
Bollgard II	Monsanto (U.S.A.)	Cry1Ac and Cry2Ab	*Heliothis virescens*, *Helicoverpa armigera*, *Pectinophora gossypiella*, *Earias* spp., *Spodoptera frugiperda*, *Spodoptera exigua*, *Trichoplusia ni*, *Pseudoplusia includens*
WideStrike	Dow AgroSciences LLC (U.S.A.)	Cry1Ac and Cry1F	*Heliothis virescens*, *Helicoverpa armigera*, *Pectinophora gossypiella*, *Earias* spp., *Heliothis zea*, *Spodoptera frugiperda*, *Spodoptera exigua*, *Trichoplusia ni*, *Pseudoplusia includens*, *Estigmene acrea*

the area cultivated with all transgenic crops (73.8 mha) are today expressing tolerance to the same herbicide molecule, glyphosate, creating a particularly favourable environment for the growth of resistance by weeds. The significance of this risk is acknowledged in places like Australia with the development of Integrated Weed Management Systems (IWMS) with the same urgency as for the IPM cotton strategy (Charles and Taylor 2004; Owen and Zelaya 2005).

Within the limits of our current knowledge, it seems likely that the transgenic cottons will eventually suffer from the same resistance issues as the sprayed insecticides. Their rational deployment within integrated management practices is therefore essential if they are to have a prolonged commercial life. The exclusive reliance on the high dose/refugia strategy may be misguided (Hilbeck 2001).

5.2 Conservation Biological Control of Cotton Pests, Another Challenge?

Classical biological control, involving the introduction and acclimation of beneficial organisms, has not produced real successes in cotton production (Greathead 1995; King et al. 1996; Russell 2004a; Sterling et al. 1989). The principal reasons for this are the fact that cotton is an annual crop, the range of pests to be controlled and the importance given to insecticide treatments early in the season, often with broad-spectrum materials. By contrast, inundative biological control with beneficial arthropods or live microorganisms has had successes, despite the technical and regulatory difficulties faced. Various programmes report success with parasitoids (esp. egg parasitoids *Trichogramma*/*Trichogrammanza* sps. and *Bracon*/*Habrobracon* larval parasitoids) and with predators (esp. the *Chrysopa*/*Chrysoperla* lacewing generalist predators) and Baculoviruses (esp. nuclear polyhedrosis viruses for control of *Heliothis*/*Helicoverpa* sps. and *Spodoptera* sps.) (King et al. 1996; Sharma 2005). Inundative releases of beneficials have been particularly widely used in China and the countries of the ex-USSR, although it is difficult to establish cost/benefit ratios in those centrally directed systems. This is further complicated by the deployment of these beneficials in systems utilising other techniques simultaneously, including reductions in the use of broad-spectrum insecticides (Greathead 1995; Wu and Guo 2005). Success in cotton has not been high, except perhaps in areas such as Uzbekistan, where severe winters and local production of *Trichogramma pintoi* parasitoids allowed immediate and local responses to the first spring emergence and egg-laying of moths.

In the Middle-East, where the pressure exerted by pest populations is locally weak (e.g. Syria and parts of Turkey), a dominant role is accorded to biological control. Over the last 25 years in Syria, the percentage of the cotton area receiving insecticide treatments has declined from c. 25% to 0.5%. This result is the outcome of a deliberate change in the phytosanitary strategy in the context of planned production, perhaps more for economic than environmental reasons (ICAC 1998, 2004, 2005). To this end, the intervention thresholds for the principle pests (including *H. armigera*) have been considerably relaxed to reduce the consumption of insecticides, access to which is strictly controlled in compliance with the new norms. From the outset, early sowing of short-stature and small-leaved cotton varieties improves the phytosanitary prospects for the crop, with improved light interception, improved air movement in the foliage, reduced humidity and strong vegetative growth before the principal pests appear. Biological control is undertaken with inundative releases of *Trichogramma principium*, multiplied in government laboratories, and of other beneficials, such as *Encarsia formosa*, *Orius laevigatus* and *Eretmocerus mundus* against white-fly, *Bemisia tabaci*. Varieties resistant to *Verticillium albo-atrum* and appropriate cultural control techniques (seed density, irrigation optimisation, manual de-topping) help to prevent the appearance of bacteriosis caused by *Xanthomonas campestris* pv. *Malvacearum*. Under these conditions, the yields obtained are among the highest in the world (around 1,300 kg of cotton lint per ha (ICAC 2006)). Comparable results have been reported from the Aegean and South Eastern Anatolian regions of Turkey (Özkan 2004).

In Australia, the focus is on the conservation management of existing indigenous beneficial organisms (Wilson et al. 2004). Apart from the lucerne/cotton companion planting system described below, this strategy is unique in the extent to which it takes into account predator/pest ratios for launching specific remedial action, such as the provision of a supplementary food spray to maintain the predator populations, insecticidal treatments based on selective biopesticides and synthetic insecticides with reduced secondary effects (such as spinosad) (Mensah and Singleton 2004). This same strategy was trialed in Texas without having the same success, but in a very different beneficial insect and agronomic context (Slosser et al. 2000).

Generally speaking, any management practice which respects the classical tenets of IPM or facilitates the use of Bt cotton and the reduction in unnecessary insecticide treatments, supports the role of the indigenous beneficial arthropod fauna, which are often insufficient by themselves (Romeis et al. 2006; Symondson et al. 2002). A new biological control strategy may then be envisaged, utilising the appropriate management of habitats relevant to the biology of natural enemy conservation (Barbosa 1998; Gahukar 2006).

5.3 Varietal Selection, Cultural Practices and New Agronomic Systems

The role of classical varietal selection needs to be acknowledged alongside that of transgenic plants and of cultural practices. By the end of the nineteenth century the growing of short season cottons was recommended in Texas to limit the effects of the boll weevil (King et al. 1996; Russell and Hillocks 1996). There are many examples of the selection of disease resistance against bacterial or cryptogamic diseases (Hillocks 2000), of which the widespread use across Africa of bacterial blight tolerant, 'Albar', varieties developed in Sudan is one of the best known. The principal characters selected for insect resistance are the gossypol gland density, nectariless, okra leaf shape, frego bract and leaf hair and their combinations (Scheffler 2004). Today the focus is on the development of cultivars which are adapted to specific growing systems, thanks to the on-going research into the interactions of genotype x growing system (Belot et al. 2005; Constable 2000; Fok 2000). Earliness remains a principal research preoccupation. Maximising the benefits of earliness requires the judicious management of agronomy, sowing dates, irrigation practices, fertilisation, and the use of chemical growth regulators. Plant architecture is another consideration, with interest in narrow-row or ultra-narrow-row cotton cultivation practices, especially in the Xinjiang Northwest inland cotton region of China where around one million hectares of cotton is grown this way using plastic film mulching to improve emergence rates and weed control. These systems are now finding favour in Argentina, Australia, Brazil, the USA and other countries, thanks to the opportunities provided by the application of herbicide on GM herbicide tolerant varieties (Rossi et al. 2004). Use of this technique shortens the growing season by 2–3 weeks, while providing superior yields; always assuming that an appropriate management system is in place, frequently with the use of growth regulators and stripper-shaker harvesters. The phytosanitary consequences of these techniques are as yet poorly understood, but the increase in total root volumes caused by the increased plant density may favour subterranean pests such as nematodes and cryptogamic diseases.

This cultural technique is often found in association with low-tillage systems, resulting in a very highly modified physico-chemical environment for cotton growth. In addition to improving the structure and porosity of soils there is an increase in the diversity and abundance of living organisms in the fields, both of vertebrates and invertebrates (Fawcett and Towery 2002; Stinner and House 1990). Following studies undertaken in various parts of the US cotton belt, pest populations do not seem to be especially favoured by these practices, with the exception of various species of cutworms, grasshoppers, the three-cornered alfalfa hopper and aphids (McCutcheon 2000). There is as yet no definitive set of phytosanitary recommendations to accompany these cultural practices (Stewart 2003). However, systematic studies have been undertaken to establish the types of cover-crops favouring the beneficial actions of natural enemies (Tillman et al. 2004).

Since the 1980s, work on innovative cultural systems has focused on one hand on the major crops, and on the other hand on plants destined to serve as permanent mulches whose application in tropical areas can ameliorate the loss of fertility of soils to erosion and the action of weeds, which are major production constraints (AFD 2006). Direct seeding plays a preponderant role, in various systems depending on the local socio-economic conditions. In the humid tropical climate of the Cerrados in Brazil, a recent spectacular development has involved appropriate rotations, direct seeding under cover crops and careful varietal selection. Two crops are grown successively, soya bean

Table 6 Putting integrated pest management (IPM) into practice: major activities for each phase of the cotton crop cycle and the 'off-season' (Deutscher et al., modified, 2005)

Phases objectives	Post harvest	Pre-planting	Planting to 1 flower per metre	One flower per metre to 1 open boll per metre	One open boll per metre to harvest
1. Growing a healthy plant	Rotation crop, fertiliser requirements, potential disease risks	Seed bed preparation, cotton variety selection, irrigation management strategy	Planting window, planned treatments, water management	Monitor for crop management, nutrient status, growth control, pest control	Final irrigation decisions, defoliation management, pest management
2. Keeping track of insects and damage	Sample cotton stubble for *Helicoverpa armigera* pupae	Risk of different pests and pest management in pre-planting	Sample for pests and beneficials in cotton and in trap crops	Sample for pests and beneficials and use thresholds and predator/beneficial ratio	Stop treatments at 30–40% bolls open
3. Beneficial insects – use them do not abuse them	Plant lucerne in autumn, discuss an IPM or AWM group	Planning diversified habitats, especially sorghum if *Trichogramma* releases are planned	If chemical control is required, refer to the beneficial impact table	Consider *Trichogramma* releases into sorghum, food sprays for beneficials, lucerne management	Encourage beneficials to reduce late season resistant pests
4. Prevent the development of resistance	Pupae bust to control *Helicoverpa armigera* and mites, plant spring trap crop, attend annual resistance management meeting	Consider Bollgard II® refuge options, choice of insecticides	Use pest and damage thresholds, follow the IRMs strategy for region for Bollgard II® management	Use pest and damage thresholds, follow the IRMs strategy for Bollgard II® resistance management	Use pest and damage thresholds, follow the IRMs strategy for Bollgard II® resistance management
5. Manage crop and weed hosts	Weeds and cotton re-growth management	Carefully consider summer trap rotation crops	Keep farm weed free	Keep farm weed free	Consider winter rotation crops, keep farm weed free
6. Use trap crops effectively	Plant spring trap crop, consider flowering date to time planting	Consider summer trap crop	Consider last generation trap crop	Monitor *Helicoverpa* populations in summer trap crop	Use biological and cultural methods to destroy *Helicoverpa* stages
7. Support IPM though communication and training	Consider becoming involved in an IPM or AWM group, consider doing the IPM short course	Communicate to discuss spray management plans, attend training courses	Meet regularly with consultant to discuss IPM strategies and attend local field days	Meet regularly with consultant to discuss IPM strategies and attend local field days	Meet regularly with consultant to discuss IPM strategies and attend local field days

and rain-fed rice as the main crops and maize, sorghum and millet as secondary crops, locally called 'safrinhas'. Cotton is introduced to the system as a secondary crop, sometimes after the two principal crops, sometimes after the cover crops have produced abundant biomass (Seguy et al. 2004). Studies are being undertaken to evaluate the phytosanitary implications of the use of the cover crops, which may favour the development of certain pests such as *Spodoptera frugiperda* (Ratnadass et al. 2006; Silvie et al. 2005). In Australia, by contrast, it is the desire to find a sustainable solution to phytosanitary problems which has principally guided the development of new agronomic techniques (Table 6). These rest mainly on the management of pests through the management of habitats (Deutscher et al. 2005). In both cases however, the importance given to participative processes with producers underlines the central role of socio-economics in determining the successful popularisation of new techniques.

6 Agro-ecology and Ecological Engineering for Cotton Pest Control

Since the 1970s, the evolution of plant protection has been driven by improved understanding of the functioning of ecosystems (Botrell 1980). At this time, the desire to explore these issues favoured the development of computer-based simulation models for risk assessment. The approach to these problems was considerably improved; taking into consideration the development of the plants in the particular soil/moisture/nutrient content and insolation context and considering the suite of pests present in the same crop – the development of an concept of integrated control and then of integrated production (integrated crop management). The UN Conference on the Environment and Development in Rio de Janeiro in 1992 drew attention to the need to preserve the biological diversity of ecosystems in general and agro-ecosystems in particular. The subsequent publication of diverse works aimed at advancing the IPM paradigm, helped in the national adoption of IPM strategies (Benbrook et al. 1996; Cate and Hinkle 1994; NRC 1996). The simultaneous elaboration of the scientific principles underlying this field of agroecology, rendered these calls more credible (Altieri 1995; Dalgaard et al. 2003). It was then necessary to move to the practical stage of conceiving growing systems which capitalised on the resilience of agro-ecosystems (Clements and Shrestha 2004). To this end, 'agro-ecosystems management' or 'agroecological engineering' is today recognised as one of the up and coming concepts in crop protection (Clements and Shrestha 2004; Gurr et al. 2004; Lewis et al. 1997; Nicholls and Altieri 2004), integrating well with current environmental considerations (Fig. 2).

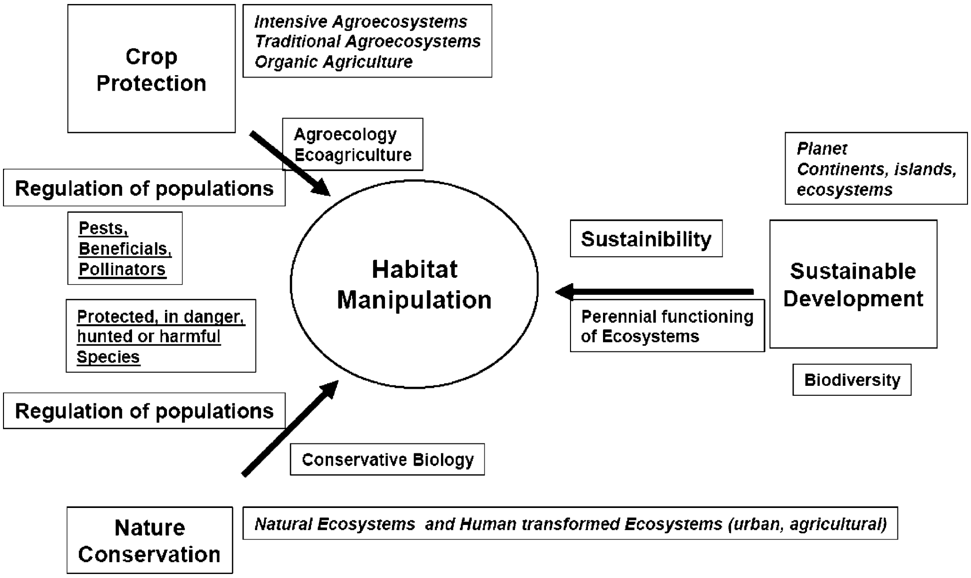

Fig. 2 Coherence and convergence of habitat manipulation from different concepts including crop protection

More generally, this development is presented in the form of an 'IPM continuum' (Jacobsen 1997), where it is clear that much of what is necessary will be a continuous evolution of traditional concepts and understanding in crop protection (Clements and Shrestha 2004). Indeed the principles of a bio-centered agriculture, developed during the last decades, have proposed new orientations to crop production. Certainly the principles of a bio-centered agriculture will require a return to the preoccupations of several decades ago. Production which is technically 'organic', in accepted sense of the certifying organic agriculture bodies, had a certain success in cotton in the mid 1990s, but it does not represent today more than a miniscule part of the market (c. 30,000 tonnes or 0.1% of global production in 2005), even if for some it seems a promising route for resource poor small scale producers (Galanopoulou-Sendouca and Oosterhuis 2004; Guerena and Sullivan 2003; Myers and Stolton 1999; Ton 2004). Organic cotton is currently produced in 22 countries, largely by Turkey (40%), India (25%), the USA, (8%) and China (7%). The number of small brands and retailers in North America and Europe interested in marketing organic cotton products is growing rapidly, but it may be argued that this is a high-price, low-volume, niche market which is unlikely to significantly expand (Haynes 2006; Swezey et al. 2007). For growers there can be a price premium but there has almost always been a yield cost to organic production. Currently there appear to be no significantly effective pest management techniques unique to organic cotton production systems, although his position may change with further research. Within this overall movement, the BASIC programme (Biological Agricultural Systems In Cotton) in operation in California for 12 years or so, illustrates a possible method for transition form traditional IPM towards a true 'biological' production system (Swezey and Goldman 1999).

6.1 Area-wide and Community-based Cotton Pest Management

As previously described, cotton crop protection was one of the earliest in the agricultural world to experiment with the application of autocidal control. Many other ways of responding to the criteria of area-wide pest management have also been envisaged, including the use of microbial control of heliothine pests in the USA with the aid of entomopathogenic viruses (Street et al. 2000), and capitalising on the long-term effects on pest populations offered by the deployment of Bt-cotton (Carrière et al. 2001, 2003).

One of the precursors of area-wide management ran in Arkansas in the mid 1970s (Hardee and Henneberry 2004; King et al. 1996). The main thrust was to gain the active support of the growers to a regional, co-ordinated, phytosanitary effort and to secure their adhesion to the agreed practices. In southern Queensland (Australia) a similar strategy has been successfully applied since the end of the 1990s in the Darling Downs region (Murray et al. 2005). This system rests on the application of the following tactics: (a) reducing the survival of over-wintering, insecticide-resistant *H. armigera* pupae, (b) reducing the early season build-up of *Helicoverpa* spp. on a district/regional scale, and (c) reducing the mid-season population pressure on *Helicoverpa*-susceptible crops. A key component of this programme was the use of early and late-season trap crops.

These new, area-wide, strategies have generally been welcomed, particularly in industrialised cotton production systems, as they form a rational response to the collective need of growers to reduce production costs. They are more difficult to implement in arid-land, small-farmer, systems where their priorities take second place to the immediate need for local food crop production. The relative complexity of these systems and technical practices proposed, and the need for a much larger number of growers to co-operate over a given cropping area, are effective barriers to adoption by small-scale producers in traditional agricultural systems. The difficulties encountered in adopting even simple scouting methods are indicative of these constraints.

Lessons learned in the Farmer Field Schools discussed earlier, have resulted in the development of learning systems better adapted to the needs of these growers (Ooi 2004; Ooi et al. 2005). The importance of genuinely participative processes is underlined by experiences in all type of production systems (for example in Australia, Dalton et al. 2004; Benin, Prudent et al. 2006; Egypt, Treen and Burgstaller 2004; Malawi, Orr and Ritchie 2004; Thailand, Castella et al. 1999; Uganda, Sekamatte et al. 2004a, b). There has been relatively little research into implanting these newer concepts into small-farming systems in ways which take into account local constraints (Castella and Deguine 2006; Lançon et al. 2004; Sekamatte et al. 2004b).

6.2 Farmscaping, Landscape Farming, Habitat Management and Cotton Intercropping

Manipulations of the cotton agro-ecosystem have been recommended since the 1970s. They have concerned both modifications of normal agricultural practices and completely novel measures. Amongst the latter, intercropping with lucerne, or deliberately maintaining residual populations of pests within cotton fields to allow the survival of their parasitoids and predators, are often cited as examples of integrated management (Smith and Reynolds 1972). Other technical solutions have been proposed: management of the vegetation in field borders, rearrangements of the spatio-temporal structure of cultures in the field themselves, and appropriate management of weeds (Altieri and Letourneau 1982; Clements and Shrestha 2004; Cook et al. 2007; Nestel et al. 2004; Wäckers et al. 2007). The expression farmscaping has been proposed to designate 'a whole-farm, ecological, approach to pest management' (Bugg et al. 1998; Dufour 2000).

Multiple cropping, where two or more crops may be taken from the field in a single year, is an example of traditional practices which are still common in tropical developing countries. They may take the form of sequential cropping, with crops succeeding each other in the same field, or intercropping – growing more than one crop in a pattern in the same field using the techniques of mixed- or multiple-, row-, strip- or relay-intercropping). For the majority of resource poor small-producers, it is often necessary to meet a significant portion of daily food requirements from the same area of land used for cash cropping and this requires a judicious understanding of the biological risks which this may engender (to soil fertility as well as pest management) (Altieri and Nicholls 2004). The abundance of the resulting pest populations naturally varies strongly between one particular case and the next. These populations are influenced by a variety of factors, amongst which are those which affect the behaviors of the pests and their natural enemies (Gurr et al. 2004; Irwin et al. 2000). The idea that crop diversification would, of itself, result in the reduction of pest impacts has now been abandoned, although the positive role of trap crops is acknowledged, and particular cropping geometries and sequences can be strongly beneficial (Altieri and Nicholls 2004; Shelton and Badenez-Perez 2006; Smith and McSorley 2000; Vandermeer 1990).

These various new practices form part of the recommendations being proposed to producers under the rubric of 'better cotton management practices' or BMPs. Again in Australia, intercrops such as sunflower, safflower, sorghum, tomato and lucerne, are considered to be favourable in their influence on the pest/predator situation, with the lucerne acting as a nursery crop for the beneficials. Having established that the abundance of natural enemies declines rapidly with the distance between the two crops, it is recommended, for example, to grow a band of lucerne 8–12 m wide, as a single median strip, between two cotton fields up to 300 m wide (Mensah 1999). Cutting parts of this medium strip and/or the spraying of food additives allows the management of movements of predators (Mensah and Singleton 2004). These same intercalated rows of lucerne may also play a role as trap crops for the pests themselves, such as the green mirid, *Creontiades dilutus* (Mensah and Khan 1997). One should not, however, underestimate the likelihood that these intercrops may also favour infestations of certain pests. This can be an obstacle to the adoption of these practices, even with the use of selective biopesticides on the intercalated crop (Duraimurugan and Regupathy 2005; Gurr et al. 2004; Mensah and Singleton 2004).

In is in China that the practice of intercropping is the most common and the most diversified. Cotton is frequently sown in spring between lines of winter wheat, which helps in the management of early-season aphids. One particular success in this area has been the growing of lucerne (*Medicago sativa* L.) around cotton field margins as a nursery crop for ladybirds (*Coccinella septempunctata*, *Propylea quatrodecimpunctata* and *Hypodama variagata*), chrysopids and other beneficial arthropods in Xinjiang province of Eastern China. The lucerne is cut several times in a season and the beneficials move from lucerne, where they have been feeding on the non-cotton aphid *Therioaphis maculata*, into the cotton, where they significantly reduce the number of cotton aphids (*A. gossypii*), which are by far he most important cotton pests in the region (Lin et al. 2003). Agro-forestry, under the name of 'alley cropping' or 'tree-based intercropping' is undertaken in some area with poplar, *Paulownia* and Elm (Yin and He 1997). Poplar acts as an oviposition attractant to *H. armigera* whose larvae are then not able to survive on the trees. This utilisation of tree intercrops, characteristic of peasant agriculture in many parts of China since the 1980s, must be seen as primarily an insurance against the risks of aeolian erosion, as windbreaks and as a local source of wood for cooking, heating and construction. The phytosanitary consequences of these systems are not very well documented (Altieri and Nicholls 2004; Clements and Shrestha 2004; Landis et al. 2000; Wang et al. 2003; Xia 1995), and then may or may not fit well into the criteria of ecological management, today gathered under the term 'ecological infrastructures', which preserve the biodiversity and so the functioning of agro-ecosystems. These 'infrastructures' attempt on the one hand to provide physical linkages between different parts of the agricultural landscape which are suitable for the survival of indigenous fauna (corridors, hedgerows etc.), and on the other hand to organise the cropping land into physical units which favour the free movement of natural enemies, particularly of generalist predators (Altieri and Nicholls 2004; Boller et al. 2004; Ferron 1999; Ferron and Deguine 2005; Rencken et al. 2004).

6.3 Biodiversity, Biocomplexity and the Future of Cotton Pest Management

The emphasis placed on respect for the sustainable development of the planet obliges the researcher to find a balance between the immediate needs of humanity and the preservation of the diversity of the living world. To this end, we have no doubt accorded too great an importance to biodiversity for its own sake, at the expense of a functional biodiversity which helps to provide a sustainable integration of human activity with the functioning of ecosystems (Altieri and Nicholls 2004; Letourneau 1998).

The term biocomplexity, is to be understood as 'properties emerging from the interplay of behavioural, biological, chemical, physical and social interactions that affect, sustain, or are modified by, living organisms, including humans' (Levêque and Mounolou 2001; Michener et al. 2001). Applied to crop protection, this implies finding the delicate balance between curative treatments applied at the level of the individual field and the management of pest systems at the level of the overall agro-ecosystem.

These agro-ecosystems are characterised by an, often considerable, reduction in their diversity at the species level because of current methods of land utilisation; monoculture in a 'naked field', cleared of all weeds (Andow 1983). Under these very constrained conditions, infestations of herbivores are favoured. The limited effects of their accompanying beneficial complexes on the dynamics of their populations comes too late, even when they are not blocked altogether by non-selective phytosanitary interventions. The generalist predatory fauna is most often neither diverse nor abundant in these systems without enough alternative prey (Altieri and Letourneau 1982). It is for this reason that crop diversification is the cultural technique generally promoted, in order to favour populations of beneficials and so to reduce the need for insecticidal treatments (Clements and Shrestha 2004; Gurr et al. 2004; Prasifka et al. 2004).

The popularisation of genetically modified plants as a response to phytosanitary problems, as with cotton, has recently added supplementary questions as to their likely role and impact in agro-ecosystems as a whole (Altieri 2000). At this stage we have only preliminary results in this area (Ammann 2005; Andow and Zwalhen 2006; Cattaneo et al. 2006; Hofs et al. 2005; Kabissa 2004b; Marvier et al. 2007; O'Callaghan et al. 2005; Torres and Ruberson 2007). Modifications of the relative importance of the different pest species within the agro-ecosystem as a whole, in relation to their specific susceptibility to the Bt toxins, are already emerging. For example, circumstantial evidence is accruing of the reduction in importance of *H. armigera* as a pest of many crops since the introduction of Bt cotton in both China (1996–1997) and India (2002). Questions on the importance of these entomotoxins in the biology of soils have been asked recently (Altieri and Nicholls 2004; Gupta et al. 2002). Positive impacts on diversity within Bt cotton fields are generally reported, but measured impacts on the diversity of arthropod populations around cotton fields, which is weak but significant in certain cases, has encouraged the pursuit of investigations in this area of whole system impacts (Head et al. 2005; Naranjo 2005a, b; Torres and Ruberson 2005; Vaissayre et al. 2005; Whitehouse et al. 2005).

These are the contexts within which the design of a new concept of sustainable crop protection in general, and sustainable cotton crop protection in particular, is emerging (Tilman 1999). This new concept implies a change of strategy, to one composed, under the structure of a total-system approach, of three major components: (a) management practices established at the level of agro-ecosystems, (b) the systematic exploitation of multi-trophic interactions among plants, herbivores and parasitoids/predators, (c) recourse to pesticide applications only as a last resort (Lewis et al. 1997; Thomas 1999; Walter 2003).

An illustration is provided by the orientation given to research under the expression 'New Cotton Cultivation (NCC)', seen as identifying the best interactions between the plant, the technical context and the natural and sociological environment pertaining in a given localised situation (Deguine et al. 2000). Control of populations of piercing–sucking insects which have risen to be of major importance in the last two decades, may be taken as an example. The recommended strategy gives priority to preventative measures through a process which is multidisciplinary, adapted and participative (Deguine et al. 2004, 2007). Several other integrated management initiatives for sucking–piercing pest control in cotton have been undertaken on similar principles in recent years (Hardee et al. 1994; Ellsworth and Martinez-Carillo 2001).

More generally, the future of cotton crop protection rests in a fruitful multi-disciplinarity, particularly in the improvement, or the genetic transformation of varieties, such as to allow the full expression of their agronomic potential under the new requirement of respecting the principles of sustainable agricultural development (Vaissayre et al. 2006). This constraint, as much technical as social, imposes a break with traditional operations in making agricultural activities a part of the functioning of ecosystems, and no longer an artificial exploitation of natural resources including large amounts of inputs (Fitt 2000a, b; Fitt et al. 2004; King et al. 1996; Russell 2001; Shea et al. 2000).

7 Conclusion

The principal industrial crop, often the sole cash source for countless small growers in developing countries, source of economic conflicts in the research into 'fair trade', cotton is also the subject of serious phytosanitary and environmental concerns. These are allied to the importance of yield and quality losses occasioned by the particularly rich, polyphagous pest complex. It is for this reason that chemical control has had genuine success since the 1950s. However, the use of synthetic insecticides in insufficiently understood production systems led to their abuse. The development of the problem of evolved resistance resulted in a stream of new insecticide active ingredients, which in time resulted in an economic impasse for growers. For crop protection specialists, cotton has for long been considered as a bad example of their discipline.

The study of numerous published works on this subject over the last 25 years, allows us to revisit this judgment and to take cotton culture as a case study of the evolution of our understanding of crop protection. The diversity of soil and climatic conditions and systems of cotton production across the world has effectively allowed experimentation with phytosanitary practices, which are now available for critical analysis. Amongst these innovations, the most conspicuous in the last 10 years has been the growing of genetically modified varieties tolerant to particular herbicides and to certain major insect pests. This change is often taken into account to contribute to the preservation of the environment and consequently, with care, to more sustainable cotton production.

At the end of the 1960s the situation was effectively critical. The intensity of public and scientific opinion against the continued use of intensive chemical pest control was increasing rapidly. In the absence of a comprehensive understanding of the factors influencing the dynamics of pest populations, this led, as in other major cropping systems, to the development of the compromise solution of 'integrated control', intended to exploit natural control systems to the maximum extent possible, supported where necessary by the judicious deployment of chemical insecticides. This proved illusory. In the best cases, it was a form of directed control which prevailed, characterised by risk evaluations on the basis of economic intervention thresholds, which were then used to justify each chemical application. The adoption of such measures is indicative of the real difficulties in the practical application of more knowledge-intensive integrated pest management systems. Focusing from the outset on the use of intervention, thresholds has had the perverse effect of re-enforcing the habitual recourse of growers to synthetic pesticides, according to their immediate efficacy, rather than supporting the investigation of the potential for preventative actions, as recommended by the principles of IPM. In this respect, we should have seen a move from the stage of controlling pests at the level of the individual field to that of population management at the level of the cropping system and eventually of whole agro-ecosystems. In practice this is far from having happened, no doubt as a result both of the lack of sufficient knowledge of the agro-ecology of the cotton system and because of the lack of the alternative technical solutions or ability to socially mobilise communities to operate at this new spatio-temporal level. Where some advance has been made in this direction, it has been amongst the major industrialised producers, where number of growers per unit area has been small and their education level and financial acumen have been high. Limited success in developing countries has largely occurred where control of inputs and extension advice remains with government, as in Turkey and Egypt until recently, or with a few major cotton companies, as in parts of West Africa.

In this context, in common with most major cropping systems, the development of insecticide resistance by the major cotton pests has played a determining role in constraining the producers to respect the rules of good agronomic practice, favourable to a genuine mastery of the employment of synthetic insecticides. One of these constraints concerns the necessity for spatio-temporal co-ordination of the control practices in a region, illustrated, for example, by the Australia 'window strategy'. The implementation of the eradication–suppression strategy for boll weevil in US cotton belt has also shown the value of a collective approach to phytosanitary problems, in drawing attention to the role of non-cultivated zones in the overall management of pest dynamics. Even modest reductions in the number of pesticide treatments, obtained by respecting good agronomic practices, have focused attention on the impact of natural population regulating processes and in particular on the role of beneficial organisms, parasites and predators. Although the exploitation of the potential of introduced beneficial arthropods in classical or inundative biological control remains limited to a few cases, the use of indigenous beneficials (and particularly generalist predators) though the implementation of conservation practices, is becoming a more promising option.

It has been the need to respond to low cotton market prices and globally stagnant yields which has, over the last 10 years, led to the spectacular adoption of Bt cotton, which has allowed very significant reductions in insecticide treatments. The global interest in the provision of refuge zones, in which susceptible insects are produced to dilute the impact of any rare resistance genes selected for in the Bt crop, has sensitised cotton stakeholders to the potential benefits of co-operative, landscape-scale, action for collective long-term benefit. More generally, there has been some movement since the 1960s on recommendations on management of the overall growing system for the benefit of cotton pest control. The use of trap crops, intercropped alternative hosts, and nursery crops for beneficials, are having some success in some specific production systems, as our understanding of basic ecological processes increases. We are however, still a long way from the objective of creating ecological infrastructures, which will support an essentially preventative pest management strategy (Bianchi et al. 2006; Boller et al. 2004).

For most authors, the movement from a 'field-by field' to a 'farm by farm' and 'agro-ecosystem by agro-ecosystem' to a 'landscape by landscape' approach is a gradual and evolutionary tendency inherent in the long-term goals of a true IPM perspective. The developments to date seem, *a posteriori*, to be steps in that direction. Others, by contrast, ask themselves whether the reality of moving to a phytosanitary system founded on these new principles, will not involve an obligatory and marked rupture with traditional practices (Deguine et al. 2000; Irwin et al. 2000). This question revisits the epistemological arguments of Kuhn (1996): when the inadequacy of a paradigm, such as chemical pest control, becomes more and more obvious, and a replacement paradigm is developed, such as agro-ecological management or 'a total systems approach to sustainable pest management' (Lewis et al. 1997), it results in a brutal scientific revolution. Some authors talk today of a 'new' green revolution or 'evergreen revolution' (Borlaugh and Dowswell 2004; Griffon 2006) to draw attention to the progress made since the 1960s, a time at which the strategy to respond to the food production needs of humanity rested essentially on the promise of varietal selection and recourse to synthetic inputs.

For agronomists, sociologists, plant protection specialists and growers, cotton production offers a rich field of experiences and large-scale experimental results. The spatio-temporal challenges provided by cotton's phytosanitary problems require a shift in thinking towards seeing agricultural production as one part of the functioning of larger agro-ecosystems. The potential ecological consequences of the actions of the industry require a re-orientation of the players towards management practices which respect the principles of agro-ecology. These will require a change in the mentality of cotton production stakeholders which may, in the end, be driven as much by consumer attitudes as by economics. In plant protection it will be necessary to move from an individual to a collective vision, giving due weight to the foreseeing of risks in the medium and long term, within an essentially preventative approach.

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Role of Nutrients in Controlling Plant Diseases in Sustainable Agriculture: A Review

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Abstract In recent years the importance of sustainable agriculture has risen to become one of the most important issues in agriculture. In addition, plant diseases continue to play a major limiting role in agricultural production. The control of plant diseases using classical pesticides raises serious concerns about food safety, environmental quality and pesticide resistance, which have dictated the need for alternative pest management techniques. In particular, nutrients could affect the disease tolerance or resistance of plants to pathogens. However, there are contradictory reports about the effect of nutrients on plant diseases and many factors that influence this response are not well understood. This review article summarizes the most recent information regarding the effect of nutrients, such as N, K, P, Mn, Zn, B, Cl and Si, on disease resistance and tolerance and their use in sustainable agriculture. There is a difference in the response of obligate parasites to N supply, as when there is a high N level there is an increase in severity of the infection. In contrast, in facultative parasites at high N supply there is a decrease in the severity of the infection. K decreases the susceptibility of host plants up to the optimal level for growth and beyond this point there is no further increase in resistance. In contrast to K, the role of P in resistance is variable and seemingly inconsistent. Among the micronutrients, Mn can control a number of diseases as Mn has an important role in lignin biosynthesis, phenol biosynthesis, photosynthesis and several other functions. Zn was found to have a number of different effects as in some cases it decreased, in others increased, and in others had no effect on plant susceptibility to disease. B was found to reduce the severity of many diseases because of the function that B has on cell wall structure, plant membranes and plant metabolism. Cl application can enhance host plants' resistance to disease. Si has been shown to control a number of diseases and it is believed that Si creates a physical barrier which can restrict fungal hyphae penetration, or it may induce accumulation of antifungal compounds. Integrative plant nutrition is an essential component in sustainable agriculture, because in most cases it is more cost-effective and also environmentally friendly to control plant disease with the adequate amount of nutrients and with no pesticides. Nutrients can reduce disease to an acceptable level, or at least to a level at which further control by other cultural practices or conventional organic biocides are more successful and less expensive.

Keywords Deficiency • Disease resistance • Integrative pest management • Metabolism • Nutrients • Plant physiology • Tolerance • Toxicity

1 Introduction

Sustainability is a term that has been used extensively in recent years in many aspects of our lives, and especially in agriculture because of the effect that certain crop production methods have on the environment (Hanson et al. 2007; Atkinson and McKinlay 1997). Sustainable agriculture is the management and

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utilization of the agricultural ecosystem in a way that maintains its biological diversity, productivity, regeneration capacity, vitality and ability to function, so that it can fulfill – today and in the future – significant ecological, economic and social functions at the local, national and global levels, and that does not harm other ecosystems (Lewandowski et al. 1999).

The sustainability of agriculture has faced some of the most significant challenges in recent years (Hanson et al. 2007; Oborn et al. 2003). Major challenges include: (1) first of all, the rapid growth of the human population and the increased demand for agricultural land and resources, (2) overdependence on fossil energy and the increased monetary and environmental costs of nonrenewable resources, (3) global climate change (Brown 2006; Diamond 2005), and (4) globalization (Hanson et al. 2007). These dominant issues are challenging agriculturists to develop more sustainable management systems like no other time in history. To meet the food and nutritional needs of a growing population, agriculture will need to move beyond the past emphasis on productivity to encompass improved public health, social well-being and a sound environment (Hanson et al. 2007). Also, it is important to find alternative measures to control plant diseases which do not harm the environment and at the same time increase yield and improve product quality (Atkinson and McKinlay 1997; Batish et al. 2007; Camprubí et al. 2007).

Nutrients are important for growth and development of plants and also microorganisms, and they are important factors in disease control (Agrios 2005). All the essential nutrients can affect disease severity (Huber and Graham 1999). However, there is no general rule, as a particular nutrient can decrease the severity of a disease but can also increase the severity of the disease incidence of other diseases or have a completely opposite effect in a different environment (Marschner 1995; Graham and Webb 1991; Huber 1980). Despite the fact that the importance of nutrients in disease control has been recognized for some of the most severe diseases, the correct management of nutrients in order to control disease in sustainable agriculture has received little attention (Huber and Graham 1999).

Nutrients can affect disease resistance or tolerance (Graham and Webb 1991). Disease resistance of the host is its ability to limit the penetration, development and reproduction of the invading pathogens (Graham and Webb 1991). On the other hand, tolerance of the host is measured in terms of its ability to maintain its own growth or yield in spite of the infection. Resistance depends on the genotype of the two organisms, plant age and changes in the environment. Although plant disease resistance and tolerance are genetically controlled (Agrios 2005), they are affected by the environment and especially by nutrient deficiencies and toxicities (Marschner 1995; Krauss 1999). The physiological functions of plant nutrients are generally well understood, but there are still unanswered questions regarding the dynamic interaction between nutrients and the plant–pathogen system (Huber 1996a). A number of studies showed that it is important with the correct nutrient management to control diseases in order to obtain higher yield (Marschner 1995; Huber and Graham 1999; Graham and Webb 1991 and reference therein). However, there is not enough information regarding the appropriate crop management practices in sustainable agriculture that can reduce yield losses of crop plants due to diseases. There are many factors that can affect the severity of plant disease such as seeding date, crop rotation, mulching and mineral nutrients, organic amendments (manures and green manures), liming for pH adjustment, tillage and seedbed preparation, and irrigation (Huber and Graham 1999). Many of these practices affect the level of nutrients available for the plant and the pathogen, which can affect the disease severity.

It is important to manage nutrient availability through fertilizers or change the soil environment to influence nutrient availability, and in that way to control plant disease in an integrated pest management system (Huber and Graham 1999; Graham and Webb 1991). The use of fertilizers produces a more direct means of using nutrients to reduce the severity of many diseases and together with cultural practices can affect the control of diseases (Marschner 1995; Atkinson and McKinlay 1997; Oborn et al. 2003).

In addition, nutrients can affect the development of a disease by affecting plant physiology or by affecting pathogens, or both of them. The level of nutrients can influence the plant growth, which can affect the microclimate, therefore affecting infection and sporulation of the pathogen (Marschner 1995). Also, the level of nutrients can affect the physiology and biochemistry and especially the integrity of the cell walls, membrane leakage and the chemical composition of the host, e.g., the concentration of phenolics can be affected by B deficiency (Graham and Webb 1991). Nutrients can affect the growth rate of the host which can enable seedlings to escape/avoid infection when they are at the most susceptible stages. In addition, fertilizers can influence the soil environment and can affect the development of the pathogen. This review aims at summarizing the most recent information regarding the effect of nutrients on disease resistance and tolerance and their use in sustainable agriculture. The main topics will be: (1) nutrition and disease control and role of nutrients in reducing disease severity, (2) nutrient management and disease control, (3) use of cultural methods in improving plant nutrition and disease resistance, and (4) systemic induced resistance or systemic acquired resistance. Also, I will discuss the need for further research on finding how nutrients can affect the mechanisms that are associated with the resistance and tolerance of plants to diseases.

2 Nutrition and Disease Control and Role of Nutrients in Reducing Disease Severity

When a plant is infected by a pathogen its physiology is impaired, and especially nutrient uptake, assimilation, translocation from the root to the shoot and also utilization (Marschner 1995). There are pathogens that can immobilize nutrients in the rhizophere, the soil surrounding plant roots, or in infected tissues such as roots, while others interfere with translocation or utilization efficiency and can cause nutrient deficiency or hyperaccumulation and nutrient toxicity (Huber and Graham 1999). Also, other organisms can utilize a significant amount of nutrients for their growth, causing a reduction in the availability of nutrients for the plant and increasing its susceptibility due to nutrient deficiency (Timonin 1965).

One of the most common symptoms of many soil-borne pathogens is root infection, which reduces the ability of the root to provide the plant with water and nutrients (Huber and Graham 1999). This effect is more serious when the levels of nutrients are marginal and also for immobile nutrients. Also, stem girdling or acropetal infection can limit root growth and affect nutrient and water uptake. Plant disease can also infect the vascular system, which can impair nutrient translocation and utilization. Pathogens can also affect membrane permeability or mobilization towards infected sites, which can induce nutrient deficiency or toxicity. *Fusarium oxysporum f. vasifectum* can increase the concentration of P in leaves, but also decrease the concentration of N, K, Ca and Mg (Huber and Graham 1999).

Fig. 1 Powdery mildew (*Erysiphe cichoracearum*) with an extensive growth of white, powdery fungal mycelium on the upper leaf surface of sunflower (*Helianthus annuus*)

One of the first observations of the effect of nutrients on disease development was that fertilization reduced disease severity when plants were under deficiency, as fertilization optimized plant growth. When N was applied to cereal crops, take-all (*Gaeumannomyces graminis*) was reduced (Huber and McCay-Buis 1993). Also, P reduced both take-all and pythium root rot infection in cereal crops (Kiraly 1976; Huber 1980). A different trend was observed in the foliar disease of cereal crops, e.g., rust and powder mildew, as increasing N application caused an increase in the incidence of the disease (Figs. 1 and 2). Since the interaction of nutrients and disease pathogens is complex, I will describe the effect of each nutrient on certain diseases and also the possible mechanism for the tolerance of or resistance to the particular pathogen.

2.1 Nitrogen

Nitrogen is the most important nutrient for plant growth and there is an extensive literature about the effect of N on diseases, because its role in disease resistance is quite easily demonstrated (Engelhard 1989; Huber and Watson 1974; Marschner 1995). Despite

Fig. 2 Damage caused by safflower rust (*Puccinia carthami*)

the fact that N is one of the most important nutrients for plant growth and disease development, there are several reports of the effect of N on disease development that are inconsistent and contradict each other, and the real causes of this inconsistency are poorly understood (Huber and Watson 1974; Büschbell and Hoffmann 1992; Marschner 1995; Hoffland et al. 2000). These differences may be due to the form of N nutrition of the host (Huber and Watson 1974; Celar 2003; Harrison and Shew 2001), the type of pathogen: obligate vs. facultative parasites (Büschbell and Hoffmann 1992; Marschner 1995) or the developmental stage of N application (Carballo et al. 1994). Also, there are no systematic and thorough studies about the effect of N supply on disease resistance, on biocontrol agents' activity, and especially on the interaction among nutrient, pathogen, and biocontrol organisms (Tziros et al. 2006).

The effect of N is quite variable in the literature. This is due to the different response depending on the type of the pathogen. Regarding the obligate parasites, e.g., *Puccinia graminis* and *Erysiphe graminis*, when there is high N supply there is an increase in severity of the infection; however, when the disease is caused by facultative parasites, e.g., Alternaria, Fusarium and *Xanthomonas* spp., high N supply decreases the severity of the infection (Table 1). However, the situation is more complex for soilborne pathogens as on the root surface there are many more microorganisms than in the bulk soil. Also, there is competition between and repression of different microorganisms, and there are chemical barriers such as high concentration of polyphenols in the rhizodermis and physical barriers such as silicon depositions on the endodermis (Huber 1980). The difference between the obligate and facultative parasites is due to the nutritional requirements of the two types of parasites. Obligate parasites require assimilates supplied directly from living cells. In contrast, facultative parasites are semisaprophytes which prefer senescing tissue or which release toxins in order to damage or kill the host plant cells. Therefore, all factors which support the metabolic activities of the host cells and which delay senescence of the host plant can increase resistance or tolerance to facultative parasites (Agrios 2005; Vidhyasekaran 2004).

In the case of obligate fungal parasites the nutritional requirements of the parasites cause changes in the anatomy and physiology of the host plant in response to N. At high rates there is a higher growth rate during the vegetative stage and the proportion of the young to mature tissue shifts in favor of the young tissues, which are more susceptible. Also, there is a significant increase in amino acid concentration in the apoplast and on the leaf surface, which promotes the germination and growth of conidia (Robinson and Hodges 1981). At high N rates the metabolism of the plant changes: as some key enzymes of phenol metabolism have lower activity, the content of the phenolics decreases and the lignin content may be lower – all these are part of the defense system of plants against infection. In addition, at high N rates Si content decreases (Grosse-Brauckmann 1957; Volk et al. 1958). Therefore, the main reason for the increased susceptibility to obligate parasites at high N rates is the various anatomical and biochemical changes together with the increase in the content of the low-molecular-weight organic nitrogen compounds which are used as substrates for parasites. It is believed that plants grown under conditions of low N availability are better defended against pathogens because there is an increase in the synthesis of defense-related compounds (Bryant et al. 1983; Herms and Mattson 1992; Hoffland et al. 1999; Wilkens et al. 1996; Hoffland et al. 2000). However, the response to the N level was different in the facultative parasites, as when the plants were grown under high levels of N they were more resistant to pathogens such as *B. cinerea*. In the case of obligate pathogens such as *Pseudomonas syringae* pv. *tomato*, *Ustilago maydis* and *Oidium lycopersicum* increased susceptibility was observed when plants were grown with high N supply (Hoffland et al. 2000;

Table 1 Effect of N level on disease severity of several diseases

Pathogen or disease		Low N	High N	References
Obligate parasite	*Puccinia graminis*	Decrease	Increase	Howard et al. (1994)
	Erysiphe graminis	Decrease	Increase	Büschbell and Hoffmann (1992)
	Oidium lycopersicum	Decrease	Increase	Hoffland et al. (2000)
	Plasmodiophora brassicae	Decrease	Increase	Kiraly (1976)
	Tobacco mosaic virus	Decrease	Increase	Singh (1970)
	Pseudomonas syringae	Decrease	Increase	Hoffland et al. (2000)
Facultative parasite	*Xanthomonas vesicatoria*	Increase	Decrease	Chase (1989)
	Alternaria solani	Increase	Decrease	Blachinski et al. (1996)
	Fusarium oxysporum	Increase	Decrease	Woltz and Engelhar (1973)

Fig. 3 Tumor-like galls that were formed in corn ears infected by common smut (*Ustilago maydis*)

Kostandi and Soliman 1991) (Fig. 3). These reports indicate that disease susceptibility depends on N supply and that the effect of N supply on susceptibility is pathogen-specific.

The form of N is also important in plant diseases, and the presence of nitrification inhibitors is important too (Huber and Graham 1999; Celar 2003; Harrison and Shew 2001). At high NO_3 disease is decreased in the case of *Fusarium oxysporum*, *Botrytis cinerea*, *Rhizoctonia solani* and *Pythium* spp. In contrast, at high NH_4 disease is decreased in the case of Pyricularia, *Thielaviopsis basicola*, *Sclerotium rolfsii* and *Gibberella zeae*. The form of N can affect the pH of the soil and also the availability of other nutrients such as Mn. Also, the level of N can affect the phenolics content of plants, which are precursors of lignin. In addition, at high levels of N there is a decrease in Si content, which can affect the disease tolerance. In this case, the subject is quite complex and more research is needed to find a specific mechanism that explains these observations because the interaction between disease and host depends on several factors, including host response, previous crop, N rate, residual N, time of N application, soil microflora, ratio of NH_4^+-N to NO_3^--N and disease complex presence.

2.2 Potassium

Potassium decreases the susceptibility of host plants up to the optimal level for growth: beyond this point, there is no further increase in resistance which can be achieved by increasing the supply of K and its contents in plants (Huber and Graham 1999). The high susceptibility of the K-deficient plant to parasitic disease is due to the metabolic functions of K in plant physiology. Under K deficiency synthesis of high-molecular-weight compounds (proteins, starch and cellulose) is impaired and there is accumulation of low-molecular-weight organic compounds. Also, K may promote the development of thicker outer walls in epidermal cells, thus preventing disease attack. K can also influence plant metabolism, as K-deficient plants have impaired protein synthesis and accumulate simple N compounds such as amides which are used by invading plant pathogens. Tissue hardening and stomatal opening patterns are closely related to infestation intensity (Marschner 1995). There were no differences in the crop response in the different sources of K. In addition, the balance between N and K affects disease susceptibility of plants.

Application of K can decrease helminthosporium leaf blight severity and increase grain yields in wheat (Sharma and Duveiller 2004; Sharma et al. 2005). It has been shown that K fertilization can reduce the intensity of several infectious diseases of obligate and facultative parasites (Table 2). It has been frequently

Table 2 Effect of K level on disease severity of several diseases

Pathogen or disease	Low K	High K	References
Puccinia graminae	Increase	Decrease	Lam and Lewis (1982)
Xanthomonas oryzae	Increase	Decrease	Chase (1989)
Tobacco mosaic virus	Increase	Decrease	Ohashi and Matsuoka (1987)
Alternaria solani	Increase	Decrease	Blachinski et al. (1996)
Fusarium oxysporum	Increase	Decrease	Srihuttanum and Sivasithamparam (1991)
Pyrenophora tritici-repentis	Increase	Decrease	Sharma et al. (2005)
Erysiphe graminis	Increase	Decrease	Menzies et al. (1992)

Fig. 4 Light gray lesions with a dark border in sugar beet (*Beta vulgaris*) leaves caused by *Cercospora beticola*

Fig. 5 Lesions caused by bacterial blight (*Xanthomonas campestris* pv *malvacearum*) in cotton (*Gossypium hirsutum*)

observed that K reduces the incidence of various diseases such as bacterial leaf blight, sheath blight, stem rot, sesamum leaf spot in rice, black rust in wheat, sugary disease in sorghum, bacterial leaf blight in cotton, cercospora leaf spot in cassava, tikka leaf spot in peanut, red rust in tea, cercospora leaf spot in mungbean and seedling rot caused by *Rhizoctonia solani* (Figs. 4 and 5) (Table 2) (Huber and Graham 1999; Sharma and Duveiller 2004; Sharma et al. 2005).

2.3 Phosphorus

Phosphorus is the second most commonly applied nutrient in most crops and is part of many organic molecules of the cell (deoxyribonucleic acid (DNA), ribonucleic acid (RNA), adenosine triphosphate (ATP) and phospholipids) and is also involved in many metabolic processes in the plant and also in the pathogen. However, its role in resistance is variable and seemingly inconsistent (Kiraly 1976). P has been shown to be most beneficial when it is applied to control seedlings and fungal diseases where vigorous root development permits plants to escape disease (Huber and Graham 1999). Phosphate fertilization of wheat can have a significant effect and almost eliminate economic losses from pythium root rot (Huber 1980). Similarly, in corn P application can reduce root rot, especially when it is grown on soils deficient in P, and in other studies it can reduce the incidence of soil smut in corn (Huber and Graham 1999; Potash and Phosphate Institute 1988). A number of other studies have shown that P application can reduce bacterial leaf blight in rice, downy mildew, blue mold, leaf curl virus disease in tobacco, pod and stem blight in soybean, yellow dwarf virus disease in barley, brown stripe disease in sugarcane and blast disease in rice (Huber and Graham 1999; Kirkegaard et al. 1999; Reuveni et al. 1998, 2000; Potash and Phosphate Institute 1988). However, in other studies application of P may increase the severity of diseases caused by *Sclerotinia* in many garden plants, *Bremia* in lettuce and flag smut in wheat

(Huber 1980). Foliar application of P can induce local and systemic protection against powdery mildew in cucumber, roses, wine grapes, mango and nectarines (Reuveni and Reuveni 1998).

2.4 Calcium

Calcium is another important nutrient that affects the susceptibility to diseases in two ways. First, Ca is important for the stability and function of plant membranes and when there is Ca deficiency there is membrane leakage of low-molecular-weight compounds, e.g., sugars and amino acids, from the cytoplasm to the apoplast, which stimulate the infection by the pathogens (Marschner 1995). Second, Ca is an important component of the cell wall structure as calcium polygalacturonates are required in the middle lamella for cell wall stability. When Ca concentration drops, there is an increased susceptibility to fungi which preferentially invade the xylem and dissolve the cell walls of the conducting vessels, which leads to wilting symptoms. In addition, plant tissues low in Ca are also much more susceptible than tissues with normal Ca levels to parasitic diseases during storage. Ca treatment of fruits before storage is therefore an effective procedure for preventing losses both from physiological disorders and from fruit rotting. Adequate soil Ca is needed to protect peanut pods from infections by *Rhizoctonia* and *Pythium* and application of Ca to the soil eliminates the occurrence of the disease (Huber 1980). Ca confers resistance against *Pythium*, *Sclerotinia*, *Botrytis* and *Fusarium* (Graham 1983). Ca can be mobilized in lesions of alfalfa caused by *Colletotrichum trifolli* and supports the growth of the pathogen by stimulating the macerating action of pectolytic enzyme polygalacturonic acid transeliminase (Kiraly 1976). A putative mechanism by which Ca is believed to provide protection against *Sclerotinia sclerotiorum* is by binding of oxalic acid or by strengthening the cell wall.

2.5 Other Nutrients

Regarding other nutrients such as sulfur and magnesium, there is not enough information about their role in plant diseases. S can reduce the severity of potato scab, whereas Mg decreases the Ca content of peanut pods and may predispose them to pod breakdown by *Rhizoctonia* and *Pythium* (Huber 1980).

2.6 Micronutrients

The effect of micronutrients on reducing the severity of diseases can be attributed to the involvement in physiology and biochemistry of the plant, as many of the essential micronutrients are involved in many processes that can affect the response of plants to pathogens (Marschner 1995). Micronutrients can also affect disease resistance indirectly, as nutrient-deficient plants not only exhibit an impaired defense response, but often may also become more suitable for feeding as many metabolites such as reducing sugars and amino acids leak outside the plant cell. For example, plants suffering from a Zn deficiency showed increased disease severity after infection by *Oidium* spp. (Bolle-Jones and Hilton 1956). It was also observed that in B-deficient wheat plants, the disease severity was several-fold higher than that in B-sufficient plants, with the fungus spreading more rapidly than in B-sufficient plants (Schutte 1967).

Systemic acquired resistance (SAR) may be involved in the suppression of plant diseases by micronutrients. Reduction in disease severity has been reported in other crops after a single foliar application of H_3BO_3, $CuSO_4$, $MnCl_2$ or $KMnO_4$, which provided systemic protection against powdery mildew in cucumber plants (Reuveni et al. 1997a, b; Reuveni and Reuveni 1998). The same authors also suggested that application of nutrients such as Mn, Cu and B can exchange and therefore release Ca^{2+} cations from cell walls, which interact with salicylic acid and activate systemic acquired resistance mechanisms.

Micronutrients play an important role in plant metabolism by affecting the phenolics and lignin content and also membrane stability (Graham and Webb 1991). Micronutrients can affect resistance indirectly, as in deficient plants they become more suitable feeding substrate.

2.6.1 Manganese

Manganese is probable the most studied micronutrient about its effects on disease and is important in the development of resistance in plants to both root and foliar diseases (Graham and Webb 1991; Huber and Graham 1999; Heckman et al. 2003). Mn availability in the soil varies and depends on many environmental and soil biotic factors. Mn is required in much higher concentration by higher plants than by fungi and bacteria and there is opportunity for the pathogen to exploit this difference in requirement (Marschner 1995).

Manganese fertilization can control a number of pathogenic diseases such as powdery mildew, downy mildew, take-all, tan spot, and several others (Brennan 1992; Huber and Graham 1999; Heckman et al. 2003; Simoglou and Dordas 2006). Despite the fact that Mn application can affect disease resistance the use of Mn is limited, which is due to the ineffectiveness and poor residual effect of Mn fertilizers on most soils that need Mn supplements, and is because of the complex soil biochemistry of Mn. In most soils that require addition of Mn such as calcareous soils, 90–95% of added Mn is immobilized within a week. Mn has an important role in lignin biosynthesis, phenol biosynthesis, photosynthesis and several other functions (Marschner 1995; Graham and Webb 1991). Mn inhibits the induction of aminopeptidase, an enzyme which supplies essential amino acids for fungal growth and pectin methylesterase, a fungal enzyme that degrades host cell walls.

Manganese controls lignin and suberin biosynthesis (Römheld and Marschner 1991; Vidhyasekaran 1997) through activation of several enzymes of the shikimic acid and phenylpropanoid pathways (Marschner 1995). Both lignin and suberin are important biochemical barriers to fungal pathogen invasion (Kolattukudy et al. 1994; Rioux and Biggs 1994; Hammerschmidt and Nicholson 2000; Vidhyasekaran 1997, 2004), since they are phenolic polymers resistant to enzymatic degradation (Agrios 2005). Lignin and suberin are believed to contribute to wheat resistance against powdery mildew and to all diseases caused by *Gaeumanomyces graminis* (Sacc.) (Rovira et al. 1983; Graham and Webb 1991; Huber 1996b; Krauss 1999). It has also been shown that Mn soil applications reduce common scab of potato (Keinath and Loria 1996), *Fusarium* spp. infections in cotton and *Sclerotinia sclerotiorum* (Lib. de Bary) in squash (Graham and Webb 1991; Agrios 2005).

2.6.2 Zinc

Zinc was found to have a number of different effects as in some cases it decreased, in others increased, and in others had no effect on plant susceptibility to disease (Graham and Webb 1991; Grewal et al. 1996). In most cases, the application of Zn reduced disease severity, which could be because of the toxic effect of Zn on the pathogen directly and not through the plant's metabolism (Graham and Webb 1991).

Zinc plays an important role in protein and starch synthesis, and therefore a low zinc concentration induces accumulation of amino acids and reducing sugars in plant tissue (Marschner 1995; Römheld and Marschner 1991). As an activator of Cu/Zn-SOD, Zn is involved in membrane protection against oxidative damage through the detoxification of superoxide radicals (Cakmak 2000). Impairments in membrane structure caused by free radicals lead to increased membrane leakage of low-molecular-weight compounds, the presence of which favors pathogenesis (Graham and Webb 1991; Marschner 1995; Mengel and Kirkby 2001). Application of Zn to the soil reduced infections by *Fusarium graminearum* (Schwabe) and root rot diseases, e.g., caused by *G. graminis* (Sacc.) in wheat (Graham and Webb 1991; Grewal et al. 1996).

2.6.3 Boron

Boron is the least understood essential micronutrient for plant growth and development, and at the same time B deficiency is the most widespread micronutrient deficiency in the world (Brown et al. 2002; Blevins and Lukaszewski 1998; Röhmeld and Marschner 1991). B has a direct function in cell wall structure and stability and has a beneficial effect on reducing disease severity. In several diseases, however, the function of B in disease resistance or tolerance is the least understood of all the essential micronutrients for plants. The function that B has in reducing disease susceptibility could be because of (1) the function of B in cell wall structure, (2) the function of B in cell membrane permeability, stability or function, or (3) its role in metabolism of phenolics or lignin (Brown et al. 2002; Blevins and Lukaszewski 1998).

Boron promotes stability and rigidity of the cell wall structure and therefore supports the shape and strength of the plant cell (Marschner 1995; Brown et al. 2002). Furthermore, B is possibly involved in the integrity of the plasma membrane (Marschner 1995; Brown et al. 2002; Dordas and Brown 2005). B has been shown to reduce diseases caused by *Plasmodiophora brassicae* (Woron.) in crucifers, *Fusarium solani* (Mart.) (Sacc.) in bean, *Verticillium albo-atrum* (Reinke & Berth) in tomato and cotton, tobacco mosaic virus in bean, tomato yellow leaf curl virus in tomato, *G. graminis* (Sacc.) (Graham and Webb 1991) and *Blumeria graminis* (D.C.) (Speer) in wheat (Marschner 1995).

2.6.4 Iron

Iron is one of the most important micronutrients for animals and humans and the interaction between Fe nutrition and human or animal health has been well studied, as it is involved in the induction of anemia. However, the role of Fe in disease resistance is not well studied in plants. Several plant pathogens, e.g., *Fusarium*, have higher requirements for Fe or higher utilization efficiency compared with higher plants. Therefore, Fe differs from the other micronutrients such as Mn, Cu and B, for which microbes have lower requirements. Addition of Cu, Mn and B to deficient soils generally benefits the host, whereas the effect of Fe application is not as straightforward as it can have a positive or negative effect on the host. Fe can control or reduce the disease severity of several diseases such as rust in wheat leaves, smut in wheat and *Colletotrichum musae* in banana (Graham and Webb 1991; Graham 1983). Foliar application of Fe can increase resistance of apple and pear to *Sphaeropsis malorum* and cabbage to *Olpidium brassicae* (Graham 1983). Also, in cabbage the addition of Fe overcame the fungus-induced Fe deficiency in the host but it did not affect the extent of infection (Graham and Webb 1991; Röhmeld and Marschner 1991). In other cases, Fe in nutrient solution did not suppress take-all of wheat and *Colletotrichum* spp. in bean. Application of Fe to disease-suppressive soils increased take-all of barley, and in soils with a high disease score Fe had no effect.

Iron can promote antimycosis or interfere with it. Fe does not seem to affect lignin synthesis, even though Fe is a component of peroxidase and stimulates other enzymes involved in the biosynthetic pathway. Fe can activate enzymes that are involved in the infection of the host by the pathogen or the defense, which is why opposite effects were found (Graham and Webb 1991). Fe can promote synthesis of fungal antibiotics by soil bacteria (Graham and Webb 1991). Rhizosphere microorganisms can synthesize siderophores which can lower Fe level in the soils. These siderophores can suppress germination of clamydospores of *Fusarium oxysporum f.sp. cucumerinum in vitro*. However, the production of siderophores and the antagonisms for Fe are not only mechanisms to limit the growth of parasitic fungus.

2.6.5 Chlorine

Chlorine is required in very small amounts for plant growth and Cl deficiency has rarely been reported as a problem in agriculture. However, there are reports showing that Cl application can enhance host plants' resistance to disease in which fairly large amounts of Cl are required, which are much higher than those required to fulfill its role as a micronutrient but far less than those required to induce toxicity (Mann et al. 2004). It has also been suggested that Cl might interact with other nutrients such as Mn. Cl has been shown to control a number of diseases such as stalk rot in corn, stripe rust in wheat, take-all in wheat, northern corn leaf blight and downy mildew of millet, and septoria in wheat (Graham and Webb 1991; Mann et al. 2004). The mechanism of Cl's effect on resistance is not well understood. It appears to be nontoxic *in vitro* and does not stimulate lignin synthesis in wounded wheat leaves. It was suggested that Cl can compete with NO_3^- absorption and influences the rhizosphere pH: it can suppress nitrification and increase the availability of Mn. Furthermore, Cl ions can mediate reduction of $Mn^{III,IV}$ oxides and increase Mn for the plant, increasing the tolerance to pathogens.

2.6.6 Silicon

Although Si is the second most abundant element in the earth's soil and is a component of plants it is not considered to be an essential element as defined by Arnon and Stout, except for members of the Equisitaceae family (Marschner 1995). However, when Si is added to the soil, plants low in soluble Si show an improved growth, higher yield, reduced mineral toxicities and better disease and insect resistance (Graham and Webb 1991; Alvarez and Datnoff 2001; Seebold et al. 2000, 2004). Also, in many countries crops such as rice and sugarcane which accumulate high levels of Si in plant tissue are fertilized routinely with calcium silicate slag to produce higher yields and higher disease resistance. Si has been shown to control a number of diseases such as blast (*Magnaporthe grisea*) in St. Augustinegrass, brown spot (*Cochliobolus miyabeanus* (Ito and Kuribayashi in Ito Drechs ex Dastur) in rice and sheath blight (*Thanatephorus cucumeris* (A.B. Frank) Donk.) in rice, and increase the tolerance of various turfgrasses to *Rhizoctonia solani*, *Pythium* spp., *Pyricularia grisea* (Cooke sacc) and *Blumeria graminis* (DC) (Carver et al. 1998; Savant et al. 1997; Alvarez and Datnoff 2001; Seebold et al. 2000, 2004; Zhang et al. 2006)

The mechanism by which Si confers disease suppression is not well understood. It is believed that Si creates a physical barrier which can restrict fungal hyphae penetration, or it may induce accumulation of antifungal compounds such as flavonoid and diterpenoid phytoalexins which can degrade fungal and bacterial cell walls (Alvarez and Datnoff 2001; Brescht et al. 2004).

Except from the essential nutrients for plant growth and development there are a number of other elements that can occur in plant tissue in trace amounts (Li, Na, Be, Al, Ge, F, Br, I, Co, Cr, Cd, Pd and Hg) and have occasionally been linked with host–pathogen relationships: Li and Cd through their marked suppressive effects on powdery mildews are the most noteworthy. Cd was found to inhibit spore germination and development at a concentration of $3\,\mathrm{mg\,kg^{-1}}$, which is not toxic but elicits a response to infection in the host. Cd and Hg can also promote synthesis of lignin in wheat (Graham and Webb 1991). The mechanism of Li is not known and it is quite possible that it catalyzes a metabolic pathway which can function in defense.

3 Nutrient Management and Disease control

Fertilizer application affects the development of plant disease under field conditions directly through the nutritional status of the plant and indirectly by affecting the conditions which can influence the development of the disease such as dense stands, changes in light interception and humidity within the crop stand. It is important to provide a balanced nutrition and at the time when the nutrient can be most effective for disease control and also for higher yield. Not only the application of the fertilizer can affect the disease development, but also anything that affects the soil environment such as pH modification through lime application, tillage, seedbed firmness, moisture control (irrigation or drainage), crop rotation, cover crops, green manures, manures and intercropping.

3.1 Examples of Disease Control by Nutrients

There are several examples of disease control through nutrient manipulation which can be achieved by either modifying nutrient availability or modifying nutrient uptake (Huber and Graham 1999). The most common way to affect the nutrient availability is by using a fertilizer; however, changing the environment through pH modification, tillage, seedbed firmness, moisture control (irrigation or drainage) and specific crop sequences can have a striking effect on nutrient availability. Use of nitrification inhibitors can increase the efficiency and availability of N in high leaching or denitrifying conditions. Addition of microorganisms such as bacteria, fungi which form mycorrhizae and any plant growth-promoting organisms can increase nutrient uptake (P, Zn, Mn) by influencing minor element availability through their oxidation–reduction reactions or siderophore release (Huber and McCay-Buis 1993). In some cases, the application of fertilizers to the soil is not always effective, such as in the case of Mn, Zn and Fe in high-pH soils with high concentrations of free $CaCO_3$, or where rapid oxidation by microorganisms makes Mn unavailable in the soil. Many times it is recommended to conduct foliar applications which relieve aboveground deficiency symptoms, but Mn is not well translocated in the phloem so that root tissues which are attacked by the pathogens remain Mn-deficient (Huber and McCay-Buis 1993). Also, addition of nitrification inhibitors with NH_4^+ fertilizers can suppress Mn oxidation as well as nitrification and increase the availability of Mn, P and Zn for plant uptake.

Nutrient uptake can be altered by changing root absorption, translocation and metabolic efficiency, and in some cases it has been shown that wheat seeds with higher Mn content produced plants with less take-all compared with the same cultivars with a lower Mn concentration in the seed (Huber and McCay-Buis 1993). Increasing the nutrient content in the grains was actively pursued as a means of improving human nutrition and may concurrently increase plant resistance to a variety of diseases (Graham 1983; Graham and Webb 1991).

Some of the most common examples of interaction of nutrients and disease have been the Streptomyces scab of potato, Verticillium wilt, take-all of wheat, stalk rot of corn, clubroot of crucifers, fusarium wilt and tissue-macerating disease (Huber and Graham 1999). *Streptomyces* spp. are strong Mn oxidizers and any cultural technique such as crop rotation, soil amendments with specific crop residues, N fertilizers, soil acidification and irrigation can increase Mn availability and reduce the incidence of the disease. Verticillium wilt caused by *Verticillium albo-atrum* and *V. dahliae* is very common and in many cases is one of the most devastating diseases of vegetables, ornamentals, fruits, herbs, field and forage crops. Verticillium wilt can be controlled by resistant cultivars, careful crop rotation, sanitation, soil fumigation and nutrient sufficiency, as N, P and K can reduce the disease. Soil fumigation and nitrification inhibitors maintain NH_4^+ in the soil, increase Mn, Cu and Zn and reduce Verticillium wilt in tomato. Green manure and flooding the soil to maintain the high moisture content of the soil (known as flood fallowing) can control Verticillium wilt in potatoes and tomatoes due to the reduction in inoculum potential and also by increasing the availability of Mn and other nutrients.

Take-all is one of the most important diseases of wheat and occurs in many countries of the world. It was found that 12 of the 14 principal nutrients required for plant growth affect take-all. Application of N fertilizer and especially NH_4^+ can reduce the losses from take-all: NH_4^+ also increases the availability of Mn, Zn and Fe. Crop rotation can decrease the incidence of the disease. Also, it was found that long-term monocropping of wheat provides a natural biological control of this disease called take-all decline. Oat can also reduce take-all of wheat. In addition, balanced nutrition, sufficient P and nitrification inhibitors, along with crop rotation, are some of the most effective strategies for reducing take-all in many areas.

Fusarium oxysporum is an important pathogen which causes vascular wilt in many crops such as vegetable, fruit, fiber and ornamental crops. Fusarium wilt is favored by warmer, low-pH soils. In contrast, application of NO_3^--N fertilizers and application of lime, which reduces the availability of Mn and Fe, increases the pH and results in the reduction of the pathogen.

4 Use of cultural methods in improving plant nutrition and disease resistance

Not only the application of nutrients as fertilizers can increase the tolerance to the disease, but any measure that can increase the availability and limit the imbalance of certain elements can affect growth and the tolerance of diseases. Most of the approaches that are used in sustainable agriculture have been found to provide a balanced plant nutrition, and at the same time to increase the availability of certain elements and improve the tolerance of plants to disease (Oborn et al. 2003). Approaches such as crop rotation, green manure, application of manures, intercropping and tillage can affect disease resistance and also plant growth. Most of these approaches can significantly increase soil organic matter, which is very important in sustainable agriculture.

4.1 Soil Organic Matter

Soil organic matter (SOM) content and quality affects many soil functions which are related to soil health such as moisture retention, infiltration, release, and also plant health. Field-applied organic residues (crop residues, cover crops and organic wastes) can affect soilborne pathogens and diseases and it is a cultural practice that can affect the availability of nutrients (Stone et al. 2004). Practices such as addition of sphagnum peat, green manures and animal manures have been shown to produce suppressive soils on which pathogens do not establish or persist and do not affect the crop plants. Addition of sphagnum peat to soil has been shown to suppress disease caused by *Pythium* spp. (Hu et al. 1997). Also, addition of different organic amendments has been shown to reduce Phytophthora root rot in a number of species (Hoitink et al. 1977; Spencer and Benson 1982; Szczech et al. 1993; Dixon et al. 1990; Hu et al. 1997). Dairy manure can suppress a number of pathogens in sweet corn (causal agents *Drechslera* spp., *Phoma* spp. and *Pythium arrhenomanes*) and snap bean (causal agents *Fusarium solani* and *Pythium* spp.). There are several mechanisms that are proposed to be involved in biologically and organic material-mediated disease suppression such as microbiostasis, microbial colonization of pathogen propagules, destruction of pathogen propagules, antibiosis, competition for substrate colonization, competition for root infection sites and induced system resistance (or systemic acquired resistance SAR). Soil organic matter (SOM) quantity and quality can affect the plant nutrient status. SOM can impact not only on the total soil nutrient content but also on nutrient availability through the activity of soil microorganisms. Therefore, nutrients can affect disease incidence by increasing plant resistance, improving plant growth (allowing the plant to escape the disease), and influencing the pathogen's environment. Although quantity and quality can have dramatic impacts on soil and plant nutrient content there are only a few studies which focus on soil properties and disease incidence which investigate the contribution of soil or tissue nutrient contents to disease-suppressive effects. Fields with a history of annual organic amendments had higher microbial activity and K contents. Lower NO_3 content and corky root incidence was positively correlated with soil NO_3 and plant tissue N and negatively correlated with soil N mineralization potential, microbial activity, total soil N and soil pH. In another study composed biosolids improved ryegrass establishment, growth and tolerance to leaf rust (caused by *Puccinia* spp.) by improving N nutrition in the amended soil (Loschinkohl and Boehm 2001).

4.2 Crop Rotation and Cover Crops

Crop rotation is the practice of growing a sequence of different crops on the same field. Long-term experiments (more than 100 years) showed that crop rotation together with other fertility management practices are fundamental to long-term agricultural productivity and sustainability (Reid et al. 2001; Stone et al. 2004). The most straightforward principle underlying rotation as a disease control strategy is that plant pathogen propagules have a lifetime in soils and rotation with nonhost crops starves them out (Reid et al. 2001). In bean crops rotation is the most powerful and effective practice to control bean diseases. Crop rotation can increase N levels and can also affect the availability of other nutrients which can affect the disease severity (Reid et al. 2001; Huber and Graham 1999). Also, crop rotation affects the survival of pathogens and it has been used extensively to reduce the severity of many diseases. A nutrient that is affected by crop rotation is Mn: it was found that crop rotation with lupins increases the availability of Mn (Graham and Webb 1991).

Not only crop rotation but also cover crops can change soil chemical, physical and biological properties, including the composition of the soil microbial community, and can therefore reduce or increase the severity of plant diseases. The effect depends on the plant species used and cultivars. Cover crops can increase the content of active OM in the soil, microbial biomass and microbial activity, and contribute to suppression. Cover crops affect the rhizosphere and also the soil microbial community composition and in that indirect way can affect plant health. Crop rotation can influence the severity of soilborne diseases by increasing the buffering capacity of the soil, denying the pathogen a host during the interim of unsuitable species and affecting nitrification, which influences the form of N predominant in the soil (Huber and Graham 1999; Graham and Webb 1991).

Green manure can affect the availability of N and also other nutrients such as P and K. Most of the green manure species that are used can fix N with N-fixing bacteria and can increase soil N levels by $459\,\mathrm{kg\,N\,ha^{-1}}$ (Cherr et al. 2006). This can have a significant effect on disease development. Also, green manures can affect the availability of other nutrients such as P, Mn and Zn, which can affect the tolerance of disease (Huber and Graham 1999; Graham and Webb 1991).

4.3 Intercropping

Intercropping systems have the potential to reduce the incidence of diseases (Anil et al. 1998). However, different responses to disease severity with different systems of intercropping have been observed. There are four mechanisms involved in an intercropping system that can reduce disease incidence, all of which involve lowering the population growth rate of the attacking organism:

1. the associate crop causes plants of the attacked component to be poorer hosts.
2. the associate crop interferes directly with the attacking organism.
3. the associate crop changes the environment of the host such that natural enemies of the attacking organism are favored.
4. the presence of nonhost or resistant plants growing in-between susceptible plants can physically block inoculum from reaching the susceptible hosts (i.e., the nonhost serves as a physical barrier to the pathogen inoculum).

Francis (1989) found that intercropping reduced pests and diseases in 53% of experiments and increased them in 18%. The reasons for this increase in pests include reduced cultivation and increased shading, favoring some pests and pathogens; associate species serving as alternative hosts; and crop residues serving as a source of pathogen inoculums. In addition, intercropping was found to improve nutrients by increasing N from legumes, or increasing the uptake of phosphorus and potassium (Anil et al. 1998, reference therein).

4.4 Soil Tillage

Reduced tillage systems or zero tillage can increase SOM content in many agricultural systems. Reduced tillage has the advantage that it conserves SOM, reduces erosion, and reduces energy consumption and production costs (Carter 1994; Fernandez et al. 1999). However, reduced tillage can alter the soil environment and these changes can result in an increase, decrease or no change in disease incidence or severity, depending on the cropping system and disease. Minimum tillage concentrates residues on the soil surface and therefore concentrates the pathogen propagule number on the soil surface: this might or might not impact on disease incidence. Minimum and zero tillage do not disrupt the plant residues in the soil as much as conventional tillage (i.e., since they tend not to bury them), thereby leaving more stubble on the soil surface. The adoption of conservation tillage by farmers has led to an increase in the incidence and severity of many stubble-borne diseases. Standing residues or residues lying on the soil surface are colonized by soil organisms much more slowly and pathogen survival and growth in the undisturbed residues are favored in these systems. Residue-colonizing pathogens are therefore favored over the reduced tillage system and can generate significant yield reduction (Bockus and Schroyer 1998). Conservation-tillage systems concentrate plant residues in the surface soil layer, and microbial biomass and activity are higher in that layer (Dick 1984).

5 Systemic Induced Resistance or Systemic Acquired Resistance

The induction of resistance reactions of plants against pathogens is a well-known phenomenon in plant pathology. It was first described as a resistance to an attack from a nonvirulent pathogen. Thus, it is an enduring, nonspecific resistance against pathogens, induced by pathogens that cause a necrotic reaction on the infected leaves, and it is called systemic acquired resistance (SAR) if the resistance is systemically distributed within the plant. SAR can be induced by avirulent pathogens but also by chemical compounds such as salicylic acid (SA), which is involved in the signal transduction pathway leading to SAR, and also structural analogues of SA can induce SAR. Wiese et al. (2003) introduced the term chemically induced resistance (CIR), which is used to describe the systemic resistance after application of synthetic compounds. This resistance is related to the formation of structural barriers such as lignification, induction of pathogenesis-related proteins and conditioning of the plants (Graham and Webb 1991).

Systemic induced resistance (SIR) has been found to be induced by foliar sprays of nutrients such as phosphates, K and N. It has been hypothesized that during SIR an immunity signal released or synthesized at the induction site of the inducer leaf is systemically translocated to the challenged leaves, where it activates the mechanisms for defense (Reuveni and Reuveni 1998). Salicylic acid (SA) has been hypothesized as a possible signal and its exogenous application induces resistance and PR proteins, which typically accompany SIR (Reuveni and Reuveni 1998). However, SA was found in the phloem sap of noninfected upper leaves when it could not be detected in the phloem sap collected from petioles of the lower leaves infected with *Pseudomonas syringae*. This indicates that SA may not be the primary systemic signal for SIR.

A single phosphate foliar application can induce high levels of systemic protection against powdery mildew caused by *Sphaerotheca fuliginea* in cucumbers (Reuveni et al. 1997a, b). A similar response was found in maize, where foliar spray with phosphates induced a systemic protection against common (caused by *Puccinia sorgi*) and northern leaf blight (NLB) (caused by *Exserohilum turcicum*). Trace elements may also play an important role in plants, affecting their susceptibility to fungal or bacterial phytopathogens (Graham 1983). Foliar spray with H_3BO_3, $CuSO_4$, $MnCl_2$ or $KMnO_4$ separately induced systemic protection against powdery mildew in cucumber plants. Similar results were found in wheat, where application of B, Mn and Zn separately increased the resistance of plants to tan spot (Simoglou and Dordas 2006). The mechanism of SIR development is still unknown and it was proposed that the chemicals trigger a release and rapid movement of the "immunity signal" from the infected leaves to the unchallenged ones (Reuveni and Reuveni 1998). The mechanism might involve an increase in both solute and ionically bound components of peroxidase activity and β-1,3-glucanase in protected leaves above those sprayed with $MnCl_2$. Mn and Cu might act as cofactors of metalloprotein enzymes such as peroxidase, for which Mn ions serve as an inducing agent (Marschner 1995; Mengel and Kirkby 2001). Peroxidase and β-1,3-glucanase are involved in the cross-linking of the cell wall components, polymerization of lignin and suberin monomers and subsequent resistance to pathogens. SA is proposed to be a translocatable signal compound in SIR and interacts with intercellular Ca^{2+} in the induction of chitinase in carrot suspension culture. Application of cations such as Mn, Cu and B can increase the Ca^{2+} cations, and interact with SA and activate SIR (Reuveni and Reuveni 1998). These findings indicate that the mechanism for resistance is present in susceptible plants and it can be induced by simple inorganic chemicals, and that this induced resistance is not pest-specific.

6 Future Perspectives

More research is needed in order to find the nutrients or nutrient combinations which can help to reduce disease severity. It is also necessary to find the best integrated pest management approaches with disease-resistant varieties which can be combined with specific cultural management techniques and can efficiently control plant disease. In addition, more research is required to find how the nutrients increase or decrease disease tolerance or resistance, what the changes are in plant metabolism and how this can be used to control plant disease.

It is also important to understand the biochemical pathways by which the nutrients can affect disease. Despite the fact that each nutrient has several functions, mild deficiency can usually be linked to one or more processes that are most sensitive and these processes are linked to the secondary metabolism, which is not immediately necessary for the survival of the organism. The secondary metabolism is involved in the defense against pathogens and some of the roles are well understood and others remain to be elucidated. Also, the evidence that an element has a role in the defense mechanisms not yet regarded as essential in higher plants could lead to recognition of their essentiality. This may require a slight modification of the criteria of essentiality to cover the situation in which yield increases, and indeed survival, are due to the element in question which is manifested only in the presence of a pathogen. This means that such essential elements would not be recognized in disease-free laboratory conditions. The requirement for a key biochemical role would remain.

Systemic induced resistance (SIR) (caused by application of nutrients) could be an alternative strategy to reduce disease severity. In addition, there is a commercially available product containing acibensolar-*S*-methyl (with the commercial name Actigard) that activates the same defense response of SAR. The best SIR will be a chemical which can minimize adverse effects on the host and has high levels of efficacy. NPK fertilizers together with disease-resistant cultivars can be used in this way; however, other nutrients can be used together with NPK in order to reduce disease. In addition, any measure such as crop rotation, application of manures, green manures and cover crops can be used to increase nutrient availability and reduce disease incidence and can be used in the IPM system in sustainable agriculture. Also, the reduction in the crop production cost, the conservation of beneficial biological enemies of pests, preservation of environmental quality and slowing the rate of development of pesticide-resistant strains are some of the benefits that the use of fertilizer can have on IPM and on sustainable agriculture.

7 Conclusion

In most of the studies reported here the addition of nutrients or application of fertilizers has decreased the incidence of disease in crop plants. This is probably because these nutrients are involved in the tolerance or resistance mechanisms of the host plant. Nutrient application had a much greater effect on reducing disease when the plants were at deficiency levels. Supraoptimal rates of nutrients can also decrease the disease incidence. In cases where the addition of a nutrient has exacerbated the disease it is possibly because of toxicity rather than deficiency; or in other cases, the addition of a nutrient can aggravate the primary deficiency. Also, in sustainable agriculture balanced nutrition is an essential component of any integrative crop protection program because in most cases it is more cost-effective and also environmentally friendly to control plant disease with the adequate amount of nutrients and with no pesticides. Nutrients can reduce disease to an acceptable level, or at least to a level at which further control by other cultural practices or conventional organic biocides are more successful and less expensive.

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Crop Protection, Biological Control, Habitat Management and Integrated Farming

Pierre Ferron and Jean-Philippe Deguine

Abstract In a context of rationalised agriculture integrating the principles of sustainable development, preventive measures for crop protection are called upon to play a dominant role. These are based on close knowledge of the functioning of agroecosystems. They are aimed at managing biological pests and their natural enemies, first through action on their habitats both in crop fields and in the non-cultivated part of the farm. A balance of the biological control possibilities available is drawn up and attention paid to the application of recent knowledge in conservation biology. The bases of pest control with a ranking of the various intervention techniques are then set out. Implementation means the development of cropping systems and therefore increased participation by agronomists and also a break with certain agronomic practices commonly used by farmers today.

Keywords Biodiversity • Biological control • Habitat management • Integrated control • Integrated farming

Résumé Dans le contexte d'une agriculture raisonnée intégrant les principes d'un développement durable, les mesures préventives de protection des cultures sont appelées à jouer un rôle prépondérant. Celles-ci reposent sur une connaissance approfondie du fonctionnement des agro-écosystèmes. Elles visent la gestion des populations de bio-agresseurs et de leurs ennemis naturels, d'abord par l'aménagement de leurs habitats, dans les parcelles de culture comme dans les zones non cultivées de l'exploitation. Un bilan des possibilités offertes par la méthode de lutte biologique est dressé et l'attention est attirée sur les applications des connaissances récemment acquises dans le domaine de la biologie de la conservation. Les bases d'une stratégie phytosanitaire, hiérarchisant le recours aux diverses techniques d'intervention, sont avancées. Sa mise en œuvre implique un aménagement des systèmes de culture et donc une participation accrue des agronomes, mais aussi une rupture avec certaines pratiques agronomiques couramment utilisées par les praticiens aujourd'hui.

Mots clés Biodiversité • Lutte biologique • Culture intégrée

1 Introduction

Agriculture has been subjected to fresh socioeconomic pressure for about a decade, in particular following the globalisation of trade and the taking into account of the sustainable development concept. Thus Europe's common agricultural policy (CAP) now favours produce quality and simultaneously recommends the adoption of agri-environmental measures. To remain competitive in this new context, farmers must limit production costs and in particular reduce the quantities of inputs that hitherto enabled a continuous increase in yields. Crop management sequences and farming systems themselves can thus be called into question.

The protection of crops from organisms that are occasionally harmful (microbes and phytopathogenic viruses, animal pests and weeds) or biological pests is particularly concerned by this development because of

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the scale of the costs involved and the unfavourable secondary effects on food chains and biological balances. Rich with various experience gained during the last century – significantly marked by the development and success of synthetics pesticides – crop protection may also experience a conceptual development allowing it to progress from the stage of control of biological pests to that of population management applied to both pests and auxiliaries within the framework of increased mastery of the perennial functioning of agroecosystems.

2 Crop Protection: Control or Management

The traditional method for controlling biological pests is curative, consisting essentially of chemical methods thanks to the discovery, synthesis and marketing of pesticides with practically instant efficacy, ease of application but a finally substantial cost and above all frequently insufficient target specificity. Application is at best determined after prior diagnosis of the real risk at field level according to economic thresholds set for each crop for a given socioeconomic situation. Its success among farmers led to forgetting the interest initially shown in other methods – usually agronomic and preventive (fallows, cropping sequences, crop rotation, etc.). Today, alternative phytosanitary solutions are nevertheless recommended within the context of so-called integrated control but they have not really changed habits that are now firmly anchored, except in a few special cases in which phenomena of resistance to synthetic pesticides had led to notorious economic and crop health impasses (Ferron, 1999).

Innovative measures were nevertheless recommended from 1967 onwards by FAO (Fig. 1) with the "integrated control" concept adopted a few years later by Californian scientists who used the expression integrated pest management (IPM). The latter phrase meant that attention was drawn significantly to the conceptual difference between the notions of control and management. Unfortunately, application of the new approach, illustrated in particular in the USA by the so-called "Huffaker IPM Project" did not have the success expected, mainly because of the difficulty of persuading farmers of the need to first reduce the quantities of pesticides generally used (Benbrook, 1996; Perkins, 1982). As a reaction and to underline the advantages of alternative biological solutions, the expressions "biologically intensive IPM", "biointensive IPM" and "ecologically based IPM" were proposed by various authors (Ferron, 1999).

In a general manner, teachers, scientists and technicians themselves did not award the desired importance to this integrated control approach, limiting it at best to its reduced meaning – the management of a single biological pest species in a given crop – whereas in the broad sense it is applied to the harmonious management of all pest populations in their agricultural or forest environment (FAO, 1967). Its meaning was often the subject of more or less erroneous interpretations causing a dispersal of the means and efforts devoted to promoting it. Furthermore, the rare supporters of IPM counted on the application of new biotechnologies and unfortunately usually limited themselves to the sometimes extremely elaborate development of pesticide preparations based on biological agents usable in the form of biological treatments comparable to those performed with synthetic pesticides. Various experiences have shown that the gamble was a risky one for agronomic, biotechnical, ecological and economic reasons. Furthermore, the international biopesticides market has stagnated for some 20 years at an insignificant level forming some 2% of the world market for pesticides of all kinds. A few rare state laboratories have remained faithful to the original principle of biological

▶ **Integrated control** is a pest management system that in the context of the associated environment and the population dynamics of the pest species uses all suitable techniques and methods in as compatible a manner as possible and maintains the pest populations at levels below those causing economic injury. It is not a simple juxtaposition or superposition of two control techniques (such as chemical and biological control) but the integration of all the management techniques suited to the natural regulation and limiting factors of the environment (FAO, 1967).

▶ **Pest management** is the reduction of pest problems by actions selected after the life systems of the pests are understood and the ecological as well as economic consequences of these actions have been predicted, as accurately as possible, to be in the best interest of mankind. In the development of a pest management programme, priority is given to understanding the role of intrinsic and extrinsic factors in causing seasonal and annual change in pest populations (Rabb and Guthrie, 1970).

▶ **Integrated pest management** (IPM) is a pest containment strategy that seeks to maximise natural control forces such as predators and parasites and to use other tactics only as needed and with a minimum of environmental disturbance (Glass, 1975).

Fig. 1 Integrated control, pest management and integrated pest management (IPM) control with the introduction-acclimatisation of exotic beneficials, but have discovered the constraints and possible secondary effects of such practices on indigenous fauna and flora. True achievements have nevertheless been made recently in this area, in particular in weed control.

Today, the supporters of so-called integrated farming (Bonny, 1997) recommend the implementation to the greatest possible extent of preventive measures and the use of alternative biological solutions (Fig. 2). However, it should be noted that except in special cases these methods are still not sufficiently operational and their inadequate results leave the field open to an organised synthetic pesticides market, at least in the developed countries, whatever the degree of supervision of registration and conditions of application.

3 The Ecological Basis of Crop Protection

Targeting the population of a biological pest species using the principles of integrated pest management was therefore a significant stage in the recent evolution of phytosanitary protection, even though this new attitude has not yet been adopted by all stakeholders. There is a tendency to move from the control of a pest in a given crop field to the rational management of its populations in the whole farm, or even in several adjoining farms (areawide integrated pest management) (Kogan, 2002). Furthermore, such management of biological pest populations is part of the framework of the overall management of populations closely associated with the same agroecosystem. Drawing up a new crop protection strategy therefore requires the taking into account of the spatio-temporal dimension of the phenomena and involves the development of cropping systems. Overall, farming must now be integrated rationally in the functions of the ecosystems; nature is no longer domesticated (Vandermeer, 1995). Production objectives in a given socio-economic framework are therefore logically associated with the environmental constraints of sustainable development of the biosphere.

From the scientific point of view, this new approach was recently enhanced by the increased knowledge of biology, genetics and population ecology. Conservation biology, a new synthetic discipline, is therefore attempting to respond to the major challenge raised by the sustainable management of the biosphere through its association of the most recent knowledge in biology with the contributions of the social sciences, economics and political science (Fig. 3). The implementation of the Convention on Biological Diversity is the preferred field of application. Among other general considerations, the Preamble to the Convention mentions (a) the recognised role of biological diversity in the general functioning of the biosphere, (b) the responsibility of humans for reducing it, (c) a general lack of knowledge for taking appropriate measures for conserving it, and (d) the interest of the *in situ* conservation of ecosystems and natural habitats and of the maintenance and recovery of viable populations of species in their natural surroundings.

It is estimated that 40–50% of land has been changed or degraded by man with, in particular in agricultural land, a decrease in biological diversity, biological activity and the proportion of organic matter in the soil (Lévêque and Mounolou, 2001). The necessary conservation of ecosystems is therefore undertaken

▶ **Integrated farming** (or integrated crop management, ICM) is 'a holistic pattern of land use, which integrates natural regulation processes into farming activities to achieve a maximum replacement of off-farm inputs and to sustain farm income' (El Titi et al., 1993).

Fig. 2 Integrated farming

▶ **Biological diversity** or biodiversity has been defined as 'the variety of living organisms considered at all levels of organization, including the genetic, species, and higher taxonomic levels, and the variety of habitats and ecosystems, as well as the processes occurring therein' (Meffe and Carrol, 1997)

▶ 'The study of biodiversity and the means to protect it fall within the domain of an emerging science called **conservation biology** (Knight and Landres, 2002)

▶ '**Biological conservation** is a more encompassing field than is conservation biology in that it addresses not only the biology, but also the planning, managing, and politics of protecting life's diversity' (Knight and Landres, 2002)

Fig. 3 Biological diversity, conservation biology, and biological conservation through protected zones or sustainable development policies. In Europe, for example, the objective of the Natura 2000 network is the conservation of biodiversity through a network of protected sites where appropriate habitat management is implemented with the taking into account of both economic, social and cultural constraints and regional and local features. Accepting such a strategy in agronomy is necessarily the result of a compromise and involves a change in traditional farming systems where productivity is favoured at the expense of quality of the environment and biodiversity.

3.1 The Population–Environment System

Ecologists defines a population as all the individuals of the same species within specified spatial limits. The population-environment concept (Barbault, 1997) is coherent with this definition by associating the state variables of a population (number, spatial distribution, age structure, genetic structure, and social organisation) with the characteristics of a given environment (physicochemical and biological features).

The noteworthy feature of agroecosystems is that humans have mastered on the one hand the domesticated animal and plant populations and on the other farming systems as well as landscape structures. The main difficulty encountered is that within the limits of a given environment all the populations present, or biocoenosis, must be taken into consideration. Ecologists must therefore restrict their fields of action to limited plurispecific sets referred to as populations or communities with a risk of having only a partial view of overall biological diversity.

In an agricultural environment, another feature of these populations is that they are made up of fragmented populations (metapopulations) as a result of the agrarian structures and cropping systems used (Hunter, 2002). In kinetics, wild populations are characterised by local processes of extinction and recolonisation related to the spatial heterogeneity of agroecosystems that disturb their movements and their natural regulation mechanisms. This is why studies of the dispersion of these populations is so successful today, especially at the interfaces of agrarian structures (Ekbom et al., 2000). Recent examination of the spatio-temporal heterogeneity of ecosystems means that movements between the various landscape components must henceforth be considered as a determinant factor in understanding ecological processes (Yoccoz et al., 2001).

3.2 The Dynamic Equilibrium of Populations

It was assumed until recently that ecosystems evolved towards a state of equilibrium defined by the characteristics of soil, climate and vegetation via a series of successive states characterised by an increase in spatial heterogeneity, species diversity and density of populations of organisms, increasing complexity of community organisation and the development of stabilisation mechanisms (Vandermeer et al., 1998). Now, the maintaining of agroecosystem equilibrium at some of these intermediate stages is in fact achieved by practices, development operations or cropping or pastoral systems that are called into question today because of their systemic secondary effects such as ploughing, drainage, crop spraying, grazing, etc. (McLaughlin and Mineau, 1995). The importance and form of these anthropic contributions determine the different types of agriculture (small farms, extensive, intensive, organic farming, etc.). As a result the application of the general laws of ecology to agroecosystems runs up against their anthropic specificity. This is why the reduction desired in the use of inputs (water, fertilisers and pesticides) implies in return the awarding of an increasing role to better mastery of their management methods and hence deeper knowledge of the functioning of agroecosystems.

The existence of a link between the regulation of these populations and the species diversity of communities is also a subject for discussion between ecologists and pest management specialists. However, it is generally agreed that outbreaks of biological pests are more limited when agroecosystems are more diversified. Entomologists have long attempted to demonstrate the influence of plant diversity on the associated phytophagous fauna. But they are more frequently interested in the abundance of antagonistic species (predators and parasites) than their true diversity. All the data thus gathered are not enough to affirm, except in rare cases, that crop diversification alone is sufficient to ensure the abundance and diversity desired (Andow, 1991; Letourneau and Altieri, 1999).

4 Biological Control: Results and Prospects

The idea of natural balance dates back to late nineteenth century naturalists approached by agronomists who wished to reduce harvest losses at a time when demand for produce was increasing rapidly. Traditional agricultural techniques (fallow, cropping sequence, crop rotation) were proving to be inadequate and crop protection procedures were delicate if not dangerous to use and in any case unsuitable for large-scale crops. The successful introduction of two Australasian beneficials, a predatory ladybird (*Rodolia cardinalis*) and a parasite cryptochetid fly (*Cryptochetum iceryae*), in Californian citrus orchards devastated by outbreaks of a scale of foreign origin (*Icerya purchasi*), marked the birth of a new pest control practice, biological control (Ferron, 2002).

Unfortunately, its definition is still subject to confusion today. A regrettable ambiguity is still maintained even at the International Organization for Biological Control (IOBC) of noxious animals and plants as some people limit its field of application to the use of live organisms only to control outbreaks of biological pests (DeBach, 1964; IOBC/OILB, Statutes, 1973); this contrasts with the interpretations of others who include both these living organisms and inert biological products extracted from them (botanical pesticides, natural products or bioproducts). The term biopesticide is thus also a source of the same confusion. The most recent syntheses concerning crop protection (Bellows and Fisher, 1999; Pimentel, 2002) try to clarify the situation by referring to the restrictive definition proposed by DeBach (1964) (Fig. 4), also used in this paper. The coherence of his agroecological reasoning centred on the management of living organisms is thus respected.

> Three types of procedure are used in the implementation of biological control: (a) classical biological control: the identification of indigenous and exotic natural enemies, the importation and release of exotic natural enemies, and the evaluation of the abilities of natural enemies to suppress a pest must be performed; (b) augmentation of natural enemies: the culture and release of natural enemies to suppress a pest when a natural enemy is present but in numbers insufficient to provide adequate suppression must be accomplished. Three subsets of this mode are distinguished: inoculation, augmentation, and inundation; (c) conservation of natural enemies: action must be taken to conserve existing natural enemies by preventing their destruction from other practices (Perkins and Garcia, 1999).

4.1 Benefits and Risks

After analysis of numerous data according to the criteria of a market economy aiming at immediate benefits, the results of classical biological control with the introduction of beneficial organisms are often considered to be inadequate in spite of a few spectacular successes that show that the return on investment can be very high (Table 1).

The future of the biological treatment method by augmentative biological control is considered to be limited for the moment. As mentioned above, the use of biopesticides has not evolved along the lines hoped for by the industry itself over the past 25 years because of the various difficulties involved in the manipulation of living material (patentability, registration, mass reproduction of identical material, specificity of effects, storage and marketing conditions, application).

These methods have nonetheless made it possible to solve frequently critical pest control situations where the problem is the control of species that have accidentally become invasive (weeds for example) or that of populations that have become resistant to synthetic pesticides (the case of the codling moth *Laspeyresia pomonella* in apple orchards or the American serpentine leafminer *Liriomyza trifolii* in greenhouse crops, for example). It must therefore be supposed that the evaluation criteria of the results achieved are not appropriate for

- ▶ **Biological control**, when considered from the ecological viewpoint as a phase of natural control, can be defined as the action of parasites, predators or pathogens in maintaining another organism's population density at a lower average than would occur in their absence (DeBach, 1964)
- ▶ **Biological control** means the use of living organisms to prevent or reduce the losses or harm caused by pest organisms (IOB/OILB, Statutes, 1973)

Fig. 4 Biological control

Table 1 Some economic assessments of classical biological control programmes (USD milli on) (Greathead, 1995)

Pest	Regions	Savings[a]	Costs of control programme
Cassava mealybug *Phenacoccus manihoti*	Africa (1984–2003)	96.0	14.8
Rhodes grass scale *Antonina graminis*	Texas (1974–1978)	194.0	0.2
Skeleton weed *Chondrilla juncaea*	Australia (1975–2000)	13.9	3.1
Wood wasp *Sirex noctilio*	Australia (1975–2000)	0.8	8.2
White wax scale *Ceroplastes destructor*	Australia (1975–2000)	0.09	1.4
Two-spotted mite *Tetranychus urticae*	Australia (1975–2000)	0.9	0.9
Potato tuber moth *Phthorimaea operculella*	Zambia (1974–1980)	0.09	0.04
Spotted alfalfa aphid *Therioaphis trifolii*	USA (1954–1986)	77.0	1.00
Water fern *Salvinia molesta*	Sri Lanka (1987–2112)	0.5	0.22

[a]The years in brackets are those of the period used by economists in calculating the discounted benefits shown in column 3 as annual "savings"

▶ **Conservation biological control** involves the use of tactics and approaches that incorporate the manipulation of the environment (i.e. the habitat) of natural enemies so as to enhance their survival and/or physiological and behavioural performance, and result in enhanced effectiveness. This approach to biological control can be applied to exotic (i.e. introduced) natural enemies used as part of classical or augmentative biological control programmes as well as to indigenous (native) natural enemies (Barbosa, 1998)

Fig. 5 Conservation biological control

the target (Gutierrez et al., 1999), a question that feeds more general debate on the economic evaluation of biological diversity (Lévêque and Mounolou, 2001).

Although knowledge is lacking about how to satisfactorily quantify the benefits of biological control, the limits have been better evaluated (Duan and Follett, 2000; Wajnberg et al., 2000). These mainly concern the environmental consequences of the acclimatisation of exotic living organisms used as biological control agents, whether such consequence are direct effects on non-target indigenous species or indirect effects on the communities concerned; the hypothesis has even been put forward that the extinction of species may be a result of biological control operations, in particular in island environments. Fortunately, these various disadvantages can be reduced or even avoided by in-depth studies of the activity spectrum of potential beneficials before they are introduced like, for example, the centrifugal phylogenetic testing recommended in biological control of weeds (Barrat, 2002; Briese, 1996). The introduction of beneficial organisms is also the subject of strict recommendations by FAO, which drew up an international Code of Conduct for the Import and Release of Exotic Biological Control Agents in 1995 for national quarantine services (Schulten, 1997).

4.2 Environmental Management, a Preliminary and Necessary Step?

The recent progress of knowledge in conservation biology fortunately gives us an opportunity to revise our traditional view of biological control by awarding priority to the conservation of populations of beneficial organisms and hence the conservation of their habitats (Ferron, 2000; Letourneau, 1998; Pickett and Bugg, 1998) (Fig. 5). A fair proportion of the failures recorded in the classical introduction-acclimatisation method can probably be ascribed to a varying degree of unsuitability of the environmental conditions of the receiving agroecosystem (Table 2). This is the defect that so-called conservation biological control

Table 2 Summary of classical biological control results using insect agents to control insect pests and weeds (Greathead, 1995)

	Insect pests (N)	Weeds (N)
Introduction	4769	692
Establishment	1445	443
Target pests	543	115
Good control	421	73
Countries or islands	196	55

'Habitat management regimes to increase natural-enemy effectiveness are directed at:

– enhancing habitat suitability for immigration and host finding

– provision of alternative prey/hosts at times when the pest is scarce

– provision of supplementary food sprays, pollen and nectar for predators and parasitoids

– provision of refugia (for mating or overwintering)

– maintenance of non-economic levels of the pests or alternative hosts over extended periods to ensure continued survival of natural enemies' (Letourneau and Altieri,1999)

Fig. 6 Objectives of habitat management with a view to the increased efficacy of biological control agents

intends to correct. Furthermore, the attention recently paid to the dynamic balance of populations was a reminder of the hitherto neglected role played by indigenous predatory faunae (Bommarco and Ekbom, 2000; Symondson et al., 2002) and stimulated its re-evaluation in the regulation of biological pest populations. However, it should be reminded that concern to develop habitats prior to the implementation of biological control procedures had not escaped the perspicacity of precursors, even if their recommendations were not followed by effects in their time (van den Bosch and Telford, 1964).

The role played by biological corridors between habitats and the edge effect on the distribution of biological pests and their parasite complexes is beginning to receive attention in crop protection (Gurr et al., 1998; Häni et al., 1998; Landis and Marino, 1999; Marino and Landis, 1996; Thomas et al., 1991; Verkerk et al., 1998). It is true that biological corridors can only really be exploited in a collective strategy – generally contractual – of conservation of biological diversity as a whole. Although the research sector has only paid limited attention to the subject so far, the preliminary conclusions generally support the idea that large, interconnected habitats are favourable for biological diversity and population stability (Kruess and Tscharntke, 2000; Tshernyshev, 2003).

In contrast, the edge effect can easily be used by the farmer himself on his own initiative and on the scale of his own farm. Thus, in Switzerland ecological compensation areas (ECA) are recommended in various forms (tracks, hedges and copses and also orchards, extensive pasture, etc.) and are subsidised if they form at least 7% of total farm area. The procedures used most frequently are wildflower strips 3-m wide, rotating fallows and field edges left unsprayed to widths of 3–12 m, referred to as conservation headlands. The technique is taken to the point at which a mixture of about 30 indigenous herbaceous species (annual, biannual and perennial) are specially chosen for these strips and intended to be left for a period of 2–6 years (Studer et al., 2003). Experimental farms are designed for testing these new techniques (Booij, 2003; van Alebeek et al., 2003; Vereijken, 1997). With the same aim of enhancing beneficial fauna, the food properties (pollen, nectar) of these strips planted for parasitoid beneficials, and also referred to as grassy banks or beetle banks, receive particularly close study (Landis et al., 2000; Wratten et al., 2003). For large-scale cultivation as used for cotton both in Australia and the United States, they can be usefully replaced by strips of rapeseed, wheat or alfalfa set within the main crop (Mensah, 1999; Parajulee and Slosser, 1999). The procedure is called strip-cropping or strip-intercropping and is part of the new cropping systems mentioned below (Lys, 1994; Trenbath, 1993). The new techniques are also used in sheltered cropping insofar as the development of the spaces between the greenhouses or shelters also contributes to the management of populations of biological pests and beneficials (Parella et al., 1999). In a more general manner, Letourneau and Altieri (1999) listed the objectives of the conservation or development of the habitats of beneficials (Fig. 6). Care is obviously taken in the choice of plant species for these habitats to avoid those that might form reservoirs of undesirable pathogenic organisms.

Multifunctional crop rotation, integrated nutrient management, minimum soil cultivation, integrated crop management, ecological infrastructure management and implementing an integrated system (Holland, 2007).

Fig. 7 The general principles of integrated farming

5 Crop Protection and Integrated Farming

In the light of the new importance thus awarded to the interactions between crop fields and their immediate biological environment, agronomists are faced with the same obligation to develop their strategies as pest management specialists (Deffontaines, 2001; Meynard et al., 2001). It is now necessary to broaden the cropping system concept and to use a less physico-chemical view of the environment than in the past. It is also noted that the concepts in English of "cropping system" and "farming system" apply to larger areas and sometimes that of a small region, thus intersecting the idea of areawide pest management mentioned above. These refinements benefit today from the use of models of the functioning of agroecosystems that use artificial intelligence or multicriterion decision aid methods (Boiffin et al., 2001; Meynard, 1998; Meynard et al., 2001).

Numerous bibliographical data make it possible to address the characterisation of agrosystems that discourage biological pest outbreaks: great spatio-temporal diversity of crops, discontinuity in monoculture (rotations, early varieties, etc.), a mosaic of small fields to ensure the juxtaposition of cultivated and non-cultivated land, the presence of a dominant perennial crop (especially orchards), crops grown with high sowing density to limit weed populations, great genetic diversity in the crops grown (varieties grown mixed or alternate rows of crops). Various recommendations concerning the management of cultivated plants and the choice of cropping techniques are then made, for example for the drawing up of crop management strategies: the spatio-temporal dimension, the composition and abundance of the indigenous flora in and around the fields, soil type, the nature of the environment and the type of farm (Letourneau and Altieri, 1999).

Stress is also laid on preventive techniques to protect crops again biological pests whereas the most commonly used technique today is the curative method using chemical pesticides. The biological control and environmental practices proposed above form part of a larger set of preventive techniques such as those generated by genetics (Durand-Tardif and Candresse, 2004) but that have the originality of addressing the sustainable exploitation of agroecosystems. In a forward-looking analysis of the evolution of crop protection, Ferron and Deguine (Ferron and Deguine) drew up the basis for a new phytosanitary strategy calling on the two methods in a structured, ranked manner without ruling out the use of curative control methods under well-defined conditions as a last resort. Given the variability of biological phenomena and the need to conserve the benefit of a harvest, the implementation of the strategy requires respect of a planned spatio-temporal conception of the management of the farm.

6 Conclusion

The adoption of such a preventive approach to crop protection forms a break with the practices recommended up to now. It therefore requires an effort in education, design and development, adaptation to local conditions, validation and extension that concerns all the sector stakeholders. The proposal follows the trend of the approach undertaken by FAO with a view to drafting good farming practices on the basis of the Common Codex for Integrated Farming as in that of a "Doubly Green Revolution" (Griffon, 1996; Ribier and Griffon, 2004). The position awarded to agronomic techniques implies that agronomists once again play a driving role in the evolution of this discipline, in particular by making cropping systems and techniques evolve according to the principles of integrated farming (Boller et al., 1997; Holland, 2002) (Fig. 7). The wish for better synergy between ecology and agronomy for the benefit of sustainable exploitation of the biosphere could thus be granted (Weber, 2004).

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Using Grassed Strips to Limit Pesticide Transfer to Surface Water: A Review

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Abstract Grassed buffer strips are one way to reduce pesticide transfer by surface runoff from farmed fields to streams. Numerous experimental studies have demonstrated that this type of development is very effective in various conditions. The results are nevertheless very variable. This variability is partially explained by the multiplicity of processes and contributive factors and by the fact that the latter are dynamic in nature and site-specific. Several results also show that the intercepted products are not systematically immobilized within the strip but can move in the subsurface, thus affecting the overall effectiveness of the system. As a consequence of this complexity, the present guidelines for the sizing and siting of grassed strips are still only qualitative or are the result of empirical approaches. The present review analyses the available results and defines needs for further research. This concerns several basic processes determining the pesticide interception by grassed strip and numerical models necessary to integrate the complexity of interacting processes and formulate reliable managing guidelines.

Keywords Best management practices • Buffer zone • Grassed strip • Modeling • Nonpoint source pollution • Pesticides

1 Introduction

Despite the implementation of new regulatory provisions and the progressive introduction of farming practices that restrict the use of the most mobile pesticides, such products are still frequently found in surface waters at concentrations above the maximum level permitted by the European Directive on Drinking Water (98/83/EC). The use of grassed strips in the prevention of nonpoint source pollution of surface water has been the subject of numerous studies over recent decades. Indeed, surface runoff coming from farmed fields is a major source of contamination of surface water. Grassed strips, acting like a buffer zone, can be an effective solution to reduce this type of transfer between the farmed field and the hydrologic reservoirs.

The first experimental results concerned the ability of grassed strips to intercept flows of sediments and nutrients transported by runoff. Two major scientific reviews should be mentioned: One discusses the retention of nitrogen, phosphorus and sediments by grassed strips following the various transport processes (surface runoff, subsurface drainage, soluble or adsorbed to particles) (Muscutt et al., 1993), and the other analyzes the contradictory results published about nutrient retention at the plot scale and the catchment scale, in order to underline the importance of locating appropriately the buffer zone in accordance with the local buffer physical characteristics or the type of pollutant of concern (Norris, 1993). The results acquired on the ability of grassed strips to capture pesticides in surface runoff are more recent, even if few isolated studies can be found before 1990 (Asmussen et al., 1977; Rohde et al., 1980). Indeed, the literature has grown

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considerably over the last 10 years, and was particularly concerned with the limitation of water pollution by herbicides. As well as evaluating the effectiveness of grassed strips in retaining these products, these studies have been designed to provide a better understanding of the processes and factors involved in pesticide retention by the strips. The ultimate aim was to determine rules for sizing and locating the strips in order to optimize their efficiency for limiting surface water pollution at the outlet of fields and watersheds. As far as we know, only one review has been published until now about pesticide retention by grassed strips (USDA-NRCS, 2000). Its main aim was practical, namely to help american engineers assisting farmers and landowners installing conservation buffers. This report deals with different types of buffers, lists the results published about the potential of buffers to limit pesticides transport by surface runoff, and gives technical and economical considerations for buffer locating, sizing and maintenance. In the end, the review of Dosskey (2001) could also be mentioned, even if only some presented results concern pesticides, because it is a recent and major review. All the environmental functions of buffers (surface runoff reduction and filtration, groundwater filtration, bank stabilization and stream water filtration) were evaluated, and information gaps were identified.

The main aims of the present review are to define which mechanisms involved in the epuration potential of grassed strips are now well understood and which need further research, in the specific case of pesticide transport by surface runoff. In this respect, four specific questions are addressed hereafter:

1. What are the main sources of variation of the effectiveness of grassed strips for intercepting pesticides in surface runoff?
2. What is the fate of the compounds intercepted by a grassed strip?
3. What are the existing modeling approaches to the simulation of grassed strips and what are their limits?
4. What are the current recommendations applied for dimensioning and locating a grassed strip in a watershed and how are they consistent with our current state of knowledge?

2 Variation in Interception Performance of Grassed Strips

Table 1 shows the latest works on pesticide retention by grassed strips, complementing the previously published litterature review (USDA-NRCS, 2000). The presented results confirm that grassed strips can be very efficient in dissipating flows of pesticides present in the surface runoff. However they also show a large variability of the observed efficiencies, as has already been emphasized elsewhere (Dosskey, 2001; Dosskey, 2002). Interpreting this variability is a complex matter.

This is primarily due to large differences in the experimental protocols, as can be seen in Table 1. A first source of differences is related to the kind of experiment, in natural or simulated flow conditions. Experiments conducted under natural conditions reproduce the configuration of a grassed strip adjacent to a cultivated plot subject to rainfall. Measurements are made continuously for a long time i.e. for a wide range of hydrologic conditions on the same plot. In contrast, experiments based on rainfall and/or runoff simulations have several restrictive features. The simulated hyetographs and/or hydrographs usually consist only of a single runoff event with constant and time-limited flow. The dimensions of the simulators are small, resulting in large *grassed surface/treated surface* ratios. The grassed part is usually not watered (except in combined runoff + rainfall systems) so dilution processes and increase in transport capacity due to rain falling directly on the strip are not taken into account. The strip is usually "dry" when the simulated runoff starts. In runoff simulations, the flow has a null velocity when it enters the strip; its concentration in pesticide is kept constant throughout the runoff event. These differences between natural and simulated flow conditions raise doubts about the representativeness of the results obtained with simulators and may explain in part the variable observed efficiencies of grassed strips.

A second source of variability is the large variation of many experimental parameters (e.g. the treated area vs. grassed area ratio or incoming flow rate, molecule type, grassed length, soil type, initial soil moisture) between the experiments, making it difficult to compare the results. Several parameters often vary

Table 1 Published results about the interception effectiveness of grassed strips

		Dimensions			Data	Interception effectiveness (%)		
Reference	Experimental method	Length (m)	Surface ratio (%)	Parameter tested	Measurement type Result type (repet.)	Pollutant	Mass	Conc.
Arora et al. (1996)	Natural rainfall	20	5	Molecule type	Instantaneous	Water	9–98	
					Total per event (6)	Atrazin tot. Metolachlor tot. Cyanazin tot.	13–100 22–100 15–100	
Webster and Shaw (1996)	Natural rainfall	2	18	Molecule type Runoff load	Total per event (24) year averaged (3)	Water Metolachlor dis. Metribuzin dis.	0–65 13–88 22–89	
Patty et al. (1997)	Natural rainfall	6, 12, 18	12–36	Molecule type Grassed length	Total per event (32) year averaged (2)	Water Isoproturon dis. Diflufenican dis. Atrazin dis. Lindane dis.	43–100 99–100 97–100 44–100 72–100	
Lecomle (1999)	Natural rainfall	3, 6	5, 10	Grassed length	Instantaneous	Water	7–100	
				Length partition Source area Molecule type Solid transport	Total per event (55) year averaged (2)	Isoproturon tot. Diflufenican tot.	76–97 60–95	
Lowrance et al. (1997)	Natural rainfall	8		Molecule type	Total per event year averaged (3)	Atrazin tot. Alachlor tot.	37–86 50–94	0–68 0–75
Tingle et al. (1998)	Natural rainfall	0, 5–4	2–18	Molecule type Grassed length	Total over 2d and 84d after application year averaged (3)	Water Metolachlor dis. Metribuzin dis.	47–69 48–68 48–69	 48–69 48–68

(continued)

Table 1 (continued)

Reference	Experimental method	Dimensions		Parameter tested	Data Measurement type	Interception effectiveness (%)		
		Length (*m*)	Surface ratio (%)		Result type (repet.)	Pollutant	Mass	Conc.
Rankins et al. (2001)	Natural rainfall	0, 3	1, 4	Molecule type	Instant. conc., total	Water	49–76	
				Vegetation	runoff over 127d	Fluometuron dis.	59–84	32–71
					year averaged (3)	Norflurazon dis.	45–86	35–66
Vellidis et al. (2002)	Natural rainfall	8	80	Molecule type	Total per event year	Atrazin tot.	92–97	61–89
					averaged (2)	Alachlor tot.	91–99	73–95
						Bromide		55–73
Chamber d'Agriculture du Vaucluse (2000)	Natural rainfall	3–6	4–8	Molecule type	Total per event (29)	Water	55–86	
				Grassed length	year averaged (3)	Dluron tot.	77–88	
						Fosethyl-Al tot.	37–100	
						Thiodicarb tot.	14–91	
Spatz et al. (1997)	Simulated rainfall	1–15	14–214	Soil moisture	Instantaneous	Pendimethalin tot.	77–100	
	60 mm/h			Grassed length	Total per event (1)	Fenproplmorph tot.	42–100	
	90–180 min			Soil type		Terbuthylazin tot.	29–100	
				Molecule type		Primicarb tot.	23–100	
						Isoproturon tot.	18–100	
Souiller et al. (2002)	Simulated runoff	3		Flow rate	Instantaneous	Water	55–92	
	117–200 mm/h			Molecule type	Constant flow (1)	Diflufenican dis.	61–98	25–70
	60–150 min			Seasonality		Atrazin dis.	60–95	<10
						Isoproturon dis.	61–79	<10
						Dluron dis.	62–81	
Syversen and Bechmann (2003)	Simulated runoff	5–7, 5		Grassed length	Instantaneous	Glyphosat dis.		24–70
	38–58 mm/h			Seasonality	Constant flow (1)	Glyphosat part.		10–80
	300 min			Molecule type		Fenpropimorph dis.		32–78
				Solid transport		Fenpropimorph part.		50–95
						Propiconazol dis.		61–73
						Propiconazol part.		0–70

Klöppel et al. (1997)	Simulated runoff 400–2,000l/h Simulated rainfall 14 mm/h	10–20		Grassed length Flow rate Molecule type Flow dispersion	Total per event (1)	Water	0–92	
						Dichlorprop-p	61–98	14–73
						Isoproturon	70–98	10–79
						Terbuthylazin	70–98	30–79
Schmitt et al. (1999)	Simulated runoff 100–200 mm/h; 25′ Simulated rainfall 50 mm/h; 30′	7, 5–15	9–19	Grassed length Molecule type Vegetation	Total per event (1)	Water	35–80	
						Permethrin tot.	45–95	5–80
						Atrazin tot.	30–90	0–40
						Alachlor tot.	40–90	5–65
Misra et al. (1996)	Simulated runoff	12	3–7	Flow rate	Total per event (1)	Water	29–34	
	190–380 mm/h; 45′ Simulated rainfall 64 mm/h; 60′			Runoff load		Atrazin dis.	26–50	0–7
						Metolachlor dis.	27–47	0–2
						Cyanazin dis.	26–47	0–2
Krutz et al. (2003)	Simulated runoff	3	10	Molecule type	Total per event (4)	Atrazin	22	9
	250 mm/h; 60′					diaminoatrazin	19	6
						deisopropylatrazin	19	6
						desethylatrazin	19	6
						hydroxyatrazin	19	6

NB: The inteception effectiveness can be expressed relatively to the entered mass and/or flux concentration (*Conc.*).

simultaneously and in turn it is difficult to detect which parameter may explain the exact origin of the observed differences in efficiency between several experiments. This is reinforced by the fact that in many papers key parameters, such as the soil water status at the time of the event or the soil hydrodynamic properties, are not indicated, or worse were not measured.

A last source is the difference in the way the grassed strip efficiencies are expressed: in term of pesticide mass, in term of mean concentration or flux, or in term of instantaneous concentration. Furthermore, the elements needed to harmonize these results are not always provided.

Finally, comparing results stemming from different experiments and different authors appears to be quite hazardous. To illustrate this, Fig. 1 compares the infiltration capacities of grassed strips as observed by several authors. The dispersion of results is tremendous and cannot be related to variations in the length of the strip, which is thought as a main parameter of grassed strips efficiencies.

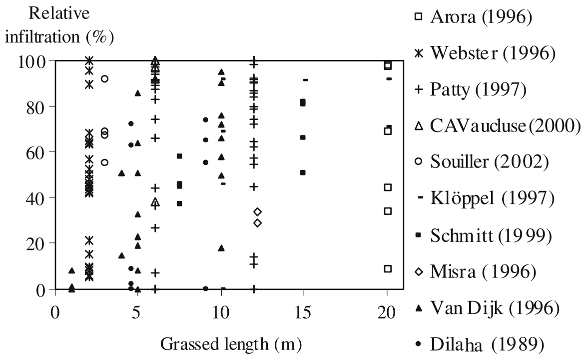

Fig. 1 Relative infiltration capacities determined at the event scale by several authors, for different grassed lengths: illustration of the results dispersion

In sum, the effectiveness variability suggests that a wide range of physical and biochemical processes are involved in the functioning of grassed strips and that their relative importance can vary from one situation to another as a function of numerous parameters. It is therefore necessary to move beyond simple recognition of the effectiveness of a grassed strip and to examine its behaviour from a mechanistic point of view, with the aim of explaining the variability of the experimental results. To this end, we review below the factors that were shown in the literature to be involved in the interception capacity of a strip.

2.1 Mechanisms of Interception

Overall, four main processes are thought to be responsible of the interception properties of a grassed strip (see Fig. 2):

2.1.1 Infiltration

Many authors have shown that the main advantage of a strip lies in its high infiltration capacity (Muscutt et al.,

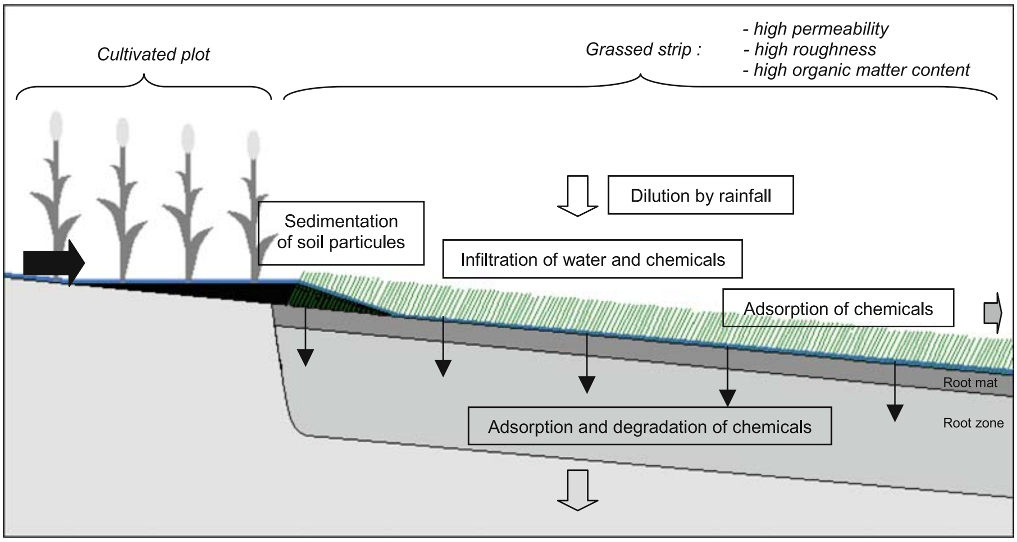

Fig. 2 Processes occurring in a grassed strip

1993; Patty, 1997; USDA-NRCS, 2000) (see Table 1 for quantitative data). This process primarily acts on the compounds in solution, a part of which infiltrates in the soil. However, fine (<0.45 μm) particles can also penetrate the soil, carrying with them molecules adsorbed to their surface (Mercier, 1998). Few in situ measurements of the permeability of grassed strips are available: they are all particularly high, ranging for example from 2 to 58 cm/h for a cover of 7-year-old Perennial ryegrass (*Lolium perenne*) (Souiller et al., 2002) and from 15 to 33 cm/h for the root zone of a 2- to 3-month-old fescue cover (Watanabe and Grismer, 2001). It has been noted that grassing can modify the pore characteristics of the soil surface layer. This can be explained by the structuring effect of the grass rooting system, which is particularly dense, and by the increase in soil organic matter (Benoit et al., 1999; Madrigal et al., 2002), the latter having a stabilizing effect on particle aggregates (Tisdall and Oades, 1982). Macrofaunal activity also plays a role: populations of rodents, moles or earthworms are usually high on grasslands, because of the presence of food, the absence of pesticide treatment and the absence of tillage (Beven and Germann, 1982), and they encourage the formation of rapid flow paths. Observations on soil cylinder samples from grassed strips confirm the presence of roots and worm holes (2–3 mm in diameter) up to 30 cm below the surface (Benoit et al., 2000; Pot et al., 2003). However, there are very few quantitative data on the impact of macroporosity of plant or animal origin on infiltration capacity.

2.1.2 Sedimentation

This process reduces the flow of suspended particles and thus the flow of pesticides adsorbed to their surface. Laboratory canal experiments have shown that particle retention occurs mainly as a result of sedimentation upstream of the strip, in the area of still water that builts up against the upper boundary of the grassed zone (Dabney et al., 1995; Ghadiri et al., 2001; Meyer et al., 1995), rather than as a result of the filtering effect (in the mechanical sense of the term) of the vegetation itself. The transport capacity in this still water area is virtually zero, leading to rapid deposit of the suspended particles (Dabney et al., 1995; Jin et al., 2000). However, the deposit formed upstream can be carried inside the strip more or less rapidly (Dillaha et al., 1989). It must be also noted that sediment deposits may lead to a strong malfunctioning of the grassed strip in erosive areas. Deposition that occurs upstream of the grassed strip can increase to the point of forming ridges that concentrate the flow in local outlets (Dillaha et al., 1989) or in extreme cases, to the point of sealing the grass roughness, which induces the formation of water pathways with high velocities.

2.1.3 Dilution

This operates at the surface of the strip when uncontaminated rainwater falls on the strip and mixes with the contaminated runoff coming from upstream. The dilution factor (*rainfall/rainfall + runoff*) is usually non-negligible (Lowrance et al., 1997; Schmitt et al., 1999; Vellidis et al., 2002). It is determined by the *area of grassed zone/area of treated zone* ratio and by the runoff and rainwater volumes. The recorded observations show concentration reductions associated to dilution ranging from 25% to 50% for an 8 m strip downstream of a 2.5 ha cultivated plot (Lowrance et al., 1997), from 30% to 67% for an 8 m strip downstream of a 10 m hillslope (Vellidis et al., 2002), and from 15% to 30% for 7 m and 15 m strips, respectively, receiving the runoff of an 80 m cultivated hillslope (Schmitt et al., 1999). However, we must underline that dilution influences only the concentration of pesticides in overland flow, but does not change the loads of pesticides crossing the grassed strip.

2.1.4 Adsorption

This concerns molecules in solution. It occurs on the soil surface, on the above ground part of the vegetation and on any other plant debris on the surface of the strip. The above ground part of the vegetation and the partially humified plant debris have the highest adsorption capacities (Benoit et al., 1999; Lickfeldt and Branham, 1995), while the soil has a smaller but still significant capacity, higher than for the same soil when cultivated or bare (Benoit et al., 1999, 2000; Madrigal et al., 2002; Mersie et al., 1999; Reungsang et al., 2001; Staddon et al., 2001). This adsorption capacity varies according to the soil organic matter content

(Benoit et al., 1999; Reungsang et al., 2001; Staddon et al., 2001). Quantification of the role of adsorption in the effectiveness of grassed strips is quite difficult experimentally: direct measurements of the amount of adsorbed pesticides on the soil and plant materials of the strip are difficult from an analytical point of view since the amounts are small. So, the values are most often estimated indirectly from the difference between pesticide concentrations entering and exiting the strip by overland flow. This makes it necessary to take into account the other processes responsible for the reduction of pesticide concentration in overland flow crossing the strip: namely dilution by rainfall but also infiltration. This explains why there are few reliable quantitative results concerning the impact of adsorption on the flows of pesticide conveyed. Nevertheless, it was shown that adsorption is a significant retention mechanism, although there are conflicting reports about the exact value of the process contribution (Arora et al., 1996; Krutz et al., 2003; Misra et al., 1996; Souiller et al., 2002; Spatz et al., 1997).

2.2 Major Properties of Grassed Strips Influencing Interception

2.2.1 Infiltration

The infiltration capacity of a strip is controlled by several factors. One is the infiltrating area of the strip, which depends on the dimensions of the system (length, width). The impact of the grassed length on infiltrated volumes has been demonstrated by many authors, who compared outflows from strips of different lengths (Vaucluse, 2000; Dillaha et al., 1989; Lim et al., 1998; Patty et al., 1997; Schmitt et al., 1999; Spatz et al., 1997; van Dijk et al., 1996). The infiltrating area also depends, however, on the uniformity of the surface flow on the strip. With most grassed strips the effective width of flow is less than the width of the strip. Channelized flow often occurs as a consequence of non-flat topography (Abu-Zreig et al., 2001; Dillaha et al., 1989; Lecomte, 1999) or of concentrated entering flow.

Other factors of control are the soil hydraulic properties, which vary according to the pore structure of the soil surface layer, and to all factors influencing it, namely the soil type, the precedent soil treatments, the age and nature of the sward since it has a structuring effect on soil porosity (Benoit et al., 1999; Benoit et al., 2000; Reungsang et al., 2001).

A last set of factors are the initial and boundary conditions of the strip. As shown by the infiltration theory (Smith, 2002), the initial soil water status, the presence of an impermeable layer close to the soil surface and the water height of surface flow during the flood event are of importance. Observations on grassed strips confirmed that the infiltration capacity is less when the soil is already close to saturation at the beginning of the runoff event. Also, the infiltration capacity will tend more or less rapidly towards zero when an impermeable boundary does exist in the soil profile. Reciprocally, there is no direct evidence whether the variation in hydraulic head is an important factor of variation of observed infiltration capacities, since it is difficult to measure accurately the flow water heights on grassed soils. Several experiments indicated that when the flow rate increases, infiltration increases virtually as much e.g. by a factor 1,7 (Arora et al., 1996; Misra et al., 1996) or 1,5 (Souiller et al., 2002) when runoff doubles. But this effect might also be due to an increase of the infiltrating area, as large flow rate will overflow the microtopography.

2.2.2 Sedimentation

Several studies have shown that the grassed length is not a determining parameter for the retention of suspended matter, even if it favours it (Vaucluse, 2000; Dillaha et al., 1989; Lim et al., 1998; Schmitt et al., 1999; Spatz et al., 1997; Srivastava et al., 1996; Tingle et al., 1998). This is consistent with in situ observations, already reported above, showing that sedimentation essentially occurs either in the first few decimetres of the strip (Tingle et al., 1998) or upstream of it (Dabney et al., 1995; Ghadiri et al., 2001; Meyer et al., 1995). Experiments using laboratory models indicate that the parameters influencing upstream sedimentation are the size distribution of the suspended particles, the velocity of the flow entering the strip and the height of water built up against the upstream boundary of the strip by dam effect (Dabney et al., 1995). The mechanical properties of the grass cover and in particular its density and its resistance to bending (linked to stem diameter) are therefore determining factors (Jin et al., 2000; Meyer et al., 1995). Vegetation density is linked to the grass age so that significant differences are observed between 2-year-old strips and denser 15- or 25-year-old strips (Schmitt et al., 1999; van Dijk et al., 1996). About the impact of the size distribution on particle trapping, in situ experiments confirm that the coarsest particles are deposited first (Lee et al., 2000), so that the relative part of finest particules ($< 20\,\mu$m) in total solid transport increases between the entry and the exit of a grassed strip (Lecomte, 1999). This is of first importance since pesticide concentration can be ten times higher in this fraction ($< 20\,\mu$m) than in coarser ones (Lecomte, 1999): thus, the sedimentation process could have only a reduced impact on the transport of pesticides, even if strongly adsorbed products are concerned.

2.2.3 Adsorption

The overall adsorption capacity of the strip is primarily determined by the contact area between flowing water and the soil and vegetation of the strip. It might therefore be correlated with the length of strip, the effective width of flow and the *surface roughness/height of runoff water* ratio. But relevant experimental results about the adsorption process in grassed strips are few in number. They show no clear correlation between length of grassed strip and reduction in concentrations by adsorption (Patty, 1997; Tingle et al., 1998). There are contradictory results on the effect of water height (Misra et al., 1996; Souiller et al., 2002) and no results at all concerning the impact of the effective flow width. A plant density effect has however been demonstrated: atrazin, alachlor and permethrin concentrations were statistically smaller at the outflow of a 25-year-old grassed strip than at that of a (less dense) 2-year-old strip (Schmitt et al., 1999).

The variation of adsorption capacities of grassed strips may also be related to the known factors of the adsorption process of organic coumpounds, namely the nature of the adsorbate, that of the adsorbents, the water content of the adsorbent, the quantity of available adsorbate, the presence of other organic molecules or mineral ions, the pH and the temperature (Calvet et al., 1980). As far as organic pesticides are concerned, laboratory studies have shown that their adsorption is almost proportional to the organic matter content of the substrate (Stoeckel et al., 1997). Studies on grassed strips confirmed it and showed large adsorption capacities due to large organic matter contents (Benoit et al., 1999; Madrigal et al., 2002; Reungsang et al., 2001). The relationship between adsorption and organic matter content is generally represented by the K_{oc} [$L^3\ M^{-1}$] coefficient that is derived from the soil/water partition coefficient K_d [$L^3\ M^{-1}$]:

$$K_d = \frac{C_s}{C_{eq}};\ K_{oc} = \frac{K_d}{f_{oc}}$$

where C_s is the adsorbed concentration [$M\,M^{-1}$], C_{eq} the concentration of the product in solution [$M\,L^{-3}$] and f_{oc} the mass fraction of soil organic carbon [–].

The K_{oc} can be regarded as intrinsic to each compound and varies considerably between the compounds. As a result, the adsorption capacity of a strip varies also very much according to the coumpound of interest (Schmitt et al., 1999; Souiller et al., 2002; Spatz et al., 1997). For instance, the concentration of diflufenican (K_{oc} of 1990 l/kg) in the liquid phase of the surface runoff can be reduced by two-thirds as it passes through a grassed strip, whereas the concentrations of atrazin and isoproturon (K_{oc} of 38–174 l/kg and 80–230 l/kg respectively) are not reduced at all (Souiller et al., 2002). The overall retention (infiltration + adsorption) of pendimethalin (K_{oc} of 5000 l/kg) in a grassed strip can be 96% while only 75% of isoproturon (K_{oc} of 122 l/kg) is retained (Spatz et al., 1997).

It must be underlined that the total organic matter content of the substrate is not the only factor defining the retention of a given compound. Laboratory studies of soil samples taken from grassed strips indicate that the nature of the organic matter and particularly its degree of mineralization can also have an impact. For example, partially humified organic matter, enriched in constituents such as lignins or cutins were shown to increase adsorption (Benoit et al., 1999). Moreover, in a carbonated and/or calcareous soil, the hydrophobic character of the organic matter declines and hence also the retention of neutral hydrophobic molecules (e.g. isoproturon) or very hydrophobic molecules (e.g. diflufenican) (Madrigal et al., 2002). Another factor determining adsorption is clay content, the second most important adsorbent of the soil after organic matter. The clay influence is considered to be significant if the *clay/organic matter* ratio is above 30 (Staddon et al., 2001).

Finally, the adsorption capacity of a grassed strip also depends on kinetics aspects since flow is transient and adsorption is not an instantaneous process. In the case of isoproturon and diflufenican, it was shown that equilibrium between the concentrations of pesticides in the adsorbed phase and in the liquid phase is reached after a delay of 5 minutes to several hours, depending on the substrates (Madrigal et al., 2002; Margoum et al., 2001). In comparison, since flow velocities on a grassed substrate can be estimated to be of the order of about 1 metre per minute (Gril et al., 1996), contact times are of the order of a few minutes depending on the length of the grassed strip. This means that equilibrium may not be reached for the specific flow conditions in a strip. Although there are too few results on the adsorption kinetics of pesticides to soil to draw any firm conclusions, it is reasonable to assume that adsorption kinetics limit significantly the adsorption on the strips for standard strip lengths and flow rates. This also shows the difficulty in interpreting results obtained under real flow conditions using adsorption coefficients obtained in batch experiments with long contact times, high concentrations and sufficient agitation to ensure optimum contact between the two phases. In turn, it suggests a need for additional studies on pesticide adsorption by grassed strips under realistic flow conditions.

2.3 Temporal Changes in Interception Effectiveness

It must be emphasized that the effectiveness of a grassed strip varies over time, according to the dynamic of each process and the factors controlling them. The evolution of the strip must be taken into account to explain the observed variations in the interception effectiveness of water and pesticide overland flows by a grassed system under natural conditions at event, year and long term scales. Several processes are involved.

First, rainfall regimes and upstream runoff impose variable supply conditions in terms of water flow rate and pesticide concentrations, and in term of variable initial soil moistures of the strip, both at the event and year scales. Since infiltration, adsorption and sedimentation processes depend on the characteristics of the incoming flux and on the initial conditions, this leads to large variations in the apparent effectiveness of the strip at the event (Arora et al., 1996) and year scales (Arora et al., 1996; Lowrance et al., 1997; Rankins et al., 2001; Tingle et al., 1998).

Second, biological processes like root development, changes in organic matter content and macrofaunal activity also influence largely the interception capacity of a strip, but mainly at annual or longer time scales. For example, they were shown to lead to changes in soil permeability by a modification of the soil poral structure or to an increase in adsorption capacity by a larger organic matter content.

Third, a major factor of evolution is the sedimentation process. It can lead to significant changes in soil permeability, slope and surface roughness which alter the infiltration rates, favor channel flow and in turn decrease the infiltration capacity of the strip (Deletic, 2000; Misra et al., 1994). These effects occur as well at the event scale as at longer scales. On the long term, the negative effects of the sediment deposits can counterbalance the positive effects of biological activity on permeability and adsorption, and may require some management of the strip (levelling, tillage, resowing).

2.4 Knowledge Needs About Interception Performance

The main processes and properties of the strips which determine their interception effectiveness are known at least from a qualitative point of view. Table 2 summarizes the processes, their control variables or parameters and the strip properties that influence them. However, the prediction of the interception effectiveness of a given strip still seems unattainable with the present state of knowledge. In our opinion, this stands in two major reasons.

The first one stems from the number of interacting processes and strip properties which is so large that the global functioning of a strip seems not to be predictable by a simple model and from a few characteristics of the strip (e.g. strip length). An evidence of this is the large variation in observed interception effectivenesses which appears difficult to relate to the variation of the strip properties. Concerning the latter, we must stress that in many papers the observed effectivenesses of grassed strips are published without

Table 2 Presentation of processes and main parameters controlling the buffer capacity of a grassed strip

Processes	Control variables and parameters	Related characteristics of the grassed strip	Related characteristics of the upstream watershed
Infiltration	Infiltrating area	Length and width Surface microtopography	Previous rainfall and run-off
	Soil permeability	Soil texture and structure Root development Macrofaunal activity	
	Initial soil moisture	Evapotranspiration Substratum depth	
Adsorption	Organic matter content	Grass age	Agrochemical practices
	Adsorption-desorption coefficient Adsorption-desorption kinetic		
	Contact time	Flow velocity Length	
Sedimentation	Particule size distribution		Erosivity Flow rate
	Flow transport capacity	Surface slope Cover roughness	
Dilution	Rainfall intensity		Weather hazard
	Runoff intensity		Hydrologic response
	Mixing area	Length and width	

any proper description of the intrinsic characteristics of the strips, of the initial and boundary conditions. As a consequence, despite the large number of available experiments, the number of relevant data is small for elucidating the sources of variation of the functioning of grassed strips.

The second reason is that some processes are clearly insufficiently described from a quantitative point of view. Among them are the channeling of surface flow within a strip, the contribution of preferential flows to the infiltration process, the fate of fine solid particles in relation to the sedimentation and infiltration processes, the adsorption process on plant and soil materials (and its variation according to the flow conditions and the type of organic substrate), and the temporal changes in strip characteristics due to biological activity and/or sedimentation process.

3 Fate of Pesticides Intercepted by a Grassed System

Little work has been done on what becomes of the products intercepted by a buffer strip. This issue is however important because grassed strips are often established close to streams and, hence, are potentially close to surface- and groundwaters. The following questions arise: Are the products adsorbed to the solid soil matrix? What is their degradation rate after adsorption? What is the risk that the products will be transferred to depth, both through direct percolation of the contaminated runoff water via macropore-type preferential flow paths and through the leaching of molecules previously adsorbed to the soil matrix of the grassed strip? Can lateral subsurface transfer occur? Are such transfers sufficiently large to affect significantly the actual effectiveness of a grassed system? Is there a large risk of contamination if the grassed strip is above a shallow water table or established on a river bank (case of riparian strips)?

3.1 Degradation of Infiltrated Products

The degradation process concerns the infiltrated compounds that remain in the soil after infiltration either in the adsorbed phase or on the liquid phase. Degradation decomposes the parent molecule into by-products, that can have an even higher reactivity with the soil components than the parent material. Such is the case with isoproturon (Benoit et al., 1999, 2000), metolachlor (Staddon et al., 2001) and atrazin (Mersie et al., 1999), molecules that have a moderate K_{oc} but are rapidly decomposed and have metabolites that are adsorbed to a much greater extent than the parent molecule. It has generally been observed that grassed strips have a high degradation potential because of their high total and readily available organic carbon content. Fungi and bacteria populations and enzyme activity are up to four times higher within a grassed soil than in bare soil (Staddon et al., 2001). This explains the faster degradation kinetics noted in grassed soils than in tilled soils (Benoit et al., 2000; Mersie et al., 1999) and the fact that the surface horizons, with the highest carbon content, are the site of the most intense microbial and enzyme activity (Benoit et al., 2000).

The estimated half-lives (corresponding to the disappearance of half of the applied quantity) for the parent molecules are short: for metolachlor, 10 days in a grassed strip and 23 days on bare soil (Staddon et al., 2001); for atrazin, between 14 and 121 days depending on the horizon and the soil in question (Reungsang et al., 2001); for isoproturon, from 72 days on cultivated soil to 8 days in the surface horizon of a grassed soil (Benoit et al., 1999). However, the measured disappearance of a compound is in fact also the result of partial decomposition into degradation metabolite. Actually, complete mineralization of pesticides still takes a long time despite the favorable conditions encountered in grassed soil. For instance, with samples of grassed soil under standard conditions, complete mineralization was measured to be less than 1% for isoproturon after 44 days of incubation (Benoit et al., 2000), less than 4% for metolachlor after 46 days of incubation (Staddon et al., 2001) and less than 6% for atrazin after 84 days of incubation (Mersie et al., 1999). Besides, it must be remembered that degradation metabolites can be more stable than the parent molecule (Benoit et al., 1999; Benoit et al., 2000; Mersie et al., 1999). For instance, atrazin degrades rapidly in the open field but its degradation products can persist in the soil for up to 9 years after its application (Stoeckel et al., 1997). So, even if it seems that degradation may rather rapidly decrease the concentrations of parent compounds infiltrated in the strip, and consequently limits the risks of accumulation and leaching, it is probably not true for degradation products. More data needs to be collected on the daughter products of the major pesticides in use to be able to analyze the specific risks of accumulation and leaching of these compounds.

3.2 Deep Percolation of the Compounds

The American guidelines relating to the development of buffer zones (USDA-NRCS, 2000) assume that the risk of deep percolation of pesticides under grassed strips is not significant. This hypothesis is based on three assumptions: (a) strongly adsorbed products have a very low leaching potential, because of the role of the strip in filtering suspended matter and the retention capacity of grassed soils; (b) weakly adsorbed products have a high leaching potential, but it cannot be expressed on grassed strips as this type of molecule is present only at small concentration in the incoming surface runoff water due to rapid infiltration of products at the application plot level; (c) whenever leaching occurs, subsurface flows are less prejudicial than allowing surface runoff to directly enter the stream, due to the adsorption and degradation capacity by soil and vegetation in buffer.

The first and third assumptions appear to be justified, even if for the first, a doubt remains about the effective retention of finest and most contaminated solid particles (Lecomte, 1999; Mercier, 1998).

But the second is not. In fact, several studies have shown the presence of pesticides with a low K_{oc} in runoff water from agricultural fields (Lennartz et al., 1997; Louchart et al., 2001; Neumann et al., 2002; Schiavon et al., 1995; Wauchope, 1978).

Furthermore, several experimental results showed the existence of significant percolation of pesticides below grassed strips. With a strip established downstream of a maize plot treated with atrazin, for instance, the annual quantities leached below 120 cm under the grassed strip relative to the area of the plot were of the order of $0.6–2.9\,\mathrm{g\,ha^{-1}}$ for an applied dose of $750\,\mathrm{g\,ha^{-1}}$ (Delphin and Chapot, 2001). The maximum concentrations measured by these authors in the soil solution at a 60 cm depth were as high as $10\,\mathrm{mg\,l^{-1}}$ for atrazin and $6\,\mathrm{mg\,l^{-1}}$ for deethylatrazin. The authors explain that in the hydrologic conditions concerned the transfer occurred in two stages: (a) rapid transfer during the period following application of the product, but limited to the first 60 cm because of the evapotranspiration of both the crop and the strip, leading to a deficit water balance and (b) transport to a greater depth through the leaching of the adsorbed molecules after the growing period of the crop, when precipitation again exceeded evapotranspiration. In this situation, the risk of contamination concerns only molecules (parent molecules or metabolites) sufficiently persistent to still be present in the soil profile once the growing period of the crop has ended. Two studies also showed the temporary and localized contamination of a shallow water table by atrazin and alachlor beyond a buffer system, both of which are attributed to infiltration of surface runoff in a grassed strip (Lowrance et al., 1997; Vellidis et al., 2002). The peak concentration of atrazin measured in the water table was $6\,\mu g\,l^{-1}$ immediately upstream of the strip and $2\,\mu g\,l^{-1}$ downstream, for a runoff concentration of $90\,\mu g\,l^{-1}$ (Lowrance et al., 1997). According to the authors, the very short transfer times can only be attributed to contamination by surface runoff and not to subsurface spread from the treated plot.

The occurrence of pesticide transport to depth under grassed strips may be linked both to the existence of macropore-type rapid transfer paths and to the leaching of previously adsorbed pesticides on the soil matrix. Macropores, which can explain the high infiltrability of grassed soils, constitute potential hydraulic by-passes with regard to the retention capacities of the porous matrix. Several observations suggest the existence of these structures:

1. In situ concentration measurements using porous ceramic cups located 60 cm below a grassed strip, indicating contamination of the soil solution with atrazin and deethylatrazin in a time incompatible with matrix transfers (Delphin and Chapot, 2001).
2. Particularly rapid bromide and isoproturon elution curves obtained on an undisturbed column (Benoit et al., 2000).
3. In situ measurements under a grassed strip that had received contaminated runoff, showing residual concentrations on the soil matrix too small to be explained by Darcy-type matrix transport which in principle presents sufficiently long contact times and large exchange areas for higher soil matrix concentrations (Souiller et al., 2002).

However, the role of the macropores should not be overrated. Not all the macropores present in the soil are necessarily active; only those hydraulically connected to the surface contribute to the transfers, while the others remain dry through capillary barrier effect. In addition, the macropore walls can also be the site of active adsorption, linked to the presence of organic substances (Edwards et al., 1992). The results concerning the contribution of macropores to infiltration in grassed strips are still too partial and too few to permit any quantitative conclusion about the risk of deep percolation due to these poral structures.

There are virtually no results on the leaching of products already trapped in a grassed strip. It has been shown that bound products on the surface of a grassed strip can be salted out during subsequent runoff episodes (Schmitt et al., 1999; Watanabe and Grismer, 2001). But it has generally been shown that adsorption in the soil of a grassed strip is less reversible than in bare soil and that reversibility decreases rapidly with time so that the risk would be limited (Benoit et al., 1999). With isoproturon, it has also been shown that salting out does not concern the parent product, which decomposes rapidly, but rather its metabolites, which are more stable and whose fate is intrinsically linked to the evolution of the soil organic matter to which they are tightly bound (Benoit et al., 2000).

3.3 Subsurface Lateral Transport

The occurrence of subsurface lateral transport in grassed strips must be questioned mainly in the case of riparian strips where this type of flow may directly contaminate the river. Subsurface transport is more likely when there is a discontinuity in the porous matrix (original stratification or stratification of anthropic origin, e.g. plough pan) that can lead to the formation of rapid saturated lateral flow during the infiltration events. An example of this was identified under a riparian buffer zone established on a soil with an impermeable horizon at 1 m depth (Bosch et al., 1994): in the wet season, a gravitational saturated lateral flow was observed above this horizon. However the risk of transfer was not very important, because of the low measured velocities, around 1.4 mm per hour (Bosch et al., 1996). It may also be stressed that a difference in conductivity between the root mat of a grassy cover and the underlying horizon (×3 in winter, ×25 in spring) is likely to favor lateral flows (Souiller et al., 2002). Actually in-situ measurements confirm that a difference does exist between the infiltration measured during runoff simulations and the theoretical infiltration deduced from direct measurements of vertical hydraulic conductivity, the theoretical value being significantly smaller than the measured value, which suggests the existence of a significant lateral flow (Souiller et al., 2002). Elsewhere, it was observed on a physical laboratory model that lateral flow representing between 4% and 7% of the rainfall takes place in the root mat (3 cm) of a fescue sward established on a 3% slope (Watanabe and Grismer, 2001). But the associated loss of diazinon was low (<0.9%) due to the adsorption process (Watanabe and Grismer, 2001).

In fact, it seems that the risk of lateral subsurface transport is limited to specific situations. Shallow flows occurring within the root zone, even if significant in terms of water flow, should induce limited pesticide transport due to the strong adsorption capacity of soil and root materials. Thus, transport could occur only for "very thin" strips located along the rivers, where contact time can be too short. But deeper transport can in theory be possible for a wider range of situation since the leaching risk has been demonstrated (see Section 3.2. "Deep Percolation of the Compounds") and because of a reduced adsorption capacity of the under-root zone. The groundwater flow velocity and the length of groundwater pathway between the infiltrating zone (i.e. the grassed strip) and the river will also be determining. Critical situations will be associated to coarse soils (because of their high permeability) overlying an impermeable layer, and particularly near the river.

3.4 *Knowledge Needs About Pesticide Fate in Grassed Strips*

The fate of pesticides intercepted by grassed strips should clearly be taken into account when evaluating the performance of the strips or when deciding the installation of strips to reduce water pollution by pesticides. As reported above there is a significant number of data indicating that the intercepted pesticides are not irreversibly fixed or fully degraded in the strip. But, because most studies on grassed strips focused in priority on the filtering processes over short time scales, little is known about the importance of pesticide losses from the strip after interception. Predicting the fate of pesticides after their interception by the strip seems even more difficult than predicting the interception itself. Our review suggests two major points that should be studied more closely.

First, the study of the fate of the intercepted pesticides should not be restricted to the parent compounds but should be extended to the degradation by-products. There is a general agreement to recognize that degradation processes are rather intense in grassed strips due to their high microbiological activity, but the available data essentially concern the parent compounds, and only few data were acquired on the degradation rate of the daughter compounds, which, as already noted, may also be a source of water contamination.

Second, subsurface flow processes, whether of preferential type or not, remain largely unknown. This is in fact a general problem in the study of subsurface hydrological processes since in most soils the poral structure and the active water pathways below the soil surface can rarely be identified and observed. But it is even more a problem in grassed strips for two reasons. One is that soils of grassed strips exhibit most often a larger macroporosity than usual agricultural soils since they are subject to larger faunal and rooting activity over long terms. This implies that the possibility of preferential flow is certainly larger in grassed strips than elsewhere. Another reason is that many grassed strips are located at the bottom of slopes and close to rivers, which correspond to wet situations that enhance the possibility of significant subsurface flow.

4 Numerical Modelling of the Functioning of Grassed Strips

Mechanistic numerical models have been produced to integrate the different processes described above, with the aim of explaining experimentally measured outflows of water and pollutants from grassed strips of given dimensions and subject to a given incoming runoff. These tools represent each process by mathematical equations. The parameters involved in these equations usually have a physical sense and can therefore be measured either directly or indirectly. We review hereafter the main modelling approaches that were published so far to our knowledge.

The VFSMOD (Muñoz-Carpena et al., 1999) and TRAVA (Deletic, 2001) are field-scale, storm-based models designed to route an incoming hydrograph and sedimentograph from an adjacent field through a grassed strip and calculate the outflow, infiltration and sediment trapping efficiency. They represent surface flow by means of the kinematic wave equation solved in 1D and allow a fine discretization of the grassed strip characteristics (vegetation roughness and density, slope, soil permeability). Infiltration is calculated using the *Green and Ampt* equation. A distinguishing feature of these models is their specific representations of solid particle transfers: physically based equations established at the University of Kentucky and the University of Aberdeen respectively, derived from laboratory experiments. They have been successfully tested on experimental data: VFSMOD was calibrated and validated on natural (Muñoz-Carpena et al., 1999) and simulated (Abu-Zreig et al., 2001) rainfall events, while TRAVA was successfully calibrated on runoff simulations (Deletic, 2000). But unfortunately, these models do not represent solute transport and adsorption-desorption processes.

The CREAMS model, initially developed to work at the plot scale and over long periods, has also been used to model the impact of a grassed strip on surface runoff and particle transfer (Flanagan et al., 1989). Like the previous two models it solves the kinematic wave equation in 1D and the Green and Ampt equation. The model has been tested on natural events, but with variable results: it has been shown that even though it reproduces more or less accurately daily runoff volumes, the occurrence of runoff events and seasonal losses of sediment, there is no correlation between simulated and measured values for sediment losses per event (Cooper et al., 1992).

Another published model is the GRAPH model (Lee et al., 1989), which can be used to simulate solute transport, more particularly phosphorus transport, through a grassed strip in association with a runoff and erosion model. It takes into account the processes of advection, infiltration, biological sink terms and adsorption/desorption on soil and suspended matter. GRAPH was validated on phosphorus transfer data from open field experiments.

Last is the Riparian Ecosystem Management Model (REMM) (Lowrance et al., 2000). This model takes into account a large number of processes, and particularly subsurface lateral flow that is not taken into account by previous models, to estimate water, nitrogen, phosphorus and sediment transfer through a buffer system. Flow is calculated on a daily time step, which involves a number of simplifications, particularly for simulating surface runoff: the latter is represented by the ratio between an empirical concentration time and the duration of the event. Moreover, the model does not allow a fine spatial discretization: only nine macro-compartments are described, with three distinct soil layers and three vegetation zones. It follows that the validation of REMM is still partial. Even though the modelling of water table heights is generally acceptable, the error on runoff volumes is far higher (Inamdar et al., 1999; Tucker et al., 2000).

Eventually, it is important to underline that none of these models tackles the question of pesticide transport across a grassed strip. In their present state of development, the existing models only simulate the transfer of water and suspended matter. The chemical elements that are considered in these models are so far phosphorus and nitrogen. Moreover, the concepts used in these models fail to recognize the true complexity of the hydrodynamic processes occurring in a strip. For instance, the *Green and Ampt* equation, widely used to represent infiltration, is fairly restrictive: it assumes homogeneous initial soil water content, one dimensional flow and ignores the phenomenon of sorptivity. In particular, it fails to take account of infiltration in a stratified soil or in the presence of a water table. Also, no reported model considers subsurface flows explicitly, which in fact would require a 2D modeling approach.

5 Recommendations for the Installation of Grassed Strips

The complexity and variability of the factors controlling the effectiveness of a grassed strip are so great that no satisfactory quantitative tool is presently available to define the optimum characteristics of a strip in a particular hydrologic situation, neither at the strip scale as explained in the previous part nor at the catchment scale. The current recommendations for the introduction of this type of system (siting and sizing) are essentially qualitative or the result of empirical approaches. This section reports the existing approaches.

5.1 Locating Grassed Systems in a Watershed

Two reports, one written in France (Comité d'Orientation pour la Réduction de la Pollution des Eaux par les Nitrates les phosphates et les produits phytosanitaires provenant des activités agricoles (CORPEN), 1997) and the other in the United States of America (USDA-NRCS, 2000), present current practices related to the development of grassed strips. Although the reasoning differs on some points, the guidelines are very similar:

1. A first very obvious guideline is that grassed systems should be sited to intercept runoff from cultivated plots.
2. A second defines the upstream to downstream management of the catchment. It is based on the fact that surface runoff, initially diffuse at the level of the plot where it appears, becomes more concentrated as it flows down the watershed in rills, gullies and channels, leaving an ever-greater mark on the landscape until cropping is no longer possible. Different systems are required to match these different types of flow (different in velocity, particle load and height): in-field or edge-of-field grassed strips to intercept diffuse runoff; the grassing of existing thalwegs or the development of grassed thalwegs to control concentrated runoff; the grassing of stream banks to intercept flows before they enter the water resource to be protected. Moreover, american guidelines take account of the fact that runoff concentration can also limit the effectiveness of buffer systems. They therefore recommend that buffer zones be located as close as possible to the source, advocating the use of in-field grassed strips parallel to the contour lines. With edge-of field strips there is a larger risk of concentrated flows because of sediment deposits. The downstream development of riparian strips needs to take this factor into account and provide for dispersion of the flow before it enters the strips. This can be done when the strip is installed, either by smoothing the terrain or by developing the buffer system to match contour lines, or via a purpose-built system. An embankment to break the flow arriving from upstream, associated with small ditches that can overflow uniformly onto the strip, can play this role (see Fig. 3).
3. The third guideline concerns only riparian strips. American authors have shown that most of the runoff water in a river comes from small streams in the upper parts of the watershed, temporary or level 1 or 2 streams. This category of stream is therefore the one that must be protected first and foremost. Protection by riparian strips of higher level (level 3 or 4) branches of the hydrographic network can have only a very limited effect on stream quality as the area with direct runoff to this zone will be negligible at watershed scale.

Until now, no experimental data has been published about the validity of such locating recommendations. Nevertheless, a distributed hydrological modelling work (Lecomte, 1999) confirms the fact that a small grassed fraction of the catchment area can significantly reduce contaminant fluxes at the outlet if the grassed strips are located on contributive waterways and at the

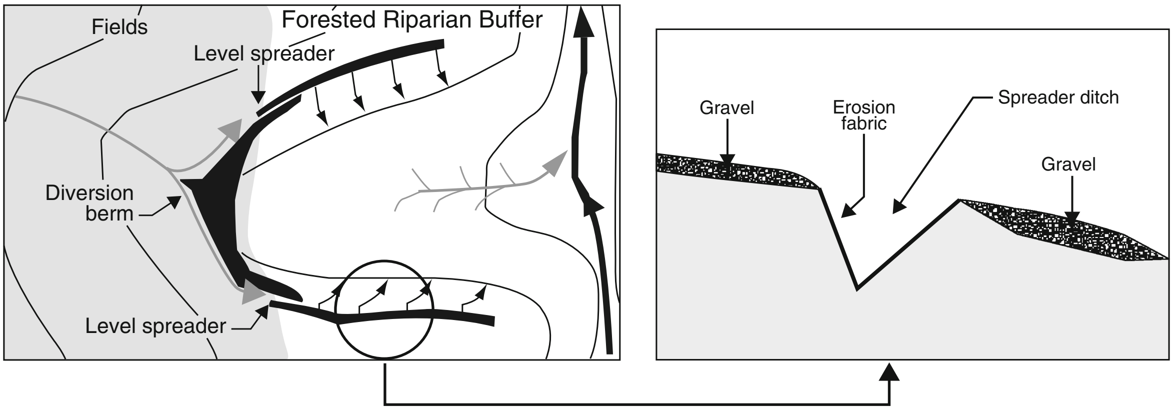

Fig. 3 Embankment proposed in the United States to break and disperse flow before entering the strip (USDA-NRCS, 2000)

low end of contributive plots. We also underline that existing recommendations do not take account of the subsurfacic processes. As stressed above, this is particularly problematic in the case of riparian strips which are close to rivers and where groundwater comes often up to the surface.

5.2 Sizing of the Strip

Although the effectiveness of grassed strips in dissipating pollutant flows has been demonstrated by numerous authors, there are few references describing the dimensions that these systems should be given for optimum effectiveness according to the particular features of the site.

In France (Comité d'Orientation pour la Réduction de la Pollution des Eaux par les Nitrates les phosphates et les produits phytosanitaires provenant des activités agricoles (CORPEN), 1997), there are no bibliographical references and only a few qualitative rules on sizing have been formulated, primarily based on the distinction between diffuse flow and concentrated flow. Quantified values based on experimental results obtained at the Arvalis site of La Jaillière in the Loire Atlantique (Patty, 1997) are provided by way of information. For diffuse runoff, strip lengths of 10 and 20 m are proposed for hillslopes of under and over 100 m respectively; if the flow is not perpendicular to the field edges, the lengths must be estimated in the direction of the flows. For concentrated runoff, the entire flow channel should be grassed or meadows created "in steps" if the rainfall area is greater than about 100 ha (see Fig. 4).

American technical guidelines do not include any quantitative rules for sizing, either (USDA-NRCS, 2000). Nevertheless, it is noted that sizing should be based on the protection objectives (a strip to stop solid particles will be smaller than one to intercept soluble products such as nitrates and pesticides), the site-specific conditions (the strip will be larger if the soil permeability is low, the soil is close to saturation, particularly in the presence of a surface water table, and the runoff area is large) and, lastly, on what is economically or even politically feasible. Despite this "mechanistic reasoning", a "standard length" is proposed: 50 feet (15 m) for a stated 50% effectiveness if the runoff is diffuse and modulo the previously mentioned parameters. As far as sediment transfer is concerned, classes of grassed strip size have been established empirically, using an externally calibrated erosion coefficient.

Obviously, such fixed recommended strip lengths are contradictory to the multiplicity of processes and variables affecting the retention capacity of a grassed strip both in space and time (as explained in the first part). Such lengths are probably satisfactory on average but they should be optimized for specific situations, which certainly require more mechanistic approaches.

Other approaches rely on empirical formulas that consider only single acting processes and compute the expected flow reduction in a grassed strip of a given size. These formulas have been established with regard to the retention of solid particles (Flanagan et al., 1985; van Dijk et al., 1996), nitrogen and phosphorus (Lim et al., 1998) or to the limitation of surface runoff (Mander et al., 1997). Their applicability is severely

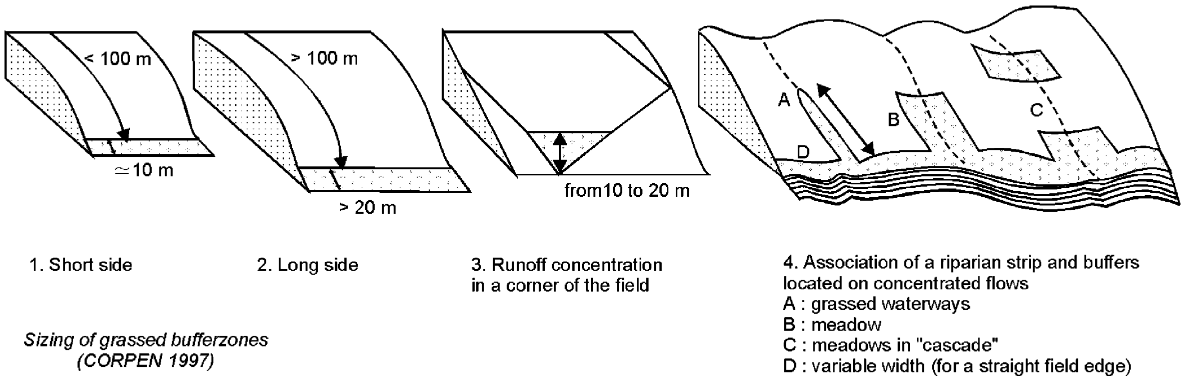

Fig. 4 Typical management schemes proposed in France by the Orientation Committee for Environment-friendly Agricultural Practices considering Environment (Comité d'Orientation pour la Réduction de la Pollution des Eaux par les Nitrates les phosphates et les produits phytosanitaires provenant des activités agricoles (CORPEN, 1997)

limited through the use of empirical coefficients without physical significance and calibrated under particular conditions. Moreover, by construction they are unable to take account of several interacting processes in a dynamic way.

Two physically-based approaches of the sizing of grassed strips can also be found in the literature. The first (Suwandono et al., 1999) uses a front-end model based on a combination of the NRCS curve number method, the unit hydrograph and the modified Universal Soil Loss Equation (based on vegetation, soil type and topography) to generate a rainfall hyetograph, a runoff hydrograph and a sediment loss from the cultivated area. The VFSMOD model (Muñoz-Carpena et al., 1999), already described, is then used to test the impact of different grassing scenarios on the contaminated flow. The second is an original approach developped for permeable soils, that is based on the criterion of minimizing the impact on the alluvial aquifer of flows infiltrating into the buffer zone (Lin et al., 2002). A probable depth of pesticide spread in the soil is calculated mechanistically, with the convection-dispersion relationship solved for a 1D permanent flow. Knowing the topography of the hillslope, the buffer zone is sized so that the upstream boundary of the buffer zone receiving the contaminated runoff is higher than the level established by the minimum distance to be observed between the watertable and the soil surface to avoid contamination.

Finally, the ideal sizing strategy should combine physically based descriptions of surface and subsurface flows, the latter being bi-dimensional to take both vertical and lateral flows into account. But new numerical models have to be developed first as the existing models described above are not satisfactory (see Section 4. "Numerical Modelling of the Functionning of grassed strips").

6 Conclusion

Experimental studies to date have identified the processes and factors determining the effectiveness of a grassed strip to intercept pesticides. Nevertheless, this review highlighted some major processes that are of importance in the functioning of grassed strips and for which insufficient data and observations are presently available: the channeling of surface flow inside the grassed strips, the sedimentation and infiltration of highly concentrated fine solid particles, the adsorption of chemicals on soil and plants as influenced by to its kinetic aspects and the characteristics of organic matters, and the temporal evolution of strip physico-chemical properties. Further research is required on the effectiveness of grass strips under natural conditions but primarily on systems for which boundary conditions, initial conditions, and intrinsic physico-chemical properties are measured.

To predict what the intercepted products become on the short and long terms, our review suggests two major points that should be studied in the future. The first is the fate of degradation metabolites, as they were found to be more stable than the parent molecules. The second is the impact of subsurface flows, of preferential type or not, on the global effectiveness of a buffer system, particularly in the case of riparian strips.

In the present situation, there is considerable uncertainty about the development of grassed systems in order to stop both surface and groundwater contamination by pesticides. Technical guidelines for the siting and sizing of these developments are still essentially qualitative and based on results obtained on few particular sites. The literature to date still does not allow quantitative rules to be formulated, so the prediction capacities need to be improved. This is not only an environmental issue but also an economic one, as creating and maintaining a buffer zone costs money. Quantitative information on the expected effectiveness of the installation of a grassed strip in terms of flow reduction and of impact on the quality of aquatic environments would be useful for managers of rural areas, but it would also be useful to convince a greater number of farmers to give up part of their cropping area for installing strips.

Improving the prediction tools involves taking the particular features of each site into account, and this requires the use of physically based numerical models capable of integrating the different processes that determine the dissipation capacity of grassed strips. Mechanistic models have already been proposed but these require further development, in particular by integrating the whole range of transport processes and chemical reactions affecting pesticides on the surface and subsurface flows.

With the development of a prediction tool at the scale of the grassed strip, the formulation of short-term recommendations could be envisaged for a wide range of standard situations, through the testing of development scenarios. But such a tool would then need to be integrated in a more general management approach at the hillslope or the watershed scale and allow greater account to be taken both of the initial situation and of the upstream conditions.

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Recycling Biosolids and Lake-Dredged Materials to Pasture-based Animal Agriculture: Alternative Nutrient Sources for Forage Productivity and Sustainability: A Review

Gilbert C. Sigua

Abstract Domestic sewage sludge or biosolids and lake-dredged materials are examples of materials that can be used to cut fertilizer costs in pasture-based animal agriculture. Sustainable biosolids and lake-dredged materials management is based upon controlling and influencing the quantity, quality and characteristics of these materials in such a way that negative impacts to the environment are avoided and beneficial uses are optimized. This article examines the following two key questions. Is the use of these materials in an agricultural setting harmless and sensible? Is the use of biosolids secure in all climates, in all soils and is it sustainable over the long term? Recycling biosolids and lake-dredged materials to pasture-based animal production is quite productive as alternative nutrient sources for forage production. Perennial grass can be a good choice for repeated applications g of biosolids and lake-dredged materials. Although biosolids and lake-dredged materials supply some essential plant nutrients and provide soil property-enhancing organic matter, land-application programs still generate some concerns because of possible health and environmental risks involved. Repeated applications of biosolids and lake-dredged materials indicate no harmful effects on soil quality and forage quality. Beneficial uses of biosolids and lake-dredged materials are both economical and environmental. The concentrations of soil nitrogen and phosphorus following repeated application of biosolids were far below the contamination risk in the environment. The residual effect of biosolids over the long term can be especially significant in many forage-based pastures where only 50% of the million hectares of pastures are given inorganic nitrogen yearly. Long-term studies have demonstrated the favorable and beneficial effects of added lake-dredged materials on the early establishment of bahiagrass in sandy pasture fields. Often these materials can be obtained at little or no cost to the farmers or landowners. Lake-dredged materials can be used as soil amendments (lime and fertilizer) for early establishment of bahiagrass in beef cattle pastures. Bahiagrass in plots that were treated with biosolids and lake-dredge materials had significantly higher forage yield and crude protein content when compared with those bahiagrass in the control plots or untreated plants.

Keywords Agriculture • Bahiagrass • Beef cattle • Biosolids • Carry-over effect • Domestic wastewater • Dredging • Ecological implication • Environment • Forage productivity • Forage-based pasture • Sewage sludge• Subtropical pastures

1 Introduction

Wastewater treatment in the United States represents a major effort to keep the nation's waters clean. Sewage sludge is the solid, semisolid, or liquid residue generated during treatments of domestic sewage. Although biosolids supply some essential plant nutrients and impart soil property enhancing organic matter, land application programs still generated apprehension because of possible health and environmental risks involved

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(Berti and Jacobs 1996; Alloway and Jackson 1991). Repeated biosolids applications are feasible in an intensive forage system, but field managers will need to adjust rates or cease applications as appropriate to avoid excess nitrogen or phosphorus concentrations in soil which may lead to undesirable accumulations of these nutrients, potential for harming the environment (Sigua 2005; Adjei and Rechcigl 2002; Cogger et al. 1999, 2001; Zebarth et al., 2000; Sullivan 1998; Sullivan et al. 1997; Fresquez et al. 1990). There are also wide public health trepidation of soil contamination with trace metals and pathogens from repeated application of biosolids. Pathogens tend to persist in less than a year, but trace or even heavy metals associated with biosolids may tend to persevere in the environment for much longer period (Henry et al. 1994; Cavallaro et al. 1993).

The continued need to dredge ports, waterways, lakes, rivers, and canal both for maintenance and environmental improvement will produce millions of cubic meters of lake-dredged materials. These bottom sediment materials are composed of upland soil enriched with nutritive organics, trace metals and contaminants. Productive disposal options of these materials may provide substantial and vital benefits that will enhance the environment, community and society. Dredged or spoil materials because of its variable, but unique physical and chemical properties are often viewed by society and regulators as pollutants, but many have used these materials in coastal nourishment, land or wetland creation, construction materials and for soil improvements as soil amendment (Sigua 2005; Sigua et al. 2003, 2004a, b, 2005, 2006). Environmental impact assessment is an important pre-requisite to many dredging initiatives. Current dredged material disposal alternatives have several limitations (Fitzgerald and Pederson 2001; MacDonald 1994). Options for dealing with lake-dredged materials include leaving them alone, capping them with clean sediments, placing them in confined facilities, disposing of them at upland sites, treating them chemically, or using them for wetlands creation and other beneficial uses (Sigua et al. 2004b; Adams and Pederson 2001; Patel et al. 2001; Krause and McDonnell 2000; Gambrel et al. 1978).

The prohibition of dumping lake-dredged materials and biosolids in streams and oceans, diminishing land fill space, higher landfill costs and concerns over air pollution from incineration of wastes have contributed to a strong public interest in finding alternative, environmentally sound solutions for disposal methods (National Dredging Team 2003; Krause and McDonnell 2000; APHA 1989). Disposal of these materials may create major economic and environmental problem, but countries around the world are committing increasing resources to find effective long-term solutions. The most important step in evaluating the biosolids and lake-dredged materials application alternatives is to determine whether these materials are suitable for agricultural land (Wenning and Woltering 2001). Therefore, the biosolids and lake-dredged materials should be analyzed carefully and thoroughly to evaluate their quality. The parameters most commonly measured must include the percentage of total solids, total nitrogen ammonium and nitrate nitrogen, total phosphorus and potassium, and total cadmium, copper, nickel, lead, and zinc. Other elements and metals like chromium and mercury are of equal importance and may also need to be measured because industry is contributing high levels of these chemicals into the sewer system. This means that before biosolids and lake-dredged materials can be used commercially in our cropping and livestock systems, they must be shown that they are safe for the environment as well as beneficial for agriculture production. Recycling biosolids and lake-dredged materials to pasture-based animal production is quite productive as alternative nutrient sources for forage production. Speir et al. (2003) investigated the application of large quantities of raw sewage sludge to poor quality pastureland developed on coastal dune sands and found little effect on soil biochemical properties, either adverse or beneficial. Perennial grass can be a good choice for repeated applications of biosolids and lake-dredged materials.

The cow–calf (*Bos taurus*) industry in subtropical United States and other parts of the world depends almost totally on grazed pasture areas. Thus, the establishment of complete, uniform stand of bahiagrass in a short time period is vital economically. Failure to obtain a high-quality bahiagrass stand early means the loss of not only the initial investment costs, but also production and its cash value. Forage production often requires significant inputs of lime, nitrogen fertilizer and less frequently of phosphorus and potassium fertilizers. Domestic wastewater sludge or sewage sludge, composted urban plant debris, waste lime, phosphogypsum and dredged materials are examples of materials that can be used for fertilizing and liming pastures. Beef cattle producers throughout the United States need better forage management systems to reduce input costs and protect environmental quality. This is particularly true in the 11 million hectares classified as grazingland in the subtropical (23.5–30°N Lat) United States due to climatically mediated dependence on tropical forages. Throughout the southeastern United States and elsewhere, grazing lands may have considerable variability in soils, climate and growing season, which not only affect the types of forage that can be grown, but also the overall biodiversity, management, and temporal/spatial nutrient dynamics. In the southeastern United States, particularly, Florida, most of the grazing areas are located on flatwood soils. Flatwood soils comprise about 81 million km^2 or about 51% of Florida soils and are dominated by forestry, beef cattle, citrus, vegetable and dairy operations (Botcher et al. 1999).

Additional research on disposal options of lake-dredged materials and biosolids are still much needed to supply information on criteria testing and evaluation of the physical and chemical impacts of these materials at a disposal site, as well as information on many other aspects of dredged and biosolids materials disposal. While preliminary efforts are underway to provide information to establish criteria for land disposal, testing procedures for possible land disposal of contaminated sediments are still in their developing stage. This paper will attempt to discuss briefly the current and future outlook of lake-dredged materials and biosolids efficacy in agriculture and environment of subtropical regions of the United States of America. The lessons learned can provide valuable insights on what could be done in similar agro-ecosystems elsewhere, including Asia and Africa where management will be different, but the principles could be a road map to more sustainable intensifications on the use of biosolids and lake-dredged materials in forage-based pastures cow–calf operations.

1.1 Lake-Dredged Materials

Rivers carry suspended sand and soil along with them as they flow toward the ocean. The higher the water velocity, the greater its energy and capacity to move soil, sand and even rocks along with it. As stream or river velocity slows, heavier materials like sand and gravel, will settle out first. Silt and clay particles, being light in weight, do not settle out until the river has lost most of its energy. Material that falls to the bottom is called sediments (Fig. 1). If enough sediment deposits to build a shallow spot on the river or ocean bottom, it forms shoals. A shoal in a navigation channel that causes the bottom to become shallower is safety hazard.

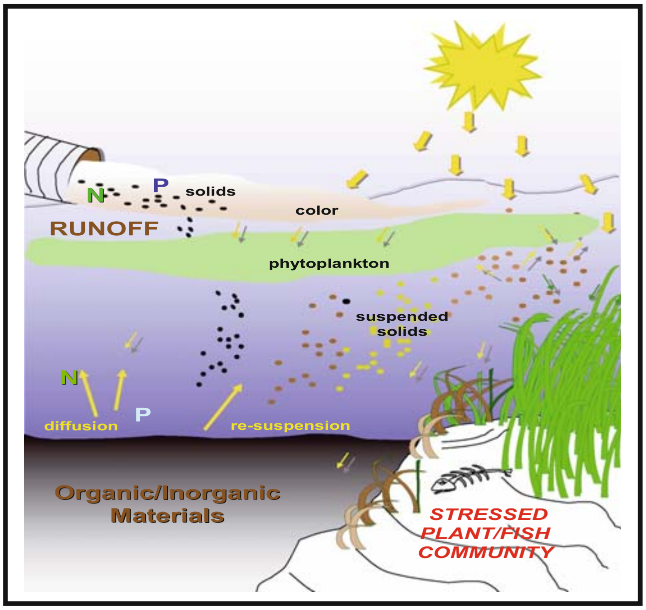

Fig. 1 Typical sedimentation process, building up shallow spot called shoals

Fig. 2 Typical dredging equipment at the middle of the lake

Fig. 3 Lake-dredged materials being delivered and deposited at temporary containment area

Dredging (Figs. 2 and 3) is the process of removing materials (sediment, debris and organic matter) from the bottom of a water body in order to make it deeper. Additional depth in estuaries is usually needed to allow for commercial and/or recreational water traffic such as oil tankers, other cargo ships, tour boats, ferries and larger power or sailboats. The build up of sediments is a natural process that is a result of weathering or erosion of the land due to rainfall. Rainfall carries small particles to streams and rivers. In a flowing stream the particles are suspended in the fresh water. However, when the particles reach quiet water the energy to keep them suspended is no longer present. Then gravity and density take over, and the sediments settle to the bottom where they collect. Over time they are eventually buried by the continuous delivery of new sediment from the watershed.

Table 1 shows some selected properties of dredged materials from Lake Panasoffkee in Florida, USA (Fig. 3). These lake-dredged sediments that are typical in most lakes in Florida had high Ca (as $CaCO_3$) content of $828 \pm 2.1\,g\,kg^{-1}$ and an average pH of 7.8 ± 0.2 (Table 1). The Mg content of the dredged sediment was about $9.0 \pm 3.0\,g\,kg^{-1}$, while OC level was about $127.0 \pm 1.5\,g\,kg^{-1}$. The TP, TKN, and K contents of the lake-dredged materials were relatively low with mean concentrations of 1.6 ± 1.2, 6.9 ± 0.3, and $4.3 \pm 1.8\,mg\,kg^{-1}$, respectively (Table 1). Average values for Pb, Zn, As, Cu, Hg, Se, Cd, and Ni of 5.2 ± 1.3, 7.0 ± 0.6, 4.4 ± 0.1, 8.7 ± 1.2, 0.01 ± 0.02, 0.02 ± 0.02, 2.5 ± 0.1, and $14.6 \pm 6.4\,mg\,kg^{-1}$, respectively, were below the threshold effect levels and the probable effect levels published by the Florida Department of Protection (McDonald 1994). The average concentration of Cd ($2.5 \pm 0.1\,mg\,kg^{-1}$) was higher than the threshold effect levels, but lower than the probable effect levels. Since Cd level was below the probable effect levels value, the use of dredged-lake materials was still warranted because Cd level would not result to adverse biological effects (Table 1). Threshold effect levels represents the concentrations of sediment-associated contaminants that are considered to cause

Table 1 Selected chemical properties of typical lake-dredged materials from Lake Panasoffkee, Sumter County, FL

Parameter	Unit	Dredged materials	Analytical method
pH	pH unit	7.8 ± 0.2	EPA150.1
Organic Carbon (OC)	$g\,kg^{-1}$	127.0 ± 1.5	EPA9060
Potassium (K)	$mg\,kg^{-1}$	4.3 ± 1.8	EPA6020
Total Phosphorus (TP)	$mg\,kg^{-1}$	1.6 ± 1.2	EPA6010
Total Nitrogen (TKN)	$mg\,kg^{-1}$	6.9 ± 0.3	EPA351.2
Nitrate-N	$mg\,kg^{-1}$	0.2 ± 0.05	EPA351.1
Nitrite-N	$mg\,kg^{-1}$	0.3 ± 0.05	EPA351.1
Ca (as $CaCO_3$)	$g\,kg^{-1}$	828 ± 2.1	ASTM C25-95
Mg (as $MgCO_3$)	$g\,kg^{-1}$	9 ± 3.0	ASTM C25-95
Lead (Pb)	$mg\,kg^{-1}$	5.2 ± 1.3	EPA6020
Zinc (Zn)	$mg\,kg^{-1}$	7.0 ± 0.6	EPA6020
Arsenic (As)	$mg\,kg^{-1}$	4.4 ± 0.1	EPA6020
Copper (Cu)	$mg\,kg^{-1}$	8.7 ± 1.2	EPA6020
Iron (Fe)	$mg\,kg^{-1}$	710.0 ± 1.3	EPA6020
Mercury (Hg)	$mg\,kg^{-1}$	0.01 ± 0.02	EPA7471
Selenium (Se)	$mg\,kg^{-1}$	0.02 ± 0.02	EPA6020
Cadmium (Cd)	$mg\,kg^{-1}$	2.5 ± 0.1	EPA6020
Nickel (Ni)	$mg\,kg^{-1}$	14.6 ± 6.4	EPA6020

Source: Sigua et al. 2004b

significant hazards to aquatic organisms, while probable effect levels represents the higher limit of the range of the contaminant concentrations that are usually or always associated with adverse biological effects. Additionally, the United States Environmental Protection Agency's pollutant concentration limit of the class B sludge for Cd is 39 $mg\,kg^{-1}$, which is about tenfold higher than the concentration of Cd in lake-dredge materials that were used in the study. The absence of contaminations and based on nitrogen and phosphorus composition of lake-dredge materials, these materials can be used as low-grade nitrogen and phosphorus fertilizers and also as source of calcium.

1.1.1 Beneficial Use Alternatives of Lake-Dredged Materials: Examples

The most common dredged material disposal methods are capping, land creation and improvement, topsoil creations and enhancement, beach and littoral nourishment, construction materials, and habitat restoration.

(a) *Capping* – is the placement of clean or relatively clean dredged materials on top of other land areas or in aquatic environments. The lake-dredged materials serve as a "caps" on top of other land materials.

(b) *Land creation/improvement* – Land creation or improvement includes the building of dikes or berm for shore protection, filling, raising and protecting submerged and low-lying areas; and applying material to areas where the quality of existing land is poor, such as mine land or brown fields' reclamation.

(c) *Topsoil creation/enhancement* – this usually involves allowing dredged materials to dry out and applying it alone or mixing it with other materials to make topsoil. Dredged materials are commonly composed of silt, clay and organic matter, which are all important components of topsoil.

(d) *Beach/littoral nourishment* – Beach/littoral nourishment is the placement of dredged materials along the shore or in the near shore area to provide a source of nourishment for natural sand (littoral) movement or recreational beach improvement and creation.

(e) *Construction materials* – Construction materials can use the sand portion of dredged materials in road construction and riprap. Dredged materials can also be used as ingredients in the manufacture of bricks, ceramics and concrete.

(f) *Habitat restoration* – Habitat restoration using dredged material can occur in aquatic, wetland, or upland environments. Strategic placement of dredged materials can also be used to restore and establish wetlands and to create aquaculture ponds for fisheries.

1.2 Sewage Sludge or Biosolids

Wastewater treatment plants commonly process domestic sewage to produce clean "effluent" water. The treatment removes solid materials from the wastewater.

These solid materials have to be removed periodically to keep the facilities operating properly. The collected materials, called "residuals" or commonly called "biosolids". Sewage sludge becomes biosolids when it undergoes pathogen control treatment that meets federal and state biosolids regulatory requirements, followed by land application to beneficially recycle it (Obreza and O'Connor 2003).

There are two types of biosolids produced in Florida based on the stabilization process: (a) lime-stabilized; and (b) stabilized by other processes (chemical, physical, or biological). The stabilization process may significantly alter the nutrient composition of the resulting biosolids (Muchovej and Obreza 2004). Most biologically stabilized materials undergo an aerobic and anaerobic digestion process. The typical compositions of lime-stabilized and anaerobically digested biosolids are shown in Table 2.

Agricultural uses of biosolids that meet strict quality criteria have been shown to produce significant improvements in crop growth and yield when applied at recommended rates. Sewage sludge or biosolids being derived from organic waste contain a variety of nutrients, which can be used by plants, and organic matter that improves the soil. Biosolids also contain small amounts of contaminants that can limit how they are used. Before biosolids can be applied to land, treatment must be provided to destroy disease-causing organisms (pathogens). Part 503 of the US EPA contain the established pollutant limits that are designed to protect both human health and the environment under worst-case exposure conditions (Smith 1997). Under both the federal and state regulations, biosolids are classified as either Class A or Class B for pathogen reduction (Table 3). Class AA received the highest degree of treatment for pathogen reduction and also

Table 2 Selected characteristics of two types of biosolids

	Type of biosolids	
Characteristics	Anaerobically digested	Lime stabilized
Solids ($g\,kg^{-1}$)	250	250
Nitrogen (N, $g\,kg^{-1}$)	56	38
Phosphorus (P, $g\,kg^{-1}$)	22	10
Potassium (K, $g\,kg^{-1}$)	2	4
Copper (Cu, $\mu g\,g^{-1}$)	566	236
Molybdenum (Mo, $\mu g\,g^{-1}$)	23	5
Zinc (Zn, $\mu g\,g^{-1}$)	1484	321
Arsenic (As, $\mu g\,g^{-1}$)	4	1
Cadmium (Cd, $\mu g\,g^{-1}$)	11	4
Chromium (Cr, $\mu g\,g^{-1}$)	91	10
Lead (Pb, $\mu g\,g^{-1}$)	195	17
Nickel (Ni, $\mu g\,g^{-1}$)	59	33
Mercury (Hg, $\mu g\,g^{-1}$)	2	2
Selenium (Se, $\mu g\,g^{-1}$)	3	1
pH	8	12

Source: Muchovej and Obreza (2001)***

Table 3 Pollutant limits in Federal and State regulations

Pollutants	Part 503 Table I ceiling conc. ($mg\,kg^{-1}$)	Chapter 62–640, FAC ceiling conc. ($mg\,kg^{-1}$)	Part 503 Table II cumulative loading ($kg\,ha^{-1}$)	Chapter 62–640, FAC cumulative loading ($kg\,ha^{-1}$)	Part 503 Table III exceptional quality* ($mg\,kg^{-1}$)	Chapter 62–640, FAC class AA ($mg\,kg^{-1}$)
Arsenic	75	–	41	–	41	–
Cadmium	85	100	39	4.9	39	30
Copper	4,300	3,000	1,500	140	1,500	900
Lead	840	1,500	300	560	300	1,000
Mercury	57	–	17	–	17	–
Molybdenum	75	–	–	–	–	–
Nickel	420	500	420	140	420	100
Selenium	100	–	100	–	100	–
Zinc	7,500	10,000	2,800	280	2,800	1,800

Source: Smith (1997)

meets the most stringent pollutant limits. “Ceiling concentrations” were established to prevent land application of residuals with excessive levels of pollutants. If the limit for any one pollutant is exceeded; the residuals cannot be applied to the land. Table 3 lists the federal and state pollutant limits for ceiling concentrations and cumulative loadings. If cumulative loading limit is reached for any pollutant, no further application is allowed at the site. Also lists in Table 3 are the limits for the highest classification of residuals. Under the federal rule, the highest classification is known as the “exceptional quality”. The highest classification in the state rule is “Class AA”.

1.2.1 Biosolids as Nutrient Source

Biosolids usually are applied at rates designed to supply crops with adequate nitrogen. They contain a substantial amount of nitrogen (typically 3–6% by weight). The nitrogen is not immediately available to crops, but is released slowly by biological activity. Since biosolids are produce and handled by different processes at different treatment plants, it is important to know if those treatment processes affect how much nitrogen becomes available to plants. Table 4 shows the typical macronutrient contents of biosolids produced by Pacific Northwest and southern states wastewater treatment facilities. Nutrients in municipal residuals produced annually in the United States account for about 2.5% of the total N, 6% of the P, and 0.5% of the K applied on farms each year (Muse et al. 1991).

1.2.2 Potential Problems: Fertilizing with Biosolids

Excess nutrients applied to crops have the potential to reach either groundwater or surface water. Nitrogen has the tendency to move towards groundwater in the form of nitrate, which moves freely with water as it flows through the soil. Phosphorus can flow into surface water as particulate matter with eroding sediments. Successful residuals application programs use several of the following nutrient management practices to keep nutrients in the root zone so they can be utilized by crop (Kidder 1995): (1) Monitoring N concentration of the residuals and accurate record-keeping of rates applied so that the capacity of the soil and crop to assimilate the nutrients is not exceeded; (2) Applying residuals shortly before the peak nutrient demands of the crop to maximize uptake of mineralized N; (3) Incorporating residuals into the soil soon after application, which greatly reduces the potential for P losses in the surface runoff; and (4) Overseeding an annual cool-season forage crop into a perennial grass to provide a crop for nutrient uptake during times when the perennial is dormant. A sample calculation for a wastewater residuals application is given below.

1. Assume:

(a) The residuals to be used contain 4% total N on dry weigh basis.
(b) The residuals are a cake material containing 25% solids.

Table 4 Properties of wastewater residuals from Pacific Northwest and from several Southern States

		Pacific Northwest States range[a]		Several Southern States range	
Property	Unit	Low	High	Low	High
Organic Matter[b]	$g\,kg^{-1}$	450	700		
pH				5.4	7.0
Nitrogen	$g\,kg^{-1}$	30	80	6	75
Phosphorus	$g\,kg^{-1}$	6	13	1	53
Calcium	$g\,kg^{-1}$	10	40	1	60
Magnesium	$g\,kg^{-1}$	4	8	1	50
Potassium	$g\,kg^{-1}$	1	6	1	10
Sulfur	$g\,kg^{-1}$			11	11
Iron	$g\,kg^{-1}$			11	11
		Source: (Sullivan D. 1998)		Sources: (King et al. 1986; Muse et al. 1991)	

[a] The usual nutrient concentration range includes approximately 80% of the biosolids analyses reported. Biosolids composts and alkaline-stabilized biosolids were not included in the calculation of the usual nutrient concentration range.

[b] Organic matter determined by loss on ignition (volatile solids).

(c) The residuals are to be surface-applied to a forage-based pasture for the first time.
(d) The desired fertilization rate is 70 kg of plant-available N per ha.

2. Calculations:

(a) 0.04 kg total N per kg dry residuals × 0.25 kg dry residuals per kg cake residuals × 1,000 kg per ton = 10 kg total N per ton of cake residuals.
(b) Only 50% of the total N is assumed to be plant-available, so there are 5 kg of available N in each ton of cake residuals.
(c) 70 kg N needed per ha/5 kg N per ton of cake residuals = 14 tons of cake residuals per ha.

2 Biosolids and Lake-Dredged Materials Recycling to Pasture-based Agriculture: Research Perspectives (Florida Experiences)

2.1 Lake-Dredged Materials

2.1.1 Experimental Design and Methods

This field study was adjacent to the Coleman Landing spoil disposal site in Sumter County, FL. Each plot (961 m^2) was excavated to a depth of about 28 cm, and existing natural soil (NS) and organic materials were completely removed. Excavated NS materials were placed at the south end of the test plots. Existing vegetation from each plot was totally removed prior to backfilling each plot with different ratios of NS and lake-dredge materials (LDM): (100% NS + 0% LDM); (75% NS + 25% LDM); (50% NS + 50% LDM); (25% NS + 75% LDM); and (0% NS + 100% LDM). These ratios of NS to LDM represent the treatment combinations of LDM0, LDM25, LDM50, LDM75, and LDM100, respectively. Natural soils that were excavated were backfilled to each plot along with lake-dredged materials that were hauled from the adjacent settling pond. The total amount of lake-dredged materials and natural soils that was placed back on each test plot was in accordance with the different ratios of lake-dredged materials and natural soils that were described above. After mixing the natural soils and lake-dredged materials, each of the test plots was disked to a uniform depth of 28 cm. Plots were disked in an alternate direction until lake-dredged materials and natural soils were uniformly mixed. Each plot was seeded with bahiagrass at a rate of 6 kg $plot^{-1}$, followed by dragging a section of chain link fence across each test plot to ensure that bahiagrass seeds were in good contact with the natural soils and lake-dredged materials. Field layout was based on the principle of a completely randomized block design with four replications.

Three sub-samples of soils (0–20 cm depth) were taken from each plot using a 15 cm steel bucket-type hand auger. Soil samples were air-dried and passed through a 2 mm mesh sieve prior to soil chemical extractions. The Mehlich 1 method (0.05N HCl in 0.025N H_2SO_4) was used for chemical extraction of soil (Mehlich 1953). Soil phosphorus and other exchangeable cations (K, Ca, Mg, Al, Fe, Zn, Mn, Cu, Si, and Na) were analyzed using an Inductively Coupled Plasma (ICP) Spectroscopy. Soil organic matter content was analyzed following the method of Walkley and Black (1934). Soil pH was determined by using 1:2 soils to water ratio (Thomas 1996).

Several measurements of soil penetrometer resistance (0–20 cm depth) were taken using the Dickey-John Penetrometer (Dickey-John Corp, Auburn, IL). The penetrometer is designed to mimic a plant root, which consists of a 30-degree circular stainless steel cone with a driving shaft and pressure gauge. This penetrometer comes with two cones, one with a base diameter of 2.03 cm for soft soils and 1.28 cm for hard soils. The driving shaft is graduated every 7.62 cm (3 in.) to allow determination of depth of compaction. The pressure gauge indicates pressure in pounds per square inch.

2.2 Highlights: Research Results and Discussion

2.2.1 Effects on Soil Compaction

Results have shown the favorable influence that lake-dredged materials had on soil penetrometer resistance or soil compaction (Fig. 4). The treatment × year interaction effect was not significant, but the average soil compaction varied widely ($P \leqslant 0.001$) with lake-dredged materials application. In 2002 and 2003, soil compaction of plots was lowered significantly as a

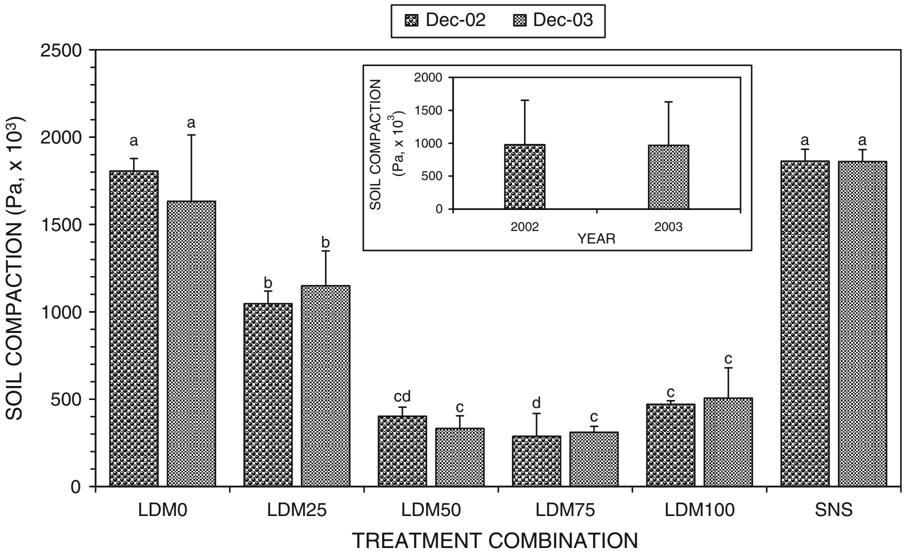

Fig. 4 Degree of soil compaction for soils with varying levels of lake-dredged materials. Soil compactions from plots with or without lake-dredged materials are significantly different ($P \leqslant 0.05$) when superscripts located at top of bars are different

result of lake-dredged materials additions (Fig. 4). The least compacted soils in 2002 and 2003 were observed from plots with 75% lake-dredged materials with mean soil compaction of 300 × 10^3 and 350 × 10^3 Pa, respectively. The most compacted soils in 2002 and 2003 were from the control plots with mean soil compaction of 1,800×10^3 and 1,600 ×10^3 Pa, respectively. The degree of soil compaction in the control plots were comparable with the surrounding natural soils (SNS), but were different and significantly higher than those plots with lake-dredged materials additions.

Penetrometer resistance of soils in plots with LDM50, LDM75, and LDM100 were all comparable among each other in 2002 and in 2003, respectively (Fig. 4). Soil compaction was lowered significantly by the application of lake-dredged materials. The least compacted soils in 2002 and 2003 were observed from plots with 75% lake-dredged materials, while the most compacted soils in 2002 and 2003 were from the control plots (0% lake-dredged materials). These results have shown the favorable influence that lake-dredged materials had on soil compaction. The higher rates of lake-dredged materials application may have had improved soil structure and soil tilth which can promote better water holding capacity, sufficient aeration, and creates more friable soils.

The compaction of agricultural soils is a serious problem and growing concern because the productive capacity of the land could be seriously reduced. A compacted layer within the soil profile may restrict root growth and access to water and nutrients (Follet and Wilkinson 1995). The structure of fine-textured (typic quartzipsamments) soils in the study area (Coleman Landing) has shown improvement as a result of lake-dredged materials addition. This is largely the result of an increase in the organic matter content and to a lesser extent to the flocculation of calcium-saturated colloids. Application of lake-dredged materials may have had promoted intense biological activity, increased nitrogen fixation by soil microorganisms, and release of component elements by the more rapid decomposition of plant residues (Follet and Wilkinson 1995; Pearson and Hoveland 1974).

Penetration resistances of soil treated with lake-dredged materials have values well within the “good” range of root development or penetration. Penetration resistance of about 1,035 × 10^3 Pa in soils could result to a root penetration reduction of about 50%, while penetration resistance of greater than 1,380 × 10^3 Pa may result to 80–90% root penetration reduction (Fig. 5). Penetration resistance of greater than 1,380 × 10^3 Pa may trigger poor root development if not corrected properly. The use of soil penetrometer, which is designed to mimic a plant root, is one way of monitoring soil compaction.

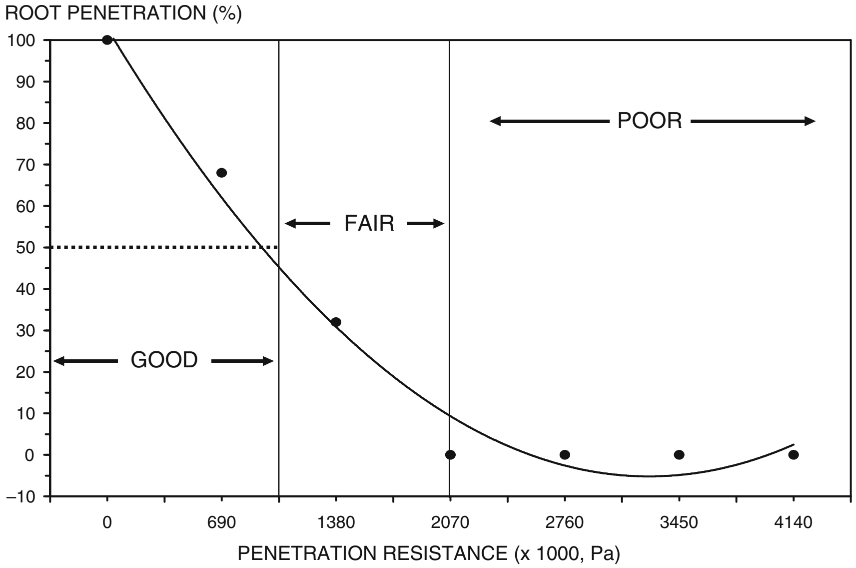

Fig. 5 Relationship of root penetration and penetration resistance in soils using penetrometer (Source: Murdock et al. 1995). RP (%) = 153.1 + 4.8x^2 – 55.2x, R^2 = 0.98

2.2.2 Effects on Soil Chemical Properties

The average soil tests values for pH, total inorganic nitrogen, total phosphorus, potassium, calcium, and magnesium varied significantly ($P \leqslant 0.001$) among plots amended with different rates of lake-dredged materials within years, but not affected by the year × treatment interaction effects (Table 5). Compared with the control plots, the soils in plots amended with lake-dredged materials exhibited an increase in soil pH, total inorganic nitrogen, calcium, and magnesium in all years. However, levels of soil calcium and magnesium from plots with lake-dredged materials addition were lower in 2005 compared with their average values in 2002, 2003, and 2004. The average calcium levels in soil (averaged across plots with lake-dredged materials) in 2002, 2003, and 2004 were 2,010, 6,503, and 1,184 mg kg^{-1}, respectively compared with 237 mg kg^{-1} in 2005. The levels of calcium show a decline in value in four years.

Addition of lake-dredged materials resulted in higher soil pH than those plots with no lake-dredged materials. Soil pH (averaged across plots with lake-dredged materials) of 7.4, 7.6, 7.7, and 7.2 were higher than plots with no lake-dredged materials (5.9, 5.5, 5.9, and 5.5) in 2002, 2003, 2004, and 2005, respectively (Table 5). Soil test values for total inorganic nitrogen in 2004 and 2005 showed an increasing trend when compared with their levels in 2002 and 2003 for soils treated with lake-dredged materials. The average increase of total inorganic nitrogen in 2004 and 2005 in soils treated with lake-dredged materials (averaged across treatments) were 34.5 and 17.1 mg kg^{-1} compared with 0.94 and 0.99 mg kg^{-1} in 2002 and 2003, respectively.

The levels of total phosphorus in soils that were treated with different levels of lake-dredged materials were consistently lower than the soil total phosphorus values in plots with no lake-dredged materials application for all years. The average soil test values for total phosphorus in soils with no lake-dredged materials were 5.1, 6.7, 2.3, and 6.9 mg kg^{-1} in 2002, 2003, 2004, and 2005, respectively. It must be noted that the soil tests values for total phosphorus should not be construed as environmental problems. Their present soil tests values are well below levels considered to be harmful to the environment. Concerns for losses of soil phosphorus by overland flow occur when soil tests values are well below levels considered being harmful to the environment. Concern for losses of soil P by overland flow occur when soil P exceeded 150 mg kg^{-1} in the upper 20-cm of soil (Johnson and Eckert 1995; Sharpley et al. 1996).

Average soil tests values for Mehlich 1 extracted Zn, Mn, Cu, Fe, and Al from plots treated with different levels of lake-dredged materials are shown Table 6.

Table 5 Average levels of soil pH, TIN, TP, K, Ca, and Mg from beef cattle pasture plots amended with different levels of lake-dredged materials in 2002, 2003, 2004, and 2005

Application rates (g kg^{-1})	pH	TIN[a] (mg kg^{-1})	TP[b] (mg kg^{-1})	K[b] (mg kg^{-1})	Ca[b] (mg kg^{-1})	Mg[b] (mg kg^{-1})
Initial (all treatments)	5.9 ± 0.01	2.9 ± 1.5	20.6 ± 8.9	39.9 ± 11.6		
2002						
0	5.9 ± 0.006d[c]	0.16 ± 0.02c	5.08 ± 0.81a	3.6 ± 0.6a	105 ± 5.4b	4.3 ± 2.6b
250	7.1 ± 0.1c	0.67 ± 0.44b	0.17 ± 0.13b	0.9 ± 0.1c	1, 962 ± 25.8a	11.9 ± 0.7a
500	7.4 ± 0.005b	0.91 ± 0.24ab	0.13 ± 0.02b	2.8 ± 1.4a	2, 040 ± 29.1a	13.6 ± 1.1a
750	7.4 ± 0.00b	1.34 ± 0.12a	0.06 ± 0.02b	1.8 ± 1.0bc	2, 008 ± 87.1a	14.6 ± 1.7a
1,000	7.5 ± 0.06a	0.87 ± 0.13b	0.14 ± 0.04b	2.5 ± 0.7abc	2, 030 ± 9.2a	14.7 ± 0.6a
LSD (0.05)	0.11	0.44	4.53	1.60	78.10	2.80
2003						
0	5.46 ± 0.08d	0.26 ± 0.01c	6.69 ± 0.01a	21.4 ± 2.8c	348 ± 40.3c	15.1 ± 0.84d
250	7.35 ± 0.10c	0.71 ± 0.44b	1.11 ± 0.21b	41.5 ± 13.4a	6, 534 ± 10.5ab	61.7 ± 5.6c
500	7.62 ± 0.08b	0.96 ± 0.19b	1.99 ± 0.14b	41.9 ± 11.4a	6, 679 ± 32.84a	86.3 ± 9.5a
750	7.67 ± 0.08b	1.40 ± 0.14a	1.69 ± 0.19b	36.5 ± 8.1ab	6, 564 ± 45.0a	92.9 ± 12.9a
1,000	7.77 ± 0.05a	0.91 ± 0.13b	1.61 ± 0.31b	27.9 ± 3.8bc	6, 236 ± 44.6b	74.9 ± 86b
LSD (0.05)	0.09	0.42	4.79	10.6	155.01	10.11
2004						
0	5.9 ± 0.69b	2.77 ± 0.19b	2.26 ± 0.82a	19.3 ± 0.1a	173 ± 9.1b	4.6 ± 1.5d
250	7.5 ± 0.08a	61.85 ± 1.5a	0.08 ± 0.01b	15.9 ± 1.2ab	1, 268 ± 7.8a	7.2 ± 0.8c
500	7.8 ± 0.06a	21.59 ± 5.26b	0.04 ± 0.01b	5.0 ± 1.0ab	1, 160 ± 9.8a	8.4 ± 1.1bc
750	7.7 ± 0.11a	28.16 ± 3.28ab	0.06 ± 0.01b	2.8 ± 0.3b	1, 155 ± 5.5a	10.4 ± 0.6ab
1,000	7.8 ± 0.04a	26.50 ± 6.58b	0.07 ± 0.04b	6.2 ± 0.4ab	1, 155 ± 10.4a	12.7 ± 2.2a
LSD (0.05)	0.57	34.56	2.29	16.2	117.0	2.5
2005						
0	5.5 ± 0.77b	2.36 ± 0.67b	6.99 ± 0.98a	3.48 ± 0.8b	44.7 ± 8.3c	1.9 ± 1.4b
250	6.8 ± 0.96ab	2.32 ± 0.57b	3.15 ± 4.31ab	1.30 ± 0.4b	174 ± 2.8b	1.9 ± 1.4b
500	7.4 ± 0.02a	24.46 ± 13.32b	0.32 ± 0.05b	2.56 ± 0.57b	156 ± 2.6b	2.7 ± 1.8ab
750	7.3 ± 0.02a	29.26 ± 3.36a	0.18 ± 0.01b	14.25 ± 0.78a	318 ± 10.5a	8.8 ± 2.7a
1,000	7.5 ± 0.02a	12.47 ± 7.83ab	0.11 ± 0.04b	3.06 ± 0.01b	301 ± 12.8a	4.8 ± 1.7ab
LSD (0.05)	1.42	18.20	5.11	10.1	313.7	6.14

[a] Extracted with 2N KCl
[b] Extracted with double acids (0.05N HCl in 0.025N H_2SO_4)
[c] Means in each column for each year with common letter (s) are not significantly different at $P \leqslant 0.05$

The levels of extractable Zn, Mn, Cu, Fe, and Al in soils were significantly reduced by lake-dredged materials application and this result was consistent for all years.

The overall results however showed that with increasing application rates of lake-dredged materials, soil test values for extractable Zn, Mn, Cu, Fe, and Al remained to be statistically comparable from 2002 to 2005. These initial data suggest that applied lake-dredged materials regardless of application rates would not be source of trace metals in the soil (Table 6).

The average levels of extractable Zn and Mn (averaged across years) in soils with lake-dredged materials treatments were significantly lower when compared to soils with no lake-dredged materials (Table 6). Similar trends and comparisons of results were noted for extractable Cu, Fe, and Al between plots with lake-dredged materials and plots with no lake-dredged materials application in 2002, 2003, 2004, and 2005. The average levels of Cu in soils without lake-dredged materials treatment were 0.45, 1.04, 0.14, and 0.02 mg kg^{-1} compared with 0.002, 0.000, 0.002, and 0.009 mg kg^{-1} in 2002, 2003, 2004, and 2005, respectively (Table 6).

2.2.3 Effects on Forage Yield

The forage yield of bahiagrass at 112, 238, and 546 Julian days after seeding are shown in Fig. 6. Forage yield of bahiagrass varied significantly ($P \leqslant 0.001$) among plots with lake-dredged materials additions.

Table 6 Average level of Zn, Mn, Cu, Fe, and Al of sandy soils from beef cattle pasture plots amended with different amounts of lake-dredged materials in 2002, 2003, 2004, and 2005

Application rate (g kg^{-1})	Zn[a] (mg kg^{-1})	Mn[a] (mg kg^{-1})	Cu[a] (mg kg^{-1})	Fe[a] (mg kg^{-1})	Al[a] (mg kg^{-1})
Initial (all treatments)	0.40±0.3	1.30±0.7	0.20±0.1	4.90±0.1	83.4±17.1
			2002		
0	0.69±0.13a[b]	2.86±0.39a	0.45±0.05a	15.81±5.59a	87.23±13.28a
250	0.01±0.006b	0.35±0.05b	0.001±0.0005b	0.03±0.01b	0.19±0.24b
500	0.006±0.001b	0.31±0.01b	0.002±0.001b	0.002±0.001b	0.03±0.02b
750	0.007±0.0006b	0.25±0.01b	0.002±0.001b	0.002±0.001b	0.01±0.00b
1,000	0.005±0.00b	0.34±0.04b	0.003±0.001b	0.003±0.000b	0.04±0.02b
LSD (0.05)	0.10	0.32	0.04	4.56	10.80
			2003		
0	0.93±0.14a	2.66±0.24a	1.04±0.08a	23.17±9.1a	46.31±9.1a
250	0.13±0.02c	0.91±0.06b	0.00±0.00b	0.19±0.06b	0.58±0.33b
500	0.23±0.02b	0.93±0.95bb	0.00±0.00b	0.32±0.06b	0.16±0.15b
750	0.29±0.04b	0.83±0.09b	0.00±0.00b	0.29±0.07b	0.13±0.03b
1,000	0.24±0.07b	1.08±0.16b	0.00±0.00b	0.36±0.08b	0.14±0.07b
LSD (0.05)	0.09	0.17	0.04	4.84	20.83
			2004		
0	0.33±0.04a	1.28±0.11a	0.14±0.11a	4.96±0.78a	41.37±7.14a
250	0.006±0.001b	0.14±0.11b	0.003±0.001b	0.000±0.00b	1.27±0.18b
500	0.000±0.000b	0.06±0.02b	0.001±0.0001b	0.000±0.00b	1.08±0.01b
750	0.000±0.000b	0.07±0.01b	0.002±0.0001b	0.000±0.00b	1.06±0.04b
1,000	0.000±0.000b	0.08±0.05b	0.002±0.001b	0.000±0.00b	1.03±0.07b
LSD (0.05)	0.04	2.23	0.09	0.63	11.77
			2005		
0	0.117±0.06a	0.23±0.15a	0.021±0.011a	0.54±0.30a	51.46±7.14a
250	0.018±0.02b	0.09±0.01b	0.010±0.007b	0.32±0.05b	11.27±9.18b
500	0.010±0.01b	0.13±0.01b	0.012±0.001b	0.02±0.01b	0.23±0.03b
750	0.002±0.00b	0.08±0.02b	0.004±0.001b	0.02±0.00b	0.52±0.01b
1,000	0.004±0.00b	0.07±0.01b	0.013±0.002b	0.01±0.00b	0.49±0.12b
LSD (0.05)	0.07	0.26	2.57	0.64	33.76

[a] Extracted with double acids (0.05N HCl in 0.025N H_2SO_4) as described by Mehlich (1953)

[b] Means in each column for each year with common letter (s) are not significantly different at $P \leqslant 0.05$

The greatest forage yield of 673 ± 233 kg ha^{-1} at Julian day 112 was from plots amended with 50% lake-dredged materials while bahiagrass in plots amended with 100% lake-dredged materials and 75% lake-dredged materials had the highest forage yield at Julian days 238 and 546 with average forage yield of 3,349 ± 174 and 4,109 ± 220 kg ha^{-1}, respectively (Fig. 6). The lowest forage yield of 89 ± 63, 1,513 ± 166, and 1,263 ± 116 kg ha^{-1} were from the control plots for Julian days 112, 238, and 546, respectively (Fig. 6). The average forage yield increase of bahiagrass in plots amended with lake-dredged materials (averaged across treatments) was 512%, 82%, and 173% when compared with bahiagrass in control plots with 0% lake-dredged materials for Julian days 112, 238, and 546, respectively (Fig. 6). These data show the favorable influence that lake-dredged materials had on forage yield of bahiagrass during its early establishment in subtropical beef cattle pastures.

Mean forage yield of bahiagrass during Julian day 112 in plots with 50% lake-dredged materials of 673 ± 233 kg ha^{-1} was not significantly different from that in plots with 75% lake-dredged materials (654 ± 106 kg ha^{-1}), but was greater than that in plots with 25% lake-dredged materials (378 ± 185 kg ha^{-1}) and 0% lake-dredged materials (Fig. 6). For Julian day 238, the greatest forage yield among plots amended with lake-dredged materials was from plots with 100% lake-dredged materials (3,349 ± 174 kg ha^{-1}). The lowest forage yield of 1,513 ± 166 kg ha^{-1} was from plots with 0% lake-dredged materials. Mean forage yield of bahiagrass in plots with 50% lake-dredged materials of 2,467 ± 320 kg ha^{-1} was not significantly different from that in plots with 75% lake-dredged materials

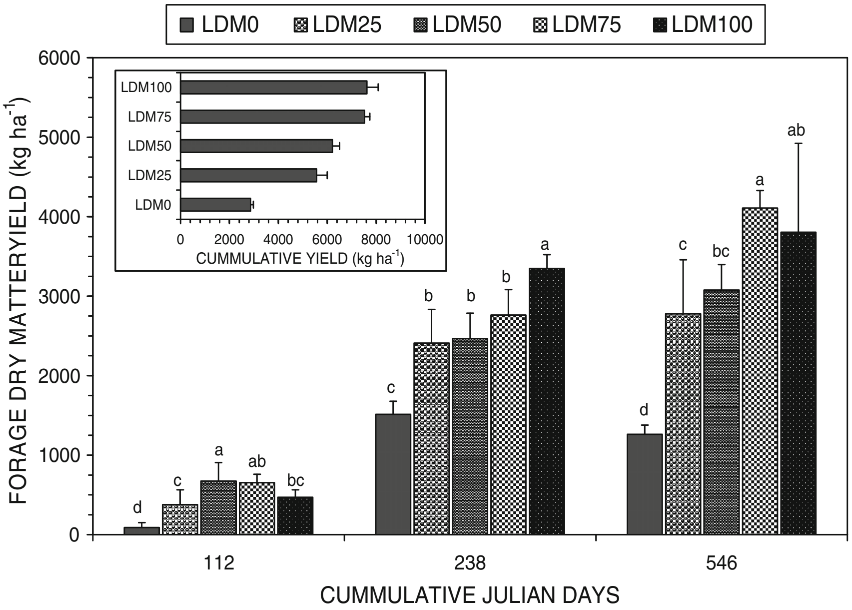

Fig. 6 Forage yield of bahiagrass (Julian days 112–546) as affected by varying levels of dredged materials application. Forage yield from plots with or without lake-dredged materials are significantly different ($P \leqslant 0.05$) at Julian days 112, 238, and 546 when superscripts located at top of bars are different (Source: Sigua et al. 2004b)

(2,467 ± 320 kg ha^{-1}) and 25% lake-dredged materials (2,409 ± 423 kg ha^{-1}), but was greater than that in plots with 0% lake-dredged materials (Fig. 6).

For Julian day 546 (78 weeks), mean forage yield of bahiagrass in plots with 100% lake-dredged materials of 3,804 ± 1,120 kg ha^{-1} was comparable with that of bahiagrass yield in plots with 75% lake-dredged materials (4,109 ± 220 kg ha^{-1}) and 50% lake-dredged materials (3,077 ± 322 kg ha^{-1}). However, mean forage yield of bahiagrass in plots with 75% lake-dredged materials was significantly higher than the mean forage yield of bahiagrass in plots with 50%, 25% (2,780 ± 678 kg ha^{-1}), and 0% (1,263 ± 116 kg ha^{-1}) lake-dredged materials. Forage yield variability (83%) of bahiagrass during its establishment can be explained by the addition of lake-dredged materials as shown by the equation below.

$$\text{Forage yield} = 25.64 \times \text{LDM} + 1{,}724.3$$
$$R^2 = 0.83^{***} \qquad P \leqslant 0.0001. \qquad (1)$$

The greatest cumulative forage yield of bahiagrass of 7,623 ± 462.3 kg ha^{-1} was from plots with 100% lake-dredged materials and the least cumulative forage yield of 2,865 ± 115 kg ha^{-1} was from the control plots (0% lake-dredged material). Cumulative forage yield of bahiagrass from plots with 100% lake-dredged materials, 75% lake-dredged materials, and 50% lake-dredged materials did not vary among each other, but was significantly greater than the cumulative yield of bahiagrass grown in plots with 25% lake-dredged materials. Interestingly, cumulative yield of bahiagrass in plots with 25% lake-dredged materials was increased by 94% over the control plots while the average yield increase of bahiagrass (averaged across 50% lake-dredged materials, 75% lake-dredged materials, and 100% lake-dredged materials) was about 145% over the untreated bahiagrass (Fig. 6).

2.2.4 Effects on Crude Protein Content

The crude protein content of bahiagrass with and without lake-dredged materials during early establishment (Julian days 546) are shown in Fig. 7. Results have shown the favorable influence that lake-dredged materials had on bahiagrass crude protein content. The crude protein content of bahiagrass varied significantly ($P \leqslant 0.001$) with varying levels of lake-dredged materials applications. The tissues of bahiagrass with 100% lake-dredged materials had the highest crude protein (151 ± 22 g kg^{-1}) and the lowest crude protein of

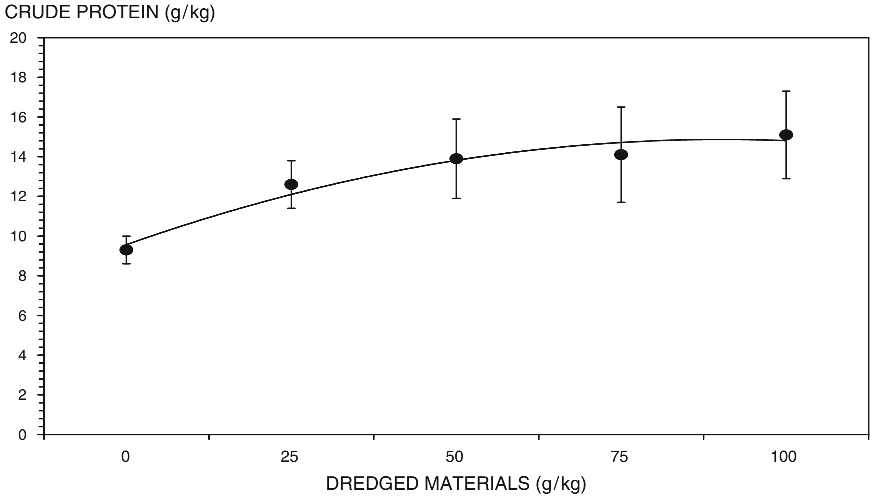

Fig. 7 Relationships of crude protein content of bahiagrass with increasing rates of lake-dredged materials application. $y = -0.4071\mathrm{DM}^2 + 3.7529\mathrm{DM} + 6.22$, $R^2 = 0.96$; $P \leqslant 0.0001$

$93 \pm 7\,\mathrm{g\,kg^{-1}}$ was from the control plots (0% lake-dredged materials). The crude protein in plots with 50% ($139 \pm 20\,\mathrm{g\,kg^{-1}}$), 75% ($141 \pm 24\,\mathrm{g\,kg^{-1}}$) and 100% ($151 \pm 22\,\mathrm{g\,kg^{-1}}$) lake-dredged materials were statistically comparable, but were significantly different from the crude protein in the control plots (Fig. 7). However, the crude protein in the control plots was not different from the level of crude protein in plots with 25% lake-dredged materials. The crude protein of bahiagrass increased quadratically with increasing rates of lake-dredged materials application (Fig. 7). The crude protein response of bahiagrass to lake-dredged materials application can be described by the equation below:

$$\begin{aligned}\text{Crude protein} &= -0.407 \times \mathrm{LDM}^2 \\ &\quad + 3.7529 \times \mathrm{LDM} + 6.22 \\ R^2 &= 0.96^{***} \quad P \leqslant 0.0001. \end{aligned} \tag{2}$$

2.3 *Biosolids*

All biosolids mostly used in research was of class B in terms of United States Environmental Protection Agency's pathogens and pollutant concentration limit (Table 7). Pathogen and chemical composition of the class B biosolids that were used in the study were all in compliance with the USEPA guidelines. Liquid sludge (SBS11) had the lowest fecal coliforms counts ($0.2 \times 10^6\,\mathrm{CFU\,kg^{-1}}$) while the cake biosolids (CBS) had the greatest coliforms counts of $178 \times 10^6\,\mathrm{CFU\,kg^{-1}}$. The fecal coliforms counts for SBS7 were about $33 \times 10^6\,\mathrm{CFU\,kg^{-1}}$. The fecal coliforms counts of all biosolids that were used in the study were below the USEPA fecal coliforms counts limit of $\leqslant 2{,}000 \times 10^6\,\mathrm{CFU\,kg^{-1}}$ (Table 7). Concentrations of As, Cd, Cu, Pb, Hg, Mo, Ni, and Zn of biosolids again, were far below the national USEPA limits (Table 7). The total P, total N, and K contents of biosolids ranged from 22 to $33\,\mathrm{g\,kg^{-1}}$, 39 to $48\,\mathrm{g\,kg^{-1}}$, and 2.5 to $3.1\,\mathrm{g\,kg^{-1}}$, respectively. Based on their N and P compositions, biosolids can be used as low-grade nitrogen and phosphorus fertilizer and also as source of calcium especially the lime-stabilized residuals (Hue 1995).

2.3.1 Research Highlights: Cumulative and Residual Effects of Repeated Biosolids Applications

The objectives of this study were to evaluate the cumulative and residual effects of repeated applications of biosolids on (i) bahiagrass (*Paspalum notatum* Flügge) production over years with (1997–2000) and without (2001–2002) biosolids applications during a 5-year period, and (ii) on nutrients status of soils that received annual application of biosolids from 1997 to 2000 compared with test values of soils in 2002 (with no biosolids application) in South Florida.

Table 7 Some soil properties and average chemical and bacteriological composition of biosolids used for the experiment in relation to USEPA concentration limit

Parameter	Soils	Liquid sludge (pH 7)	Liquid sludge (pH 11)	Cake biosolids	USEPA concentration limit[a]
Fecal coliforms CFU kg^{-1} [b]		33.3×10^6	0.15×10^6	177.5×10^6	$\leqslant 2{,}000 \times 10^6$
Total solids, mg L^{-1}		47,000	20,500	500,000	
Organic matter, g kg^{-1}	12				
Total P, g kg^{-1}	0.0014	25	22	33	
Total N, g kg^{-1}		48	40	39	
Total K, g kg^{-1}	0.0020	2.5	2.6	3.1	
Ca, mg kg^{-1}	221				
Mg, mg kg^{-1}	45				
As, mg kg^{-1}		6.1	2.8	7.5	41
Fe, mg kg^{-1}	18				
Cu, mg kg^{-1}	0.15	362	301	532	1,500
Cd, mg kg^{-1}		2	10	4	39
Cr, mg kg^{-1}		6.5	34	48	1,200
Mo, mg kg^{-1}		7.7	8.0	10	18
Pb, mg kg^{-1}		15.3	35	46	300
Zn, mg kg^{-1}	0.32	1,022	973	1,590	2,800
Hg, mg kg^{-1}		1.1	0.24	0.66	17
Ni, mg kg^{-1}		18.3	38	44.6	420

[a] Concentration limits as defined in USEPA (1993)
[b] Colony forming units kg^{-1} of residuals

The field experiment was conducted at the University of Florida Agricultural Research and Education Center, Ona, FL (27°26’N, 82°55’W) on a Pomona fine sandy soil. With the exception of the control, bahiagrass plots received annual biosolids and chemical fertilizers applications to supply 90 or 180 kg total N ha^{-1} $year^{-1}$ from 1997 to 2000. Land application of biosolids and fertilizer ceased in 2001 season. In early April 1998, 1999, and 2000, plots were mowed to 5-cm stubble and treated with the respective N source amendments. The experimental design was three randomized complete blocks with nine N-source treatments: ammonium nitrate (AMN), slurry biosolids of pH 7 (SBS7), slurry biosolids of pH 11 (SBS11), lime-stabilized cake biosolids (CBS), each applied to supply 90 or 180 kg N ha^{-1}, and a nonfertilized control (Control). Application rates of biosolids were calculated based on the concentration of total solids in materials as determined by the American Public Health Association SM 2540G methods (APHA 1989) and N in solids (see Sect. 1.1.2.2). The actual amount of biosolids applications was based on the amount required to supply 90 and 180 kg N ha^{-1}. Sewage sludge materials were weighed in buckets and uniformly applied to respective bahiagrass plots. Soil samples were collected in June 1997, June 1999, and in June 2002 from 27 treatment plots. In 1997 and 1999, soil samples were collected using a steel bucket type auger from the 0- to 20-, 20- to 40-, 40- to 60-, and 60- to 100-cm soil depths.

Forage was harvested on 139, 203, 257, and 307 day of year (DOY) in 1998; 125, 202, 257, and 286 DOY in 1999; 179, 209, 270, and 301 DOY in 2000; and on 156 and 230 DOY in 2002 (no biosolids applications) to determine the residual effect of applied biosolids following repeated application. Forage yield and soils data were analyzed using analysis of variance procedures with year and treatment as the main plot and sub-plot, respectively (SAS 2000). As a result of significant year effects on forage yield, data were reanalyzed annually (i.e., 1998, 1999, 2000, and 2002).

Effects on Forage Yield

Forage yield of bahiagrass was significantly ($P \leqslant 0.001$) affected by the different biosolids in all years (1998–2002), but not by the interaction effects of year × treatments. Although yield trend was declining from 1988 to 2002, forage yield of bahiagrass that received biosolids were consistently and significantly ($P \leqslant 0.05$) greater than the forage yield of the unfertilized bahiagrass (Table 8). The bahiagrass fertilized with SBS11-180 had the greatest forage yield in 1998 (5.1 ± 0.4 mg ha^{-1}), 1999 (4.6 ± 0.2 mg ha^{-1}), 2000

Table 8 Comparison on forage yield (mg ha^{-1}; mean ± S.D.) of bahiagrass among years with repeated application of biosolids (1998, 1999, and 2000) and with no biosolids application (2002)

Nitrogen sources	With sewage sludge 1998	1999	2000	Without sewage sludge 2002
Control	2.4 ± 0.5d[a]	1.8 ± 0.2c	1.4 ± 0.3d	1.2 ± 0.2c
AMN-90	4.3 ± 0.2ab	3.7 ± 0.1b	2.1 ± 0.1cd	2.1 ± 0.3bc
AMN-180	4.7 ± 0.4a	4.7 ± 0.02a	3.2 ± 0.3b	2.2 ± 0.4b
SBS7-90	4.4 ± 0.4ab	3.1 ± 0.3b	2.2 ± 0.4bcd	2.5 ± 0.5ab
SBS7-180	5.0 ± 0.5a	5.1 ± 0.2a	2.6 ± 0.2bc	2.3 ± 0.5b
SBS11-90	4.1 ± 0.5abc	3.3 ± 0.3b	1.9 ± 0.3cd	1.9 ± 0.2bc
SBS11-180	5.1 ± 0.4a	4.6 ± 0.2a	4.5 ± 0.2a	3.3 ± 0.6a
CBS-90	2.9 ± 0.4cd	2.2 ± 0.2c	1.8 ± 0.6cd	2.5 ± 0.5ab
CBS-180	3.3 ± 0.3bcd	3.3 ± 0.1b	2.7 ± 0.2bc	2.5 ± 0.5ab

[a] Mean values in each column followed by the same letter(s) are not different ($P > 0.05$) according to the Duncan's multiple range test

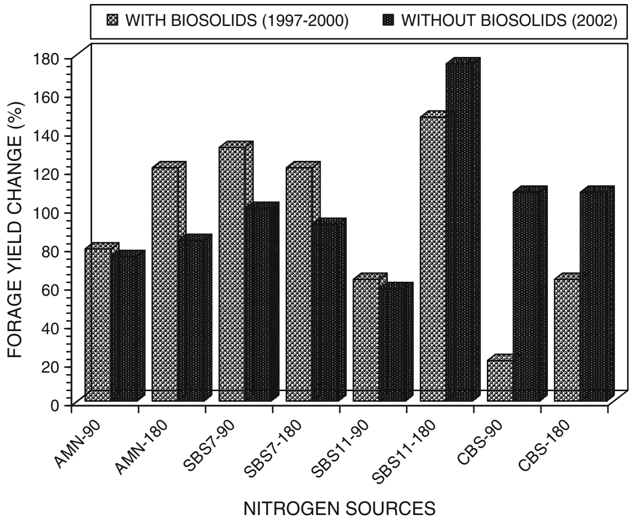

Fig. 8 Residual effects of applied sewage sludge and ammonium nitrate fertilizer on forage yield change over the unfertilized bahiagrass with repeated sewage sludge application (1997–2000) and without sewage sludge application (2001–2002)

(4.5 ± 0.2 mg ha^{-1}), and in 2002 (3.3 ± 0.6 mg ha^{-1}). Forage yield of bahiagrass fertilized with AMN-90 and AMN-180 was significantly greater than those of the unfertilized bahiagrass in 1998 and 1999, but not in 2000 and in 2002. Although SBS11-180 had the greatest residual effect (170%) in 2002, CBS-90 and CBS-180 had more pronounced effects when compared with the other sewage sludge sources because their relative impact on forage yield compared with the control between years with (1997–2000) and without (2002) sewage sludge applications increased from 30% to 110% and 70% to 110%, or net increases of 267% and 57% in forage yield change, respectively (Fig. 8).

The residual effects of applied sewage sludge on bahiagrass yield expressed as percent forage yield change over the unfertilized bahiagrass are shown in Fig. 8. Residual effects of AMN-90 (–6%), AMN-180 (–31%), SBS7-80 (–21%), and SBS7-180 (–17%) declined (negative) with time, but the residual effects of applied SBS11-180 (+13%), CBS-90 (+267%), and CBS-180 (+57%) were positive over time although sewage sludge application ceased after harvest in 2000. The percent forage yield change of bahiagrass fertilized with SBS11-180, CBS-90, and CBS-180 during years when sewage sludges were applied (1998–2000) were 150%, 30%, and 70% compared with percent forage yield change of 170%, 110%, and 110% in 2002 (when sewage sludge applications ceased), respectively.

The residual effects on forage yield of applied CBS-90 (+267%) and CBS-180 (+57%) relative to the control increased with time although biosolids applications ceased after the 2000 harvest season (Fig. 8). This was probably due to the higher concentration of organic nitrogen in addition to the liming property of CBS. Liming the field could have some direct and indirect effects on forage productivity and on the nutrient status of the soils. Perhaps the single direct benefit of liming is the reduction in acidity and solubility of aluminum and manganese (Peevy et al. 1972). Some of the indirect benefits of liming pasture fields among others would include: enhancing P and microelement availability, nitrification, nitrogen fixation, and improving soil physical conditions (Nelson 1980; Tisdale and Nelson 1975; Russell 1973). Dried and composted biosolids have slower rates of N release and in case of CBS with much higher solids concentration (500,000 mg L^{-1}); more N will be released in the second, third, or even the fifth year after the initial application due to higher amount of organic nitrogen than ammonium nitrogen. The proportions of ammonium and organic N in biosolids vary with the stabilization process.

Under an intensive management condition, bahiagrass maintained high forage yields through years with repeated biosolids applications and through years without biosolids application. Although the average bahiagrass forage yield in 2002 was slightly lower than in 2000, yield differences between the control and treated plots were indicative of a positive carry over effect of applied biosolids in 2002. Lime stabilized biosolids (SBS11, CBS) had the highest residual effects on bahiagrass forage yield and had enhanced overall soil characteristics. The carry over effect of these biosolids over the long term can be especially significant in many areas of Florida where only 50% of the one million hectare of bahiagrass pastures are given inorganic nitrogen yearly. These biosolids if processed and applied according to USEPA rules (EPA 1993) have the potential to boost and maintain production because they are inexpensive, environmentally safe, and could act as liming and organic matter amendment as well.

2.3.2 Effects on Soil Chemical Properties

Total Inorganic Nitrogen and Total Phosphorus

Average soil test values in June 2002 exhibited: (i) decrease in total inorganic nitrogen (NO_3-N + NH_4-N), TP, K, Ca, Mg, Al, Mn, and Fe concentrations; and (ii) slight increase in Zn and Cu concentrations when compared with the June 1997 soil test results (Table 9). Levels of total inorganic nitrogen in June 1997, three months after initial biosolids applications, varied significantly with nitrogen sources, but total inorganic nitrogen concentrations leveled off in June 2002. As expected, plots with AMN (180 kg ha^{-1}) had the greatest concentration of total inorganic nitrogen (2.7 mg kg^{-1}) initially. Although the concentrations of total inorganic nitrogen in plots with different sources of nitrogen (DSS + AMN) were significantly higher than the concentrations of total inorganic nitrogen for the unfertilized plots in 1997, the concentrations of total inorganic nitrogen declined in 2002, suggesting no soil accumulations of nitrogen over time.

The concentrations of soil total phosphorus declined by almost 50% in plots with different nitrogen sources from June 1997 to June 2002 (Table 9). Plots with different sources of nitrogen had higher concentrations of total phosphorus than the unfertilized plots in June 1997 and in June 2002. However, the concentrations of total phosphorus in 2002 for plots with biosolids were not much higher than the concentrations of total phosphorus in the unfertilized plots. Again, application of biosolids did not result to any total phosphorus build up in the soil over time (1997–2002). Distribution of total phosphorus (averaged across treatments) also did not show significant build up with soil depth (Table 9). The levels of soil total phosphorus showed declining trend from June 1999 to June 2002. In June 2002, soil total phosphorus did not significantly vary with soil depth. The level of total phosphorus in the surface soil (0–20 cm) was about 5.4 mg kg^{-1} and 4.7 mg kg^{-1} at soil depth of 60–100 cm in June 2002. Likewise, soil test values of total phosphorus in June 1999 did not vary significantly with soil depth, i.e., 0–20 cm: 7.8 mg kg^{-1}; 60–100 cm: 7.8 mg kg^{-1} (Table 9).

K, Ca, and Mg

Similar to total phosphorus and total inorganic nitrogen, soil test values for K, Ca, and Mg declined significantly from June 1997 to June 2002 (Table 9). The concentrations of K, Ca, and Mg in June 1997 ranged from 13.5 to 32.5, 173.8 to 287.7, and 35.4 to 64.1 mg kg^{-1}, respectively. Soil test values of K, Ca, and Mg in June 2002 ranged from 1.3 to 2.2, 22.8 to 53.9, and 3.5 to 9.9 mg kg^{-1}, respectively. Although the concentrations of soil K, Ca, and Mg (averaged across treatments) did decline over time, the surface soil (0–20 cm) had the greatest concentrations and tended to decrease with soil depth (Table 9). Again, biosolids application did not result to any build up of K, Ca, and Mg in the soils over time.

Table 9 Comparative distribution of soil TP, K, Ca, Mg, Zn, Mn, Cu, Fe, Al, and Na with soil depth in June 1999 and in June 2002 (Source: Sigua et al. 2005)

Soil depth (cm)	TP[a]	K[a]	Ca[a]	Mg[a]	Zn[a]	Mn[a]	Cu[a]	Fe[a]	Al[a]	Na[a]
June 1999										
0–20	7.8a[b]	11.5a	382.1a	55.9a	0.7a	0.26a	0.37a	11.4b	58.1a	10.6a
20–40	3.8a	2.3a	56.4bc	12.5c	0.3b	0.05b	0.05b	6.2b	30.4a	5.7b
40–60	6.7a	6.4a	93.1b	30.4b	0.4b	0.11b	0.03b	36.1a	17.7a	8.6ab
60–100	7.8a	3.4a	38.8c	12.2c	0.3b	0.05b	0.01b	14.3b	32.4a	5.2b
June 2002										
0–20	5.4ab	2.0a	56.6a	8.7a	0.5a	0.11b	0.25a	6.8a	54.8ab	14.9a
20–40	4.6a	2.1a	73.3a	11.2a	0.5a	0.26a	0.30a	2.9b	34.7b	14.4a
40–60	3.1a	0.9b	16.9b	3.4b	0.3b	0.03b	0.26a	1.9b	89.9a	14.4a
60–100	4.7a	1.1b	15.4b	3.6b	0.4ab	0.02b	0.29a	4.5ab	95.9a	15.2a

[a] mg kg^{-1}.

[b] Mean values in each column followed by the same letter(s) are not different ($P > 0.05$) according to the Duncan's multiple range test.

Zn, Mn, and Cu

Slight increase in the concentrations of Zn and Cu were noted in June 2002 while the concentration of Mn tended to decrease from June 1997 to June 2002 (Table 9). The concentrations of Zn, Mn, and Cu (averaged across treatments) in June 1999 and in June 2002 did not vary much with soil depth, again with decreasing trends with soil depths, respectively. Concentrations of Zn and Cu in June 2002 did not change at all with soil depth (Table 9). The soil test values of Mn in 2002 decreased from 0.11 mg kg^{-1} at the soil surface (0–20 cm) to 0.02 mg kg^{-1} at soil depth of 60–100 cm. The concentrations of Zn and Cu in plots with AMN-90, AMN-180, CBS-90, CBS-180, and the unfertilized plots remained unchanged between June 1997 and June 2002, but slight increase in the concentrations of Zn and Cu were observed from plots with SBS7-90, SBS7-180, SBS11-90, and SBS11-180. The concentrations of Mn across treatments showed a general decline in June 2002, but their concentrations were not significantly different from the control (Table 9).

Al, Fe, and Na

The concentrations of Al across treatments tended to decline significantly from June 1997 to June 2002 while the concentrations of Fe and Na remained unchanged from June 1997 to June 2002 (Table 9). In June 2002, the concentrations of Al across treatments declined by about 88% (274–32 mg kg^{-1}). The concentrations of Fe and Na in June 1997 ranged 4.8–15.2 mg kg^{-1} and 6.2–11.1 mg kg^{-1} compared with their concentrations of 1.7–9.7 mg kg^{-1} and 13.5–15.5 2.1 mg kg^{-1} in June 2002, respectively (Table 9). The concentrations of Fe and Al (averaged across treatments) in June 1999 did not vary with soil depth. The level of Na decreased from 10.6 mg kg^{-1} (0–20 cm) to 5.2 mg kg^{-1} (60–100 cm). In June 2002, the concentrations of Fe and Al (averaged across treatments) at 0–20 cm were comparable with their concentrations at 60–100 cm, suggesting no build up of Fe and Al within soil profile The concentrations of Na in June 2002 likewise did not vary with soil depth (Table 9).

All sources of N (domestic biosolids and AMN) gave better forage production than the unfertilized control during years with domestic biosolids application (1997–2000) and also during years with no domestic biosolids application (2001–2002). Although the average bahiagrass forage yield in 2002 (2.3 ± 0.7 mg ha^{-1}) was slightly lower than in 2000 (3.5 ± 1.2 mg ha^{-1}), yield differences in 2002 between the control (1.2 ± 0.2 mg ha^{-1}) and treated plots (2.3 ± 0.5 mg ha^{-1} to 3.3 ± 0.6 mg ha^{-1}) were indicative of a positive carry over effect of applied domestic biosolids. The favorable carry over or residual effects of applied domestic biosolids in 2002 may have had received additional boost from the amount of rainfall in the area. Rainfall varied between years, which caused the initial forage harvest in 1998 and forage harvest in 2002 to differ. It should be noted that applications of domestic biosolids and AMN fertilizers ceased after the 2000 harvest season. The total annual rainfall in the area was 1,735, 1,253, 801, 1,643, and 1,756 mm in 1998, 1999, 2000, 2001, and 2002, respectively. Additionally, domestic biosolids (especially the SBS and

CBS) supported forage production at a similar rate and to the same extent as the inorganic AMN fertilizers during the first three years (1997–2000) of repeated land application and during the succeeding years with no domestic biosolids (2000–2001) application.

The residual effects on forage yield of applied CBS-90 (+267%) and CBS-180 (+57%) relative to the control increased with time although domestic biosolids applications ceased after the 2000 harvest season (Fig. 8). This was probably due to the higher concentration of organic nitrogen in addition to the liming property of CBS. Liming the field could have some direct and indirect effects on forage productivity and on the nutrient status of the soils. Perhaps the single direct benefit of liming is the reduction in acidity and solubility of aluminum and manganese (Peevy et al. 1972). Some of the indirect benefits of liming pasture fields among others would include: enhancing P and microelement availability, nitrification, nitrogen fixation, and improving soil physical conditions (Nelson 1980; Tisdale and Nelson 1975; Russell 1973).

Dried and composted domestic biosolids have slower rates of N release and in case of CBS with much higher solids concentration (500,000 $\mathrm{mg\,L^{-1}}$); more N will be released in the second, third, or even the fifth year after the initial application due to higher amount of organic nitrogen than ammonium nitrogen. The proportions of ammonium and organic N in domestic biosolids vary with the stabilization process. The lime-stabilized and anaerobically digested CBS would normally contain 25% ammonium nitrogen and 75% organic nitrogen (Evanylo 1999). Organic nitrogen must be broken down initially to $\mathrm{NH_4^+}$ and $\mathrm{NO_3^-}$ by soil microorganisms before this form of nitrogen becomes available for plant use; therefore, organic nitrogen can be considered to be a slow release form of nitrogen. The organic nitrogen not mineralized during the first year (1997) after application of our study is mineralized slowly in succeeding years even domestic biosolids applications ceased in 2001 season. Since CBS was applied annually (equivalent to 90 and 180 $\mathrm{kg\,N\,ha^{-1}}$) on the same site that began in 1997 and ceased in 2000, mineralization of organic nitrogen may still be occurring even after the 2000 harvest season. Because of the slow mineralization process from CBS with higher organic nitrogen fully supported our results (i.e., residual effects of applied CBS-90 of +267% and CBS-180 of +57% relative to the control). Additionally, CBS may have had provided essential micronutrients including copper, boron, molybdenum, zinc, and iron to bahiagrass. Of these micronutrients, molybdenum and iron are playing vital roles during plant's photosynthetic activities.

Repeated applications of domestic biosolids indicate no harmful environmental or plant effects. Results support the hypothesis that repeated land application of domestic biosolids to supply 90 and 180 $\mathrm{kg\,N\,ha^{-1}}$ would not increase soil sorption for P, trace, and heavy metals. Results have indicated that the concentrations of soil TIN and TP declined by almost 50% in plots with different nitrogen sources from June 1997 to June 2002 suggesting that enrichment, mobility, or leaching of nitrogen and phosphorus to ground water is insignificant. The concentrations of soil nitrogen and phosphorus in 2002 following repeated application of domestic biosolids were far below the contamination risk in the environment (EPA 1993). Sewage sludge contains both organic and inorganic forms of nitrogen and phosphorus. After land application, the residual-derived nitrogen and phosphorus enter the soil nitrogen and phosphorus cycle, respectively (Basta 1997; Stevenson 1982, 1986). Mineralization of organic phosphorus will convert it to plant-available dissolved phosphate and shortly after release as dissolved phosphate; soil chemical adsorption and precipitation processes decreased dissolved phosphorus to a low concentration in soil solution. The rate of nutrient release, or mineralization, is affected by the stabilization process used to treat domestic wastewaters. Amounts of nitrogen mineralized under laboratory conditions depend heavily on the domestic biosolids treatment process and ranged from 10 to 40% (Gilmour et al. 1985, 2003; EPA 1983) while mineralization rates under field conditions are more variable and ranged from seven to 55% of the organic nitrogen mineralized (Gilmour et al. 2003; Evanylo 1999; Basta 1997; Gilmour and Clark 1988).

The levels of trace metals in the soils after repeated applications of biosolids did not indicate Mn, Al, and Fe enrichment in the soils (Table 9). Previous work of Chang et al. (1987) collaborated with previous research results. Chang et al. (1987) reported that the mobility of metals is greatest in the first year and decreases with time which is in direct contradiction to the "time bomb effect" suggested by Beckett and Davis (1979). Beckett and Davis (1979) claimed that decomposition of residuals would release metals that would eventually result in metal toxicity (especially heavy metals) in the soil. Research results presented in this paper do not support the "time bomb" theory, but likely supporting the concepts postulated by Chaney (1973), where soil chemical process reduce heavy metals availability in residuals-amended soils with time. These heavy metals may occur in residuals as insoluble precipitates, surface adsorbed mineral complexes, and insoluble organic matter chelates (Corey et al. 1987). The concentrations of Cu and Zn reported for June 2002 in soils with SBS7-180, SBS11-180, and CBS-90 were slightly elevated, but not toxics. The concentrations of Cu and Zn in 2002 were still within the norms for sludge heavy metals (Table 9) as reported by the US Environmental Protection Agency (EPA 1993).

3 Summary and Conclusions

Land application of lake-dredged materials and biosolids may provide substantial benefits that will enhance the environment, community, and society. The trace metal contents of these materials were below the threshold effect levels. As such, the agricultural or livestock industry could utilize these materials to produce forages. Although results have demonstrated the promising effects of added lake-dredged materials and biosolids on the early establishment of bahiagrass in pasture fields, further studies are still needed not only in pastures of south Florida, but also in other areas of the world with similar climatic conditions to determine whether the environmental and ecological implications of these materials are satisfied or not either in shorter or longer term.

The ability to reuse lake-dredged and domestic sewage sludge materials for agricultural purposes is important because it reduces the need for offshore disposal and provides an alternative to disposal of these materials in landfills that are already overtaxed. Often these materials can be obtained at little or no cost to the farmers or landowners. Thus, forage production offers an alternative to waste management since nutrients in the lake-dredged materials and biosolids are recycled into crops that are not directly consumed by humans. Results have shown the favorable influence that biosolids and lake-dredged materials had on bahiagrass during its early establishment in sandy subtropical beef cattle pasture areas in south central Florida. Some of the promising effects of added biosolids and lake-dredged materials on soil quality and on early establishment of bahiagrass are summarized below.

- Favorable influence that lake-dredged materials had on soil penetrometer resistance. Higher rate of application may have had improved structure and tilth of sandy soils, which can promote water holding capacity, sufficient aeration and creates more friable soils. This is largely the result of an increase in the organic matter content and to a lesser extent to the flocculation of calcium-saturated colloids.
- The lake-dredged materials have provided the benefits that we normally would obtain from liming the field using commercially available lime. Compared with the control plots, the soils in plots amended with lake-dredged materials exhibited an increase in soil pH, total inorganic nitrogen, calcium, and magnesium.
- Forage yield (mg ha^{-1}) variability (83%) of bahiagrass during its establishment can be explained by the addition of lake-dredged materials as shown by this equation (forage yield = $25.64 \times \text{LDM} + 1724.3$).
- The crude protein of bahiagrass increased quadratically with increasing rates of lake-dredged materials application. The crude protein response of bahiagrass to lake-dredged materials application can be described by this equation (crude protein $= -0.407 \times \text{LDM}^2 + 3.7529 \times \text{LDM} + 6.22$).
- Forage yield of bahiagrass was significantly affected by the different sewage sludges in all years (1997–2002). Forage yield of bahiagrass that received biosolids were consistently and significantly greater than the forage yield of the unfertilized bahiagrass. Although the average forage yield of bahiagrass in 2002 ($2.3 \pm 0.7\,\text{mg ha}^{-1}$) was slightly lower than in 2000 ($3.5 \pm 1.2\,\text{mg ha}^{-1}$), yield differences in 2002 between the control ($1.2 \pm 0.2\,\text{mg ha}^{-1}$) and treated plots ($2.3 \pm 0.7$ to $3.3 \pm 0.6\,\text{mg ha}^{-1}$) were indicative of a positive carry over effect of applied biosolids.
- Repeated applications of sewage sludge indicate no harmful environmental or plant effects. Excessive build up of plant nutrients (e.g., total N, total P, and trace metals) as expected did not occur in beef cattle pastures that repeatedly received sewage sludge materials while favoring long-term increased forage yield of bahiagrass.

- Biosolids if processed and applied according to the USEPA rules have the potential to boost and maintain production because they are inexpensive, environmentally safe, and could act as liming and organic matter amendment as well.
- Successive land application of biosolids for at least three years followed by no sewage sludge application for at least two years would be a good practice economically and environmentally because it will boost and/or maintain sustainable forage productivity and at the same time minimize probable accumulation of nutrients, especially heavy metals.

4 Research Direction and Outlook

Forage production offers an alternative to waste management since nutrients in the waste are recycled into crops that are not directly consumed by humans. Establishment of an excellent, uniform stand of bahiagrass in a little time period is essential and economical. Failure to obtain an early good stand means the loss of not only the initial investment costs, but production and its cash value. Forage production often requires significant inputs of lime, nitrogen fertilizer, and less frequently of phosphorus and potassium fertilizers. Bahiagrass is a good general-use pasture grass that can tolerate a wide range of soil conditions and close grazing, and withstands low fertilizer input (Burson and Watson 1995; Kidder 1995, 2001; Kincheloe et al. 1987). It has the ability to produce moderate yields on soils of very low fertility and easier to manage than other improved pasture grasses (Chambliss 1999).

Land application of biosolids for at least three (3) years followed by no biosolids application for at least two (2) years may be a good practice economically and environmentally because it will enhance and/or maintain sustainable forage productivity and at the same time minimize probable accumulation of nutrients to a certain degree, especially heavy metals. However, in the longer term, consecutive applications of biosolids may result in build up of toxic metals in soils. The possibilities for environmentally and economically sound application strategies are encouraging, but more and additional research is required to find most favorable timing and rates that minimizes negative impacts on the environment. For proper utilization of biosolids, knowledge of the biosolids composition and the crop receiving it are crucial, so that satisfactory types and rates are applied in an environmentally safe manner.

Land application of lake-dredged materials and biosolids may not only provide substantial benefits that will enhance the environment, community, and society in south Florida, but also in other parts of the world especially those areas having tropical and subtropical climate with forage-based beef cattle pastures. As such, the agricultural or livestock industry could utilize biosolids and lake-dredged materials to produce forages. Lake-dredge materials and biosolids should be regarded as valuable resources, as part of the ecological system.

Is the use of biosolids and lake-dredged materials in an agricultural setting protected and realistic? Is the use of these materials safe and sound in all climates, in all soils and is it sustainable over the long term? Since the benefits of forages in cropping system are sometimes understated and do not manifest themselves immediately, the use of biosolids and lake-dredged materials as alternative sources of nutrients in forage-based pastures research needs should be conducted over the longer term. Perhaps, the greatest research needs is to maintain long-term, field-based forage research programs, and establish new programs that address new questions.

There is still much to be learned whether the environmental and ecological objectives are satisfied over the longer term. Additional research on disposal options of lake-dredged materials and biosolids are much needed to supply information on criteria testing and evaluation of the physical and chemical impacts of biosolids and lake-dredged materials at disposal sites. The first necessary step in evaluating the sludge and lake-dredged materials application alternatives is to determine whether these materials are suitable for use on agricultural land. Therefore, the biosolids and lake-dredged materials should be analyzed carefully and thoroughly to evaluate their quality. The parameters most commonly measured would include percentage total solids, total nitrogen, ammonium and nitrate nitrogen, total P and K and total cadmium, copper, nickel, lead, and zinc, chromium and mercury.

Despite of the relative success of recycling biosolids and lake-dredged materials as reported in this paper, one of essential areas of future research would be on the effect of recycling of biosolids and lake-dredged materials on the cost-effective performance of pasture-based agriculture, and the market awareness of animal products produced from land receiving biosolids and lake-dredged materials.

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Symbiotic Nitrogen Fixation in Legume Nodules: Process and Signaling: A Review

Neera Garg and Geetanjali

Abstract The Green Revolution was accompanied by a huge increase in the application of fertilizers, particularly nitrogen. Recent studies indicate that a sizeable proportion of the human population depends on synthetic nitrogen (N) fertilizers to provide the 53 million t N that is harvested globally in food crops each year. Nitrogen fertilizers affect the balance of the global nitrogen cycle, pollute groundwater and increase atmospheric nitrous oxide (N_2O), a potent "greenhouse" gas. The production of nitrogen fertilizer by industrial nitrogen fixation not only depletes our finite reserves of fossil fuels, but also generates large quantities of carbon dioxide, contributing to global warming. The process of biological nitrogen fixation offers an economically attractive and ecologically sound means of reducing external nitrogen input and improving the quality and quantity of internal resources. Recent studies show that in irrigated cropping systems, legume N is generally less susceptible to loss processes than fertilizers. Biological nitrogen fixation (BNF) has provided a number of useful paradigms for both basic and applied research. Establishing a fully functional symbiosis requires a successful completion of numerous steps that lead from recognition signals exchanged between the plant and bacteria to the differentiation and operation of root nodules, the plant organ in which nitrogen fixation takes place. The initial sensing of the two organisms by each other starts with the release of root exudates by the plant that include flavonoids and nutrients such as organic acids and amino acids. Flavonoids secreted by the host plant into the rhizosphere function as inducers of the rhizobial *nod* genes. *nod* gene induction results in the secretion of lipochitin oligosaccharides that are thought to bind to specific plant receptor kinases that contain LysM motifs, such as NFR1 and NFR5 in *Lotus japonicus* and LYK3 and LTK4 in *Medicago truncatula*. This initiates a complex signaling pathway involving calcium spiking in root hairs. The result is that the root hairs curl and trap the rhizobia, which then enter the root hair through tubular structures known as infection threads that are formed by the plant. The infection threads then grow into the developed nodule tissue. Ultimately, the invading bacteria are taken into the plant cell by a type of endocytosis in which they are surrounded by a plant-derived peribacteroid membrane (PBM). The resulting symbiosomes fill the plant cell cytoplasm and as plant and bacterial metabolism develops, the bacteria become mature bacteroids able to convert atmospheric nitrogen to ammonium. To increase knowledge of this system of particular importance in sustainable agriculture, major emphasis should be laid on the basic research. More work is needed on the genes responsible in rhizobia and legumes, the structural chemical bases of rhizobia/legume communication, and signal transduction pathways responsible for the finely orchestrated induction of the symbiosis-specific genes involved in nodule development and nitrogen fixation. This review unfolds the various events involved in the progression of symbiosis.

Keywords Flavonoids • Nitrogen fixation • Nod factors • *Rhizobium* • Sustainable agriculture

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1 Introduction

A key component to the success of the "green revolution" in improving the yields of crops like rice and wheat was the increased input of synthetic fertilizers. Nitrogen fertilizers today are an indispensable part of modern agricultural practices and rank first among the external inputs to maximize output in agriculture. However, N fertilizer contributes substantially to environmental pollution. The continued and unabated use of N fertilizers would accelerate the depletion of stocks of nonrenewable energy resources used in fertilizer production. The removal of large quantities of crop produce from the land depletes soil of its native N reserves (Peoples and Crasswell 1992). There are vast areas of the developing world where N fertilizers are neither available nor affordable due to weak infrastructure, poor transportation, and high cost. Even in wealthier nations, economic and environmental considerations dictate that biological alternatives, which can augment and in some cases replace, N fertilizers must be sought (Bohlool et al. 1992). Thus, emphasis should be laid in developing new production methods that are sustainable both agronomically and economically. Biological nitrogen fixation (BNF) can act as a renewable and environmentally sustainable source of N and can complement or replace fertilizer inputs (Peoples et al. 1995). Its use can mitigate the need for fertilizer nitrogen, with concomitant benefits accruing in terms of effects on the global nitrogen cycle, global warming, and ground- and surface-water contamination. Intercropping legumes and other species capable of symbiotic N_2 fixation offer an economically attractive and ecologically sound means of reducing external inputs and improving the quality and quantity of internal resources. Nitrogen from this source (biologically fixed N_2) is used directly by the plant, and so is less susceptible to volatilization, denitrification and leaching. BNF is a kind of beneficial plant–microbe interaction that provides a restricted range of plants with the often-limiting macronutrient-nitrogen. This type of symbiosis evolved some 60 million years ago and is an archetypal example of a monospecific association (Bonfante 2003; Hirsch 2004).

Biological nitrogen fixation is done by both free-living organisms (e.g., *Azotobacter, Beijerinckia, Clostridium, Bacillus, Klebsiella, Chromatium, Rhodospirillum*) and those that form symbiotic associations with other organisms. In agricultural settings, perhaps 80% of this biologically fixed N_2 comes from symbiosis involving leguminous plants and α-proteobacteria, order Rhizobiales, family Rhizobiaceae, including species of *Rhizobium, Bradyrhizobium, Sinorhizobium, Azorhizobium* and *Mesorhizobium* (Willems and Collins 1993; Farrand et al. 2003). Recently, it has been shown that β-proteobacteria may also participate in this kind of relationship (Sawada et al. 2003). The legumes are a diverse and important family of angiosperms. With more than 650 genera and 18,000 species, legumes are the third largest family of higher plants and are second only to grasses in agricultural importance (Doyle 2001). Legumes provide largest single source of vegetable protein in human diets and livestock feed. Legumes are divided into three subfamilies, Mimosoideae, Caesalpinoideae, and Papilionoideae. Most cultivated legumes are found within the Papilionoideae, the subfamily with largest total number of genera. Of the three subfamilies of legumes, over 90% of the Papilionoideae and Mimosoideae, nodulate, whereas less than 30% nodulate in the more ancient Caesalpinioideae (Doyle 2001). Worldwide, legumes are grown on approximately 250 Mha and they fix about 90 Tg of N_2 per year (Kinzig and Socolow 1994). Legume productivity is theoretically independent of soil nitrogen status and they provide important grain and forage crops in both temperate and tropical zones (Cooper 2004).

2 Invading the Plant

2.1 Detection of and Response to Host-released Signals by Members of Rhizobiaceae

Bacterial chemotaxis towards plant root exudates is a crucial event in legume-*Rhizobium* interactions. Plants exude high levels of nutrients, and many of these act as chemoattractants for the bacteria. Different strains have been described to be positively chemotactic to sugars, amino acids, various dicarboxylic acids such as succinate, malate, fumarate, and aromatic compounds (Bergman et al. 1991; Robinson and Bauer 1993).

Binding of rhizobia to plant surfaces is essential for establishing a long-term interaction of the bacteria with their hosts. Plant lectins (proteins that possess at least one non catalytic domain that binds reversibly to mono- or oligosaccharides) could serve as receptors for bacterial exopolysaccharides (EPS). Lectins might mediate specificity in the *Rhizobium* – legume symbiosis (Ridge et al. 1998; Heeb and Haas 2001; Rudiger and Gabius 2001). Binding of rhizobia to plant surfaces is thought to take place in two steps. The first is a rather weak and reversible binding step that may involve a variety of bacterial polysaccharides. The products of the *ndvA* and *ndvB* genes in *Sinorhizobium meliloti* are involved in the synthesis of a cyclic glucan (Stanfield et al. 1988; Ielpi et al. 1990), which could act as an adhesin via gelling interactions with host polysaccharides or could interact with plant lectins (Heeb and Haas 2001). The second binding step requires the synthesis of bacterial cellulose, which causes a tight, irreversible binding and formation of bacterial aggregates on the host surface (Robertson et al. 1988).

2.2 Host Detection During Nodule Formation

Nitrogen fixation can only occur when the plants are in the symbiotic state and the Rhizobia invade the root or stem cortex (Cooper 2004). The progression to the symbiotic state by two initially independent, free-living partners is governed by reciprocal signal generation and perception, which has been described as "molecular dialogue" (De'narie et al. 1993). This leads to a gradual and coordinated differentiation and adjustment of physiology and metabolism in both partners (Schultze and Kondorosi 1998; Bladergroen and Spaink 1998; Broughton et al. 2000; Perret et al. 2000; Spaink 2000).

2.3 Early Signals from Legume to Rhizobia

Detection of the signals leads to altered patterns of gene expression that culminate in specific and adaptive changes in bacterial physiology that are required for these associations (Brencic and Winans 2005). Symbiotic interaction is initiated by micromolar or nanomolar concentrations of flavonoids or isoflavonoids in legume root or seed exudates. These compounds may initially assist rhizosphere colonization by acting as chemoattractants or less likely, as growth enhancers for rhizobia (Schultze and Kondorosi 1998; Copper 2004).

2.4 Structure and Function of Flavonoids

Flavonoids are secondary metabolic products of the central phenylpropanoid pathway and the acetate–malonate pathway of plants. Thus, all falvonoids are derivatives of phenylalanine from the shikimic acid pathway and malonyl CoA from the acetyl CoA carboxylase reaction. They are polycyclic aromatic compounds, released by plants into the rhizosphere (Barbour et al. 1991; Kape et al. 1991). These are 2-phenyl-1,4-benzopyrone derivatives. Their structure is defined by two aromatic rings, A and B, and a heterocyclic pyran or pyrone ring the C ring. Specific modifications of this basic structure produce different classes of flavonoids including chalcones, flavanones, flavones, flavonols, isoflavonoids, coumestans, and anthocyanidines (Harborne and Williams 2000, 2001). So far more than 4,000 different flavonoids have been identified in vascular plants (Perret et al. 2000). Not all of them, however, are active as inducers of the nodulation genes. Comparison of structure of different *nod*-inducing flavonoids revealed that hydroxylation at the C-7, and C-4 positions are important for *nod*-inducing activity (Cunningham et al. 1991). Host legumes are thought to be discriminated from nonhosts partly on the basis of the specific flavonoids that they release (Hirsch et al. 2001; Parniske and Downie 2003).

Flavonoids acting as primary signals to rhizobia have been found in legume seed coat and root exudates (Hartwig and Phillips 1991). Flavonoids are released in their greatest amounts near root tips (Hartwig et al. 1990; Graham 1991), and optimal concentrations occur near the emerging root hair zone, which is most favorable site for rhizobium infection (Zuanazzi et al. 1998). Their main role in the initiation of a rhizobial symbiosis is an interaction with the constitutively expressed *nodD* gene product(s) of the microsymbiont to form a protein–phenolic complex – a transcriptional regulator of other rhizobial nodulation (*nod*) genes that are responsible for synthesis of reciprocal signals to the plant root. The combination of Nod D proteins with appropriate plant flavonoids triggers the production of highly specific reverse signal molecules by rhizobia – the chitolipooligosaccharide (CLOS) Nod factors – by means of the transcriptional activation of common and host specific *nod* genes (Cooper 2004).

In addition to the flavonoids, several nonflavonoid *nod* inducers have been identified. Stachydrin (*N*-methylproline methylbetaine) and trigonelline (nictotinic acid *N*-methylbetaine) were identified from exudates of alfalfa seedlings as inducers of *nod* genes in *S. meliloti* (Phillips et al. 1992). These molecules are quaternary ammonium compounds collectively known as betaines (Chen and Murata 2002). Both trigonelline and stachydrine have been found in seeds, roots, and root exudates of various legumes (Rozan et al. 2000, 2001). The concentrations (in low millimolar range) of betaines required for *nod* induction are much higher than those of flavonoids (low micromolar range (Phillips et al. 1994). Two aldonic acids (tetronic acid and erytronic acid), as well as some simple phenolics (vanillin, coniferyl alcohol, chlorogenic acid, and ferulic acid) were also identified as natural inducers of *nod* genes in certain rhizobial species (Kape et al. 1991; Gagnon and Ibrahim 1998). The concentrations required for their activity are similar to those of betaines (Kape et al. 1991).

2.5 Reverse Signals from Rhizobia to Legume Roots – the Chitolipooligosaccharide Nod Factors

The key event in nodule formation is the synthesis and release by the bacteria of small molecules that are detected by the plant and that trigger the formation of the nodule (Long 1996; Downie 1998; Hadri and Bisseling 1998; Cullimore et al. 2001; Geurts and Bisseling 2002). These molecules are called Nod factors. Detection of Nod factors by a legume host induces major developmental changes in the plant, which are required for entry of the rhizobia into the host (Downie and Walker 1999; Geurts and Bisseling 2002). The tip of a root hair, to which rhizobia are bound, curls back on itself, trapping the bacteria within a pocket, from which they are taken up into a plant made intracellular infection thread. Nod factors also induce cell division and gene expression in the root cortex and pericycle, where they initiate development of the nodule (Truchet et al. 1991; Horvath et al. 1993; Spaink 1996; Cullimore et al. 2001).

2.6 Structure and Function of Nod Factors

The structure of Nod factors was first determined in 1990 for *Sinorhizobium meliloti* (Lerouge et al. 1990). Nod factors usually comprise four or five β-1-4-linked *N*-acetyl glucosamine residues with a long acyl chain that is attached to the terminal glucosamine. Many Nod factors from different rhizobia species have been identified and shown to differ with regard to the number of glucosamine residues, the length and saturation of acylchain and the nature of modifications on this basic backbone (Denarie et al. 1996; Downie 1998). These host specific modifications include the addition of sulphuryl, methyl, carbamoyl, acetyl, fucosyl, arabinosyl and other groups to different positions on the backbone, as well as differences in the structure of the acyl chain. These variations define much of the species specificity that is observed in the symbiosis (Perret et al. 2000). Proteins encoded by bacterial genes *nodA*, *nodB*, and *nodC* are involved in the biosynthesis of the basic LCO structure (John et al. 1993; Geremia et al. 1994; Rohrig et al. 1994; Brencic and Winans 2005). Many different *nod* genes are involved in modifying the basic LCO structure specifically for different rhizobia. For instance, *nodH* encodes a sulfotransferase that transfers a sulfate group to the reducing end of Nod factors of *R. meliloti* (Roche et al. 1991; Bourdineaud et al. 1995; Ehrhardt et al. 1995).

2.7 Transcriptional Regulators of Nod Genes

Plant-released flavonoids are detected by rhizobia through a variety of Nod D proteins (Schell 1993). The Nod D proteins of several species are believed to be membrane associated (Schlaman et al. 1989). Many species of rhizobia have more than one copy of the *nodD* gene, and the properties of different *nodD* genes vary within the same strain as well as from one *Rhizobium* species to another. Some strains possess two to five copies of *nodD* (Gottfert et al. 1992; van Rhijn et al. 1993; Fellay et al. 1995; Schlaman et al. 1998), and may in addition possess one or two copies of another LySR-type regulator gene called *syrM* (symbiotic regulator) (Mulligan and Long 1989; Michiels et al. 1993, 1995; Swanson et al. 1993; Hanin et al. 1998). *SyrM* is a Nod D homolog and also acts as an activator of *nod* genes (Brencic and Winans 2005).

Chitolipooligosaccharide Nod factors are vital signals for rhizobial entry into legume roots (Relic et al. 1994) and the success or otherwise of the infection process is in large part determined by their structural features. Application of nanomolar or femtomolar concentrations of purified rhizobial Nod factor to the roots of an appropriate legume host elicits responses like deformation of root hairs (Lerouge et al. 1990) accompanied by root hair plasma membrane depolarization (Ehrhardt et al. 1996); rapid increases then oscillations in intracellular free calcium in root hairs, referred to as calcium spiking (Ehrhardt et al. 1996; Gehring et al. 1997; Wais et al. 2000, 2002; Walker et al. 2000); change in the root hair cytoskeleton (Cárdenas et al. 1998; Timmers et al. 1998); preinfection thread formation in deformed root hairs (van Brussel et al. 1992); and localized cortical cell division at the sites of root nodule primordia (Spaink 1992; Spaink et al. 1993; Lopez-Lara et al. 1995). Nod factors alone can induce some of the plant genes (nodulins) that are expressed in the preinfection, infection, nodule development, and nodule function phases of symbiotic interaction, some examples of the more rapidly expressed genes being *enod12* (Scheres et al. 1990), *enod40* (Kouchi and Hata 1993), *rip1* (Cook et al. 1995) and *dd23b* (Crockard et al. 2002). Nod factors also control the number of nodules formed on a root system by inducing an autoregulation response in the host plant (van Brussel et al. 2002). A symbiosis receptor-like kinase (SYMRK) gene in *Lotus* (Stracke et al. 2002) and a nodulation receptor kinase (NORK) in *Medicago* (Endre et al. 2002) that is required for early signal transduction in both rhizobial and mycorrhizal symbioses have recently been discovered. More recently, two genes that encode LysM receptor-like kinases that function upstream of SYMRK and could be direct receptors for rhizobial Nod factors were discovered in *Lotus japonicus* (Madsen et al. 2003; Radutoiu et al. 2003).

2.8 Nod Factor (NF) Signaling in Root Epidermis

Two genes in *L. japonicus*, *LjNFR1* and *LjNFR5*, have been predicted to function in Nod-factor perception. Both encode receptor-like kinases with LysM domains in the predicted extracellular domain (Madsen et al. 2003; Radutoiu et al. 2003). In *M. truncatula,* two additional receptor-like kinase genes (*MtLYK3* and *MtLYK4*) that encode LysM domains have been identified, and they are thought to be orthologous to *PsSYM2A* (Limpens et al. 2003); both of these show strong similarity to *LjNFR1*. The LysM domains are the binding sites for peptidoglycan and binding seems to be the N-acetyl-glucosamine-N-acetylmureine backbone (Steen et al. 2003). LysM domain is the peptidoglycan-binding motif found in many bacterial peptidoglycan-binding proteins (Stacey et al. 2006). In addition, LysM domains are present in two proteins that are known to bind chitin (Ponting et al. 1999), which is chemically identical to the Nod factor N-acetylgluscosamine backbone. Furthermore, chitin oligomers can induce Ca^{2+} spiking in legumes (Walker et al. 2000; Oldroyd et al. 2001). The analogy to Nod-factor binding is striking and the LysM-receptor-like kinases seem excellent candidates for Nod-factor receptors (Oldroyd and Downie 2004).

Several other components that are essential for most of the early steps in NF signaling have been identified, and these are active directly downstream of the NF receptors. In *M. truncatula,* these genes are named *DOESN'T MAKE INFECTIONS1 (DMI1), DMI2 and DMI3*, and *NODULATION SIGNALING PATHWAY1 (NSP1)* and *NSP2* (Catoira et al. 2000; Oldroyd and Long 2003). All of these genes are essential for the NF-induced changes in gene expression showing their importance in NF signaling (Mitra et al. 2004). *MtDMI1* and *MtDMI2* are positioned upstream of NF-induced Ca^{2+} spiking, whereas, *MtDMI3* and the *MtNSPs* are active downstream of Ca^{2+} spiking (Wais et al. 2000; Oldroyd and Long 2003). The oscillations of Ca^{2+} concentration known as Ca^{2+} spiking occurs in the perinuclear region of epidermal cells and induced within a few minutes of exposure to NFs (Ehrhardt et al. 1996). Pharmacological studies show that Ca^{2+} spiking is essential for NF-induced gene expression as visualized with the marker gene *EARLY NODULIN 11* (*ENOD11*) (Engstrom et al. 2002; Charron et al. 2004; Geurts et al. 2005).

Rhizobial Nod factors induce in their legume hosts the expression of many genes and set in motion developmental processes leading to root nodule formation. Smit et al. (2005) have reported the identification of the *Medicago* GRAS-type protein Nodulation Signaling Pathway 1 (NSP1), which is essential for all known Nod factor induced changes in gene expression. Rhizobial Nod factors induce in their legume hosts the expression of many genes and set in motion developmental processes leading to root nodule formation. Smit et al. (2005) have reported the identification of the *Medicago* GARS-type protein Nodulation signaling pathway 1 (NSP1), which is essential for all known Nod factor-induced changes in gene expression. Kaló et al. (2005) have shown that *nodulation signaling pathway* genes (NAP2) from *Medicago truncatula* encodes a GARS protein essential for Nod-factor signaling. NSP2 functions downstream of Nod-factor-induced calcium spiking and a calcium/calmodulin-dependent protein kinase. Their work has provided evidence that a protein transduces calcium signal in plants and provides a possible regulator of Nod-factor-inducible gene expression.

2.9 Ion Fluctuations

A very early response induced by Nod factors in root hairs is the transient activation of ionic fluxes (Crespi and Galvez 2000). A number of studies using calcium dyes and ion-selective electrodes have indicated considerable Nod factor-induced calcium changes in the root hair cells (Cardenas et al. 2000). Nod factors, when added to legume roots, induce two phases of ionic changes that can be observed in root-hair cells. One is a rapid influx of Ca^{2+} (Ca^{2+} flux), which is immediately followed by membrane depolarization. Some minutes later, oscillations in the cytosolic Ca^{2+} concentration (calcium spiking) are induced (Oldroyd and Downie 2004).

2.10 Calcium Flux and Spiking

Felle et al. (1998, 1999) used ion-specific microelectrodes and observed a rapid Nod-factor-induced Ca^{2+} influx followed by the efflux of Cl^-, then K^+ and an alkalinization of the cytoplasm (Felle et al. 1996), within one minute of adding Nod factor. Membrane depolarization was induced over a range of Nod-factor concentrations (10^{-10}–10^{-7} M) with half maximal induction at 10^{-9} M and no response at 10^{-11} M (Felle et al. 1998, 1999, 2000). The Ca^{2+} influx might trigger the activation of an anion channel that allows Cl^- efflux and K^+ might serve as a charge balance, which eventually stops the depolarization and initiates repolarization (Felle et al. 1998). Increased Ca^{2+} concentrations at the tip of growing root hairs establishes a gradient of Ca^{2+} down the root hair. Adding Nod factor accentuates this gradient (Cardenas et al. 1999) and induces a wave of Ca^{2+} that migrates down the shaft of the root-hair cell towards the nucleus (Shaw and Long 2003). Isolated regions of high Ca^{2+} concentrations are observed in a diversity of legumes (*Pisum sativum*, *Medicago sativum, Medicago truncatula, Phaseolus vulgaris*) (Ehrhardt et al. 1996; Cardenas et al. 1999; Walker et al. 2000; Shaw and Long 2003). Together, the data can be incorporated into a model in which Nod factors activate a Ca^{2+} flux at the tip of root hair cells, with at least some of this Ca^{2+} originating from the external medium (Oldroyd and Downie 2004).

Oscillations in cytosolic Ca^{2+} (Ca^{2+} spiking) have been observed in legume root-hair cells following the addition of Nod factor (Ehrhardt et al. 1996; Cardenas et al. 1999; Wais et al. 2000; Walker et al. 2000; Harris et al. 2003). Nod-factor-induced Ca^{2+} spiking occurs with a lag of approximately 10 min following the application of either Nod factor or rhizobia (Wais et al. 2002). The Ca^{2+} spikes are predominantly restricted to the region of the cytosol that is associated with the nucleus (Ehrhardt et al. 1996; Walker et al. 2000). Individual Ca^{2+} spikes have a very rapid initial Ca^{2+} increase, followed by a more gradual decline.

2.11 Rhizobial-induced Gene Expression in Plants for Nodule Organogenesis

During the nodule development, many plant genes, the so-called nodulin genes (van Kammen 1984), need to be coordinately induced in the different steps of the process. Rhizobial induced genes fall into two major classes: the early nodulins (*ENOD*) and the late nodulins (Nap and Bisseling 1990). The late nodulins represent genes that are induced many days after the application of bacteria, during a period of nodule maturation. Early nodulins are induced within the first few days of the infection, a period prior to and during the initiation of nodule primordia. *ENOD12, ENOD40,* *RIP1* are all activated within a few hours of application of Nod factor or rhizobia (Scheres et al. 1990; Pichon et al. 1992; Cook et al. 1995; Minami et al. 1996). *ENOD12* and *RIP1* are initially activated in the epidermis of the root in a zone where the root hairs are most responsive to the bacteria (Pichon et al. 1992; Cook et al. 1995). *ENOD40* genes code for RNAs (around 700 base pairs) that contain only short ORFs in their sequences (10–37 amino acids) (Crespi et al. 1994). *ENOD40* expression, on the other hand, is associated with the nodule primordium and other mitotically active cells throughout the plant, suggesting a role for *ENOD40* in a plant meristematic program (Kouchi and Hata 1993; Yang et al. 1993; Asad et al. 1994; Crespi et al. 1994). The fact that *ENOD12* and *RIP1* are induced rapidly upon application of low concentrations of Nod factor suggests that these genes are candidates for activation directly from the Nod factor signal transduction pathway. However, *ENOD12* and *RIP1* have nonsymbiotic expression, including root meristematic expression of *ENOD12*, suggesting additional nonsymbiotic regulation (Cook et al. 1995; Bauer et al. 1996). *ENOD40*, however, is activated by high concentrations of Nod factor that are sufficient to induce cortical cell division (Hirsch et al. 1989; Cooper and Long 1994; Hirsch and Fang 1994). Apart from these nodulin genes, other related to nitrogen fixation and assimilation have been detected, such as sucrose synthase, GS, GOGAT, PEPC, carbonic anhydrase, and aspartate aminotransferase (Vance and Gantt 1992; Shi et al. 1997). These latter genes are mainly expressed in the symbiotic zone and induced late in nodule development except for carbonic anhydrase whose transcripts accumulate specifically in the inner cortical cells (Coba de la Pena et al. 1997), the same cells, involved in oxygen permeability.

3 Infection and Nodule Organogenesis

3.1 Root Hair Curling

The symbiotic interaction starts when the bacteria colonize the root surface and induce curling of the root hair tips (Schultze et al. 1994; Long 1996). Root hair curling is thought to be caused by a gradual and constant reorientation of the growth direction of the root hair (Emons and Mulder 2000). The bacteria become entrapped within the pocket of the curl, where the plant cell wall is locally degraded, the cell membrane invaginated and new material deposited by both plant and bacteria (Limpens and Bisseling 2003). Simultaneously, pericycle and cortical cells are activated for division, usually in front of a xylem pole, close to the infection point. The cortical cells actively divide to form the nodule primordium wherein large amounts of amyloplasts accumulate (Crespi and Galvez 2000). Root-hair curling only occurs in a few hairs in the root zone that is susceptible to rhizobia, whereas most root hairs within this zone show altered behavior, resulting in so called root hair deformations (Heidstra et al. 1994). These deformations are the result of isotopic growth, by a reinitiation of tip growth in an altered growth direction (De Ruijter et al. 1998). Although many bacteria can be attached to a single hair, it is probable that one or only a few bacteria induce the curling (Gage and Margolin 2000; Gage 2002). The growth direction of the hair needs to be constantly redirected towards the bacteria in order for them to become entrapped.

At the root surface, rhizobia caught in the root hairs, locally degrade the plant cell walls, and infection develops that grows within the root hair. Before the infection thread reaches the base of the root hair cell, the root cortical cells are induced to dedifferentiate, activating their cell – cycle and causing them to divide to form the nodule primordium. In addition to the cortical cells, pericycle cells are also activated and undergo some cell divisions (Stacey et al. 2006). The infection thread, (only 1–5%) (Crespi and Galvez 2000), traverses the outer cell layers to reach the nodule primordium. The infection threads penetrate and ramify into primordium cells traversing their walls, they then enter cortical cells, initiating a differentiation process that is heralded by cell enlargement (Crespi and Galvez 2000). Within the infection thread, the rhizobia multiply, but remain confined by the plant cell wall. As the primordia develops into a nodule, bacteria are released from the tip of the infection thread by endocytosis and differentiate into bacteroids surrounded by peribacteroid membrane (Hirsch 1992).

3.2 Bacteroid Formation

The release of bacteria into plant cells is initiated by the formation of an infection droplet. Infection droplets can form at the tip of short intracellular infection threads (Cermola et al. 2000) or, more usually at positions in the infection threads where the cell wall gets disrupted and rhizobial cells come into direct contact with the host cell plasma membrane (Brewin 2004). The plant cell membrane then outgrows and bacteria are taken up into the plant cell lumen by endocytosis. The newly formed structure, which consists of bacteria that are differentiating in bacteroids enclosed in a plant cell membrane, is called a symbiosome. Differentiated bacteroids present an important physiologic adaptation with respect to their enzymatic capacity, notably the production of nitrogenase (Crespi and Galvez 2000). In determinate nodules, individual symbiosomes fuse and/or bacteroids further divide within the symbiosome, which results in symbiosomes that typically contain several bacteroids. However, in indeterminate nodules, individual symbiosomes further divide, together with the bacteroid, which mostly result in single bacteroids within a symbiosome (Prell and Poole 2006). The bacterial carbon storage compound polyhydroxybutyrate (PHB) accumulates in rhizobial cells in infection threads (Lodwig et al. 2003, 2005). This suggests that a plentiful carbon supply is available for bacteria during growth in the infection threads.

3.3 Symbiosome

The symbiosome is critical for biological nitrogen fixation, (Catalano et al. 2004). While being released from the infection thread into the plant cytoplasm, a plasmalemma-derived symbiosome membrane forms an uninterrupted envelope around each bacterium and delineates the symbiosome space between the symbiosome membrane and the bacterial outer membrane. The symbiosome membrane, bacteroid, and symbiosome space form the basis of the symbiosome (Roth et al. 1988). Specifically, the symbiosome membrane serves both as a physical interface and as a mediator of metabolite exchange between the symbionts, both functions being essential for nodule formation. In mature root nodule cells, the symbiosome membrane represents a mixture of proteins that resemble most closely the protein constituents of the plasma membrane and the tonoplast. Proteins involved in transport, energy, metabolic processes, nodule formation and function, signaling, pathogen response, and protein destination have been identified from the symbiosome membrane. Also, channels and bacterial proteins have been identified for this membrane (Udvardi and Day 1997; Lodwig et al. 2003). The symbiosome membrane must proliferate in enlarged infected root nodule cells to accommodate bacteroid growth and division. During this process of symbiosome membrane proliferation, a large amount of lipid and protein synthesis is required since infected root nodule cells typically consist of hundreds of bacteroids, each enclosed by their own symbiosome membrane. Symbiosome membrane biogenesis and demand in infected plant cells is 30 times greater than that required for plasma membrane synthesis (Catalano et al. 2004).

4 Conclusions and Future Prospects

Legumes form novel plant organs, the “root nodules,” in response to lipooligosaccharide signals, “Nod factors,” delivered by specific soil bacteria called rhizobia. The adoption of model legumes for genetic analysis of nodulation has led to major advances in our understanding of initial steps in Nod signal recognition and subsequent signaling, however, a complete picture of the genetic interplay involved in rhizobial symbiosis is yet to appear. There are still a number of genes, with a role in Nod-factor signal transduction that remain to be cloned. Detangling of this system (legume-*Rhizobium* symbiosis) would help in better understanding of the molecular mechanisms governing nodule differentiation. With a complete understanding of early signaling pathways, quest like which genes are responsible for nodule formation and which genes are missing from crop plants such as wheat and rice that do not form endosymbiosis with nitrogen-fixing bacteria will be answered.

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Factors Responsible for Nitrate Accumulation: A Review

Anjana, Shahid Umar, and Muhammad Iqbal

Abstract Leafy vegetables occupy a very important place in the human diet, but unfortunately, constitute a group of foods, which contributes maximally to nitrate consumption by living beings. Under excessive application of nitrogen fertilizer, these vegetables can accumulate high levels of nitrate and, on being consumed by living beings, pose serious health hazards. Therefore, efforts are warranted to minimize the accumulation of nitrate in leafy vegetables and its ingestion by human beings. This review focuses on (a) the contribution of vegetables towards dietary nitrate intake by humans, (b) the nutritional, environmental and physiological factors affecting nitrate accumulation in plants, (c) the harmful and beneficial effects of nitrate on human health, and (c) the strategies that may be followed for minimizing the nitrate content in plants and its subsequent consumption by human beings. The risk to human health due to nitrate consumption may be minimized by harvesting vegetables in the noon, removal of organs rich in nitrate content and cooking of vegetables with water having a low nitrate content. The European Commission (EC) Regulation No. 1822/2005 needs to be followed in order to ensure safe levels of nitrate in plants for human consumption.

Keywords Human health • Leafy vegetables • Nitrate reductase • Nitrate toxicity • Spinach

1 Introduction

The anthropogenic activities aimed at enhancing food production may facilitate accumulation of undesirable substances in plants and affect the quality of the soil and water resources adversely. Excessive amounts of nitrogenous fertilizers are applied to crops considering that it is a reasonable insurance against yield losses and their economic consequences. However, when input of nitrogen exceeds the demand, plants are no longer able to absorb it, and nitrogen then builds up in the soil, mostly as nitrates (Nosengo, 2003). This causes imbalance of nutrients in the soil and increases the nitrate level in groundwater supplies (NAAS, 2005) which influences the nitrate content of plants (Dapoigny et al., 2000; Vieira et al., 1998), especially the leafy vegetables. Nitrate has long been one of the highly emotive anions, always being talked about, whether with pride or with horror (Hill, 1999). There are conflicting evidences regarding the potential long-term health risks associated with nitrate levels encountered in the human diet. That reduction in dietary nitrate is a desirable preventive measure (Santamaria, 2006), stands undisputed.

Vegetables are the major source of the daily intake of nitrate by human beings, supplying about 72–94% of the total intake (Dich et al., 1996). Therefore, European Union prescribed, almost a decade ago, the maximum limits for nitrate in lettuce and spinach which became the foundation stone for the subsequent European Commission Regulation (No. 1822/2005). Investigations have indicated that a high nitrate accumulation in plants results in nitrite production which is converted into nitric oxide (NO) which, together with O_2^-, could be rapidly catalyzed by nitrate reductase

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(NR) into peroxynitrite ($ONOO^-$) which is highly toxic to plants (Durner and Klessig, 1999; Lamattina et al., 2003). Therefore, high nitrate accumulation in plants is harmful to human health (Ikemoto et al., 2002; Ishiwata et al., 2002) as well as to plant growth (Reddy and Menary, 1990).

The factors responsible for nitrate accumulation in plants are mainly nutritional, environmental and physiological. Nitrogen fertilization and light intensity have been identified as the major factors that influence the nitrate content in vegetables (Cantliffe, 1973). Diurnal changes in light intensity lead to a diurnal pattern of nitrate accumulation in plants. Many nutrients, such as chloride, calcium, potassium, sulphate and phosphorus, are involved in the nitrate accumulation process in plants. The nitrate content varies in various parts of a plant (Anjana et al., 2006; Santamaria et al., 1999) and with the physiological age of the plant (Anjana et al., 2006; Maynard et al., 1976). A reduction in nitrate content can add value to vegetable products already so popular for their nutritional and therapeutic properties (Santamaria, 2006). Therefore, it is important to adopt appropriate strategies and determine the role of individual physiological factors in the process in order to limit accumulation of nitrate in vegetables, optimize the use of fertilizer nitrogen and reduce the potential degradation of soil and water resources.

This review focuses on contribution of vegetables towards the dietary nitrate intake, factors responsible for nitrate accumulation in plants, and effects of nitrate on human health. It also suggests ways for minimizing the nitrate content in plants and its subsequent consumption by human beings.

2 Vegetables as a Source of Nitrate

Vegetables, an important component of human diet and a major source of nitrate, constitute nearly 72–94% of the average daily human dietary intake (Dich et al., 1996). Unfortunately, leafy vegetables grown under different agro-ecological conditions accumulate nitrate to potentially harmful concentrations. Generally, nitrate-accumulating vegetables belong to the families Brassicaceae (rocket, radish, mustard), Chenopodiaceae (beetroot, Swiss chard, spinach), Amaranthaceae (Amaranthus), Asteraceae (lettuce) and Apiaceae (celery, parsley) (Santamaria, 2006).

Nitrogenous fertilizers, mainly of nitrate variety, are used widely in vegetable agriculture, resulting in accumulation of nitrate in plants, if the rate of its uptake exceeds the rate of its reduction to ammonium (Luo et al., 1993). As suggested by McCall and Willumsen (1998), high rates of nitrate application increase the plant nitrate content without increasing the yield. Therefore, growers who apply excessive fertilizers to ensure that nitrogen is not limiting for plant growth are unlikely to achieve any gain in terms of yield but increase the nitrate content of crops to the levels potentially toxic to humans. Ysart et al. (1999) estimated for the adult human population a total nitrate intake of 93 mg/day, normally through potatoes (33%), green vegetables (21%), other vegetables (15%), beverages (8.5%), meat products (4.2%), fresh fruit (3.5%), dairy (3.1%), milk (2.9%), miscellaneous cereals (2.1%), bread (1.6%) and others (5.1%).

The European Commission's Scientific Committee for Food (SCF) established, in 1995, the Acceptable Daily Intake (ADI) of nitrate ion as 3.65 mg kg^{-1} body weight (equivalent to 219 mg/day for a person weighing 60 kg) (SCF, 1995), whereas the Joint Expert Committee of the Food and Agriculture (JECFA) Organisation of the United Nations/World Health Organisation (WHO) established the Acceptable Daily Intake of nitrate as 0–3.7 mg kg^{-1} body weight (Speijers, 1996). Therefore, assuming a 60-kg body weight, ingestion of only 100 g of fresh vegetables with a nitrate concentration of 2,500 mg kg^{-1} fresh weight exceeds the Acceptable Daily Intake for nitrate by approximately 13%. For a real assessment, however, nitrate content in all other sources as well as their average daily consumption amount must be taken into account. On the other hand, the USA the Environmental Protection Agency (EPA)'s Reference Dose (RfD) for nitrate is 1.6 mg nitrate-N kg^{-1} body weight per day (equivalent to about 7.0 mg NO_3 kg^{-1} body weight per day) (Mensinga et al., 2003). On November 8, 2005, the European Commission adopted EC Regulation No. 1822/2005 (Table 1) and set the harmonized maximum levels for nitrate in lettuce, spinach, baby foods and processed cereal-based foods. The limits vary depending on the season, with higher nitrate levels permitted in winter-grown vegetables.

Table 1 Summary of the maximum levels in European Commission (EC) Regulation No. 1822/2005

Product	Harvest period	Maximum level (mg NO_3/kg)
Fresh spinach[a] (*Spinacia oleracea*)	1 October to 31 March	3,000
	1 April to 31 September	2,500
Preserved, deep-frozen or frozen spinach		2,000
Fresh lettuce (*Lactuca sativa* L.) (protected and open-grown lettuce) excluding "iceberg" type lettuce	1 October to 31 March	
	– Grown under cover	4,500
	– Grown in open air	4,000
	1 April to 30 September	
	– grown under cover	3,500
	– grown in open air	2,500
"Iceberg" type lettuces	Lettuce grown under cover	2,500
	Lettuce grown in the open air	2,000
Baby foods and processed cereal-based foods for infants and young children[b]		200

[a] The maximum levels for fresh spinach do not apply for fresh spinach to be subjected to processing and which is directly transported in bulk from field to processing plant

[b] The maximum levels shall apply to the products as proposed ready for consumption or as reconstituted according to the instructions of the manufacturers

3 Factors Responsible for Nitrate Accumulation

Plant nitrate content is commonly viewed as an outcome of imbalance between its net absorption and assimilation rates (Cardenas-Navarro et al., 1999). The effect of endogenous and exogenous factors seems to be straightforward: on one hand, both uptake and assimilation systems are genetically determined (Ferrario-Mery et al., 1997; Ourry et al., 1997), explaining the variability of plant nitrate content among species and cultivars, whereas on the other hand, nitrate absorption depends on availability of nutrition, and nitrate assimilation depends independently on climate, as it is partly a photosynthetic process in many plants (Ferrario-Mery et al., 1997). Alternatively, plant nitrate content might be either fixed through osmotic potential regulation (McIntyre, 1997) or regulated per se through negative feedback on its transport systems (Cardenas-Navarro et al., 1999).

3.1 Nutritional Factors

Nazaryuk et al. (2002) have studied the role of agricultural chemicals in regulating the nitrate accumulation in plants and shown that the process of nitrate accumulation depends on three major groups of factor: application of mineral fertilizers, treatment with physiologically active substances and sorbents, and the natural and anthropogenic changes in the soil environment. With respect to their impact on nitrate accumulation, these factors may be arranged in the following descending order: fertilizers > physiologically active substances > soil. For exploring a possibility of controlling the nitrate accumulation in plant tissues, it is important to estimate the effect of an exogenous nitrogen supply on the degree of utilization of nitrogen from soil as well as fertilizers (Nazaryuk et al., 2002).

Nitrogen fertilization facilitates accumulation of nitrate in plant tissues as a result of an excess of nitrogen uptake over its reduction. When taken up in excess of immediate requirement, it is stored as free nitrate in the vacuole and can be remobilized subsequently when nitrogen supply is insufficient to meet the demand (van der Leij et al., 1998). Nitrate accumulation in vegetables often depends on the amount and kind of nutrients present in the soil and is closely related to the time of application, and the amount and composition of the fertilizers applied (Zhou et al., 2000). An adequate fertilization programme may ensure sufficient plant growth without any risk of plant nitrate levels going too high (Vieira et al., 1998). Plants accumulate more nitrate as the nitrogen fertilization level increases (Chen et al., 2004; Nazaryuk et al., 2002; Santamaria et al., 1998a, b) whereas limiting the nitrogen availability reduces nitrate content significantly (McCall and Willumsen, 1999). Applying nitrogen once at the beginning of the cropping cycle is effective in controlling nitrate accumulation, since the plant and soil nitrate concentrations decrease as plants reach a marketable size (Vieira et al., 1998). The use of fertilizers based on ammonia or a mixture of nitrate and ammonium can reduce the nitrate content in plants (Inal and Tarakcioglu, 2001; Santamaria et al., 2001); it is worthy of further consideration in soilless growth systems because ammonium reduces the need of adding acids to the nutrient solution to lower its pH (Santamaria and Elia, 1997). Depending on which plant part is to be consumed, appropriate fertilizer can be selected (Zhou et al., 2000), e.g. order of nitrate accumulation in the cole leaves, on application of different nitrogenous fertilizers, is: urea > ammonium carbonate > ammonium nitrate > ammonium sulfate; for nitrate accumulation in petioles, it is: urea > ammonium nitrate > ammonium sulfate > ammonium carbonate.

Vegetables supplied with organic fertilizers have a low nitrate content, compared with the minerally fertilized (Raupp, 1996) or conventionally grown vegetables, and this effect is independent of site conditions. Nitrate accumulation in products is a function of increasing supply of nitrate by fertilization and mineralization of soil organic matter on the one hand, and the reduced availability of assimilates on the other hand. Therefore, the higher the nitrogen availability (mineral fertilizer > liquid manure = slurry > manure > compost) and the lower the assimilation intensity (e.g. by site conditions and season effects), the greater would be the nitrate accumulation. Moreover, the poorly controlled flux of soil nitrogen resulting from active mineralization of organic matter may lead to excessive accumulation of nitrate in plants (Nazaryuk et al., 2002).

To have a quality yield with low nitrate content is possible by manipulating the nitrogen nutrition of plants (Izmailov, 2004). Apart from nitrogen fertilization, a balanced nutrition of plants is particularly important in intensive agricultural production where high fertilizer applications are aimed at improving the yield and quality. Nutrient balance may be a critical factor affecting the nutrient status of plants (Ahmed et al., 2000). Proper application of nitrogenous, phosphate, potassium fertilizers, as also the green and farmyard manures could materially reduce the nitrate accumulation in vegetables (Zhou et al., 2000).

Seginer (2003) developed a dynamic lettuce model, NICOLET, for predicting plant growth and nitrate content. This was valid for the initial stage of plant growth, where composition of plants with respect to reduced-nitrogen and water contents is time-invariant. The model was later modified (Seginer et al., 2004) to extend its validity to a late stage of vegetative growth to accommodate the ontogenetic changes in the reduced-nitrogen and water contents. According to the modified model, prediction of nitrogen uptake for the substantial nitrate pool of lettuce depends on the water content. These models have the potential to improve the estimates of nitrogen uptake, thus leading to a more accurate calculation of fertilizer needs.

Nitrate concentration in plants can also be manipulated by stopping nitrogen supply for some days before crop harvesting (Santamaria et al., 2001). In this way, nitrate will be removed from vacuoles and plants will guard the organic vacuoles needed to make up for the decreased osmotic value. Nitrate concentration can also be reduced by replacing nitrate-N with chloride, sulfate, ammonium or amino acids few days prior to crop harvesting (Inal and Tarakcioglu, 2001; Santamaria et al., 1998b).

Chloride (Cl^-) and nitrate ($NO_3{}^-$) ions play an interchangeable role in osmoregulation, the former are able to prevent excessive nitrate concentration by replacing the latter and may have a positive effect on nitrogen content in plant organs (Dorais et al., 2001). Interaction of chloride uptake with the uptake of nitrate and other nutrients has been reviewed by Xu et al. (2000). Chapagain et al. (2003) have shown that fruit nitrate decreases and chloride increases on increasing the chloride concentration in the nutrient solution; this agrees with the earlier findings on antagonism between the chloride and nitrate uptake in plants, especially in the foliar tissues. As the total nitrogen content of plants did not decrease in response to chloride treatment, Liu and Shelp (1996) believed that chloride absorption did not compete directly with nitrate absorption. Addition of moderate amounts of chloride to the growing medium of broccoli plants decreased their nitrate content by increasing accelerating nitrate reduction. The authors suggested that chloride application could be used as a strategy to decrease nitrate content of vegetables, particularly of those like spinach, lettuce and cabbage which are classified as nitrate accumulators (Maynard et al., 1976). However, inhibition of nitrate uptake by chloride depends on the plant species and the concentrations of both nitrate and chloride in the medium (Cerezo et al., 1997). In root cells, the high-affinity, saturable system for nitrate uptake that operates at small nitrate concentrations (Siddiqi et al., 1990) is inhibited by high external chloride, whereas the low-affinity, linear system that operates at high nitrate concentrations seems to be inhibited by internal chloride (Cerezo et al., 1997). Urrestarazu et al. (1998) have suggested that replacing nitrate with chloride in the nutrient solution during the last week before the crop harvest decreases nitrate content in leafy vegetables.

Increase in the rate of potassium application facilitates uptake and transport of nitrate towards the aerial parts of the plant, promotes metabolism and utilization of nitrate and, ultimately, reduces nitrate accumulation in some vegetable crops (Ahmed et al., 2000; Ruiz and Romero, 2002). However, some studies have indicated that the soil potassium resources have no effect on nitrate accumulation (Drlik and Rogl, 1992).

Ahmed et al. (2000) have shown a reduction in nitrate content due to increasing phosphorus fertilization. Inorganic phosphorus within the plant is necessary for metabolism and storage of nitrate, but high concentrations inhibit enzyme reactions, create abnormal pressure in the cell, and accelerate senescence, and the advancing plant age is accompanied by a reduced nitrate uptake and accumulation (Ahmed et al., 2000). Growth reduction associated with limiting phosphate results in increases in root–shoot ratio, dry matter content, concentrations of sugars and organic acids, and reduction in the concentration of nitrate in the shoots (Buwalda and Warmenhoven, 1999).

Nitrate accumulation has also been studied in relation to some other chemicals. Foliar application of salicylic acid (Ahmed et al., 2000), molybdenum fertilizers (Zhou et al., 2000) and nitrification inhibitors (Xu et al., 2005; Zhou et al., 2000) and calcium (Tzung et al., 1995) can materially reduce nitrate content of plants. In lettuce leaves sugar levels and free amino acids concentrations increase under high supply of calcium and replace nitrate in the vacuole of the lettuce cell (Ahmed, 1996). There exists a negative correlation between nitrate content and sulphate content in lettuce plant (Blom-Zandstra and Lampe, 1983) and, therefore, sulphur deficiency might lead to increase in nitrate content (Maynard et al., 1976). The contents of nitrate in most crops (cabbage, carrot, beet, tomato, onion, potato) decreased significantly under the influence of humic acid as well as zeolite (Nazaryuk et al., 2002).

Supply of boron in concentrations that were either deficient or just sufficient for plant's normal metabolism did not affect the nitrate content, but the toxic boron rate significantly increased the nitrate content of plants (Inal and Tarakcioglu, 2001). Cultivation of vegetables on soil contaminated with sewage sludge may also lead to accumulation of nitrate in edible plant parts due to very high levels of soil nitrogen caused by the sludge (Nazaryuk et al., 2002).

Accumulation of salt in the soils can alleviate nitrate accumulation in vegetable crops (Chung et al., 2005). Due to high fertilization and poor water supply, water potential of the soil may become so negative that salination takes place and plants take up and accumulate nitrate as an osmoticum to adapt to water conditions. In such a situation, potassium compensates the increased nitrate in maintaining the electroneutrality of the plant.

3.2 Environmental Factors

Nitrate accumulation in plants is affected greatly by environmental factors. Santamaria et al. (2001) observed an interaction between light intensity, nitrogen availability and temperature on nitrate accumulation in rocket. Under conditions of low light availability, an increase in temperature increases the nitrate accumulation. Under high light intensity, an increase in temperature increases the nitrate content mainly when nitrogen supply is high. Chadjaa et al. (2001) have studied the effect of artificial lighting in greenhouses on nitrate accumulation in lettuce. High pressure sodium vapour lamps were more effective than metal halide lamps in increasing the nitrate reductase activity and reducing the nitrate accumulation. Effect of climate on nitrate accumulation was studied by Grzebelus and Baranski (2001) who found that nitrate content was lesser in the year that had a high rainfall. In warm and wet years, increased accumulation of nitrate is possible, regardless of whether nitrogen originate from organic or mineral sources (Custic et al., 2003). Plant nitrate levels were influenced by weather conditions more significantly than by the form and application rates of fertilizers (Custic et al., 2003). Nitrate accumulation varies with season (Vieira et al., 1998), being higher in autumn-winter than in spring (Santamaria et al., 1999). It is accepted that the plants in winter are not able to use all the nitrogen available in the soil due to less favourable light and temperature conditions. Gruda (2005) has extensively reviewed the effect of different environmental factors on the nitrate content of greenhouse vegetables.

3.3 Physiological Factors

3.3.1 Genotypic Variability

The nitrate content varies markedly with plant species, cultivars of the same species, and even genotypes with different ploidy (Anjana et al., 2006; Grzebelus and Baranski, 2001; Harada et al., 2003). The shoot nitrate content is genetically determined and likely to be controlled by several genes (QTLs) (Harrison et al., 2004). The causal factors might include genetic differences among genotypes in enzymes of the nitrogen metabolic pathway (nitrate reductase/nitrite reductase), the rate of nitrate uptake, the rate of uptake of other elements needed for enzyme activity, or differences in generation of electron donors needed in the assimilative pathway that might lead to the observed variation in nitrate accumulation. In contrast, Blom-Zandstra and Eenink (1986) found no evidence to conclude that nitrate accumulation was caused by a low assimilation rate of nitrate in lettuce genotypes which differed significantly in nitrate content. The differing capacities of nitrate accumulation can also be correlated with differing locations of nitrate reductase activity (Andrews, 1986), and with differences in photosynthetic capacity (Behr and Wiebe, 1992), ability to generate and translocate respiratory substrate and reducing equivalents, or differences in capacity to translocate the absorbed nitrate to reduction sites. Nitrate accumulation decreases with increasing carbohydrate concentration in the vacuoles. Nitrate accumulation is negatively correlated to sugar concentrations (Blom-Zandstra and Lampe, 1983) and dry matter content (Reinink et al., 1987), while the latter two parameters are positively correlated to each other in different genotypes. Thus, genotypes with high dry matter content could have a high carbohydrate content in their vacuoles and thus need little nitrate to maintain their osmotic value (Reinink et al., 1987). All these factors need to be studied intensively to determine the causal factors for differences in nitrate accumulation between genotypes.

As discussed by Harada et al. (2004), studies using mutants and transgenic plants have revealed a number of genes that can affect concentration of nitrate in a plant, e.g., genes encoding nitrate reductase (Scheible et al., 1997a), a putative anion channel, At CLC-a (Geelen et al., 2000), glutamine synthetase, and ferredoxin-dependent glutamate synthase (Hausler et al., 1994). However, other genes must also be involved. Loudet et al. (2003), using *Arabidopsis* Bay-O and Shahdara recombinant inbred lines (RILs), identified eight quantitative trait loci (QTLs) for nitrate content on a dry matter basis. In maize, Hirel et al. (2001) identified five quantitative trait loci for nitrate content in dry matter, one of which included a gene encoding glutamine synthetase. Nitrate storage in vacuoles is affected by multiple processes including the relative rates of nitrate uptake, nitrate reduction and assimilation, nitrate transfer to the vacuole and its export from there. Therefore, numerous gene products can potentially influence the naturally occurring variation in free nitrate levels at the whole tissue level (Harada et al., 2004).

Harrison et al. (2004) have studied genotypic variability in shoot nitrate content in *Lotus japonicus* and found that it was mainly due to an increase in the ion uptake regardless of biomass production. The positive correlation between the shoot nitrate content and the steady state level of mRNA encoding high affinity nitrate transporters suggests that the higher nitrate flux is due to enhanced expression of transporters. In contrast, neither the level of nitrate reductase mRNA, nor the potential enzyme activity in vivo in the different lines was correlated with shoot nitrate content. This indicates that nitrate transport is one of the main checkpoints controlling shoot nitrate accumulation and that it is possible to lower the nitrate content through breeding strategies without affecting the biomass production. According to Harrison et al. (2004), the concept that nitrogen accumulation in crops under suboptimal nitrogen feeding conditions is highly related to crop growth rate and biomass accumulation via internal plant regulation (Gastal and Lemaire, 2002) needs to be reconsidered. It is necessary to take into account that the control of nitrate uptake and its accumulation in the plant may be subjected to genetic variability regardless of the plant's demand. This variability allows for an adaptive regulatory control mechanism depending on soil nitrate availability.

Thus, selection of genotypes that accumulate less nitrate may contribute significantly to reduction in the nitrate consumption by humans through vegetables and the subsequent risk of nitrate poisoning. Another method of circumventing the problems associated with nitrate accumulation may be to breed cultivars that do not accumulate nitrate even under heavy nitrogen fertilization.

3.3.2 Nitrate Distribution Within the Plant

In general, vegetables that are consumed with their roots, stems and leaves have a high nitrate accumulation, whereas those with only fruits and melons as consumable parts have a low nitrate accumulation (Zhou et al., 2000). Nitrate content of various parts of a plant differs (Santamaria et al., 1999). Indeed, the vegetable organs can be listed by decreasing nitrate content as follows: petiole > leaf > stem > root > inflorescence > tuber > bulb > fruit > seed (Santamaria et al., 1999). In lettuce and "head chicory", inner leaves accumulate less nitrate than outer leaves and in parsley and spinach, leaf blades accumulate less nitrate than petioles (Santamaria et al., 1999; Santamaria et al., 2001). Nitrate concentration in the petiole was more than double the one in the lamina of rocket leaf (Elia et al., 2000); the difference was as high as 6.6 fold in spinach (Anjana et al., 2006). Similarly, nitrate concentration in the petiole-stems was higher than in the leaves, with the lowest in the roots in leafy vegetables (Chen et al., 2004). Therefore, increasing the blade/petiole ratio of spinach plants at harvesting (Santamaria et al., 1999) and separation of those parts of a vegetable that accumulate high nitrate concentrations, prior to processing or preparation of vegetable food, may appreciably reduce the extent of nitrate consumption by humans. Chen et al. (2004) have also shown that nitrate supply has a significant effect on nitrate distribution both in the metabolic pool and the storage pool of leaf blades. The comparison of the nitrate storage pool with the nitrate concentration in the whole leaf (nitrate metabolic pool + nitrate storage pool) suggests that about 90% of the nitrate accumulates in the storage pool. Functional diversity of nitrate compartmentation in the cells of various species has been discussed by Izmailov (2004).

The concentration of nitrate in various plant parts could be a measure to evaluate the balance between adequate and excessive available nitrogen for optimum growth of plants during a growth season. Typically, however, this balance assumes two markedly different modes depending on vegetable crop under consideration. Plants that develop fruit or storage organ, such as potato and tomato, usually have low nitrate-N concentrations in petioles as the crop approaches the harvest. The decline in the petiolar nitrate concentration depends on the translocation of soluble-nitrogen to the developing storage organ, and on the gradual decrease in the available soil nitrogen. Vegetables that do not develop storage organs as the foodstuff have a different pattern of nitrate accumulation wherein nitrate often continues accumulating with the growing plant age (Maynard et al., 1976). Similar were the findings of Anjana et al. (2006), showing that nitrate concentration in spinach was higher at later stages of plant growth. Therefore, optimum physiological age for harvesting needs to be standardized for different leafy vegetable crops. Since nitrate continues to accumulate in spinach plant till maturity, the extent of nitrate intake by humans through spinach leaves may be reduced by harvesting the crop during vegetative stage.

3.3.3 Diurnal Effects

Nitrate accumulation is a complex process involving many physiological steps. Being involved in photosynthesis as well as in uptake, translocation and reduction of nitrate, intensity of light has a crucial role in the regulation of nitrate accumulation (Merlo et al., 1994). Light intensity is inversely correlated to nitrate content of plants; therefore, diurnal changes in light intensity might cause a diurnal nitrate accumulation pattern. The diurnal variation in nitrate content can be kept low by selecting a proper harvest time. That a high light intensity reduces nitrate accumulation has been shown in several studies (Blom-Zandstra et al., 1988; van der Boon et al., 1990). Marked diurnal patterns showing a decrease of nitrate content during the day period followed by an increase during the night have also been demonstrated (Cardenas-Navarro et al., 1998; Delhon et al., 1995a, b). Various explanations, depending on uptake and assimilation of nitrate are put forth for the diurnal changes in the nitrate content of plants.

Nitrate uptake and accumulation. Light intensity can have a profound effect on nitrate acquisition by roots and restrict the rate of nitrate uptake in darkness

(Le Bot and Kirkby, 1992). The differences in nitrate content of butterhead lettuce cultivars are due to differences in nitrate uptake (Behr and Wiebe, 1992). On the contrary, several studies have indicated that nitrate uptake does not modify under dark conditions (Macduff and Wild, 1988). These discrepancies demonstrate that characterization of the effect of light intensity on nitrate uptake is still incomplete. In particular, it is not yet clear whether inhibition of net nitrate uptake in darkness results from a decreased influx (Macduff and Jackson, 1992) and/or an increased efflux (Pearson et al., 1981) across the plasmalemma of the root cells. Beevers et al. (1965) described the role of light in nitrate accumulation to be indirect in that light enhances nitrate uptake as a result of increased permeability of the tissue and this, in turn, stimulates enzymatic activity and assimilation of nitrate. There exists a strong correlation between the net uptake of nitrate by the roots and the vacuolar nitrate concentration in the leaf blades (Steingrover et al., 1986a). The increase in nitrate concentration of leaf blades during the nights results from increased nitrate uptake by roots, and from reduction of nitrate to organic solutes that are metabolized in the cytoplasm. During the daytime, nitrate leaves the vacuoles partly to be replaced by soluble carbohydrates and organic acids synthesized in the light. When light is limiting, synthesis of organic acids and soluble carbohydrates declines and their role as an osmoticum is taken up readily by nitrate.

Scaife (1989) and Scaife and Schloemer (1994) have developed a model, assuming a concentration-dependent nitrate efflux in which net nitrate uptake (influx minus leakage) and nitrogen assimilation (considered as a constant fraction of photosynthetic rate) can be calculated. This could provide precise details of the diurnal variation of nitrate uptake dependent on radiation. Another similar model assumes that nitrate influx is adjusted to reduction flux by means of a negative feedback control, which is proportional to internal nitrate concentration (Cardenas-Navarro et al., 1998). In both these models, the internal nitrate concentration itself is involved directly in the regulation mechanism as the regulatory signal. By this hypothesis, low nitrate concentrations in plants result from an increased nitrate demand, associated with higher growth rates at high daily integrals, and correspond with a low level of feedback control over nitrate influx in the Cardenas-Navarro et al. (1998) model, with a low rate of concentration-driven efflux in the Scaife model (1989). Thus, these models suggest that nitrate concentration would result from a simple proportional regulation mechanism. This hypothesis is supported by experiments in which nitrogen nutrition or assimilation was altered (King et al., 1993), and the negative correlation obtained between uptake rate and endogenous nitrate (Laine et al., 1995; Cardenas-Navarro et al., 1998, 1999). Furthermore, a direct measurement of cytosolic nitrate concentration shows that it is regulated in root cortical cells (Miller and Smith, 1996) and there is no such evidence of homeostasis of nitrate in other compartments/organs, or at the whole-plant level. The recent simulation models based on this hypothesis seem to be able to predict the whole-plant nitrate content adequately (Cardenas-Navarro et al., 1998).

Macduff and Bakken (2003) have provided a framework for considering diurnal co-regulation of nitrate uptake by the amino acid and nitrate levels in species assimilating nitrate predominantly in the shoots. According to this model, diurnal regulation of nitrate influx is affected by nitrate levels throughout the dark period (downregulation) and the first half of the light period (upregulation), but by amino acids levels during the second half of the light period (downregulation). The sudden light/dark transitions affect transpiration rate and hence xylem nitrogen flux, which in turn affects the concentration of nitrate in the cytoplasmic compartment of the roots, the rate of nitrate assimilation in the shoot and the phloem amino acid flux, once the shoot demand for amino acids associated with protein synthesis and vacuolar storage has been met.

On the other hand, according to the turgor maintenance concept, nitrate level is negatively correlated with the level of soluble, non-structural carbon compounds (SNC; mainly sugars and organic acids) as observed in lettuce and some other vegetables (Blom-Zandstra et al., 1988; Steingrover et al., 1986a, b). Veen and Kleinendorst (1985) demonstrated that nitrate and soluble carbohydrates play complementary roles in the maintenance of cell turgor. This concept was generalized by Seginer et al. (1998) who assumed that the pool size of soluble, non-structural carbon compounds is determined by the balance between source activity (supply by photosynthesis) and sink activity (demand by growth and maintenance). They also hypothesized that a regulation mechanism adjusts the nitrate concentration to the soluble, non-structural carbon compounds level according to the plant's requirements for osmotically active solutes to maintain turgor.

This means the low summer nitrate level in lettuce is caused by the increased rate of photosynthesis at high daily integrals, which leads to accumulation of soluble, non-structural carbon compounds, thereby diminishing the need for nitrate as a cellular osmoticum. In this concept, maintaining a constant level of turgor by adjusting the nitrate concentration to fluctuating levels of soluble, non-structural carbon compounds would require a rather complex regulation. Therefore, the carbon balance, rather than nitrate homeostasis, is the primary factor that determines nitrate accumulation in lettuce (Buwalda and Warmenhoven, 1999).

Light intensity determines the production of carbohydrates and affects nitrate assimilation (by supply of NADH and induction of nitrate reductase). This may also affect the leakage of nitrate from the vacuoles (Aslam et al., 1976). Organic acids and sugars play an important role in the osmotic adjustment. There is an inverse relationship between nitrate compounds and organic compounds, suggesting that nitrate also has a role as osmoticum, which may explain its relative unavailability for reduction in the metabolic pool (Blom-Zandstra, 1989). Concentrations of nitrate, organic acids, and sugars change significantly with varying light intensity. Variation of nitrate concentration is fully compensated for by a change in the concentration of organic acids and sugars to maintain electroneutrality and osmotic pressure. At low light intensities, production of organic acids is low due to low rate of photosynthesis (Blom-Zandstra and Lampe, 1985) and less organic compounds are thus available for storage in the vacuoles and, therefore, nitrate can serve as an alternative. This will require low energy costs, because nitrate uptake, transfer through the xylem, and storage in the vacuoles do not require as much energy, derived from ATP, as do the production and accumulation of organic compounds (Lambers and Steingrover, 1978). The plant seems to shift the balance from carbohydrates to nitrate so as to maintain turgor for cell expansion during growth, and the availability of organic compounds for storage affects the need for nitrate as an osmoticum, which in turn affects nitrate uptake. Thus, the ratio between accumulation of organic compounds and accumulation of nitrate is light dependent.

Under light-limiting conditions, assimilate production increases with increasing light intensity which is invested in structural growth rather than in osmoregulation if nitrate is available at a higher level than is required to meet the plant's demand for reduced nitrogen. It, therefore, appears likely that increasing light intensity will result in a substantial replacement of nitrate with organic osmotica only where growth is not limited by light intensity or where nitrate availability becomes growth limiting (McCall and Willumsen, 1999). Moreover, a negative correlation exists between nitrate accumulation and dry matter content, as reported for different genotypes (Reinink et al., 1987). It implies that when a plant accumulates nitrate as an osmoticum, it can utilize more carbohydrates for growth since the osmotic requirement is fulfilled by nitrate.

Nitrate content can be reduced significantly both by limiting the nitrogen availability and by increasing the light intensity (McCall and Willumsen, 1999). Reducing the nitrogen availability significantly reduces the contribution of nitrate to the osmotic potential and increases that of chloride, glucose and sucrose. Furthermore, contribution of nitrate to the osmotic potential becomes significantly low and that of sucrose significantly high at a high light intensity. As the reduction in nitrate content with supplementary light becomes relatively slight, growth is greatly enhanced. The reduced nitrate content under high light intensity is accompanied by an increased content of sucrose, suggesting an increased rate of photosynthesis under high light intensities (McCall and Willumsen, 1999).

Nitrate assimilation and its accumulation. As discussed in detail by Stitt et al. (2002), nitrogen metabolism undergoes drastic diurnal changes, which are driven by a transient imbalance between the rate of nitrogen assimilation and the rate of nitrate uptake and ammonium assimilation in the first part of the light period. It has been shown in tobacco plants growing in a high nitrate and favourable light regime that their leaves contain high levels of the *NIA* transcript at the end of the night. Illumination stimulates translation of the transcript and inhibits degradation of NIA protein (Kaiser et al., 1999), leading to an approximately threefold increase of NIA protein during the first hours of the light period (Scheible et al., 1997b). A rapid post-translational activation of NIA and a high rate of nitrate assimilation are achieved during the first part of the light period. These exceed the rate of nitrate uptake by a factor of 2 (Matt et al., 2001), leading to a rapid depletion of the leaf nitrate pool. During the second part of the light period, nitrate assimilation is progressively inhibited by mechanisms that act at several levels to decrease NIA activity. These include a dramatic decrease of the *NIA* transcript level, which commences soon after illumination (Matt et al., 2001; Scheible et al., 1997b) and results in a decline of NIA protein and activity as well as post-translational activation of NIA after darkening (Scheible et al., 1997b). In consequence, the rate of nitrate reduction falls about twofold in the second part of the light period, and is negligible during the night (Matt et al., 2001). By contrast, nitrate uptake in the roots and movement of nitrate to the shoot remain high during the entire light period and fall by only 30% during the night. The nitrate that is taken up during the night is used almost exclusively to replenish the leaf nitrate pool (Matt et al., 2001).

Nitrate reductase activity and nitrate accumulation. It is noteworthy that during a diurnal cycle, nitrate reductase activity in leaves often decreases during the late light phase although sugar level in the leaves is still increasing or at least is not decreasing (Kaiser et al., 2002). This "afternoon depression" of nitrate reductase may reflect its degradation (Man et al., 1999) and/or a block in its synthesis, and is usually paralleled by decreasing nitrate concentrations in the leaves. This effect of light reflects in fluctuations in the carbohydrate level and in the corresponding supply of reducing equivalents (ferredoxin and NADPH). In addition, however, leaves of plants grown under low light intensity usually have low levels of nitrate reductase; the enzyme activity increases when the plants have been transferred to conditions of high light intensity. Light intensity also affects the stability of the enzyme. The rate of nitrate reduction in leaves is thus affected by light in a variety of ways.

Anjana et al. (2006) have reported that nitrate concentration was lowest in the noon of a sunny day in the spinach leaves. However, the time at which plants contain lowest nitrate concentration may vary with the environmental conditions in different geographical regions of the world. Therefore, harvesting schedules need to be standardized for different geographical regions and the farmers advised to harvest vegetables at the recommended point of time and supply them to the market as soon as possible so that deterioration in their quality due to nitrite formation does not take place during the post-harvesting period. Moreover, by cooking vegetables in water (with low nitrate concentration), at least 50% of accumulated nitrate can be removed (Meah et al., 1994).

4 Effect of Nitrate Ingestion on Human Health

4.1 Adverse Effects

Several human health hazards due to nitrate toxicity have been identified. The toxicity of nitrate is thought to be due to its reduction to nitrite and conversion to nitrosamines and nitrosamides through reaction with amines and amides, whose carcinogenic action is well known (Walker, 1990). The principal mechanism of nitrite toxicity is the oxidation of the ferrous iron (Fe^{2+}) in haemoglobin to the ferric (Fe^{3+}) valence state, producing methaemoglobin. As a consequence of methaemoglobin formation, oxygen delivery to human tissues is impaired (Knobeloch et al., 2000; Mensinga et al., 2003). The methaemoglobin that is formed is reduced by the following reaction:

$$\mathrm{Hb}^{3+} + \mathrm{Red\ cyt\ b}_5 \rightarrow \mathrm{Hb}^{2+} + \mathrm{Oxy\ cyt\ b}_5$$

Reduced cytochrome b_5 (Red cyt b) is regenerated by the enzyme cytochrome b_5 reductase:

$$\mathrm{Oxy\ cyt\ b}_5 + \mathrm{NADH} \xrightarrow{\text{cyt b5 reductase}} \mathrm{Red\ cyt\ b} + \mathrm{NAD}$$

Enzyme cytochrome b_5 reductase plays a vital role in counteracting the effects of nitrate ingestion.

The percentage of total methaemoglobin in oxidized form determines the clinical picture of oxygen deprivation with cyanosis, cardiac dysrhythmias and circulatory failure, and progressive central nervous system (CNS) effects. The CNS effects can range from mild dizziness and lethargy to coma and convulsions (Agency for Toxic Substances and Disease Registry, 2001). Clinical findings vary with methaemoglobin concentrations (Table 2). Methaemoglobinemia, earlier believed to occur in infants only, has been reported by Gupta et al. (2000a) in people of different age having a high nitrate ingestion, the infants and the above 45 age groups being most susceptible to nitrate toxicity.

Of the total nitrate absorbed from the diet or produced endogenously, 25% is taken up by salivary glands and secreted into the mouth (McColl, 2005). Bacteria on the dorsum of the tongue convert 10–90% of this nitrate in saliva to nitrite. On being swallowed,

Table 2 Signs and symptoms of methaemoglobinemia (Dabney et al., 1990)

Methaemoglobin concentration (%)	Clinical findings
10–20	Central cyanosis of limbs/trunk; usually asymptomatic
20–45	Central nervous system depression (headache, dizziness, fatigue, lethargy, syncope), dyspnea
45–55	Coma, arrhythmias, shock, convulsions
>70	High risk for mortality

when saliva meets acidic gastric juice, it is converted to nitrosating species (that is N_2O_3 and NOSCN) and by further reacting with ascorbic acid in the gastric juice to nitric oxide (McColl, 2005), which are potentially mutagenic and carcinogenic (Iijima et al., 2003; Moriya et al., 2002). Excessive local production of nitric oxide leads to functional abnormalities associated with the gastro-oesophageal reflux diseases (McColl, 2005).

In the early weeks of life, before establishment of the gastric acid barrier, the infant gut is colonized by bacteria throughout its length and, therefore, the nitrate in the feed is readily reduced to nitrite in the stomach and small intestine by the action of bacterial nitrate reductase (E.C. 1.6.6.1). This is then absorbed and reacts with haemoglobin to form methaemoglobin; the latter greatly reduces oxygen-binding capacity of blood and consequently the infant tissues are starved of oxygen and ultimately cyanosis results (Hill, 1999). In pregnant women, the level of methaemoglobin increases from the normal (0.5–2.5% of total haemoglobin) to a maximum of 10.5% at the 30th week of gestation and subsequently declines to normal after delivery. Thus, pregnant women might be more sensitive to the induction of clinical methaemoglobinemia by nitrites or nitrates in or around the 30th week of gestation (Agency for Toxic Substances and Disease Registry, 2001).

Another concern pertains to human cancer as a result of the bacterial production of N-nitroso compounds in the colonized hypochlorhydric stomach. N-nitroso compounds have been shown to be carcinogenic in more than 40 animal species tested, including mammals, birds, reptiles and fish (Hill, 1999) and in human (Michaud et al., 2004). Endogenously formed nitrogen and oxygen free radicals are believed to be involved in human cancer etiology (Szaleczky et al., 2000). Elevated risks for the cancer of urinary bladder (Michaud et al., 2004), esophagus, nasopharynx and prostate have been reported due to high nitrate ingestion (Eicholzer and Gutzwiller, 1990). Other health problems associated with nitrate toxicity include oral cancer (Badawi et al., 1998), cancer of the colon, rectum or other gastrointestinal regions (Knekt et al., 1999; Turkdogan et al., 2003), Alzheimer's disease, vascular dementia of Biswanger type or multiple small infarct type (Tohgi et al., 1998), multiple sclerosis (Giovannoni et al., 1997), spontaneous abortion or congenital defects (Fewtrell, 2004), anencephaly (Croen et al., 2001), non-Hodgkin's lymphoma (Michal, 1998) and cardiovascular disorders (Morton, 1971).

A high percentage of acute respiratory tract infection with history of recurrence has been reported in children consuming high nitrate content (Gupta et al., 2000b). Recurrent diarrhoea in children up to 8 years of age (Gupta et al., 2001) and recurrent stomatitis (Gupta et al., 1999) are also associated with high nitrate ingestion. It also affects human immune system (Ustyugova et al., 2002). Some other reported effects are infant mortality, early onset of hypertension, hypothyroidism, diabetes and adverse effect on cardiac muscles, alveoli of lungs and adrenal glands (Gupta, 2006).

In animals, nitrate toxicity varies according to species. In general, ruminant animals develop methaemoglobinemia while monogastric animals exhibit severe gastritis (Bruning-Fann and Kaneene, 1993).

4.2 Beneficial Effects

Various studies suggest that nitrate is harmless and rather beneficial. It has been postulated as a useful nutrient (Dykhuizen et al., 1996). Bjorne et al. (2004) have suggested that dietary nitrate may serve important gastroprotective functions. Dietary nitrate is converted into nitrite by a symbiotic relationship involving nitrate-reducing bacteria on the tongue surface. This relationship is designed to provide a host defense against microbial pathogens in the mouth and lower gut (Duncan et al., 1995). The host provides nitrate, which is an important nutrient for many aerobic bacteria. In return, bacteria help the host by generating the substrate (nitrite) necessary for generation of nitric oxide in the stomach (Bjorne et al., 2004). High concentration of nitrite present in saliva may, upon acidification, generate nitrogen oxides in the stomach (Duncan et al., 1995). Nitrite-derived nitric oxide and related compounds play an important role in gastric host defense by enhancing the acid-dependent killing of swallowed pathogens (Benjamin et al., 1994; Duncan et al., 1995; Dykhuizen et al., 1996; Xu et al., 2001). The high plasma nitrate levels in patients suffering from infective gastroenteritis may protect against the faecal-oral route of reinfection via increased generation of salivary nitrite (Dykhuizen et al., 1996). According to McKnight et al. (1999), nitrate in the diet is an effective host defense against gastrointestinal pathogens. It acts as a modulator of platelet activity, gastrointestinal motility and microcirculation.

It has also been suggested that the reactive nitrosating chemistry that occurs when saliva meets acidic gastric juice may also have biological benefit (McColl, 2005). Since, this chemistry possesses antimicrobial activity (Duncan et al., 1997), it appears to be primarily designed to kill pathogenic microbes entering the body via the upper gastrointestinal tract (McColl, 2005). The beneficial effects of nitrate also include reduction of hypertension and cardiovascular diseases (McKnight et al., 1999). The various effects of nitrate on human health have been covered extensively by L'hirondel and L'hirondel (2002) in their book "Nitrate and man: Toxic, harmless or beneficial?"

Thus, evidences regarding the effect of nitrate on human health are conflicting. However, in view of the large number of its harmful effects on human health; it seems reasonable to take preventive measures so as to reduce nitrate accumulation in plants and its subsequent consumption by human beings.

5 Conclusions

Vegetables are the major source of dietary nitrate intake of humans and nitrate has many detrimental and some beneficial effects on human health. Our understanding of the specific role of nitrate and its derivatives with respect to health is incomplete for which further studies need to be carried out. The various approaches that may be adopted for reducing nitrate level in vegetables are summarized below:

- A balanced fertilization program for vegetable crops should be chalked out to ensure an adequate, but not excessive supply of nutrients for optimum yield and quality, and avoid or minimize nitrogen losses to the environment. The supply and release of nutrients must be in synchrony with the need of the plant. Therefore, to find out an exact adjustment of a balanced fertilizer application to nitrate uptake and reduction capability of plants should be the priority for research by agronomists. For this purpose, strategies can be evolved with simulation models. Moreover, agronomic indicators need to be implemented at farm level to provide yardsticks for nutrient management by the farmers.
- Nitrate concentration in plants may be reduced by partial replacement of nitrate in the nutrient solution with ammonium, urea, mixed amino acids, chloride or sulfate few days prior to crop harvesting. Rational application of organic manure instead of inorganic nutrients, use of physiologically active substances, proper spray of nitrification inhibitors and molybdenum fertilizers, and growing plants under controlled environmental conditions may materially reduce nitrate accumulation in plants. Effects of other micronutrients and cultural conditions also need to be investigated. Selection among the available genotypes/cultivars and breeding of new cultivars that do not accumulate nitrate even under heavy fertilization may also limit human consumption of nitrate through vegetables.
- While there exists a large body of published research on strategies for reducing nitrate accumulation in plants, it remains to be translated into practice due to lack of information with farmers. Therefore, there is a need to bridge the gap between research laboratories and farmer's fields. Decision makers must formulate relevant agricultural policies encompassing education and training of farmers to make them understand the effects of nitrate on human health and the importance of nutrient management and other strategies in minimizing the nitrate content in plant tissues. Consumers also need to be educated regarding the nitrate content in vegetables and its health implications. They must be motivated to adopt practices that help in minimizing the nitrate consumption, e.g., harvesting of leafy vegetables in the noon, removal of the plant organ rich in nitrate content, cooking vegetables in water containing less nitrate content, and use of fresh vegetables, etc.

- It is important to determine he exact physiological mechanism responsible for nitrate accumulation in plants. Moreover, effect of fertilization and other cultivation practices on the physiologicalo parameters, and their significance for enhancing crop production and quality need to be investigated. Molecular tools may be applied to reduce nitrate concentration in plants. Even though the literature suggests that genetic manipulation of activities of nitrogen assimilation enzymes may not increase yield and/or nitrogen use efficiency of plants, we understand that overexpression of nitrate reductase genes may prove helpful in reducing the nitrate content of plants and, in consequence, improve the quality of plants for human consumption.

Therefore, an integrated collaboration among agronomists, physiologists, molecular biologists, farmers, consumers and policy makers is the need of the hour and may yield satisfactory results towards minimizing the nitrate accumulation in plants and its subsequent consumption by human beings.

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Role of Phosphate Solubilizing Microorganisms in Sustainable Agriculture – A Review

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Abstract Compared with the other major nutrients, phosphorus is by far the least mobile and available to plants in most soil conditions. Although, phosphorus is abundant in soils in both organic and inorganic forms, it is frequently a major or even the prime limiting factor for plant growth. The bioavailability of soil inorganic phosphorus in the rhizosphere varies considerably with plant species, nutritional status of soil and ambient soil conditions. To circumvent the phosphorus deficiency, phosphate solubilizing microorganisms (PSM) could play an important role in supplying phosphate to plants in a more environment friendly and sustainable manner. The solubilization of phosphatic compounds by naturally abundant PSM is very common under in vitro conditions; the performance of PSM in situ has been contradictory. The variability in the performance has thus, greatly hampered the large-scale application of PSM(s) in sustainable agriculture. Numerous reasons have been suggested for this, but none of them have been conclusively investigated. Despite the variations in the performance, the PSM(s) are widely applied in agronomic practices in order to augment the productivity of crops while maintaining the health of soils. This review presents the results of studies on the utilization of PSM(s) for direct application in agriculture under a wide range of agro-ecological conditions with a view to fostering sustainable agricultural intensification in developing countries of the tropics and subtropics.

1 Introduction

As the world human population continues to increase, the demands placed upon agriculture to supply future food will be one of the greatest challenges facing the agrarian community. In order to meet this challenge, a great deal of effort focusing on the soil biological system and the agro-ecosystem as a whole is needed to better understand the complex processes and interactions governing the stability of agricultural land. At the present time, food is (generally) not in short supply (rather there is a lack of timely distribution of foods to areas of need) due, in part, to high-input agriculture, which in turn caused the green revolution. The green revolution has been one of the profound successful human activities resulting in global food security and, consequently, transformed some of the developing countries, such as India, from being food deficient to having a food surplus. However, the consistent and alarming increase in the human population has again threatened the world food security. There is therefore, urgent need of a second green revolution to increase the food production by around 50% in the next 20 years in order to sustain the population pressure (Vasil, 1998; Leisinger, 1999).

Chemical fertilizers e.g., manufactured water-soluble phosphatic (WSP) fertilizers (super phosphates) have played a significant role in the green revolution and are commonly recommended to correct phosphorus deficiencies. Most developing countries,

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however, import these fertilizers, which are often in limited supply and represent a major outlay for resource-poor farmers. In addition, intensification of agricultural production in these countries necessitates the addition of phosphate not only to increase crop production but also to improve soil phosphatic status in order to avoid further soil degradation. Moreover, the use of chemical fertilizers is reaching the theoretical maximum use beyond which there will be no further increase in crop yields (Ahmed, 1995). However, it is becoming increasingly clear that conventional agricultural practices can not sustain the production base, a healthy plant soil system for too long. While, to augment crop productivity, agronomist depends heavily on chemical fertilizers. In this context, after nitrogen, phosphorus is an essential plant nutrient whose deficiency restricts crop yields severely. Tropical and sub tropical soils are predominantly acidic, and often extremely phosphorus deficient (Gaume, 2000) with high phosphorus sorption (fixation) capacities. On an average, most mineral nutrients in soil solution are present in millimolar amounts but phosphorus is present only in micromolar or lesser quantities (Ozanne, 1980). The low levels of phosphorus are due to high reactivity of soluble phosphate with other elements. For instance, in acidic soils phosphorus is associated with aluminium and iron compounds (Norrish and Rosser, 1983) whereas calcium phosphate is predominant form of inorganic phosphate in calcareous soils (Lindsay et al., 1989). Organic phosphate may also make up a large fraction of soluble phosphate, as much as 50% in soils with high organic matter content (Barber, 1984). Since the indiscriminate and excessive application of chemical fertilizers has led to health and environmental hazards, agronomists are desperate to find alternative strategies that can ensure competitive yields while protecting the health of soils. This new approach to farming, often referred to as sustainable agriculture, requires agricultural practices that are friendlier to the environment and that maintain the long-term ecological balance of the soil ecosystem. In this context, use of microbial inoculants (biofertilizers) including PSM in agriculture represents an environment friendly alternative to further applications of mineral fertilizers. A continued exploration of the natural biodiversity of soil microorganisms and the optimization/manipulation of microbial interactions in the rhizosphere represents a prerequisite step to develop more efficient microbial inoculants with phosphorus solubilizing ability.

2 Urgent Need for Phosphate Solubilizing Microorganisms in Plant Phosphate Nutrition

The production of chemical phosphatic fertilizers is a highly energy intensive process requiring energy worth US$4 billion per annum in order to meet the global need (Goldstein et al., 1993). The situation is further compounded by the fact that almost 75–90% of added phosphatic fertilizer is precipitated by metal cations complexes present in the soils (Stevenson, 1986). Further, it has been suggested that the accumulated phosphates in agricultural soils is sufficient to sustain maximum crop yields worldwide for about 100 years (Goldstein et al., 1993). Thus, the dependence of fertilizer production on fossil energy source and the prospects of diminishing availability of costly input of fertilizer production in years to come, have obviously brought the subject of mineral phosphate solubilization (mps) in the forefront. Hence, it is imperative to explore alternative phosphatic sources. Under diverse soil and agro-climatic conditions, the organisms with phosphate solubilizing (PS) abilities have proved to be an economically sound alternative to the more expensive superphosphates and possess a greater agronomic utility. The microbial system can siphon out appreciable amounts of nutrients from the natural reservoir and enrich the soil with the important but scarce nutrients. The crop microbial ecosystem can thus, be energized in sustainable agriculture with considerable ecological stability and environmental quality. The organisms with phosphate solubilizing potentials increase the availability of soluble phosphate and can enhance the plant growth by increasing the efficiency of biological nitrogen fixation or enhancing the availability of other trace elements such as iron, zinc, etc. and by production of plant growth promoting regulators (Sattar and Gaur, 1987; Kucey et al., 1989; Ponmurugan and Gopi, 2006).

3 Nature of Phosphatic Biofertilizers

Majority of the crop plants have been found to be positively affected from the association with rhizospheric microorganisms under phosphorus deficient conditions. This association could result either in improved uptake of the available phosphates or rendering unavailable phosphorus sources accessible to the plant. The arbuscular mycorrhizae (AM) belong to the former category while the later category includes numerous bacteria and fungi capable of solubilizing insoluble mineral phosphate. In the present section, an attempt is made to identify such natural phosphate solubilizing organisms.

3.1 Phosphate Solubilizing Microorganisms

Microorganisms are important component of soil and influence directly or indirectly the soil health through their beneficial or detrimental activities. Rhizospheric microorganisms mediate soil processes such as decomposition, nutrient mobilization and mineralization, storage release of nutrients and water, nitrogen fixation and denitrification. Furthermore, the organisms possessing phosphate solubilizing ability can also convert the insoluble phosphatic compounds into soluble forms (Kaang et al., 2002; Pradhan and Sukla, 2005) in soil and make it available to the crops. The role of rhizospheric organisms in mineral phosphate solubilization was known as early as 1903. Since then, there have been extensive studies on the mineral phosphate solubilization by naturally abundant rhizospheric microorganisms. Important genera of mineral phosphate solubilizers include *Bacillus* and *Pseudomonas* (Illmer and Schinner, 1992) while *Aspergillus* and *Penicillium* forms the important fungal genera (Motsara et al., 1995). Recently, the nematofungus *Arthrobotrys oligospora* was tested in vitro and in vivo for its ability to solubilize kodjari rock phosphate (Burkina Faso, KRP), togolese rock phosphate (Senegal TRP) and tilemsi rock phosphate (Mali TIRP). All three types of rock phosphates were solubilized by the fungus and demonstrated the ability to solubilize additional phosphate from rock phosphates in vivo as well (Duponnois et al., 2006). Most of the impact with the use of microorganisms as biofertilizers has been directed towards understanding the biological nitrogen fixation. In contrast, the fundamental work on phosphate solubilization by nodule bacteria has been substantially less; though it is known that phosphorus is the most limiting factor for nitrogen fixation by *Rhizobium*-legume symbiosis. There are only a few reports of phosphate solubilization by *Rhizobium* (Halder et al., 1991; Abd-Alla, 1994; Chabot et al., 1996) and non-symbiotic nitrogen fixer, *Azotobacter* (Kumar et al., 2001). Phosphate solubilizing microorganisms are ubiquitous whose numbers vary from soil to soil. In soil, phosphate solubilizing bacteria constitute 1–50% and fungi 0.5–0.1% of the total respective population. Generally, the phosphate solubilizing bacteria outnumber phosphate solubilizing fungi by 2–150 fold (Kucey, 1983). The high proportion of PSM is concentrated in the rhizospheres and is known to be metabolically active than those isolated from sources other than rhizosphere (Vazquez et al., 2000). Conversely, the salt, pH and temperature tolerant phosphate solubilizing bacteria have been reported maximum in rhizoplane followed by rhizosphere and root free soil in alkaline soils (Johri et al., 1999). The PSM strains with these stressed properties should therefore serve as an excellent model to study the physiological, biochemical and molecular mechanism(s) of phosphate solubilization under stressed ecosystems. Further, it has been observed that the phosphate solubilizing bacteria upon repeated sub culturing loose the phosphate solubilizing activity but such losses have not been observed in phosphate solubilizing fungus (Kucey, 1983). In general, the phosphate solubilizing fungi produce more acids and consequently exhibit greater phosphate solubilizing activity than bacteria in both liquid and solid media (Venkateswarlu et al., 1984). The phosphate solubilizing ability of PSM also depends on the nature of nitrogen source used in the media, with greater solubilization in the presence of ammonium salts than when nitrate is used as nitrogen source. This has been attributed to the extrusion of protons to compensate for ammonium uptake, leading to a lowering extra-cellular pH (Roos and Luckner, 1984). In some cases, however, ammonium can lead to decrease in phosphorus solubilization (Reyes et al., 1999).

3.1.1 Search for Phosphate Solubilizing Microorganisms

Phosphate solubilizing microorganisms can be isolated using serial dilutions or enrichment culture technique on/in Pikovskaya medium (Pikovskaya, 1948) from non rhizosphere and rhizosphere soils, rhizoplane, and also from other environments, such as rock phosphate deposit area soil and marine environment (Gaur, 1990). Upon incubation of the organisms on to the solid plates containing insoluble phosphate, PSM are detected by the formation of clear halos around their colonies (Fig. 1). Recently, a few other methods for the isolation and selection of PSM have been suggested (Gupta et al., 1994; Nautiyal, 1999). Since certain strains of phosphate solubilizing organisms exhibit many fold variation in phosphate solubilizing activity and instability with regard to their phosphate solubilizing activity (Illmer and Schinner, 1992), they are repeatedly sub-cultured to test the persistence of phosphate solubilizing potential. Once the efficient phosphate solubilizing organisms are selected, they are tested for their ability to solubilize insoluble phosphate under liquid culture medium. Finally, the selected efficient phosphate solubilizing cultures are used for making the inoculants and their performance under pot/field conditions is tested against various crops.

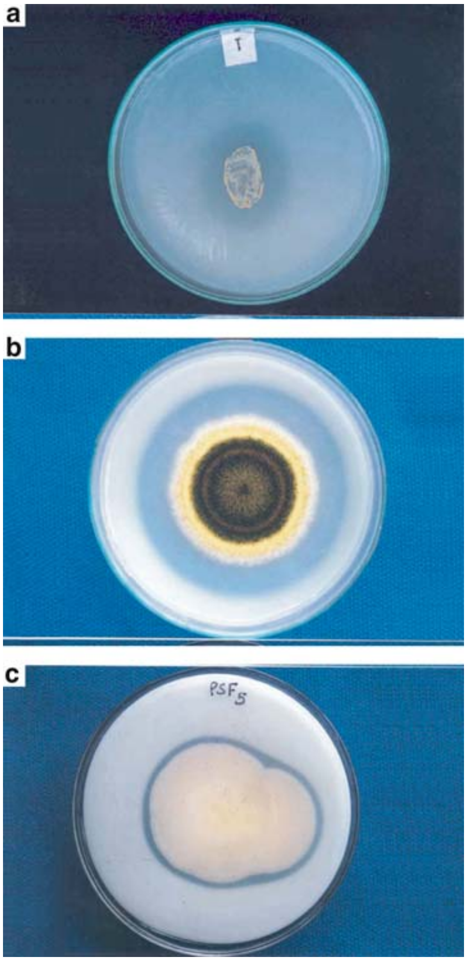


Fig. 1 Solubilization of insoluble P on Pikovskaya medium by phosphate solubilizing bacteria (**a**) and fungi (**b** and **c**)

3.1.2 Mechanism of Phosphate Solubilization – An Overview

Many researchers have quantitatively investigated the ability of PSM to solubilize insoluble phosphate in pure liquid culture medium (Whitelaw, 2000; Narula et al., 2000). The microbial solubilization of soil phosphorus in liquid medium has often been due to the excretion of organic acids (Table 1). For instance, oxalic acid, citric acid, lactic acid, etc. in liquid culture filtrates were determined by paper chromatography or thin layer chromatography or by high performance liquid chromatography and certain enzymatic methods to allow more accurate identification of unknown organic acids (Gyaneshwar et al., 1998). Such organic acids can either directly dissolve the mineral phosphate as a result of anion exchange of PO_4^{2-} by acid anion or can chelate both iron and aluminium ions associated with phosphate (Omar, 1998). While, in certain cases phosphate solubilization is induced by phosphate starvation (Gyaneshwar et al., 1999). However, no definite correlation between the acids produced by PSM and amounts of phosphate solubilized are reported (Asea et al., 1988). The role of organic acids produced by PSM in solubilizing insoluble phosphate may be due to the lowering of pH, chelation of cations and by competing with phosphate for adsorption sites in soil (Nahas, 1996). Inorganic acids e.g., hydrochloric acid can also solubilize phosphate but they are less effective compared to the organic acids at the same pH (Kim et al., 1997). However, acidification does not seem to be the only mechanism of solubilization, as the ability to reduce the pH in some cases did not correlate with the ability to solubilize mineral phosphates (Subba Rao, 1982). The chelating ability of the organic acids is also important, as it has been shown that the addition of 0.05M EDTA to the medium has the same solubilizing effect as inoculation with *Penicillium bilaii* (Kucey, 1988). Among nodule bacteria (e.g., *Rhizobium*/*Bradyrhizobium*), the phosphate solubilizing activity of *Rhizobium* was associated with the production of 2-ketogluconic acid which was abolished by

Table 1 A brief summary of production of principal organic acids by phosphate solubilizing microorganisms

Organism	Predominant acids	References
Phosphate solubilizing fungi and actinomycetes		
Aspergillus flavus, A. niger, *Penicillium canescens*	Oxalic, citric, gluconic succinic	Maliha et al. (2004)
A. niger	Succinic	Vazquez et al. (2000)
Penicillium rugulosum	Gluconic	Reys et al. (1999)
Penicillium radicum	Gluconic	Whitelaw et al. (1999)
Penicillium variable	Gluconic	Vassilev et al. (1996)
A. niger	Citric, oxalic,gluconic	Illmer et al. (1995)
A. awamori, A. foetidus, A. terricola, A. amstelodemi, A. tamari	Oxalic, citric	Gupta et al. (1994)
A. japonicus, A. foetidus	Oxalic, citric gluconic succinic, tartaric	Singal et al. (1994)
Penicillium bilaji	Citric, oxalic	Cunningham and Kuiack (1992)
A. niger, P. simplicissimum	Citric	Burgstaller et al. (1992)
A. awamori, P. digitatum	Succinic, citric, tartaric	Gaur (1990)
Penicillium sp.	Oxalic, itaconic	Parks et al. (1990)
Scwaniomyces occidentalis	Succinic, fumaric, citric, tartaric, α-ketbutyric	Gaur (1990)
A. niger	Succinic	Venkateswarlu et al. (1984)
Aspergillus sp., *Penicillium* sp., *Chaetomium nigricoler*	Oxalic, succinic, citric, 2-ketogluconic	Banik and Dey (1983)
Streptomyces	Lactic, 2-ketogluconic	Banik and Dey (1982)
A. fumigatus, A. candidus	Oxalic, tartaric, citric	Banik and Dey (1982)
Phosphate solubilizing bacteria		
Enterobacter intermedium	2-ketogluconic	Hoon et al. (2003)
Bacillus amyloliquefaciens, B. licheniformis, B. atrophaeus, Penibacillus macerans, Vibrio proteolyticus, xanthobacter agilis, Enterobacter aerogenes, E. taylorae, E. asburiae, Kluyvera cryocrescens, Pseudomonas aerogenes, Chryseomonas luteola	Lactic, itaconic, isovaleric, isobutyric, acetic	Vazquez et al. (2000)
Pseudomonas cepacia	Gluconic, 2-ketgluconic	Bar-Yosef et al. (1999)
Bacillus polymyxa, B. licheniformis, Bacillus spp.	Oxalic, citric	Gupta et al. (1994)
Pseudomonas striata	Malic, glyoxalic, succinic, fumaric, tartaric, α-ketobutyric	Gaur (1990)
Arthrobacter sp.	Oxalic, malonic	Banik and Dey (1982)
Bacillus firmus	2-ketogluconic, succinic	Banik and Dey (1982)
Micrococcus spp.	Oxalic	Banik and Dey (1982)
Bacillus subtilis, Bacillus spp.	Oxalic, succinic, citric, 2-ketgluconic	Banik and Dey (1983)

the addition of NaOH, indicating that phosphate solubilizing activity of this organism was entirely due to its ability to reduce the pH of the medium (Halder and Chakrabarty, 1993). However, the detailed biochemical and molecular mechanism of phosphate solubilization by symbiotic nodule bacteria are not known.

3.1.3 Production of Phosphate Solubilizing Microorganism Inoculants

The efficient PSM cultures are mass-produced for supply to the farmers as microphos. The production of microphos i.e., the preparation having microorganisms with phosphate solubilizing activity, includes three phases: the first concerns selection and testing phosphate solubilizing strains; secondly, inoculant preparation, including selection and processing of material carrier and mass culture of PSM; and thirdly, quality control procedures and distribution. For microphos production, peat, farmyard manure (FYM), soil and cow dung cake powder has been suggested as the suitable carrier (Kundu and Gaur, 1981). Finally, the cultures are packed in polybags and can safely be stored for about three months at 30 ± 2°C. In India, a microbial preparation termed Indian Agricultural Research Institute (IARI) microphos culture (Gaur, 1990) was developed that contained two efficient phosphate solubilizing bacteria (*Pseudomonas striata* and *Bacillus polymyxa*) and three phosphate solubilizing fungi (*Aspergillus awamori, A. niger* and *Penicillium digitatum*).

3.2 Mycorrhizae

Phosphorus is an essential element for plant nutrition and it can be only assimilated as soluble phosphate. However, in natural conditions, most of phosphatic soil content (soil mineral phosphate i.e., rock phosphate and organic phosphorus) is poorly soluble. Moreover, among soil microorganisms, arbuscular mycorrhizal fungi have been found to be essential components of sustainable soil- plant systems (Schreiner et al., 2003). The arbuscular mycorrhizal fungi increased plant uptake of phosphate (Bolan, 1991), micronutrients (Burkert and Robson, 1994), nitrogen (Barea et al., 1991), soil aggregation (Tisdall, 1994) and act as antagonists against some plant pathogens (Duponnois et al., 2005). Moreover, it has been demonstrated that plants inoculated with arbuscular mycorrhizal fungi utilize more soluble phosphate from rock phosphate than non-inoculated plants (Antunes and Cardoso, 1991). The main explanation is that mycorrhizas developed an extramatrical mycelium, which increased the root phosphate absorbing sites (Bolan, 1991). Since arbuscular mycorrhizal fungi are obligate endosymbionts and live on carbohydrates obtained from the root cells, all soil factors affecting plant growth and physiology will also modify fungal activity and, in turn, influence the structure and functioning of bacterial communities (Azaizeh et al., 1995). It is now well established that arbuscular mycorrhizal fungi modify root functions (i.e., root exuadation) (Marshner et al., 1997), change carbohydrate metabolism of the host plant (Schachar-Hill et al., 1995) and influence rhizosphere populations (Hobbie, 1992). Microorganisms in the hyphosphere of arbuscular mycorrhizal fungi may thus affect mycorrhizal functions such as nutrient and water uptake carried out by the external hyphae of arbuscular mycorrhizal fungi.

An intimate relationship between arbuscular mycorrhizal fungi and plants has existed for at least 350 million years (Remy et al., 1994). This co-evolution has involved numerous interactions at the ecological, physiological and molecular levels between these organisms during the long development of the symbiosis. Today, about 95% of the world's extant species of vesicular plants that are typically mycorrizal, making this association of fundamental importance in all ecosystems (Trappe, 1987). During the intergeneric interactions, the arbuscular mycorrhizal fungi are believed to enhance phosphate nutrition of plants by scavenging the available phosphorus due to the large surface area of their hyphae, and by their high affinity phosphate uptake mechanisms (Hayman, 1983). The role of arbuscular mycorrhizal fungi in improving plant phosphate nutrition and their interaction with other soil biota have been investigated with reference to host plant growth, but little is known about how these fungi affect the phosphorus status of soils. However, there are reports of organic acid production by arbuscular mycorrhizal fungi (Lapeyrie, 1988) that could solubilize the insoluble mineral phosphate. Production of organic acids by arbuscular mycorrhizal would certainly affect the availability of acid-labile insoluble phosphate and the whole issue of arbuscular mycorrhizal mediated increase in available phosphate needs re-examination. Among the mycorrhizal fungi, ectomycorrhizal fungi posses phosphate solubilizing activity (Lapeyrie et al., 1991) and are capable of utilizing phosphate from inositol phosphates. They also possess phosphatase activity, through which they affect the release of phosphate from soil organic matter (Koide and Schreiner, 1992). In addition, the arbuscular mycorrhizal fungi can exert a selective influence on soil microbial communities through a multiplication of α-ketoglutarate catabolising microorganism (Duponnois et al., 2005). However, more researches are required to precise the ecological relevance of arbuscular mycorrhizal symbiosis and their associated microbial communities. In addition, the arbuscular mycorrhizal fungi can also make iron phosphates available to developing crops (Bolan et al., 1987). However, the arbuscular mycorrhizal fungi in general, do not colonize plant roots strongly under phosphorus sufficient conditions (Amijee et al., 1989) and consequently the growth of certain plants have been found to be decreased by arbuscular mycorrhizal colonization in the presence of available phosphate (Son and Smith, 1995).

4 Phosphate Solubilizing Microorganisms as Inoculants for Sustainable Agriculture

The word "sustain," from the Latin *sustinere* (*sus*-, from below and *tenere*, to hold), to keep in existence or maintain, implies long-term support or permanence. As it pertains to agriculture, sustainable describes farming systems that are "capable of maintaining their productivity and usefulness to society indefinitely. Such systems must be resource conserving, socially supportive, commercially competitive, and environmentally sound. Thus the term sustainable agriculture means an integrated system of plant and animal production practices having a site-specific application that will, over the long term: satisfy human food and fiber needs, enhance environmental quality and sustain the economic viability of farm operations. It is achieved through management strategies, which help the producer select hybrids and varieties, soil conserving cultural practices, soil fertility programs, and pest management programs. The goal of sustainable agriculture is hence, to minimize adverse impacts to the immediate and off-farm environments while providing a sustained level of production and profit. However, the most important constraint-limiting crop yields in developing nations worldwide, and especially among resource-poor farmers, are soil fertility. Unless the soil fertility is restored in these areas, farmers can gain little benefit from the use of improved varieties and more productive cultural practices. Soil fertility can be restored effectively through the concept of integrated soil fertility management (ISFM) encompassing a strategy for nutrient management based on natural resource conservation, biological nitrogen fixation and increased efficiency of the inputs. In general, crop yields could be improved by enhancing the phosphate availability through the application of phosphatic fertilizers. However, the global energy crisis and dwindling resources have increased the cost of the chemical fertilizers and this trend is expected to continue. Increasing the level of food production without affecting cost benefit ratio is thus a challenging task ahead of the scientists worldover. Agronomists are therefore looking vigorously for an alternative source of phosphatic ferilizer to supplement or to replace in some cases the chemical fertilizers to ensure competitive yields of crops. So, alternate to chemical phosphatic fertilizers is the exploitation of various microbial processes encompassed in soil-root interface (rhizosphere). Microorganisms that colonize the rhizosphere, are actively engaged in phosphorus transformation in soil and transport phosphate to the plants. The use of phosphate solubilizing organisms in agronomic practices is advocated due to several reasons. For example, improve soil fertility through their sustained activities in the soil, increase plant growth and crop yield through increased nutrient availability, do not cause environmental pollution, improve soil heath and conditioning, protect plants against some soil borne pathogen and involves low cost technology for its production with high cost benefit ratio.

For agronomic purposes, phosphorus is second only to nitrogen as the most limiting element for plant growth. Phosphorus promotes nitrogen fixation in legume crops and is essential for photosynthesis, energy and sugar production (Saber et al., 2005). Microbial involvement in the solubilization of inorganic phosphate is well documented. Most of the studies on phosphate solubilization were however, centered on the isolation of the microorganisms from the rhizospheric soil and then evaluating their phosphate solubilizing activity under in vitro conditions. The investigations on solubilization of phosphorus under field conditions and its uptake by plants were however, started later. In this context, beneficial effects of the inoculation with PSM to many crop plants have been described (Zaidi and Khan, 2005; Zaidi et al., 2003, 2004). Nitrogen fixing bacteria are, perhaps, the most promising group of PSM on account of their ability to fix nitrogen symbiotically (legumes) or asymbiotically (non legumes) together with the ability of some strains to solubilize inorganic phosphatic compounds. Several publications have demonstrated that phosphate-solubilizing strains of *Rhizobium* and *Bradyrhizobium* and *Azotobacter* increase growth and phosphorus content in both nonleguminous and leguminous plants. An alternative approach for the use of PSM as microbial inoculants is either the use of mixed cultures or the co-inoculation with other microorganisms. In this regard, some results suggest a synergistic interaction between arbuscular mycorrhizal fungi and phosphate solubilizing bacteria, which allows for better utilization of poorly soluble phosphatic sources. Phosphate-solubilizing bacteria have already been applied as effective inoculant in agronomic practices for raising the crop productivity. For example, in the former Soviet Union a commercial biofertilizer under the name "phosphobacterin" was first prepared using *Bacillus megaterium* var. *phosphaticum* and later on was frequently applied in East European countries and India. In the following section how PSM are applied in sustainable agriculture will be discussed.

5 How is Phosphate Solubilizing Microorganisms Applied?

Since the introduction of PSM inoculants, the application of PSM inoculant to the seed surface prior to sowing is traditionally the most commonly used and easiest means of inoculation. When properly applied, this method ensures that each seed receives the introduced microphos. Disadvantages include a limitation of the quality that can adhere to the seed surface, direct contact with any seed applied chemicals, movement of PSM away from rooting zone and exposure to environmental stress after planting. However, the use of sticker solution e.g., gum arabic improves the adherence of the inoculant PSM on the seed. There are however, certain situations where seed applications may be an ineffective means of applications e.g., with seed dressed with pesticides incompatible with PSM. Under such circumstances, soil application may be followed. Inoculants applied to the soil have following advantages – greater population of PSM per unit area, direct contact with chemically treated seeds is minimized, elimination of seed mixing, and apparent ability to withstand low moisture conditions better than powder form. Thus in accordance with these considerations, two approaches can be applied for PSM inculation – firstly, the single culture approach (SCA) where phosphate solubilizing organisms can be used alone, and secondly, the multiple or mixed culture approach (MCA), often called as co-inoculation, where PSM are used along with the other beneficial rhizosphere microorganisms

6 Factors Affecting the Survival of Phosphate Solubilizing Microorganism Inoculants

The competitiveness of a phosphate solubilizing microorganism in natural environments will depend upon its ability to survive and multiply in soil. However, understanding of this part of the use of PSM is the most limiting factor and it is difficult, if not impossible, to predict the behavior and efficacy of the inoculated PSM in a particular location. In general, the population size or density of the artificially introduced PSM decline rapidly upon the introduction in soils (Ho and Ko, 1985). The survival of the inoculant strain thus, depends upon various factors, such as soil composition (Bashan et al., 1995), physiological status, temperature, pH, moisture content (Van Elsas et al., 1991) and the presence of recombinant plasmids (Van Veen et al., 1997). The biotic factors that affect the survival of the inoculated PSM include competition, predation and root growth that provide the substrates to the microbes.

7 Crop Response to Composite Inoculations

The soil is a habitat for a vast, complex and interactive community of naturally occurring soil organisms, whose activities largely determines the physicochemical properties of the soil and consequently promotes the growth of the crop plants. From seed germination until a plant reaches maturity, it lives in close association with soil organisms. The association is termed as rhizocoenosis (lynch, 1983) and hence, the rhizosphere that harbors heterogeneous microbial communities with sufficient phosphate solubilizing abilities becomes an important soil habitat. Therefore, the focus of attention has now been shifted from plant microbe interactions to plant microbe-microbe interactions. Recently, some instances have been reported where plant growth has been markedly enhanced using two or three member association of rhizospheric organisms and such syntropic associations are of paramount agronomic utility. In the present section, an attempt is made to address and discuss the interactions of PSM with other rhizospheric microorganisms and their impact on crop yield.

7.1 Interaction between Phosphate Solubilizing and Nitrogen Fixing Organisms

Nitrogen and phosphorus are the two major plant nutrients and combined inoculation of nitrogen fixers and PSM may benefit the plants better than either group of organisms alone. Interaction studies have been done both in vitro and in vivo (Sarojini et al., 1989). Phosphate solubilization was observed by the mixed cultures (e.g. *Pseudomonas striata*, *Bradyrhizobium* sp., *Mesorhizobium ciceri*) suggesting that they could be used as mixed microbial inoculant (Fig. 2). Further, no antagonistic behavior of one organism towards other was noted (Fig. 3a, b). Thus the mixed cultures or co-inoculation with other microorganisms are generally preferred over single inoculation treatments. In this context, nitrogen fixers and PSM when inoculated together colonized the rhizosphere and enhanced the growth of legumes by providing it with nitrogen and phosphate, respectively (Gull et al., 2004). The nitrogen fixing organisms, not only provide nitrogen to the plants but also improve nitrogen status of soil, alone or in combination with PSM. Accordingly, the application of phosphate solubilizing bacteria (*Pseudomonas striata*) and nodule bacteria gave significantly higher yield in greengram (Khan et al., 1997) and chickpea (Algawadi and Gaur, 1988) than obtained by the use of *Rhizobium* alone. Furthermore, *Rhizobium* and phosphate solubilizing fungi (*Aspergillus awamori*) when used as seed inoculant, increased the grain yield of chickpea under field conditions (Dudeja et al., 1981). Similarly, the effect of interactions between three phosphate solubilizing fungi namely *Aspergillus niger, A. fumigatus* and *Penicillium pinophilum* and nitrogen fixing *Rhizobium leguminosarum* biovar viciae showed significantly greater positive effect on growth, nutrient uptake (nitrogen and phosphorus) and consequently the yield of *Vicia faba* under field conditions (Mehana and Wahid, 2002). While a long term (10 years) trials using phosphate solubilizing bacteria and nitrogen fixing organism enhanced the seed production in soybean crop and was found more effective compared to superphosphate alone (Dubey, 2001). In a similar study, single or combined inoculation with PSM and nitrogen fixers had a positive effect on the yield and nutrient uptake of cereals and legume crops (Sarojini and Mathur, 1990; Kumar et al., 2001; Whitelaw, 2000).

A beneficial effect of phosphate solubilizer alone and in combination with nitrogen fixer on cotton in field (Kundu and Gaur, 1980) and wheat (Zaidi et al.,

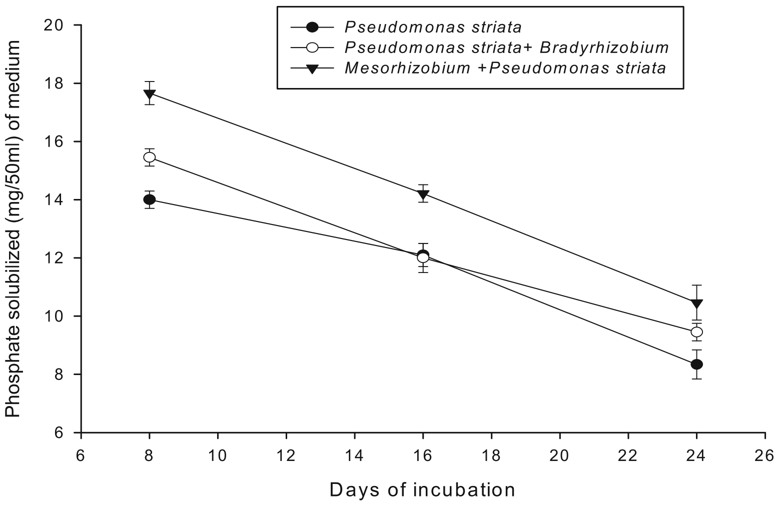
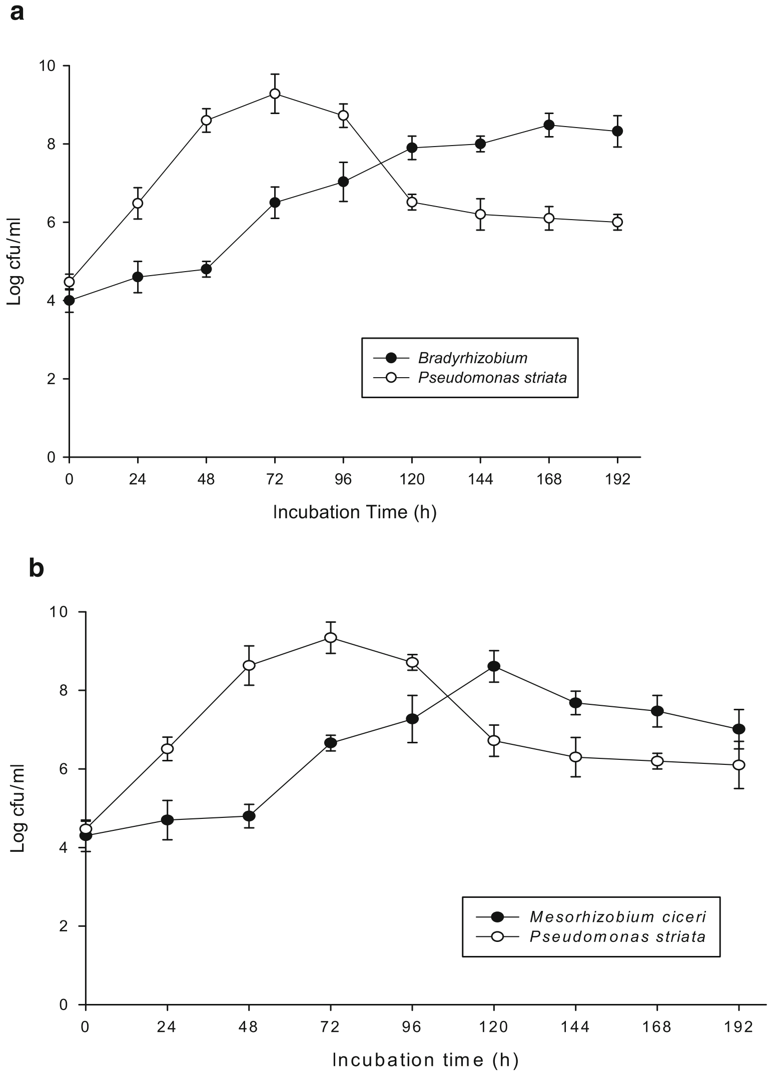


Fig. 2 *In vitro* solubilization of tricalcium phosphate by *Pseudomonas striata* alone and as influenced by *Bradyrhizobium* sp. (vigna) and *Mesorhizobium ciceri* in liquid Pikovskaya medium at different incubation days

Fig. 3 Synergistic relationship between *Bradyrhizobium* sp.(vigna) and *Pseudomonas striata* (**a**) and *Mesorhizobium ciceri* and *Pseudomonas striata* (**b**) in liquid Pikovskaya medium



2005) has been reported. Co-inoculation studies using *Rhizobium* and phosphate solubilizing bacteria have been carried out by many workers (Kucey, 1987; Downey and Van, 1990). Similar results were obtained for frenchbean (*Phaseolus vrisulga*) when inoculated with *Agrobacterium*, a phosphate solubilizer. In contrast, beans grown in autoclaved soil inoculated with phosphate solubilizing *Penicillum baliji*, and *R. phaseoli* showed no significant increase in dry matter or total uptake of phosphate (Kucey, 1987). Further, a decrease in total nitrogen fixation in field peas due to dual inoculation of *Penicillium bilaji* and *Rhizobium leguminosarum* have also been reported (Downey and Van, 1990). It was suggested that the adverse effect of *Penicillium bilaji* on nitrogen fixation might be explained by the mode of action of fungus. The phosphate releasing fungi produce more organic acids (Venkateswarlu et al., 1984), which enhance the solubilization of phosphate. However, most rhizobia prefer neutral or alkaline conditions during nodulation. These results suggest that before carrying out in vitro studies, the compatibility between the two associates must be checked in vivo.

Combined inoculation of *Rhizobium* and phosphate solubilizing *Pseudomonas striata* or *Bacillus polymyxa* with or without added fertilizers on chickpea yield and nutrient content was studied under greenhouse conditions. *Rhizobium* inoculation alone increased nodulation and nitrogenase activity, whereas the phosphate-solubilizing organism increased the available phosphorus content of the soil. The combined inoculation increased nodulation, available phosphate of soil as well as dry matter of the plants, grain yield and phosphorus and nitrogen uptake by the plants. The inoculation effects, however, were more pronounced in the presence of added fertilizers (Algawadi and Gaur, 1988). In a pot experiment, lentil seeds were inoculated with *Rhizobium leguminosarum* along with increasing doses (50, 100, 200, 400 kg/feddan, 1 feddan = 0.42 ha) of rock phosphate with or without a 1: 1 mixture of elemental sulphur and rock phosphate in the presence or absence of phosphate solubilizing bacteria. Plant dry weight and nitrogen, phosphorus, iron, zinc, manganese and copper uptake increased with rock phosphate, sulphur and phosphate solubilizing bacteria compared with untreated control. Dry matter yield and nutrient uptake was slightly higher with sulphur application (Saber and Kabesh, 1990). A combination of *Azotobacter chroococcum* GA-1 and GA-3 with *Penicillium* HF-4 and HF-5 and *Aspergillus* GF-1 and GF-2 increased radicle and plumule length but the remaining culture combinations decreased radicle/plumule length. A significant increase in mungbean yield and groundnut yield was observed with the inoculation of *Rhizobium* spp. and phosphate solubilizing bacteria along with phosphatic fertilizers (Khan et al., 1997, 1998). Moreover, the microbes that are involved in phosphate solubilization as well as better scavenging of soluble phosphate can enhance plant growth by improving the efficiency of biological nitrogen fixation, accelerating the availability of other trace elements and by production of phytohormones

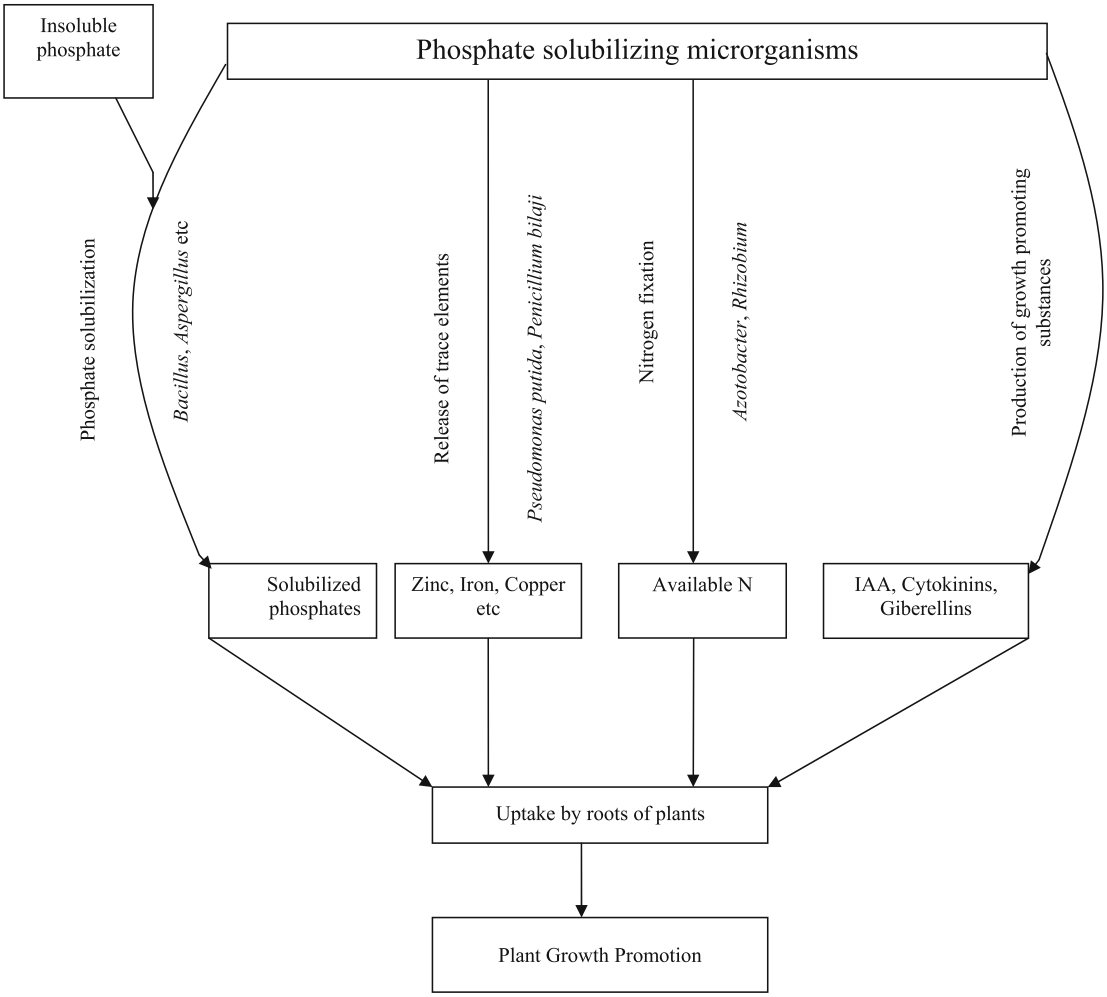


Fig. 4 Mechanism of plant growth promotion by phosphate solubilizing microorganisms

(Fig. 4). Accordingly, increase in yield of various legumes have been observed following seed or soil inoculation with nitrogen fixing organisms and PSM (Perveen et al., 2002) or PSM(s) and arbuscular mycorrhizal fungus (Mukherjee and Rai, 2000). It has further been suggested that about 50% of phosphatic fertilizer requirement could be saved by the combined inoculation of *Rhizobium* strain Tt 9 with *Bacillus megaterium* var. phosphaticum in groundnut. *Rhizobium* strain Tt 9 along with phosphobacteria at 75% phosphate level recorded higher nodule number; root length, shoots length and increased pod yield than the dual inoculation at 100% phosphorus level in groundnut (Natarajan and Subrammanian, 1995). However, no significant increase in phosphate contents in pigeonpea plant inoculated with *Rhizobium* (CCI) with *Bacillus megaterium* var. phosphaticum was observed (Gunasekaran and Pandiarajan, 1995). Similarly, about 37% increase in the grain yield of blackgram was reported following the inoculation of *Rhizobium* and *Bacillus megaterium* (Prabakaran et al., 1996). Gaind and Gaur (1991) reported improved nodulation, available $P_2 O_5$ content of soil, root and shoot biomass, straw and grain yield and nitrogen and phosphorus uptake by the moongbean [*Vigna raiata* (L.) wilczek] plants upon inoculation with thermo-tolerant species of phosphate solubilizing *Bacillus subtilus, B. circulans* and *Aspergillus niger*. Dubey and Billore (1992) showed an increase in yields of legumes after inoculation with rock phosphate and phosphate solubilizing bacteria, *Bacillus megaterium*, *Pseudomonas striata* and phosphate solubilizing fungus *Aspergillus awamori* and suggested to use low-grade rock phosphate for both neutral and alkaline soils with phosphate solubilizing inoculants. Effect of inoculation with phosphate solubilizing bacterium *Bacillus firmus*, irrigation schedules and phosphorus levels on lentil (*Lens esculentus*) and black gram (*Vigna mungo*) resulted in significantly greater seed yield in field trials during the winter seasons (Tomar et al., 1993). While, in a number of field trials carried out during 1991 and 1992 using soybean as test crop under rainfed conditions on vertisol, a significantly greater yield and phosphorus content in the plants were observed when seeds were bacterized with *Pseudomonas striata* and rock phosphate than compared to single super phosphate application (Dubey, 1996).

7.2 Symbioses between Phosphate Solubilizing Microorganism and Arbuscular Mycorrhizal Fungi

Mycorrhizal fungi are ubiquitous soil inhabitants and form symbioses with terrestrial plants (Jeffries, 1987). However, in association with nitrogen fixers, the arbuscular mycorrhizal fungi increase nitrogen and phosphatic nutrients of the plants, especially in phosphorus deficient soil. In addition, the PSM interact well with the arbuscular mycorrhizal fungi in phosphorus deficient soils or soils having rock phosphate (Poi et al., 1989). The PSM can release some phosphatic ions from otherwise sparingly soluble phosphorus sources (Barea et al., 1983), which is tapped and translocated by the arbuscular mycorrhizal fungal hyphae to the plant (Azcon-Aguilar et al., 1986). Moreover, the PSM survives longer around mycorrhizal roots compared to non mycorrhizal roots and acts synergistically with the arbuscular mycorrhizal fungus leading to increased plant growth, especially where rock phosphate is applied to soil (Singh, 1990). Simultaneous dual inoculation of arbuscular mycorrhizal fungi and PSM has been shown to stimulate plant growth more than inoculation of either microorganism alone in certain situations when the soil is phosphorus deficient (Piccini and Azcon, 1987). The simplest interpretation of this fact is that mycorrhizal endophyte could be stimulated in quantity, efficiency, and longevity. The main effect of this mycorrhiza in improving plant growth is through improved uptake of nutrients, especially phosphorus due to the exploration by the external hyphae of the soil beyond the root-hair zone where phosphorus is depleted. Besides root exudation and plasticity might change by PSM inoculation, which could also affect arbuscular mycorrhizal development. The arbuscular mycorrhizal fungi also produce plant hormones and increase the activity of nitrogen fixing organisms in the root zone (Bagyaraj, 1984). However a more thorough understanding of interactions between soil microorganisms is needed for an optimal utilization of these interactions with respect to growth and development of plants. When *centrosema macrocarpum* plants were inoculated with *Rhizobium* strains and arbuscular mycorrhizal fungi *Glomus manihotis* or *Acaulospora longula*, a significantly greater dry matter production, mineral absorption, nodulation and infection by arbuscular mycorrhizal fungi was recorded (Satizabal and Saif, 1987). However, a small amount of nitrogen fertilizer was suggested for application at the time of sowing. The dual inoculation of *Azotobacter chroococcum* and *Glomus fasciculatum* enhanced root infection of arbuscular mycorrhizal fungi, stimulated plant growth, and increased nitrogen, phosphorus and zinc contents in maize and wheat (Elgala et al., 1995). Similarly, a significant increase in dry matter yield of wheat plants following dual inoculation of rock phosphate solubilizing fungi (*Aspergillus niger* and *Penicillium citrinum*) and *Glomus constrictum* was recorded (Omar, 1998). Combined inoculation of *Rhizobium* and *Glomus etunicatum* and application of rock phosphate or PSM and arbuscular mycorrhizal fungus gave greatest yield and had variable effects on nodulation in clovers (Leopold and Hofner, 1991), mungbean (Zaidi et al., 2004), cowpea (Thiagarajan et al., 1992) and chickpea (Poi et al., 1989). The experiments have revealed that the establishment of arbuscular mycorrhizal fungus on to the root system can alter the rhizospheric microbial populations (Ames et al., 1984), which in turn affects the competitive interaction between introduced and native rhizobia for nodulation sites.

7.3 Tripartite Symbioses between Nitrogen Fixers, Phosphate Solubilizers and Arbuscular Mycorrhizal Fungi

In the earlier section the inoculation effects of single and dual cultures of nitrogen fixing and PSM on crop plants were discussed. However, very few reports are available on the effect of combined inoculation of crop plants with nitrogen fixers and PSM in the presence of arbuscular mycorrhizal fungus. During the inter-generic interaction, nitrogen fixing microorganisms provide nitrogen to the plants and consequently improve the nitrogen status of the soil while PSM enhance plant growth by providing it with phosphates. Where nitrogen and phosphorus are limiting, arbuscular mycorrhizal fungi may improve phosphate uptake for plants, the higher phosphorus concentration in plant benefits the nitrogen fixers and the functioning of its nitrogenase leading to increased nitrogen fixation, which in turn promotes root and mycorrhizal development. In a study Singh and Singh (1993) investigated the associative effect of *Bradyrhizobium japonicum*, arbuscular mycorrhizal fungus and phosphate solubilizing microbes on soybean in a mollisol. Inoculation with endophyte alone resulted in 70% root colonization. Addition of rock phosphates or inoculation with phosphate solubilizing bacteria except *Bacillus polymyxa* stimulated root infection of native as well as introduced arbuscular mycorrhizal endophytes. Application of rock phosphate with triple inoculation significantly increased grain yield, nodulation, nitrogen uptake and available soil phosphate. Further, Bethlenfalvay (1994) demonstrated the impact of *Glomus mosseae*, *Bacillus* sp. and *Rhizobium* sp. on plant growth and soil aggregation under *Pisum sativum* cultivation. Complex interaction was found among the three microorganisms, which in turn dramatically promoted plant growth and soil aggregation. It is evident from the earlier studies that a positive interaction exists between root colonization, phosphorus uptake and growth promotion as observed by a few workers (Zaidi et al., 2003; Zaidi and Khan, 2005). Inoculation of *Rhizobium*, *Bacillus polymyxa* and *Glomus faciculatum* resulted in significantly greater dry matter production and $PO4^-$ uptake as compared with single or double inoculation (Poi et al., 1989). However, no significant response of soybean to dual inoculation was observed (Kloepper et al., 1980).

8 Why Phosphate Solubilizing Microorganism Inoculations Fail?

Doubts have been raised on the ability of PSM to liberate phosphate under soil conditions (Tinker, 1980). Growth promotion and crop yields following superphosphate supplementation despite the abundance of PSM in the rhizosphere and bulk soils, raises important issues on the variations in the effectiveness of PSM inoculations. Why is phosphorus limited for plants when PSM(s) are abundant in soils? Many reasons were proposed to account for the variations in the effectiveness of PSM inoculations on plant growth and crop yields (Kucey et al., 1989): (1) survival and colonization of inoculated PSM in the rhizosphere, (2) competition with native microorganisms, (3) nature and properties of soils and plant varieties, (4) insufficient nutrients in the rhizosphere to produce enough organic acids to solubilize phosphorus, and (5) inability of PSM to solubilize soil phosphorus. It has been shown that at least two PSM showing phosphate solubilization in laboratory conditions could not release phosphate from alkaline vertisols even when supplemented with other nutrients (Gyaneshwar et al., 1998). Similarly no soluble phosphate was liberated when the non-sterilized bulk and rhizospheric soil samples were supplemented with carbon and nitrogen. The ability of the PSM to release phosphate from mineral phosphorus was significantly decreased possibly due to the high buffering capacity of the soils, coupled with PSM inability to secrete high concentrations of organic acids.

Moreover, despite the promising results, PSM based biofertilizers has not got widespread application in agriculture mainly because of the variable response of plant species or genotypes to inoculation depending on the bacterial strain used. Differential rhizosphere effect of crops in harboring a target phosphate solubilizing bacterial strain or even the modulation of the bacterial phosphate solubilizing capacity by specific root exudates may account for the observed differences. On the other hand, good competitive ability and high saprophytic competence are the major factors determining the success of a bacterial strain as an inoculant. Therefore, studies to know the competitiveness and persistence of specific microbial populations in complex environments, such as the rhizosphere, should be addressed in order to obtain efficient inoculants. In this regards, the efforts in order to obtain appropriate formulations of microbial inoculants, which protect the inoculant organism against environmental stresses and at the same time enhance and prolong its activity, may help in promoting the use of such beneficial bacteria in sustainable agriculture.

9 Application of Genetic Engineering in Developing Super Phosphate Solubilizing Microbial Inoculants

The performance of PSM inoculants depends heavily on their ability to colonize a particular rhizosphere habitat. The study of colonization of phosphate solubilizer provides useful information to solve the deficiency of phosphorus of soils. Plate counting and most probable number techniques have been used for the study of PSM communities in rhizosphere. However, in general, less than 1% of the microorganisms in the environment can be usually cultured by standard techniques because these techniques fail to reproduce in artificial media. The niches of many microorganisms are found in high diversity environment such as rhizosphere. However, using a strain specific DNA probes we can decipher a considerable number of heterogenous group of microorganisms in the rhizosphere (Cooper et al., 1998; Frederic et al., 2000). Thus the recent development of molecular biology techniques, which do not rely on cultivation methods, allows microbial ecologists to reveal inhabitants of natural microbial communities, which have not yet been cultured (Hugenholtz et al., 1998; Snaidr et al., 1998). As a result, these techniques are now widely applied to characterize microbial community structures in different environments (Holben et al., 1998; Nozawa et al., 1998).

Two of these techniques, cloning and sequencing, allows us to determine which microorganisms are present in the community, but they are time consuming. Hybridization and probing are faster, but requires a sufficient knowledge of the community to choose the appropriate target sequence (Amann, 1995). However, the other molecular biology techniques such as amplified ribosomal DNA restriction analysis (ARDRA) or ribosomal intergenic spacer analysis (RISA) can be applied to study the PSM colonization or community structure. Even faster than hybridization and probing, ARDRA and RISA have been used in the analysis of mixed bacterial population from different environments (Moyer et al., 1994; Martinez-Mercia et al., 1995). Although ARDRA gives little or no information about the type of microorganisms present in the sample, it can be used for a quick assessment of genotypic changes in the community over time, or to compare communities subject to different environmental conditions. In comparison, RISA is a method of microbial community analysis that provides a means of comparing differing environments or treatment impacts without the bias imposed by culture dependent approaches. In brief, RISA involves polymerase chain reaction amplification of a region of the rRNA gene operon between the small (16S) and large (23S) subunits called the intergenic spacer region (ISR). By using oligonucleotide primers targeted to conserved regions in the 16S and 23S genes, RISA fragments can be generated from most of the dominant bacteria in an environmental sample. While the majority of the rRNA operon serves as a structural function, portions of the 16S-23S intergenic regions can encode tRNAs depending on the bacterial species. However, the taxonomic value of the ISR lies in the significant heterogeneity in both length and nucleotide sequence. In RISA, thus we attempt to exploit the length heterogeneity of the ISR. The use of such molecular techniques having greater quantitative efficiency can be extended to study the colonization and characterization of PSM inoculants under diverse environmental conditions.

Moreover, the solubilization of mineral phosphate is carried out by organic acids, either by reduction in pH, or chelating the cations associated with phosphorus. A better understanding of the genetic basis of the release of organic acids could pave the way for transferring the mineral phosphate solubilizing (mps) ability to various bacteria that are competent to colonize a particular rhizosphere. Among the various factors, the rhizosphere competence is a focal point that decides the success or failure of the inoculant. Rhizosphere has tremendous amounts of carbon sources that are available to the heterogeneous microbial communities in soil to produce different kinds of organic acids. In addition, the solubilized phosphates can be utilized by the plant before it gets precipitated again. Among the various organic acids, gluconic acid seems to be the major mechanism of phosphate solubilization by Gram negative bacteria (Goldstein et al., 1993; Kim et al., 1998). Gluconic acid is produced by the oxidative metabolism of glucose by glucose dehydrogenase (GDH) which requires pyrroloquinoline quinine (PQQ) as a cofactor. Thus the genes involved in the biosynthesis/transport of PQQ can be cloned from various bacteria and be transferred to other bacteria (Babu-Khan et al., 1995). For instance, the rhizosphere competent bacteria (RCB) like *Rhizobium* posses apo-GDH, it would be interesting to transfer the genes involved in PQQ biosynthesis to *Rhizobium* to make an effective and better PSM. The resulting *Rhizobium* strains will thus have phosphate solubilizing activity in addition to its natural nitrogen fixing ability. Another important rhizosphere competent bacteria (*Pseudomonas* spp.) can form gluconic acid through the oxidative glucose metabolism and over expression of PQQ biosynthesis and GDH genes could also make them better PSM (Fig. 5). The alternative approach is to screen the mineral phosphate solubilizing (mps) genes directly in the target bacteria by over/under expression of genes followed by the selection of transformants with mineral phosphate solubilizing ability. Such an approach has been used to obtain mineral phosphate solubilizing genes from *Synechosystis* PCC 6803 in *E. coli* (Gyaneshwar, 1998). However, it remains to be seen if this will also be effective in other bacteria. Genetic engineering could also help in increasing the survival of the inoculant strains by incorporating the abilities to utilize certain nutrients better than the rest of the microbial populations (Glick and Bashan, 1997). Also genes for utilization of salicylate were transferred to a growth promoting bacteria and the recombinant bacterium was able to survive and enhance plant growth better than the wild type (Colbert et al., 1993).

10 Conclusion

The phosphatic fertilizer in current use requires a greater input that can not be afforded by the farmers of the developing nations. Microbiologists and soil scientists thus have a responsibility to the society to find ways and means of making phosphorus available to the crops, an economically efficient substitute for fertilization of crops. Since most soils are deficient in plant available phosphorus and chemical fertilizers are cost effective, there is interest in using rhizosphere competent bacteria or soil microorganisms endowed with phosphate solubilizing ability as inoculants to mobilize phosphate from poorly available sources in soil. Although potential clearly exists for developing such inoculants, their widespread application remains limited by a poor understanding of microbial ecology and population dynamics in soil, and by inconsistent performance over a range of environments. Furthermore, promotion of growth of agronomically important plants, as a consequence of microbial inoculation, may not necessarily be associated with characteristics such as phosphate solubilization, which are manifest under laboratory conditions. Further, in order to ensure food security in developing countries, there is an urgent need for the sustainable intensification of agricultural production systems towards supporting productivity grains and income generation. In this context, novel, genetically modified soil and region specific PSM(s)

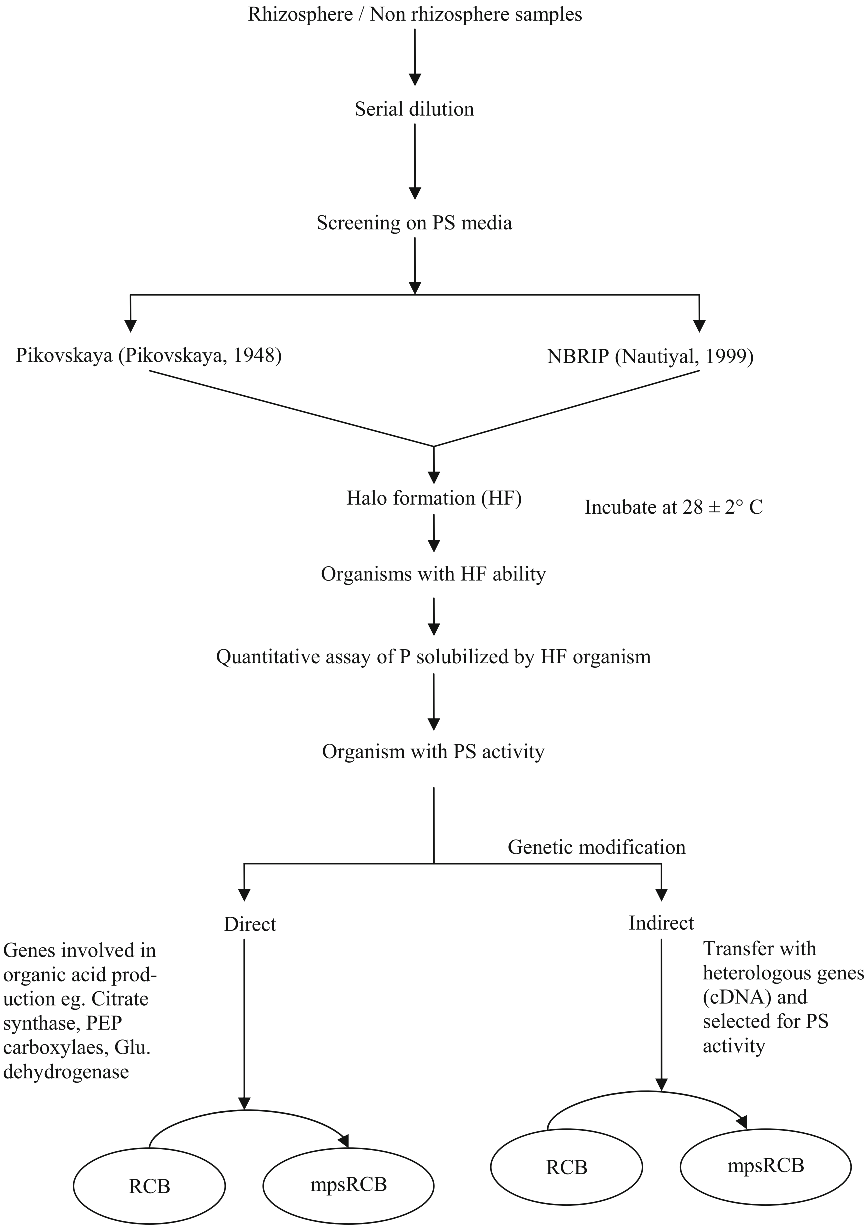


Fig. 5 Selection and genetic modification of phosphate solubilizing microorganism

and technologies for their ultimate transfer to the fields have to be developed, pilot tested and transferred to farmers in a relatively short time.

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Iron and Zinc Biofortification Strategies in Dicot Plants by Intercropping with Gramineous Species: A Review

Y. Zuo and F. Zhang

Abstract The lack of micronutrients such as iron and zinc is a widespread nutrition and health problem in developing countries. Biofortification is the process of enriching the nutrient content of staple crops. Biofortification provides a sustainable solution to iron and zinc deficiency in food around the world. Reports have highlighted the current strategies for the biofortification of crops, including mineral fertilization, conventional breeding and transgenic approaches. Any approach which could increase root growth and result in a high transfer of Fe and Zn from the soil to the plant is crucial for biofortification. In addition to these approaches, we draw attention to another important aspect of Fe and Zn biofortification: intercropping between dicots and gramineous species. Intercropping, in which at least two crop species are grown on the same plot of land simultaneously, can improve utilization of resources while significantly enhancing crop productivity, whereas monocropping is a traditional cropping system of only one crop growth. Monocropping has maintained crop productivity through heavy chemical inputs including the application of fertilizers and pesticides. Monocropping has therefore resulted in substantial eutrophication, environmental pollution, a food security crisis and economic burdens on farmers. Monocropping has also reduced the plant and microorganism diversity in the ecosystem. Compared with monocropped plants, intercropped plants can use nutrients, water and light better due to the spatial and temporal differences in the growth factors and a variety of species-specific mechanisms of physiological response to environmental stress. Intercropping is common in developing countries such as China, India, Southeast Asia, Latin America and Africa. In particular, interspecific interaction facilitates the iron and zinc nutrition of intercropping systems such as peanut/maize, wheat/chickpea and guava/sorghum or maize. Intercropping also increases iron and zinc content in the seeds. In a peanut/maize case study, the Fe concentrations in peanut shoots and seed were 1.47–2.28 and 1.43 times higher than those of peanut in monocropping, respectively. In intercropping of chickpea and wheat, the Fe contents in wheat and chickpea seed were increased 1.26 and 1.21 times, respectively, and Zn concentration in chickpea seed was 2.82 times higher than that in monocropping. In this review, we focus on exemplary cases of dicot/gramineous species intercropping that result in improved iron and zinc nutrition of the plants. We present the current understanding of the mechanisms of improvement of iron and zinc in intercropping. The available literature shows that a reasonable intercropping system of nutrient-efficient species could prevent or mitigate iron and zinc deficiency of plants. Here, we propose that intercropping can potentially offer an effective and sustainable pathway to iron and zinc biofortification.

Keywords Biofortification • Dicots • Gramineous species • Intercropping • Iron and zinc

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1 Introduction

The World Health Organization states that the lack of micronutrients such as iron and zinc represents a major threat to the health and development of populations in the world. Two billion people are anemic, many due to iron deficiency (WHO 2007). Billions of individuals are also at risk for zinc deficiency (Prasad 2003). Although food supplementation or fortification efforts have been effective in some countries, their overall success remains limited in developing countries. Biofortification, the process of enriching the nutrient content of crops as they grow, provides a sustainable solution to malnutrition in the world (Jeong and Guerinot 2008). Biofortification can be achieved by utilizing crop and soil management with plant breeding to increase micronutrient concentrations in the edible parts of crops. The concept of biofortification is attractive not only for improving the growing conditions of crops but also for exploiting a plant's potential for micronutrient mobilization and utilization. There have been several recent reviews on the current strategies for the biofortification of crops, including mineral fertilization, conventional breeding and transgenic approaches (Zhu et al. 2007; Mayer et al. 2008). In addition to these approaches, we would like to draw attention to another important aspect of Fe and Zn biofortification: intercropping between dicots and gramineous species, which are strategy I and Strategy II plants, respectively, in their response to iron deficiency. Relatively little attention has been paid to the effects of intercropping on crop micronutrient status. However, considering the importance of intercropping systems in nutrient acquisition and crop production processes, the management of intercropping would be the key to Fe and Zn biofortification.

Intercropping, which is the intermingled growth of two or more crops, is practiced in >28 million hectares of annually sown area in China (Liu 1994) and is also common in other parts of the world, such as India, Southeast Asia, Latin America and Africa (Vandermeer 1989). Multiple cropping, i.e., intercropping or intercropped cropping, plays an important role in agriculture because of the effective utilization of resources, significantly enhancing crop productivity compared with that of monocultured crops (Li et al. 1999, 2007). Facilitative root interactions in mixed cropping systems are most likely of importance for the nutritional improvement of crops grown in nutrient-poor soils and low-input agroecosystems (Zhang and Li 2003; Li et al. 2004). Recently, some research groups have reported that interspecific root interactions and rhizosphere effects could be linked to improved Fe and Zn nutrient uptake in dicot plants by intercropping with graminaceous species in pairings which included maize/peanut, guava/sorghum or maize and chickpea/wheat. For instance, maize/peanut intercropping was shown to improve Fe and Zinc nutrition of peanut (Kamal et al. 2000; Zuo et al. 2000; Gunes et al. 2007; Inal et al. 2007). In particular, as one of the important staple crops with high consumption, any increase in mineral nutrient content might have a significant effect on human nutrition (Graham et al. 1998; Graham and Welch 2001; Cakmak 2002). Presumably, if the nutritional quality of such staple crops can be improved by intercropping it would benefit human nutrition, particularly for the important micronutrients iron and zinc.

In this article, we concentrate on reviewing the literature on how intercropping dicots and gramineous species has been applied to advancing our knowledge specifically related to iron and zinc improvement in plants, and speculate on its future potential impact on biofortification. Hopefully, it will provide a significant component of integrated approaches, which include conventional plant breeding, transgenic approaches and mineral fertilization. The combined use of multiple strategies for iron and zinc improvement will offer a more effective and sustainable pathway to alleviating micronutrient malnutrition.

2 Improvement of Fe and Zn Uptake by Intercropping

2.1 Improvement of Fe and Zn Uptake in Peanut by Rhizosphere Effects from Maize in Intercropping

Iron deficiency is a common nutritional disorder in crop plants in China, particularly in northern China where aerobic and calcareous soils are widespread. Peanut (*Arachis hypogaea* L.) is the major oilseed crop in China, accounting for 30% of the total oilseed production in the country. Iron chlorosis is one of the most common yield-limiting nutrient problems in peanut grown in monocropping systems in the calcareous soils

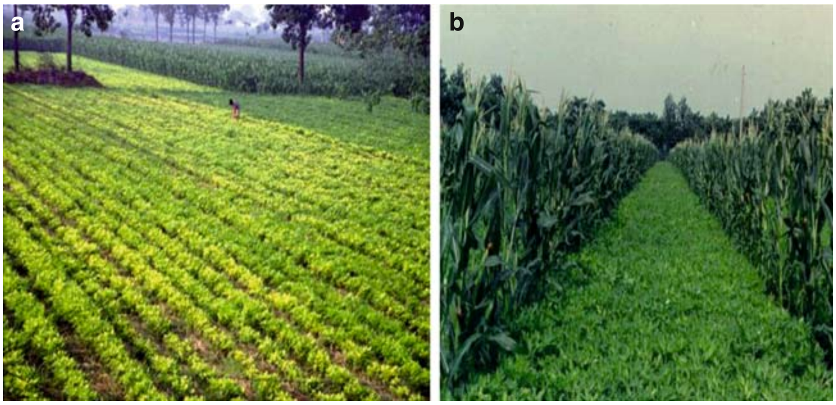

Fig. 1 Peanut growing in monoculture in the field with symptoms of Fe deficiency chlorosis (**a**), Peanut intercropped with maize in the field without symptom of Fe deficiency chlorosis in particular in the vicinity of maize (**b**)

Table 1 The effects of peanut intercropped with maize plants on Fe and Zn contents (mg kg^{-1} DW) in the shoot of peanut at the flowering stage in the field

Cropping systems	Distance from maize (Rows)	Fe	Zn
Monocropping peanut		205.8 ± 23.3^{c}	43.6 ± 5.2^{b}
Peanut/maize (danyu13)	1	302. 6 ± 21.4^{a}	54.8 ± 5.3^{a}
	2	290.0 ± 29.3^{a}	52.1 ± 6.1^{b}
	3	279.8 ± 40.2^{a}	50.6 ± 4.2^{b}

Columns with the same letter are not significantly different at 0.05, using the LSD multiple range test

of northern China (Zuo et al. 2000). In about 50% of these soils, the DTPA-extractable Fe concentration is lower than 5.5 mg kg^{-1} (DTPA: diethylenetriaminepentaacetic acid). For example, in most parts of Henan province iron chlorosis is very severe in peanut grown in monocropping systems in calcareous soil. (Fig. 1a). Calcareous soils are characterized by low organic matter, high pH (7.5–8.5) and high levels of bicarbonate. Soil amendment and foliar application of Fe fertilizers are usually ineffective or uneconomic measures for correction of Fe deficiency chlorosis. There is therefore considerable interest in devising practical approaches for the correction or avoidance of Fe deficiency in crops in Chinese agriculture. Chlorosis in peanut was much less pronounced when this species was intercropped with maize. This is a much more common cropping system than peanut monoculture in the region (Fig. 1b). The extent of improvement in the Fe nutritional status of intercropped peanut was found to be closely related to the distance between the peanut plants and the neighboring maize plants. The nearer the peanut plants to the maize, the less Fe chlorosis in peanut plants was observed. The severity of iron deficiency chlorosis in young leaves of peanut in the intercropping systems was closely related to the distance of the peanut plants from the maize roots when treatments were assessed during the peanut flowering period. In the unrestricted intercropping treatment, where neighboring roots of peanut and maize intermingled freely, the young leaves of peanut plants in rows 1–3 from the maize grew without visible symptoms of iron deficiency (Table 1), while those in rows 5–10 showed variable degrees of chlorosis. These results indicated that the comprehensive rhizosphere effects of maize played an important role in the improvement of the Fe nutritional status of peanut intercropped with maize under field conditions.

Based on the phenomena and evidence from the field, a greenhouse experiment was designed to test whether interaction between roots of maize and peanut has any effect on the Fe nutritional status of peanut in rhizoboxes. The only difference between the monocropping and intercropping systems in the rhizobox experiment was due to separation versus interaction between maize and peanut roots. The taller maize plants would have shaded the peanuts in both treatments, with or without root barriers, but chlorosis developed only in the former treatment. Since the

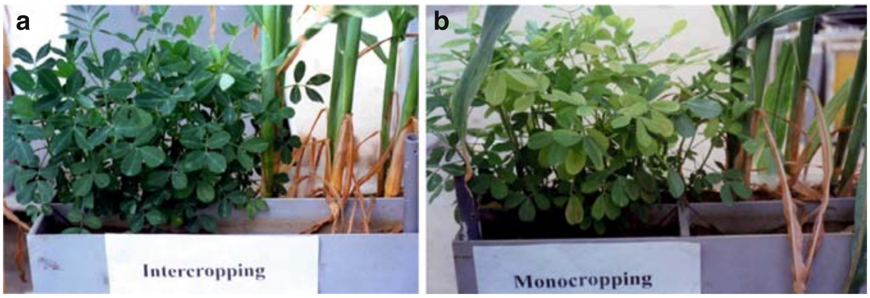

Fig. 2 Peanut grown in intercropping with root interaction of maize and peanut without symptom of Fe deficiency chlorosis. (**a**) Peanut grown in monocropping without root interaction of maize and peanut with symptom of Fe deficiency chlorosis (**b**)

Table 2 The effects of intercropping peanut with maize on Fe and Zn concentrations in peanut (mg kg^{-1} DW) grown on calcareous soil

Plant tissue	Monocropping	Intercropping	References
Fe			
Shoots	28.0 ± 7.0^b	65.5 ± 8.9^a	Zuo et al. 2000
Roots	159.5 ± 13.1^b	203.1 ± 16.8^a	
Seeds	22.2 ± 2.9^b	31.8 ± 3.9^a	
Zn			
Shoot	10.4	26.2	Inal et al. 2007
F values	14.01*		

All data were analyzed using SAS software, expressed as means of three replicates with standard deviation, and the means were subjected to another test by using the least significant difference (LSD) method at the 5% probability level (Zuo et al. 2000). Statistical significance of difference was determined by analysis of variance (ANOVA) and the LSD test at $P \leqslant 0.05$ for multiple comparisons (Inal et al. 2007) $^*P < 0.05$

peanut plants in both the monocropping and intercropping systems shared the same lighting conditions, it seems unlikely that the major interaction between the two species in rhizoboxes can be explained by a shading effect. The younger leaves of peanut plants remained green when the roots of maize and peanut interacted in the intercropping system (Fig. 2a), whereas chlorosis appeared on the youngest leaves when root interaction between the two species was prevented using a PVC barrier in the monocropping system (Fig. 2b). This indicates that maize could markedly improve the Fe nutrition of peanut plants. A more likely explanation for enhanced Fe nutrition was root interaction between maize and peanut. The Fe concentrations in various parts of peanut plants whose roots were allowed to mix with those of maize were generally higher than those whose roots were kept separate (Table 2). The Fe concentration in roots, shoots and seeds of peanut plants grown in the intercropping system without root barriers were 1.3, 2.3, and 1.4 times higher, respectively, than those of peanut plants grown with root barriers (Table 2). The chlorophyll concentration increased about threefold and the HCl-extractable Fe concentration doubled in the intercropping system (Zuo et al. 2000).

It was noteworthy that the maize not only improved the Fe status of peanut in the intercropping system, but intercropping also enhanced Zn content in the peanut (Table 2): this indicates that agronomic intercropping helps mobilize and uptake the limiting nutrient elements Fe and Zn as well as providing benefits through effects on plant growth, development and adaptability to adverse environments.

2.2 Improvement of Fe and Zn Uptake in Plants in Intercropping of Chickpea/Wheat by Interspecific Root Interactions

Malnutrition and micronutrient deficiencies, especially Fe and Zn, can be prevalent in many chickpea- and wheat-consuming regions, even though chickpea and wheat seeds are good sources of essential mineral nutrients (Graham et al. 1999; Welch and Graham 1999; Wang et al. 2003; Cakmak et al. 2004). However, in regions where wheat and chickpea are a significant component of the human diet, there appears to be considerable variation in the Fe and Zn concentrations present in the edible portions of the two crop species and their cultivars.

The ranges in Fe and Zn concentrations of wheat germplasm seeds grown in Mexico were 28.8–56.5 mg kg^{-1} and 25.2–53.3 mg kg^{-1}, respectively (Graham et al. 1999). Reported Fe and Zn concentrations (mg kg^{-1}) of chickpea seeds for more than 20 cultivars varied between Fe 39–98 and Zn 25–35 (Williams and Singh 1987). A breakthrough study was published in 2007 in which intercropping of wheat and chickpea improved the concentrations of Fe in wheat seeds, and Fe and Zn in chickpea seeds in the field experiment (Gunes et al. 2007). The concentrations of Fe and Zn in intercropped wheat shoots were significantly higher than in monocrop wheat. In chickpea, the Zn concentration was higher in intercropped chickpea than in the monocropped chickpea (Table 3). Intercropping could overcome potential Fe and Zn nutrient deficiencies, particularly in harvested seeds.

In another chickpea/wheat study under a different P supply (Li et al. 2004), the Fe content in wheat shoots was significantly increased by the complete interspecies root interactions (intercropping) between wheat and chickpea, compared with treatments without the root contact (roots being separated by a solid root barrier, monocropping). The Fe content was increased by the free interspecies root interactions compared with the treatment with the root barrier and inorganic P, suggesting mobilization of Fe from inorganic $FePO_4$ by chickpea (Table 4). Zinc content in wheat and chickpea shoots supplied with inorganic P was increased by interspecies root interactions. Compared with the treatment with the root barrier, however, complete interspecies root interactions (no barrier) between two species increased Zn content in wheat shoots, but decreased Zn content in shoots of chickpea supplied with organic P (Table 4). This study indicated that the Fe and Zinc concentrations vary in response to both genetic and environmental factors. The results from this glasshouse study need to be verified in the field.

According to those studies, we propose that wheat/chickpea intercropping can potentially offer a more effective and feasible method both for increasing Fe and Zn in grain/seed of wheat and chickpea and producing human foods with a higher micromineral nutrient content than when the two species are grown as monocrops. However, considering the importance of different intercropping systems in nutrient

Table 3 Shoot and seed iron and zinc concentrations per dry mass of wheat and chickpea grown as monocropping and intercropping in field conditions (modified from Gunes et al. 2007)

Cropping system	Fe (mg kg^{-1})	Zn (mg kg^{-1})
Shoot		
Wheat	28.69	5.71
Wheat intercropped	40.31	9.45
F test	**	**
Chickpea	70.65	5.01
Chickpea intercropped	80.11	13.63
F test	ns	**
Seed		
Wheat	36.58	25.09
Wheat intercropped	46.13	27.10
F test	*	ns
Chickpea	18.75	10.67
Chickpea intercropped	22.75	30.05
F test	**	**

$^{**}P < 0.01$, $^{*}P < 0.05$, and *ns* nonsignificant. Means within each column followed by different letters are significantly different by Duncan's multiple range test at $P = 0.05$

Table 4 Iron and zinc content of wheat and chickpea grown with two P sources in monocropping and intercropping treatments (modified from Li et al. 2004)

Treatments		Wheat		Chickpea	
		Fe (μg Fe pot^{-1})	Zn (μg Zn pot^{-1})	Fe (μg Fe pot^{-1})	Zn (μg Zn pot^{-1})
Organic P (phytate)	Monocropping	218b	209b	286a	138a
	Intercropping	475a	339a	193b	97b
Inorganic P ($FePO_4$)	Monocropping	240b	327b	321a	168b
	Intercropping	340a	440a	343a	204ab

Mean values of the three intercropping treatments with the same *P* source followed by different letters (a, b) are significantly different ($P \leqslant 0.05$)

acquisition and crop production processes, the management of intercropping would be the key to Fe and Zn biofortification.

3 Strategies for Fe and Zn Uptake in Plants

3.1 Physiological Responses to Increase Fe and Zn Uptake in Plant Species

In general, plant species develop physiological responses to increase iron uptake under a Fe-deficient environment which are characterized as "Strategy I" and "Strategy II" systems (Curie and Briat 2003; Hell and Stephan 2003; Schmidt 2003; Grotz and Guerinot 2006). It is well known that peanut and maize have distinctly different response mechanisms to Fe deficiency stress. Peanut and chickpea are 'strategy I' plants, while maize and wheat belong to the 'strategy II' group. Specifically, peanut displays Strategy I mechanisms, under conditions of Fe deficiency, where reductase activity is increased and release of protons and reductants is enhanced from the roots. Furthermore, plants use the reduction strategy to mobilize iron from the rhizosphere and ferric chelate reductase activity has been shown to be the rate-limiting step for iron uptake (Guerinot 2007). In calcareous soils, the high pH and large bicarbonate buffering capacity may render this strategy ineffective in the peanut due to decreased expression of a ferric chelate reductase (Bienfait 1988; Guerinot 2007). This strategy might not succeed even if Fe-efficient varieties of peanut were used in an attempt to overcome the iron chlorosis problem.

Strategy II plants are characterized by a higher Fe acquisition efficiency in soils with high pH and, in particular, high bicarbonate content through the excretion of phytosiderophores (PS) into the rhizosphere (Römheld and Marschner 1986), and thus have a high resistance to Fe deficiency stress compared with strategy I plants. Graminaceous plant species respond to Fe and Zn deficiency by exudation of phytosiderophores to increase the availability of Fe and Zn and transport PS-Fe(III) or PS-Zn from the rhizosphere to the root cell for uptake (Marschner et al. 1989; Cakmak et al. 1994). Grasses, which exude phytosiderophores in response to Fe deficiency, may also use this chelation strategy in order to obtain Zn from the soil. The mugineic acid family phytosiderophores (MAs) play a major role in iron (Fe) acquisition, and may also contribute to the acquisition of Zn and other metal nutrients by graminaceous plants (Römheld 1991; Welch 1995; Wirén et al. 1996). Therefore, exudate phytosiderophores from gramineous species have important ecological significance in calcareous soil.

3.2 Molecular Regulation of Fe and Zn Homeostasis in Plants

Significant progress on molecular aspects of Fe and Zn homeostasis in plants has been made in recent years in our understanding of how metals are obtained from the soil and distributed throughout the plant. For instance, in strategy I plants, Fe is first reduced on the root surface from ferric to ferrous ion by a plasma membrane-bound Fe(III) chelate reductase (FRO gene family) and subsequently translocated across the rhizodermal plasma membrane barrier by a high-affinity Fe(II) transporter such as the IRT gene family into the root cell (Varotto et al. 2002; Vert et al. 2002; Connolly et al. 2003; Mukherjee et al. 2005). Other Fe transporter genes, such as AtNRAMP3, AtNRAMP4, and AtVIT1, are all expressed in the vascular system of the roots and shoots, and the proteins that these genes encode appear to play a role in vacuolar Fe homeostasis (Thomine et al. 2003; Lanquar et al. 2005). Vacuolar Fe storage is also critical for seedling development that will ultimately aid to increase the chances of obtaining a nutrient-rich seed, benefiting both human health and agricultural productivity (Kim et al. 2006). Recent results confirmed that the FRD3-mediated efflux of citrate into the root vasculature is necessary for efficient Fe translocation; this process is important for the translocation of Fe to the leaves. FRD3 transports a small, organic iron-chelator that is necessary for the correct localization of Fe throughout the plant into the xylem (Durrett et al. 2007).

In strategy II plants, the first YS1 gene was identified from maize roots that could transport PS-Fe(III) from the rhizosphere (Curie et al. 2001). Recently, 18 YSL genes have been identified in rice, many of which are expressed in both the roots and the shoots

(Koike et al. 2004). In fact, the YSL genes also play a role in Strategy I plants. The YSL family, consisting of eight members in *Arabidopsis*, has been implicated in the intercellular transport of Fe chelates, specifically Fe complexed into nicotianamine (NA), which regulates Fe and Zn homeostasis and plays a role in Fe and nicotianamine seed loading (Le Jean et al. 2005; Schaaf et al. 2005). A number of small, organic molecules have been implicated in metal ion homeostasis as metal ion ligands to facilitate uptake and transport of metal ions with low solubility and also as chelators implicated in sequestration for metal tolerance and efficient storage of metals in fruits and seeds (Haydon and Cobbett 2007). A fuller understanding of the role of mugineic acid, nicotianamine, organic acids (citrate and malate), histidine and phytate as ligands for iron (Fe), zinc (Zn), copper (Cu), manganese (Mn) and nickel (Ni) in plants could make a significant contribution to our understanding of metal homeostasis in plants.

Although the molecular mechanisms for Zn efficiency are not understood, it has been suggested that an increased secretion of phytosiderophores by Zn-efficient plants would be involved. Furthermore, several ZIP proteins have been characterized in the Strategy II rice plant, suggesting that this protein family plays a role in the grasses (Ramesh et al. 2003). Genetic engineering approaches have been applied to increasing plant tolerance to low-Zn soils. At present, knowledge of the genes controlling specific steps of the Zn network in soil–plant systems is still rudimentary, but increasing rapidly. Transformation and overexpression of known Zn transporters from Arabidopsis to barley (Hordeum vulgare cv. Golden Promise) can increase plant Zn uptake and seed Zn content (Ramesh et al. 2004). Recently, a NAC gene was identified in wheat, which can accelerate senescence and increase Zn and Fe remobilization from leaves to developing grains (Uauy et al. 2006). These results clearly show the contribution of molecular genetic tools to manipulating Zn and Fe efficiency in crops and the potential for enrichment of the food supply with Zn and Fe. Novel breeding strategies, combined with improved agronomy practice, have been developed based on these genetic findings. However, there is no information on how Fe and Zn nutrient content of seeds is affected by intercropping at a molecular level. The fact that many of the molecular and biochemical changes in response to Fe and Zn deficiency occur in synchrony suggests that genes involved in Fe or Zn uptake and translocation are co-ordinately expressed in intercropping. Therefore, systematic studies are needed to understand the molecular mechanisms of improvement of Fe and Zn content in the seeds of staple crops.

4 The Mechanism of Improvement of Fe and Zn Uptake in Intercropping

4.1 The Potential Role of Phytosiderophores from Graminaceous Plants in Improvement of Fe and Zn Nutrition of Dicot Plants

Cropping systems such as intercropping may have numerous advantages in terms of increasing availability of micronutrients such as Fe and Zn. In the peanut (*Arachis hypogaea* L.)/maize (*Zea mays* L.) intercropping case, the excretion of phytosiderophores by maize into the rhizosphere plays an important role in improving Fe nutrition of peanut crops (Zuo et al. 2000). For strategy II plants, iron accumulation can be enhanced by the production of higher levels of phytosiderophores (Suzuki et al. 2006). There seems to be some cross-talk between the iron and zinc transport pathways because transgenic plants and mutants with overexpressed iron transporters also show increased zinc accumulation (Schaaf et al. 2005). Therefore, the possible reason for such differential effects on Zn concentrations of peanut plants caused by intercropping could be root exudates from gramineous species. Specifically, production and release of phytosiderophores from gramineous species may improve solubility of Fe and Zn by chelation, which helps plants obtain those essential elements from the soil (Rengel 2002; Schmidt 2003; Inal et al. 2007). In a study of peanut intercropping with different gramineous species, it was clearly shown that the incidence of chlorosis of peanut could be eliminated in intercropping with gramineous species such as two maize genotypes, barley, oats and wheat (Fig. 3). Moreover, there

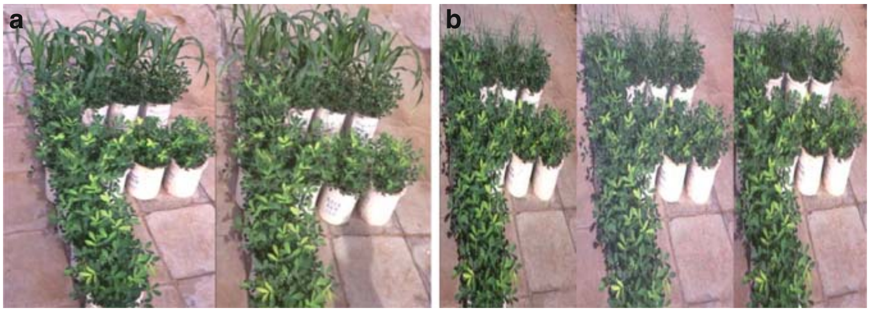

Fig. 3 The effects of six cropping systems on iron deficiency chlorosis symptoms in the young leaves of peanut plants at 60 days growth. (**a**) Peanut intercropping with two maize genotypes from left to right (danyu13, zhongdan2). (**b**) Peanut intercropping with barley, oats, or wheat from left to right. There are seven pots of monocropped peanut and four pots of intercropped peanut and gramineous species in each picture

is a strong, positive correlation between the amounts of phytosiderophores and the resistance of plants to iron deficiency (Mori et al. 1987; Takagi et al. 1988; Zhang et al. 1990).

Generally speaking, the release rates of phytosiderophores of barley, oats and wheat are much higher than those of maize under solution culture conditions (the order is barley > oats > wheat > maize). Plants that released more phytosiderophores positively correlated with improved growth in alkaline soils (Awad et al. 1994; Marschner and Römheld 1994; Shen et al. 2002). The results indicated that the effect of improved iron nutrition of the peanut by the two genotypes of maize (danyu13 and zhongdan2) is similar to that of barley, oats and wheat in intercropping, and the iron content in shoots of peanut plants intercropped with maize was lower than that of peanut plants intercropped with barley, oats or wheat (Table 5). In a greenhouse study, peanut intercropping with different gramineous species not only improved the iron nutrition of the peanut, but also enhanced zinc content in the peanut shoot. This suggests that the lower phytosiderophore levels produced by maize could be enough to improve iron nutrition of peanut in calcareous soil. It was not technically feasible to determine in the field the rates of synthesis and release of phytosiderophores of those gramineous species in different cropping setups, mostly because they cannot be recovered after release into the rhizosphere in soil conditions. It is difficult to answer directly the question of whether phytosiderophores play an important role in the improvement of iron nutrition in peanut, but there is some evidence to support the hypothesis.

Table 5 The effects of peanut intercropping with five gramineous plants on Fe and Zn contents (mg kg^{-1} DW) in the shoot of peanut at 60 days' growth in the greenhouse experiment

Treatments	Iron and zinc concentration in peanut
	Fe content
Monocropping peanut	190.5 ± 13.1^{c}
Peanut/maize(danyu13)	313.6 ± 16^{ab}
Peanut/maize(zhongdan2)	280.8 ± 24.3^{b}
Peanut/barley	330.3 ± 10.5^{a}
peanut/oats	345.7 ± 24.0^{a}
Peanut/wheat	362.9 ± 30.3^{a}
	Zn content
Monocropped peanut	109.8 ± 3.1^{c}
Peanut/maize(danyu13)	124.4 ± 7.2^{b}
Peanut/maize(zhongdan2)	119.9 ± 4.3^{b}
Peanut/barley	121.0 ± 6.9^{b}
peanut/oats	132.3 ± 2.6^{a}
Peanut/wheat	122.3 ± 10.3^{ab}

Columns with the same letter are not significantly different at 0.05, using the LSD multiple range test

4.2 *Ferric Reductase Capacity for Improvement of Fe and Zn Uptake in Intercropped Dicot Plants*

For strategy I plants, the inducible activity of Fe^{3+} chelate reductase reduces $Fe^{3+}$to Fe^{2+}, which is the rate-limiting step for Fe acquisition from soil (Ishimaru et al. 2007), so enhancing the Fe^{3+} chelate reductase activity of peanut plants renders those plants resistant to Fe deficiency. In a maize/peanut study in China (Zuo et al. 2003), the reducing capacity of peanut roots in monoculture increased in conjunction

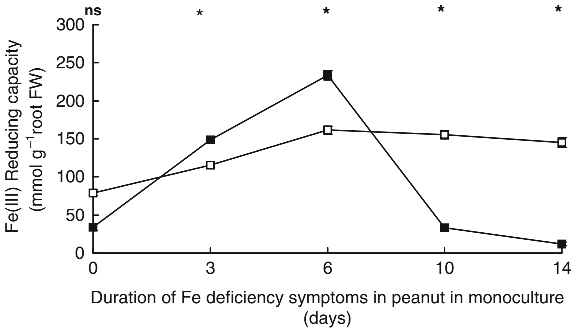

Fig. 4 Reducing capacity of peanut roots in monocropping and intercropping during the period of Fe deficiency symptoms in peanut in monocropping. Significance of difference between monocropping and intercropping by paired *t*-test: $*P < 0.05$; *ns* not significant. cropping type: *filled square*, monocropping; *open square*, intercropping. *Bars*: standard error of the mean ($n = 4$)

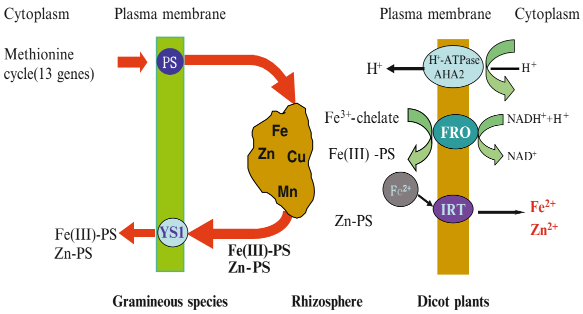

Fig. 5 Summary of the possible molecular and physiological mechanisms of improvement in iron and zinc nutrition of dicot plants intercropped with gramineous species

with the appearance of Fe deficiency chlorosis symptoms in young leaves. The maximum Fe(III)-reducing capacity of roots in monoculture occurred at 6 days and subsequently decreased rapidly. By the fourteenth day, when peanut showed severe Fe deficiency in monoculture, the reducing capacity of the roots was lower than that of peanut that had no Fe deficiency symptoms from the intercropped culture system. In contrast, the reducing capacity of peanut roots grown in intercropping with maize increased very slowly, and was greater than that of peanut roots from monoculture after the appearance of Fe deficiency chlorosis in monoculture at 10 days (Fig. 4). In another maize/peanut case in Turkey, the results also indicated that the root Fe(III)-reducing capacity of peanut was found to be significantly higher in intercropping (0.56 mmol Fe g^{-1} FW h^{-1}) than that of monocropped peanut (0.29 mmol Fe g^{-1} FW h^{-1}) (Inal et al. 2007). Those studies confirmed that maize/peanut intercropping could keep a higher ferric reduction capacity of peanut roots for a longer time period than that of monocropping, indicating that intercropping could enhance Fe^{3+}chelate reductase of peanut, which helps peanut plants tolerate Fe deficiency in calcareous soil.

Based on available research evidence, Fig. 5 shows the possible mechanisms of improvement of iron and zinc nutrition of dicot plants in this review. In dicot plant/gramineous species intercropping systems in calcareous soil, the release of phytosiderophores by strategy II plants not only acquires Fe to meet their demand, but also improves Fe and Zn uptake of strategy I plants. Compared with monocropping dicot plants, one possible explanation is that gramineous species released phytosiderophores into the rhizosphere of dicot plants and helped to make much more phytosiderophore-Fe available to dicot plants in intercropping; however, there is no phytosiderophore-Fe available to dicot plants in monoculture. Although peanut does not produce phytosiderophores (PS) under Fe deficiency, phytosiderophore-Fe chelates from maize should exist in the rhizosphere of peanut intercropped with maize. For strategy I peanut plants, it has to reduce solubilized Fe(III) by a membrane-bound Fe(III) chelate reductase and subsequent transport of the resulting Fe(II) into the plant root cell by a Fe(II) transporter. Phytosiderophore-Fe is one of the Fe(III) states which are more easily reduced and taken up by dicots than other Fe(III) forms (Hopkins et al. 1992; Jolley and Brown 1994), We infer from those studies that intercropping provides more phytosiderophore-Fe, which is easily reduced and absorbed by peanut. Furthermore, a higher ferric reduction capacity of dicot plant roots for a longer time period in intercropping may have assisted in the mobilization of sparingly soluble Fe(III) compounds from the rhizosphere so that the dicot plants remained green. A noteworthy advance in Fe and Zn research in plants is that there seems to be cross-talk between the iron and zinc transport pathways, because transgenic plants and mutants with overexpressed Fe(III) reductases and iron transporters also show increased zinc accumulation (Zhu et al. 2007). Those combined factors may thus have contributed to the improvement in Fe and Zn nutrition of dicot plants in intercropping with gramineous species.

5 Conclusion

Biofortification of iron and zinc content in plants is an emerging international research area of plant nutrition. Anemia as a result of iron deficiency afflicts more than two billion people worldwide, especially in developing countries including China (http://www.harvestPlus.org/iron.html). Biofortification of iron and zinc content and availability in plant foods could be an economical solution to this problem (Nestel et al. 2006; Yan et al. 2006). Enriching the nutrition contribution of staple crops through plant breeding, transgenic crops and mineral fertilization are significant tools in the fight against human malnutrition. Micronutrient-dense crop varieties are being developed using the best traditional breeding and modern biotechnology methods to achieve increases in nutrient concentrations. However, feasible and cost-effective approaches are needed, especially to reach the rural poor in developing countries. In this review, maize/peanut, chickpea/wheat and guava/sorghum or maize intercropping could overcome iron and zinc nutrient deficiencies, particularly in harvested seeds. The development of ecologically and economically viable strategies to prevent iron zinc deficiency represents the goal of the biofortification of crops.

The studies suggest that a rational intercropping system of nutrient-efficient species should be considered to prevent or mitigate iron and zinc deficiency of plants in agricultural practice. It will be one of a number of approaches to produce more biofortified crops. More researchers are becoming aware that increasing bioavailability of micronutrients in the edible parts of staple crops through agricultural management is a cost-effective and sustainable way to alleviate micronutrient malnutrition. Although significant progress has been made in recent years in our understanding of how metals are obtained from the soil and distributed throughout the plant, there is still a lack of knowledge of how Fe and Zn micronutrients behave in intercropping systems of strategy I and strategy II plants. Substantial efforts are being made aimed at increasing plant Fe and Zn nutrient efficiency in intercropping at the molecular, cellular and whole-plant levels. This requires a multidisciplinary research approach, a willingness among scientists to communicate across disciplinary boundaries, and innovative funding strategies to support the research and ultimate dissemination of the biofortified seeds. Strategies for intercropping dicot plants and gramineous species could potentially contribute to iron and zinc biofortification in a more practical, effective and sustainable manner in developing countries.

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Soil Exploration and Resource Acquisition by Plant Roots: An Architectural and Modelling Point of View

Claude Doussan, Loïc Pagès, and Alain Pierret

Abstract We provide a brief overview on soil exploration and resource extraction by roots during the course of plant development. First, we examine how roots explore the soil volume in relation with the heterogeneity of soil conditions. We then consider resource acquisition by roots and root systems, taking into account the root system's heterogeneous functioning and its variable degree of plasticity. In extensive, complex and dynamic systems such as root systems, processes of soil exploration and resource acquisition can be analysed through the unifying point of view of root system architecture. We exemplify how a modelling approach based on the concepts of functional architecture has potential to provide sharper insight into the soil exploration/utilization processes.

Keywords Architecture • Model • Review • Root system • Soil exploration

Résumé Nous présentons une courte revue décrivant les grandes étapes de colonisation et d'exploitation du sol durant le développement de la plante. Nous montrons tout d'abord comment les racines explorent le sol en interagissant avec l'hétérogénéité des conditions de sol (du point de vue des nutriments ou des contraintes à la croissance). De cette vision essentiellement géométrique (exploration), nous passerons à l'exploitation des ressources par les racines en considérant l'hétérogénéité de fonctionnement du système racinaire ainsi que son plus ou moins fort degré de plasticité. Ces processus d'exploration et d'exploitation du sol seront présentés sous l'angle unificateur de la prise en compte de l'architecture racinaire. Dans des systèmes complexes et très dynamiques, comme un système racinaire, nous montrerons, avec des exemples récents, comment la modélisation, fondée sur une architecture fonctionnelle, permet d'aller plus loin dans la compréhension des processus d'exploration/exploitation du sol par les plantes.

Mots clés Architecture • Exploration du sol • Modélisation • Système racinaire

1 Introduction

Root systems of terrestrial plants have a functional importance in resource storage, synthesis of growth regulators, and propagation. However, the primary functions of root systems are anchorage of the plant in the soil and the acquisition of soil resources, i.e. mainly water and mineral nutrients (Fitter, 2002). In soils, roots are exposed to highly heterogeneous and variable conditions both in space and time. Constraints to root growth often originate from this variability in resource availability (water and nutrients) and physico-chemical properties. The development and propagation of an extensive and structured root system represents plants' evolutionary response to the spatio-temporal variability of resources availability and the associated constraints to growth (Harper et al., 1991). The extension in space and time of the root system obeys developmental rules susceptible of modulation by interaction with the environmental conditions. Moreover, the root

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system should be viewed as a population of roots with varying, although coordinated, morphological, and physiological (particularly uptake) properties. In addition, physiological heterogeneity also exists along single root axis in relation to ageing.

In this review, we first examine how roots explore the soil volume in relation to the heterogeneity of soil conditions. From this purely geometrical viewpoint of soil exploration, we then consider the impact of roots' functional heterogeneity and plasticity on the root system's overall ability to use soil resources. In extensive, complex and dynamic systems such as root systems, processes of soil exploration and resource mining can be analysed through the unifying point of view of root system architecture. We exemplify how a modelling approach based on the concepts of functional architecture, has potential to provide sharper insight into the soil exploration/utilization processes. We also show how such a modelling approach represents a way to quantify soil exploration by roots at scales ranging from the individual root to the entire root system.

This review is specifically focused on root architecture, the influence of mycorrhizae on nutrient uptake is not discussed. The influence of mycorrhizae on nutrient acquisition by plants is well know and has been widely reported (see Marschner, 1990; Wilcox, 1996). In addition, it has been shown that mycorrhizae can modify the overall root system architecture (Hooker et al., 1992). Likewise, the modification of rhizospheric soil by root-induced chemical processes, e.g. modifications induced by specialised roots such as cluster roots (Skene, 2000), is not discussed in this review (see Hinsinger [1998] for a review of these processes).

2 Roots Systems as a Response to the Heterogeneous Distribution of Resources and Soil Constraints to Root Growth

2.1 *Soil Water and Nutrients Heterogeneity in Time and Space in Soils*

The availability of hydro-mineral resources is variable at spatial scales ranging from a few mm ("hot spots", Parry et al., 2000), a few centimeters (gradients in O_2, water availability, pH, nutrient status [Lynch, 1995]) to several meters. This variability in resource availability can be related to e.g. variations in soil texture (Zobel, 1992), topography, climate, vegetation, or soil management. The subterranean activity of soil fauna can create localised zones of fertile loose soil in otherwise poor soil horizons (Lynch, 1995; Pierret et al., 1999). Heterogeneous patterns may persist in time from a few days to several years. For example, a burst of mineralization may occur for some days and induce nitrate leakage into deeper soil horizons (Van Vuuren et al., 1995), while phosphate enriched patches may remain immobile within a few centimetres for years (Lynch, 1995). Snow thawing in spring flushes mobile nutrients accumulated in surface horizons. In such situations, roots must respond rapidly to the temporal pulse of resources before they are leached away from the root zone.

Examples of variations in nutrient availability under natural soil conditions, at the root scale, are given by Jackson and Caldwell (1993) and Robinson (1996). These authors studied the distribution of mineral nutrients (extractable phosphate, nitrate and ammonium) in a sagebrush-steppe (dominated by *Artemesia Tridenta*) in 0.5 × 0.5 m plots. Phosphate exhibited a strong spatial variability with concentrated patches (around 31 mg kg^{-1}) and less concentrated zones (around 20 mg kg^{-1}; Fig. 1) and strong positive associations

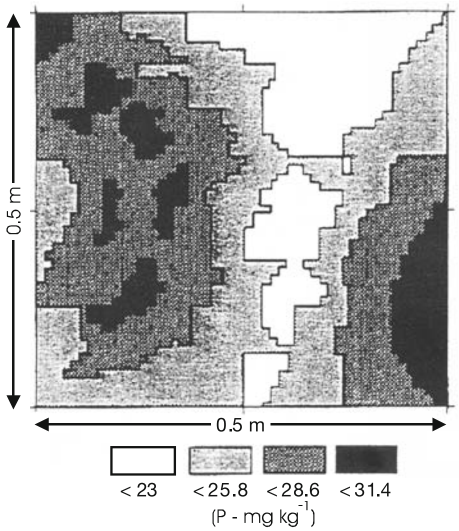

Fig. 1 Spatial distribution of phosphate in the topsoil of a 0.5 × 0.5 m area of sagebrush steppe. Phosphate concentration scale in mg kg^{-1} is shown as shaded boxes (from Jackson and Caldwell, 1993)

with other variables such as soil organic matter, potassium, nitrification and net N mineralization. Nitrogen compounds (NH_4 and NO_3) varied substantially too (386% and 116% respectively) but were less spatially dependent to other variables than P. As Robinson (1996) pointed out, patchiness is more likely to occur in soils for less mobile ions, such as P. In a situation of P availability such as the case illustrated in Fig. 1, different parts of a single root system and even single roots may experience very different P concentrations. This natural variability may be enhanced in cultivated soils, depending on agricultural practices. At the field scale, in a highly managed (highly fertilised and irrigated) salad crop (*lactuca sativa*), $N - NO_3$ concentrations ranged between 20 and 200 kg ha^{-1} (Bruckler et al.) with mostly non-normal distributions at different sampling dates. Depending on the sampling date, spatial patterns of concentrations were more or less expressed. In particular, these authors showed that spatial variability of nitrate was time dependent; the locations with the highest initial concentrations retained the highest values with time (and conversely), but this correlation weakened and finally vanished after 2–3 months under these particular crop/agricultural and climatic conditions. Tillage in cultivated systems seems to have no effects on variability (Robinson, 1996), but tends to reduce soil patchiness by increasing the patches size.

In conjunction with resource heterogeneity, root systems have to cope with the contrasting behaviour of soil nutrients. Some ions are relatively mobile (such as nitrate) and can move for some distance (i.e. centimetres) from the bulk soil towards the roots. They can be taken up by mass flow and diffusion. Others nutrients (e.g. phosphate) diffuse much more slowly in soil, due to interactions with the solid phase, and uptake necessitate that roots (or mycorrhizae) intercept the nutrient (depletion zone around roots within the millimetre range – Marschner, 1990; Jungk, 2002; Hinsinger, 1998). For fast growing species (such as annual crops) this implies a continuous exploration of new soil domains where less mobile nutrients have not already been depleted by root uptake (Lynch and Brown, 2001).

Last but not least, the pattern of variability of the hydro-mineral resources also differs in time and space between the different resources. For example, on the one hand, water might be available at depth, while shallow horizons are relatively dry. On the other hand, mineral nutrients (e.g. phosphate, Liao et al., 2001) are often more available in the surface horizons.

2.2 Heterogeneity of Soil Constraints to Root Growth

Physico-chemical conditions in soils may adversely affect root exploration of the soil. As for nutrients, adverse conditions vary in time and space, and roots need to develop strategies to adapt or avoid such conditions.

Among chemical stresses, it seems that strongly acidic subsoils, with pH less than 5, represent one of the most important limitations to root penetration (Foy, 1992). In this case, in relation with pH dependent solubility of ions and geochemical reactions, roots may be exposed to severe H, Al, Mn toxicity and Ca, Mg, Mo, P deficiencies. Root growth will then be restricted to the thin topsoil.

Aeration of the soil can also restrict soil exploration by roots. Temporary flooding (e.g. heavy seasonal rainfall on poorly draining soils) can induce a dramatic drop of soil oxygen concentrations (from $\sim$20% to a few percent) within a few hours to a few days, depending on the temperature and biomass respiration (Drew, 1983). Except for wetland plant species, when roots get trapped in oxygen depleted soil, they stop growing and eventually die (Amstrong and Drew, 2002). Fine textured subsoils with a shallow water table are characterised by permanent more or less anoxic/reducing conditions. Roots of most plant species are unable to colonise such soils. Soil temperature is also of major importance to soil colonisation by roots (Cooper, 1973). Temperature gradients with soil depth exist throughout the year and the highest range of temperature variations is in the surface layers (Chanzy, 1991). Management of cultivated soils (e.g. tillage or irrigation) modifies the soil thermal regime. Optimal, minimum and maximum (Bowen, 1991) temperatures for root growth vary depending on species and their origin ($\sim$17°C to 35°C, McMichael and Burke, 2002). The downward penetration rate of the rooting front varies with soil temperature and a good correlation between this penetration rate and a particular soil isotherm is sometimes observed (Kaspar and Bland, 1992). Temperature and temperature gradients affect not only root growth but also root initiation, branching and orientation (Bowen, 1991; Kaspar and Bland, 1992; Klepper, 1992).

Finally, one of the most common physical limitations to soil exploration by roots is the presence of zones of high mechanical resistance (Hoad et al., 1992). Zones of high soil strength can originate from compaction, which induces an increase in bulk density (plow pan or subsoil compaction due to tillage or wheel traffic), or from a decrease in water content (Bengough, 1997). High soil strength can also be related to specific, genetic, soil horizons (indurated zones, duripans, fragipans, ortstein, . . .) (Bennie, 1996). In cultivated soils, the location and thickness of high strength zones vary during the growing season (Castrignano et al., 2002). Increases in soil strength reduce root growth and the average number of laterals on primary axes (Bennie, 1996). Soil structure of high strength zones is of prime importance to root penetration. In such zones, roots tend to follow cracks and (bio) pores in which they can fit. It was shown that roots of a new crop (corn) re-colonised pores from the preceding crop (alfalfa) (Rasse and Smucker, 1998). In hardsetting soils of clay B-horizon, Stewart et al. (Stewart et al.) showed that roots grew in macropores, but a large proportion (80%) was also located in the soil within 1 mm of macropores, a zone defined as the macropore sheath. Pierret et al. (1999) and Pankurst et al. (2002) showed that the chemical and biological properties of this *macropore sheath* and of small aggregates associated with roots located within macropores differed largely from the bulk soil (for example, bacteria were 5–10 times greater in the macropore sheath and showed a greater metabolic activity; C, N, and P concentrations showed higher levels in the macropore sheath).

2.3 Roots and Root System Architecture

As they grow, plants must adjust to the spatio-temporal variability of resources availability and constraints to root growth. The root system development represents plants' evolutionary response to this spatio-temporal variability (Harper et al., 1991). The overall length of mature plants' root system might be quite important and values of 1 to more than 10 kilometres have commonly been reported (up to 500 km for 16-week-old winter rye plant) (Dittmer, 1937; Krasil'Nikov, 1958). Maximum rooting depths are also highly variable among species and biomes (from 0.3 m for some tundra species up to 68 m for *Boscia albitrunca* in Kalahari Desert): trees, shrubs and herbaceous plants, (bulked as functional groups for a wide range biomes) have been reported to have an average rooting depth of 7, 5 and 2.6 m respectively (Smit et al., 2000).

It has been recognised for quite a long time that the appearance of root systems *in situ* (e.g. dominance of the main axis, branching pattern. . .) can vary greatly, even within species, and be quite complex (Fig. 2; Kutschera, 1960; Weaver, 1919; Cannon, 1949). The 3-dimensional, dynamic, development of the root system within the soil volume is on the one hand, genotypically driven, and on the other hand, environmentally influenced, as the heterogeneity of resource availability constrains root growth.

Roots can be classified into three main categories according to their ontogenesis: primary, nodal and lateral roots (Pagès et al., 1998; Klepper, 1992; Harper et al., 1991). A single-axis root system, or taproot system, with dominant vertical growth (gravitropism), emerges first. Then, a primary root differentiates from the seed's radicle. Adventitious (or nodal) roots differentiate from organs other than roots (e.g. rhizomes, stems, leaves, . . .) and are initiated at precise locations (near stem nodes for example) with a defined temporal pattern. They are often abundant and give rise to a fibrous root system. Adventitious roots are much less sensitive to gravitropism than primary roots (Klepper, 1992). The ability to produce adventitious roots is a genotypic feature (Schiefebein and Benfey, 1991). Lateral roots originate from the branching of a parent axis, generally at right angle, and differentiate from parent roots younger tissues, at some distance from the apex. This process results in a branching front which follows the parent root's apex (acropetal branching). Even when acropetal branching is the dominant branching process, some lateral roots may appear out of the main sequence, differentiating from older tissues near the base of the tap root for example. The maximum number of branching orders seems to be a genotypic feature. Branching can also develop by reiteration. In this process, the parent axis duplicates into two (or three) axes of the same morphological type and creates forks

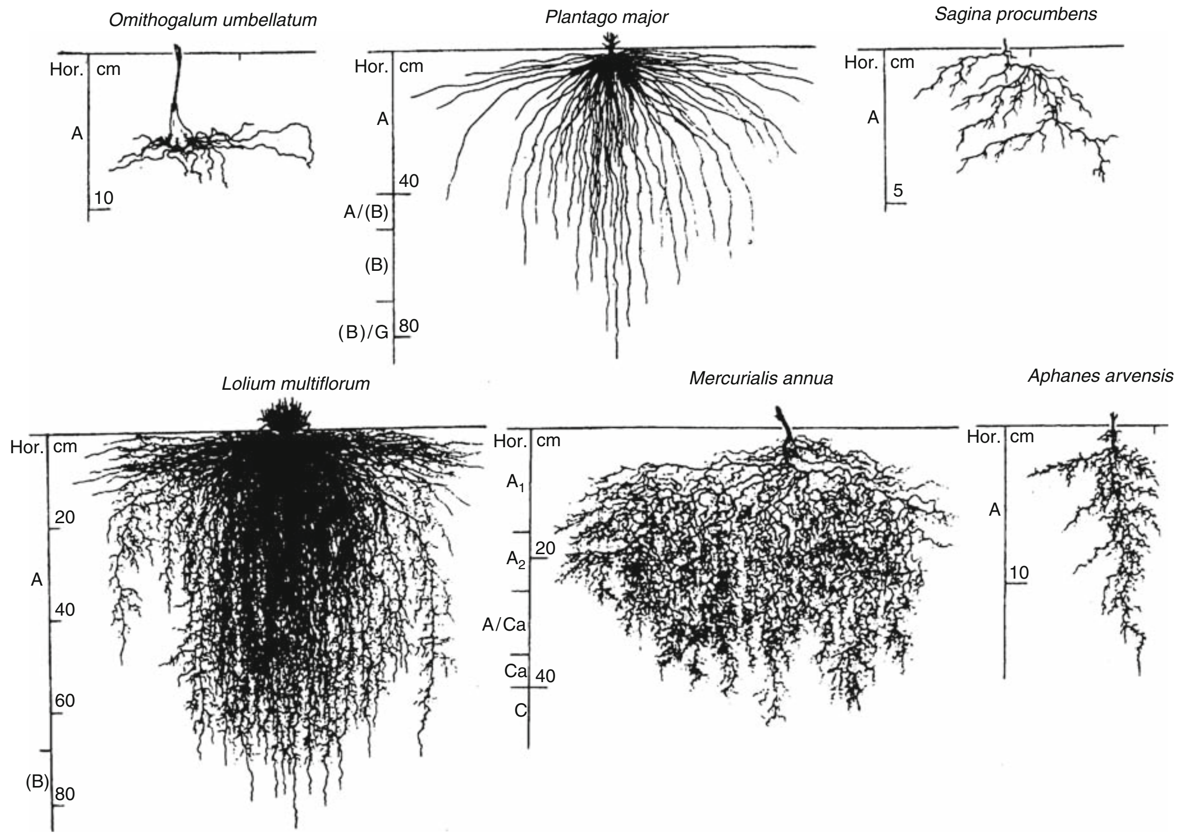

Fig. 2 Example of contrasted root systems. On the top row, root systems are little branched, while more profusely branched on the bottom row. Increasing dominance of a single main axis from left to right (from Kutschera, 1960)

in the root system. Vercambre et al. (Vercambre et al., 2003) observed that, in the plum tree, reiterations occurred periodically and profoundly affected the architecture of the root system.

The primary root system evolves from the growth and branching (first, second, third. . .order laterals) of the primary vertical root. Depending on the extension of laterals relative to the primary axis, the morphology of the root system will vary between taprooted and diffuse or fibrous (Fig. 2). These root system types are often found in dicotyledon species. Adventitious root systems are characterised by a large number of root axes originating from the stem base, or from a portion of the stem. They are generally not strongly gravitropic but quite sensitive to water and temperature tropism. Adventitious root systems are typical of monocotyledon species. In a number of plant species (e.g. cereals), the primary root system will dominate the early growth stages while the adventitious root system will take over in older plants.

The respective importance of the primary and adventitious root systems, i.e. the relative growth rates of main axes and laterals, the number of branching orders, etc. . . . , varies across plant species. Hence, different plant species develop different soil exploration strategies. Figure 3 exemplifies such differences between a monocotyledon and a dicotyledon species (maize and alfalfa). As maize emits nodal roots throughout its vegetative phase, shallow soil horizons are repeatedly explored and mined by these new roots and their branches. In the case of alfalfa, shallow soil horizons are explored by a single generation of branch roots, with much less new roots emitted in time than for maize.

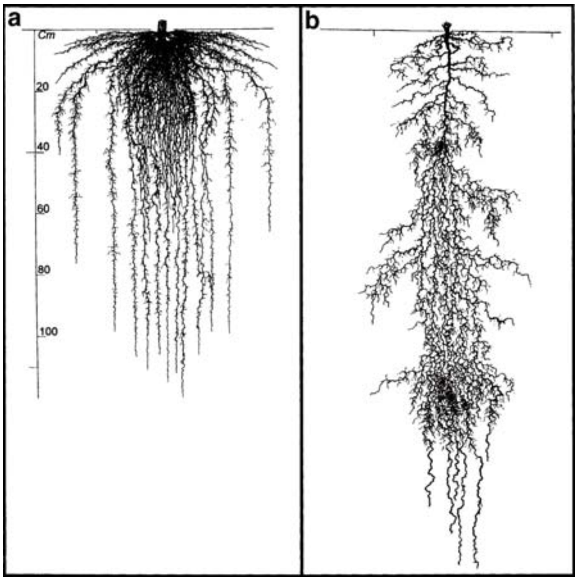

Fig. 3 Difference of rooting pattern between maize (**a**) and alfalfa (**b**) (from Kutschera, 1960)

3 A Quantitative View of Soil Exploration by Root Systems

3.1 Modelling of Root System Architectures

Conceptually, the modelling of root system architecture is based on describing root systems as sets of connected axes in a 3D space. Existing models of root system architecture include variable degrees of dynamic complexity.

3.1.1 Static Modelling of Root Systems

The static models describe the branching pattern within the root system architecture by means of synthetic descriptors. Fractal geometry is an example of this approach. This non-Euclidian geometry permits to describe how roots fill the soil space using a non-integer dimension (the fractal dimension D). This approach's underlying assumption is that the root system is self-similar across a large range of space scales, a property often exhibited by root systems (Tatsumi et al., 1989). D has been shown to vary between genotypes, plant age and growth conditions (Tatsumi, 2001). Fractal geometry also proved useful to estimate the total size of root systems based on measurements of basal roots (Van Noordwijk and Mulia, 2002). Topological modelling is another synthetic approach. It takes into account and globally describe the way root systems branch (Fitter, 2002). In this case, the root system is described by a set of links (a link connects an apex to a branching point or two branching points) and characterized by some topological parameters (for example, the number of links along the path between an apex and the collar of the root system). The topology of root systems will vary from a herringbone pattern (i.e. all branches derives from the same main axis) to a dichotomous pattern (i.e. equiprobable branching on all links). Topological modelling is a fruitful approach for describing the global implications of contrasted branching patterns (herringbone vs dichotomous) in relation with functional properties and root system optimisation (i.e. trade-off between carbon allocation to roots and efficiency of a root system process).

3.1.2 Dynamic Modelling of Root Systems

Dynamic models of root system architecture simulate growing root systems on the basis of simple production rules (for a review see Pagès, 2002; Pagès et al., 2000; Lynch and Nielsen, 1996). This approach puts the emphasis on the invariance of basic developmental processes. Such a modelling approach really emerged about a decade ago (Diggle, 1988; Pagès et al., 1989; Lynch et al., 1997) and became possible with the increasing power of computers. Virtually grown root systems are represented by a set of connected root segments. The root system development is simulated in discrete time steps by applying the basic morphogenetic production rules to the existing root system. These rules are: (a) emergence of new main axes (radicle, seminal or adventitious roots), (b) growth of the axes (including elongation and growth direction – gravitropism...), and (c) branching (new lateral axes). Apart from these three basic processes, other processes can be taken into account: (d) decay and abscission of roots and (e) radial root growth.

The dynamic modelling approach considers different root categories which generally, correspond to the branching order. Different root types will show very different developmental characteristics (e.g. appearance, growth and branching). The classification of root types is based upon several criteria reflecting morphogenetic properties (Pagès, 2002): growth rate, growth duration, branching ability and density, tropism, radial growth… For example, Vercambre et al. (2003) considered 6 root types to dynamically model the root architecture of plum trees. The distinctions they made were based on the nature of roots (woody or not), the axial growth (finite or indefinite), radial growth (presence or absence), the maximal length, and the decay (abscission or perennial).

Basic developmental rules need to be parameterized for each root type and depend on the species investigated. For example, continuous emission of nodal roots by cereals is a highly organized sequence in space and time that can be described by thermal time or leaf number (Pagès et al., 1989; Klepper, 1992). Growth direction may be largely influenced by gravitropism or plagiotropism, the influence of which is highly variable among root branching orders or nodal/seminal roots (Lynch and Brown, 2001; Pagès et al., 1998). Branching is generally represented by an acropetal sequence (with a fixed distance of emergence behind the apex or a maturation time lag of the primordia), but some species show lateral roots growing out of this sequence. These late lateral roots generally appear near the base of the tap root (e.g. rubber tree, blue lupin). They have a long life span and are important in the re-colonisation of superficial soil layer. Branching can also develop by reiteration, a process that should be considered particularly for trees.

These dynamic models include a stochastic description of some processes (e.g. growth direction and soil strength, intrinsic variability of growth rates, radial branching angles…). Dynamic architectural models provide realistic 3D visualizations of root systems (Fig. 4). Each segment of the simulated root system contains some information about its 3D co-ordinates, age, diameter, root type, etc, … Both annual and perennial plants have been modelled and parameterised using dynamic architecture models: Lupin (Dunbabin et al., 2002b), wheat (Diggle, 1988), Maize (Pagès et al., 1989), bean (Lynch et al., 1997), Plume tree (Vercambre et al., 2003), Oil-Palm (Jourdan and Rey, 1997), rubber-tree (Pagès et al., 1995), pine (Japanese red pine) (Tsutsumi, 2003).

3.1.3 Modelling of the Interactions between Root Systems and Their Environment

Root system models offer an opportunity to integrate, from the root segment to the root system levels, the impact of heterogeneous soil conditions on root growth. Hence, interactions between root systems and their environment can be modelled. The models developed by Somma et al. (1998) and Dunbabin et al. (2002b) integrate a great diversity of environmental conditions and allow to simulate their impact on root system development.

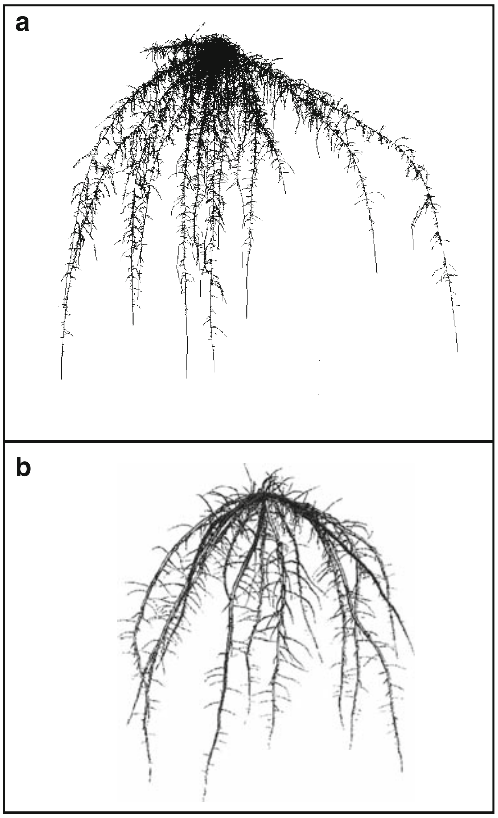

Fig. 4 Examples of 3D root systems simulated by architectural models. (**a**) Maize (From Pagès et al., 1989) and (**b**) Bean (from Lynch, 1995)

Some models incorporate the influence of soil temperature on root growth or root emission, using a thermal time scale (Diggle, 1988; Pagès et al., 1989) or a reduction coefficient applied to optimal root growth rates (Clausnitzer and Hopmans, 1994). The effect of soil strength has also been included (Fig. 5), by means, generally, of indirect variables such as soil bulk density or water content (Pagès, 1999; Clausnitzer and Hopmans, 1994) combined with empirical functions which reduce optimal growth rates and alter root growth direction. Somma et al. (1998) introduced the effect of nutrient concentration using growth rates linearly correlated to an optimal range of con-

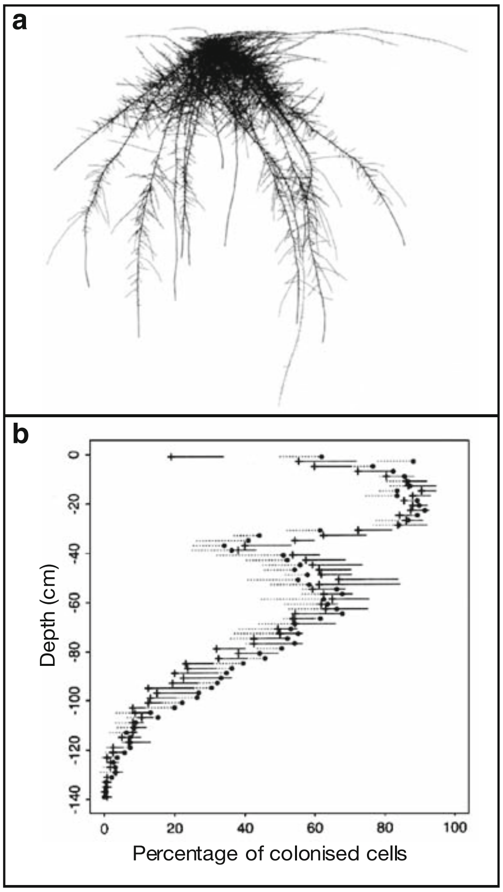

Fig. 5 Simulation of maize root system architecture interacting with the environment. A plough pan layer impedes root growth at 35 cm depth. (**a**) General morphology of the simulated maize plant. (**b**) Simulated (+) and observed (•) root profiles, obtained by counting the number of colonised cells (2 × 2 cm) on vertical grids. The horizontal bar represents one standard deviation (from Pagès, 1999)

centrations. Recently, Tsutsumi (2003) introduced the influence of hydrotropism by including a sensing of the water flux at the root tip which modulates the bending of the root.

3.2 Using Architectural Models to Quantify Soil Exploration by Root Systems

Most commonly, root uptake potential, is assessed on the basis of synthetic descriptors such as the root density (in term of length, biomass or surface area … per unit soil volume). This descriptor is indicative of soil exploration by roots and, if one can assume that roots are regularly distributed within the soil, an average distance between roots can be derived from root density measurements (Table 1). The average distance between roots is often used in water and nutrient uptake simulations to define the outer cylinder of soil accessible to the roots (De Willigen and van Noordwijk, 1994; Yanai, 1994; Silberbush, 2002). However, in real situations, the assumption of a regular distribution of roots does not hold. Hence, global parameters such as root depth or root density are not sufficient to investigate in detail the development and functioning of root systems. Root distribution within the soil has a strong influence on resource acquisition (Pagès, 2002; Pagès et al., 2000; Lynch and Nielsen, 1996). Consequently, it is necessary to include detail of root architecture and growth dynamics to gain sharper insight into the soil exploration/utilization processes.

Models of root system architecture can help to better understand soil exploration by plant roots by taking into account environmental constraints to growth, as previously discussed, but also by giving a quantified view of the soil volume that the roots can access or influence. This is possible since architectural models include full parameterisation of the 3D geometry.

In a first approach, the soil influenced by roots can be investigated by assuming that it is a cylinder of fixed radius centred on the root. This provides a crude estimate of the geometrical properties of a root system independently from any process (i.e. the zone

Table 1 Root distribution and average distance between roots of three crop species (Adapted from Jungk, 2002)

	Root length density (cm cm^{-3})			Average half distance between roots (cm)		
Soil Depth	Wheat	Maize	Spinach	Wheat	Maize	Spinach
0–30	8.2	3.8	2.3	0.2	0.3	0.4
30–60	1.7	1.5	0.06	0.4	0.5	2.3
60–90	1	0.4	–	0.6	0.9	–
90–120	0.7	0.1	–	0.7	1.8	–
120–150	0.27	0.01	–	1.1	5.6	–
150–180	0.03	–	–	3.2	–	–
Total root length (km m^{-2})						
0–180	36	17	7			

influenced could be a "rhizospheric" zone, a zone of nutrient depletion, of water depletion . . .). An example of calculation of the soil volume accessed by maize roots as a function of time of growth and radius of influence is shown in Fig. 6. This figure shows that if larger radii of influence globally lead to a bigger zone of influence, they also give rise, with time, to increasing overlap between depletion zones of the different roots. This means that more branch roots will penetrate a zone already influenced by another root as the radius of influence increases. This pattern of soil exploration and interaction between roots derives from interactions between growth rates (i.e. the rate at which a branch root can escape from a zone already exploited), branching density and angle of emission, and the way main axes (nodal roots) are emitted and grow (i.e angle of emission, gravitropism).

A more dynamic view of soil exploration, focused on nutrient acquisition, can be gained by considering the diffusion of nutrients to the roots (Robinson, 1991). In this case, the radius of the depleted volume changes with time (the elapsed time since the root first appeared at some location within the soil volume) and is expressed as (Ge et al., 2000):

$$R_d = r + 2\sqrt{D_e \cdot t} \tag{1}$$

where R_d is the radius of the depleted zone, r the radius of the root segment, D_e the effective diffusion coefficient of the ion in soil and t the time period of root growth.

At the root system scale, using topological modelling, Fitter (2002) predicted that for mobile nutrients ($D_e > 10^{-7}\,\mathrm{cm}^2\,\mathrm{s}^{-1}$), such as nitrate, herringbone topologies would be more efficient in exploiting the nutrient (Fig. 7). This result is related to the fact that herringbone type root systems produce a few order of laterals, which are characterised by low growth rates and limited extension from the zone depleted by the parent root. For less mobile ions (e.g. phosphate, $D_e < 10^{-8}\,\mathrm{cm}^2\,\mathrm{s}^{-1}$), the impact of the topology of the root system would be negligible because depletion zones for such ions are very narrow and all roots could access fresh, unexploited soil zones. These modelling experiments led Fitter (2002) to suggest some ecological implications of root topology. Hence, differences in the topology of root systems (herringbone to dichotomous) could correspond to overall plant growth rates and their ability to adapt to various degrees of soil fertility (especially for mobile nutrients).

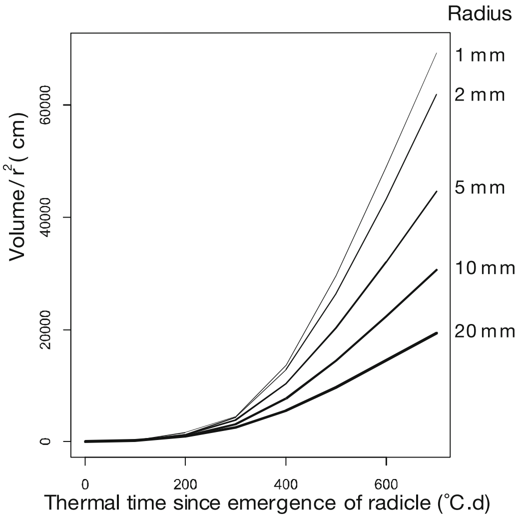

Fig. 6 Volume of soil explored by a simulated root system of maize (cf. Fig. 4) for an hypothetical radius of root influence (from 1 to 20 mm) in the soil. The soil volume explored by the roots is normalised by the (radius)^2 in the figure and represents an effective length of roots in soil not influenced by other roots. The decrease of the normalised prospected zone with increasing radius of influence is due to the increasing overlapping of prospected soil zones by different roots

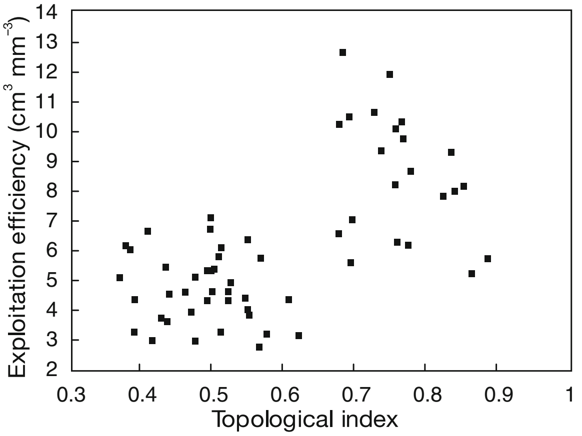

Fig. 7 Exploitation efficiency of a root system (defined as the volume of soil exploited per unit root tissue volume) increase with the increase of topological index of simulated root systems. High values of the topological index represent little-branched (herringbone) root systems; low values represent dichotomous branching pattern. The zone of soil exploited increases with time according to diffusion of the mobile resource (diffusion coefficient = $10^{-7}\,\mathrm{cm}^2\,\mathrm{s}^{-1}$, similar to nitrate). The simulation was performed with a range of other architectural characteristics, which explains the scatter of the points (from Fitter, 2002)

Phosphorus acquisition has been the most extensively studied process, at scales ranging from the root segment to the root system, which can be addressed via architectural modelling. In particular, elegant studies by Lynch and co-workers, combining experimental work and architectural modelling, gave a detailed portrait of soil exploration in relation with P acquisition for bean (for a review see Lynch and Brown [2001]).

Combining architectural modelling and the time dependent expansion of the P depleted zone (eq. 1, with $D_e = 10^{-8}\,\mathrm{cm}^2\,\mathrm{s}^{-1}$), Ge et al. (2000) studied the effect of altered gravitropism of basal roots (that varied from shallow to deep) on P acquisition efficiency. In the case of homogeneous P distributions in the soil profile, shallower root systems explored more soil (per unit root biomass) than deeper systems because less inter-root competition occurred in the former case (i.e. less overlapping depletion zones between neighbouring roots – Fig. 8a, b). In the case of stratified soil P concentrations, with high P concentrations in the first 10 cm, shallower root systems were also able to get more P because of increase foraging of the topsoil and less inter-root competition (Fig. 8a, c). Figure 9 shows the extent of depletion zones around bean roots and gives a visual idea of overlapping zones.

An example of the use of architectural modelling to dynamically predict uptake of more mobile nutrients is given by (Somma et al., 1998). In this model, soil water and nitrate transport, nitrate uptake and the influence of nitrate availability on root growth are simulated (cf. 2–1–3). Figure 10 shows the simulated root system of a 25 day-old barley plant. Water and NO_3 are supplied through drippers at the soil surface. In one scenario, NO_3 is applied continuously (Fig. 10a), in the other NO_3 is applied for a finite time at the beginning of the simulation. The total amounts of applied N are equal in the two cases. In the first case, simulations show that N concentrations are higher in the upper part of the soil and root density decreases with depth. In the second case, the NO_3 plume moved downwards when application stopped and caused a greater root density in the central part of the soil. Interestingly, the maximum of root length density and NO_3 concentration are shifted. This is linked to the relative rates of root growth and downwards percolation of NO_3.

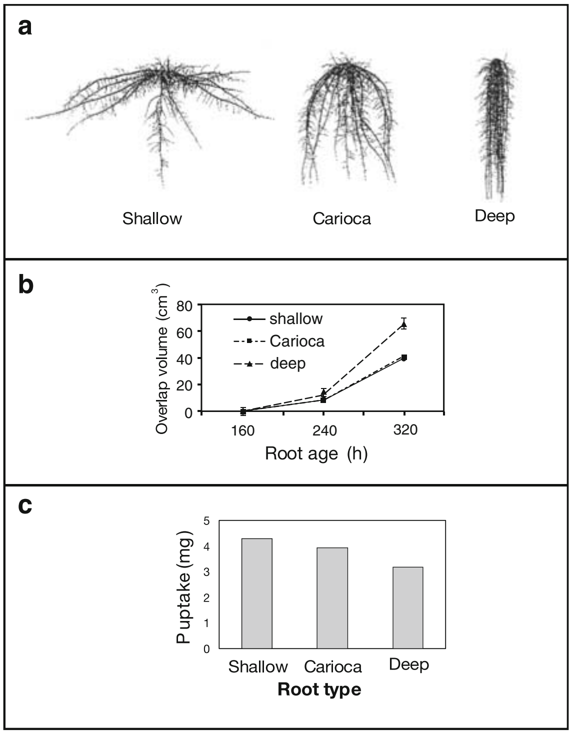

Fig. 8 Simulation of the influence of different degree of basal root gravitropism on the exploitation of P by bean root systems. The depletion zone of P is represented by diffusion of P to the root with time (Diffusion coefficient = $10^{-8}\,\mathrm{cm}^2\,\mathrm{s}^{-1}$). (**a**) Bean root systems simulated with different rooting pattern (shallow; Carioca, an actual cultivar, and deep). (**b**) Volume of the overlapping exploited zones for the three root system types. (**c**) P uptake by the three simulated root systems at the end of simulation (320 h), in the case of a stratified soil profile of P (P concentration is higher in the first 20 cm of soil (from Ge et al., 2000)

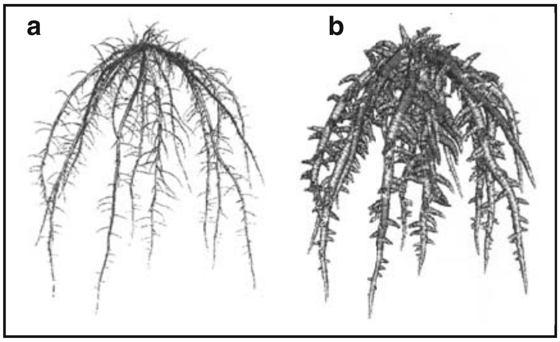

Fig. 9 3D representation of a simulated root system of bean and depletion zone of P around the roots (cf. Fig. 8) (from Lynch and Nielsen [1996])

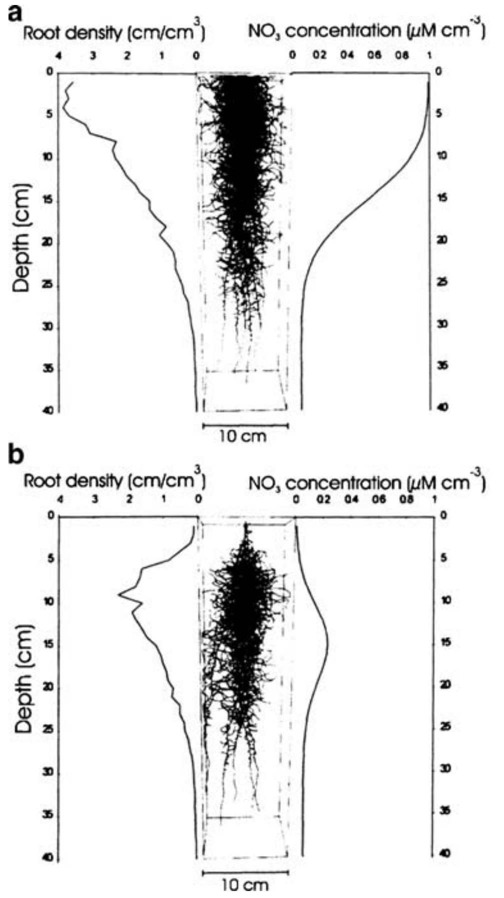

Fig. 10 Simulated 3D root architecture (coupled with water and nitrate transfer and uptake by the root system) with corresponding root density and nitrate concentration distribution for (**a**) continuous supply of nitrogen by drippers and (**b**) the same amount of nitrogen, but supplied only at the beginning of the simulation period (from Somma et al., 1998)

4 From Soil Exploration to Resource Acquisition

Assessing soil exploration by plant roots would represent a convenient way to estimate resource acquisition if it could be assumed that all roots were equally and constantly involved in resources uptake (whatever the process investigated). However, it has been shown that for water or nitrate uptake, considering that all roots behave identically (a common assumption) and using ex-situ measured rates, would lead to over-estimating the actual uptake rates (Robinson, 1991). Based on actual uptake rates, it can be inferred that only 10% of the total root system length would be effectively involved in nitrate uptake and 30% in water uptake. But, as pointed out by Robinson (Robinson, 1991), an important question is to determine which 10% and 30% parts of the root system are active?

As was shown in 3–1–2 for root growth and branching, a root system, should, from a functional viewpoint be regarded (a) as a population of individual roots behaving differently from each other (Waisel and Eshel, 2002), (b) as a function of tissue differentiation and (c) in response to changing environmental conditions (plasticity).

4.1 Variations in Root Properties

4.1.1 Variations among Root Types

Many reports show that different root types are functionally different. Leaf expansion of wheat is more impaired when drought affects seminal roots than nodal roots (Volkmar, 1997), and contribution of the seminal roots to the whole plant exceeds what could be expected from their fractal mass (Waisel and Eshel, 2002). Navara (1987) showed that radicle and seminal roots of maize play a dominant role in the water supply during a significant part of the plant life span. Nodal roots of maize were able to take up more phosphate from soil than radicle and seminal roots (Mistrik and Mistrikova, 1995). While the maximum uptake rate of barley for nitrate globally decreases between vegetative and reproductive growth stages, the nitrate uptake rate by nodal roots remains constant (Mattson et al., 1993). Lazof et al. (1992) showed that nitrate uptake rates (per unit of dry weight) of the primary axis of young maize plant amount for 68% of the lateral uptake rate. Waisel and Eshel (1992) showed variations in the uptake of Cl or K between taproot and laterals in pea. Mature lateral roots of maize lowered the pH at the soil-root interface, while their parent root made it more alkaline (Marschner, 1990).

4.1.2 Variations along Roots

Large variations in physiological properties exist along roots. These variations can be related to ontogenesis as root tissues get older, mature and differentiate, at increasing distances from the root tip (Clarkson, 1996).

Variations in growth rates between different root types and the way their tissues differentiate can also explain some of the variations in physiological/metabolical properties between root types. Many processes were shown to vary along roots as a function of age, tissue structure and anatomical differentiation. High variations in root respiration were found along primary roots of *Prunus persica* (Bidel et al., 2000), not only in the vicinity of the apex but up to about 20 cm from the tip. Parts of roots can release H^+ (acidification) while others release OH^- depending on the available nitrogen resource (Jaillard et al., 2000). NO_3 and NH_4 uptake was found to vary along roots, with defined zones of active (generally the apical zone) and passive uptake (Cruz et al., 1995; Lazof et al., 1992). Variations in the uptake and translocation of other ions (P,K,Ca. . .) along roots were also demonstrated (Clarkson, 1996). In the field, cortical senescence in older root parts seems relatively common in cereals and other grasses (Robinson, 1991). Cortical senescence may weaken uptake because of physiological decrepitude but also by disrupting the transport pathway between the soil and the root.

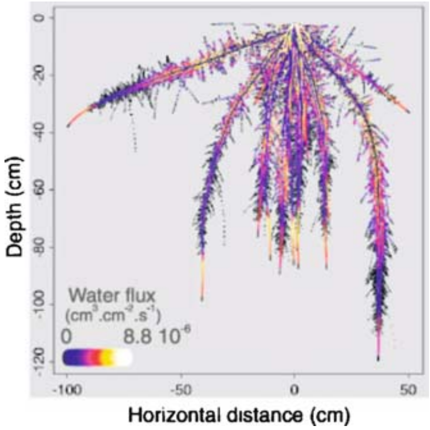

Fig. 11 Distribution of water uptake within a simulated maize root system (43-day-old – 3D root system projected on a vertical plane). Water uptake is simulated by taking into account the variability of the root hydraulic conductance within the root system. The imposed transpiration is 5 10^{-3} cm^3 s^{-1} and the water is freely available in the outside medium: water potential $= 0$ MPa. Even if water availability is uniform within the outside medium, uptake is heterogeneously distributed in the root system, showing hot-spot of uptake (from Doussan et al., 1999)

The case of water uptake provides an illustrative example of how root functional heterogeneity can be taken into account in root system modelling. Water uptake along roots is related, for the radial pathway (water transport from soil to xylem vessels), to the differentiation of relatively impermeable structures (suberization) and for the axial pathway (water transport along roots), to xylem maturation (Steudle, 2000). In some monocot species, late metaxylem vessels (able to carry much of the water) were shown to open at large distances from the root tip (around 20–30 cm for main axes of maize (Wenzel et al., 1989)). Based on experimental data on water flow in maize roots (Varney and Canny, 1993) and architectural/water transport modelling of the root system, Doussan et al. (1998a, b) derived the variations of hydraulic conductance along maize primary and lateral roots, and showed the impact of these variations on the distribution of water uptake within the maize root system for the case of a uniform water avaiblity (Fig. 11). This figure shows the high heterogeneity of water uptake within the maize root system, even if water is uniformly available. Another type of pattern of water uptake was found for the perennial root system of a tree (*Prunus*), with most of the uptake located in the basal part of the root system (Doussan et al., 1999). Heterogeneity in the water uptake capacity along the root will have an impact on the way roots extract water from soil (Fig. 12; Doussan, 1998) and on variations of the environment (water potential and moisture content) in the vicinity of roots. This variations along a root may impact the rhizospheric activity and diversity.

4.2 Root System Plasticity and Uptake Optimisation

It is well known that plant roots systems are continuously subject to modifications following interactions with the environment. The function of this plasticity could be an adaptation to their sedentary life style to better explore their surroundings (Leyser and Fitter). Root system plasticity should be considered in relation to soil conditions, but also in relation to competition with other species. Indeed, as shown for nitrate uptake under a wheat crop monoculture (Robinson, 2001), plasticity does not necessarily imply a greater uptake: it can simply be triggered when competition with another species exists. Nutrient availability induces morphological variations of the root system. Parameters which can be affected are (Ford and Lorenzo, 2001):

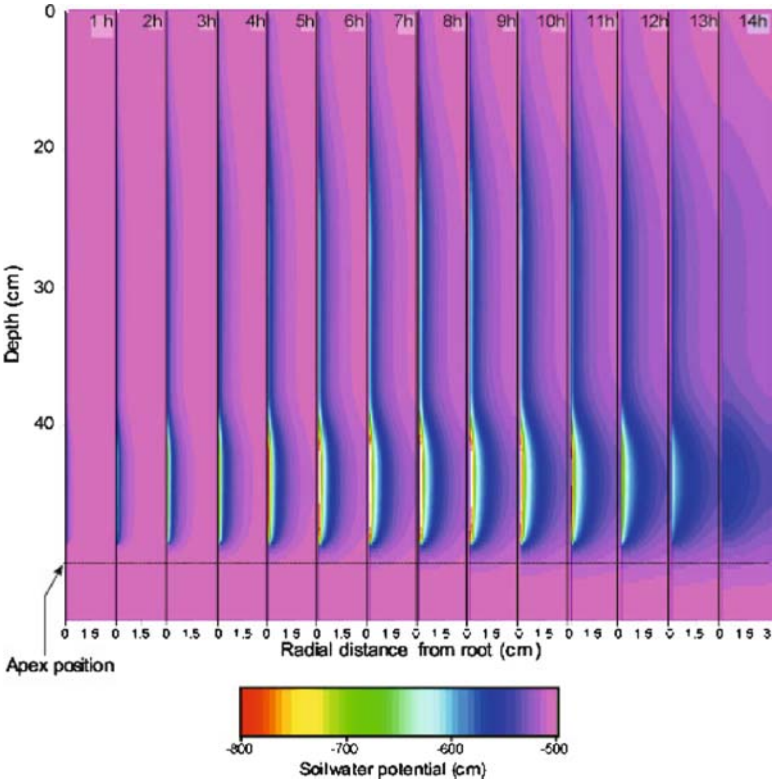

Fig. 12 Simulated variation of water potential in soil for a single maize root axis (50 cm length, no laterals) during a 14h simulation period. A sinusoidal variation (between −0.1 and −1.2 MPa) of the xylem water potential is imposed at the root collar (similar to leaf water potential). The simulation couples water transfer in the soil and into/along the single root. The variations of the hydraulic conductance (axial and radial) along the maize root are included in the simulation and generate an heterogeneous pattern of uptake and water potential in the soil along the root, with greater variations near the root tip. The root axis is located at the left axis of each figure. The soil is a clay loam and the initial soil water potential is −0.05 MPa (from Doussan, 1998)

root branching, root growth (with growth of main axes generally less affected by nutritional effects than higher order axes), root diameter, root angle (for example, low P availability decreases the angle of emission of basal roots in bean, soybean and pea [Liao et al., 2001]), root hair length and density, emission of specific root types (cluster roots [Skene, 2000]; drought-induced roots [Vartanian, 1996]).

Plants response to spatial heterogeneity of nutrients has received much attention (see review by Robinson, 1994). The influence of temporal variations in nutrient concentrations on root plasticity has been less studied. Experimental observations generally relate to conditions in which a small part of the root system has access to sufficient nutrients while the other is deprived (Drew and Saker, 1975). Plant response in such a heterogeneous system happens at two time scales (Robinson, 1996): (a) a rapid and reversible physiological response within hours, which consists of an increase in nutrient inflow rate (high affinity transport), (b) a slow morphological response within days, resulting in an increased root growth, and sometimes increased lateral density, towards and within the nutrient rich patch, associated with a reduced growth in the other part of the root system. However, if these responses are the global trend for plants, considerable variations in the intensity of these responses exist between species. The intensity of the response varies between no response to an order of magnitude variation (for growth or nutrient inflow) for the roots in the nutrient patch (see Robinson, 1996; for review). Stimulation of the inflow varies depending on the nutrient considered (and the duration of starvation). Root proliferation seems to depend less on the nutrient considered (except for K in some species). Localised responses such as root proliferation were generally explained in terms of nutritional effects (Zhang et al., 1999): roots directly exposed to the nutrient benefit the most from the increased N supply, or alternatively, the increased metabolic activity in those same roots increase the influx of carbohydrates and auxines. Recently, Zhang et al. (1999) proposed a dual pathway for NO_3 in *Arabidopsis thaliana*: a systemic inhibitory effect with NO_3 accumulation and a localised stimulating effect, under genetic control, depending on NO_3 concentration at the lateral root tip, where NO_3 is directly the signal.

Interactions between nutrient availability and growth in architectural models have been presented by Somma et al. (1998) and Dunbabin et al. (2002b). In Somma et al.'s model (1998) nitrate affects root growth out of an optimal range of concentration and elongation is scaled according to the amount of biomass allocated to the root system. The model by Dunbabin et al. (2002b) includes a more subtle description of root system plasticity. It uses the plant demand for individual resources and the ability of the various components of the root system to supply individual resources to drive the allocation of assimilates and subsequent architectural variations (root growth, branching) as well as nutrient uptake rates. This reproduces the dual pathway of a local "sensing" response and a whole-plant response. Inflow plasticity and root proliferation plasticity are modelled. The model is based on Diggle's root architecture model ROOTMAP (Diggle, 1988). Nitrate is the nutrient taken into account and the model performance was assessed against laboratory data and validated in field experiments with Lupin species (Dunbabin et al., 2002a, b). An example of the results yielded by this model, applied to the extreme topological types of root systems (herringbone and dichotomous), is shown Fig. 13 (Dunbabin et al., 2001). Nitrate is distributed in soil in random patches, with the same random profile (static supply) or a new re-randomised profile (dynamic supply), and applied every two days.

Interestingly, Fig. 13 shows that incorporation of root and inflow plasticity in the root system behaviour makes the dichotomous system more efficient than the herringbone one in the case of static (heterogeneous) supply of nitrate. This contrasts with results of homogeneous supply (Fig. 7). On the contrary, in the case of dynamic N supply, the herringbone system is more efficient than the dichotomous one, and the latter gains almost no efficiency in uptake from plasticity.

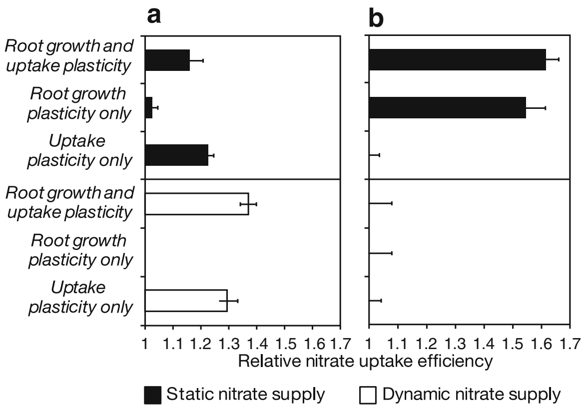

Fig. 13 Simulation of nitrate uptake efficiency with an architecture model taking into account both inflow and morphological plasticity of the root system. Nitrate is distributed in the soil as small patches. The efficiency of uptake with plasticity is relative to the same root system with no plasticity response. Root systems are (**a**) herringbone system and (**b**) dichotomous system. In the dynamic supply case, the nutrient patches are randomly redistributed in space, which is not the case for static supply (from Dunbabin et al., 2001)

5 Conclusion

Soil exploration and resource acquisition by plant roots result from both the dynamic expansion of the root system in space and the temporal variability of the root function/activity (between and along roots). Soil environmental conditions can also continuously modulate the pattern of exploration and exploitation by roots because of the constraints it imposes on root growth or because of root plasticity induced by resources heterogeneous availability. Plant adaptative strategies to locally sense environmental changes result in local responses but are co-ordinated at the whole plant level. All these plant-soil processes, with different time and space scales, can be integrated within the unifying framework of root system architecture.

Such integration is now possible because root system architecture models are widely available and become increasingly powerful tools which enable the simulation of root functioning, plasticity and interactions of roots with their environment. Such modelling tools provide a quantified view of soil-plant interactions, from the single root to the root system level, and can link local processes to global behaviour.

On the other hand, breakthrough technologies give more and more spatially detailed data on the functioning of root systems *ex and in situ* (e.g. non destructive imaging of the root system and its soil environment [Pierret et al., in press]; Cryo-scanning of soil-root system [McCully, 1999], microsensors [Portefield, 2002]; root-pressure probes [Steudle, 2000]). These new experimental data give new insight into root functioning and a sound basis for parameterisation and validation of distributed modelling of root systems at a range of scales.

As Lynch and Nielsen (Lynch et al., 1997) pointed out, not all the interactions and processes of the plant-soil system can be simulated with the same degree of accuracy (however, this should be moderated considering the evolution of experimental devices and computing power). Progress can be achieved by focusing root models on some specific process, while approximating the general trend of other factors or interactions. Another challenging way for operational applications is to degrade the sophisticated root models, looking at the more important parameters, in order to get some simple but robust relationships (such as root – sink terms) adapted to some particular crop and soil settings.

Among the numerous processes which, so far, have only received little attention and which could be tackled via architectural modelling in relation with root exploration are

- Interactions between plants, arising either from intra or inter-specific competition including allelopathy, and resulting in profound modifications of root morphology (Vaughan and Ord).
- Interactions with mycorrhyzae, which can alter root system architecture (Hooker et al., 1992) and are able to access a very different range of soil pore sizes than roots (with possibly specific biogeochemical environments).
- Mobilisation of nutrients by roots and modification of the soil environment (mucilage, release of acids, complexing agents... [Hinsinger, 1998]) and their implications for soil properties near the roots.
- Occurrence and functioning of new root types: Cluster roots, drought-induced roots, hairy roots...

Finally, much is to be understood about "how real roots work" (McCully, 1995). The *in situ* environment of roots may be quite different from what is generally accepted. For example, much research is need to improve our understanding of the development of rhizosheaths (McCully, 1999) and root colonisation of a relatively specific range of soil pores having specific biologic and physico-chemical properties (soil macropores and cracks, preceding root biopores [Rasse and Smucker, 1998; Pierret et al., 1999; McCully, 1999]).

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Methods for Studying Root Colonization by Introduced Beneficial Bacteria

Elisa Gamalero, Guido Lingua, Graziella Berta, and Philippe Lemanceau

Abstract Some free-living rhizobacteria are considered as potential biocontrol and plant growth-promoting agents. Successful application of beneficial bacteria as microbial inoculants requires their presence and activity at the appropriate level, but even more, at the right time and place. Various markers are described in the literature to differentiate introduced bacteria from indigenous microflora and to visualize them. These markers are presented together with the methods currently applied to quantify bacterial densities and to characterize the distribution of introduced bacteria. The methods to quantify bacterial densities are either based on bacterial cultivation or not. Different types of microscopical observations, allowing the characterization of the bacterial distribution and location in the rhizosphere, are also described. The respective advantages and limitations of these markers and methods are discussed.

Keywords Bacterization • Methodology • Rhizosphere

Résumé Certaines rhizobactéries libres sont considérées comme des agents potentiels de lutte biologique et de stimulation de la croissance des plantes. Le succès de leur application nécessite la présence et l'activité des bactéries à un niveau suffisamment élevé, mais également au bon moment et au bon endroit. Différents marqueurs permettant la différentiation des bactéries introduites de la microflore indigène ainsi que leur observation dans la rhizosphère, sont décrits dans la littérature. Ces marqueurs ainsi que les méthodes, appliqués pour quantifier la densité et pour caractériser la distribution des bactéries introduites, sont présentés. Les méthodes de quantification de la densité bactérienne sont basées ou non sur la culture des bactéries. Différentes méthodes d'observations microscopiques permettent de caractériser la distribution et la localisation bactérienne dans la rhizosphère. Les avantages et les inconvénients respectifs des marqueurs et des méthodes décrits sont discutés.

Mots clés Bactérisation • Méthodologie • Rhizosphère

1 Introduction

The rhizosphere was defined in 1904 by Hiltner (1904) as being the volume of soil, influenced by the presence of living plant roots, whose extension may vary with soil type, plant species, age and other factors (Foster, 1988). Plant roots release an enormous amount of root exudates that may represent up to 10–20% of the photosynthethates (see the review by C. Nguyen in the present issue), leading to a significant stimulation of the microbial density and activity. Specific populations are more favoured than others in the rhizosphere due to the level of adequation of their metabolic activities with the composition of the root exudates. The structure and diversity of microbial populations in rhizosphere differ then significantly from that of soilborne populations (Lemanceau et al., 1995; Maloney et al., 1997; Mavingui et al., 1992). These quantitative and qualitative variations of the soilborne microflora

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are described as the rhizosphere effect. This rhizosphere effect varies according to the root exudate composition which is affected by the plant physiology (De Leij et al., 1994), the stage of plant development (Waisel et al., 1991) and the position on the root system (Liljeroth et al., 1991).

The microflora associated with the roots affects plant growth and health. Indeed, some bacterial populations are pathogenic, whereas others are beneficial. Beneficial rhizobacteria include both symbiotic and free-living microorganisms. Among the latter, a special attention has been given to the fluorescent pseudomonad group. Positive effects on plant growth and health of inoculations of bacterial strains belonging to this bacterial group have been reported since the late 1970s (Bakker et al., 1987; Burr et al., 1978; Glick, 1995; Kloepper and Schroth, 1981; Lifshitz et al., 1987; Weller, 1988). However, overall biological control of soilborne diseases achieved by microbial inoculants is often inconsistent (Schippers et al., 1987). This has been specially illustrated for fluorescent pseudomonads. This inconsistency has been partially associated with inefficient root colonization by the introduced bacteria (Lemanceau and Alabouvette, 1993; Weller, 1988). Indeed, a clear relationship has been established between suppression of the wheat root disease take-all and that of fusarium-wilts by different strains of fluorescent pseudomonads, and the densities of these bacteria in the corresponding host-plants (Bull et al., 1991; Raaijmakers et al., 1995). In order to make biological control more consistent, there is then a need for a better knowledge of bacterial traits promoting rhizosphere competence.

Biocontrol of soilborne diseases is ascribed to microbial antagonism and/or to induced resistance of the host plant (Cook et al., 1995; Van Loon et al., 1998). Microbial antagonism results from the suppression of saprophytic growth of plant pathogens mediated by antibiotics and siderophores. The concentration of these metabolites in the rhizosphere is expected to be related to the density of active bacteria. Even more, the synthesis of some of these metabolites (phenazines, pyoverdines) was demonstrated to be regulated by quorum-sensing (Pierson et al., 1994; Stintzi et al., 1998). Beside the total bacterial density, which is related to the survival kinetic of the introduced strain, the antagonistic metabolites and then the bacterial cells should be located at the infection courts of the soilborne pathogens. To summarize, the expression of the beneficial effects by the introduced bacteria requires their presence at a density high enough and at the time and location that are favourable for the root infection by the pathogens.

The methods required for analysing bacterial traits involved in the rhizosphere competence and plant microbe interactions must allow then the quantification of bacterial density but also the characterization of the bacterial cell distribution and location. Even more, these methods should take in account not only culturable, but also total bacterial cells since the frequency of viable but non culturable cells would vary according to the environmental conditions (Troxler et al., 1997).

The aim of the present review is to present methods of quantification and characterization of the distribution along the roots of introduced bacteria in soil and rhizosphere.

2 Markers Used for Tracking Introduced Bacteria

Tracking bacteria introduced in complex environments such as soils requires to be able to discriminate them from the indigenous microflora. Markers used for that purpose should then fulfil several prerequisites. These markers should obviously be specific. This specificity must be checked in the environment in which bacteria are introduced. The markers should be also stable in soil and with time. The relative stability of the marker is required both to avoid its loss and/or its transfer to other bacteria. Since the aim of the markers is to perform ecological studies on the introduced bacteria, they should affect as little as possible the behaviour of these bacteria. Surprisingly, there are quite few studies comparing the fitness of the marked and wild-type strains (Blot et al., 1994; Devanas et al., 1986; Glandorf et al., 1992; Orvos et al., 1990; Philippot et al., 1995; Van Elsas et al., 1991). In the same way, since the perturbation of the system should be kept as low as possible, the expression of the marker should avoid substrate amendment. More generally, markers chosen should be easy to track in a wide range of soils, and environmental conditions (pO_2, pH, etc...) favourable for their expression should be considered (Jansson, 1998).

In this section different markers are presented together with their properties.

2.1 Serological Markers

The primary immunologic tool used in environmental microbiology is the antibody. Immunoassays are analytical methods used to detect and/or quantify the antigen–antibody interaction. The conjugation of a signal molecule (fluorochromes, enzymes, radioisotopes) to the antibody is required to visualize the antigen–antibody interaction. Immunological techniques are relevant especially for the detection, enumeration and localization of introduced bacterial strains in soil and rhizosphere. The critical aspect of serological methods is the specificity of the antibodies used. Polyclonal or monoclonal antibodies may be applied according to their specificity. Monoclonal antibodies are obviously more expensive to raise but are more specific. Specificity of the antibodies, especially for polyclonal ones, should be checked to decrease occurrence of possible cross-reactions. Usually, a specificity high enough may be obtained for fluorescent pseudomonad strains with polyclonal antibodies raised against membrane proteins (Glandorf et al., 1992).

2.2 Molecular Markers

Various molecular markers such as antibiotic resistance (Glandorf et al., 1992; Lemanceau et al., 1992; Van Elsas et al., 1986), chromogenic (*xyl*E, *gus*A, *lac*Z) (Kozaczuk et al., 2000; Weller, 1988), luminescent (*lux*AB, *luc*) (Kragelund et al., 1997; Ma et al., 2001; Ramos et al., 2000; Räsänen et al., 2001; Rattray et al., 1995; Turnbull et al., 2001) and fluorescent markers (gfp and unstable gfp) (Bloemberg et al., 2000; Lowder and Oliver, 2001; Normander et al., 1999; Suarez et al., 1997) have been developed and widely applied to study root colonization.

2.2.1 Antibiotic Resistance

Antibiotic resistances have been widely used as markers in microbial ecology. Although various plasmids and transposons have been used (De Lorenzo, 1994; Mirleau et al., 2001; Orvos et al., 1990; Prosser, 1994; Van Overbeek et al., 1997), most of the studies on bacterial survival kinetics are based on the use of spontaneously occurring antibiotic-resistant mutants. Rifampicin resistance is commonly used as a marker to study survival kinetics of introduced bacteria in the rhizosphere (Glandorf et al., 1992; Mirleau et al., 2000; Van Elsas et al., 1986). Stability of rifampicin resistance was checked with *Pseudomonas putida* WCS358 in field conditions (Glandorf et al., 1992). Kanamycin and streptomycin resistance obtained by Tn*5* mutagenesis with the suicide plasmid method of Simon et al. (1983) was also described as a possible marker (Van Elsas et al., 1986). The maintenance of Tn*5* in the mutant JM218 was ascertained by comparing bacterial densities of this mutant in root suspensions, estimated by serology, with bacterial density estimated by plate count on King's B medium supplemented with kanamycin (Lemanceau et al., 1992). As stressed above, the level of resistance of the indigenous microflora to the antibiotic used as a marker must be determined in order to check the specificity of the marker used. As an example, Wilson (1994) have estimated that the background of naturally kanamycin resistant bacteria in a dutch soil was 2×10^4 cfu per gram.

Antibiotic resistance is often used for studies on survival kinetic of introduced bacteria (Glandorf et al., 1992; Mirleau et al., 2000; Prosser, 1994; Van Elsas et al., 1986) since the corresponding detection method (plate counting, see Section "Culture-Dependent Methods") is quite sensitive, cost effective, reliable and easy to perform. However, possible genetic changes associated with chromosomal-mediated antibiotic resistance may affect several ecologically important traits (Blot et al., 1994; Devanas et al., 1986; Mahaffee et al., 1997; Smit et al., 1995). Moreover, the use of antibiotic tagged bacteria carries with it the risk of contributing to indesiderable spread of antibiotic resistance in nature (Jansson, 1995).

2.2.2 Chromogenic Markers

Several genes encoding metabolic enzymes have been used as markers to detect, quantify and localize introduced bacteria. The *xyl*E gene encodes for a catechol 2,3-dioxygenase catalysing the formation of hydroxymuconic acid that, reacting with a catechol, forms a yellow semialdehyde derivative. The *gus*A and *lac*Z genes encode for a β-glucuronidase and β-galactosidase, respectively: in presence of the adequate substrates (5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside and 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside), they produce a blue pigment (Jansson, 1995).

Although the use of these chromogenic markers is simple, not expensive and well defined, several disadvantages have been widely discussed (Errampalli et al., 1999; Jansson, 1998; Sørensen et al., 2001). For example, the *xyl*E gene applicability is restricted due to inactivation of cathechol 2,3-dioxygenase by oxygen (Roger et al. 2004). The *gus*A and *lac*Z markers have limited application in soil because of the presence of high background (10^5 cfu/g of soil) from the indigenous microflora (Flemming et al., 1994; Wilson, 1994). Moreover, these chromogenic markers require substrates or reactives to be expressed or detected; the stability of these gene products is usually measured by hours or days (Jansson, 1998).

2.2.3 Luminescent Markers: luxAB and luc genes

Another very sensitive approach is the transfer of bioluminescence marker genes to bacteria, providing them the capability to emit light. Prokaryotic bioluminescence genes have been cloned from *Vibrio fischeri* and *Vibrio harveyi* (Belas et al., 1982; Engebrecht et al., 1983), while those eukaryotic (*luc* genes) have been cloned by the firefly *Photinus pyralis* (De Wet et al., 1985). The *lux* operon of *V. fischeri* includes five genes: two genes (*lux*AB) encode the subunits of the luciferase enzyme and the other three (*lux*CDE) encode enzymes involved in the synthesis of the aldehydic substrate (n-decanal) (Jansson, 1998).

The requirement for molecular oxygen limits the use and the interpretations of this kind of physiological reporter system, but if oxygen is present, the monitoring of light may result in very useful information on bacterial activity and distribution in different environments such as plant rhizosphere (Molin and Givskov, 1999). The minimum detection limit for fully active cells has been reported as 445 cells per gram of soil (Rattray et al., 1995). Although eukaryotic *luc* genes show some advantages in comparison to the prokaryotic counterpart (i.e. higher specificity, absence of background in native microflora), the substrate luciferin is expensive and sometimes not readily taken up by the cells. The stability of the gene product is usually measured by minutes or hours (Jansson, 1998).

2.2.4 Fluorescent Markers: Stable and Unstable Green Fluorescent Protein

Another attractive marker system to monitor bacterial cells in the environment is the green fluorescent protein (GFP). The GFP is a 27 KDa polypeptide which converts the blue chemiluminescence of the Ca^{+2}-sensitive photoprotein (aequorin from the jellyfish *Aequorea victoria*) into green light (Chalfie et al., 1994). A series of red shifted GFP mutants, 20–35 times stronger than the wild type, with various excitation and emission wavelengths such as the ECFP (enhanced cyan), EGFP (enhanced green) and EYFP (enhanced yellow), have been recently developed (Tsien, 1998).

The advantages and disadvantages of this marker have been extensively discussed by Errampalli et al. (1999). Some of the most relevant advantages are that GFP is extremely stable and resistant to proteases, is easily detectable, and does not require exogenous substrate and allows the monitoring of single cells even in real time. Moreover, GFP is continuously synthesized and there is no background in indigenous bacterial populations. However, the interference of soil particles, the variability of GFP expression in different species, the inability to work in anaerobic conditions and the instability of the plasmid should be considered. In order to overcome the latest limitation and reduce the risk of a plasmid transfer to other microorganisms, bacterial strains used are preferentially chromosomally marked. For that purpose, several Tn*5* transposon suicide delivery vectors have been developed (Suarez et al., 1997; Tombolini et al., 1997). The stability of the GFP varies according to the variants and plasmid constucts in the range of hours or days (Jansson et al., 2000; Suarez et al., 1997).

Recently, Andersen et al. (1998) developed a new variant of GFP characterized by its short half-life. The unstable GFP has been constructed by the addition of a short peptide sequence to the C-terminal end of the intact GFP: this modification allows its degradation by bacterial endogenous proteases. Since the GFP produced during bacterial growth does not accumulate, it is possible to perform real-time analysis of the bacterial metabolic activity (Lowder and Oliver, 2001; Sternberg et al., 1999). However, different levels of proteases may be expressed depending on the microorganisms, the growth phase and environmental factors and care must be applied in the interpretation of the results (Jansson et al., 2000).

2.2.5 Specific Primers and Oligonucleotidic Probes

Introduced bacteria can be monitored using primers or probes that allow amplification or hybridization of sequences which are strain specific. Specific probes can be used to hybridise bacterial colonies after *in vitro* growth (Werner et al., 1996) or bacterial cells for in situ studies. Probes are usually covalently linked to a fluorochrome such as fluorescein, rhodamine, Texas red, Cy3 and Cy5 (Amann et al., 2001).

Specific sequences may be introduced by a genetic construction. As an example, a specific primer amplifying across *nptII*-*lacZ* junctions on the Tn5B20 construct was used to follow the survival kinetic in soil and rhizosphere of the strain *P. fluorescens* R2f tagged by *lacZ*-*nptII* marker gene (Van Overbeek et al., 1997). However, as stressed before, genetic constructs may affect the ecological behaviour of bacterial strains.

Another strategy consists in identifying sequences specific of the strains in order to design primers and probes. Different approaches have been proposed to develop this identification. One is to compare homologous nucleic acid sequences of ribosomal RNA (rRNA) sequences to sequences available in databases. Since rRNA are present in all living microorganisms in high copy number and are quite stable, oligonucleotidic probes can be applied (Amann et al., 2001). They are either species specific or even strain specific in some cases (Assmus et al., 1995; Christensen et al., 1999). *Pseudomonas* specific primer has been designed by Braun-Howland et al. (1993). This PSMg primer was applied to describe the dynamic of indigenous populations of *Pseudomonas* in soil hot-spots (Johnsen et al., 1999) and to characterize the succession of *Pseudomonas* on barley root in a perturbed environment (Thirup et al., 2001). Analysis of the 16S rDNA of *Paenibacillus azotofixans* strain with that of 2000 bacteria also enabled Rosado et al. (1996) to identify the presence of three highly variable regions that were used to design primers for studying the kinetic of this bacterial strain in soil and wheat rhizosphere. Another approach to define primers and probes is (a) to characterize the diversity of populations belonging or not to the same group by Random Amplification of Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR), in order (b) to identify discriminating bands, then (c) to pick them from the gel, (d) to re-amplify and test them for specificity.

Monitoring introduced bacteria on the basis of its specific RAPD-PCR pattern has also been proposed but is very time consuming (Chapon et al., 2002; Latour et al., 1999).

3 Methods to Quantify Densities of Introduced Bacteria

Methods to quantify introduced bacteria can be classified in two major types upon the fact that they are based or not the cultivation of the bacteria. Obviously, the culture-dependent methods will not allow the detection of viable but not culturable bacteria (VBNC). Since microorganisms introduced in soil can go through different processes (conversion to non culturable state and phase changes) doubts have raised about the representativity of the view given by data yielded with culture-dependent methods of the real processes in soil. Despite this limitation, culture-dependent methods remain widely used mainly because they are easy to apply. The culture-independent methods can provide a more complete picture of the kinetic of the total number of microbial cells (Van Elsas et al., 1986). However, the major limitation of these methods is that they may not allow differentiating viable and not-viable cells.

In this section, major culture-dependent and culture-independent methods of microbial quantification in the rhizosphere are presented.

3.1 Culture-Dependent Methods

These methods are based on the suspension-dilution of soil and/or root samples and on inoculation of growing media (solid or liquid) with adequate dilutions. They require then the use of labelled strains (see Sections "Serological Markers" and "Molecular Markers"). The culture-dependent methods differ according to the type of marker used giving the specificity to the growing media. This type of method is quite simple to perform, not too expensive and quite sensitive (10^2–10^3 cfu/g), but labour-intensive and shows some limitations (Jansson et al., 2000). This type of method underestimates the number of bacteria present in soil or in rhizosphere. Bacteria may remain physically attached to the soil particles or may be killed in the dilution medium or may fail to grow on growth media (Kloepper and Beauchamp, 1992). Some of them may keep aggregated even during the dilution process in such way that a cfu may be originated by more than one cell. Suspension-dilution can either be plated on solid media or introduced in liquid media with various dilutions in order to determine from which dilution there is no more bacterial growth. This last method named Most Probable Numbers (MPN) requires the use of probability tables to process data that contribute to reduce the sensitivity of the analysis compared to plating (MacCrady, 1915).

The most basic method consists in plating mutant resistant to antibiotic on solid growth medium supplemented with the corresponding antibiotic and with an anti-eucaryotic compound such as cycloheximide. This method is widely used especially for survival kinetic of introduced bacteria and for competition studies between wild-type strains and mutants impaired in specific phenotypes (Mavingui et al., 1992; Mirleau et al., 2000; Orvos et al., 1990; Van Elsas et al., 1991).

The sensitivity of this type of plating method may be significantly lowered by combining plating and serological approaches with the immunofluorescence colony-staining (IFC) technique. Detection limits as low as 20 cfu of *Erwinia* spp. per gram of soil have been reported by van Vuurde and van der Wolf (1995). The IFC technique, developed by these authors, is based on the use fluorescein isothiocyanate (FITC)-conjugated IgG antibodies specific of the introduced bacterial strain to discriminate target from non-target colonies. Bacterial colonies remain viable and IFC positive colonies can be subcultured to confirm their identity by other biochemical or molecular methods. Direct IFC has been used by several investigators (Leeman et al., 1991; Lemanceau et al., 1992; Mahaffee et al., 1997; Raaijmakers et al., 1994; Van Vuurde and Roozen, 1990) to track and quantify bacterial strains introduced into soil or onto plants. Since the IFC does not require any alteration in the phenotype or genotype of the wild-type strain, this technique allows the comparison of an unaltered wild-type strain to a genetically modified derivative of the wild-type strain. The main restriction of direct IFC is the necessity of a good quality fluorescent conjugate against the target bacteria. However, outside the medical field, specific conjugates are usually not commercially available. For this reason, Veena and van Vuurde (2002) recently developed an indirect IFC using diluted specific antiserum and commercial conjugate to detect bacterial pathogens on tomato seeds. Indirect IFC is suitable for routine applications with facilities for fluorescence microscopy and does not require much expertise. As for any serological methods, the main limitation is the risk of false positive reactions due to cross-reacting bacteria.

Reporter genes may also be applied for culture-dependent methods. *lux-luc* tagged bacteria can be detected and enumerated by plate counting, luminometry or scintillation counter and by imaging. Luminometry is an easy and sensitive method that has been applied to evaluate the density of luminescent bacteria on root surface (Beauchamp et al., 1993) and rhizosphere (Rattray et al., 1995). These two instruments are sensitive but they are not specifically designated for bioluminescence application (Burlage and Kuo, 1994). Bioluminescent colonies can be counted directly by color photography (autophotography), by exposure to X-ray film, by direct microscopy with a CCD camera enhancement (imaging) or alternatively, if the amount of light emitted is high, it is possible to visualize them by eyes (Jansson, 1995).

Green fluorescence due to a GFP tagged bacteria can be observed in colonies cultured on agar media under a hand-held long wave UV lamp. This is a simple and cheap way to enumerate colonies but the potential DNA damage of UV over time may be a limitation (Errampalli et al., 1999). Fluorimetric detection of GFP labelled bacteria is useful for screening or confirmation of cell growth. A detection limit of 10^3 cells per ml of *P. putida* in soil has been reported by Burlage and Kuo (1994). Recently, Cassidy et al. developed a MPN method to evaluate cell density of GFP marked *Pseudomonas* in soil, rhizosphere and rhizoplane (Cassidy et al., 2000).

Finally, colonies grown on solid media can be hybridised with specific oligonucleotidic probes (Werner et al., 1996).

3.2 Culture-Independent Methods

Culture-independent methods can be distributed in three different categories: serological, molecular and cytological methods.

3.2.1 Serological Methods

Among the serological methods, the enzyme-linked immunosorbent assay (ELISA) is a very sensitive immunoassay for the detection of antigens. ELISA is based on direct or indirect sandwich methods. The ELISA method has been used to study and quantify the external and internal root colonization of maize by two *P. fluorescens* strains (Benizri et al., 1997) and the distribution of two diazotrophic enterobacterial strains, *Pantoea agglomerans* and *Klebsiella pneumoniae*, on cereals shoot and root (Remus et al., 2000). ELISA method is quite sensitive (10^3 cells per ml in pure cultures and 10^4–10^5 cells per g of soil) and associated to a standard curve relating the amount of signal given to the direct counts by microscopic enumeration, can provide quantitative information. Disadvantages are related to possible cross reactivity and non-specific signal production.

3.2.2 Molecular Methods: Detection of Nucleic Acids

Detection methods based on nucleic acids extracted from the soil offer the possibility to monitor specific bacterial genotypes (gene or genomic markers), providing a picture of the dynamics of total numbers of microbial cells (Johnsen et al., 1999). The polymerase chain reaction (PCR), primarily used as a qualitative method to confirm the presence or absence of a specific DNA sequence, has been recently applied to obtain quantitative information. Up to now, PCR is the most sensitive method for detection of specific DNA in environmental samples; sensitivities of 1–100 cells per gram of soil have been reported (Jansson and Leser, 1996).

Before amplification, microbial DNA is extracted from soil. Various methods have been described for this extraction (Martin-Laurent et al., 2001). All DNA extraction methods present potential bias depending on the soil properties (i.e. humic substances) (Van Loon et al., 1998). The final goal of DNA extraction methods is to obtain DNA having a quality good enough for PCR amplification and for yielding consistent data.

Three PCR methods for quantification have been developed and applied to evaluate bacterial population in soil or rhizosphere: the most probable number-PCR (MPN-PCR), the competitive PCR (C-PCR) and quantitative PCR (Q-PCR).

In the case of MPN-PCR, the quantification of target sequences is based on the serial dilution of the PCR products in order to identify from which dilution the target sequence is not anymore detectable by electrophoresis. The detectable limit of product is calibrated by an external standard and the initial amount of the target molecules is estimated using the dilution factor of the positive sample. This method was first developed by Picard et al. with *Agrobacterium tumefaciens* and *Frankia* spp. (Picard et al., 1992). Using this method, Picard et al. were able (a) to detect *A. tumefaciens* strain when inocula ranged from 10^3 to 10^7 cells and (b) to estimate the indigenous populations of *Frankia* spp. at 0.2×10^5 genomes per gram of soil (Picard et al., 1992). A detection limit of 10^2 cfu of *Paenibacillus azotofixans* per gram of rhizospheric soil was reported by Rosado et al. (1996). However, as indicated by Jansson and Leser (1996), a limit of the MPN-PCR method is the probabilistic evaluation, based on several dilutions and replicates, which contribute to reduce the precision of the estimation.

In C-PCR, a DNA fragment containing the same primer sequences (internal standard) as the target fragment is allowed to compete in the same tube with the target for primer binding and amplification. Experimentally, PCR reaction tubes containing the target samples are spiked with a dilution series of the competitor fragment. When the molar ratio of PCR products generated from target and competitor is equal to one, the amount of target is equal to competitor. Since the amount of competitor is known, the amount of target can be determined. However, this technique is labor-intensive and its accuracy is dependent on an internal competitor, which must possess the same amplification efficiency as the target (Jansson and Leser, 1996). Thirup et al. (2001) applied this type of PCR to study the effects of *P. fluorescens* DR54 and the fungicide Imazalil on the succession of indigenous *Pseudomonas* spp. and *Actinomyces* on barley roots. Martin-Laurent et al. (2001) have quantified with C-PCR the amount of *atzC* gene known to be involved the atrazine mineralization in a soil treated with this herbicide.

Finally, Q-PCR is the direct measure of the amount of products generated from different samples by a calibrated instrument. Initial amount of the target molecules in the samples is estimated by the determination of the PCR amplification efficiency defined by the amplification of a known amount of the same target (external standard). Q-PCR has certain limitations, such as accounting for the variation between samples during the reaction and the fact that quantification is only possible during the exponential phase of the amplification (Jansson and Leser, 1996).

The major advantages of the PCR based methods are their sensitivity and specificity. Theoretically, a single copy of the target nucleic acid sequence can be detected; normally, at least 10^3 copies of target are required for PCR methods in nucleic acids isolated from environmental samples. These methods allow to take in consideration both cultivable and not cultivable but do not allow differentiation between viable and non-viable organisms.

3.2.3 Cytological Method: Flow Cytometry

Flow cytometry is a cytological tool valid for evaluating root colonization by introduced bacteria. This instrument measures and analyses the optical properties of hundreds of single cells per second, passing through a focused laser beam. As each cell or particle passes through the flow cytometer, it is monitored by forward scatter (detects each particle according to its size), side scatter (measures simultaneously the size and the shape of the particle) and fluorescence (evaluates the fluorescence intensity) detector. Flow cytometry allows the detection and quantification of both the individual fluorescent cells within a population and the fluorescent intensity from more than one bacterial group. For environmental samples, bacteria from the particulate matter and an internal standard must be used to quantify the cell number (Jansson et al., 2000). A limitation of the technique is related to its low sensitivity due to the abundance of fluorescence particles present in most environmental samples. However, the assay is rapid and simple and thousand of cells can be analysed in a short time allowing the processing of the data by various statistical procedures.

Flow cytometry can be applied to evaluate the density and characterize the kinetic of introduced bacteria tagged by fluorescent antibodies (Chitarra et al., 2002), GFP (Tombolini et al., 1997) and specific oligonucleotidic probes (Thirup et al., 2001). Detection limit of 10^3 cells per ml has been recorded evaluating the density of fluorescent antibody-tagged *Xanthomonas campestris* in *Brassica oleracea* (Chitarra et al., 2002), and of 3×10^4 cells per gram of dry soil in the characterization of the kinetic of the fluorescent oligonucleotidic probe-tagged *Sphingomonas* spp. strain 107 in soil (Thomas et al., 1997).

The use of different dyes or fluorochrome provides an extremely powerful way to characterize the physiological state, activity or degree of viability of bacteria (Gregori et al., 2001; Von Caron et al., 2000), and then to quantify by flow cytometry the viable and non-viable bacterial cells. Various criteria have been proposed to discriminate the viable from non-viable cells. Impermeability of the bacterial membrane to dyes is the basis of the dye exclusion test. Propidium iodide (PI) and other dyes characterized by the presence of quaternary ammonium groups and two or more positive charges, such as Sytox green, TO-PRO-1 and TO-PRO-3, are membrane-impermeant. Cells retaining these dyes are usually considered as non-viable cells. Moreover, using simultaneously permeant (SYBR green) and non-permeant (PI) dyes makes possible the discrimination of cells having a compromised, slightly damaged or intact membrane (Gregori et al., 2001). The use of a membrane permeant substrate such as fluorescein diacetate (FDA) and 5-cyano-2,3-ditolyl tetrazolium chloride (CTC), that are cytoplasmic enzymatically cleaved to form a fluorescent impermeant product (fluorescein and formazan, respectively) allows the discrimination of cells with intact (retaining the product of the reaction) and damaged membrane (loosing the product of the reaction) (Veal et al., 2000). Membrane potential (MP) is the most used vitality parameter in microbial flow cytometry. A 100 mV bacterial transmembrane electrical potential gradient, due to selective permeability and ionic transport is usually reported. Variations in the MP measurement can be recorded using lypophilic charged dyes that can be accumulated or excluded by the cell (Shapiro, 2000).

4 Methods to Characterize Distribution and Localization of Introduced Bacteria

Distribution and localization of introduced bacteria require the use of fluorescent antibodies, fluorescent markers and oligoucleotic probes (see Sections "Markers Used for Tracking Introduced Bacteria," "Fluorescent Markers: Stable and Unstable Green Fluorescent

Protein," and "Specific Primers and Oligonucleotidic Probes"). These studies are sometimes only possible in gnotobiotic conditions.

Immunolocalization is based on the use of fluorescent signal molecules conjugated to the antibodies; the emission of fluorescent light indicates the presence of a specific antigen. The basic procedure consists in the reaction between a fluorescent specific antibody with the antigen attached to a slide, and in the observation of the sample using a fluorescence microscope. To enhance the signals and the specificity of the reaction, an indirect immunolocalization, using a fluorescent secondary antibody, is usually performed. Simultaneous localization of different antigens can be obtained using antibodies coupled to different fluorochromes. Immunolocalization has been successfully used studying root colonization. Examples include the analysis of the spatial competition between *P. fluorescens* Ag1 and *Ralstonia eutropha* (formely *Alcaligenes eutrophus*) during barley root colonization (Kragelund and Nybroe, 1996), the cells distribution of *P. fluorescens* DF57 on barley roots (Hansen et al., 1997), the autoecology of the biocontrol agent *P. fluorescens* CHA0 in the rhizosphere of different crops (Troxler et al., 1997) and the endophytic colonization of spruce by *Paenibacillus polymyxa* (formely *Bacillus polymyxa*) Pw-2R and *P. fluorescens* Sm3-RN (Shishido et al., 1999). Advantages of immunolocalization are the simple use and the short time required to obtain results. On the other hand, several problems, such as autofluorescence of the sample, non-specific staining, antigen instability and the inability to check viability have to be considered.

Lux genes have been widely used in the study of bacterial root colonization and activity (Kragelund et al., 1997; Ma et al., 2001; Ramos et al., 2000; Roberts et al., 1999; Unge et al., 1999), while *luc* genes have been only recently applied for monitoring activity of *P. fluorescens* 31K3 in forest soil (Björklöf and Jørgensen, 2001) and of *Sinorhizobium arboris* in *Acacia senegal* rhizosphere (Räsänen et al., 2001).

GFP and its derivatives have been applied to characterize the distribution of the biocontrol agent *P. chlororaphis* MA342 on barley seeds (Tombolini et al., 1999), the localization, the viability and the activity of *P. fluorescens* DR54 on barley rhizosphere (Normander et al., 1999) and the colonization pattern of *P. fluorescens* WCS365 on tomato root (Bloemberg et al., 2000). The use of different GFP colour variants, allowing the simultaneous monitoring of multiple bacterial species, opens new perspectives in the study of complex microbial community (Bloemberg et al., 2000). The visualization of GFP-tagged cells using microscopy assures a single cell detection level.

Fluorescent in situ hybridisation (FISH) involves the use of fluorescence-labelled oligonucleotidic probes, constructed on the basis of 16S rRNA sequence, to target rRNA within morphologically intact cells (Amann and Ludwig, 2000). The FISH technique can be used to detect all bacterial cells, using a universal probe, or a single population, using a strain-specific probe. The in situ localization of *Azospirillum brasilense* in the wheat rhizosphere was described by Assmus et al. (1995), the distribution of *P. syringae* and *Rhodococcus fascians* on tomato root surface was characterized by Macnaughton et al. (1996) and the potato tissue infection by *Ralstonia solanacearum* was studied by Wullings et al. (1998). The in situ hybridization method is further detailed in the present issue by Schumpp et al. (in press).

Bacteria marked by fluorescent antibodies, fluorescent markers and by oligonucleotic probes, can be detected by direct microscopy using an epifluorescent microscope with an adequate filter kit. The method is simple and the counts are rapid and precise, but the limit of detection is related to field of view and to the matrix of the sample. Interfacing an epifluorescent microscope with a charged-coupled device (CCD) camera and image analysis software can enhance the sensitivity to a single cell level (Fig. 1). The main problem using direct microscopy is the high background of fluorescence coming from the root, the soil particles and the contaminants.

However, the recent development of confocal laser scanning microscopy (CLSM) has significantly reduced some of these limitations. CLSM is a powerful apparatus for visualizing with high-resolution microbial cells labelled by fluorescent antibodies, GFP or oligonucleotidic probes. Because three-dimensional views can be generated, the CLSM readily lends itself to digital processing, by which images of thin optical sections can be reassembled into a composite, 3D image. The major advantage of CLSM is that the confocal imaging system allows the detection of signals only from the focused plane, limiting background fluorescence arising from materials such as plant tissue, soil particles or organic debris. Moreover, by using different fluorescence channels, CLSM allows the simultaneous detection of different bacterial populations

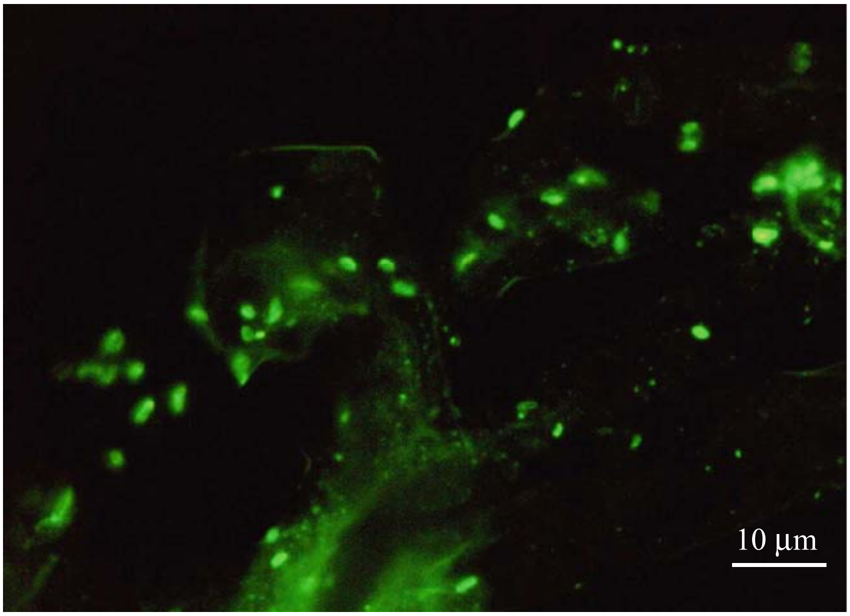

Fig. 1 Epifluorescence image of FITC-antibody-labelled *Pseudomonas fluorescens* strain A6RI colonizing root hairs of 7-day-old root of tomato grown in gnotobiotic conditions

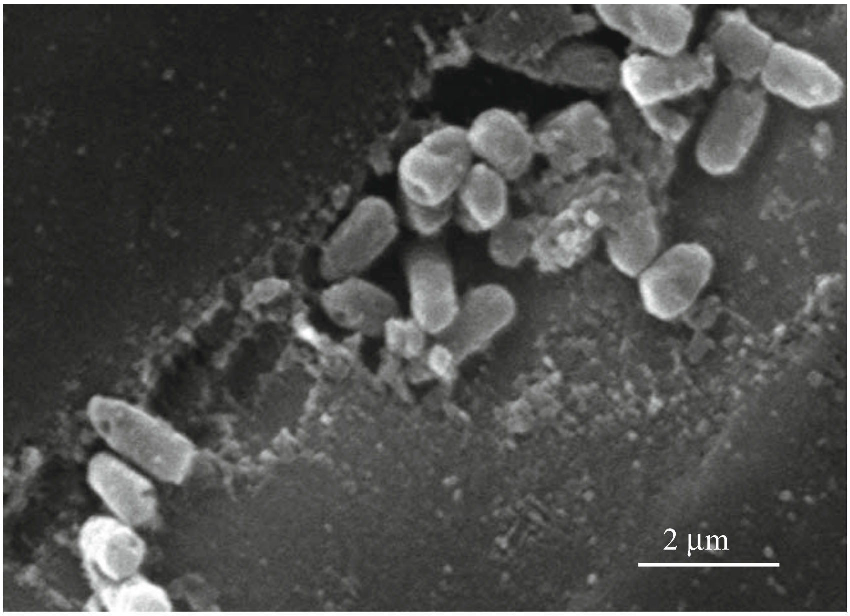

Fig. 2 SEM image of *Pseudomonas fluorescens* A6RI colonizing root surface of 7 day-old root of tomato grown in gnotobiotic conditions

and/or secondary metabolites. For all these advantages, there is an increasing use of CLSM to localize introduced microorganisms on plant roots (Assmus et al., 1995; Bloemberg et al., 2000; Hansen et al., 1997; Tombolini et al., 1999). The limitation in the use of CLSM is the cost of the instrument.

Single-cell distribution of rhizobacteria along plant root, grown in gnotobiotic conditions, can be also characterized by electron microscopy. The scanning electron microscope (SEM) has been widely used. For example Chin-A-Woeng et al. (1997) described by SEM, the spatial-temporal tomato rhizosphere colonization pattern by the biocontrol agent *P. fluorescens* WCS365. More recently, Bacilio-Jimenez et al. (2001), observed, by SEM analysis, the presence of two endophytic strains in rice seeds identified as *Bacillus pumilus* and *Corynebacterium flavescens*. SEM provides an excellent resolution and allows exact localization of microorganisms in relation to the root structure (Fig. 2). However, sample preparation is expensive in time and needs care to avoid the production of artefacts. Transmission electron microscope (TEM) has also been widely applied as for example to study (a) the inter- and intracellular colonization of tomato roots by the biocontrol agent *P. fluorescens* WCS417r (Duijff et al., 1997) and (b) the cell colonization and infection thread formation in sugar cane roots by *Acetobacter diazotrophicus* (Bellone et al., 1997). Sample preparation for the TEM is much more time consuming than for SEM and requires other instruments (i.e. ultramicrotome) to obtain sections with the adequate thickness.

Information regarding the internal root colonization by two different bacterial strains can be obtained by immunogold labelling. In addition, it has to be stressed that, both by SEM and TEM, only very small root samples can be analysed. To allow the investigation of a more general root bacterial distribution, electron microscopy should be combined to others methods (Achouak et al., 1994).

5 Conclusions and Perspectives

During the present review, different methodologies to quantify and localize introduced bacterial strains have been presented. These methods have their own advantages and limitations. Some only allow the quantification of cultivable bacteria putting aside the so called VBNC (culture-dependent methods). Others allow the quantification of the cultivable and non-cultivable cells of the introduced bacterial strain however they do not allow the discrimination of the viable and nonviable cells (immunofluorescence, PCR). Taking in consideration the limitations of both types of methods, combination of culture dependent methods and serological methods (IFC) or combination of culture dependent and molecular method (colony hybridisation) have been proposed. Combining colony counts of an antibiotic resistant strain with immunofluorescence technique has been successfully applied to monitor distribution and dynamic of bacteria in soil or on the root (Hase et al., 1999; Kragelund and Nybroe, 1996).

Taking in account the advantages and the limitations of the different methods, a polyphasic approach based on the use of different enumeration methods (conventional plate counting, luminometry, fluorimetry, flow cytometry, quantitative PCR) has been proposed by Cassidy et al. (2000) to discriminate the total number, and the number of viable and cultivable bacterial cells.

A polyphasic approach could also be proposed to both characterize the localization and the activity of the cells of the introduced bacterial strain. As an example, Lübeck et al. (2000), applied the combination of fluorescent antibodies and FISH studying sugar beet root localization of *P. fluorescens* DR54 by CLSM; this dual staining protocol allowed cellular activity to be recorded in both single cells and microcolonies during the bacterial establishment on the root. Similarly, Unge et al. (1999) developed a dual *gfp-lux*AB marker system to monitor simultaneously the cell number and activity of specific bacterial populations. They recently applied this dual marker to characterize the population size, the metabolic activity and the distribution pattern of *P. fluorescens* SBW25 along wheat root by luminometry, flow cytometry and CLSM (Unge and Jansson, 2001).

Over the last years, there has been an increased interest for microscopical observations of the microflora in the rhizosphere (Bloemberg et al., 2000; Hansen et al., 1997; Tombolini et al., 1997) renewing with the early studies of Foster (Foster, 1988). This revival of interest is related to the progresses made in the microscopy apparatus and in the molecular and serological markers.

In conclusion, different techniques combining multiple staining/tagging methods should provide more insight on the reciprocal interactions between the plant and the microorganisms in the rhizosphere and about the spatial-temporal colonization pattern and the physiological status of a microbial inoculant along the root.

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Sustainable Urban Agriculture in Developing Countries: A Review

Hubert de Bon, Laurent Parrot, and Paule Moustier

Abstract The population living in cities is continuously increasing worldwide. In developing countries, this phenomenon is exacerbated by poverty, leading to tremendous problems of employment, immigration from the rural areas, transportation, food supply and environment protection. Simultaneously with the growth of cities, a new type of agriculture has emerged; namely, *urban agriculture*. Here, the main functions of urban agriculture are described: its social roles, the economic functions as part of its multi-functionality, the constraints, and the risks for human consumption and the living environment. We highlight the following major points. (1) Agricultural activity will continue to be a strong contributor to urban households. Currently, differences between rural and urban livelihood households appear to be decreasing. (2) Urban agricultural production includes aquaculture, livestock and plants. The commonest crops are perishable leafy vegetables, particularly in South-east Asia and Africa. These vegetable industries have short marketing chains with lower price differentials between farmers and consumers than longer chains. The city food supply function is one of the various roles and objectives of urban agriculture that leads to increasing dialogue between urban dwellers, city authorities and farmers. (3) One of the farmers' issues is to produce high quality products in highly populated areas and within a polluted environment. Agricultural production in cities faces the following challenges: access to the main agricultural inputs, fertilizers and water; production in a polluted environment; and limitation of its negative impact on the environment. Urban agriculture can reuse city wastes, but this will not be enough to achieve high yields, and there is still a risk of producing unsafe products. These are the main challenges for urban agriculture in keeping its multi-functional activities such as cleansing, opening up the urban space, and producing fresh and nutritious food.

Keywords Freshness • Livelihoods • Marketing chains • Multi-functional • Urban and peri-urban agriculture • Vegetables

1 Introduction

While urban agriculture occurs in all cities of the world, there are still many questions about whether and how to develop research and development activity for this particular type of agriculture. The tremendous and continuing urbanization process in Asia, Africa and Latin America raises questions about the employment of the new "urban" manpower, feeding the growing urban population, and the management of the continuously moving fringes of the cities of developing countries. Different definitions of urban agriculture have been developed that stress the relationships between agriculture and the city both in terms of resources and outputs (Lourenco–Lindell 1995; Moustier and Mbaye 1999; Moustier and Fall 2004; Mougeot 1995). In this paper, the words "urban agriculture" will be used as defined by the growing of plants and the raising

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of animals for food and other uses within and around cities and towns (from Van Veenhuizen 2006).

The major question asked of agronomists, agro-economists and agro-sociologists is whether urban and peri-urban agriculture are genuinely distinct from rural agriculture and, if so, what are their main distinguishing characteristics? Does this type of agriculture then require specific research work? Literature on the subject is rather extensive, belonging both to the life sciences and the social sciences, and including also a large number of technical documents, technical bulletins and project reports. Since the end of the 1980s, CIRAD has developed research programs on urban agriculture in Africa and in South-east Asia (Parrot et al. 2008a,b; Moustier 2007). This paper makes the following assertions about the future of urban agriculture: the continuing population growth of cities in developing countries will not decrease the economic and social importance of urban agriculture, if governments are made aware of its multi-functional role, and if the safety of its products and environment can be guaranteed.

To give answers to this hypothesis, three characteristics of urban agriculture in developing countries will be developed and commented on: (1) the social roles of urban agriculture in relation to the urban population growth; (2) the economic functions of urban agriculture and the emergence of its multi-functionality; (3) the constraints and the risks of developing an urban agriculture for human consumption. CIRAD's experiences in developing country projects will be presented and complemented by a review of the literature.

2 Urban Agriculture and Urban Population Growth

Half of Africa's population already lives in cities, a proportion that will continue to increase (UN 2006), though it is also recognized that agriculture still provides employment and income for the majority of the population (World Bank 2007). If national data and predictions are correct, a significant part of the African population will live in cities, but will rely on agriculture for income. This situation may cause serious sanitary and environmental challenges for all agricultural activities conducted in an urban area (Cohen 2004; Ruel and Haddad 1999; Haggblade and Hazell 1989).

2.1 Farmers Will Live in Towns

According to The 2005 Revision of World Urbanization Prospects (UN 2006), by 2030, more than 50% of the African population is expected to live in cities. For example, in Cameroon today, 50% of the population already lives in cities; by 2030, this number is expected to be more than 70%. With the rise of mega-cities, secondary towns and small urban settlements will spread into rural areas, increasing population densities even in remote areas. The traditional distinctions between urban and rural lifestyles are becoming redundant, and we can reasonably expect a convergence in developing countries between these two lifestyles (Cohen 2004).

The concept of urban agriculture involves the notion of both urban and rural areas, but the definition of what constitutes an urban or a rural area varies between countries. No clear consensus seems to prevail in the literature (Cohen 2004; Tiffen 2003; Frey and Zimmer 2001). For example, in Cameroon, the 1976 and 1987 censuses considered as urban population anybody living in a locality with a district, a division, a department or a Province and/or having at least 5,000 inhabitants and including a secondary or post-primary school, a healthcare center, a water conveyance and a daily market. A locality with less than 5,000 inhabitants and without any of the cited infrastructures was considered as rural (INS 2004). In Benin, localities with 10,000 inhabitants or more are classified as urban in UN data, while in Angola, Argentina and Ethiopia, all localities with 2,000 inhabitants or more are considered urban. Such disparities pose problems when making international comparisons.

Between 1960 and 2020, the number of West African cities, Cameroon included, with a population over 100,000 will rise from 17 to 200 (Cour 1995, 2001). Most of the urban growth may in fact not occur in the larger towns, but in secondary towns or in the hinterlands. For example, the respective populations of Douala and Yaoundé in Cameroon are projected to increase from a little less than 2 million inhabitants in 2005 to a little more than 2 million inhabitants in 2030 (UN 2006). These two cities will therefore have lower urban growth rates in the future than during previous decades: 5–6% between 1990 and 1995 compared with 2.5% projected between 2010 and 2015. The percentage of the urban population living in these two towns should stabilize at 20% in 2015 after reaching a peak in 2005 of about 22% (UN 2006). The missing millions of the population will therefore be located in the hinterland, in secondary towns.

In 2030, over 70% of the population of Cameroon is expected to live in cities. If we assume that 60% of the population will still derive income from farming, this means that at least 30% of farmers will live in towns. For instance, according to the UNDP, 80% of families in Libreville (Gabon), 68% of urban dwellers in six Tanzanian cities, 45% in Lusaka (Zambia), 37% in Maputo (Mozambique), 36% in Ouagadougou (Burkina Faso) and 35% in Yaounde (Cameroon) are involved in urban agriculture. In their study of Kampala (Uganda), Maxwell and Zziwa (1992) estimated that 36% of the population was involved in urban agriculture. The involvement of so many people in urban agriculture indicates its centrality in informal sector activities. There seems to be no signs in Sub-Saharan Africa today that the number of people involved in farming activities as a primary or as a secondary source of income will significantly decline in the near future (World Bank 2007). But this trend will induce strong urban-rural linkages, as rural households progressively combine employment and incomes from the two sectors (Ruel and Haddad 1999; Haggblade and Hazell 1989). Therefore, an increasing share of farmers' income will derive from off-farm activities (Reardon 1997; Ellis 1998; Parrot et al. 2008c).

2.2 Urban Agriculture Will Provide Employment

The social impact of agriculture is still predominant in Africa. In the absence of formal employment opportunities from other sectors of the economy, industries and services, agriculture remains a necessary contributor to livelihoods (Ellis and Sumberg 1998). However, the economic impact of agriculture at the country level is not always so significant. In Cameroon, the primary sector (food crops, livestock and fisheries) accounts for only 20% of the gross domestic product (GDP) (MINADER 2006).

Though agriculture in Cameroon accounts for less than 20% of GDP, in 2004, it still provided income for almost 60% of the population (MINADER 2006). The social impact of agriculture is therefore very important, especially for small-scale farming. As much as 80% of all farms are family farms, accounting for most rural employment. Following the rise in demand from cities for food, small-scale farming is gradually shifting from subsistence farming to a mix of subsistence and capitalistic farming (Cour 2001). At household level, the social impact of agriculture is still predominant in terms of employment opportunities and survival strategies (Corral and Reardon 2001; Berdegue et al. 2001; Reardon et al. 2001; Parrot et al. 2006). The lack of employment opportunities in the industrial sector or in the service sector makes agriculture essential to the livelihoods of millions of people in developing countries (World Bank 2007).

Trends in urban growth and the rise of urban farmers will affect productivity in agriculture by reducing the area of arable land, especially in regions of high population density. They will also influence environmental issues, such as reduced fallow time and multiple cropping cycles in one year (Keys and McConnell 2005). Larger proportions of farmers will live in towns or in their peri-urban belts, using more chemical products than before and therefore increasing sanitary risks (Reardon et al. 1999).

2.3 Livelihoods and the Informal Sector

Rural-urban linkages are increasing and the distinction between the two sectors is already causing conceptual problems for national statistics institutes. Very little is known about local economic activities and livelihoods. Local economic activities are difficult to assess, because of (1) underground production such as registered traders with deliberately concealed production; (2) illegal production such as fuel smuggling; (3) informal production, "unregistered traders" mostly at household level; and (4) household production for autoconsumption, e.g. food. Investigations of livelihoods will prove to be necessary in order to cover all the dimensions of households and understand the continuing structural and social changes among them. Agricultural and non-agricultural activities will have to be analyzed simultaneously and not separately for a better understanding of household strategies and income portfolios.

The informal sector also needs to be taken into account, because it impacts most other sectors of an economy and the methodological frameworks for surveys. In 2004, the informal sector accounted for more than half of the gross domestic product (GDP) of Cameroon (Fig. 1). As much as 90% of all workers in the country did not have a signed and formal contract with their employer (INS 2005). As stated by Schneider and Enste (2000), "a prospering shadow economy may cause severe difficulties for politicians, because official indicators – on unemployment, labor force, income, consumption – are unreliable". The lack of proper information, or statistics between the macro- and the micro-level of analysis, in a decentralization process, can lead to dramatic policy implications (Bahiigwa et al. 2005; Ellis and Bahiigwa 2003).

Fig. 1 The informal sector accounted for more than half of the gross domestic product (GDP) of Cameroon. In agriculture industry, it includes agricultural production activities as well as marketing of agricultural products as in Muea next to Douala. Credit: Laurent Parrot / CIRAD

Urban agriculture is one of the traditional activities conducted by African households as a risk-sharing strategy, but also as a significant part of their culture and tradition of urban gardening. As stated by Page (2002) in the case of Cameroon: "Far from being a technical practice, urban agriculture has often been a culturally and politically important aspect of urbanism in Africa". Urban growth and the consecutive structural changes in landscape and livelihoods affect urban agriculture (Cofie et al. 2008). In Africa, the institutional interactions between the ministries of Agriculture and the ministries of Urban Planning often turn into conflicts of interest as urban agriculture can be considered on one side as a necessary contributor to livelihoods or, on the other side, just as an illegal scheme for squatters. Urban agriculture is also practiced by the urban poor or newcomers, and partly in non-constructible areas of towns, in swamps and lowlands. The lack of property rights and the illicit nature of its practice make any investigation very difficult to implement. All in all, the traditional and cultural aspects of urban agriculture in Africa are confronted with the structural challenges faced by the towns in which they have been evolving for decades.

A major feature of Urban Agriculture (UA) is the diversity of the socio-economic profiles of the actors involved, and their varying income and livelihood

Table 1 Summary of typology of urban agriculture socio-economic profiles

	Home subsistence farmers	Multi-cropping peri-urban farmers	Family-type commercial farmers	Entrepreneurs
Location[a]	U	P	UP	P
Outlets	Home	Home + urban markets	Urban market	Urban market + export, additional income, leisure
Objective	Home consumption	Home consumption and income for subsistence	Income for subsistence	
Size	Usually <100 m^2	Usually >5,000 m^2	Usually <1,000 m^2	Usually >2,000 m^2
Products	Leafy vegetables, cassava, plantain, maize, rice, goats and sheep, poultry, fruits	Staple food crops, local vegetables,	Leafy vegetables temperate vegetables, poultry, (sheep), (milk)	Temperate vegetables, fruits, poultry, livestock, fish
Intensification (inputs/ha)	2	1	2–3	4
Gender	F	F + M	F + M	M
Limiting factor	Size	Access to inputs, fertility	Size, land insecurity, access to inputs, water and services, marketing risks	Technical expertise, marketing risks

Source: Moustier and Danso (2006)

[a] U within the urban districts of the city, P in the peri-urban districts of the city

strategies, a reflection of the diversity of the labor and capital basis in urban areas. A typology was established Gura in 1996 (Gura 1996) and since that time some other research has provided some attempts at classification (Bakker et al. 2000; Temple and Moustier 2004; Moustier and Danso 2006) that are summarized in Table 1. The first category, home subsistence farmers, refers to urban residents who farm on small plots around their homes, mostly for subsistence purposes. The second category also refers to farmers with predominant subsistence strategies, but whose location in peri-urban areas makes it possible to associate multiple food crops on large plots, without use of chemical inputs or irrigation. This type is especially observed in the rain-fed agricultural systems of Central Africa. The third type refers to commercial urban and peri-urban farmers who are involved in agriculture to earn a monetary income for basic family expenditures, while the "entrepreneurs" (fourth type) have diversified sources of income and are able to invest in a larger scale of production than farmers in the other categories. For these farmers, agriculture not only represents a source of income, but also a source of leisure. This dimension is also present in the other categories, although it may not be the major driver of the activity.

3 Marketing and Multi-functionality of Urban and Peri-Urban Agriculture

3.1 The Food-Supplying Role of Urban Agriculture

The contribution of urban agriculture (UA) to the livelihoods of the urban poor is obvious. In the second part of this review, the specificity of UA will be described in terms of marketing, products and multi-functionality. Urban agriculture is the subject of intense debate as regards its viability and the necessity for political support. In a challenging paper, Ellis and Sumberg (1998) provided a number of reasons why scarce public resources should not target urban agriculture: in particular, the high cost of land in urban areas and the pollution it can attract and generate. Nevertheless, more and more data is becoming available to demonstrate the unique advantages of urban agriculture that advocate for well-targeted public support.

Urban agriculture is a source of food for urban dwellers both in terms of self-consumption and in terms of purchased food. The share of self-consumption in urban agriculture ranges from 10% to 90% according to the availability of land in the city, the nature of the staples, and urban purchasing power. With increasing land pressure, home consumption tends to decrease and recourse to the market increases. Peri-urban areas play a central role in the supply of perishable products, especially vegetables.

The importance of urban agriculture in supplying fresh, perishable products, while rural areas supply more bulky and easier-to-store products, is in line with Von Thünen's predictions in the first analysis of agricultural land use according to location done in 1826. According to Von Thünen's model, land is allocated according to the use which brings the highest rent, and can be sketched as concentric circles relative to the city center. The rent is defined as the share of the output by area after deduction of production and transport costs. The most profitable and intensive land use by unit area, and commodities with high value relative to transport costs are found near the city center (Huriot 1994). This is typically the case for perishable fruits and vegetables.

The available data in Asia and Africa confirm the importance of urban agriculture in the provision of perishable food commodities, including fresh vegetables, dairy products and plantain banana. Figures on the importance of urban agriculture in urban food markets using market surveys have been gathered by CIRAD in case studies conducted between 1990 and 1995 in Central Africa (Mbaye and Moustier 2000; Moustier and Danso 2006). The International Development Research Center (IDRC) supported similar studies in Ghana via the International Water Management Institute (IWMI) (Keraita and Drechsel 2004). Other similar studies providing data on UA market share include Mai Thi Phuong Anh et al. (2004) for Hanoi; Yi-Zhanh and Zhang (2000) for Shanghai; and various sources quoted in the Urban Agriculture Magazine 2002 special edition for the World Food Summit. The CIRAD studies involved in-depth interviews with a sample of farmers and traders on the relationships between buyers and sellers, particularly the regular nature of the relationship and possible commitments in terms of quality.

Fresh vegetables supplied by urban agriculture are leafy vegetables such as amaranth (*Amaranthus* *hybridus*), water convolvulus (*Ipomea aquatica*), sorrel (*Hibiscus sabdariffa*), morel (*Solanum aethopicum* and *S. nigrum*), cabbages (various species of Brassicacea), lettuces and chives (*Allium fistulosum*) (Figs. 2, 3). These vegetables top the list of vegetables consumed in Africa and in Asia, along with onions and tomatoes. They are well known for their fragility (after one day they are no longer fresh) in countries where freshness is an important criterion for consumers who often do not have refrigerators. These leafy vegetables are mostly brought into town from distances of less than 30 km from the city centers, be it in Africa or in Asia. The urban agriculture origin represents more than 70% of the contributions in all the cities investigated. In Hanoi in 2002, more than 70% of all leafy vegetables came from a production radius of 30 km around the city. Ninety-five per cent to 100% of all lettuce came from less than 20 km away, while 73–100% of water convolvulus was harvested less than 10 km from the city (Moustier et al. 2004). In Phnom Penh, urban areas, i.e. those located inside the municipality, supply all of the water convolvulus marketed in the city. This is a vegetable particularly important for consumption by the poor (Sokhen et al. 2004).

Fig. 2 The freshness of urban leafy vegetables, as water convolvulus (*I. aquatica*) in ponds in inner districts of Hanoi, is one the reason of their cultivation in urban area. Credit: Paule Moustier / CIRAD

Fig. 3 Amaranthus and cabbages are two worldwide leafy vegetables grown in tropical urban and peri-urban areas, mainly in developing countries due to the high adaptation of some varieties to high temperature and humidity. Credit: Hubert de Bon / CIRAD

In the case of less perishable vegetables, such as tomato and cabbage, which can stay fresh for a few days, supply varies from peri-urban to rural production. Dry onion originates only from rural areas or from imports in the African cities investigated. As regards staple foods, such as rice, plantain banana and maize, the situation is highly variable according to the city. In Asia, the share of rice supplied by the city to urban residents ranges from 7% in Phnom Penh, to 100% in Vientiane, where pressure on land is low; Hanoi being an intermediate case with 58% (Mai Thi Phuong Anh et al. 2004; Ali et al. 2006), and a steady decrease in the production of rice in favor of vegetables.

3.2 The Characteristics and Advantages of Proximity in Market Supply

Urban-produced commodities are distributed through short marketing chains relative to rural commodities. The extreme case is direct producer involvement in retail sales. This is the case for 30% of all transactions in Bangui (David 1992) and 70% in Bissau, where private trade had recently been legalized at the time of surveys (David and Moustier 1993). Generally, the producer sells to retailers. This transaction takes place in the field or at nighttime wholesale markets in, for example, Brazzaville, Bangui, Bissau, and in Hanoi, Phnom Penh and Vientiane. In Hanoi, more than 40% of all wholesale market sellers are also producers. This percentage increases to 100% for water convolvulus (Moustier et al. 2004).

In Phnom Penh, the marketing chains of kangkong, i.e. water convolvulus, are short, and 57% of retailers are directly supplied by farmers, who receive more than 50% of the final price. Hence, the water convolvulus-growing areas are important with respect to poverty for both farmers and consumers (Sokhen et al. 2004). On the other hand, tomato, which mostly originates from Vietnam, is traded through collectors and wholesalers for more than 60% of transactions.

Short marketing chains enable low price differentials between the farm and final consumption. Such differentials are 30% for leafy vegetables traded in Hanoi, 35–50% of cabbage, and 75–80% of tomato, while they are more than 100% for vegetables brought from Dalat or China, and more than 200% for vegetables traded from the Red River Delta to Ho Chi Minh City (Moustier et al. 2004). In Havana, Cuba, the prices of tomato, onion, pork and fruits fell threefold between 1994 and 1999, the period when the urban agricultural program was launched (Novo 2002). The government provided free land access for more than 26,000 gardeners, and technical training in organic and hydroponic cultivation methods (Moskow 1999). Peri-urban areas have transport cost advantages compared with rural areas that translate into lower final prices. Rising oil prices will make local food supplies even more valuable than at present.

While food safety risks may be higher in urban production areas than in rural areas, because of various sources of pollution, e.g. heavy metals in water used for irrigation, and limited land area, forcing farmers to use excess fertilizers and pesticides, the proximity of production areas to consumers provides them with advantages for easier quality control. In Hanoi, supermarkets, shops and restaurants are mostly supplied by three cooperatives located in the peri-urban areas where production following Integrated Pest Management (IPM) or organic standards is certified by government bodies (Moustier et al. 2006). Proximity enables frequent contacts between farmers, traders and consumers, and monitoring of the production process. In India, farmers located around Aurangabad sell their vegetables through urban organic bazaars organized on a fortnightly basis. Local certification is obtained through "eco-volunteers", people usually working in the vicinity of the vegetable farmers (Braber 2006). The irregular nature of vegetable production is a major drawback of all direct sales by organic or IPM farmers, as they are tempted to buy from sources other than their own, which then creates further difficulties in guaranteeing product safety (Braber 2006).

Factors other than distance also give specific advantages to urban agriculture. In certain cases the hinterlands of cities are especially favorable for agriculture, and there are cases when a city was established in a given location because of a rich agricultural hinterland. Furthermore, compared with rural areas, farmers are motivated to earn regular cash income year-round from small plots in order to be able to buy food and ensure a regular livelihood, while in rural areas some land can be reserved for subsistence food production. This explains why urban production tends to be less seasonal than rural production, which is an important factor for guaranteeing food security in urban areas (Moustier and Danso 2006).

The possibility for citizens to exert control over the way food is produced can indeed be considered as a legitimate right (Koc et al. 1999). Yet the development of international trade, as well as the globalization of capital in food distribution, is now widely documented (Mc Michael 1994; Reardon and Berdegué 2002). This creates risks of growing distance between producers and consumers. Durability of food is developed at the expense of its sustainability (Friedmann 1994). Growing distances between production and consumption areas reinforce consumers' anxieties about food safety, which some authors have called "anxiogenic distanciation" (Bricas and Seck 2004).

3.3 The Case for Public Support for Multi-functional Urban Agriculture

In addition to its role in urban food supply, urban agriculture plays a number of environmental, social and economic functions that are still to be recognized by urban authorities. Multi-functionality, usually defined as the multiple roles or objectives that society assigns to agriculture, including economic, social and environmental roles, is a typical characteristic of urban agriculture (Vollet 2002; Véron 2004; Duvernoy et al. 2005; Ali et al. 2006). Urban agriculture creates landscape, i.e. a public good, in which users cannot be excluded. This makes land management of little interest to the private sector (Donadieu and Fleury 1997). In both Southern and Northern countries, as well as with family gardens, urban agriculture produces other things of value to the public, such as food security, social insertion and employment. Within cities, other sectors create landscape, such as parks, to which urban agriculture can be compared. The advantage of urban agriculture over other "landscape producers" is that its functioning is supported by market forces, even if these markets are imperfect. It is thus a less expensive landscape producer than a public park. It also provides jobs and social inclusion. Based on research in France, Russia and Brazil, it has been argued that urban agriculture is a key component of sustainable human development, including therapy, culture and identity (Boukharaeva et al. 2005; Boukharaeva and Marloie 2006). The multi-functionality of urban agriculture makes it a "cheap" producer of public good (Moustier and Danso 2006).

Increasing distances between urban centers and agriculture is, however, irreversible, if market forces are given a free hand. This is due to the fact that it is more economically sound to develop land than farm it, other than for exceptions such as swamps. Access to land is always quoted among the first constraints by farmers, together with excess or deficient water, flooding and humidity, resulting in various diseases (Temple and Moustier 2004; Midmore and Jansen 2003; Prain 2006). Hence, from a political economy viewpoint, it is legitimate for the public sector to support urban agriculture. In fact, for urban agriculture to be successfully maintained in the city, farmers and non-farmers should share some objectives: duties and rights to examine from the urban residents' side, landscape and environment, and from the farmers' side, protection from land development. Instead of claiming a specific space for urban agriculture, farmers have to negotiate sharing it with other users (Mbiba and Veenhuizen 2001). In Delft, a city in the south of Holland, a farmer was able to negotiate a 12-year term lease for 35 ha of land with the municipality, thanks to his commitment to producing organic vegetables and milk, and also the setting aside of 5 ha of land for nature preservation (Deelstra et al. 2001).

Four areas are particularly relevant for public support of urban agriculture: (1) integration in urban planning; (2) financial support; (3) research and extension for more profitable and sustainable intensive commercial vegetable and animal systems (Midmore and Jansen 2003); and (4) innovative marketing, including quality labeling. The municipality has a crucial role to play in organizing such support in collaboration with national and international programs.

As for the provision of other urban services, in a context of scarce public resources and concern for long-term sustainability of employment, public–private partnerships are advocated by UN agencies as a promising strategy of public support. Cuba is a successful illustration. In return for providing the land, the government receives a proportion of the produce – usually about one-fifth of the harvest – to use at state-run day-care centers, schools and hospitals (Cruz and Medina 2003).

Multi-stakeholder processes dealing with urban agriculture were amongst others developed by UN-Habitat city development strategies, especially in Ecuador, Argentina and Tanzania. In Quito, the local government, several NGOs, UN-Habitat and community representatives signed an inter-actor agreement for carrying out a participatory diagnosis and developing an action plan on urban agriculture. In Dar-es-Salaam, a multi-stakeholder consultation held in 1992 resulted in the protection of specific areas for agriculture (Dubbeling and Mertzthal 2006). Growing attention and increasingly positive attitudes towards urban agriculture are reflected in a number of "declarations on urban agriculture" in which local and national level policy-makers have stated their formal commitment to developing policies on urban agriculture. These include the forum in Harare in 2003 attended by local governments from Kenya, Malawi, Swaziland, Tanzania and Zimbabwe, and the Quito declaration signed in 2000 by city mayors from 22 countries in Latin America and the Caribbean (Veenhuizen 2006).

In Benin, talks between the government and the Cotonou communal producers' union resulted in the allocation of 400 ha to market gardeners (Deguenon 2008). In Uganda, the Mayor of Kampala passed by-laws in 2005 to allow urban dwellers to cultivate land and rear animals within the city (Cofie et al. 2003). In 2005, these various experiences prompted the Cameroonian farmers to set up a coalition for the promotion of urban and peri-urban agriculture in Africa, with the support of researchers. The coalition, named *Coalition pour la promotion de l'agriculture urbaine et périurbaine en Afrique* (CAUPA) intends to foster dialogue between farmers and town councils.

4 Urban Agricultural Production Techniques

The different products of urban agriculture include many different plant crops (vegetables, cereals, tree fruits, ornamentals, spices, seedlings and plants, and flowers) and animal products (dairy, pigs, poultry, livestock and aquacultural products). This review has focused on vegetables, as already mentioned, which are typical urban crops due to their short cycles, for example, 30 days for choysum in Hanoi, short shelf-life, high manpower needs and high value (Bon et al. 2002) (Fig. 4).

4.1 *Technical Agricultural Requirements for Production in Urban and Peri-Urban Areas*

Urban agriculture faces severe competition with non-agricultural economic activities, habitat, transportation, etc. There is strong competition for access to manpower, but also to inputs (water, fertilizers) and land. In addition, urban and peri-urban environments are often highly polluted by industry, domestic activities such as domestic and office heating and cooling, for example, and transport. At the same time, agriculture is known to pollute the environment through the use of pesticides, and chemical and organic fertilizers. Thus, the challenge for urban agriculture is to demonstrate that it does not pollute the city environment, but rather that it produces safe food products despite a sometimes polluted urban environment (Fig. 5). One difficulty for the agronomist is that the "field" in peri-urban and urban areas can vary from 1 ha for rice in Taiwan to 1 m^2 for organoponic beds in buildings in La Habana; the "field" can also be a pond to grow aquatic vegetables, as in Hanoi. As Deffontaines (1991) has shown for rural areas, the field is increasingly a piece of the landscape that is located in an environment, the city in the case of urban and peri-urban agriculture, and is the center of multiple interests for the grower, and also for all the population living around the field. So the concept of sustainability as defined in rural areas (Meynard et al. 2001) must be used with all its social, economic and environmental dimensions to propose cropping systems adapted to the city environment.

Fig. 4 The cultivation of short-cycles leafy vegetables, from 25 to 40 days, as choysum (*Brassica rapa* cvg. *Choysum*) in Hanoi, is one of the main characteristics of the urban and peri-urban production. Credit: Hubert de Bon / CIRAD

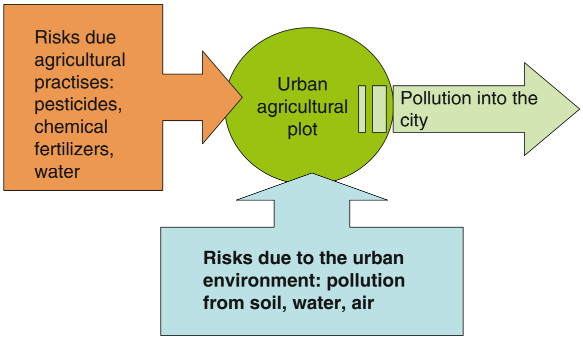

Fig. 5 The risks in the relation between city and urban agricultural production are various and reciprocal. Evaluation has to be done for each couple city/crop

4.2 *What Inputs Are Used in Urban Agriculture?*

Despite many efforts to increase productivity, to provide disease- and pest-resistant varieties, and to develop techniques for small areas, water and fertilizers are the major inputs used in agricultural production (Bon 2003). In addition, horticultural production requires pesticides, and livestock production needs animal feed. The proximity of cities may provide opportunities to get part of them by the uses of solid and liquid wastes of the cities, the available quantities of which increase with the growth of the cities.

The different sources of nutrients for urban crop production are chemical fertilizers, plant compost, animal manures and solid city wastes. Chemical fertilizers are used by all the urban farmers in all the cities and cases cited in this review. Solid wastes are also used for fish ponds. The use of organic matter, although very frequent, is not so widespread. Organic wastes are used fresh or composted. In Hanoi, the different manures that are used include chicken dung, cow manure, pig manure and various mixes of these. The percentage of farmers using manure in Hanoi increases from 16% for the inner urban agriculture farms of the city to 75% for those at the district limits. The manures are produced on the farm or are bought. Average annual usage is 9.8–12.7 t ha^{-1} (Mai Thi Phuong Anh et al. 2004). Vagneron (2006) cites the use of 5 t ha^{-1} on vegetables in Bangkok. In Antananarivo, organic matter is provided by manure, which is sometimes on-farm compost, straw from uncultivated lands and compost from solid city wastes (N'Dienor 2006). In Lomé, organic matter and chemical fertilizers are used in all the city gardens (Tallaki 2005). In Dakar, it has been estimated that 25% of the nutrients for horticulture crops come from plant compost and another 25% from animal manure (Fall et al. 2002). The integration of livestock and horticulture with horticultural residues being used as livestock feed has been promoted (Akinbamijo et al. 2002). Most surveys of urban areas have shown links between horticulture and livestock production by the means of purchase of animal manure by farmers rather than by integration of the two activities. And the manure and crop residues are not sufficient for a complete urban nutrient cycle.

The use of solid waste in urban agriculture is common in the cities of developing countries. In these cities, kitchen wastes and paper are the major components of refuse, accounting for 42% and 19%, respectively, in Metro Manila (Ali and Porciuncula 2001). The nutrient content of these wastes is rather low; for example, just 0.29% nitrogen and 0.16% phosphorus in organic waste in Ougadougou and Bamako (Eaton and Hilhorst 2003). Numerous projects have been implemented to encourage the use of different wastes from municipality projects by establishing compost plants for community and individual growers in cities using specific compost chambers, containers, heaps, trench composting and vermiculture systems. In composting urban solid wastes, the risks to human health for both consumers and the farmers handling the compost must be considered. This includes the survival of pathogenic organisms (*Salmonella*, *Entamaeba coli*, *B. cereus*), zoonosis, disease vectors, and chemical pollution by heavy metals and persistent organic compounds. A sorting of wastes based on a house-to-house source-separated waste-collection system with a good composting process for the correct raw materials should be used to minimize these risks (Cofie et al. 2006).

The use of wastewater for crop production, e.g. ornamentals, vegetables, tree fruits and fodder, as well as for aquaculture, occurs in developing country cities and those of emerging countries such as China and Mexico. The generation and the use of wastewater is rising in peri-urban and urban areas together with increasing population. The IWMI estimates that 16,000 ha in Hyderabad are irrigated with wastewater (Buechler et al. 2006). In Kumasi (Ghana), the area irrigated with wastewater is about 11,900 ha in a catchment of 12,700 households, and in Nairobi (Kenya) 2,220 ha and 3,700 households (Cornish and Kielen 2004). In arid and semi-arid areas, such as Nouakchott in Mauritania, this is the only source of water for crops. Wastewater provides nutrients for crops and for fish in aquaculture. The water needed to produce, for example, 1 kg of tomatoes can vary from 50 to 100 l depending on the climatic conditions. Thus, as domestic and industrial demands for freshwater resources increase, it becomes unreasonable to consider irrigating crops with potable water. The use of wastewater brings benefits for growers. In Nairobi, the average annual revenue per hectare from irrigated plots is US $1,770, but only US $544 in Kumasi during the dry season. So urban wastewater can contribute to the livelihoods of the irrigators using it (Cornish and Kielen 2004), but the implications for public health of wastewater use are serious. Fecal coliforms and streptococcus as well as Ascaris, Giardia and *E. coli* parasites are present in wastewater. Lagoon sewage treatment with *Psitia stratiotes* can improve the quality of the water by reducing the presence of parasites, but not the fecal coliforms (Gaye and Niang 2002).

The use of solid and liquid wastes is thus an opportunity for developing agricultural production and for cleansing the polluted urban environment. These wastes could supply a part or all of the nutrients needed for urban agriculture, but the human health concerns are still to be addressed.

4.3 Pollution of the Environment

The soils, water resources and air of the urban environment are polluted. Analyses indicate that city soils are more polluted that those in rural areas. In one study, organic pollutant (benzo(a)pyrene) contents were more than $0.05\,\mathrm{mg\,kg^{-1}}$ in all of the urban soils sampled compared with only 15% of those sampled from rural areas (Konig, 1991, cited by Barriuso et al. 1996). Similar observations have been made in various studies on PCB and PAH contamination (PCB: polychlorobiphenyls, PAH: polycyclic aromatic hydrocarbons). The heavy metal contents of urban agriculture soils are frequently above allowable limits. Cu, Zn, Pb, Ni, Cd, Co, Mn and Cr were found in a survey of various cities in Eastern Europe. The largest sources of this contamination are heavy industry and run-off from highway drains (Lungu 2002). This type of contamination is also often found in the irrigation water and water used for aquaculture in Asian cities.

Air pollution is due mainly to transportation, domestic heating and industry. In Hanoi, the average contents of NO_2, CO_2 and NO_3 in the air have reached levels of $0.04–0.09\,\mathrm{mg\,m^{-3}}$ and the level of CO $2–5\,\mathrm{mg\,m^{-3}}$. The concentrations of SO_2 and CO_2 in urban districts are higher than the permissible limits (Mai Thi Phuong Anh et al. 2004).

4.4 The Use of Pesticides

A major constraint to the development of agriculture in and around cities is the use of synthetic chemical pesticides. Technical protocols for vegetable, ornamental and flower crop production typically recommend frequent pesticide applications. Various active ingredients from all the principal chemical families – organophosphates, carbamates, pyrethroids and organochlorines – are commonly used in urban vegetable production (Tallaki 2005; Mai Thi Phuong Anh et al. 2004; Cissé et al. 2002) as well as the biological insecticide *Bacillus thuringiensis*. In Lomé, neem seed juice from *Azadarichta indica* is used by 70% of farmers alone or in combination with chemical insecticides (Tallaki 2005). Pesticides are applied with small individual sprayers at rather high frequencies of up to once or twice a week throughout the year. These practices can have negative effects on the health of farmers and consumers, and on the environment. An extensive study on the contamination of the watershed in Niayes in Dakar showed chemical pesticide contamination of the 20 wells surveyed (Cissé et al. 2002). Different studies have shown toxicity symptoms due to pesticides in Dakar (Cissé et al. 2002) and Hanoi (Trong Khac Thi, unpublished). Despite numerous projects on Integrated Production Management in the urban agriculture of large cities around the world, there is still much to do in training farmers, extension workers, and chemical retailers and traders in the areas of pest and disease identification, correct use of pesticides and their application, and promotion of less toxic pesticides. Research on how to enhance the natural control of pests and diseases needs to be developed.

4.5 Is There a Future for Specific Techniques in Urban Agriculture?

To avoid the problems of pollution due to chemical pesticide use, organic agriculture has been suggested and pushed in some cities of Germany, the Netherlands and Slovenia. This kind of production is seen as a way to reinforce the role of agriculture in maintaining biodiversity. Interesting initiatives have been encouraged in some Eastern European cities (e.g. Romania, Bulgaria and the Czech Republic) (Yoyeva et al. 2002). The integration of different agricultural production systems such as livestock, aquaculture, vegetables and tree fruits could be a way to reduce input costs. However, animal husbandry in the city is problematic because of its relation to unpleasant smells and noise, as well as health risks and need to manage manure. It must therefore be strictly regulated in relation to population density and distance to the city center in terms of animal numbers and types, the cleaning of stalls, disease control and water use. The risk due to heavy metal contamination in water, and solid wastes used for compost and soil can be decreased by phytoremediation or specific land uses (e.g. flowers, ornamentals and recreational areas). Nevertheless, the use of waste to produce agricultural products for human consumption must still be improved to assure consumer safety.

In Asia, the SUSPER project (AVRDC/CIRAD) has enabled cities (Hanoi, Ho Chi Minh City, Phnom Penh and Vientiane) to respond better to local demand for vegetables and to make the switch to

Table 2 Some characteristics of urban / peri-urban vs. rural agriculture in developing countries

Characteristics	Urban agriculture	Rural agriculture
Employment	Agricultural labor is low related to non-farm employment in the city	Agriculture is the main employer in the rural area
Farmers' income	Agriculture may be a temporary or partial source of income	Agriculture is the main source of income
Farm profile	Informal and often illegal use of the land	Traditional access to land
Market supply	Urban markets and self-consumption	Self-consumption, urban and rural markets, exports
Product types	High value and perishable products	All types, mainly staple food
Commodity chain	Short marketing chain	Long marketing chains
Multi-functionality	High	Low
Access to inputs	Close to the sellers	Far from sellers
Food safety risks	Risky (polluted inputs and environment)	Low risk
Access to natural resources	Strong competition with other urban economic activities	Little competition with other uses
Public policy	Ambiguous. Generally in favor of other urban activities and land uses	Priority for policy-makers in charge of rural areas

commercial production. Technical solutions have been found in order to satisfy market demand and boost farmers' incomes, such as out-of-season production. New vegetable sanitary quality certification systems have been tested, and a system for gathering and disseminating daily price information has been developed to facilitate negotiations between producers and traders (Moustier 2007).

5 Conclusion

In Table 2, the components of urban agriculture that have been analyzed in the paper are compared with rural agriculture. These specificities have to be taken into account in the development of research related to urban agriculture.

Urban growth in Africa and urban food requirements will induce significant changes in African agriculture. Two types of farmer already coexist on the continent and this trend will continue for the next few decades. At one extreme there is the traditional farmer, living in a rural or an urban context, with low productivity, low income and off-farm incomes. At the other extreme is the capitalistic farmer, specialized in agriculture, with high productivity and strong market integration (Cour 1995, 2001). In urban agriculture, the family-type, commercial farmer, is still widely represented, but his options for economic accumulation are still limited. Export crops and high added-value production, such as horticultural crops, are part of two strategies which will develop in the near future (Jayne et al. 2006; Oliver and Spencer 2005). These structural changes will require specific analyses and specific policy actions. Urban agriculture is often tolerated by governments, but rarely encouraged despite its vital contribution to employment and livelihoods, although this is reported to be changing. The urban farmers must be more determined in promoting their agriculture and in proposing services to the urban dwellers and city authorities, including landscape preservation and social inclusion. The promotion of the multiple functions of urban agriculture is a major challenge for the future. Hence, there is a growing need for documentation of the successful integration of urban agriculture in urban development, and on the conditions necessary for its social, economic and environmental sustainability.

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Nitrogen, Sustainable Agriculture and Food Security: A Review

J.H.J. Spiertz

Abstract The impact of modern agriculture on natural resources has become a major global concern. Population growth and expanding demand for agricultural products constantly increase the pressure on land and water resources. A major point of concern for many intensively managed agricultural systems with high external inputs is the low resource-use efficiency, especially for nitrogen. A high input combined with a low efficiency ultimately results in environmental problems such as soil degradation, eutrophication, pollution of groundwater, and emission of ammonia and greenhouse gases. Evidently, there is a need for a transition of current agricultural systems into highly resource-use efficient systems that are profitable, but at the same time ecologically safe and socially acceptable. Here, opportunities to improve nitrogen-use efficiency in cropping and farming systems are analyzed and discussed. In the past and present, increased productivity of the major plant production systems has been derived from genetic improvement, and from greater use of external inputs such as energy, fertilizers, pesticides and irrigation water. Aiming at improving resource-use efficiencies, in high-input systems the focus should be on more yield with less fertilizer N. In low-input systems additional use of N fertilizer may be required to increase yield level and yield stability. Developing production systems that meet the goals of sustainable agriculture requires research on different scales, from single crops to diverse cropping and farming systems. It is concluded that N supply should match N demand in time and space, not only for single crops but for a crop rotation as an integrated system, in order to achieve a higher agronomic N-use efficiency. A combination of quantitative systems research, development of best practices and legislation will be needed to develop more environmentally-friendly agricultural systems. The growing complexity of managing N in sustainable agricultural systems calls for problem-oriented, interdisciplinary research.

Keywords Biodiversity • Cropping systems • Environment • Land use • Nitrogen-use efficiency • Productivity

1 Introduction

Population growth and expanding demand for agricultural products constantly increase the pressure on land and water resources. Today, global agriculture feeds a population of approximately 6.4 billion and delivers a wide range of additional services such as rural employment, bioenergy and biodiversity. The world's population is increasing by about 1 billion people every 12 years. In 2050, the population is projected to be about 9 billion (UNEP 2007). However, the main question is not if we can feed 9 million people in 2050, but can we do it sustainably, equitably and on time in the face of the growing demand for biofuel and the probable changes in climate? Agriculture has to meet at a global level a rising demand for bio-based commodities such as food, feed, fiber and fuel, while satisfying even tighter constraints with respect to the safety of products, the environment, nature and the landscape.

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Currently, policy-makers in countries of the European Union are focusing strongly on the concept of multifunctional land use. Indeed, besides agriculture other economic activities such as recreation, producing regional products with a special brand, and ecological services such as maintenance of landscape, biodiversity and water harvesting, contribute to employment and income (Tait 2001). Furthermore, high standards for food safety and quality are imposed to control food scares (Knowles et al. 2007).

Sustainability is based on the principle that we meet the needs of the present without compromising the needs of the future. Sustainable agriculture combines three main objectives: economic profitability, environmental health and ethical soundness. It is often presented as a conceptual 3-P framework: People–Planet–Profit (Fig. 1). The changes in agriculture from a purely profit-oriented activity into a triple-P-based production sector, trying to meet productivity, efficiency and efficacy aims, have been of considerable importance during the last few decades.

The concerns of scientists and consumers about the large-scale use of chemical external inputs such as fertilizer nitrogen and pesticides from the 1950s onwards led to movements that searched for alternatives to conventional agricultural practices (Matson et al. 1997). Besides the organic movement, the agricultural research community invested in the development of systems, such as: integrated agriculture, and low-input and sustainable agriculture (Altieri 1995). The aim is to reduce the environmental impact and to enhance food quality while maintaining acceptable yields. Generally, the use of pesticides is controlled by legislation and inspection services. However, the use of N is mainly determined by economic incentives such as profitability and subsidies, and less by environmental costs. An ecosystem-based approach to manage nutrients and productivity of agroecosystems was proposed by Drinkwater and Snapp (2007). They stated that N losses would be minimal in systems where yields and soil reserves are maintained with nutrient inputs approximately equal to harvested exports. The critical question remains, what productivity levels can be supported by these technologies? Generally, low external inputs of nutrients will result in reduced yields when soil nutrients cannot buffer the gap between demand and supply. Sustainable crop management cannot be a "blue-print", but best practices should be adjusted to the specific agroecological conditions such as land availability, soil quality, water resources, weather patterns, labor requirements and markets. Scarcity of land and water is becoming a dominant factor for major cropping systems (Bouman et al. 2007a).

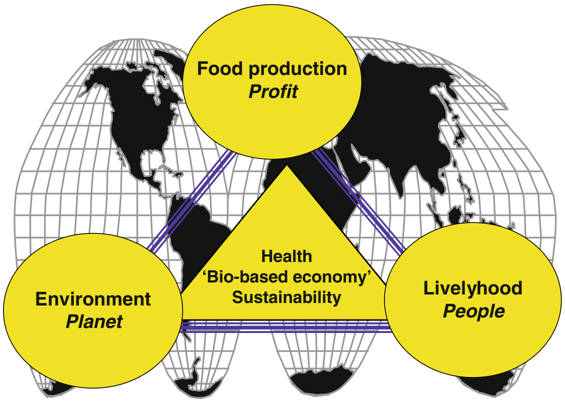

Fig. 1 Framework to relate substainability, bio-based economy and health goals boundaries for objectives in the domains of *People*, *Planet* and *Profit*

Land that is suitable for agricultural production is a finite and vulnerable resource on a global scale; however, there are big contrasts between regions. The availability of arable land per capita amounts currently to about 0.45 ha as a global average; however, a more severe decline to <0.10 ha has taken place in densely populated regions of China (Zhao et al. 2008). China is becoming more dependent on import of commodities, because it has to feed over 20% of the world's population with only 7% of the arable land. Globally, the agricultural land suited for growing crops can be expanded by some 180 million ha, especially in South America, North America, and Central and Eastern Europe (Hansen and De Ridder 2007). In Argentina and Brazil vast acreages of semi-natural grassland are reclaimed to grow arable crops, mainly soybeans.

Historically, increases in crop yield potential, intensification of cropping systems and expansion in the area of cultivated land have all contributed to the enhancement of world food production. The levelling off per capita grain production during the last two decades means that increases in grain production are only keeping pace with population growth. At the same time the rise in meat consumption causes a sharp increase in the use of cereals for feed. Currently, the world market of cereals has become volatile due to a growing demand and declining stocks of major commodities: rice, maize and wheat. The increase in grain prices will lead to an expansion of the acreage sown with cereals, especially maize and wheat. To avoid expansion of cultivation into fragile natural ecosystems, Cassman et al. (2003) concluded that raising yields should get more priority. Improved resource-use efficiencies are pivotal components of a sustainable agriculture that meets human needs and protects natural resources. The excess use of N fertilizers, available at low cost, causes environmental pollution (Eickhout et al. 2006).

In this review I will discuss the driving forces for intensification and diversification of major food production systems. Furthermore, options in strategic and tactical crop management to meet sustainability goals will be presented. The following topics are addressed:

- Food security, and land and nitrogen use
- Nitrogen use, crop growth and yield
- Primary productivity and biodiversity
- Nitrogen use at the farm and global levels
- Environmental boundaries and N management

The review will be concluded with some recommendations for improving cropping systems and management practices.

2 Food Security, and Land and Nitrogen Use

Agriculture has broadened and diversified its objectives. Good agricultural practice, where farmers aim at an ecologically and economically sustainable use of resources, should be the guiding principle in achieving sustainability goals. Furthermore, there are a range of technologies and practices that aim at resource conservation, such as: agroforestry, conservation agriculture, integrated aquaculture (fish-rice systems), integrated cropping management (ICM), integrated nutrient management (INM), integrated pest management (IPM) and water harvesting (Pretty 2008; Hobbs et al. 2008; Gupta and Seth 2007; Oehme et al. 2007; Tipraqsa et al. 2007; Yang H.S. 2006). Consequences of climate change for the occurrence of heat or drought stresses should also be taken into account (Olesen and Bindi 2002).

A general consensus exists that sustainable agriculture should focus primarily on:

- Maximization of the use of ecological processes, such as plant–microbial interactions, biological pest and disease control (IPM), crop–weed competition, and cycling of organic matter and nutrients (INM) within farming systems agroecosystems
- Optimal use of natural resources, e.g. soil fertility, soil water content, above- and below-ground biodiversity, and of genetic diversity in plant traits
- Restricted use of external resources such as synthetic chemicals, fossil energy and fresh water

Given the growing population and the limited area of fertile cropland globally there is an urgent need for further increasing the yields of crops combined with a sustainable use of non-renewable resources.

The question if the world is approaching the biophysical limits of food production was addressed by Penning-de-Vries et al. (1997). They analyzed the potential supply of food in different parts of the world. It was shown that there still exists a huge potential for expanding and increasing crop production, especially in Latin America. Within less than 10 years after this study, Brazil and Argentina have become major exporters of soybean and cereals to Asia and Europe (Hansen and De Ridder 2007). Globally, food production has risen since the 1960s by 65% (in Europe) to 280% (Asia), while world population has grown from 3 million to 6.4 million (Pretty 2008; Hazell and Wood 2008). Thus, per capita food production has outpaced population growth. The per capita grain production increased up to 1980 and has kept stable since then. Borlaug (2007) showed that a tripling of the world cereal production since the 1950s was achieved through only a 10% increase in area planted to cereals. The majority of the gain came from yield increase per unit land area resulting from the introduction of high-yielding cultivars that responded very well to supply of fertilizer nitrogen and irrigation water (Fig. 2). It was concluded that intensification of cereal production saved about one billion ha of agricultural land. Therefore, in regions with scarcity of land agronomic intensification will no doubt continue, with more nutrients, water and other inputs applied to crops in an

Fig. 2 High-yielding wheat cultivars grown under temperate climatic conditions in the Flevopolder, The Netherlands (photo taken by the author). For details, see Spiertz and Ellen (1978)

appropriate form, with a better timing and a more targeted site-specific application (Evans 1999).

In food crop systems nitrogen plays a key role, because it is the main yield-determining nutrient. The rise in food production did have a price: more external N inputs and more environmental harm. Until 1960 N fertilizer was used at a relatively low rate; then, crop nitrogen uptake depended strongly on manure applications, biological N fixation and indigenous N supply through mineralization of soil organic matter. Since the early 1960s, the use of nitrogen fertilizers has grown approximately sevenfold and nowadays 30–80% of nitrogen applied to farmland is lost to surface and ground-waters, and to the atmosphere (Goulding et al. 2008). In the atmosphere the non-reactive form (N_2) is already present abundantly; only the reactive N forms (NH_3, NO_x) are harmful. Goudriaan et al. (2001) analyzed that in 1990 fertilizer N accounted for 60% of the net primary production (NPP) and since 1980 has exceeded the amount taken up by crops globally. The economic response in crop yield far outweighs the cost of fertilizer, which led to over-applications when only yield improvement was considered and not environmental sustainability.

The role of nitrogen in future world food production and environmental sustainability was explored by Eickhout et al. (2006). Based on FAO projections they concluded that despite improvements in nitrogen-use efficiency of food production systems in developed countries, total reactive N loss will grow strongly towards 2030 because of the intensification of animal and crop production systems in developing countries. Herridge et al. (2008) estimated the global inputs of nitrogen fixed biologically in agricultural systems by pulses, oilseed legumes and other cropping systems at 50–70 Tg N (Tg: million tons). Soybean represents 50% of global crop legume area and contributes to about 75% of the N fixed by crop legumes. The increasing global trade of commodities such as soybean is also accompanied by a flow of nutrients. The annual total N flow in traded cereals from exporting to importing countries was estimated to amount to 11.5 Tg N in 2004; mainly from Brazil and the US to China and Europe (UNEP 2007).

So, there is a need for a great leap forward in a balanced use of fertilizer N. Only economic optimization of fertilizer use does not control over-use; therefore, communication about the risks of N excess in agro-ecosystems and legislation should be implemented. Unfortunately, in many developing countries adequate environmental policies are still in their infancy.

3 Nitrogen Use, Crop Growth and Yield

Long-term experiments, such as at Rothamsted Research in the UK, show that wheat yields with fertilizers exceed those without external N input by a factor of 2–3 (Rasmussen et al. 1998). It was found for on-farm and research station experiments that grain yields of maize increased from 3 to 14 $\mathrm{t\,ha^{-1}}$ with a rise in plant N accumulation from 50 to 300 $\mathrm{kg\,N\,ha^{-1}}$, while rice yields increased from 2 to 8 $\mathrm{t\,ha^{-1}}$ with a rise in plant N accumulation from 25 to 200 $\mathrm{kg\,N\,ha^{-1}}$, respectively (Dobermann and Cassman 2002). The increased use of fertilizers, especially nitrogen, strongly enhanced crop growth and yield, and as a consequence, the associated resource-use efficiencies such as light-use efficiency ($\mathrm{g\cdot MJ^{-1}}$) and water productivity (kg dry matter per unit of evapotranspiration).

In complex cropping systems, such as multiple cropping and relay intercropping, the response of the component crops to N is less than for sole crops (Van Noordwijk and Cadish 2002). This was clearly shown for rice–wheat cropping systems (Fan et al. 2007). The total N efficiency of relay intercropping systems of wheat and cotton was assessed by comparing the relative nitrogen yield to the relative yield total (Zhang et al. 2008). The relative nitrogen yield varied from 1.4 to 1.7, while the relative yield total ranged from 1.3 to 1.4, indicating that intercrops used more N per unit produce than monocrops. Thus, component crops in intercropping systems should get less fertilizer N than a monocrop.

Higher yields of cereal crops (e.g. rice and wheat) were derived from the breeding of high-yielding and N-responsive cultivars and a greater use of agrochemical inputs such as fertilizer and pesticides, and irrigation (Evans and Fischer 1999; Peng et al. 1999). The optimal N use for growth and maximizing yields is determined by plant traits, physiological processes, environmental conditions and nutrient management. The most significant increases in nitrogen-use efficiency (NUE) have come from improved plant genotypes and agronomic practices. Opportunities for improving NUE are:

- *Improved genotypes*; modern plant biotechnology and classical plant breeding show opportunities to improve NUE by selection for specific traits [root architecture, integrative traits (Laperche et al. 2006; Van Ginkel et al. 2001) and adaptation to stress conditions (Cabrera-Bosquet 2007)].
- *Improved resource use*; a better timing of nitrogen and water supply by time- and site-specific management can avoid stress at critical growth stages. More productivity per unit of water will lead to increased yields and thus higher NUE (Peng and Bouman 2007).
- *Improved cropping systems*; farmers can vary the timing of sowing/planting and the choice of crops in a cropping sequence to make a better match of the genetic make-up of a crop and the growing conditions determined by climate, soil and pests (Hobbs et al. 2008; Ladha et al. 2005).

Under high land pressure the emphasis will be on growing high-yielding genotypes and optimizing the management of external inputs, while under low-input conditions adapting cropping systems, including cultivar choice, to the variability and resource availability of contrasting agroecological conditions will become more important.

3.1 Nitrogen, Photosynthesis and Plant Growth

Nitrogen is the key element in plant nutrition limiting plant growth and crop yields in many agroecosystems, rainfed as well as irrigated systems. Crop photosynthesis is closely associated with light capture by the canopy and leaf N content depending strongly on the availability of nitrogen (Lemaire et al. 2007; Hikosaka 2004). Leaf N content is strongly associated with the rate of photosynthesis (Cabrera-Bosquet 2007; Dreccer et al. 2000; Sinclair and Horie 1989). An early canopy closure and a delay of canopy senescence will enhance the amount of light intercepted, while a very high leaf area index (LAI) increases mutual shading and therefore decreases light-use efficiency (Russell et al. 1989). In reproductive crops, such as cereals and pulses, the duration of canopy photosynthesis is also determined by the functional balance between sink strength and source capacity (Yin and Van Laar 2005; Sinclair and De Wit 1975) and by the ability of roots to capture N at the end of the growing season (Kichey et al. 2007; Spiertz and De Vos 1983).

The amount of N in the harvested part of the crop is determined by the sink strength of the storage organs and expressed as N harvest index. This value is usually high in cereals and tuber crops, e.g. 0.60–0.80 for wheat (López-Bellido et al. 2008; Spiertz and Ellen 1978) and 0.70–0.80 for potato (Biemond and Vos 1992)), but somewhat lower in legumes (Chapman et al. 1985). Cereals do reallocate N from the leaves to the grains, while in root crops most N is retained in crop residues. Generally, dry matter and nitrogen partitioning in wheat differ between old and modern cultivars; however, both parameters are not always genetically associated (Van Ginkel et al. 2001). Martre et al. (2007) found that variations in weather and N treatments also affected the nitrogen harvest index of wheat. Further improvement will require a good understanding of genotype × environment interactions.

3.2 Synchronization of N Demand and N Supply

To secure crop yields and avoid N losses the N supply should match the crop N demand in dose and time. The concept of *synlocation* and *synchronization* in plant nutrition was proposed by De Willigen and Van Noordwijk (1987) some 20 years ago. However, implementation of this concept seems to be difficult. A more generic approach to achieve a demand-based N supply of crops is based on the functional relationship between N uptake and carbon acquisition through canopy photosynthesis of a sole crop or a multiple cropping system. This relationship can be illustrated with the following simple equations:

(1) Total C acquisition = $\sum$ LI × LUE, where $\sum$ LI is the total amount of light intercepted by the canopy of a sole crop or intercrop (MJ m^{-2}) and LUE is the light-use efficiency (g MJ^{-1}). The $\sum$ LI is mainly determined by the amount of incoming radiation and the growth duration of a sole crop or a sequence of crops (Keating and Carberry 1993).

(2) Total N uptake = f × C-acquisition, where f is a parameter determined by the maximum N content of the biomass. This parameter depends on species- or cultivar-specific plant traits (Lemaire et al. 2007).

(3) Total N-supply = $a \times$ (N soil reserve + manure + mineralization) + $b \times$ (N fertilizer) where a and b are parameters determined by plant traits, such as: root length, rooting depth, etc., that affect the recovery of applied N (Van Delden 2001).

More sophisticated algorithms were developed for describing the relationship between crop dry weight and nitrogen uptake (Van Delden 2001; Booij et al. 1996). To get an optimal match of N demand and N supply crop growth models can assist in predicting the yield potential under specific climatic conditions and take into account the risk of growth-limiting (e.g. drought, heat) and growth-reducing (e.g. weeds, pests, diseases) conditions.

In irrigated rice systems (Fig. 3), the use of water and fertilizers, especially nitrogen, has increased dramatically; a major point of concern for these systems is the agronomic efficiency of the use of water and nutrients (Belder et al. 2005a,b). In a study on strategies for increasing rice yield potential using ORYZA models, Aggarwal et al. (1997) found that only with growth-rate driven N management the yield potential of high-yielding (9–10 t ha^{-1}) cultivars can be realized. By applying a simple rice-nitrogen model, MANAGE-N, it was possible to improve the timing of nitrogen dressing (ten Berge and Riethoven 1997). For each user-defined fertilizer N dose, the model identifies the timing and amount of applications, associated with maximum grain yield and maximum agronomic N efficiency (kg grain per kg N applied). Improved timing of nitrogen on irrigated rice resulted in yield increases of 4–10%, at a fixed total dose. Changing the N dose to the predicted economic optimum rate resulted in additional increases up to 13%. Thus, in rice it appeared essential to match the seasonal pattern of N supply to the N demand of the crop at each stage of development to achieve maximum yields, but also to minimize N losses to the environment. Integrated nutrient management and precision farming have shown to be effective tools to improve NUE (Pierce and Nowak 1999). Smart farming technologies aiming at both productivity and efficiency gains have been promoted. A vast number of experiments have been carried out with site-specific nutrient management (SSNM) in rice, but this method did not change fertilizer use significantly and therefore N losses continue to harm the environment (Ladha et al. 2005). To facilitate site-specific decision-making long-term multiple crop yield-map datasets have been transformed into profit maps that contain economic thresholds (Massey et al. 2008). However, environmental thresholds are still lacking, and as a consequence farmers are not informed about the risk of N losses.

Fig. 3 High-yielding rice cultivars grown under subtropical conditions in Jiangs Province, China (photo taken by the author)

Generally, the emphasis in N management is too strong on tactical fine-tuning of the N dose and too weak on strategic choices to make the cropping system less leaky. The transformation of flooded to aerobic rice systems (Bouman et al. 2007a,b) in regions with water scarcity is one of the best examples of a strategic approach to achieve a more sustainable use of limited natural resources. Benchmarking of low- and high-input cropping systems is needed to make a full assessment of economic benefits and environmental harm; an example for various rice ecosystems is presented in Table 1. Rice yields vary from 2,000 to 12,000 kg ha^{-1} with an associated N input ranging from 50 to 260 kg ha^{-1}.

Surprisingly, the variation in parameter values for nitrogen-use efficiencies does not differ much between

Table 1 Estimated rice yields and N-use parameters for different rice cropping systems. *NUE* nitrogen use efficiency

Parameters	Irrigated lowland rice[a]	Irrigated rice–wheat systems[b]	Aerobic rice systems[c]	Rainfed rice systems[d]
1. Rice yield ($kg\,ha^{-1}$)	9–12.000	5–9.000	4–6.000	2–4.000
2. Grain N uptake ($kg\,ha^{-1}$)	100–150	60–100	50–85	30–60
3. Apparent N recovery ($kg\,kg^{-1}$)	0.30–0.40	0.25–0.40	0.30–0.50	0.40–0.60
4. Physiological NUE ($kg\,kg^{-1}$)	50–80	50–70	40–60	30–50
5. Agronomical NUE ($kg\,kg^{-1}$)	30–60	20–30	25–50	25–30
6. Crop N demand ($kg\,ha^{-1}$)	150–200	100–150	80–125	40–80
7. Recommended fertilizer N supply ($kg\,ha^{-1}$)	200–260	150–200	120–160	50–80

Sources:
[a] Samonte et al. (2006), Belder et al. (2005a), Jiang et al. (2004), Cassman et al. (1993)
[b] Becker et al. (2007), Ladha et al. (2005), Pande and Becker (2003)
[c] Belder et al. (2005b), Yang et al. (2005)
[d] Saito et al. (2007), Boling et al. (2004)

low- and high-input systems. Genetic improvements of rice are most effective in enhancing physiological N-use efficiencies (Peng and Bouman 2007), while applying best management practices can raise apparent N recoveries (Campbell et al. 1995; Cassman et al. 1993)

It is concluded that N supply should match N demand in time and space – not only for single crops, but for a crop rotation as an integrated system – to achieve a higher agronomic NUE. Alleviating factors that limit growth – such as drought, flooding, pests and diseases – will be most effective in increasing the apparent N recovery.

4 Primary Productivity and Biodiversity

Net primary productivity (NPP) in terrestrial temperate ecosystems is generally limited by N availability. However, excessive levels of reactive N in the soil, water and atmosphere constitute a major threat to biodiversity in natural ecosystems (Suding et al. 2005). There is increasing evidence that diversity of soil biota as well as plants contributes to ecosystem functioning and improved nutrient-use efficiency (Brussaard et al. 2007; Barrios 2007;Van Ruijven and Berendse 2005). Diversity and functional complementarity leads to greater soil C and N accumulation on agricultural degraded soils (Fornara and Tilman 2008). Most of the research on improved ecosystem functioning by an increased diversity were carried out in vegetations with relatively low external inputs and biomass yield. However, Oerlemans et al. (2007) studied the impact of long-term nutrient supply on plant diversity in grassland; they found that increased N fertilization reduced the number of species site-independently. A unimodal relationship was observed between productivity and species number. The highest number of species was found when N and K were co-limited.

The challenge for the future is how can we combine a high land productivity – the capacity of agricultural land to produce biomass on a sustainable long-term basis – with the provision of ecosystem services and soil biodiversity (Barrios 2007)? The use of crop genetic diversity in maintaining ecosystems services was reviewed by Hajjar et al. (2008). They concluded that crop genetic diversity can enhance agroecosystem functioning and provide ecosystem services, especially by contributing to stability in crop and soil health. As a consequence, crop productivity and resource-use efficiencies will also become more stable.

There are still many gaps related to methodological, experimental and conceptual approaches that prevent quick progress in the guidance for policy- and decision-making on changes needed in developing highly productive, sustainable agricultural systems. Swift et al. (2004) concluded that maintaining ecosystem services and biodiversity outside conservation areas lies in promoting diversity of land use on the landscape and farm rather than field scale. Good examples are arable and grassland field margins (Sheridan et al. 2008; Asteraki et al. 2004); these field margins can provide multiple ecological services, such as biodiversity and pest control (Olson et al. 2007). Biodiversity effects can be managed (Storkey and Westbury 2007). It was reported that these effects increase linearly with biotope space (Dimitrakopoulos and Schmid 2004). By spatially allocating land (2–5% of the area) for ecosystem services complementary to land used for crop cultivation, biodiversity can be enhanced within intensive cropping systems without a severe loss of production potential. In areas with hilly or rolling land, strips or banks dedicated to developing biodiversity richness may also reduce run-off of nutrients and soil erosion.

5 Nitrogen Use at the Farm and Global Levels

A high nitrogen-use efficiency is no guarantee that N losses do not exceed critical environmental thresholds. The most important factor determining the risk of potential N losses is the total amount of mineral N left over after the harvest in crop residues and in the soil. To assess the environmental impacts the N dynamics should not be studied in one crop, but in a diversity of cropping cycles and in mixed plant–animal systems (Table 2).

5.1 Arable Cropping Systems

The main objectives for ecologically and economically sustainable agriculture are maintaining soil fertility and improving crop productivity and stability. Management options are: site- and time-specific nutrient and water management, crop protection measures and the choice of adapted, high-yielding cultivars. The effects of the various measures that are of importance for the maintenance and use of the resource base cannot easily be assessed within one growing cycle but should be evaluated over a sequence of crops. Crop rotation is an important component of an integrated approach of sustainable agriculture and resource conservation. Short- and long-term effects of a cropping sequence and related management practices can be expressed in physical soil properties such as water-holding capacity and bulk density; chemical soil properties such as *p*H,

Table 2 Sustainability parameters for benchmarking of contrasting food production systems in temperate, non-water-limited regions

Parameters	Technological high-input systems	Ecological low-input systems	Mixed animal–plant systems	Dairy-grazing systems
A. Quantitative parameters[a]				
1. Biomass – NPP ($\mathrm{t\,ha^{-1}}$)[b]	12–20	8–12	10–18	12–20
2. Total N supply ($\mathrm{kg\,ha^{-1}}$)[c]	150–300	100–150	200–350	200–400
3. N use (NUE) (N_{output}/N_{input})[d]	0.30–0.60	0.40–0.70	0.25–0.50	0.15–0.35
B. Qualitative parameters[e]				
1. Marketable yield ($\mathrm{kg\,ha^{-1}}$)	High	Low	Moderate	High
2. Ecological stability / diversity	Low	Moderate	High	Moderate
3. Nutrient recycling	Low	Moderate	Moderate	High
4. Environmental N load	Moderate	Low	Moderate	High
5. Profitability (net returns)	High	Moderate	High	Moderate
6. Sustainability (planet issues)	Low	High	Moderate	Moderate
7. Ethical acceptance	Moderate	High	Moderate	High

[a] Best guesses of the author

[b] Aboveground biomass or net primary productivity (NPP) , expressed as ton dry matter per hectare

[c] N supply by manure and fertilizer use

[d] N use defined as overall system recovery, expressed as the ratio between output (N in harvestable crop parts, meat or milk) and input (N in manure and fertilizers)

[e] After Pretty (2008); Principles of agricultural sustainability

carbon content and nutrient contents, and biological soil properties such as microbial activity (Lal 2008; Shibu et al. 2006).

Growing special crops in a rotation can improve the sustainability of the cropping system (Struik and Bonciarelli 1997); examples are:

- Legumes for improving the nitrogen availability
- Green manure crops for improving the physical and biological soil fertility
- Cover crops to prevent soil erosion and to store nutrients prone to leaching or run-off

The potential of legumes can be established by comparing yields and N uptake under the same agroecological conditions. Sibma and Spiertz (1986) carried out field experiments with three forage crops – grass (*Lolium perenne*), lucerne (*Medicago* sativa) and maize (*Zea mays*) – over 3 years on a fertile clay soil under temperate climatic conditions. It was found that above-ground DM yields ranged from 13.4 to 19.8, from 13.4 to 18.1 and from 13.7 to 17.1 t ha^{-1} for grass, lucerne and maize, respectively (DM: dry matter). The associated N yields ranged from 413 to 452, from 392 to 577 and from 188 to 220 kg ha^{-1} (Spiertz and Sibma 1986). Grass showed the highest productivity in the first year and lucerne in the last year. N fixation by lucerne varied between 462 and 507 kg ha^{-1} without and between 107 and 195 kg ha^{-1} with a N fertilizer application. The after-effects from soil N reserves of a 1-, 2- and 3-year cropping sequence of lucerne (no N fertilizer) and grass (300 kg N ha^{-1}), measured as N uptake by an unfertilized maize crop, amounted to 140–175 and 110–140 kg N ha^{-1}, respectively. For comparison, the N after-effect of a preceding maize crop centered around 110 kg N ha^{-1}. Even higher DM yields were reported by Lloveras et al. (2008) for irrigated lucerne under Mediterranean conditions; they found a range from 16 to 21 t ha^{-1} averaged for three years. These data show the high potential of a legume crop for N fixation and DM production under favorable growing conditions.

5.2 Mixed Farming Systems

Besides crop rotation, integration of crop and animal production on the farm and regional scales may be an opportunity to increase *eco-efficiency* (Wilkins 2008). Nitrogen is mobile in the soil–plant–animal system and with the required N inputs for high crop yields and intensive livestock production the risk of N losses increases (Van Keulen et al. 2000). Traditionally, nutrient management has been concerned with optimizing the economic return from nutrients used for crop production. The main emphasis was on the expected crop response from adding nutrients to the soil. In practice, however, nutrients, particularly manure, are not always applied to optimize plant nutrient use. Such practice or the improper or untimely application of manure and fertilizer may release nutrients into the air and water. The problems are most significant in regions with an intensive animal production (Aarts et al. 1992). The excess of nitrogen compounds in manure has become an issue of major concern in many European countries and will also become an increasing problem in other countries, such as India and China, with high stocking rates of animals. The problems with nutrient pollution are not generally the result of mismanagement by farmers, but are a result of how agricultural systems have evolved, with no direct costs associated with environmental quality and conservation of natural resources. Beegle et al. (2000) concluded that nutrient management strategies will not be the same for all farms. They classified farms on a nutrient balance basis into three groups:

- Nutrient-deficient farms; nutrient imports are less than exports. Thus, additional nutrients in the form of purchased fertilizer or other sources are required for achieving optimum crop yields. A well-planned nutrient management program emphasizing economic and agronomic efficiency should reduce the need for purchased inputs and thus should improve farm profitability.
- Nutrient-balanced farms; nutrient imports are approximately equal to exports. Because these farms are often at the upper limit of being able to safely handle all the nutrients in the production system, nutrient management planning may offer potential environmental benefit.
- Nutrient-surplus farms; nutrient imports significantly exceed exports. The nutrients in the manure generally exceed those required for crop production on the farm. A significant component of a nutrient management program involves acceptable off-farm uses for the excess manure.

Some countries, such as Denmark, have given priority to agro-environmental schemes, restricting the use of fertilizers and manure. The impact of these measures was studied by evaluating farm gate nutrient balances (Kyllingsbaek and Hansen 2007). It was shown that nutrient surpluses at the farm gate were reduced; however, the effects on water quality were small. It is still not clear if there is no direct relationship or that the lag time of reducing the N load is longer than considered in the study.

5.3 Organic Agriculture

In Western countries with an affluent society, organic farming has got increasing support from citizens and governments during the last three decades, because of the perceived ecological services, environmental benefits and human well-being and health (Rembialkowska 2007). "The ethos of organic farming is that it forms the basis of a production system that is environmentally, socially and economically sustainable" (Topp et al. 2007). However, consumers are reluctant to buy organic food, because of the much higher prices than of conventional products. A debate is going on if organic farming can feed a growing world population and to what extent organic farming outperforms conventional high-input farming systems in sustainability. In contrast with recent claims by Badgley et al. (2007) that organic agriculture does have the potential to produce enough food for a growing world population, Connor (2008) concluded that organic agriculture cannot feed the world. He noticed a major overestimation of the potential for N fixation by legumes, the availability of organic nutrients and the productivity of organic agriculture in a comparison with conventional low- and high-input agriculture. Furthermore, the study of Topp et al. (2007) on resource-use efficiencies made clear that the delineation of system boundaries in both space and time is critical in the compilation of resource-use budgets. The expression of output per unit of land area tends to favor low-input systems, because the impact on the regional scale may be less but may result in the need for additional land elsewhere (Van der Werf et al. 2007). Thus, on a national or even a global scale the total impact of food production may increase.

Evaluation of the advantages in sustainability performance of organic agriculture and other low-input systems, such as SRI (System of Rice Intensification, McDonald et al. 2006), should be carried out on an eco-regional and global scale over a time-span of at least 10 years. Then, the full benefits of improved soil processes and crop health as well as the costs in terms of land productivity, nutrient depletion and weed competition can be taken into account.

6 Environment and N Management

Concerns about the environmental impact of intensive agricultural systems require an improvement in production technologies to maximize resource-use efficiencies, and to minimize the environmental impact. Nitrogen (N) fertilizers comprise almost 60% of the global reactive N load attributable to human activities; especially in China (UNEP 2007). N use has a major impact on the functioning of the ecosystems and human well-being. In Europe, agriculture is responsible for 40–80% of the N loading to surface waters (OECD 2001). Nitrogen losses associated with the application of N fertilizer can result in nitrate contamination of water resources and increased emissions of ammonia (Erisman et al. 2007; Bussink and Oenema 1998), nitrous oxides (N_2O), a potent greenhouse gas (Stehfest and Bouwman 2006), and NO_y, with negative human health effects (Wilkinson et al. 2007; Van Egmond et al. 2002). Today, the agronomic and economic requirements of nutrient management remain central, but in addition we must consider the potential impact of these nutrients on environmental quality. Total reactive loss will grow in the period explored until 2030, because of an increase in fertilizer consumption in developing countries to feed the growing population and concurrently a steep rise in dairy and meat consumption in emerging industrialized countries (China, India, etc.), despite improvements in overall system N recovery in developed countries (Eickhout et al. 2006).

Since land is a finite and fragile resource, its sustainable management depends on the husbandry of its different components, of which soil fertility and water availability are key factors for agricultural production. At the moment, agronomic N-use efficiencies are often very low (Spiertz and Oenema 2005). Agronomically, farmers should aim at the minimum input of each production resource required to allow maximum utilization of all other resources (de Wit 1992). Consequently, above a certain minimum, higher inputs of yield-increasing factors such as water and nutrients result in higher yields per unit area and are associated with higher efficiencies of other resources. Higher efficiencies, expressed as output per unit of input, might coincide with larger emissions per unit of area.

6.1 Plant–Soil–Atmosphere

Mathematical modeling has strongly contributed to a more quantitative understanding of the soil–plant N cycle and the soil, plant and environmental factors which govern it (Galloway 1998). Environmental concerns are focused on nitrogen losses from soils, which may pollute the environment (Fig. 4). Leaching is the major route by which nitrate enters the ground- and surface waters, while denitrification and nitrification are significant sources of N_2O, an important greenhouse gas. Improved efficiency of N use on a field and farm scale, both increasing crop yield and quality and reducing losses, is dependent upon dynamic optimization to match supply of N and the N requirements of the crop on a field scale. This optimization requires measurement and prediction of soil-N supply, crop uptake and their variability (Stockdale et al. 1997). Models of crop growth, the soil-N cycle and plant–soil models have been developed (Bouman et al. 1996). However, these are little used in current fertilizer and farm management recommendations. Farmers cannot wait for our understanding of plant–soil dynamics to be perfect, but need researchers to put their current knowledge to use.

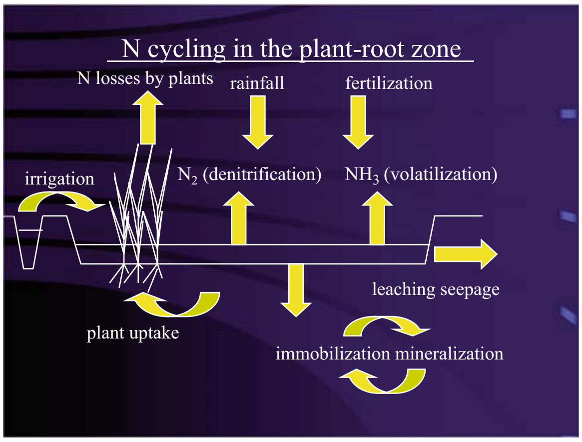

Fig. 4 N cycling schema for irrigated rice systems (modified after Bouman 2007)

6.2 Scale and Systems

A system approach can be used as a research tool, but also as an instrument for training of students and scientists. Research and training networks enable the necessary development of a common language of concepts, models and databases, and allow frequent interaction among actors (Ten Berge and Kropff 1995). System approaches were introduced in the 1970s and are used increasingly in research on food production studies, natural resource management, land use options and rural development (Van Keulen 2007). The system approach can be described as the systematic and quantitative analysis of agricultural systems. Agricultural systems are defined as well-delineated parts of the real world, consisting of many interacting elements (Neeteson et al. 2002). The system approach uses many specific techniques, such as simulation modeling, expert systems with databases, linear programming and geographic information systems. A system approach has been applied for studies at different aggregation levels, such as:

- *The plant level*; analyses and evaluation of genotype × environment interactions in breeding programs
- *The crop level*; optimization of nutrient and water management
- *The farm level*; prototyping of integrated farming systems and analyzing the flows of nutrients on farms
- *The watershed and landscape level*; optimization of water use and water saving
- *The eco-regional level*; *ex-ante* assessment of the possible impacts of changes in technology or in the socio-economic environment on agricultural development

Actually, in the last few decades we have witnessed the integration of process-specific knowledge into very precise, widely accepted relationships between processes in the system and driving factors from outside the system (Van Keulen et al. 2000). This applies mainly for water management at the field and catchment levels (Bouman 2007). Mathematical quantification of N flows in space and time is more complex, because of the dynamic nature of N in the plant, soil, water and atmosphere (Erisman et al. 2007; Galloway et al. 2003). The growing complexity of managing N in sustainable agricultural systems calls for problem-oriented, interdisciplinary research. Key disciplines are: agronomy, crop science, soil science, conservation biology, environmental sciences and systems modeling.

The current assessment of the impact of climate change on agriculture and the options for adaptation relies heavily upon both crop and climatic modeling. The output of the models is greatly limited by the extent of our understanding of short- and long-term crop adaptation to changing environmental conditions, especially in soil traits and weather patterns. Howden et al. (2007) stated that complex problems require multidisciplinary solutions, with a focus on integrated rather than disciplinary science.

6.3 Policy-Making and Regulation

A more environmentally sensitive nutrient management on the field and farm levels can reduce nitrogen losses to a level that meets the standards (Goulding et al. 2008; Aarts et al. 1992). Decision-making related to nutrient management occurs at the strategic, tactical and operational levels. In Europe society demands more and more accountability from farmers; therefore, more legislation has been implemented by the EU and the national governments. Legislation is inevitable whenever “profit” and “planet” goals conflict. In Europe and especially in The Netherlands, there exists a set of regulations set up by the government to protect the environmental compartments – soil, water and air – against nutrient losses from agro-production. Legislation can only be successful when clear motivations and regulatory tools are provided to farmers (Schroder et al. 2003). There are two approaches to nutrient management planning in a regulatory situation. One approach is to specify what should be done on all farms as a recipe for nutrient management. Another approach is to establish performance criteria or goals for farmers to meet as part of their farm nutrient management plan.

Langeveld et al. (2007) concluded that agro-environmental indicators can be used for design, implementation and testing of farming systems, but it should be kept in mind that indicators are not perfect, because of the complexity and highly variable processes involved in N cycling. Transitions occur at some cost; for example, large savings on one limited resource, such as irrigation water, may have a trade-off on yield. A methodology for an integrated analysis of trade-offs between economic and environmental indicators is available (Stoorvogel et al. 2004). Integrative modeling approaches to evaluate the impact of multifunctional agriculture have been developed (Rossing et al. 2007). New approaches are needed that will integrate biophysical processes and ecological processes at the crop, farm and landscape levels (Pretty 2008; Giller et al. 2006). A policy that will lead to N applications of manure and fertilizer balanced with crop N demand is urgently needed, not only in developed, but also in developing countries. An intensive communication between all stakeholders (environmental agencies, policy-makers, researchers and farmers) and a controlled implementation of indicators and guidelines may contribute to a balanced application of nutrients (Delgado et al. 2008). Such an approach will be needed to meet “planet” and “profit” objectives and to prevent nitrate levels in groundwater from exceeding the standards for human consumption (a “people” objective).

7 Conclusion

Two strategies to meet sustainability goals in food production, with a safe and profitable use of N, can be followed:

(a) Developing *low-input*, *high-diversity* agricultural systems.

Within these systems diversity in crop choice and crop rotation minimizes the risks of yield reductions by abiotic and biotic stresses. Furthermore, the stability of the agroecosystems is enhanced by combining genetic diversity with functional biodiversity at the farm and landscape levels. The supply of nutrients, especially N, relies strongly on maintaining high levels of soil organic matter (SOM). Crop output levels will range from low to moderate; therefore, these systems require more land.

(b) Developing *high-input*, *low-diversity* agricultural systems.

Within these systems high-yielding, high N-responsive crop cultivars are chosen to achieve a maximum productivity per unit of land. The stability of these agroecosystems depends strongly on the management of genotype × environment

× management interactions and soil quality. An optimized N management during the whole crop cycle will control N losses. The advantage of these agroecosystems is a high productivity per unit of land and therefore, less land is needed for food production. As a result, virgin and fragile soils can be saved.

For Southeast Asia the "high-input low-diversity" approach will be unavoidable due to scarcity of land. However, in other regions where more land per capita is available the "*low-input high-diversity*" approach is recommended. It would environmentally even be more effective when the different strategies are not applied on a global but on a regional scale.

A balanced sequence of crops with complementary functions can help to improve the N-use efficiency and to maintain the profitability and sustainability of the cropping system in the long run. Contrary to the widespread view that high-input agrosystems are homogeneous, many researchers have found large spatial and temporal differences in nutrient levels and fertilizer efficiencies, even on similar soil types. Differences between fields are in part due to historical differences in management. However, the major cause of low and varying fertilizer-use efficiency, particularly for N, is that the supply of nutrients from soil reserves and fertilizers is not well synchronized with the demands of the crops, and managing fertilizers to improve this synchrony is complicated. Despite many attempts, there has been little success correlating spatial grain yield with spatial patterns in soil fertility (Pierce and Nowak 1999). An increasing body of knowledge suggests that spatial variation in soil water relations may be an important factor in causing spatial variation in grain yield.

More advanced diagnostics could be used to increase the specific nature of recommendations or to adjust model recommendations during the growing season. This would enable a greater use of dynamic optimization strategies in the field. An "ecological modernization" requires *synlocation* and *synchronization* of crop nutrient demand and supply by fertilizers. Precision farming methods will implement these concepts in practice, but at some cost. Management of the environment has moved from a command and control paradigm to a much wider perspective of regulatory means, including economics, participatory approaches and ethics. Ignorance and uncertainty still play an important role in decision-making on environmental consequences of modern farming and cropping systems. Therefore, research should not only be focused on productivity and profitability of food production systems, but also on agroecosystem functioning (nutrient cycling, stability, resilience) and ecosystem services such as biodiversity, carbon sequestration and water harvesting.

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Conversion to Organic Farming: A Multidimensional Research Object at the Crossroads of Agricultural and Social Sciences – A Review

Stéphane Bellon and Claire Lamine

Abstract Literature on the conversion to organic farming is scattered. However, both the conversion of farmers to organic farming and of consumers to organic food are the driving forces for the development of the organic sector. In this review, we combine agricultural and social scientists' viewpoints for a critical appraisal of literature on conversion to organic food and farming. First, a brief historical retrospective enables us to refer the scientific production to the institutional and economic context over the past decades. Secondly, we review the methods used to analyse conversion in agricultural and social sciences, and show that emphasis is most often laid upon the effects of conversion and the motivations to convert, on the basis of comparative approaches with so-called conventional agriculture. Therefore, the literature minimises the importance of transitional aspects and trajectories, and rarely approaches conversion as a longer process than its legal duration and from a wider point of view. Thirdly, we examine the paradigms of input efficiency and system redesign, which frame discussions about transitions in agriculture, beyond organics, and therefore helps shed light on sustainability issues. We suggest that analysing conversion and more generally transitions in agriculture as multidimensional issues, involving both production and social practices, entails interdisciplinary approaches and the redefinition of some central research topics.

Keywords Organic food and farming • Conversion • Transition • Conventionalisation thesis • Redesign • Interdisciplinarity

1 Introduction and Short Retrospective

Conversion to organic production is most often defined through regulations. It involves a given time span as well as specific constraints and procedures, whereby organic principles must be applied to parcels and animals for 2 or 3 years. For farmers though, conversion does not restrict itself to this period and these technical procedures codified by regulations, but entails transformations which transcend this legal period and definition. These transformations also go beyond the technical level and concern farmers' conceptions, values and inscription in social networks. Therefore, the first objective of this literature review focused on the English- and French-speaking worlds is to show how agronomy and sociology have dealt with the issue of conversion, to present the approaches and methods used in each field, to enhance more extensive approaches and to capture the different versions of organic farming in practice, in opposition to a sometimes homogenising vision of organics. For this latter reason, we will refer to organics when we need to encompass organic farming and organic consumption in their diversity. Beyond organics itself, our second objective is to assess conversion to organic farming as a more general transition model towards more sustainable agriculture and to point out relevant approaches and needs for further research.

Firstly, a short historical retrospective of both agronomical and sociological literature during the last three

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decades, that is, a generation of organic farmers and consumers, is suggested. Secondly, we present the methodological approaches of conversion developed both in agronomy and in sociology, and find that conversion is mainly analysed in terms of effects and motivations, at the expense of more comprehensive approaches. Finally, we show how some recent studies on organics, mainly centred on questions of conventionalisation and paradigm changes, enrich the debates on the different forms of sustainable agriculture and enhance the need for more comprehensive and interdisciplinary approaches.

Conversion, both of a given farmer or on a larger scale, must be related to the general dynamics of organic farming. Converting to organic farming in the 1970s, when there was no label, is completely different from converting in the twenty-first century. The process of "institutionalisation" of organic farming started very progressively from the 1960s and lead to a relative regulatory harmonisation on a European scale with the 2092/91 regulation, despite the national specificities due to the national policies to support organic farming (Lampkin and Stolze 2006) or to the organisation of specific professional and associative structures, as acknowledged for the USA (Vos 2000), the UK (Reed 2001), Belgium and Denmark (Lynggaard 2001) or France (Piriou 2002).

How does the literature reflect this evolution of organics? The founding fathers of organic farming published their work from the 1920s (R. Steiner's Spiritual Foundations for the Renewal of Agriculture dates back to 1924) to the 1940s (An Agricultural Testament by A. Howard is from 1940) (Besson 2007). Then there was a gap in publications until the 1970s, where most of the research work in organics was carried out by private institutions. From the 1970s until today, the literature can be organised in three main blocks: (1) pioneering studies, mainly from the 1970s and 1980s, seeing organic farming as an alternative model or criticising its feasibility; (2) from the mid-1990s on, articles analysing the specialisation of organic production and debating its conventionalisation; and finally, (3) in the recent period, promising approaches which appear to be more extensive.

In the 1970s, several pioneering studies, in agronomy as well as in sociology, strove to describe this new fact that was the development of organic farming on the agricultural scene. In the field of agronomy, organic farming has been studied through its performances (Sebillotte 1972, 1974), leading to a critical vision disregarding organic farming as a genuine agricultural alternative. Although this vision remained relatively dominant, other authors tried to rehabilitate organic agriculture and the farmers who practised it by analysing its technical foundations and subsequent balances, i.e. organic and mineral fertilisation, labour, assets, and liabilities (Bellon and Tranchant 1981; Lockeretz 1981), by looking at it as a social and economic practice and by acknowledging its technical and regional diversity (Cadiou et al. 1975). In this literature, organic farming is found to be a feasible alternative to conventional models (Viel 1979; Gautronneau et al. 1981). In the USA, in this period of questioning over the limits of so-called modern agriculture, organic agriculture was considered a possible model (USDA 1980), and other qualifications and definitions for agriculture also emerged, in particular "sustainable" (Harwood 1990) and "alternative" agriculture (National Research Council 1989). These French and American studies favoured a holistic approach enhancing the relationships among crop rotations, tillage methods, pest control and nutrient cycling, which is part of a more general and lasting shift in research programmes towards the use of systemic analysis (Bellon et al. 1985; Norman and Malton 2000). In the field of sociology, several studies, in the USA and in France, tackled organic farmers' attitude and practices (Harris et al. 1979; Barrès et al. 1985; Le Pape and Rémy 1988). While later studies on conversion were mostly centred on the analysis of farmers' motivations, these pioneering studies suggested a more extensive vision of conversion which put forward the social and biographical factors that led farmers to convert in a professional context that was largely reluctant.

If the study of organic farming seriously declined in the 1980s, it came back onto the scientific scene in the 1990s when it became codified and acknowledged by laws and institutions. A bibliometric analysis built from the "ISI expanded" base created in 1991 shows a multiplication of publications related to organic farming between 1992 (47 publications) and 2004 (224 publications), i.e. a significantly higher increase than literature concerning agriculture in the same database. The journals having published more than 40 articles about organic farming in this period are "Agriculture Ecosystems & Environment" (71 articles), "Biological Agriculture &

Horticulture" (70) and "Biology and Fertility of Soils" (41). These articles deal mainly with assessments of organic farming (the effects on biodiversity, nutrient cycles and energy consumption) and its spatial relations to the environment (e.g. the role of hedges and grass strips).

Other authors describe the increasing specialisation of organic farms (Allard et al. 2001), the consequent accentuation of technical problems such as weed control and fertilisation management and the parallel specialisation of research, development and extension, leading to a focus on a commodity approach to organics, as for livestock (Hovi and Garcia Trujillo 2000; Roderick 2004) and fruit production (Gigleux and Garcin 2005). Social scientists rather put the accent on the processes of "conventionalisation", whereby organic production and markets are being overcome by large-scale structures and becoming more and more vertically integrated, just like in conventional agriculture.

In recent years, more comprehensive approaches to organic farming but also, broadly speaking, to organic food systems have been developed. In the social sciences several authors point out the necessity to study the links between production and consumption, when talking about the evolution of agro-food systems, as it is discussed within the "production–consumption debate" (Lockie 2002; Goodman 2003). Biological and agricultural scientists contributed to the enlargement of this debate by suggesting an approach that, beyond a restricted agronomic vision limited to the field or at best to the farm, would encompass natural phenomena such as climate change (Flessa et al. 2002) and social aspects linked to food consumption (Gliessman 1997; Francis et al. 2003).

This brief retrospective as well as the increasing number of publications in agricultural journals and the richness of organic workshops in several recent rural sociology congresses (Holt and Reed 2006) reveal that, particularly since it became framed by law, organic farming has become a scientific subject in both agricultural and social sciences. However, despite the large number of studies concerning organic farming, and despite the diversity of approaches, conversion in itself is not a subject of study in agronomy. Social scientists seem to have been more prolific on this subject, even though we will see that their analyses often stick to the classical market-orientated/values-orientated opposition.

2 Methods Applied to Analysing Conversion in Agricultural and Social Sciences

The majority of studies published in agronomical journals approach conversion in terms of its effects much more than in terms of a dynamic process. These effects are usually analysed through two approaches. The first one uses a conventional reference so as to assess possible differences among situations and to understand underlying processes. The second approach uses intra-organic comparison without referring to conventional agriculture. Case studies are favoured, arguing that organic farming is site-specific. Case studies also enable a better identification of research hypotheses according to real farm dilemmas (Lampkin 1986; Loes 1990). Methods in both approaches entail on-farm surveys and experiments in agricultural stations so as to analyse the effects of conversion on particular environmental compartments or on agronomical and sometimes economic performances. The review of these two types of approaches points out the necessity of long-term and farm-scale studies and the interest of interdisciplinary studies to take into account the internal dynamics of organic farming.

2.1 Studies Comparing Organic Farming with Other Forms of Agriculture

Several studies evaluate the effect of organic farming on various environmental compartments with balances, indicators and models, or based on scenarios. Most of them rely on comparisons with conventional agriculture (Table 1).

Studies comparing organic farming with other forms of agriculture gave rise to numerous articles, but few of these directly deal with the conversion period. Yield differences between a reference in conventional agriculture and/or one in integrated production are often a focus of attention. For example, Lotter et al. observed that the economic margin of an organic maize was better than for a conventional one in 4 out of 5 years, but those years were affected by drought (Lotter et al. 2003). Above all, when compared with farms which are poorly managed, organic agriculture will

Table 1 Main topics and approaches identified on organic farming and environmental issues

Environmental topics	Approach	References
Soil quality		
Physical, chemical and biological measurements	Long-term trial (as from 1978, study of biodynamic, organic, and conventional)	Mäder et al. (2002)
Chemical and biological properties	Two adjacent fields (7 years of organic certification)	Marinari et al. (2006)
Soil organic carbon accumulation rates	Ten cropping systems and native ecosystems (LTER site)	Grandy and Robertson (2007)
Nutrient management		
N leaching and balances	Modelling approaches (function and balance) at system level	Hansen et al. (2000)
N balance at farm level and reduction of potential N losses	Three scenarios with relative conversion in a local area	Dalgaard et al. (2001)
Effect of cash crops on sulphate leaching	3-year trial in low-input systems	Eriksen and Thorup-Kristensen (2002)
Input–output balances for macronutrients (P, K, Mg) and trace elements (Cd, Cu, Zn)	Field trials in adjacent dairy systems on experimental farm	Bengtsson et al. (2003)
Biodiversity		
Activity density and diversity of carabids and staphylids	Two field experiments over periods of 6 and 8 years during conversion	Andersen and Eltun (2000)
Structural and functional diversity at farm level	Indicators of cropping system biodiversity on 33 farms (18 organic) in 2002	Caporali et al. (2003)
Species richness and abundance	Meta-analyses based on 66 publications with paired comparisons between the two systems	Bengtsson et al. (2005)
Impacts on biodiversity of organic farming	Review of 76 comparative studies of the two systems, across a broad range of taxa	Hole et al. (2005)
Relations between weed communities, management variables and site conditions	Comparison between two weed surveys (in the 1960s and late 1990s)	Hyvönen (2007)
Greenhouse gas emissions		
Aggregate greenhouse gas emission (CO_2, CH_4 and N_2O)	Two farming systems compared (in Germany)	Flessa et al. (2002)
Energy consumption		
Energy use as an indicator of the intensity of production processes	System modelling of energy prices based on data from farm studies	Refsgaard et al. (1998)
Several environmental compartments		
Soil properties, ecosystem biodiversity, water quality, use on non-renewable resources	Relative ranking, based on experts' survey from 18 EU countries and international databases (300 publications)	Stolze et al. (2000)
Soil organic matter, N and P leaching and balances, biodiversity	Review, based on indicator framework and empirical studies	Hansen et al. (2001)
Strengths and risks of organic farming (soil, water, landscape diversity, water utilisation)	Literature review and case study (Martinique, FWI)	Blanchart et al. (2005)

generally give better yields, and vice versa. This raises the question of the comparability and the representativity of studied situations.

Comparative studies can therefore lead to contradictory results concerning the evaluation of organic agriculture as compared with other modes of production. Their assumptions and their specific experimental conditions, as well as the generalisation of their results, have been largely criticised (Elliot and Mumford 2002; Kirchman and Ryan 2004; Martini et al. 2004; Trewavas 2004). Another limit of comparative tests is that they do not really take into account either the interactions between management, crop varieties and site-specific effects, the externalities (environment, energy, health) or the systemic properties (autonomy, resilience, stability). Organic agriculture is often interpreted – in experimental conditions – through the absence of chemical products. Moreover, conversion is restricted to its legal duration. Conversely, the few experimental studies which have taken into consideration the dynamics of organic conversion through the construction of successive balances over longer periods of time were published in renowned journals (Reganold et al. 2001; Mäder et al. 2002). In their literature review on biodiversity, Hole et al. (2005) suggest that there may be a time lag in the response of wildlife communities to any benefits generated by a switch from conventional to organic farming. They also assume that those farmers who choose to convert may be pre-disposed to environmentally-friendly farming practices in the first place or may farm land that has previously been managed less intensively and is therefore easier to convert successfully to organic. They finally advocate the need for longitudinal studies that assess the capacity of organic conversion to reverse previous biodiversity losses caused by intensification.

In these comparative studies, the diversity and the internal dynamics of organic agriculture are often ignored (Sylvander et al. 2006), as if it were a homogeneous whole, except in the case of a few recent articles (Petersen et al. 2006; Rasmussen et al. 2006). Recognising the diversity of situations is actually difficult for comparisons over several years. Does the experimental comparison of several organic systems allow a better accounting of this internal diversity and these dynamics? Such an approach has been put in place for the case of livestock, where two systems were compared: a grassland system with limited production objectives and a mixed cropping-livestock system with higher production objectives (Benoît et al. 2005; Coquil et al. 2006). In a similar perspective, Benoît and Veysset (2003) tested the notion of conventionalisation through the application of a conventional sheep-breeding pattern (three lambing periods per ewe every 2 years) in organic sheep meat production. This option, which aimed at maximising the productivity, finally appeared as inadequate due to its complex implementation, in particular with dependence on external inputs, variability in performances and lower margins of security in an accelerated sheep production system. In short, implementing such an intensive breeding pattern in organic agriculture is difficult because it introduces a supplementary constraint in a system already highly constrained. Taking the farm into consideration over time can render this conclusion more specific, though. If farmers were already following an intensive breeding pattern before conversion, switching to organic management might be easier; however, they will not have the same ways as in conventional agriculture to face difficulties (Cabaret et al. 2002). If farmers convert from a grazing-based and seasonal breeding system, then switching is all the more difficult.

In field crop production, other ways to approach the subject are considered so as to improve the methods; for example, through comparisons between cropping systems (Vereijken 1997) and through the integration of the environment of compared plots and farms (Marshall and Moonen 2002). A more radical proposal favours comprehensive studies of organic agriculture to comparative studies which are dominantly descriptive. This enables the design of subsidiary hypotheses, and the identification of topics for further studies of underlying biotechnical processes in order to derive more general principles (Wynen 1996; Kristiansen 2006). A clear identification of the specific objectives and constraints of organic agriculture – rarely formalised in research projects – could help with rethinking organic agriculture but also other forms of agriculture. We agree that it is a major stake for organic agriculture, i.e. to construct and characterise innovations which can serve other forms of sustainable agriculture.

2.2 Longitudinal Studies Specific to Organic Farming

These studies develop comparisons between organic farms after their conversion, through case studies (Bellon and Tranchant 1981) or typologies (Langer 2002). The central subject is the effect of conversion on production and the inter-annual variability of the yields. Several studies reveal that yields do not always increase several years after conversion (Stanhill 1990; Stolze et al. 2000). This finding is controversial, as results may vary according to the cropping systems and the regions under consideration. In the mid-term, when a different soil functioning has been put in place, yields are somewhat higher or comparable with those obtained before switching from conventional to organic management, as shown by Zundel and Kilcher (2007) in a bibliographical review. They also suggest that a decrease in yields during the legal period of conversion depends on the previous intensification level of cropping systems. The "depressive effect" would be stronger in formerly high-input systems.

The role of soil in conversion has been pointed out by several authors (Liebhardt et al. 1989; MacRae et al. 1990) who mention an "organic transition effect". This effect would lead to a reduction in technico-economic performances early in the organic conversion (ecological processes being inadequate to supply nutrients, to control pests and diseases, or to provide essential functions previously provided by chemical inputs). Afterwards, soil properties and biological activity would improve after 3 or more years of organic management, which in turn would give higher yields.

Martini et al. (2004) discuss this "soil-quality hypothesis". They compare identically managed organic systems, differing only in the number of years since being converted to organic farming (less than 1 year and more than 5 years), with a 2-year crop rotation of processing tomato and maize. No significant differences appeared in tomato yields as related to the year of conversion. Organic tomato yields were also superior to a conventional reference. On the other hand, soil chemical properties (P, K, total N and total C) were superior in the plots which had been converted for more than 5 years. Such properties might have little effect on yield once some minimum threshold is achieved, for instance due to past practices. These authors suggest that the increasing experience of farmers after conversion can have a determining effect, which highlights that previous yield comparisons among years may also reflect learning processes in organic farming. However, they point out that the extrapolation of such results beyond specific experimental conditions is risky without additional controlled comparisons between replicated plots.

In accordance with organic principles, crop rotations are also particularly focused on (Bulson et al. 1996; Bertsen et al. 2006; Papadopoulos et al. 2006). In the process of converting, indeed, soil fertility has to be built up through the effects of preceding crops, particularly on stockless farms. Crop sequences influence soil fertility and nutrient cycles; they also protect crops from risks of infestation by weeds, pests and diseases; and they contribute to seedbed preparation. Huxham et al. (2004) tested in specialised field crop systems the effects of seven conversion strategies, defined by couples of preceding crops, on a subsequent winter wheat and on two different kinds of soils. They noticed significant yield differences in wheat (2.8–5.3 $\mathrm{t\,ha^{-1}}$) according to the strategy. Higher wheat yields were obtained after a Red clover–Ryegrass (*Lolium perenne* L.) green manure. These authors conclude on the importance of the crop establishment phase of the first wheat crop following conversion, and show that preceding effects on wheat yields are mediated by soil structure.

The evaluation of organic farming performances solely through yields, which is the focus of most studies, is questionable, as this production mode also targets other objectives (effects on the environment, quality of products and new relations with consumers). Indeed, some authors argue that organic farming is multi-targeted and add criteria concerning agricultural labour, product quality or environmental friendliness (Niggli et al. 2007).

Moreover, some case studies, most often carried out on the regional scale, deal with economic or environmental results of conversion and adaptations after conversion of livestock farms. The range of situations which is studied is enlarged in comparison with monitoring on commercial farms or experimental stations. In this perspective, other authors suggest using methods adapted to the assessment of longer-term consequences of conversion, such as simulation (Dabbert and Madden 1986; Dalgaard et al. 2001; Benoît and Veysset 2003) or modelling (Rosegrant et al. 2006).

2.3 The Necessity of Long-term and Farm-Scale Studies to Analyse the Dynamics of Conversion

The notion of stability, which is an underlying concept in agronomical studies that compare organic and conventional systems, does not suit the analysis of conversion. Such studies rely on a Cartesian scientific paradigm which reduces the factors of variation in the environment and circumscribes the subject to a controllable system, whereas conversion, on the contrary, enhances natural regulation processes and an evolutionary relationship to techniques.

When conversion is studied in itself rather than through its effects, its time span is formal and fixed, whereas its duration actually depends on specific situations, and its term is not a given. Therefore, the transitional dynamics are neglected. To take them into account, the construction of new balances should be described, with the identification of new combinations of productions in space and over time, of subsequent states in the field and its environment (Sjursen 2001), and of techniques to manage or master pests and diseases (Zehnder et al. 2007); all consistent with a revision of agronomical and economic performances.

For example, based on comprehensive studies of 29 converting farmers, Bonnaud et al. (2000) identify three types of trajectories: (1) reinforcement of a farm transformation which was already undertaken, with environmentally-friendly and close-to-organic practices; (2) direct conversion to organic farming, often with strategies of direct marketing; and (3) branching out towards a new orientation, whether breaking with past practices and experiencing alternative management methods into new networks, or searching for new combinations of farm activities. The first type can be considered as an organic agriculture "by default" and does not really imply a profound change of mind. It represents probably a larger potential base for the development of organic farming than the two other types, but supposes that producers and advisers take seriously the principles of organic farming and really apply them for the further evolution of the converted farms.

From a methodological point of view, farm or field pluri-annual monitoring and modelling can contribute to this objective. However, monitoring is costly and not always achieved in this perspective. The reference to a situation preceding the conversion relies more often on a reconstitution than on the analysis of collected data, except in the few cases where monitoring had been started before a conversion that was actually not anticipated. Modelling can also be useful to simulate potential farm evolutions and changes in scales.

Besides the importance of long-term studies, the scale on which these studies are carried out is also fundamental. The farm scale is considered as relevant in most research work (MacRae et al. 1990) and in European regulations (EEC 2092/91). In the new regulation adopted (EEC 834/2007), the general rules concerning conversion are unchanged. Both the implementation and exception procedures of this regulation still favour the scale of the plot, the crop or the animals as elementary units. However, several authors highlight the advantages and the constraints of system approaches on the farm scale (Lockeretz 1985, 1987; David 1999). In particular, Lockeretz and Stopes (1999) analysed on-farm research in organic farming spread over different regions, and listed the reasons motivating these approaches as well as their limits. Some arguments in favour of on-farm research are also relevant for the study of conversion: to work over a large range of situations of production, to study the long-term effects of a production method, to shed light on farmers' experience and to anticipate the relevance of new technologies. These on-farm system approaches also exhibit limits: difficulties of coordinating on-farm monitoring and exploring highly variable situations, as compared to experimentations in stations. The authors suggest how to link on-farm research and experiments in controlled environments. Lastly, they point out the existing gap between the methodological intentions of these on-farm research projects, which enhance a "systemic approach", and actual research practices.

2.4 In the Social Sciences: Towards the Analysis of Trajectories and Transitional Processes

In the social studies, three main approaches to conversion can be identified: (1) quantitative analyses of motivations, generally based on the study of farmers' attitudes; (2) identification of decision-making processes during conversion, generally restricted to its administrative period; and (3) qualitative approaches considering conversion over a longer time period and from a wider point of view.

The first series of studies use quantitative methods to analyse attitudes towards organic farming or food, such as Likert scales, which offer contrasted items to which respondents are asked to agree or disagree. Such studies compare organic and conventional farmers' attitudes, "potential converters" and organic farmers, or recent and more experienced converters (Best 2005; Koesling et al. 2005; Lockie and Halpin 2005). Most analyses of motivations for conversion reveal that economic motivations surpass environmental as well as food quality motivations, even though the latter two are more often expressed by organic farmers than by conventional ones. Therefore, studies of motivations often lead to a classical opposition between market-orientated farmers and values-orientated ones, even in qualitative studies (Lund et al. 2002). This opposition does not acknowledge the complexity of farmers' motivations. Alroe and his colleagues suggest that there is a third perspective, besides the two quite common perspectives of, respectively, organics as a market niche and organics as a "heterogeneous protest" bringing together diverse reactions against mainstream practices and developments, which corresponds to organic agriculture seen as an "autopoietic movement" in the sense that it is rendered coherent by way of a common meaningfulness, as expressed in the core values, worldviews and alternative practices of organic actors (Alroe and Kristensen 2002; Alroe 2005). Padel also suggests another way to categorise farmers' motivations, by distinguishing technical and financial motivations linked to the farm itself from personal motivations which can be called ethics- or values-orientated, including health, environment and rural development, and finally, lifestyle motivations (Padel 2001).

We identified a second type of approach. It is based on the conversion decision process, and seems to provide a more suitable method, as it considers that the decision to convert entails several kinds of intricate motivations, whereas attitude studies generally consider the motivations as quite independent from each other. Indeed, these approaches analyse the chain of motivations mainly through the use of decision-tree methods (Gladwin 1989). The method is comprehensive and aims at identifying farmers' rationale for their actions as well as taking into account the heterogeneity of decision criteria. The results are often presented through farmers' typologies, as for attitude studies. This way, pragmatic organic farmers and committed organic farmers can be distinguished – where the same kind of opposition between market and values can be found again – the first ones being able to go back to conventional farming if price premiums were to diminish (Fairweather 1999; Darnhofer et al. 2005).

These two first types of approaches consider farmers as relatively isolated rational actors, whereas they are, of course, involved in complex social and professional networks. In addition, conversion is seen as a limited period of time, just like in many agronomical studies, and the real length of the transitional process of conversion, as well as the possible antecedents preceding conversion and the adjustments following it, are all often neglected.

The third type of sociological approach addresses these shortcomings better. It involves studying conversion through qualitative methods based on comprehensive interviews, allowing the identification of biographical events progressively leading to conversion as well as the analysis of farmers' conceptions of their work. Instead of typologies, this third approach favours the tracing of organic farmers' trajectories and the study of their practices (Guthman 2000). These approaches also analyse learning processes throughout the period of conversion considered over time and study the networks in which farmers are possibly involved. As pioneering studies of the 1970s have already highlighted, farmers are not alone and their commitments to various networks, such as organic farmer groups but also local farmer groups, as well as their meaningful relationships to a variety of actors, i.e. other farmers, consumers, advisory services, neighbours, etc., have to be analysed.

The question of local reputation and the nature of the links with other local farmers are particularly important to assess. Indeed, sociological studies since the beginning of organics often inquire whether organic farmers form a marginal professional group or are, on the contrary, well linked to the rest of the profession. Some authors tend to consider them as a marginal group, because their conceptions of farming are clearly opposed to mainstream agriculture and its modern scientific rationality characterised by the growing use of fertilisers and pesticides (Michelsen 2001). Organic farmers, especially biodynamists, would have weak relationships with their peers, precisely because they are very critical towards their own profession (MacMahon 2005). Other authors, though, observe an improvement in organic farmers' relations to the agricultural profession and a willingness to be implied in its networks, even though relationships are often analysed as being closer to consumers than to non-organic farmers (Padel 2001; Piriou 2002; Storstad and Bjørkhaug 2003).

Lastly, the role of advisory services in conversion processes is quite underestimated in the literature even though it often appears as determining when studying organic farmers' trajectories (Ruault 2006). Are they specific to organic farming or integrated into the "ordinary" services so as to facilitate conversions, as in Denmark (Kaltoft 1999)? What is the role of suppliers or clients (such as cooperatives) or organic certification inspectors (Sepannen and Helenius 2004)?

Despite their differences, these sociological approaches to conversion all point to an important problem. This is the fact that farmers' motivations are generally analysed after conversion, as if they had not changed after that point in time. Indeed, motivations

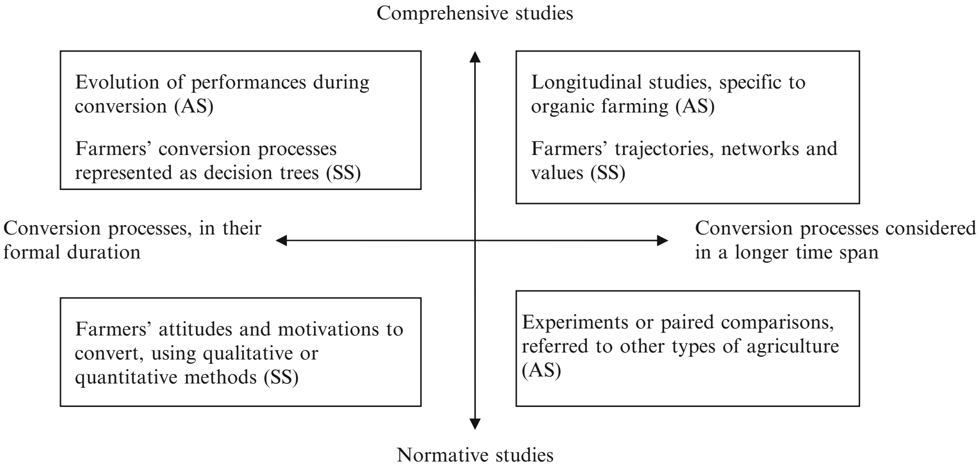

Fig. 1 Main approaches to conversion in agricultural sciences (AS) and social sciences (SS). The *horizontal axis* opposes studies on formal conversion itself (a few years) to those considering a longer time span (beyond the formal duration of conversion). The *vertical axis* opposes normative assessments of the effects of conversion to comprehensive studies of conversion as a multidimensional issue (*top*)

which are given a few years after conversion might well not be those that would have been expressed at the time of converting. It makes it all the more interesting to study conversion through farmers' trajectories, keeping in mind the idea that time and experience change the interpretation one has of one's own trajectory.

What can we conclude from this examination of agronomical and sociological methodological approaches to conversion? In agronomy, as the focus is generally on the effects of conversion, the motivations are more or less pushed into the background (Fig. 1). When they are taken into account, motivations are considered as answers to technical problems (sanitary problems in livestock farming, a failure in agricultural methods, low yields and difficulties in soil tillage) and economic drawbacks (low livestock productivity due to diseases or the high cost of inputs) (Berthou et al. 1972). Moreover, we have seen that in agronomical approaches, the dynamical and transitional aspects are often underestimated in favour of comparisons of stabilised situations, either between organic and conventional farming, between before and after conversion, or during the conversion period vs. a few years later. This weakness of a dynamical approach may seem paradoxical as systemic approaches as well as case studies focused on human activities both entail a teleological vision (Padel 2002), and because at the farm level conversion often leads to a revision of initial objectives.

On the other hand, sociological studies, which favour the study of the motivations and conceptions of organic farming, often ignore the characteristics of production systems (Morel and Le Guen 2002), which makes it difficult to analyse spurs and brakes for the development of organic farming. However, some sociological approaches take into consideration better the dynamical and transitional aspects of conversion.

The construction of interdisciplinary approaches of conversion combining agricultural and social sciences might help acknowledge the diversity of initial situations and the diversity of trajectories and help analyse the multiple dimensions of these trajectories. This has been attempted in several recent studies (O'Riordan and Cobb 2004; Darnhofer et al. 2005; Noe and Alroe 2003; Noe et al. 2005; Flaten and Lien 2006; Bellon et al. 2007).

3 Conversion as a Transition Model for Agriculture

This examination of agronomical and sociological literature does not only lead to the identification of the different methods used to approach conversion in each field and the promise held by interdisciplinary studies. It also sheds light on the main paradigms of organic farming. Even though they have done it each on their own, agricultural and social sciences have both dealt with the diverse conceptions of organics and the paradigms that sustain these conceptions. Beyond the case of organic farming, these scientific debates offer relevant conceptual tools to approach any and all transitions to sustainable agriculture.

3.1 Organic Farming Paradigms: Between Input Substitution and System Redesign

The identification of the main conceptual paradigms of organic farming is central both to social and agronomical scientists, as several studies assess it in different contexts. Based on the case of Denmark, Kaltoft identifies four paradigms: the paradigm of nutrients (or conventional point of view), the paradigm of soil fertility (ecological point of view), the biodynamic paradigm and a paradigm of communication which involves aspects of the three others. In the case of the Netherlands, other authors distinguish three conceptions within organic farming: natural as the organic, natural as the ecological and natural as referring to nature as a holistic entity (Verhoog et al. 2003). They relate these concepts to three main approaches to organic agriculture: a no chemicals approach, an agro-ecological approach and an integrity approach. This last one entails a spiritual dimension neighbouring the holistic biodynamic principles. A third study analyses the organic farmers' relations to nature and places them between an anthropocentric pole and a "natural-pragmatic" pole (Tress 2001). In the first case, productivity is the guideline for farmers' actions and nature has only an instrumental value; farmers aim for a direct control of agricultural production and performances in an unchanged technological frame of reference. In the second case, humans and nature co-exist, nature is recognised as having a certain value and as being an autonomous entity, interactions between techniques and the components of the "agroecosystem" have to be built so as to enhance natural regulation processes and partial or indirect effects.

All these suggestions of distinct categories of organic conceptions can be related to the binary distinction between an "input substitution paradigm" and a "system redesign" paradigm first suggested by biologists (Hill 1985; Hill and MacRae 1995; Altieri and Rosset 1996) and afterwards used by many authors. The first one defines organic farming as the ban of certain inputs and/or the recommendation of others (list of eligible inputs to "fight against" pests and diseases, although with biological methods), whereas the second one defines it through more qualitative and global principles such as crop rotation or means to overcome sanitary problems. This holistic paradigm refers more broadly to the construction of diversified production systems following the ecological model considered as the "natural" one, where interactions between components guarantee fertility, productivity and resilience properties. For Rosset and Altieri (1997), the basic components of sustainable agroecosystems are as follows: (1) reliance on locally available resources and enhancement of positive interactions among plants, animals and soils, (2) organic matter management and nutrient cycling, (3) maintenance of vegetative cover with reduced tillage, cover crops and mulches, and (4) habitat management favouring natural pest regulation. In the perspective of a transition towards a more sustainable agriculture, the authors (Hill and MacRae 1995; Rosset and Altieri 1997) differentiate three approaches: increased efficiency of input use, input substitution or the replacement of agrochemical inputs by environmentally more benign inputs (e.g. botanical or microbial insecticides), and system "redesign" arising from the transformation of agroecosystem functions and structure. According to them, the prevalence of input substitution drastically limits the potential solutions to the socio-economic and ecological crisis of modern agriculture, in the sense that this substitution does not call into question monoculture or the dependency on external inputs. The transition of a farm inside organic agriculture, once converted, could be defined through these three paradigms considered as successive steps. Indeed, by definition, organic agriculture relies on an input substitution. Once this substitution is accomplished, it is possible to aim for a better efficiency of inputs inside the organic framework, or to go even further and to aim for a "redesign".

Does this notion of redesign also reflect the parallel evolution of farm and landscape structures? Organic farms are supposed to be diversified and this diversification is to be seen in landscape changes. Conversion can then be considered as a case study to test land-use options and crop combinations.

Several studies conducted in Denmark shed light on this aspect. Langer used a database composed of 448 conversion projects in which farmers describe their situations and their projects regarding crops and livestock production (Langer 2002). Over a 4-year period, no tangible evolution was perceived in production orientations. The regional distribution of cropping systems would therefore not be strongly modified in the short term. Conversely, land-use patterns change with conversion: a 20% decrease in the acreage for cereal production, a doubling of the acreage for grassland in rotation, a decrease in acreages for set-aside, row crops and oilseed rape. However, these farmers' intentions have not been verified through monitoring after conversion.

In addition, Levin studied the consequences of conversion on landscape composition (Levin 2007), based on four components as suggested by Kuiper (2000): density of uncultivated landscape elements, diversity of land uses per unit area and mean field size. The study relies both on national agricultural surveys and on regional analyses and photo-interpretations. The national analysis shows a greater diversity of soil uses and a smaller size of plots in organic farms. Conversion to organic farming lowers or inverses the general trend towards an increase in farm size. However, the regional analysis does not reveal any direct relation between organic agriculture and non-cultivated landscape elements on this scale. This could be due to the absence, in organic standards, of specific rules concerning the farm integration of the functionalities of woodlands or ecological infrastructures, which differs from other specifications such as integrated production (Girardin and Sardet 2003).

Economists (Lampkin and Padel 1994) as well as sociologists and geographers have also discussed this distinction between substitution and redesign. We find this reflects the ability of such paradigms to circulate as well as bring diverse disciplines to the same table. Studies from an interdisciplinary point of view might well offer the most promising conclusions.

From the analysis of comprehensive interviews with 150 farmers, the geographer Guthman (2000) used methods based on indicators elaborated by biotechnical sciences (Van der Werf and Petit 2002), so as to classify the farmers according to their degree of adoption of agro-ecological principles (Altieri 1995), and to the dynamics of conventionalisation. The analysis, which looks both at the differences between organic farmers and at the gaps between agro-ecological principles and farmers' practices, leads to a double distinction:

- Between mixed farmers who partially converted to organic farming and restrict themselves to following the rules which they interpret in terms of input substitution, and farmers who fully converted.
- Between large farms and smaller farms. The large farms depend more on external inputs, whereas the small ones rely on compost-making and intercropping.

However, few producers really approximate an agro-ecological ideal integrating crops and livestock, companion crops, and/or an intense mosaïc of cropping design combined with a high degree of on-farm input development. As for pest and disease management, the methods used range from proactive prophylactic measures and habitat maintenance enhancing beneficial insects to an organic agriculture "by default", without an explicit strategy to manage or by-pass biotechnical issues (apart from using eligible inputs). In between these polarities, a "wait and see" behaviour can be identified, quite similar to an IPM-based strategy.

This distinction between the two paradigms of input substitution and system redesign converges with the distinction identified in the conventionalisation debate between two distinct trends in organics, opposing industrial farmers and farmers involved in a more ideological and agro-ecological vision of organic farming. The concept of "conventionalisation" was developed by political food economists in a founding article in 1997 (Buck et al. 1997). According to them, organic production and markets were being overcome by large-scale structures and were becoming more and more vertically integrated, just like in conventional agriculture. Conventionalisation is characterised by the concentration of capital among fewer and larger growers and intermediaries more equipped to deal with retailers, the erosion of organic standards, the generalisation of the substitution of allowable inputs for proscribed inputs, and a growing dependence of farmers on input suppliers and supermarkets. This evolution led to a bifurcation into two distinct trends: a main conventionalised one and a minor resistant one.

This conventionalisation thesis was developed and is still debated over California, where large-scale growers and monoculture are prevalent. In the UK, the growth of the organic sector has also generated a decline in farm gate prices and in farmers' income which has called into question the sustainability of current levels of organic production (Smith et Marsden 2004). This phenomenon is due to the conjunction of private-sector initiatives (consumption-orientated) and government incentives to increase conversion (production-orientated), which lead the authors to highlight the limits of government intervention when it only deals with production and neglects the downstream supply chains. Other studies in other areas such as Australia (Lockie and Halpin 2005), Canada (Hall and Mogyorody 2001) and southern countries (Halberg 2007) show that more diverse evolutions should be acknowledged. If the conventionalisation thesis reveals how the boundaries between conventional and organic agricultures might be contested and mobile (Goodman 2000), it is therefore commonly agreed that the analyses have to be clearly related to their specific geographical context.

Several authors (Langer and Frederiksen 2005; Darnhofer 2006) estimate that conventionalisation goes hand in hand with an increasing dependency on external inputs (equipment, fertilisers and animal feed) and with a substitution of work by capital. These tendencies towards a bipolarisation are also visible in the construction of rules (Goodman 2000), as the organic label, according to some authors, reduces the natural-social complexity of organics to a simple question of inputs (Allen and Kovach 2000). For others, it is more largely the impact of the conditions imposed by the process of agro-industrialisation which impedes real "alternative" farming practices (Guthman 2004). A few recent studies focus on the phenomenon of "de-conversion" or "reversion", which they relate to this conventionalisation trend (Campbell et al. 2006; Kaltoft and Risgaard 2006).

From the point of view of environmental sociology, this distinction of these two paradigms could lead to asking if organic farming is more a phenomenon of ecological modernisation (Spaargaren 1997) or of ecologisation of agriculture (Deverre 2004; Obach 2007). In Brazil, Caporal and Costabeber studied the transition toward an agro-ecological model and its implementation, which might proceed through different paths and steps (Caporal and Costabeber 2004). This model is presented in sharp opposition to the industrialisation of agriculture and to the green revolution, but also to certain forms of traditional agriculture. For these authors, the choice of such an agro-ecological model is far from being only an answer to a market which demands a greening of food products through a green intensification: it involves environmental values and new ethics in terms of man–nature relations (Brandenburg 2002).

Therefore, the two paradigms of input substitution and system redesign not only correspond to specific types of relations to techniques and market, but can also be compared with the two notions of ecological modernisation and ecologisation in environmental sociology (Table 2). The input substitution paradigm remains in the technical framework of conventional agriculture, in which agricultural performances rely on a notion of control over natural phenomena and irregularities. Regarding crop protection, the central idea is still to fight against pests even if more ecological means such as biological control are necessary, whereas the system redesign paradigm highlights natural regulation processes and partial or indirect effects.

These two paradigms also differ in terms of temporalities and means devoted to conversion. Input substitution is nearer to the administrative time of conversion (with a possibility of reversion in the case of technical failure or economic difficulties), whereas the systemic conception supposes a more lasting commitment. Finally, the two paradigms differ regarding the role of extension services and certification bodies.

Table 2 Two main paradigms of organic farming and their consequences in terms of development

The two paradigms of organic farming	Corresponding concept in Environmental sociology	Relation to techniques	Market trends
Input substitution paradigm	Ecological modernisation	Direct control of the agricultural production through techniques, in an unchanged reference frame	Conventionalisation and Greening of food products
System redesign paradigm	Ecologisation	Construction of interactions between agricultural techniques and ecosystem components	Recomposition of marketing towards shorter circuits

Indeed, compliance with the first paradigm can be controlled through ordinary inspection procedures such as check-lists, at the cost of a more comprehensive approach: it is easier to inspect input purchases than the farming system as a whole.

3.2 The Case of Organic Farming as an Indicator of Society Questioning Agriculture and Food Models

If social sciences take an increasing part in this debate around the paradigms sustaining the conceptions and practices of organic farming, their specific contribution also involves the impact of the organic movement on civil society and the links to a broader debate over the future of agriculture and food systems.

The role of the organic movement is surely different today than in its beginnings, when the mission of agriculture was to feed the nations and when productivism was therefore legitimate, with hardly any place for social criticism and movements in agricultural decisions (Michelsen 2001; MacMahon 2005). In the French case, it seems that organic pioneers failed to create a real social movement that would have brought together producers and consumers (Piriou 2002). According to this author, farmers followed the logic of their corporatist lobby and could not go beyond the boundaries of their profession. About 20 years ago, however, from the analysis of the studies of organic farming carried out in the 1970s and 1980s, Barrès et al. identified a progressive shift towards a willingness to be accepted and approved by society and to develop links between cities and the countryside (Barrès et al. 1985). However, it is only quite recently that an alliance between producers and consumers really began to visibly take shape, even though the importance of pioneering signs and experiences such as organic cooperative networks has to be recognised.

Today, organic networks greatly contribute to getting the civil society involved in debates over agriculture and food. However, certain authors denounce a repression of the movement's ideological content and a betrayal of organics' initial ideals through which the dominating definition of organic agriculture reduces relations to nature to a question of allowed inputs. Both the institutionalisation and the conventionalisation of organics go hand in hand with a decline in its idealistic vision and a decline in farmers' environmental concerns (Tovey 1997; Kaltoft 1999). Many authors, though, have a more optimistic vision of the possible place of organics in political and social change. This place would rely on the capacity of organics to transform the way people look at their food, in particular through unveiling the conditions of production, instead of hiding them. Alternative networks invite consumers to consider products through the way they are grown, the kind of labour involved, the relationship to nature or even the public research investments which are implied in this production, and not only through the material characteristics of products. Thus organics could be an answer among others to larger concerns about the rising centralised control of society's relations with agricultural nature (Allen and Kovach 2000; Goodman 2000).

These suggestions imply bridging the classical gap between production and consumption. The evolution of organics relies on the conversion of both farmers to organic farming and consumers to organic food. Indeed, organic consumption is an area of research that was investigated by economic and marketing science (Sylvander 1997; Codron et al. 2006; Holt 2006) as well as by sociology and anthropology (Ouedraogo 1998; César 1999). The specificity of more recent studies in social sciences is that they tackle the interactions between production and consumption, that had received little attention (Lockie et al. 2000, Tovey 2002). Many authors shed light on these links between production and consumption through the analysis of some recent developments in agro-food systems, and specifically the study of alternative food networks centred on organic but also local production (Murdoch and Miele 1999; Marsden 2000; Goodman 2002, 2003; Goodman and DuPuis 2002; Guthman 2002; Lamine 2005; Moore 2006). The capacity of organics to have a larger impact on the visions and evolutions of agro-food systems also relies on the idea that consumption could be considered as a possible form of political action. This has already been suggested by several authors focusing on alternative food systems, as seen before, as well as on reflexive consumption (DuPuis 2000).

3.3 Beyond Disciplinary Divisions: The Study of Transitions in Agriculture

Today, it is becoming more and more evident that it is necessary to explore and develop intermediary forms between a conventional agriculture whose productivism appears to be socially and environmentally unsustainable and an organic agriculture that is not suitable for every soil, region and farmer. In this context, the two paradigms of input substitution and system redesign, that were identified on the base of organic studies, might help enrich and frame the discussion over future agricultural models. Beyond organic farming, many studies use these paradigms so as to analyse the transformations of agriculture.

In the Brazilian state of Rio Grande do Sul, these paradigms help to classify all farmers into three categories: conventional farmers (who reduce their use of chemical input for economic reasons), farmers in transition (who substitute chemical inputs with biological ones), and farmers in a "redesign" type of transition (who adopt an ecological and systemic approach incorporating soil ecological management, crop rotation and diversification, mixed crop-livestock integration, reforestation and management of agro-forestry systems) (Caporal and Costabeber 2004). According to certain authors, integrated production also corresponds to a mid-term and fluid transition between conventional and organic agricultures (Niggli 1999), with strategies such as integrated protection (IPM), reasoned crop fertilisation and priorisation of biological control methods (Hodges 1981).

Taking into account the complexity of transitions in agriculture supposes going beyond a restricted agronomical vision that would ignore the social aspects linked to food systems as well as the natural phenomena such as climate change. This is what some authors try to do by defining agro-ecology broadly as an ecology of food systems (Francis et al. 2003), based on the notions of ecosystem (Lowrance et al. 1984) and agroecosystem (Gliessman 1990), and which can be opposed to a vision of agro-ecology as an ecology applied to agricultural production (Altieri 1983). Interdisciplinarity is considered by these authors as a prerequisite to the development of agro-ecology, as economic and social aspects have to be included (Altieri 1989; Dalgaard et al. 2003).

Interdisciplinary approaches are more generally of great interest when considering conversion to organic farming as an exemplary case of larger transitions in agriculture, as they allow the taking into consideration of these transitions by surpassing their sole technical aspects, and by paying attention to their temporal dimension. With this perspective in mind, approaching conversion to organic farming from the crossroads of several different disciplines holds promise.

To cross history with agronomy would allow an analysis over time of the transformation of the historical paradigms of agronomy, which can be identified as successively chemical, physical, biological and finally, ecological paradigms (Robin and Aeschlimann 2007). Specifically for organic agriculture, a historical approach can help to identify the founding paradigms and their differences, mainly between Steiner's organic vision of a farm integrating breeding as a key component, and Howard's vision favouring soil fertility and humus management. This leads to renouncing the idea of a unique original paradigm for organic farming and contributes to the necessary acknowledgement of organics' internal diversity (Besson 2007).

Collaboration between agronomy and sociology allows the study of the transformations of farmers' practices over time. The notion of a trajectory allows the consideration of conversion over a longer time period and from a wider point of view than in its official definition. Organic farmers' trajectories encompass at the same time technical trajectories, social trajectories, learning trajectories and network trajectories Bellon et al. (2007). Compared with classical typological methods, such studies, involving agronomists and social scientists, prove that interdisciplinary approaches are more adequate to reflect the actual diversity of organic farming, as well as the evolutionary potential of farming systems and the transitional nature of conversion trajectories. In these socio-technical trajectories, the questions of plant protection practices, of input use and of farmers' representations of these issues, are central. It may seem paradoxical to note that these questions are not often raised in studies of organic farming, maybe because these problems are supposed to be solved, whereas they are more often central to comparative studies of conventional and organic farming. Pesticides have been proved to be not only a crop protection means but also a way to maintain a visible standard (Fairweather 1999), in the sense that conventional farmers are often proud to have clean fields, which is acknowledged as a major bottleneck regarding the adoption of low-input practices.

The study of organic farming as an innovation is of prime interest when thinking more generally about the transitions towards more sustainable agricultures. Some authors have applied to the case of organic farming the classical theories of adoption and diffusion, which identify innovators, as well as early and late adopters throughout the diffusion of any innovation (Padel 2001). As organic farmers do not always come from an agricultural background and are often linked to scientists and/or environmental and consumer movements, other types of innovation than classical top-down processes can be expected (Michelsen 2001). The construction of knowledge and the co-production of innovations is still a quite unexplored area of research (Gibbons et al. 1994), even though there are pioneering works which it might be useful to go back to (Salmona 1994).

Finally, interactions between agricultural and social sciences are necessary to enlarge the scope from agriculture to food chains, and thereby consider the sustainability of organic agriculture or any other form of agriculture (Stassart and Jamar 2005). Other authors also suggest considering the different development models of organic agriculture. Sylvander et al. (2006) propose to identify these development models through two axes, the first one opposing individual logics to collective organisation forms linked to markets and territories, the second one opposing the mere compliance with organic rules to a re-conception of systems.

4 Conclusions

This review of agronomical and sociological literature available in the English- and French-speaking worlds, with some incursions into other social sciences, reveals that conversion, far from being limited to an administrative period with its codified phases, is a multidimensional subject. Conversion can be considered as a programme whose temporalities vary from 2 years to a farmer's generation. This approach would ensure the achievement of the dynamic equilibrium necessary to establish an ecological basis for sustainability. Beyond the bio-technical aspects of production, conversion supposes transformations in farmers' marketing strategies as well as in their representations, values and links to various social networks. Therefore, the study of conversion invites new definition of research topics, i.e. to switch from the plot scale to the farm or even the landscape scale, from production to food chains and food systems, from the notion of changes in the cropping system to the notion of trajectories along which the relations to techniques, nature, territory, markets and consumers are redefined. From this point of view, the decisive contribution of social sciences is to suggest methods that go beyond the administrative and individual conversion and focus on processes, temporalities and networks.

Crossing over disciplinary boundaries has allowed us to identify a series of oppositions which structure the debates on organics and relations to techniques and market according to two paradigms of input substitution and system redesign, which can, respectively, be compared with the two notions of ecological modernisation and ecologisation in environmental sociology. The input substitution paradigm remains in the framework of conventional agriculture, based on the notion of control over natural phenomena and irregularities, whereas the system redesign paradigm relies on natural regulation processes and partial or indirect effects. Beyond organic farming, these paradigms prove useful to consider changes towards more sustainable agricultural practices, especially in terms of plant protection.

Indeed, conversion to organic farming is an exemplary and well-informed case to think more broadly about changes in agricultural systems. This entails approaching conversion as a more general figure of transition of agriculture (Sangar and Abrol 2004), from a perspective which can refer to the notions of agroecology (Gliessman 1997) and sustainability (Kates et al. 2001; Rigby and Cáceres 2001; Elzen and Wieczorek 2005). Such a perspective should be based on the development of fruitful interactions between agricultural and social sciences so as to encompass the aspects of consumption, markets, public policy and the conceptions and practices of agriculture and nature. This ambition raises further questions such as the capacity of organic farming to "feed the world" in the case of conversion of large areas (Griffon 2006; FAO 2007) as well as several subjects quite ignored in the literature, such as questions concerning the place of women in organic farming, developed only in rare studies (Barrès et al. 1985; Chiappe and Flora 1998); work organisation and relationships; and social justice from the production and consumption side, with a focus on fair access to healthy food (Goodman 2000).

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Triggering Transitions Towards Sustainable Development of the Dutch Agricultural Sector: TransForum's Approach

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Abstract TransForum is an innovation program which aims to make a substantial contribution to the transition towards more sustainable development of the Dutch agricultural sector. This article describes the scientific foundation and architecture of this program. TransForum operates on the basis of five working hypotheses which together constitute one integrated analytical framework. These hypotheses are: (1) sustainable development is a dynamic system property; (2) sustainable development needs system innovation; (3) system innovation is a non-linear learning process; (4) system innovation requires active participation of relevant key players from knowledge institutes, governmental bodies, civil society organisations and the business community; (5) the program requires transdisciplinary collaboration of all players. TransForum identifies three new innovation strategies: (1) vital clusters; (2) regional development; (3) international agro–food networks; as alternatives to the current arrangements. Innovative projects are organised in these innovation strategies. The aim of the scientific program is threefold: (1) it addresses research questions raised in the innovative projects; (2) it investigates the need for system-innovations and the way in which they can be realized; (3) it designs research projects to test the five main working hypotheses of the program. The scientific program is organised in four themes following a cyclic innovation process which is constantly monitored. The cycle starts with people's preferences and images, followed by studies on which inventions are required to achieve a successful innovation. Subsequently, it is investigated how to organize new innovations and transitions and finally, how citizen/consumers behaviour and preferences mobilizes sustainable development, closing the loop.

Keywords Networks • Sustainable development • System innovation • Transition

1 Introduction

Dutch agriculture and rural areas have changed dramatically during the last century (Maris and de Veer 1973). Productivity soared due to new technologies, mechanization, increased chemical use, specialization and government policies that favoured maximizing production (van Dijk and Mackel 1991). These changes allowed fewer farmers with reduced labour demands to produce the majority of the agricultural products. This development gave the Netherlands a strong agro-food sector but also changed the face of Dutch and European landscapes (Verburg et al. 2006a; Pedroli et al. 2007). The post-war development of knowledge was also directed towards high-productivity agriculture. As a result a knowledge infrastructure developed which focussed on new technology development for production maximisation. The implementation is still rather linear and top-down (Leeuwis 2000). Research results are communicated to farmers by means of an extension service telling the farmer how to improve their production. While

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this approach was successful, it is now leading to overspecialization, environmental pressures and encroachment on public spaces (Wiskerke and van der Ploeg 2004). These developments caused the Dutch agro-food sector to run into its ecological and social barriers (Dunn 2003). Moreover, the Dutch agro sector is currently at risk of losing its "license to produce" (social problem), "license to operate" (policy problem) and "license to deliver" (market problem).

TransForum was established in November 2004 in order to encourage the development of innovation oriented knowledge that would contribute towards more sustainable development in agriculture. TransForum is an innovation program that aims to provide a more sustainable development perspective for the Dutch agro-sector and rural areas by searching for and experimenting with new value propositions. TransForum's analytical framework is based on a set of new working hypotheses leading to a rather unconventional scientific program. It is the aim of this paper to demonstrate the unique features of the TransForum scientific program.

1.1 Analytical Framework of TransForum

TransForum operates on the basis of five hypotheses, which together constitute an analytical framework for both developing a more sustainable development perspective for the Dutch agro sector and an adaptation of the current knowledge infrastructure. These hypotheses are:

- Sustainable development is a dynamic system property
- Sustainable development needs system innovation.
- System innovation is a non-linear learning process.
- System innovation requires a multi stakeholder approach.
- The TransForum approach requires transdisciplinarity.

These hypotheses deal with the concept of sustainable development, the relationship between this development and the need for innovation, the relevance of learning to achieve these innovations, the necessity of the involvement of all relevant stakeholders and the consequence of dealing equally with the knowledge that all the stakeholders bring with them, including tacit knowledge, and subsequently operating in a transdisciplinary mode. All five hypotheses will be explained below.

1.1.1 Sustainable Development is a Dynamic System Property

Sustainable development rests on the principle that we must meet the needs of the present without compromising the ability of future generations to meet their own needs. Definitions of "sustainable" agriculture are generally concerned with the need for agricultural practices to be economically viable, to meet human needs for food, to be environmentally positive, and to be concerned with quality of life. Sustainable development is characterised as the effort of finding a better balance between the Triple P (People, Planet and Prosperity) triangle of relevant values (Elkington 1998).

Since this better balance can be achieved in a number of different ways, sustainable development is not automatically linked to any particular technological practice or vision. Rather, sustainable development in agriculture can be looked upon as adaptability and flexibility over time when it comes to responding to changing demands and perceptions. These changes are typically related to food and other commodities, but also to shifts in socio-economic demands. In the Dutch context these shifts are apparently even more important than changes in demand for agricultural produce. Finally, it is important to realise that agriculture and regional development (green space) are all part of the same complex adaptive system, making it essential to address sustainable development as a system dynamic property (Fiksel 2006). This view of agriculture as a complex adaptive system is a rather recent development but now a widely accepted model in system analysis (Liu et al. 2007).

1.1.2 Sustainable Development Needs System Innovation

The current highly specialised agricultural system has become unable to cope with large-scale changes and disturbances. Examples are the recent crises caused by foot and mouth disease (Thomson 2002) and the continuous political and societal debate regarding its license to produce. To overcome this barrier, there is a need to not only do things better, but to do better things. This calls for so-called system innovations or even transitions that enable the agro sector to develop apt alternatives.

Innovation is implementing a new value-proposition by means of a new, unique value chain. System innovation is the same, but for a system with multi-actor involvement (Porter 1990). While innovation typically adds value, innovation may also have a negative or destructive effect as new developments do away with or change old organisational forms and practices (Driel and Schot 2005). Innovation therefore typically involves risk. If system innovations are successful they may even lead to so-called transitions (Rotmans 2003), a radical and structural change of the system as a whole.

1.1.3 System Innovation Is a Non-linear Learning Process

The motto of the 1933 World Expo in Chicago was "science finds, industry applies, man conforms". This is the classic linear model of innovation: the idea that if you put enough money into science that after a while it will automatically lead to successful products, services and solutions for social problems. This way of thinking is referred to as "Mode-1". TransForum feels that system innovation, which includes both technical and social innovations, is not such a linear process. Science should interact more with users in order to generate successful products. This implies an innovation process with a lot of feedback. All this implies that "learning" as feedback is crucial in innovation processes (Gibbons et al. 1994). Learning about technical capabilities, their possible applications, how to realise these applications and what the consequence could be. You can learn by searching, by doing, by using and by interacting. The learning process that follows these lines is referred to as "Mode-2" (Gibbons et al. 1994; Nowotny et al. 2003). TransForum thinks that this Mode-2 approach is essential to develop the needed system innovations. Characteristically, Mode 2 research groups are less firmly institutionalised; people come together in temporary work teams and networks, which dissolve when a problem is solved or redefined. Members may then reassemble in different groups involving different people, often in different loci, around different problems. The experiences gathered in this process create a competence which becomes highly valued and which is transferred to new contexts. Though problems may be transient and groups short-lived, the organisation and communication pattern persist as a matrix from which further groups and networks, dedicated to different problems, will be formed (Gibbons et al. 1994). TransForum meets these typical Mode 2 characteristics it is a temporary organisation with temporary projects with a steady increase in human capital and experience along the way.

1.1.4 System Innovation Requires Multi Stakeholder Approach

Since sustainable development deals with finding a better balance between people, planet and prosperity, and since learning should take place in a Mode-2 fashion, the selection of participants in an innovation effort is essential. To ensure that all relevant values can play a part in attaining this better balance, representatives of differing views on sustainable development should be involved.

Within Dutch agriculture the current knowledge infrastructure (Agro-KIS) still plays an important role but with decreasing success. Knowledge is typically developed and disseminated by a top-down approach. It has now been recognized that this one-way top-down approach is insufficient to allow bottom-up interactions and feedback necessary for "natural" diversification and system adaptation (Carpenter et al. 2001). This top-down approach also dominates current visions of sustainable development of the agro-sector. Consequently indicators of sustainable development have typically been defined by scientists and policy makers only. It is important to emphasise that reaching toward the goal of developing sustainable agriculture is the responsibility of all participants in the system, including farmers, labourers, policymakers, researchers, retailers, and consumers. Each group has its own part to play, its own unique contribution to make to strengthen the sustainable agriculture community.

TransForum therefore stimulates and organises collaboration between the four main players that together must take responsibility in the system innovation: knowledge institutes, governmental bodies, civil society organisations (including consumer organisations) and the business community (including farmers). This combination is referred to as KOMBI

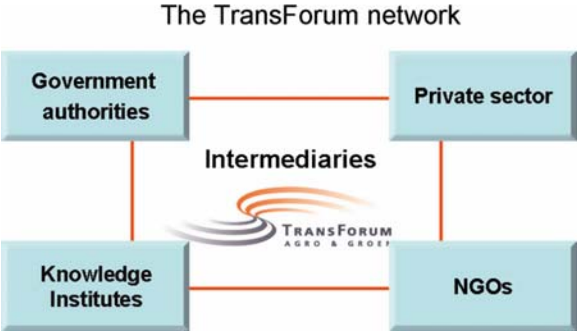

Fig. 1 The TransForum network: knowledge institutes, governmental authorities, civil society organisations (including consumer organisations) and the private sector (including farmers). This network is referred to as KOMBI (= Dutch acronym) partners. TransForum is acting as an intermediary in the network

(= Dutch acronym) partners (Fig. 1). The process of involving all four partners is directed by TransForum by actively inviting and involving selected individuals from KOMBI organizations. This selection is based on individual experience of project directors who create and facilitate the necessary conditions for the projects, act as a link with the organisations involved and encourage the transfer and use of knowledge.

1.1.5 TransForum Approach Requires Transdisciplinarity

Transdisciplinarity is seen as an integrative form of research, relating scientific knowledge and extra-scientific experience and practice in problem-solving. In this understanding, transdisciplinary research addresses issues of the real world, not issues of origin and relevance only in scientific debate. A further important feature in understanding transdisciplinary research is whether and to what extent an integration of different scientific perspectives is addressed. This aspect is often used to distinguish between trans-, inter- and multi-disciplinarity (Pohl and Hirsch Hadorn 2007).

We have used the four criteria, Lawrence and Després (2004) used to characterise transdisciplinarity research: (1) It tackles complexity in science and it challenges knowledge fragmentation. It goes beyond complexity and heterogeneity, this mode of knowledge production is also characterised by its hybrid nature, non-linearity and reflexivity, transcending any academic disciplinary structure; (2) It accepts local contexts and uncertainty; it is a context-specific negotiation of knowledge; (3) It implies intercommunicative action, which is a research process that includes the practical reasoning and knowledge of individuals [often refereed to as tacit knowledge (Polanyi 1966)] with the constraining nature of social, organisational and material contexts; (4) It is often action-oriented, making linkages not only across disciplinary boundaries but also between theoretical development and professional practice (Giller et al. 2008). Transdisciplinary contributions frequently deal with real-world topics and generate knowledge that not only address societal problems but also contribute to their solution.

Given the complexity of the innovation problems encountered, the context specificity of the projects, the many stakeholders and their different roles and interests, and the action-oriented approach, and the different scientific disciplines involved the TransForum approach requires transdisciplinary research.

2 Transforum's Practice Program

2.1 Three Main Innovation Strategies

TransForum's overall strategy is to let practice leading! In order to link firmly to current reality and practice, TransForum has organised >20 practice or innovative projects, in which the KOMBI partners attempt to overcome obstacles (real problems) concerning system innovation which prevent the current agro-sector from becoming a more sustainable system. There are three main clusters of practice projects aimed at different innovation strategies which intend to resolve pressing problems in the current system.

2.1.1 Innovation Strategy "Vital Clusters"

The signalled over-specialisation and segregation of different agro-sectors and society forms an obstacle for innovation in the agro sector. In this innovation strategy projects are developed that create new value propositions by linking different systems in so-called vital clusters. Aside from the technical challenges, the concentration of many different functions at a single location is currently almost impossible in the Netherlands

Table 1 The project portfolio of innovation strategy Vital Clusters comprising of eight practice projects

New mixed farm	2005–2008	Agropark. Second, final phase almost concluded. Implementation of enterprise will start in 2008. Government regulation determines the speed of the innovation process. Entrepreneurs are now setting up small scale business school on sustainable development
Biopark Gent-Terneuzen	2006–2007	Agropark. Second, final phase almost concluded. Implementation of enterprise will start in 2008. Tuning of process flows and business models between different enterprises proofs very difficult
Greenport Shanghai	2006–2007	Agropark. Start in 2006. Strongly building on network of New Mixed Company. Master plan finished. Implementation starts with business planning. Getting the innovative combination of Knowledge Institutes, Entrepreneurs, Governmental and Non-governmental organisations working is the is pre-dominating problem
SynErgie	2005–2008	Learning network on Energy in Greenhouses. Second phase almost concluded. Created a learning network now evolving into a Community of Practice. Strong emphasis on communication with early adapters via seminars. Successful programming of scientific projects
Unsolicited proposal 3rd ring of Amsterdam	2007	Regional approach to accessibility of Greenport. Formulating "Problem as conceived by all stakeholders" is major effort in first phase. Result of successful co-operation between TransForum and Transumo
Drive	2005–2007	Quality control in pig chain. Project has been closed after first phase. Efforts concentrated almost fully on optimization of internal operational aspects of slaughterhouse company
Healthy pip fruit chain	2005–2007	Introduction of cis-genetic modification to speed up race development in fruits. Efforts concentrate on technical innovation, which gives the project the character of a scientific project in stead of an innovative practice project
Dairy adventure	2007–2010	Three regional specific experiments with dairy farming beyond the scale of family farms, which are characteristic for the Netherlands. Application of knowledge and experiences from Dutch emigrants that started large dairy farms all over the world. Set up of an international Community of Practice

due to the (over)regulations related to environmental and zoning plans. Table 1 gives an overview of the practical projects of Vital Clusters.

2.1.2 Innovation Strategy "Regional Development"

The traditional, dominant agricultural focus when dealing with rural areas is also blocking innovations. The majority of the Dutch people view rural areas as recreational and settlement areas. Subsequently their demands with regard to the landscape are different from the demands farmers have. As a result current regulations have started increasingly restricting farmers in the development of new agricultural activities. To facilitate these changing spatial claims by different users, a transition towards a economically viable, yet socially acceptable agriculture is required. Projects in the innovation strategy Regional Development are characterised by an integrated systems approach to rural regions. Table 2 gives an overview of the project portfolio.

2.1.3 Innovation Strategy "International Agro-Food Networks"

The traditional focus on primary production comes into conflict with the reality that nowadays many products are cheaper to import then to produce. In this innovation strategy projects are stimulated that focus on the transition of the Dutch agro-sector towards adopting a role of knowledge broker and organiser in the international agro sector. Therefore the strategy is referred to as international agro-food networks, since it has to deal with the position that Dutch businesses can attain in the international arena.

In the innovation strategy TransForum wants to develop alternative ways of creating added value in international agro food networks. Table 3 gives an overview of the project portfolio.

2.1.4 Practice Projects in Each Innovation Strategy

Project portfolios with innovative practice projects (Tables 1–3) are linked to each innovation strategy.

Table 2 The project portfolio of the innovation strategy “Regional Development” comprises of six practice projects which can be regarded as regional niche experiments

Northern Frisian Woods	2004–2006	Project concluded and results published in Working Paper 6. Supported the farmers’ organisation NFW in their aim for self regulation in environmental and landscape management. A “regional contract” was developed and space for experiments created
De Sjalon	2006–2008	Phase 1 is concluded. The development of a large scale arable enterprise in the NoodOost Polder, by merging arable farms in collaboration with dairy farms and chain partners. A business plan has been developed; the start of the new company is foreseen in 2008
Green Valley process evaluation	2006	The project Green Valley never started. This evaluation investigates success and failure factors and lessons learned. Results published in a report
Brackish Agriculture	2006–2010	Experiments and research of new Brackish Crops both in laboratory and field circumstances. Focus on plant properties and cultivation techniques, product and market development. Field experiments have been started in a brackish polder (the Island Texel)
Green Care Amsterdam	2006–2009	Developing of a cooperation of Care Farms and Educational Farms, in collaboration with care organisations and schools. The cooperation, now consisting of 20 farmers, has been recognized by the national health insurance
Streamlining Greenport Venlo	2005–2008	A network of entrepreneurs and local governments is supported who are developing the “Greenport Venlo” a dynamic region in Northern Limburg, on “food, feed and flowers”. Focus on learning processes by organising and facilitating Communities of Practice
New markets and vital coalitions South Limburg (“Heerlijkheid Heuvelland”)	2006–2010	New value propositions and coalitions were developed. Important lessons were learned about regime aspects. Based on the lessons learned in phase I, the second phase received a complete “make over”. Results of phase 1 will be published in a Working paper

The aim is to have representatives of all KOMBI partners involved in these projects, facilitated by TransForum. The approach in all projects is based on learning by doing whereby practical, real world problems are the drivers. In these projects together with entrepreneurs and other stakeholders TransForum tries to discover new pathways to more sustainable development of agriculture and thriving rural areas.

The innovative practice projects not only produce practical knowledge that can be applied instantaneously, but also usable methods for generating that knowledge. This creates a new knowledge network that satisfies two requirements: (1) close partnership between research and practice; (2) cooperation between divergent disciplines in order to come up with integrated practical solutions. As a consequence the Practice Programme features strong interaction between science and practice.

The three innovation strategies fit well with the ideas presented in “The Information Age: Economy, Society and Culture” of Manuel Castells (e.g. 1996, 1997), proclaiming the arrival of a network society in which the dynamics of a space of flows were thought to supersede the space of places. Regional development represents new combinations of activities for rural areas, representing the “space of places” following Castells (1997) (Fig. 2).

International agro-food networks represent new trans-frontier production and trade networks in which the Dutch can have their own specific niche, representing the “space of flows”. Vital clusters are new combinations of economic sector in spatially concentrated clusters, were the “space of places” and the “space of flows” meet each other. Regional development resembles the “regions” discussed by Porter (2003), while the vital clusters are in line with the “local clusters” of Porter (2000).

2.2 The Supporting Role of Science and Knowledge

Firstly, the Scientific Programme contributes by research activities that are aimed at delivering solutions and instruments to develop new value propositions.

Table 3 The project portfolio "International Agrifood Networks" comprises of eight projects that together deliver new insights in how to develop alternative ways of creating added value in international agro food networks. The projects span a wide range of topics and also differ greatly in focus

Calendula	2004–2007	An innovative, international agro industry chain for renewable raw materials is developed. Concrete spin-off of the project is the start of a new company "Calendula Oil BV" that will bring Calendula Oil on the market. FEM-Business Magazine recently selected Calendula Oil BV as one of the 25 "most promising" start ups
Everything about food	2005–2008	This project has resulted in the development of the website http://www.meerovereten.nl. Consumers can find production information to easily compare sustainability performances of various food products. The consumer information thus generated can be used by participating industries and retailers for new, sustainable value propositions
Sustainability in retail	2006–2008	This project offers TransForum the possibility to find out whether a pull strategy through retail can speed up the transition towards a more sustainable agro food production and how a transition approach of strategic stakeholder partnership works out in practice
Regional food chains	2007–2008	A new chain will be developed in which the traditional hypermarket chain is no longer the only company that is communicating with consumers. The primary producers are also responsible for merchandizing their products in the retail to the consumers. In this project the hierarchies in the chain are changed. The consequences of this change will be analysed both for supermarket and for producers
Healthy with oats (phase 1)	2007–2008	A new chain will be developed with high quality products on the basis of guaranteed gluten free oats. (At this moment a chain with 100% gluten free does not exist). Gluten free oats will contribute to the reduction of an important social healthy problem of a growing group of celiac patients (2% of the population)
Laying hen husbandry (phase 1)	2007–2008	The project aims to realize a system innovation in the table egg sector. The actual laying hen production systems still have many veterinary, environmental and animal welfare problems. The desired system innovation is triggered by building a totally new chain which is able to quickly adapt to changing market demands
International livestock coordinating role	2005–2006	This project was focusing on transforming the entire livestock farming chain into sellers of knowledge and services in international markets. The aim of the project to develop a strategy to exploit this knowledge has not been obtained in this project. Therefore, TransForum requested a transition analyses. From this analysis, it was concluded that the different companies did not have a shared vision on a sustainable pig sector
Flor-i-Log orchestration	2004–2008	Aim of the project was to investigate how international orchestration of the horticultural sector could be achieved. In this project Dutch flower auctions and wholesalers are looking for new organisational and logistic models to maintain the Dutch leading position in the international floricultural sector. Goal is to seriously diminish unnecessary transport of floricultural products. Scientist and chain members have analysed international market possibilities and developed a logistic model for efficient transport. At the moment the challenge is to develop an international business model from this

The relationship between the innovative practice projects and the scientific projects in these cases is fairly straightforward. In the practice projects the research questions are formulated and the results of the scientific project will contribute directly to the success of the developed value propositions. The approach in these research activities is not so much transdisciplinary as well as interdisciplinary: in formulating the research question, in exchanging information with the practice project and in the application of the results a tight connection between scientific knowledge and the domain of practical experience is made.

Secondly, within the scientific programme the need for system-innovations and the way in which they can be realized is an object of research. For that reason the scientific programme uses the practice projects – amongst other things – as the primary source of information. In almost all practice projects some experimenting is going on with needed system-innovations. The scientific projects use this experimental information to reflect on the findings and combine the practical experiences with scientific insights on sustainable development, on inventions, innovations and transitions. So, learning from practice leads to obtaining insights in the needed knowledge, the required competencies and the necessary transitions. Based on this learning approach the four themes for the scientific programme have been identified, which will be discussed later.

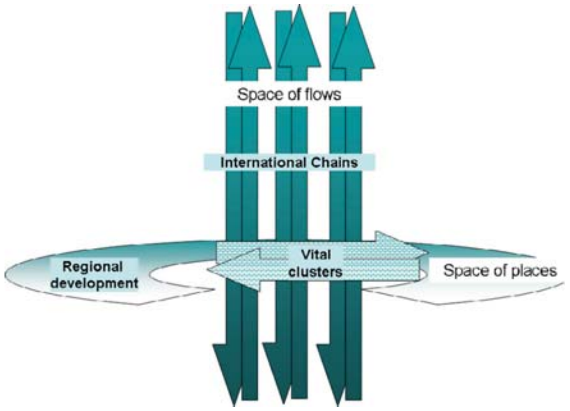

Fig. 2 Illustration of how TransForum's three innovation strategies fit with the ideas presented in "The Information Age: Economy, Society and Culture" (Castells 1996, 1997). Regional development represents new combinations of activities for rural areas, representing the "space of places" of Castells (1997). International agro-food networks represent new trans-frontier production and trade networks in which the Dutch agro sector can have its own specific niche, representing the "space of flows". Vital clusters are new combinations of economic sector in spatially concentrated clusters, were the "space of places" and the "space of flows" meet each other

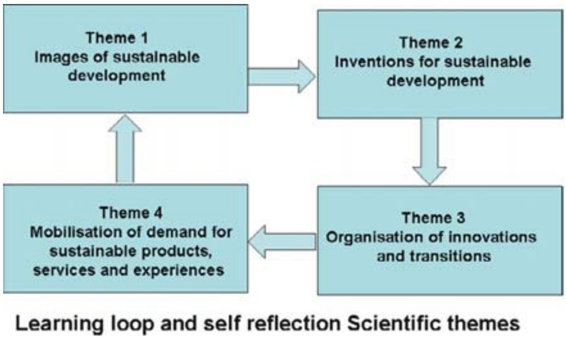

Fig. 3 TransForum's scientific program is organised in four themes following a cyclic innovation process which is constantly monitored. The cycle starts with (1) people's preferences and images, followed by (2) studies on which inventions are required to achieve a successful innovation. Subsequently, (3) it is investigated how to organize new innovations and transitions and finally, (4) how citizen/consumers behaviour and preferences mobilizes sustainable development, closing the loop. The *box* represents the fifth theme on self reflection providing the means to evaluate the effectiveness and efficiency of the learning system

Thirdly, the scientific programme is also aimed at testing TransForum's analytical framework. And following up on that, the assumption is tested that working along this framework will indeed lead to new value propositions and will indeed lead to the desired influence on current knowledge infrastructure. To focus the attention on these issues a fifth overarching theme in the scientific programme has been set up. The main aim of this fifth theme is self reflection and learning within the scientific program.

In these three ways, the scientific programme intents to look upon the issue of sustainable development and innovations in multiple ways:

- By the different emphasis in strategy on doing and learning
- By discerning between a number of different themes and research questions
- By working in multi- and interdisciplinary modes
- By striving towards an open-source approach by sharing knowledge and insights in a self reflection loop (Fig. 3)

2.3 Knowledge Development

Central in knowledge development is the way we learn. TransForum has organised the learning in a triple loop perspective. Single loop learning is done by directly serving of technical questions from the practice projects, given incremental improvement. The reflection in the double loop learning (Argyris 1994) is organised in the scientific program, causing reframing concepts and change in opinions and values. The triple-loop learning is organised in specific learning programs. Triple loop learning involves transforming who we are by creating a shift in our context or point of view about ourselves (Argyris 1999).

The learning programme is a combined learning and reflection programme. The focus is not on the classical building up and transferring knowledge, but rather on organising reflection and learning, developing the competencies and skills for innovation, and giving back and embedding these insights by means of learning arrangements and training programmes

3 Transforum's Scientific Program

The scientific programme was developed in 2005 and 2006. The aim was to develop a programme that was innovative in terms of scientific progress as well as societal usefulness. Knowledge issues arising from innovative practice projects form the basis for the agenda-setting of the scientific programme.

In several working sessions the scientific directors of TransForum have developed a thematic programme that enables the production of useful insights in the innovation process needed for a more sustainable development. A general observation in the practice projects was that the linear idea of innovation, where knowledge creation precedes valorisation, did not apply. In stead, the projects almost without exception demonstrated that knowledge creation and valorisation (or value creation) occur simultaneously in a cyclic non-linear innovation process. In this process, focussing on visions of sustainable development lead to inventions, inventions lead to innovations and innovations influence consumer behaviour. To close the loop this consumer behaviour influences the perception of issues related to sustainable development.

3.1 Science Process

Making the transition to more sustainable development of agriculture is a process. For scientists and farmers alike, this transition will require a series of small, realistic steps. It is important to realize that each small decision can contribute to advancing the entire system further on the "sustainable agriculture development continuum". The key to moving forward is the will to take the next step. Also TransForum learns by doing. Initially, TransForum was trying to give direct answers, such as improved designs, drafting new structures and methods. After organizing and executing several practice innovation projects the emphasis has shifted towards investigating the (boundary) conditions for more sustainable development. It is now the aim to promote the interaction between different types of knowledge, ensuring that this leads to joint innovations, and working to ensure people, organizations and systems get the knowledge and skills to work together and create innovations. In this way, TransForum intends to achieve more relevant, permanent results than it would achieve if it were to strive for technically substantive results which have been defined as sustainable on the basis of normative choices.

The quality of research is guaranteed through a classic scientific quality assessment system with external project and paper review. We are still developing a quality assessment system to evaluate the effectiveness of the research for the stakeholders in the practice projects.

3.2 Science Contents

A main scientific content requirement is that TransForum has to determine what it means with sustainable development. Within many different projects and its participants there are different concepts of sustainable development. Discussion about how sustainable a product or process is, often leads to a never ending debate on the "best" sustainable development indicators. This illustrates that the debate about sustainable development is normative and that the ultimate sustainable system doesn't exist. In order to stimulate more sustainable development of the agricultural sector TransForum investigates the generic dimensions of sustainable development that play a role in the expression of subjective preferences. This approach is phrased in hypothesis 1.

TransForum identified as generic dimension of sustainable development the resilience or adaptive capacity of the agricultural sector. Adaptive capacity in ecological systems is related to genetic diversity, biological diversity, and the heterogeneity of landscape mosaics (Carpenter et al. 2001). In social systems, the existence of humans, institutions and networks that learn and store knowledge and experience, create adaptive capacity in problem solving (Berkes et al. 2003; Lebel et al. 2006). Systems with high adaptive capacity are able to re-configure themselves without significant declines in crucial functions in relation to primary productivity, hydrological cycles, social relations and economic prosperity. A consequence of a loss of resilience, and therefore of adaptive capacity, is loss of opportunity, constrained options during periods of re-organisation and renewal, an inability of the system to change. Transitions are needed in order to allow social-ecological systems to emerge from an undesirable trajectory. Resilience is seen as the key to enhancing adaptive capacity and facilitates system transitions. Resilience reflects the degree to which a complex adaptive system is capable of self-organization, vs. lack of organization or organization forced by external factors, and the degree to which the system can build capacity for learning and adaptation (Carpenter et al. 2001). But self-organization is often constrained by institutional factors, particularly policies operating an National. supra national (EU) (e.g. CAP) and international levels (Cafiero 2007). By focussing on the generic dimensions of resilience and/or adaptive capacity TransForum expects it will be possible to depict and create alternative acting perspectives and value propositions how to deal with preferences instead of becoming part of the discussion (Anderies et al. 2004).

Innovation is the key to how actors in a coupled socio-ecological system, like the Dutch agricultural sector, can adapt. It is therefore essential to combine the insights and knowledge of innovation studies in the resilience concept (Newman 2005). Resilience and innovation are intrinsically linked. Only in a resilient system, change has the potential to create opportunity for development, novelty and innovation. Sustainable development is dynamic and needs to build on the notion of resilience. Innovation is essential to maintain resilience in changing environments where the future is unpredictable and surprises are likely.

Since adaptive learning is a central concept in the scientific program of TransForum the scientific themes are organised in a co-learning or co-production cycle (Martens 2006) (Fig. 3). Each theme is oriented towards answering questions which aim to help the KOMBI partners to achieve more sustainable development in practice by means of a causal learning loop. The box in Fig. 3 represents the fifth overarching theme on self reflection providing the means to evaluate the effectiveness and efficiency of the learning system.

Each theme has one or two main research questions:

- Theme 1: "Images of sustainable development": How to deal with the preferences of the various actors in the field, rather than normative statements about what sustainable development is or should be?
- Theme 2: "Inventions for sustainable development": What inventions are needed in order to make a breakthrough in the practical projects, and how to encourage the KOMBI partners to work towards this and deploy their knowledge to achieve it (= content and process aspects)?
- Theme 3: "Organisation of innovations and transitions": What encourages or hinders the cooperation of KOMBI partners?
- Theme 4: "Mobilisation of demand for sustainable products, services and experiences": How does the behaviour of citizens and consumers affect the likelihood of innovations being achieved? How can this behaviour be harnessed to promote sustainable development?

Knowledge issues arising from innovative practical projects are taken further in scientific projects. This is done with the aid of (multi)-disciplinary alliances. Only in this way new insights can be gained for solving practical problems. Every scientific project comes under one of the following themes:

3.2.1 Theme 1: Images of Sustainable Development

Goal: explore the subjective visions of sustainable development by introducing the concept of resilience (adaptive capacity).

In order to stimulate more sustainable development of the agricultural sector TransForum investigates the generic system dimension of resilience in the context of sustainable development. It is a main hypothesis that when an innovation leads to an increase in system resilience this will contribute to more sustainable development. In order to test this hypothesis theme 1 will translate the concept of resilience into:

- Triple P (People, Planet, Prosperity) context
- Different "currencies" of the main stakeholders (money, power, scientific standing and impact)
- Different scientific paradigms

Theme 1 will make an inventory of the current practical innovation projects answering the following questions:

- Which (un)sustainable development images are aimed for?
- Which dimensions are considered relevant/important for success?
- Which dimensions can be linked to resilience?
- Which dimensions are linked to the three innovation strategies of TransForum?

By making these steps the scientific theme "Images of sustainable development" will develop an operational sustainable development concept that will give entrepreneurs and other stakeholders inspiring and guiding action perspectives. At the level of the whole system it is expected that this operational concept will contribute to a more resilient agro-system. The complex adaptive system properties of the system imply that this can only be done in inter- and transdisciplinary research setting.

3.2.2 Theme 2: Inventions for Sustainable Development

Goal: Identify and stimulate inventions that will overcome the barriers towards sustainable development.

In the practical innovative projects of TransForum different innovations are implemented based on various images of sustainable development. The demanded changes often require new inventions. These inventions can be hardware, software or org-ware (organisations and institutions) oriented. This requires a clear articulation of the inventions desired on the one hand and on the other hand how to stimulate the development of such new inventions. It is important to emphasize that all available knowledge should be used and considered from high tech academic knowledge to tacit knowledge of entrepreneurs.

All inventions aim at solving sustainable development demands raised by ongoing practical projects. Development and implementation of inventions doesn't follow the linear top-down process of research, application and valorisation. Current institutional arrangements such as patents and breeding rights tend to obstruct joined development of new inventions.

Theme 2 will address the following research questions:

- Which type of inventions can contribute to breakthroughs in the three innovation strategies?
- How can inventions be stimulated which require participation of all KOMBI partners?
- How can we get effective access to all knowledge and experience of all relevant stakeholders?

3.2.3 Theme 3: Organisation of Innovations and Transitions

Goal: Developing conceptual scenarios for organizing innovations and transitions in the three innovation strategies.

System innovation can only happen when the KOMBI partners collaborate. In the real world this collaboration is not that self-evident. Why is this not the case? Which images, values, laws, rules, power distribution, etc., hampers this collaboration towards more sustainable development of the Dutch Agro-sector? Are these obstructions the same for all three innovation strategies? And when we know the obstruction are we able to define concrete action perspectives in order to resolve these innovation blockades?

Central in theme 3 is the "regime" concept. A regime is a system of systems, an interplay of structure, culture and approaches which lead towards a set of rules and resources which maintain the current status quo. Regimes can be influenced by developments in niches, which eventually lead to transitions of the regime (Stoker and Mossberger 1994). The main research questions of theme 3 are:

- What are the characteristics of the current regime which leads to self-enforcing of the current agricultural system?
- How does the current regime stimulate or obstruct the desired innovations toward more sustainable development within the three innovation strategies?
- What design criteria for a more innovative regime can we deduct from the current experience in the practical innovative projects?
- Which concrete action perspectives of the KOMBI partners lead to these design criteria?

3.2.4 Theme 4: Mobilisation of Demand for Sustainable Products, Services and Experiences

Goal: Understanding the triggers and barriers for the expression of individual social responsibility in actual purchasing and consumption behaviour with respect to sustainable development

Sustainable development is also stimulated by the demands of consumers and civilians. Civilians have a direct cumulative effect by setting political agendas that demand for example public goods such as a clean environment and a healthy working environment. But as consumers people make choices for certain products and services as offered by the market. Both types of demand are often not in agreement and have a strong impact on the achievability of sustainable development.

Within the three innovation strategies of TransForum different experimental projects are set up to study civilian and consumer demands. The final goal is to combine these two demands and address the issue of Individual Social Responsibility. Theme 4 investigates how the consumers can be more involved in the development of the individual social responsibility as a method to enhance sustainable development of the agro-sector. The main research questions are:

- How do stakeholders mobilize the consumer demand for sustainable development?
- How do stakeholders mobilize the civilian demand for sustainable development?
- Which unifying concepts can be developed to visualize consumer/civilian demands for sustainable development?
- Does the combination of the consumer and civilian approaches lead to a useful concept of "Individual Social Responsibility" which can contribute to more sustainable development?

4 Conclusion

TransForum aims to be a learning organisation. In order to learn (double loop learning) from our scientific themes and projects a Community of Scientific Practice (CSP) is organised (Bouma 2005). A CSP consists of a close, interacting team in which some do fundamental, applied or strategic research, while others participate in Communities of Practice (COP) at TransForum the practice projects, and still others focus on design, policy issues, management and communication. Interaction among team members leads to joint learning and, as a consequence, a more effective team effort. Team members will contribute their specific skills to education as needed.

The overall lines of the innovation program TransForum have been sketched. The ultimate goal is to stimulate transitions towards sustainable development of the Dutch agricultural knowledge infrastructure. TransForum is a learning reflective organisation, which means that we are willing reformulate our goals when there are sufficient indications to do so. Preliminary results from our practical projects indicate that most obstructions for innovations have an institutional character. Many current structures and regulations are hampering effective and quick changes in the current agro-sector, a finding not uncommon in Europe (Cafiero et al. 2007).

One important dimension currently not discussed in the research plan is the European and international dimension. More and more regulations and subsidies from the EU influence and affect the agro-sector and rural development. Preliminary studies have indicated (Verburg et al. 2006b) that these policies have potentially large impacts in the landscape development of the EU member states. It is the goal of TransForum to elaborate more on this international dimension. Internationally TransForum tries to seed similar initiatives in other settings and conditions. That is why TransForum has made a Memorandum of Understanding with KOMBI partners in Michigan (USA) and KOMBI partners in Shanghai (China) to stimulate similar transitions in global key countries for the agro-food sector.

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Spatialising Crop Models

Robert Faivre, Delphine Leenhardt, Marc Voltz, Marc Benoît, François Papy,
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Abstract There are many incentives for applying a crop model at a regional scale, i.e. over an area larger than that for which it has been developed. This is what we call "spatialising" a crop model. These large areas can have very heterogeneous soil, climate and management practices. Consequently, spatialising a crop model can pose serious problems. One set arises from the fact that the basic concepts, hypotheses and validity domains of crop models are derived at the plot scale and may not apply at a larger scale. Another set arises from the lack of adequate and sufficient data to run the model at a regional scale. The workshop held in Toulouse (France) on 14–15 January 2002 dealt with the topic of spatialising crop models. The present paper is a comprehensive summary of the thoughts we had before, during and after the workshop.

Keywords Crop modelling • Scale change • Spatial variability

Résumé De multiples raisons conduisent à utiliser les modèles de culture sur des surfaces supérieures à celles pour lesquelles ils ont été mis au point. C'est ce que nous désignons sous le terme de "spatialisation" des modèles de culture. Ces vastes étendues présentent de fortes hétérogénéités spatiales, relatives au sol, au climat et aux pratiques culturales. En conséquence la spatialisation pose un certain nombre de problèmes, qu'ils soient dus au fait que les concepts de base, les hypothèses et le domaine de validité des modèles de culture sont établis à l'échelle de la parcelle agricole et ne conviennent plus lorsque les modèles sont appliqués à une échelle régionale, ou au fait que les données d'entrée nécessaires manquent quand les surfaces considérées deviennent très vastes. Le séminaire de Toulouse des 14 et 15 Janvier 2002 a abordé ce thème de la spatialisation des modèles de culture. Le présent article est un résumé détaillé des réflexions conduites avant, pendant et après ce séminaire.

Mots clés Changement d'échelle • Modélisation des cultures • Variabilité spatiale

1 Introduction

The recent development of genetically modified organisms (GMO) which poses the problem of their dissemination, the new European Water Directive (2000) emphasising water quality, or climatic change and its impact on crop development, runoff and irrigation demand raise new scientific issues. The answers require in many cases the application of crop models at a regional scale, with concomitant large heterogeneities in soil, climate and management practices between fields. Using a crop model over areas larger than those over which it was developed is what we will call "spatialising the crop model". When all information needed by the model (input data, parameters) is available, this can be done quite easily from a computational point of view but it still raises questions that need to be answered. Some of them arise from the fact that the basic concepts, hypotheses and validity domains of crop

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models were derived at the plot scale and are too restrictive when applying the models at larger scales. Others arise from the lack of adequate and sufficient data to run the model at regional scales. To clarify and attempt to answer these questions, a workshop was held in Toulouse (France) on 14–15 January 2002 on the topic of spatialising crop models. The authors of the present paper are the scientific organisers of the workshop and the sessions chairmen. The present paper is a comprehensive summary of the thoughts we had before, during and after the workshop. In particular, it is based on a summary of concluding notes taken by the various session chairmen at the end of the workshop. The analysis in terms of scale change arisen from discussions held during the workshop, and continued after the workshop in a summer school entitled "for a good use of crop models" and organised by INRA at Le Croisic (France) on 14–18 October 2002.

In the next section, we first describe the main characteristics of crop models and we define what we call the scale of a crop model, pointing out on examples some specific questions that have to be answered before spatialising a crop model. In the third section, we summarise some spatialisation techniques that were presented during the workshop or in the literature. A presentation using the viewpoint of scale change is presented in the fourth section, before discussing alternative approaches as a conclusion to the paper.

2 Crop Model and Scale

2.1 Main Characteristics of Crop Models

Crop models are mathematical representations of the soil-plant-atmosphere system (SPA system), involving interactions between biological factors and environment (Hoogenboom, 2000). They calculate crop growth and yield, as well as the soil and plant water and nutrient balances, as a function of environmental conditions and crop management practices. The equations used in crop models represent the elementary processes of the SPA system. Three main modules or processes can then be identified. The soil module represents water transfer within the soil, which includes infiltration, drainage and redistribution (Leenhardt et al., 1995). The infiltration of water into the soil derives from the input of water, mainly by rainfall or irrigation, and results in a heterogeneous distribution of water in the soil profile. The soil module can also represent nutrient transfer, and specifically nitrogen transport and transformations (Brisson et al., 1998). The plant module describes two mechanisms: (a) the growth of the canopy, i.e. the production of biomass, based on interception and transformation of the photosynthetically active radiation and modulated by senescence, and (b) the development of the crop, that simulates the main stages of crop life (germination, flowering, production of seeds and senescence) and drives growth by organising, throughout development, the opening and closing of sinks, and by acting on sources (Brisson and Delécolle, 1991). The atmosphere module links the soil and plant modules. It represents evapotranspiration, which corresponds to two processes: (a) the evaporation from the soil and (b) plant transpiration or root extraction. These two processes can be simulated as a whole or separately. It allows interactions between the plant and the soil module: when water supply in the soil becomes limiting, the main physiological processes such as photosynthesis or leaf expansion are reduced, depending on the intensity of the stress. Infiltration, drainage, redistribution and evapotranspiration are generally assumed to be one-dimensional and vertical.

Mathematically speaking, crop models consist of a series of equations f. These equations contain parameters (or internal coefficients), θ, which, once the model has been calibrated, remain unchanged from one simulation to another. The models work with input data, v, variable in time (including weather data such as precipitation and temperature, and management practices such as dates and quantities of irrigation or fertilisation), or fixed, specific to the simulated crop (soil characteristics of the plot in question: soil type, soil depth cultivar or variety, etc.). They output variables y (yield, evolution of leaf area index or dry matter, water requirements, leached nitrate, etc.). One can thus adopt the following notation: $y = f(\theta, v)$. The parameters θ of a crop model can be numerous (ex: 26 for 2CV [Wallach et al., 2001] and more than 100 for STICS [Brisson et al., 1998]). Almost all process-based crop models (e.g. CERES [Jones and Kiniry, 1986], EPIC [Williams et al., 1989], CROPSYST [Stöckle et al., 1994], STICS [Brisson et al., 1998], SUCROS [Spitters et al., 1989]) are deterministic: differences in output variables y are only due to variations in input data.

Crop models suppose that the simulated plot is homogeneous as to input data: only one soil type, the same weather, the same agricultural practices (irrigation, fertilisation, ...) whatever the size of the plot. Usually, crop models are designed for a specific use and therefore parameters are estimated and calibrated on a sample of small plots. Furthermore they are validated in a limited number of conditions. However, in practice, these crop models are used on wider areas (for large plots) and often they are used to evaluate new practices (the potential of a particular cultivar in certain locations and so on). In precision agriculture, the same crop model is used when considering an inhomogeneous field plot. Thus we need to analyse the use of crop models on units or scales outside their domain of validity with respect to the hypotheses and the dedicated scale of the model.

2.2 Some Examples to Illustrate the Whys and Wherefores

Examples of the application of crop models to large and heterogeneous areas are numerous (cf. Table 1 in addition to the examples quoted by Hansen and Jones (2000), Russell and Van Gardingen (1997), or Hartkamp et al. (1999)). In most instances crop models are used not only to predict crop yields but also to estimate the impact of crop growth and management on the environment; especially on water resources or on the greenhouse effect. The ultimate objectives of crop model predictions are very diverse and depend on the end users targeted. For example, crop models are used for prognosis by managers (Leenhardt et al., 2004a), while administrative decision-makers use them rather for diagnostics, but also for tests of scenarios. Indeed, crop models used as tools for testing scenarios ("if ... this agricultural practice changes ... then ... this event occurs") are aids for crop system management and for policy analysis (Boote et al., 1996). Scenario testing uses hypothetical input data, but for diagnosis the crop model input data must correspond to an accurate description of reality (existing soils, past weather data, past agricultural practices). For prognosis, the use of crop models implies in addition some specification of future weather and practices.

In many applications, the aim is not only to study the spatial variation in crop model predictions between the fields of a simulation domain, but also to estimate global crop production and water and nutrient flows of the domain. Consequently, the simulation units cover essentially the whole region of interest. In general, the model is run independently from one simulation unit to the other, and thus the possible interactions between the simulation units, such as flows between the units, are not taken into account. The output data processing then simply of summing or averaging the predictions over the simulation area: average production (Donet et al., 2001), summation of local water consumption (Leenhardt et al., 2004a). However, when the spatial interactions are important and need to be considered, interfacing the crop model with a spatial model becomes necessary: for example, the coupling of a crop model with a hydrological model makes it possible to obtain the simulated result at the watershed outlet (Beaujouan et al., 2001; Gomez and Ledoux 2001). In some examples, simulation units are discontinuous: they do not cover the whole region. Then some kind of interpolation of the output data is required to obtain the information for the whole area (Sousa and Santos Pereira, 1999).

2.3 Characteristic Scales of Crop Modelling Applications

The characteristic scales of a crop model are both spatial and temporal. We will only define those relative to spatial aspects since the focus of this paper is on the spatialisation of crop models. We present below some definitions based in part on Bierkens et al. (2000), and then discuss related scale change issues.

The "extent" is the area concerned by the study: this can be a region, a watershed, an irrigated area or a farm. The extent is divided into a finite number of smaller areas called "support units". Information is collected on some or all support units. The "support" is defined by the total area covered by the observed units. The "coverage rate" (or coverage) is the ratio between the support and the extent. The term "resolution" should be used with precaution because it sometimes means "support" and sometimes "coverage rate".

Table 1 Some examples of use of crop models implying a change of scale

Reference	Spatial extent	Support unit for simulations	Treatments on inputs	Treatments on outputs	Type of results presented	Utilisation of the predictions	Temporal extent
Agricultural production							
Priya and Shibasaki (2001)	Sub-continent	10 km × 10 km or 50 km × 50 km cells	Generation of weather and slope data by downscaling; agricultural management data obtained at state level	None	Yield maps for different crop under interest : one value per cell	Test of scenarios	Decades
Donet et al. (2001)	Country	Spatial unit homogeneous for soil characteristics, climate and fodder practices	Soil characteristics derived from soil map; interpolation of climate data; regional sampling of fodder practices	Outputs averaged by fodder region and over the whole country	Fodder production maps : 1 value per fodder region or 1 value for the whole country	Diagnostic	Year
Faivre et al. (2000)	Region	Pixel (1 km^2)	Weather data from nearest meteorological station Soil data of predominant soil type from soil map Standard agricultural practices Crop area obtained from land use map Crop reflectance determined from disaggregation of remote-sensing data	Outputs integrated over each district or the whole region	Wheat production maps: 1 value per district or 1 value for the whole region	Prognostic	Crop cycle
Launay (2002)	Region	Spatial unit homogeneous for soil characteristics within each agricultural field	Weather data from nearest meteorological station Soil characteristics from soil map	Outputs integrated over each agricultural field	Sugar beet yield map: 1 value per field	Prognostic	Crop cycle

			Agricultural practices obtained from assimilation of remote-sensing information averaged on spatial unit				
Nicoullaud et al. (1999)	Field 20–100 ha	Spatial unit homogeneous for soil characteristics and agricultural practices	Soil characteristics and agricultural practices were mapped	None	Production maps: 1 value per plot	Test of scenarios	Crop cycle
Lal et al. (1993)	Areas of about 3,800 ha	Spatial units homogeneous for soil and climate	Weather data from nearest meteorological station Soil data derived from soil map	None	Yield maps for various crop and management combinations: 1 value per spatial unit	Test of scenarios	Crop cycle
Water quality							
Gomez and Ledoux (2001)	Large watershed 100,000 km^2	Spatial unit homogeneous for crop rotation, climate and soil	Weather data derived from a map of homogeneous meteorological zones Soil characteristics sampled in ramdom Distributions derived from a soil map Crop rotations randomly sampled in distributions, characteristic of each small agricultural region, and determined by mining data techniques	Outputs are used as upper limit conditions for hydrological models	Map : 1 value of leached nitrate flows per spatial unit; values of nitrate concentration at defined outlets	Diagnostic	Decades

(continued)

Table 1 (continued)

Reference	Spatial extent	Support unit for simulations	Treatments on inputs	Treatments on outputs	Type of results presented	Utilisation of the predictions	Temporal extent
Beaujouan et al. (2001)	Watershed (application to a virtual watershed of 0.64 km^2)	Regular cells (application to virtual 40 × 40 m cells)	not specified for soil, climate, crops and agricultural practices because theoretical application At each time step (day) lateral nitrate flows simulated by the hydrological model are inputs of the crop model	At each time step (day) vertical nitrate flows simulated by the crop model are inputs of the hydrological model	Map: one value per cell; one value at outlet	Test of scenarios	Crop cycle
Irrigation requirements							
Sousa and Santos Pereira (1999)	Region	Meteorological stations	Weather data measured at the meteorological station Typical values of soil data	Outputs interpolated over the whole region by kriging	Interpolated map of net irrigation water requirements for a potato crop	Diagnostic	Crop cycle
Heinemann et al., (2002)	Region	Spatial unit homogeneous for climate and soil, within a county (administrative stratification)	Weather data from nearest meteorological station Soil data derived from soil map Standard agricultural practices Crop area obtained for each county	Output of each unit affected to the centre of the unit, then interpolation over the whole area	Interpolated yield maps per crop : several values per spatial unit	Diagnostic	Crop cycle

Leenhardt et al. (2004a)	Irrigated perimeter 1,000–15,000 km^2	Spatial unit homogeneous for crop and climate	Crop and irrigated area determined from statistical surveys or remote-sensing Interpolation of climate data Sowing date calculated from meteorological data Irrigation practices determined by interviews of sampled farmers	Outputs aggregated over the whole area	1 value of irrigation consumption for the whole irrigated perimeter	Prognostic	Irrigation campaign (≅ crop cycle)

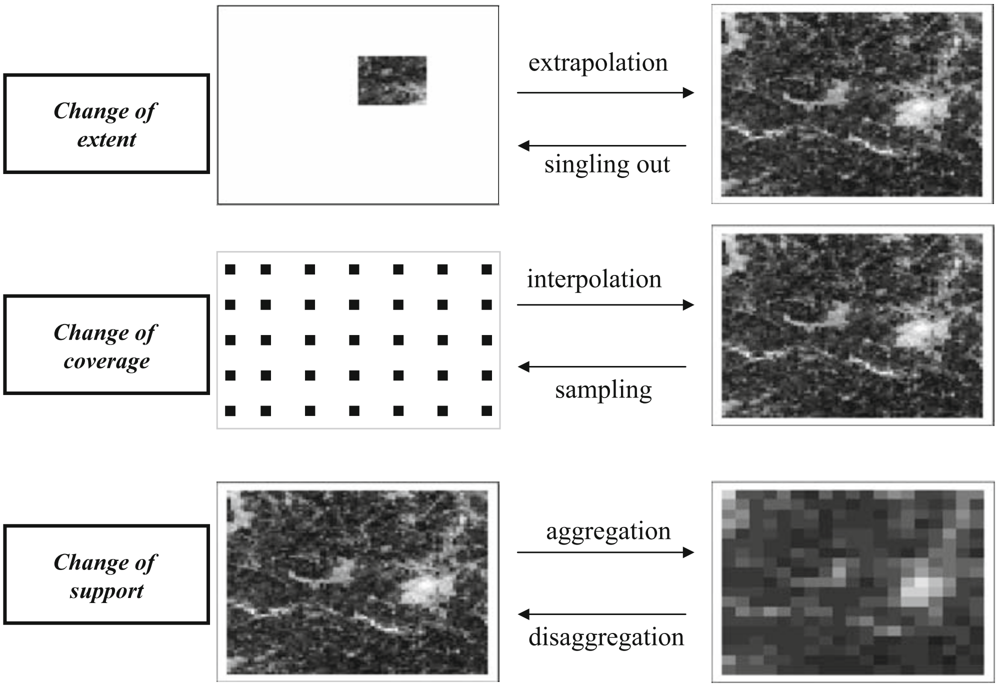

Fig. 1 Basic operations involving extent coverage and support (From Bierkens et al., 1997)

The term “scale” has a colloquial sense, in which “large scale” refers to large areas, and a cartographic sense, for which “large scale” would be associated with “high resolution” and therefore, very often to “small extent”. In the following we use the colloquial sense of scale.

When crop models are involved, the support units typically correspond to the simulation units (the field plot), i.e. the spatial units considered as homogeneous on which the model is applied to get simulated values.

Spatialisation of crop models needs to link different scales: for example, the scale at which the processes are described by the model, the scale at which input data or information (model parameters and input variables) must be available, the scale at which output results are expected or sought. Thus, spatialisation often requires some kind of change of scale, for example from the scale of validity of the model to the support unit of the model predictions or from the support unit to the extent.

Often instead of “scale change” one talks of “upscaling” (or “downscaling”). Nevertheless, for Bierkens et al. (2000), “upscaling” specifically means increasing the support, which we refer to as “aggregation” (and “downscaling” by “disaggregation”) (Fig. 1). On the other hand, expressions such as “a crop model is scaled-up from the field to the regional scale” (Russel and Van Gardingen, 1997) associates “upscaling” to increase in extent.

Often crop models are calibrated at the field scale and then used to estimate the evolution of some variables for this field. In this instance, the field is both the extent and the support of the study. When these models are used to make decisions at a regional level (for example to map nitrate leaching), the extent of the study is no longer the field. The input data, at least in part, can no longer be determined for each field of the region. It is then often supposed that many fields have the same characteristics and therefore that the same input data can be used to run the crop model for all of them. The support unit can then be considered as a set of fields, although the measurement unit of input data remains unchanged. The scale change here corresponds to the passage from a small support unit (a field) to a bigger one (a set of fields). This is the aggregation or “upscaling”, and this change of support is accompanied by a change of extent (from the field to the region). On the other hand, the scale change involved in using a crop model for precision agriculture corresponds only to a change of support (one passes from a field to a homogeneous zone within the field), without any change of extent (which remains the field). Thus spatialisation of a crop model is linked to scale change analysis.

Scale change corresponds to two opposite problems, the passage from a local to a global scale and vice versa. When the modelling scale changes, it may imply both a change in the scale of data observations (input data, output data or "validation" data), and a change in the structure of the modelling approach. The latter change is well known in the field of hydrodynamics, where water flow is described by the Navier-Stokes equations at the soil pore scale, and by Darcy's equation at the scale of the soil column. Regarding specifically crop models, the equations of the crop models are established on elementary surfaces (plots of the order of one m^2) or even under controlled conditions at the laboratory. But often crop modelling is seeked at the field scale. In this instance, there is a change of modelling scale. Most approaches assume that the structure of the model can remain similar and that it is possible to estimate effective values of the model parameters at the upper scale. In practice, the parameters of the model are estimated by calibrating the model with data observed at the scale of an agricultural field. In applications over a region, there is no such calibration step because the objective is not to erase the heterogeneity of the region. Similarly, in precision farming, homogeneous zones within the field are identified in order to take into account the within-field variability. The model can be applied to each of these zones. The models are then kept unchanged, and the scale change concerns mainly the input and/or output data.

3 Main Aspects of Spatialisation Methods

Assuming that the natural spatial scale of a crop model is a field plot and that the extent of the study is a collection of field plots (a drainage basin, an administrative region, . . .), spatialisation and/or spatial aggregation can be situated at three levels: (a) determining input data, (b) accounting for the interactions between field plots and (c) evaluating the results. The first point deals with the problem of being able to simulate crop development for all individual fields. It requires the input data specific to each simulated field. Because crop models do not take into account specific processes concerning a larger scale than the field, the second point involves coupling the crop model with a model that explicitly simulates the spatial determinism of some processes like, for example, those involved in a watershed hydrological functioning. An other solution is to control or update the simulated values using observations of the entire region, with satellite data for example. Because crop models are validated at the field plot level, the third point concerns the problem of evaluation of the spatialisation process when the results concern a large area.

3.1 Determining Input Data Throughout the Extent

As a simplified representation of the soil-plant-atmosphere system, a crop model refers to a locally limited environment (local scale of the homogeneous field) and in particular soil and climate conditions and crop management. In the case of the application of such a model at the scale of a heterogeneous field, a farm, a region or a country, the environment of the SPA system becomes variable, not only in time but also in space. The soil varies in depth, texture, and slope, climate, in particular rainfall, is variable and finally management practices (soil tillage, irrigation, fertilisation, choice of cultivar, etc.) also vary. It has been shown that the validity of crop model predictions, summed or averaged over a region, depends on the quality of the representation of the spatial variability of the input data (Hansen and Jones, 2000). Thus moving from a homogeneous field to a larger scale or to a heterogeneous field requires incorporating additional environmental heterogeneity.

Two main types of input data can be distinguished. The first includes the environment characteristics. They are essential since the basis of a crop model is to represent the interactions between biological factors and environment. A second type of data includes the technical details of management practices.

3.1.1 Environmental Data

The major environmental data necessary for running crop models are climatic variables (temperatures, precipitation, radiation, potential evapotranspiration) and soil properties. In general these quantities are not known everywhere and at every scale. They are measured or estimated on given spatial supports (soil profiles, meteorological stations) at a limited number of sites within the study area. For crop modelling purposes it is therefore necessary to estimate their values at the scale of every simulation units within the simulation area. This implies some kind of spatial estimation. Various techniques and methods have been developed and applied for soil and climate data. They are very diverse and may be distinguished by the kind of model of spatial variation they are based on. Three groups of approaches may be roughly distinguished.

- A first group of methods is based on models of spatial variation that can be classified as traditional and that do not consider random components. This is the case of many traditional choropleth mapping methods based on terrain inventories and surveys which define and map classes of soils, vegetation, climates and assume that the property of interest is best estimated by the class mean at all sites within a given class. Examples of choropleth mapping are the classical soil mapping techniques of which a review was proposed by Legros (1996). Other techniques such as Thiessen polygons, trend analysis or arbitrarily weighted averages of data also belong to this first type of methods. They have been extensively used for mapping soil and climate variables (e.g. Creutin and Obled, 1982; Laslett et al., 1987).
- A second group of methods assumes statistical models of spatial variation. The most well known set of methods of this kind are the geostatistical methods. They are well described in several textbooks, as for example those by Journel and Huijbregts (1978) Webster and Oliver (1990) and Goovaerts (1997). Their main advantages are to provide sound spatial estimates from a statistical point of view (unbiasedness and minimum variance), to take into account the spatial dependence between the data and to propose an estimate of the prediction error. The most popular of the geostatiscal methods is kriging whose predictor of a property at a given site is no more than a weighted average of the observed values at neighbouring sites. Several forms or extensions of kriging have been developed to cope with different kind of variables: continuous and categorical, with normal, log-normal and undefined density distributions. In many applications to soil and climate variables, geostatistical methods have been shown to perform better than most other methods (e.g. Creutin and Obled, 1982) for rainfall mapping (Voltz et al., 1990); and (Van Meirvenne et al., 1994) for soil texture mapping). They best apply to variables that exhibit stationary and continuous spatial variations. But in the case of discontinuous spatial variations, their performance was shown to be poorer (e.g. Voltz et al., 1990).
- The third group of methods relies on process-based models of spatial variation. In this case, the spatial estimation of a variable is made by simulating the processes that control the variable. For example, the simulation of soil formation at the landscape scale can provide with a prediction of the actual spatial variation of soil properties (e.g. Minasny and McBratney, 2001). Atmospheric 3D modelling that accounts for lateral energy fluxes between fields can be used to predict the spatial variation of the local atmospheric boundary conditions of a crop model (e.g. Courault et al., 2003). But the development of this kind of process-based approaches is still at a very early stage and cannot be considered operational yet for providing input data to crop models.

In many situations, the number of available measurements of soil and climate input data is very small and insufficient to allow for accurate spatial predictions over the simulation area whatever the performance of the mapping method used. This is so especially because of the large costs involved in measuring these data. Consequently, several approaches have been developed to investigate whether surrogate data, that are already available in existing data bases or easily measurable at high spatial densities, can help in spatially predicting the variation of the required soil and climate input data to environmental models. They are of two kinds. The first one corresponds to the development of empirical or theoretical models that use the surrogate data at a site to predict the data of interest at the same site. This enables to increase the spatial set of data for subsequent mapping. Examples of this are pedotransfer functions that use basic soil data from soil surveys, including soil morphology, soil texture, structure, organic matter content, etc., to predict more difficult to measure soil data like soil water retention curves or soil hydraulic conductivity. A review of pedotransfer functions is available in McBratney et al. (2002). Although these functions provide only approximate results, they are often used at regional scales to parameterise crop models (e.g. Donet et al., 2001; Leenhardt, 1995). Another example is the assimilation of remote sensing data in a 1D model describing the transfers between soil-vegetation-atmosphere (SVAT) for estimating the local climatic data taking into account the effect of land-use (Courault et al., 1998). The second approach is to use the information about the spatial structure of the surrogate data to improve the spatial estimation of the variables of interest. Several techniques exist; they are most often extensions of the spatial estimation methods described above. For example, the AURELHY method (Benichou and Le Breton, 1987) improves the spatial estimation of precipitation by taking into account the landscape relief through a multivariate analysis. Similarly, Monestiez et al. (2001) used a special form of kriging, namely kriging with an external drift, to account for local environmental conditions of the measurement sites in the mapping of air temperature. A last example is the use of outputs of large scale numerical weather prediction (NWP) to take into account the weather types when mapping precipitation (Bardossy and Plate, 1992; Merlier, 1997). The use of surrogate data, especially remote sensing data, for overcoming the lack of required input data when running environmental models is a very promising.

A last issue when mapping soil and climate input data is the problem of change of support when the measurement support unit differs from the crop model support unit (simulation unit). Because the measurement units are generally smaller than the simulation units, the problem is the upscaling of the observed or mapped input data. This requires some knowledge about the way the variables can be averaged in space, which most often raises difficult theoretical problems. For example, how calculating the mean temperature over a squared kilometer when land use heterogeneities occur? It is a problem of upscaling by aggregation. But in some cases, the change of scale can also be the other way round (downscaling), particularly when the simulation area is very large. For example, to obtain predictions at the Indian sub-continental scale, Priya and Shibasaki (2001) estimated the necessary local information (climate, slope on a 1 km grid) by using information available at a wider scale (meteorological stations of the national network, digital terrain model with a large grid), using a purely statistical approach.

Finally, it is important to stress that, whatever the method used, the fact of estimating model input data throughout the extent introduces errors in the model inputs, as illustrated by several examples reported by Hansen and Jones (2000) and Russell and Van Gardingen (1997). One example (Russel and Van Gardingen, 1997) concerns climatic zoning that can be used to determine weather data at various locations of a region. Weather data from the reference meteorological station of each zone are used for sites located within the zone. If the zoning is drawn up for cereals, it may not be adapted to other crops (forage, for example): (a) because conditions after cereal harvest have not been taken into account in the classification, although they influence forage growth and (b) because the choice of the representative meteorological station is based on the spatial distribution of cereal crops, which may be different from that of forage because of different climatic requirements. Errors in the model inputs are likely to be propagated to outputs (Leenhardt and Voltz, 2002; Leenhardt et al., 1994).

3.1.2 Management Data

Management data include crop species, variety, sowing date and density, irrigation, fertilisation and possibly crop protection and soil tillage information. They are discontinuously distributed in space. In fact, it is the spatial distribution of management practices that determines the boundaries of the fields. The management data also vary from year to year. In a given field, different crops follow one another. Finally, management data result from decisions taken at different scales, including the farm, the cooperative, the collective organisation for irrigation, etc.. At each of these levels, management decisions for to the various fields are interdependent (Biarnès et al., 2004; Papy, 2001).

This complexity is the reason that spatial representations of management data are rare. Often one simply uses values corresponding to typical or recommended practices to run the models (Donet et al., 2001; Hansen and Jones, 2000). Nevertheless, it is very important to distinguish irrigated zones from non-irrigated ones, to include the distribution of sowing dates and the range of varieties used in a region. If the spatial distribution of crops within the considered area has no effect on the simulated process, and if the analysis of the determining factors suggests it, it is possible to simply distribute the management practices in space according to a law of probability (Moen et al., 1994). This approach was adopted by Leenhardt and Lemaire (2002) for the sowing dates and by Mignolet et al. (2003) for crop rotations. These two studies combine segmentation of the geographic space and sampling from probability distributions.

An alternative approach to collect information regarding agricultural practices relies on the use of remote-sensing. Historically, remote sensing has widely been used to obtain land use maps, which provide a description of the spatial distribution of crops. More recently, it has provided a means to estimate the exhaustive distribution of techniques difficult to observe because of their transient nature (e.g. sowing date, nitrogen applications) and their cost of acquisition. The principle consists in re-estimating parameters (and/or input data). Example is given for sowing and emergence dates by Guérif and Duke (2000) and Launay (2002), where they compare LAI values simulated by the crop model and the outputs of a reflectance model applied to the remote-sensing data.

3.2 Accounting the Interactions Between Field Plots

Scale change implies the consideration of new processes and properties, emerging at the scale considered and revealed by the extension of the system considered. Such emerging processes or properties influence the SPA system but are not represented by the models developed at the local scale for homogeneous field. These processes can concern physical transfers between neighbouring units, including: water transfer between fields, pathogen propagation, weed or GMO diffusion, etc.. The interactions between fields can also result from the multiplicity of actors in a region and from the decisions they make. They arise because, at this scale, human and economic sub-systems cannot be neglected. For example, at the scale of an irrigated area, the water resource must be allocated between farmers. At the farm scale water allocation but also other management decisions are interrelated between fields due to the constraints of labour and equipment. Thus, when a model, developed at the local scale, is used at a larger scale, the results become even more error prone because they do not take into account the phenomena appropriate for this scale.

Although the two approaches presented hereafter can provide solutions, the difficulty of spatialising crop models when strong spatial interactions exist between fields must be emphasised. When runoff or propagation of pathogens are considered, the relative locations of fields, as well as their sizes, is essential. For example, in an area where the proportion of different crops are fixed runoff will be different depending on the sizes of the fields. This will also affect biological diversity. The spatial structure gives to the system properties that cannot be directly accounted for by the crop models.

To account for spatial interaction between fields, the most natural approach is to interface the crop model with a model that represents these spatial interactions. Nevertheless such an interface is not without difficulties. First, it requires an exchange of data between the two models at each time step during the simulation process, while existing crop models are generally structured to simulate directly an entire growing season (Beaujouan et al., 2001). Second, at larger scales (farm, watershed, region), several crops are concerned, while crop models are generally developed for one species. Embedding crop models into models of three-dimensional hydrology, intercrop competition or farm operations would require restructuring the models so that different crops could be simulated in parallel and that information exchange could be possible at each time step of simulation between the models. Although this is possible, the difficulty of reorganising model code and the need to repeat the exercise after each model revision suggests that combined models of these higher-level systems will not be sustainable without a commitment on the part of the crop modelling community to develop and maintain an appropriate modular structure (Hansen and Jones, 2000).

An indirect approach to account for interactions between field plots consists in accounting for the spatial variability of the model state variables at particular moments of the crop cycle, rather than modelling explicitly spatial interactions. Injecting remote-sensing information into the model is the most common way to do this. This refers to data assimilation reviewed by Pellenq and Boulet (2004). But here, the information is used to force the crop model to be consistent with the observed data over the course of the growing season (Faivre et al., 2000). Data assimilation implies that communication between the data and the model occurs during the course of the simulation (that is during the course of the crop cycle), which poses computer problems similar to those evoked above. However, this technique does not require a complete reorganisation of the model.

3.3 Evaluating the Simulated Results

When a crop model is applied to a large area, the overall precision of the predictions results not only from the quality of the model itself, but also from the quality of the methods of acquisition of the input data, of the choice of the units of simulation, and of the calibration of the model.

In the previous paragraphs, we underlined the importance of errors affecting input data because of the necessity of involving estimation methods over large areas. It is also important to note that these methods (pedotransfert functions, interpolation methods, remote sensing assimilation data or sampling from distributions without any spatial constraint) can generate a spatial structuration of crop model output prediction errors.

Evaluation of the overall results is possible in principle but is often problematic in practice. The most common problem is the quality of the so-called "validation" data. These data are observed data, but generally their reliability can be questioned (as it is the case for the input data), which reduces the pertinence of the comparison of observed and simulated data. For example, to validate the ISOP model which estimates grass production for every forage region, Rabaud and Ruget (2002) used estimations of production made a posteriori by local experts. There arises thus a problem of reliability of data (varying from one expert to another, depending on its own memory). Similarly, Faivre et al. (2000) simulated wheat production but, for validation, they had only at their disposal statistics of the part of the production collected for commercialisation. In addition, they have a problem of discordance between spatial units: simulations are performed for 1 km^2 support units (satellite pixels), while validation data are available on a communal basis. Besides, to estimate the quality of forecasts of total water demand for irrigation in a region, Leenhardt et al. (2004b) use data relative to farmers who subscribe to a collective irrigation system, while in the study area, there are also farmers who irrigate from their own reservoirs. There is therefore a discordance between the simulations (relative to the whole extent of the study area) and the observations (corresponding to that part of the area cultivated by farmers who irrigate from a collective system). Furthermore, within these "collective" irrigators, some receive water from collective pumping plants whose daily volume can be obtained, while others use individual pumps and only the total volume to the end of the campaign is available. Thus none of the validation datasets covers by itself the full spatial extent considered, that is the whole irrigated region including both collective and individual irrigators, nor the full temporal extent, that is the entire irrigation campaign with a daily time step. Actually, the term "evaluation" is more appropriate than "validation". Rather than evaluating spatialised models by comparing predictions with imprecise observations, it is possible to evaluate them by considering if the objective is attained. In particular, does the model allow one to make decisions correctly? For example, Leenhardt et al. (2004b) verified, for a past year for which water management decisions failed, that a regional irrigation demand prediction model was able to predict the delay in irrigation demand that was the cause of wrong decisions. The model would then have been able to allow the water manager to anticipate the difficulties and would have helped him to make better decisions by improving the estimation of the remaining potential irrigation demand.

A global evaluation gives an idea of the reliability of the results, but does not indicate how to improve them since the sources of error are not identified. To go further, it would be possible to study each individual source of error and its propagation through the model. The contribution of each type of error to the total error of the simulation result could then be identified. This type of analysis would also make it possible to choose the most adequate method of data acquisition taking into account the effect of error in each type of data on the precision of the results. Analytical methods of decomposition and of propagation of error exist for linear models (cf. for example Heuvelink et al., 1989). For crop models, which are strongly nonlinear, these methods do not apply. The procedure then becomes very complex. Indeed, no complete analysis of sources of error and of their propagation has been conducted for spatial applications of crop models. Nevertheless, the procedure has been illustrated by Leenhardt and Voltz (2002) for one kind of crop model input data, namely soil water properties. The aim was to choose the method of acquisition of such data, that is what resolution for the soil map, what estimator of the water properties within the mapping units? A more complete approach but without specific application to crop models is given by Crosetto et al. (2000) and by Tarantola et al. (2000). They propose an application of uncertainty and sensibility analyses to GIS-based models in order to estimate the precision needed for the various types of data. The objective is to obtain results with a precision within limits acceptable to the user, thus allowing decision-making.

4 With Regard to Scale Change

Since extent and support are not at the same scales (homogeneous plot for the latter region for the former), analysis in terms of scale change seems to be a good means for presenting the approaches of spatialisation of crop models. The decision-tree proposed by Bierkens et al. (2000) (Fig. 2) can be a good support for discussing the different studies presented in the previous section.

One of the most common strategies is to look for exhaustive information all over the extent at the crop model support unit level (field) in order to simulated everywhere and, then, to aggregate results to obtain an information at the extent scale. This methodology corresponds to class 1 of the decision-tree. It is represented in Fig. 3, way A, corresponding to a "calculate first, average later" strategy as mentioned by Bierkens et al. (2000).

In Section "Environmental Data", we described the methods for spatialising environmental input data, with a first view of the problem of scale change. These methods mainly correspond to a change of coverage (see Fig. 1) where the most common methods are interpolation (Fig. 3, way A_u). For characterising the soil typology at the necessary crop model support unit, one needs to proceed, from the original databases, to different scale change modes (Carré, 2003, ways A_d or A_u).

When technical input data are involved (see Section "Management Data"), interpolation methods are inadequate when local information concerning management is available. When the coverage rate of these technical information is not high enough, the use of assimilation technique of remote sensing data allows the modeller to estimate actual technical input data as Guérif and Duke (2000) for the emergence dates, which corresponds to class 1 of the decision-tree (Fig. 2). In general, local information concerning management is not available. Representative management practices are used in the concern of spatialising crop model. This corresponds to class 2 in the decision tree.

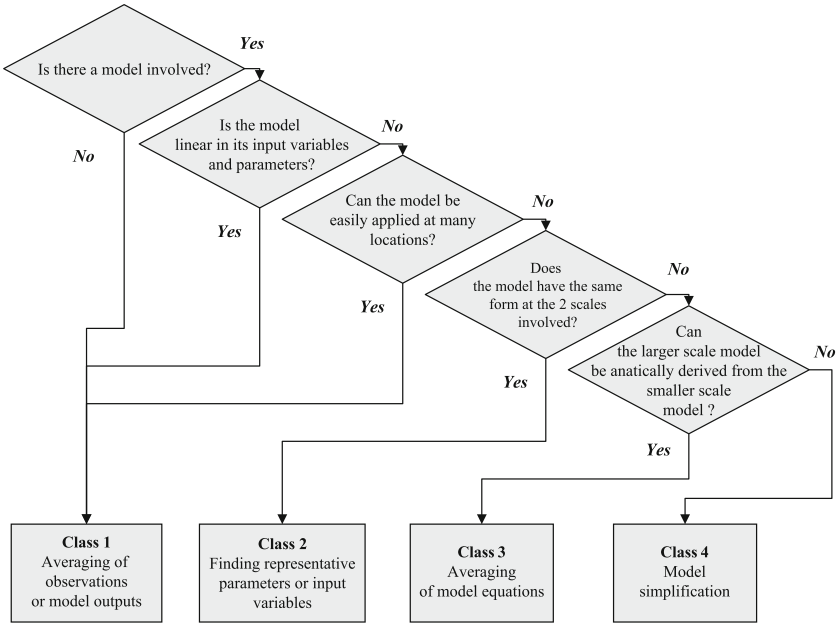

Fig. 2 Decision tree with four major classes of upscaling methods (From Bierkens et al., 1997)

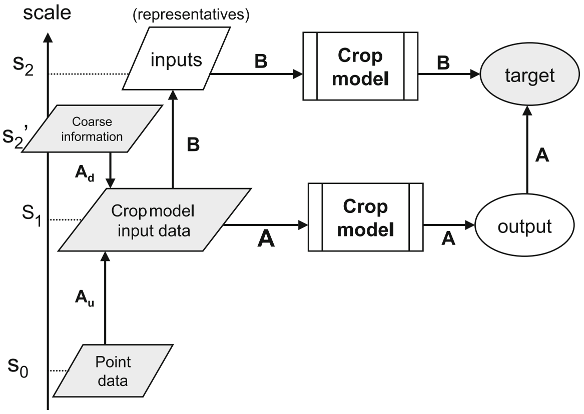

Fig. 3 Strategies for upscaling a crop model: upscaling outputs (way A), upscaling inputs first (way B)

The meaning of "easily" in the question of Bierkens et al. (2000) ("Can the model be easily applied at many locations?"), should be clarified. In the case of technical input data, it is numerically easy to apply the model but information is missing. Depending on other input data, two main strategies are used. If environmental data are also classified in different soil types, generally no spatialisation is performed (Donet et al., 2001): all combinations of soil types and management types are simulated at the crop model scale and upscaled by weighting the results of each combination (Fig. 3 way A). If environmental input data are spatialised, i.e. if available for all the plots of the extent, the recommended management practice is used for all plots (Faivre et al., 2000), i.e. scale change does only concern environmental input data while technical input data is constant over the extent. An alternative could be to spatialise management data by drawing at random from a known distribution of practices (generally available at an agricultural administrative region [see Leenhardt and Lemaire, 2002; Mignolet et al., 2003]); such a strategy should be used when spatial dependencies exists between plots (e.g. water flux between field plots).

Strategies presented above consist in spatialising input data for all the support units of the extent, then in running the crop model for all these support units and finally in aggregating the outputs over the extent. Another strategy, implying strong hypotheses, consists in applying the model at some support units and then in extending the results to the extent by some method of interpolation (Fig. 1, Change of coverage). This is the approach implemented by Sousa and Pereira (Sousa and Santos Pereira, 1999) to get water requirements for potatoes for a region. The outputs obtained by simulation at the locations of meteorological stations were then extended by spatial interpolation using kriging. Interpolation is based on the assumption that outputs are spatially structured (often varying continuously in space). In this application, water requirements are assumed to depend on climatic factors only. However, in most applications of crop model, such an assumption is not realistic: as noticed before, agricultural practices vary in space discontinuously with no known spatial structure. Therefore there is no reason that outputs vary continuously.

We presented above the spatialisation of crop model input data in order to predict outputs for all crop model support units. It could be also possible to spatialise crop model state variables. The same hypotheses of spatial structuration are necessary. More, spatialising intermediary (state) variables is very time consuming. Assimilation from exhautive information over the extent is a means of overcoming these problems. They allow to update some of the simulated state variables in the course of the simulation. It is another method of assimilation, different from that used by Guérif and Duke (2000) which estimate input data only.

Scale change is often necessary due to a gap between observed data support unit and crop model support unit. We are in the same configuration as in Fig. 3, but replacing "crop model input data" by "crop model state variables". Faivre et al. (2000) are concerned with scale change to match the support unit of observed data and the crop model support unit. They first unmixed data to recover the specific value of the considered crops (Faivre and Fischer, 1997): at the coarse satellite data of 1 km^2 support unit (pixel), data consists in aggregated values of different types of crops. The scale change (way A_d in Fig. 3) is done when the average value of the observed data at the pixel is affected to each field (crop model support unit).

Another problem relative to scale change is the spatial adequation between evaluation data and crop model outputs over the extent. In Rabaud and Ruget (2002), the validation is performed on the same support; validation data and output data are aggregated over the same extent. In Faivre et al. (2000), validation data is available at an intermediary support unit (an administrative communal support unit), lower than the extent (region) but larger than the crop model support unit (field). Here, for communal and regional evaluations, a change of support by aggregation is necessary. In Leenhardt et al. (2004b), there is a difference of coverage: validation is based on a sample of the extent (Fig. 1, change of coverage).

In term of scale change, all works relative to spatialisation of crop model concern only data, but never the model itself. Besides, simulations are always performed at the scale of the crop model (way A in Fig. 3), never at the scale of the target (way B in Fig. 3). Consequently input data spatialisation methods (cf. A_u et A_d in Fig. 3) and the upscaling methods of output data should benefit of the scale change point of view. Therefore, the general principles of change scale methods can be useful.

The specificity of crop model spatialisation is that, most often, two scale changes occur: one on the input data, the other on the outputs. Input data scale change can be downscaling or upscaling. Generally output data scale change is upscaling only (by aggregation to have a global information). The common strategy consisting in simulating everywhere should be chosen either because its better efficacy or because it respects spatial structurations that are difficult to account for differently. A way to check the effective opportunity of this strategy would be to compare simulated and observed spatial variations. This would need information over the whole extent at the support unit scale, which is rarely the case. An alternative would be to disaggregate global information (often concerning the entire extent) taking into account some spatial dependence model. This is specifically addressed in the downscaling methods issues.

5 By Way of Conclusion

The "spatialisation of crop models" has been implemented in a number of applications. Different techniques have been used but it appears that there is a lack of analysis of the methods and strategies and of the requirements.

Spatialising crop models require large amount of geographical information. This is why Heinemann et al. (2002) and Nicoullaud et al. (1999), for example, coupled crop models to a geographical information system (GIS). We can see, with this analysis in term of scale change, that this need in data can be decreased. A first solution is to use spatialisation techniques such as interpolation methods. Another one is to consider another strategy, such as the way B described in Fig. 3. This new strategy would consist in calculating a mean temperature or using representative types and then simulate with these synthetic data. This is possible because a crop model has no dimension: it simulates the outputs (for example, the mean yield) per unit of area, that is, it can be used whatever the support unit of input data. In fact, the processes represented in a crop model (and assumed to be exact) are considered as being applicable only for an homogeneous support unit of the size of a field plot.

A crop model is developed for an homogeneous simulation unit, generally the field plot. It takes into account only the process that are significant at such scale (the field). When the crop model is used on a larger extent, we have to deal with emerging processes, for example fluxes between fields. In this case, it is possible to interface the crop model with another kind of model: hydrological model to account for lateral water flows (Beaujouan et al., 2001), farm system model to account for constraints due to work organisation, etc. Contrary to what proposed Bierkens et al. (2000) (class 4 on Fig. 2), the crop model is complexified. The corresponding question in the decision-tree should be: "shall we continue to apply the crop model as it is or shall we create a new model in which the crop model is only a sub-part ?" An alternative to complexification is the use of assimilation, as noticed in §4. The proposition of Bierkens et al. (2000), that is, simplifying the crop model, is in fact never considered.

Despite there exist a number of applications of crop model spatialisation, there is a crucial lack of operational and transferable tools adapted to this problem. Efforts exist but are not coordinated. In most examples, the interfaces are partial and not automated, which does not make the tool easily transferable to other researchers. These studies are often specific to one application that is, they consider one particular model, they use one particular set of data from one study site, they address one specific question. In order to a general spatialisation approach, one idea would be to combine the different points of view in terms of processes to be modelled, which implies having a multidisciplinary team working at and observing the same study site. The existence of a common study site where researchers of different disciplines would work could be a good opportunity (a) to test advanced techniques,

(b) to evaluate the impact on predictions of different sources of error (corresponding to different methods of input data acquisition), (c) to study the techniques that should be combined for developing decision support systems. The recent creation of long term observation experiments by research organisations in France (the Environnemental Research Observatories, “ORE”, and the regional working zones, “zones atelier”) could provide the opportunity for achieving progress in crop model spatialisation. These studies would need to be viewed through the prism of the scale change analysis.

All the scientific questions evoked here are under study and in the present paper we attempt to provide a framework for analysing the spatialisation of crop models. To that extent, this paper is to be considered as a contribution to the great debate concerning the use of crop and more generally vegetation models at a large scale.

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Iterative Design and Evaluation of Rule-Based Cropping Systems: Methodology and Case Studies – A Review

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Abstract The economic and regulatory context of crop production changes rapidly, but concerns about agricultural sustainability, including environmental impacts, are increasing steadily. To cope with complexity and uncertainty, innovative methodologies are required for designing, managing and evaluating prototype cropping systems. A generic approach combining iteratively design of cropping systems and evaluation of their performances is presented in this review article. It includes five main steps: (1) defining the set of goals and constraints for each cropping system, (2) identifying a suitable agronomic strategy, (3) formulating the consistent set of technical decision rules, (4) applying and evaluating the rule-based system, and (5) validating or refining the strategy and the rules. This methodology was applied to a range of environmental and production contexts, in a perspective of integrated crop production (ICP) prototyping. Three cropping system experiments conducted in France were brought together to demonstrate the potentialities of this system approach and discuss the methodological bottlenecks to address. The three case studies differed by the context of crop production and resource use: adaptation to limited irrigation water (Toulouse), introduction of innovative cropping systems (Versailles), and substitution of herbicides by non-chemical methods (Dijon). The consequences of the specific objectives in each case study on the experimental design and the evaluation process were discussed. Special attention was paid to the time step of the evaluation process, the duration of the improvement loops when prototyping cropping systems, the global evaluation of the systems and the evaluation of individual decision rules.

Keywords Agronomic evaluation • Decision rules • Integrated cropping systems • Long-term experiment

1 Introduction

The huge diversity of soil, climatic, agronomic, economic and social contexts in an uncertain world requires a wide range of cropping systems characterised by local adaptation and flexibility in technical decision-making (Meynard and Girardin 1991; Boiffin et al. 2001). For this reason, standard technical packages, ready to use in practice, are no longer appropriate in the current agricultural context.

To develop sustainable cropping systems, several objectives have to be considered, apart from yield or gross margin. For instance, new evaluation criteria define the technological and sanitary quality of harvested crops and require adherence to environmental norms, e.g. air and water pollutants, energy use, and the simplification of crop management systems, e.g. reduction of labour input, staggering of field operations. Sustainable cropping systems should accept strong environmental constraints such as limited water resource management, reduced reliance on pesticides, decreasing emissions of greenhouse gases, and conservation of biodiversity. Consequently, under low-input management, limiting factors and sub-optimal yields are expected, in contrast to intensive production

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systems. To mitigate yield decreases and compensate for the reduction in pesticide use, biological control should be promoted (Altieri 1995; Posner et al. 1995; Meynard et al. 2003). Positive interactions between cultural practices are sought and the potential benefits of crop rotation are promoted as much as possible. Sustaining a balance in biological, physical and chemical fertility is identified as a strategy for medium- to long-term management. Based on these principles, the concept of Integrated Crop Production (ICP) was proposed as a reasonable trade-off between profitability and environmental protection (El Titi 1992; Frangenberg 2000). For instance, according to Integrated Pest Management (IPM) principles, several technical options are suggested to control disease inocula or the weed seed bank in soil, although none of them is as effective as the best chemical programme. Only a combination of techniques, each giving partial control, could replace chemical protection, and such a combination might involve major changes in the nature of the cropping system (Mortensen et al. 2000). Other environmental objectives, such as the reduction of energy costs and erosion risks, and the improvement of soil biodiversity and carbon storage might induce major changes in the whole cropping management system (sowing dates, cultivars, crop protection). Mulch-based cropping systems with direct seeding into a cover crop have been suggested as an innovation likely to solve some problems associated with crop production (Scopel et al. 2005). However, the consistency of the whole cropping system and the interactions between the techniques should be carefully considered at the beginning of the innovation process. The prototyping and evaluation of innovative cropping systems require specific methodologies accounting for this complexity.

Empiricism, on-farm surveys and field experimentation have long been the main methods used by agronomists to develop and evaluate cropping systems (Drinkwater 2002). The literature abounds in long-term trials comparing the effects of crop rotations, mineral or organic fertilisation regimes and soil tillage programmes on yield, economic return, and biotic and abiotic components of soil fertility (e.g. Varvel 1994; Johnston 1997; Soane and Ball 1998; Richter et al. 2007). These trials are generally of factorial design, with pre-determined sequences of crop operations. They suffer from a major defect: systems differing by only one technique (for instance, soil tillage) are compared without checking the consistency of the whole crop management system. For example, in experiments comparing the effects of different soil tillage methods, sowing date seldom differs among them, although the optimal sowing date should probably be different for direct sowing and deep ploughing, for trafficability reasons (Buhler 1992).

From the late 1980s, environmental concerns began to grow and experiments were set up to evaluate production systems less dependent upon fertilisers and pesticides, either at field or farm level. In European networks (Holland et al. 1994; Jordan et al. 1997; Vereijken 1997; Korsaeth and Eltun 2000), but also in the USA (Poudel et al. 2002; Reganold et al. 2001), the conventional approaches were compared with innovative systems, either called "organic", "ecological", "integrated" or "low-input". Cropping systems were compared for their ability to reach predefined objectives (production level, gross margin, input level, environmental impacts) whilst meeting labour and input level constraints (e.g. Capillon and Fleury 1986; Debaeke and Hilaire 1997).

Studies on the farmer's decision processes clearly indicated that his reasoning could be represented by the concept of decision (or action) rules (Sebillotte and Soler 1988; Chatelin et al. 1993; Leroy et al. 1997; Aubry et al. 1998). Usually a rule is made up of (1) a function which links the decision to the targets or the constraints, (2) a solution which displays the possible actions as a function of the context in conditional form ("If… then… ; else"), and (3) an evaluation criterion to check whether the objectives were reached or not (Reau et al. 1996). Decision rules are applied on the basis of soil or plant indicators which are clearly formalised and accessible to the decision-maker (such as water and nitrogen balances, and visual records of diseases and weeds from crop inspections).

The consequence is that the cropping system is not simply defined by a logical and consistent sequence of crops and technical operations but results from the application of decision rules (including crop choice) depending on environmental factors and working organisation constraints (Papy 2001).

From an experimental point of view, the cropping system research moved from the comparison of "crop management sequences", where some technical operations were fixed (because their effect on production was studied) and the others were decided by the trial manager, to the evaluation of "crop management systems" where the decision rules were formulated explicitly and became the main objects of evaluation (Reau et al. 1996). Using the concept of decision rules, the agronomist can thus account for the flexibility of cultural decisions and crop choices as a function of agronomic and economic indicators. Technical operations (N fertilisation, irrigation and crop protection) are decided with reference to plant and soil status. This is a major change in the methodology of cropping system experimentation either for management, evaluation or extension.

The experimental approach at field level (from several hundreds of m^2 to several hectares) could be extended by a micro-farm evaluation where different management methods are compared in parallel on a practical scale, simplifying both the evaluation of the feasibility and the dissemination of innovation (Vereijken 1986, 1992; Viaux et al. 1994).

Meynard et al. (1996) considered several testing levels for the evaluation of cropping systems: (1) a global multi-criteria comprehensive evaluation level, to test if the management system fits generally with the assigned objectives (economic, environmental), on the basis of data collected at harvest or during the cropping season; (2) an evaluation of agronomic strategies, which consists of testing the validity of the assumptions which were formulated to design the cropping system; and (3) an analytical evaluation of single decision rules which may result in very detailed studies. In practice, these three levels of evaluation are combined in a cropping system experiment, and their relative weight depends on the specific objectives of the study. An important feature, however, is that data collected for decision-making should be clearly distinct from those used for evaluation; otherwise the decision-making process, based on extra information usually not available for farmers, would be biased. The ideal solution would be that the people in charge of decision-making would be different from those doing the evaluation.

The aim of this paper is to highlight and discuss how a common methodology for prototyping and testing cropping systems in field experiments (Nolot and Debaeke 2003; Debaeke et al. 2006; Lançon et al. 2007) was applied to different sets of objectives and constraints, in relation to different research priorities in the context of integrated and sustainable crop production. The report is based on three cropping system experiments carried out by INRA in France during the last 10 years. The case studies were chosen because they share some methodological aspects while illustrating (1) the range of management options for conducting the experiment and (2) the range of potential outputs.

The main shared features of the three experiments are presented first, before developing the objectives and a brief description of the experimental design for each site. Then the specificities of each experiment are discussed, considering among others the time step of the evaluation process, the duration of the improvement loops in the iterative design of cropping system prototypes, the global evaluation of the systems and the evaluation of individual decision rules. Detailed results cannot be given in this summary paper; therefore the reader is invited to consult the following references: Nolot and Debaeke (2003), and Debaeke et al., (2005, 2006) for the Toulouse experiment, Bertrand et al. (2005a,b) for the Versailles experiment, and Munier-Jolain et al. (2004) for the Dijon experiment.

2 A Common Approach of the Rule-Based Cropping System Experiments

The three experiments were designed according to the five main steps described by Nolot and Debaeke (2003), combining iteratively design and evaluation phases, namely: (1) defining the set of goals and constraints for each cropping system, (2) identifying a suitable agronomic strategy, (3) formulating a consistent set of decision rules in accordance with the strategy, (4) applying and evaluating the rule-based system, and (5) validating or refining the strategy and the rules.

The experimental designs were composed of large plots allowing rational use of farm machinery. The plot size (from 0.5 to 2 ha) is justified by the dispersal of pests and the limitation of neighbourhood effects between adjacent cropping systems. The large size of the experimental unit limits the number of replications possible. It is our opinion that, unlike in factorial trials, the objective is not to demonstrate statistically the effects of single factors nor to compare the relative performances of the different systems, but to evaluate how often the expected result is obtained. Hence, the purpose of replications differs between factorial and system experiments. In a system experiment, a sufficient number of plots is required to estimate the probability of obtaining the expected result whatever the system. However, the size of plots is not guided by work organisation concerns (labour peaks, or conflicts between operations when soil and weather conditions dictate priorities for field operations) because a typical field experiment is not suited to answering such questions. The consequences of applying innovative systems on a field scale to farm organisation should be explored by other means, such as models (Vocanson 2006).

In the three case studies, the main objective of the system experiments was not to deliver one or several cropping systems directly applicable by farmers. The experiment demonstrated the feasibility of systems resulting from the application of decision rules, derived from the agronomic knowledge at a given time. Its purpose was not to look for the best cropping system: as the tested systems were defined according to different sets of objectives and constraints, their performances could not be compared on the basis of any common objective. Instead, the purpose was to test how frequently the relative or absolute objective assigned to the cropping system was reached. The evaluation criteria were the gross margin, the grain quality, the energy balance, the amount of resources used (water, other inputs), the labour use and the environmental impact on air, soil and water.

All three experiments addressed the issue of the environmental impacts of crop production, and especially the public's desire to reduce pesticide use (Aubertot et al. 2005). The reduction of the reliance on pesticides is a question arising in most European countries, where environmental concerns are increasing steadily; water quality is to be maintained or improved according to the 2000 Water Framework Directive, which aims at achieving a "good" water quality for all water across the European Union by 2015. However, the relative contribution of the sustainability components in the evaluation of cropping systems was different between the case studies, while addressing the trade-off between contradictory objectives was a common concern.

3 Application to Three Case Studies: Objectives, Treatments and Layouts of the Corresponding "Cropping System" Experiments

3.1 The Toulouse Experiment (1995–2002)

A range of three agronomic contexts (A, B and C) were defined as a function of both the amount of water available for irrigation and the labour available for field work (Nolot and Debaeke 2003; Debaeke et al. 2005, 2006). In system A, defined as productive yet environmentally-friendly, labour and water resources were not limited: up to 240 mm water was applied to summer crops (maize, soybean). Such a system is adopted in the valleys and terraces in south-western France by farms with 80 ha per full-time worker, where cereal and oil-protein crops are the major sources of income and where the environment (water quality) is a major concern. In system B, water and labour were both limited: a maximal rate of 120 mm was allocated to summer crops and there was one full-time worker per 160 ha, with crops as the main source of income, as in system A. The challenge was to optimise the ratio between the use of limited resources and the level of crop production. System C had no irrigation, and labour availability was restricted, corresponding to a farm with one part-time worker looking for a system easy to manage and robust.

For each level of water and labour availability, a range of species and cultivars was available and a yield goal was fixed, which formed a rational basis for calculating input rates. In system A, the highest possible yield and/or high crop quality were expected. The most productive crops and cultivars under full irrigation were used – generally late-maturing varieties. Water, nitrogen and plant protection requirements were satisfied at the highest level but not at an insurance level (for environmental and economic reasons). The crops grown were maize, soybean, spring pea and durum wheat. In system B, crops requiring low inputs were selected in order to reduce labour use, water consumption and crop management costs. For that reason, tolerance to diseases and low water and nitrogen requirements were the basis of choices, resulting in the growing of sorghum (instead of maize), sunflower (instead of soybean), winter pea (instead of spring pea) and durum wheat (as a winter crop). Reduced vegetative growth was expected from limited plant densities, reduced nitrogen fertilisation and reduced pre-anthesis irrigation in order to restrict leaf area index and prevent excessive water demand and disease development. Temporary N deficiencies were tolerated because reaching the maximum possible yield was not the goal. In system C, crop rationing was combined with escape strategies, by choosing a crop rotation reducing the risks of weeds, pests and diseases. The same crops as in B were chosen, with moderate water and N requirements or tolerant of unsatisfied demand (sorghum–sunflower–pea or faba bean–durum or soft wheat), but N amounts and crop densities were reduced, decreasing the risk of diseases. At the same time, the soil fertility was preserved over the medium to long term (as phosphorus, organic matter, soil structure and weed seed banks).

The experiment was located at the INRA-Auzeville experimental farm near Toulouse (43.62°N, 1.45°E) and started in 1995. The experiment had 24 plots covering an area of 33 ha: 12 plots in a fixed rotation (R) with all the crops of the rotation being present every year and 12 plots in a flexible system (F), where the crop choice depended on agronomic considerations, such as soil structure, residual mineral nitrogen and weed infestation, together with economic considerations (i.e. the target gross margin). During the 8 years of the experiment, durum wheat was grown each year on the three flexible systems, while soybean was predominant in the A and B systems, and sunflower in C. The rotation (two summer crops followed by two winter crops) was fixed as regards the management of weeds, nitrogen and soil-borne diseases.

3.2 The Versailles Experiment (1999–)

The general objective of this experiment was to suggest and evaluate profitable cropping systems in accordance with technical and environmental concerns in the context of cereal cropping in the Parisian Basin. Four cropping systems were tested in this experiment, that included deeply innovative techniques and strategies:

- "High Production": This system was close to the current regional practice. The crop rotation was unchanged since the beginning of the trial in autumn 1998: oilseed rape-winter wheat-spring pea-winter wheat. The yield should only be restricted by soil and climate local conditions without any other limiting factors. The most effective inputs were used in order to reach the potential yield. Preventive sprayings were used against pests (insurance strategy). Crop cultivars were chosen according to their yield productivity and stability in the region, and, for wheat, according to high bread-making characteristics. The soil structure was preserved in order to maximise root development, and water and nutrient uptake. The fields were mouldboard-ploughed each year except after pea. After pea they were ploughed only occasionally when the soil structure was damaged.
- "Low Input": The crop rotation was the same, but the objective was to minimise environmental damage by reducing the chemical and mineral inputs while retaining a gross margin similar to the "High Production" system. The yield losses were compensated for by saving inputs because of reduced pest attacks with lower nitrogen and plant density. Reduced yield goals were fixed, and the pest management operations were decided when a damage threshold was reached. The pesticides were chosen according to both their environmental impacts and their efficacy/cost ratios. The varieties were chosen for their disease resistance, and wheat for its bread-making characteristics. Ploughing was done every two years before oilseed rape and pea, both to restore the soil structure and to prevent weed and disease attacks.
- "Direct seeding mulch-based": Crop management differed from the previous system only by the absence of soil tillage. To protect the soil surface, a permanent cover was maintained with commercial crops and/or cover crops (in association with the main crop or as catch crops). The crop rotation was maize–wheat–pea–wheat. The commercial crop was direct-seeded under the cover crop (living mulch) after a chemical desiccation or a partial restriction of the cover by either cutting or the application of a herbicide at low dose.
- "Organic": This system was applied following organic farming specifications: no mineral fertiliser, no synthetic chemicals. Wheat was grown every two years. Crop protection against pathogens was based on the use of resistant varieties and on escape strategies (delayed sowing date). Weed control was based on mechanical weeding (harrowing or hoeing). Legumes were used as green manures to enhance the

soil nitrogen resource, and top-dressed organic fertilisers (guano, feather meal) were applied sparingly because of their prohibitive cost.

The experiment (8 ha) was located in Versailles (48.81°N, 2.14°E), on a deep loamy well-drained soil (17% clay in surface, 30% in depth), prone to soil crusting but highly productive. The experimental design was composed of two blocks, divided into four plots corresponding to the four cropping systems. Each plot was divided into two sub-plots corresponding to shifted sequences of the rotation, in order to have a wheat crop present each year in each system. Sub-plots were 0.5 ha in area, which gave the opportunity to include several sub-trials (e.g. alternative variety, control strip with no fungicide, control strip with no nitrogen).

3.3 The Dijon Experiment (2000–)

The main goal of the cropping system experiment in Dijon was to evaluate the performance of prototypes of cropping systems based on the principles of Integrated Weed Management (IWM). The experimental design was composed of five cropping systems (CS): a reference system, close to the local practices, and four systems, differing in their objectives and constraints, but all of them incorporating IWM principles (Munier-Jolain et al. 2004). These principles are based on diversified crop rotation, suitable soil tillage tools, false seedbed preparation, sowing dates chosen to escape weed emergence periods, weed-suppressing cultivars, mechanical weeding, and herbicide decisions optimising the trade-off between efficacy and environmental impact.

CS1 – Local standard: the objective was to maximise the economic profitability. Weed control relied mainly on chemicals. The crop rotation (oilseed rape / winter wheat / winter barley) resulted primarily from economic considerations.

CS2 – IWM, Minimum tillage. This cropping system mimicked large farms with a small workforce, and therefore excluded time-consuming operations, such as ploughing and mechanical weeding. The crop rotation was diversified by introducing a spring crop (spring barley) and a summer crop (soybean) with oilseed rape and several winter cereals within a 6-year rotation.

CS3 – IWM, No mechanical weeding: all the principles of IWM (including the diversified rotation as in CS2) were applied except mechanical weeding, which was considered time-consuming and inappropriate on some farms.

CS4 – IWM, with mechanical weeding: in this system, all the principles of IWM were used, including mechanical weeding, and chemical control was restricted to situations where the combination of prophylactic methods and mechanical weeding did not succeed in controlling weeds. In crops grown in wide rows such as sugar beet, which was introduced as a summer crop in the rotation, herbicides were sprayed only on the rows, while hoeing was used in between rows.

CS5 – Zero herbicide: only non-chemical weed control methods were accepted in this system.

The environmental impacts of herbicides were expected to decrease from CS1 to CS5. Most of the decision rules were driven by weed management but should not increase the development of animal pests and diseases. Economic thresholds were used to trigger pesticide applications against animal pests and diseases.

The experiment was set up at the INRA-Epoisses experimental farm near Dijon (eastern central France, 47.33°N, 5.03°E) on a productive and drained clay soil (35–45% clay). The experiment was composed of two blocks of five 2-ha fields about 1 km apart. Consequently, the five systems were replicated twice. The decision rules were the same in the two blocks, but did not always result in the same management, as weed flora may vary between the blocks as a result of cropping history. Crop rotations were not fixed at the beginning of the experiment but resulted from a combination of rotational principles to prevent the seed-set of some specific weeds, the ability of some crops to be mechanically weeded, and the weed composition of the field.

The main characteristics of the three cropping system experiments are summarised and compared in Table 1. The three experiments took account of a range of concerns regarding regional potentialities but all aimed at reducing the use of inputs in a context of integrated crop production (ICP).

Table 1 Main characteristics of the three cropping system experimental

	Toulouse	Versailles	Dijon
Objectives			
Agronomic	Adaptation to variable irrigation availabilities	Feasibility and sustainability of innovative systems	Long-term weed control in IWM systems
Environmental	Optimising water use, minimising N leaching	Minimising N leaching, minimising the use of pesticides	Minimising the use of herbicides
Economic (GM: Gross Margin)	Maximising GM, minimising labour time	GM equal to conventional	No GM objective assigned to systems: the experiment makes it possible to evaluate the cost of IWM
Constraints			
General	Summer and winter crops	Wheat every 2 years	
Specific to a system	Irrigation availability	Direct seeding in mulches Organic Low input	Minimum tillage, with or without mechanical weeding, with or without herbicide.
Agronomic strategy	Diversified rotation, crop canopy rationing, stress escape	Spray or pests and diseases escape	Diversified rotation, soil tillage, false seedbed and competitive crop canopy
Rule building	Simulation + regional expertise + local references (factorial trial)	Expert knowledge Experimental references	Simulation + expertise + experimental references + decision support system
Degree of rule explanation	+ + + for nitrogen, water, cultivar choice + other decisions	Complete	+ + + for weed management + other decisions
Lay-out	Plot size = 1.5 ha 4 replicates each crop, each year 1 fixed rotation trial (12 plots) + 1 flexible trial (12 p)	Plot size = 0.5 ha 2 replicates wheat each year Other crops: every 2 years	Plot size = 2 ha 2 replicates 1 crop per year
Evaluation			
Global	Agronomical, environmental (water use, nitrate, pesticide use), gross margin, labour	Agronomical, environmental (nitrate, pesticides, energy, earthworms), gross margin, labour	Weed control, physical soil fertility, environmental (herbicides, other pesticides, energy, GGE,[a] nitrate), gross margin
Intermediate states of the systems	Disease reduction, weeds, water saving	Numerous	Canopy competitiveness
Rules	Agronomic diagnosis + factorial trials: varieties, fungicides, plant density	Agronomic diagnosis + check plots	Check plots
Major revisions of systems and rules during the prototyping	Revision of thresholds (N fertilisation, irrigation) Crop changes in low-input system (less durum wheat, less faba bean)	Crop changes in the organic system (less oilseed rape, more alfalfa)	Increasing the proportion of legumes in the rotation

[a] GGE: Greenhouse gas emission

4 Specific Methodological Choices in Each Experiment

4.1 Toulouse Experiment

The Toulouse experiment was the oldest of the three presented here. The methodology itself was a great concern for the scientific team supervising the experiment and was one of the addressed issues. The main focus was the flexibility of the cropping systems according to the agricultural context, defined by the economic objectives, the regulatory context and the availability of water and labour. The tested systems were not regarded as particularly innovative, as they were defined according to sets of objectives and constraints really existing for farmers in the Toulouse region. A practical case study (adaptation to irrigation availability), covering a large part of the agricultural context of south-western France, was chosen to initiate a generic approach associating methods to design, manage and evaluate cropping systems. However, the nature of the 4-year rotations, which were not practised by the farmers, was a response to the need for diversification in Integrated Crop Production.

The evaluation was mainly focused on the potential to optimise the cropping systems using a set of scientific knowledge- and expertise-based decision rules; so much attention was given to the formulation and evaluation of the decision rules and to developing new decision support tools (models and indicators) in order to put the rules into action. Although it was practically impossible to predict all the possible events which might affect decisions, the tested systems were consistent because most of the technical operations were based on rules.

Every rule was composed of an objective (its justification), a solution and an evaluation criterion (Table 2). Some rules were simple ones, written according to the following syntax: "if plant or soil status is greater, equal to or lesser than [threshold], then [action 1: sow, fertilise, spray, irrigate…], else [action 2: wait, withdraw…]". However, some decisions about water and nitrogen supply were too complex to be summed up by such a rule. In such cases a decision support system specifically designed for the experiment was necessary to produce a good decision. Irrigation scheduling in systems A and B resulted from the application of a multi-species water balance model (Bil-H) (Nolot and Debaeke 2003). On each irrigated plot, a water balance (Precipitation + Irrigation +/− Ą Soil Water Storage − Drainage − Evaporation − Transpiration) was run on a weekly step. The model estimated a satisfaction rate for crop water requirement (Ta/To: ratio between actual and potential transpiration, which was a function of soil volume colonised by roots and of easily transferable water). The rule was summarised by a curve of Ta/To plotted against thermal time, which fixed the threshold below which irrigation should be triggered. When irrigation was triggered, the amount of water needed depended on the soil water content. The curve changed according to both the crop species and the level of crop rationing which was desired. Weather forecasts were used to bring forward irrigation in the case of wind or to defer it if rain was expected. Bil-H gave a dynamic estimate of Ta/To over the growing season which was useful for characterising water stress

Table 2 Examples of simple decision rules

Topic (experiment location)	Rule objective	Rule formulation	Evaluation criteria
Weed management (Dijon)	Reduction of potential weed emergence in autumn for winter cereals through: + escaping the periods of weed emergence + false seedbed	*If* autumn-emerging weeds were observed during the previous years … *then* shallow tillage (5 cm) end of Sept.–early Oct., and from 25 Oct., shallow tillage with a goose-foot tine cultivator (5 cm) and cereal sowing as soon as possible	– Working depth of each tool – For autumn-emerging weeds, density on 24 Oct. > density "end of winter" – Weed growth stage in early winter < cereal stage (no plants emerging before 25 Oct. should survive after cereal sowing)
Sowing density (Toulouse)	Choosing sowing density according to yield goal, varietal earliness, sowing date and seedbed structure	Varying sowing density around a standard for each crop (system A): ± 10% per 100 GDD before or after the optimal sowing + correction factors for yield goal, cultivar earliness and expected seed loss	– Counting plant emergence – Factorial trial with 3 density levels – Diagnosis of the factors responsible for non-optimal plant density
Disease management in wheat (Versailles)	Reduction of the risk of foliar disease in wheat crop	Low density rate and late sowing (15 Oct.) Mixture of 4 wheat cultivars with complementary susceptibility profiles to foliar diseases	– Comparing sprayed and unsprayed sub-plots – Disease assessment on the 3 last expanded leaves 300 GDD after anthesis

GDD Growing degree-days

in rainfed crops and discussing the differences between actual and recommended irrigation schedules. As Bil-H was connected to Bil-N, a model to decide on optimal N fertilisation, a dynamic estimate of N leached was provided for the assessment of environmental impacts (Nolot and Debaeke 2001).

During the course of the field experiment, the sets of rules piloting the three cropping systems were continuously improved. Most modifications were minor, because most of the knowledge supporting the rules had been available since the beginning of the experiment. Some rules were based on results of simulations using the EPIC-Phase crop model: the choice of varieties was based on crop phenology prediction for a range of cultivars. Debaeke et al. (2006) showed that early cultivars of sorghum should be grown under rainfed management, while late-maturing ones were recommended to be irrigated. Other rules were based on the application of common budget models, calibrated by real-time observations on the crop canopy (for N fertilisation, irrigation), or were based on regional data updated each year (variety choice, crop protection thresholds). However, these data are only available for conventional systems, and therefore they might have to be adapted for innovative low-input cropping systems and integrated pest management. “Grey” knowledge and individual or collective expertise were needed to design some decision rules such as, for example, the adaptation of sowing density as a function of sowing date and water availability (Table 2), or the adaptation of crop rotations to enhance natural regulation of pests and diseases and reduce the need for pesticides. However, analytical tests within the experimental design made it possible to check the validity of these decision rules by comparing variants on small plots.

Minor changes in the decision thresholds were introduced if enough evidence was found for these modifications. However, sometimes, the whole rule had to be drastically altered or the decision thresholds had to be changed to cope with changes in the socio-economic or technical context, or simply because the intermediate objectives had not been reached. We identified two situations where the initial rule (rule structure or thresholds) had to be modified: (1) a given rule could be updated due to the technical (for instance, new cultivars with innovative traits) or economic context (for instance, crop prices, input costs); however, the annual evaluation of a given rule could lead to growing the same cultivar or crop species for 2–3 years for agronomic evaluation; (2) some components of the cropping system were changed when they obviously did not suit the chosen objectives. For example, substituting durum wheat by bread wheat in low-input systems was required because of poor grain quality. Only the introduction of a new durum wheat cultivar with a high protein concentration despite a low N fertility level could have resulted in a return of durum wheat to the low-input system.

Some decision thresholds were index-linked to the price environment. The relationship between the threshold value and the economic context defined a meta-rule, i.e. a rule for adjusting the parameters of a function used for decision-making. The irrigation thresholds changed with the crop type but also with the crop price, for a given water cost (Fig. 1). If the crop price was high, irrigation was triggered for higher

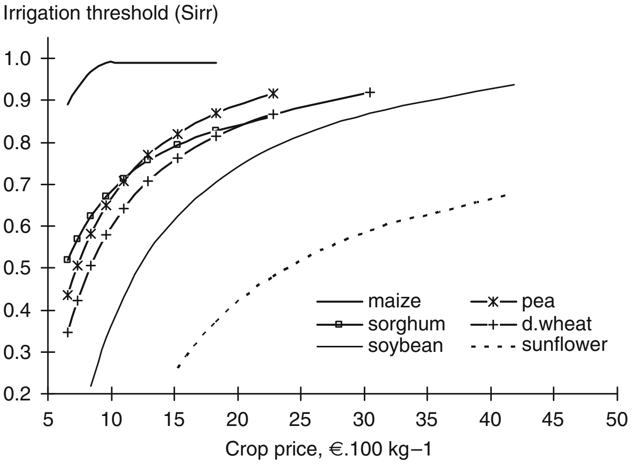

Fig. 1 An example of rule improvement. Type (1): evolution of the irrigation-triggering threshold (Sirr = Ta/To) as a function of crop prices for six major crops in Toulouse; water cost was fixed at 1.35 € mm^{-1}

levels of soil water content. Enough knowledge was available to build operational relationships between decisions and the economic context.

These decision tools were the main outputs of the experiment, along with evidence demonstrating their ability to optimise management at the cropping system level in contrasting situations. However, the data collected also clearly demonstrated that a significant reduction in inputs (when correctly decided) may not reduce the net income in the economic context of the late 1990s (Nolot and Debaeke 2003), while improving indicators of environmental impact. The evaluation of the economic return of the three cropping systems accounted for the year to year variability of yields and input levels due to the variability in the weather. As the considered variables (water balance, air-borne leaf diseases, crop yields) were only slightly affected by long-term processes (except long-term soil N availability as affected by the cropping system), the 8-year duration of the experiment was mainly justified by the analysis of the effects of weather on the rule sensitivity and on the likelihood of reaching the production targets.

As the evaluation was usually done annually, increasing the number of years for system testing improved the evaluation by allowing a risk assessment to be included. The experimental design also included all the phases of the rotation each year, which helped in interpreting the inter-annual variability (Cady 1991). This was possible only because the number of systems and the rotation length were limited: 12 plots were necessary for this objective (3 systems × 1 rotation × 4 terms). The replication applied to the set of decision rules which generated the cropping systems, but not to the annual choices which were produced by the application of the rules. The rules resulted in a range of different sequences of technique implementation, because of differences in the soil status and the weather.

In this experiment, considerable effort was put into agronomic diagnosis, which progressively added to the knowledge supporting the decision rules. The within-field heterogeneity was used for this purpose by considering several areas for crop and soil monitoring within a field of 1–2 ha. Each field included different areas dedicated to different evaluation levels: (1) evaluation on the field scale (1.5 ha), where the experiment manager applied the decision rules and evaluated the results with simple methods (*Were the decision rules feasible? Did the final results reach the expected targets?*); (2) evaluation using data collected from six to nine agronomic georeferenced stations (100 m^2) for agronomic diagnosis (time-course of leaf area index, above-ground biomass, N uptake, yield components, weed population and disease damage); and (3) evaluation using results from an analytical area (one-third of the field area, up to 500 plots of 10 m^2) where alternative management options (variety, plant population, crop protection, and their major interactions) were tested. This experimental layout was possible only because of the large size of the fields and because variety trials were included within the fields; in return, these variety trials benefited from the diversity of cropping systems and the environmental characterisation.

4.2 Versailles Experiment

Unlike the Toulouse experiment, the cropping systems tested in the Versailles experiment were not chosen according to constraints currently encountered by actual farms. The purpose was to design new cropping systems to cope with likely future constraints to crop production, such as reducing the release of pesticides into the environment and improving the energy balance of cropping systems. The extension of organic farming in areas with cereal-based cropping systems was also considered. Innovative techniques in the region of the Parisian Basin, such as direct crop seeding under a permanent living mulch in a zero-tillage system, were introduced in the cropping system experiment. As initial knowledge was lacking at the beginning of the experiment for some of the systems to be tested, namely mulch-based, Integrated and Organic without any cattle manure, the sets of decision rules were continuously improved during the experiment. In this approach, the experiment is part of the cropping system prototype design, that included several improvement loops that were used to modify the rule-sets towards a system satisfying the requirements and the objectives (Fig. 2). This iterative approach was similar to problem-solving methods used in industrial production chains for quality control and continuous improvement, such as the well-known Plan-Do-Check-Act (PDCA) cycle (Meynard and Savini 2003), a four-step model which is repeated for introducing changes into practices (Fig. 2). Using this learning process for setting up new cropping systems, the set of agronomic strategies and of decision

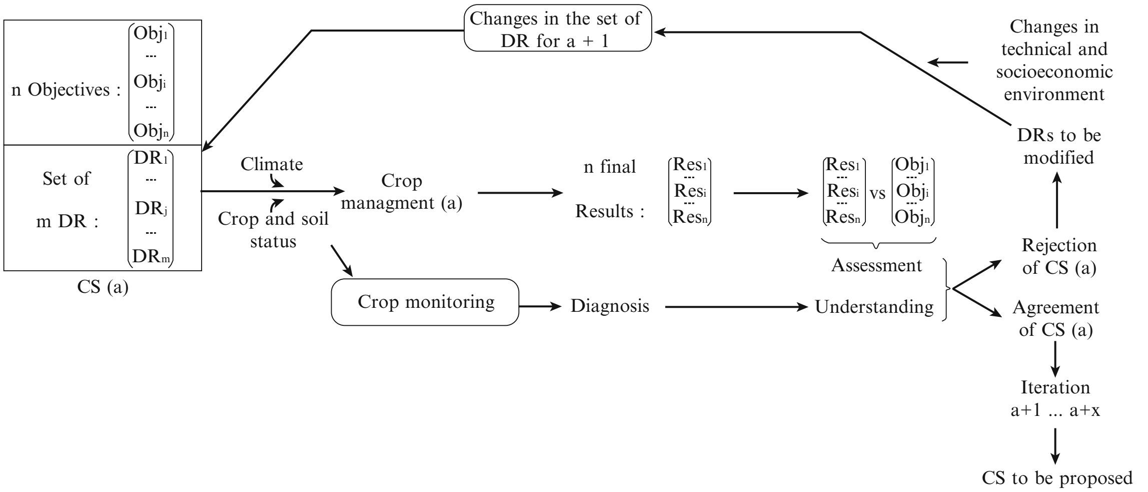

Fig. 2 The approach used for setting up cropping systems: the annual improvement loop. *CS* cropping system, *DR* decision rule, *a* annual index

rules (including thresholds for decision triggering, and crop and cultivar choices) may be revised during the course of the experiment, while the objectives and constraints assigned to cropping systems should remain fixed throughout.

Over the years the cropping system gradually changed from a prototype to a realistic system, and finally, to a system in accordance with the set of objectives. The periods of building up and evaluation alternated with time. As the cropping systems were very innovative, with unpredictable impacts on the environment and a real difficulty in suggesting suitable and robust decision rules, the time step of the improvement loop was short (typically one or a few years). As these cropping systems were not fully controlled, frequent modifications of the decision rules were necessary before getting a consistent set of rules addressing the objectives. Compromises were discovered progressively as the experts had only a partial vision of each system. For example, growing oilseed rape in the organic cropping system was rapidly abandoned because pollen beetle attacks were not adequately controlled. After 5 years of experimentation, the crop sequence in the organic cropping system had to be changed with the introduction of alfalfa to control thistles. The nature of permanent cover in the mulch-based cropping system was changed several times (red fescue, white clover and then alfalfa) to limit the adverse effect of mulch on succeeding crops. The techniques to control the early growth of the living mulch were also modified. Glyphosate (non-selective herbicide) rates were adapted to the objective, either to depress or suppress the permanent cover.

Each individual improvement loop could be short (from 1 year to 5 years to define the crop rotation in the organic system), but the sequence of successive improvement loops for different sets of techniques required a long-term experiment for designing/evaluating innovative cropping systems (permanent soil cover, stockless organic systems requiring a 2-year conversion period). This approach required specific attention to the evaluation of individual decision rules, with experimental designs including checking plots for rule evaluation. For example, different wheat cultivars including mixtures of up to four cultivars were tested in the Integrated Production cropping system in parallel strips. The data obtained over several years demonstrated that the combination of cultivar resistances to a range of diseases within a mixture reduced the risk of damage due to leaf diseases, and improved the mean yield level in cropping systems with reduced reliance on fungicides.

The long experimental duration is also required because the innovative cropping systems were likely to modify some soil properties, with cumulative effects during the course of the experiment. In the mulch-based cropping system, the organic matter content in the soil surface layers was modified and had probably still not reached its new equilibrium after 8 years. The absence of soil tillage and the changes in the organic matter status of the soil probably interacted to affect the soil structure, and thus indirectly the crop behaviour and the associated decision rules. In the organic cropping system, N availability for crops relies on N mineralisation, and therefore on the soil N content, that is likely to be affected after cessation of the fertilisation regime at the beginning of the experiment.

The main output of the Versailles experiment was to deliver different relevant and validated sets of decision rules corresponding to innovative cropping systems to suit the requirements expected for future cropping systems. Unlike the Toulouse experiment, the focus was not at this early stage to develop decision support systems or simple models for supporting crop management, but to demonstrate the technical feasibility of those systems. However, as soon as the prototype systems became stable and robust, the data collected also provided significant information about the technical, economic and environmental performances of the proposed cropping systems. The difficulty was to separate the prototyping function (which assumes a progressive tuning of the prototype) from the sustainability evaluation function, which requires a stable, unchanging system under test. In fact, some errors in the management, due to an insufficient evaluation of some risks or an inadequate understanding of innovative technology, could also have a significant influence on the system's success. The assessment of the risks associated with a wrong application of the set of decision rules is also an output of the experiment.

In addition, the experimental site offered conditions for interdisciplinary studies: each year an area was sown with wheat cv. Charger (a cultivar susceptible to fusarium) to study the conditions for mycotoxin production and accumulation in wheat grains. The amount and quality of organic matter in the soil was also analysed because the systems differed in their organic matter accumulation and tillage practices. These analytical studies, carried out by scientific teams not involved in the supervision of the cropping system experiment, benefited from the special conditions offered by the tested systems, and were possible in spite of the limitations related to the experimental layout.

4.3 Dijon Experiment

In the Dijon experiment, the main research focus was the cumulative effects of combining different techniques for weed management, each of which having only limited potential efficiency. The tested cropping systems were undoubtedly new in French conditions and were proposed to address the major environmental concern of the concentration of herbicides in both the surface and ground-waters (Aubertot et al. 2005). However, the innovation introduced arose mainly from new combinations of common cultural practices: in IWM systems, ancestral techniques such as mouldboard ploughing were combined with mechanical control based on rotary hoes, finger weeders or flex-tine harrows, which have been used for decades in organic agriculture. The experiment provided new data to judge the performance of these cropping systems, which fitted in between organic farming and conventional systems relying mainly on chemicals for weed control.

Unlike the Versailles experiment, the set of decision rules for each system had to be fixed on the basis of expected results before starting the field evaluation, and remained unchanged (or only slightly changed) over a period long enough to assess the cumulative effects of the cropping system components on the weed community. Indeed, the long-term control of weeds is a major aspect of the assessment of sustainability: one system using very few herbicides and generating high financial return would not be judged sustainable if the weed infestation were to increase over the considered period. The set of decision rules had to be kept stable in order to relate the sustainability indicators to well-identified systems. Hence, the time step of the iterative loop was long. The role of the experiment was mainly to evaluate the system as a whole.

A set of IWM principles was fully described before field testing. The different criteria to assess the performance of cropping systems were as follows: (1) weed control – *did the decision rules succeed in controlling weeds at a stable level with no severe damage to crop production?* (2) ecological – *was the floristic biodiversity promoted?* (3) agronomic – *did the decision rules for weed control provoke unexpected agronomic side-effects, such as disease increase?* (4) environmental – *did the systems result in a significant reduction in herbicide applications and related environmental impacts, did they improve energy balances, did they result in decreases in greenhouse gas emission and N leaching?* and (5) socio-economic – *did the IWM systems result in an increase in labour use, did the input reduction compensate for the yield losses and extra input costs?* These criteria covered most of the range of sustainability indicators.

As for the other two experiments, the methodology was based on formal decision rules. The knowledge available for writing formal rules came from very diverse sources. Model simulations supported the structure and parameterisation of some decision rules. For instance, the rule defining the sowing date for winter cereals (sowing after 25 October) was justified by simulation studies performed with the germination-emergence module of AlomySys (Colbach et al. 2005), a demography model for blackgrass (*Alopecurus myosuroïdes* Huds.), a typical weed species in winter cereals. These simulations suggested that delaying sowing until the end of October would result in a reduction of 75% in blackgrass emergence in wheat as compared with a normal sowing date in the region (i.e. at the beginning of October). The escape strategy (mechanical control of early blackgrass seedlings shortly before sowing) was responsible for this positive effect. Other rules were based on expert knowledge when simulation models failed to help decision-making. For example, weed demography models at the community level still do not make predictions of the effect of crop rotation on weeds. Thus, the principles for defining a typical 6-year rotation were derived from available knowledge about the timing of emergence and the seed persistence in the soil for the most frequent weeds. These principles suggested (1) spreading out the sowing dates over the crop rotation as far as possible, but also (2) limiting the frequency of spring-sown crops, because the seed persistence of spring-emerging species in the soil seed bank tends to be longer than that of the autumn-winter-emerging species (Barralis et al. 1988). Finally, as in the other two experiments, some rules were based on results from within-field testing of different options. Such a subsidiary experiment supported, for example, the choice of wheat cultivars competitive with weeds, as little information was available on this feature in the description of local varieties.

As in the Toulouse experiment, a specific tool for supporting the decision-making was developed for one complex decision, i.e. decision-making for chemical weed control (*to spray or not to spray? using which herbicide?*), that could not be formulated by an “if... then” statement. Theoretical studies showed that the concept of an economic damage threshold is not applicable to weed flora management (Munier-Jolain et al. 2002). In addition, the analysis of farmers’ decision-making for weed control, based on farm surveys, demonstrated the multi-criteria nature of this decision (Macé et al. 2007). The decision results from a weighting of different criteria, including the efficacy of the weed control method according to the weed composition, the cost of the strategy, and its suitability for the farmer’s labour plan. For managing a cropping system experiment on weeds, the formalisation of decision rules required the development of a specific decision support system based on multicriteria choice (Munier-Jolain et al. 2005). An environmental impact criterion was included in the decision-making process to account for the sustainability of the tested cropping systems. The complex decision was governed by the recommendations of the software as soon as this was reliable enough. Testing the recommendations led to the improvement of the decision support system, especially its ergonomics and ability to support decisions in real time.

Unlike the Toulouse experiment, the experimental design in Dijon did not include the principle of growing each year all the crops of the rotation, because IWM required long crop rotations. Moreover, the rotation was not fixed but flexible, as it could be modified on a given field according to the weed composition (e.g. as a function of the relative contribution of autumn- and spring-emerging species). The analysis of yield variability was not a main issue considered anyway. The two replicates of a given system had shifted rotation sequences in order to result in two different climatic sequences for each system.

The main output of the Dijon experiment is a global and multicriteria assessment of innovative cropping systems over an appropriate time period in a particular climate. The reliance on herbicides over six years was reduced in IWM cropping systems as compared with the standard one: the number of treatments was reduced by 65% and the amount of applied active ingredients by 90% in a typical IWM cropping system (Fig. 3), while the weed infestation remained stable or decreased over the 2001–2006 period. Other potential environmental impacts are currently being evaluated to check that the high frequency of shallow tillage in IWM cropping systems (false seedbed and mechanical weeding) does not worsen the energy balance. The feasibility and economic performance of the most promising system on the whole area of a given farm is also evaluated by modelling labour organisation. The data collected on the experiment improved our knowledge of the effects of cropping systems on

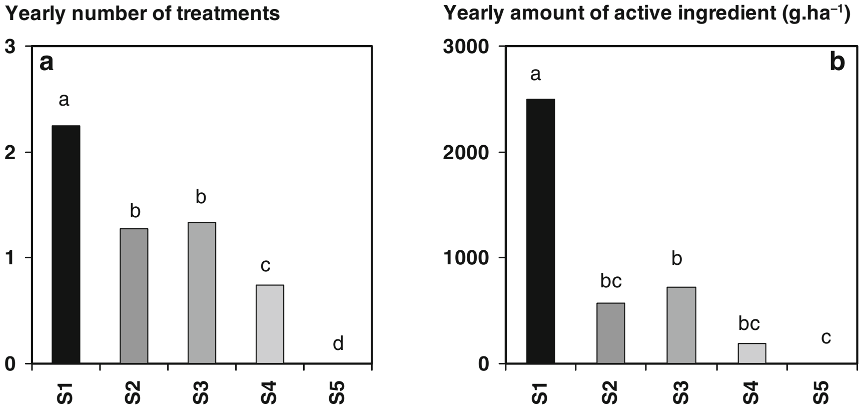

Fig. 3 Two criteria for assessing the reliance of cropping systems on herbicides: comparison of (**a**) the mean yearly number of herbicide treatments and (**b**) the mean yearly amount of herbicide active ingredients applied on cropping systems in the Dijon experiment. *S1* standard system; *S2* IWM, reduced tillage; *S3* IWM, no mechanical weeding; *S4* IWM with mechanical weeding; *S5* no herbicide. Average values were computed over the 2001–2006 period, except that the year with a sugar beet crop grown only in S4 was excluded from the analysis to avoid the bias due to this particular crop. Bars with the same letters are not significantly different according to LSD ($P < 0.05$)

weed communities. The results are also expected to provide significant inputs to the controversial debate about the future of agriculture in the European Union, addressing the important issue of the trade-off between the two components of sustainability, namely, the economics and the environmental concerns. From this point of view, although it shares part of the methodological approach with the Toulouse and the Versailles experiments, the Dijon experiment's philosophy remains closer to previous cropping system experiments comparing conventional, integrated and/or organic cropping systems (e.g. Reganold et al. 2001; Poudel et al. 2002).

5 Common Methodological Bottlenecks and Ways of Improvement

Considering soil fertility change, experiments over less than 10 years are qualified as short-term, and long-term records (several decades) are recommended for C cycling evaluation (Richter et al. 2007). Poor management can hamper the academic and practical value of such long-term experiments. Rule-based cropping system experiments are shorter (8–12 years), but because of repeated and heavy observations, strong and stable scientific and human resources are also committed. Beyond their financial and human costs, some methodological difficulties should be pointed out.

The first limitation is due to the restricted soil and climatic representativeness of these experiments, although a tested system may have a regional relevance in terms of the types of crops and type of resource management, for instance. A large part of south-western France is affected by limited water availability, which justified the Toulouse experiment. In Versailles, winter wheat was grown every two years as a component of cereal crop systems in the Parisian Basin, while no local animal waste was available for the organic system because local farms no longer rear cattle. Most of the conversions to organic agriculture were observed in stockless farms during the last decade in this region. However, the experimental results come from a few fields in only one location. The large size of the unit plots in a cropping system experiment (from 0.5 to 2 ha) limits the opportunities for ample replication. Unlike normal factorial trials, the objective is not to demonstrate statistically the effects of single factors or to compare the relative performance of the different systems, but to evaluate how often the expected result was reached (and why, if not). Hence, the purpose of replication differs between factorial and system experiments. In a system experiment, a sufficient number of plots is required to estimate the probability of obtaining the expected result whatever the system. For instance, in Dijon, eight fields managed under IWM were monitored, and this made it possible to evaluate the weed control in IWM over eight fields. However, as each system was replicated only twice in this experiment, one could argue that the satisfactory results obtained might be due to chance, and this could hinder the dissemination of the results for wider use.

Two approaches might be used to expand the results obtained from local cropping system experiments. On the one hand, modelling the effects of cropping systems on a range of variables considered in the evaluation process might make it possible to explore wide ranges of climatic scenarios on different soil types (Wallach et al. 2006). Field results obtained from a limited number of data sets would be more robust if they were confirmed by biotechnical models based on the processes involved in the complex behaviour of the system. On the other hand, farm networks managed by extension services for testing promising sets of decision rules are another way of broadening the assessment of their validity domain. This is in line with "step 5" of the methodology proposed by Vereijken (1997) for prototyping farming systems. During this step, the prototype variants tested on pilot farms are disseminated to a growing number of farms with a gradual shift in supervision from scientists to extension workers. In France, the results obtained from those experiments on experimental farms that demonstrated the efficiency of systems with few chemical inputs are currently playing an important role as precursors for field testing of cropping systems based on IPM principles on farm fields supervised by a network of extension workers (ICS, the Innovating Cropping System project funded by the French Ministry of Agriculture) (Reau and Landé 2006; Debaeke et al. 2008).

The second limitation of the cropping system field experiment is related to the field scale, which might limit a realistic evaluation of systems for some aspects. On the field scale, the evaluation cannot take into account the spatial dimension, which should be considered for some issues. For example, crop attacks by mobile pests depend on the landscape structure and the distribution of cropping systems on spatial scales far larger than the field. The ecological balance of animal pests and auxiliaries might also be affected by the landscape structure and the management of crops and other components of the landscape. In the same way, the farm organisation constraints (competition for equipment, labour, water and other resources) cannot be considered directly from data collected on the field scale. However, as previously, modelling could help in evaluating the consequences for labour organisation on the farm scale of modifying the crop rotation (and therefore the crop distribution over the farm), and of delaying or anticipating the application of technical operations (Attonaty et al. 1993; Chatelin et al. 1993).

In the previous experiments, the agronomists intended to introduce innovations able to satisfy rising environmental concerns. Which innovation is to be introduced into the cropping system and to what extent it is acceptable or reasonable are two complex questions to address. As an example, the cropping systems in Dijon did not include forage crops because of the absence of livestock farming in the region. This choice was questionable as it is agreed that sowing temporary grasslands is a good way of reducing weed seed banks from annual crops, thus reducing the use of herbicides. Introducing forage crops in a stockless region would have required finding profitable outlets for them. For that reason, such a solution, although agronomically efficient, was not considered as relevant in the Dijon experiment. Searching for the innovation(s) likely to fit best with the objectives and constraints ascribed to a cropping system is also questionable. Where should the innovation come from? From the scientist's creative brain? From cropping systems already implemented in other parts of the world? From the current practices of individual farmers searching for new solutions by themselves? In Versailles, the idea of testing direct seeding in living mulch under temperate conditions was imported from previous successful experiences in tropical regions and from organic farming experiences. In Brazil, for instance, the adoption of a direct seeding mulch-based cropping system (DMC) was introduced 30 years ago to increase carbon levels in the topsoil and reduce erosion (e.g. Bernoux et al. 2006). In organic farming, temporary living mulches in which the main crop is directly sown could suppress weeds efficiently with minimum competition to the main crop.

The methodology to build and evaluate cropping systems in experimental stations on a field scale could be profitably used in on-farm programmes where innovative systems are proposed and tested with the help of volunteer farmers on pilot farms. In the Netherlands, cropping systems previously validated on experimental farms were tested in a pilot farm network (Langeveld et al. 2005). In France, such a network is currently being established by a group including scientists, extension workers and farmers wishing to innovate in their crop management systems (Reau and Landé 2006). The prototyping approach does not plan to transfer the sets of decision rules from the experimental farms to the network directly. The method scheduled should rather begin with a discussion of the sets of decision rules with each farmer to account for the specific objectives and constraints of the farms in the network. Candidate systems will be proposed by collective expertise and discussed, they will be evaluated using forecast agro-ecological and economic indicators, and the most promising will be tested on-farm. The method follows previous attempts for prototyping crop management systems in tropical and temperate agriculture (see, for example, Lançon et al. 2007).

6 Conclusion

The cropping system experiments presented in this article differ from previous long-term experiments because they supported studies of the complexity of the crop production system: first of all because the consistency of the systems was accounted for by considering (1) the consistency between the techniques used within a system, and (2) the consistency between the techniques and the environmental conditions, through the formalisation of the system management by sets of decision rules; secondly, because the assessment of the performances of the cropping systems involved various criteria covering most of the indicators of crop production sustainability. They shared a common methodology requiring first that the context, objectives and constraints of each tested system be defined, followed by the strategies and sets of decision rules, before field implementation. However, beyond this shared methodology, the three experiments had their own specific features and research focuses. Roughly, the Toulouse experiment focused on methodological developments, ex post agronomical diagnosis and the development of decision tools to adapt the strategies to the environmental and economic context. The mean feature of the experiment in Versailles was to test very innovative strategies requiring frequent tunings of the sets of decision rules and an improvement loop with a short time step. In contrast, the Dijon experiment tested cropping systems on a criterion subjected to cumulative effects, therefore requiring stable sets of decision rules during a long period.

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Agri-Environmental Indicators to Assess Cropping and Farming Systems: A Review

Christian Bockstaller, Laurence Guichard, David Makowski, Anne Aveline, Philippe Girardin, and Sylvain Plantureux

Abstract Environmental impacts of agriculture cannot be always assessed by using direct measurements. Since the 1990s, numerous agri-environmental indicators were developed to assess the adverse effects of cropping and farming systems in the environment, such as water pollution, soil erosion, and emission of greenhouse gases. Here we present the different types of indicators developed during the last decade and review the progress of the methods used for their development. The application of different groups of indicators is discussed and illustrated by examples in the fields of nitrogen losses and pesticide risk: (1) indicators based on a single or a combination of variables related to farmer practices, (2) indicators derived from operational or more complex simulation models assessing emissions of pollutants, and (3) measured indicators linked directly to environmental impacts. The nitrogen indicator (IN) of the INDIGO method and the MERLIN indicator will be presented and used to illustrate the methodological discussion. We show that a good identification of the end-users, of the practical objectives of the indicator, and of the spatial and temporal scales is essential and should be done at a preliminary step before designing the indicator itself. The possibilities of deriving an indicator from a model and of setting a reference value are discussed. Several methods are also presented to study the sensitivity and the validity of agri-environmental indicators. Finally, several practical recommendations are made. As only few data are usually available at the regional level, several simple indicators should be used for assessing a given impact at this level. When more detailed information is available, indicators based on operational models can be useful to analyse the effects of several factors related to soil, climate, and cropping system on an environmental impact. In experimental studies, we suggest using both measured indicators and model-based indicators.

Keywords Environmental assessment • Indicators • Nitrogen • Pesticide • Simulation model • Validation

1 Introduction

Direct measurements of impact due to agriculture are often difficult to implement. Since the 1990s, numerous agri-environmental indicators and indicator-based methods were developed to assess environmental impacts of agriculture and the sustainability of agricultural systems (Rigby et al. 2001; Rosnoblet et al. 2006). Riley (2001a) spoke about an "indicator explosion", which could be explained by the growing concern for environmental issues and sustainability. The use of indicators constitutes an alternative to direct impact measurement (Mitchell et al. 1995) which presents several methodological difficulties such as impossibility of measurement and complexity of the system or practical constraints, e.g. costs and time. According to Gras et al. (1989): "Indicator is a variable which supplies information on other variables which are difficult to access".

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Assessment methods based on a set of indicators have been developed at national or international levels (e.g. EU, Delbaere and Serradilla 2004) but, also, at regional (Payraudeau and van der Werf 2005), farms (Eckert et al. 2000; van der Werf and Petit 2002; Hülsbergen 2003; Meyer-Aurich 2005), or, field and cropping system levels (Bockstaller et al. 1997; López-Ridaura et al. 2005). Studies on specific thematic indicators are also available, for examples for nutrients (ten Berge et al. 2002; Goodlass et al. 2003), or for pesticides (Maud et al. 2001; Reus et al. 2002; Devillers et al. 2005). The generic term of "indicator" represents a large diversity of tools and needs some clarification (Riley 2001b).

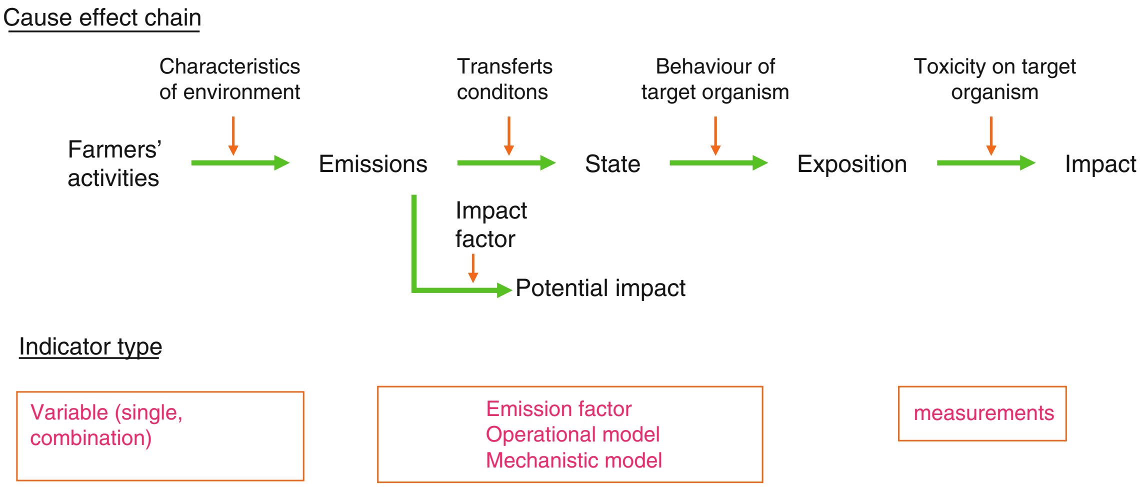
Investigations on methodological issues regarding the development of indicators were also published. Some authors have focused on specific questions like the selection of indicators (Mitchell et al. 1995), their aggregation (Nardo et al. 2005; Jollands 2006), and their validation (Bockstaller and Girardin 2003; Cloquell-Ballester et al. 2006). Girardin et al. (1999) identified five steps in the development of an indicator: (1) preliminary definition of the objectives and identification of the end-users, (2) construction of the indicator, (3) selection of a reference value, (4) sensitivity analysis, (5) validation. All steps are not always clearly addressed in articles presenting a specific indicator or an assessment method based on indicators. The purpose of this article is to present the diversity of existing indicators and to review methodological progress in each of the five steps defined by Girardin et al. (1999). The article focuses on the agri-environmental indicators, mainly at field scale (cropping system) and farm scale (farming system) with some references to higher levels. Our concepts will be illustrated with two indicators assessing nitrogen losses.

2 Overview of Agri-Environmental Indicators

Maurizi and Verrel (2002) present a series of definition for the notion of indicator. These definitions are strongly influenced by the background and disciplines of their authors. Authors working on the assessment of ecosystem health or biodiversity refer to a set of biophysical measurements like physico-chemical properties of soil, or abundance of species for a given taxon (Carignan and Villard 2002; Clergué et al., 2005). Other authors working in agronomy or/and economy based their indicators on model outputs (Meyer-Aurich 2005) whereas, for some agronomists, the use of indicators is justified by the impossibility of using dynamic models or direct field measurement (Bockstaller et al. 1997).

We based our typology on a cause–effect chain defined from the works of Hertwich et al. (1997), and, Payraudeau and van der Werf (2005), as illustrated in Fig. 1. According to this conceptual framework, the environmental impact results from a chain of processes, beginning with human activity like agricultural practices which can result in emissions of pollutant, depending on the characteristics of the environment, soil, climate. If transfer conditions are favourable, those emissions may cause a change of state for a given environmental compartment. If living beings are exposed to the pollutant, a biological or economic impact can occur, depending on the behaviour of the target organism and the toxicity of the pollutant. It should be noticed that the concept of potential impact is derived from a simplification of the assessment pathway (Freyer et al. 2000). For example in some Life Cycle Analysis methods (Brentrup et al. 2004), the impact is assessed by combining emissions of pollutant and the toxicity without data on the behaviour of living beings determining exposition. Other authors use the concept of risk, especially to deal with the environmental impact of pesticides (Levitan 2000). Many pesticide risk indicators are based on an assessment of pesticide fate and hazard (toxicity) although the term of risk in its proper meaning is a probability of occurring of a hazard and the magnitude of its effect (Flemström et al. 2004).

Several types of indicators can be distinguished in Fig. 1. They are based on the cause–effect chain although exceptions can be found. (1) The first group consists of simple indicators based on the use of one type of variable obtained by survey, databases and not directly measured. In many cases, they are derived from statistics on farmers' practices which are assumed to be causes of the impact according to the knowledge of their developer. Some are also based on environmental characteristics, e.g. soil, climate. They can be based on one or a simple combination of variables, like the calculation of balances, e.g. for nutrients, or ratio, e.g. for energy. Indicators of this group provide an indirect



Examples of indicators :

Variable Single	combination	Emission factor, operational models, mechanistics models	Measurement
Nitrogen Rate N % soil cover in winter % non fertilized area	Farm gate N balance Surface N balance Leaching index (=drainage/ available water capacity)	NH3 emission coef. MERLIN* Nitrogen indicator IN* Output of leaching model	Soil mineral nitrogen in winter Nitrate concentration in dwell
Pesticide Number of treatments Amount of active ingredient	IFT (sum of rate/registrated rate)	I-Phy P-ema (using output of the Macro model)	pesticide concentration in dwell, river, number of death due to pesticides

Fig. 1 A typology of indicators based on the cause–effect chain of impact: examples of indicators, variable based indicator, indicator derived from a model, and measured indicators are presented for assessing nitrogen losses and risks induced by pesticides. Examples of indicator with *asterisk* will be detailed later in text

assessment of the environmental impacts and are often qualified as "proxy", having a poor quality of prediction (Riley 2001c). (2) The second group of indicators includes indicators based on calculation and integrating more than one type of factors, e.g. farm practices and soil conditions. This category covers a great diversity of indicators with different levels of complexity, from emission coefficient used in Life Cycle Analysis, to indicators based on mechanistic simulation models. Those indicators are often used to assess emissions of pollutant or the pollution of an environmental component like water compartments. Advantages and limitations depend on the type of tools from which the indicator is derived. Conceptual or mechanistic simulation models integrating well processes may be preferred from the scientific point of view. They allow to link the predicted effect to causes. However their complexity is a major limitation to use in many cases. Solution can be found as shown in Sect. 3.2.2. (3) The third group includes indicators based on one or several measurements. Biodiversity indices belong to this category (Carignan and Villard 2002; Clergué et al. 2005). They are used when users focus on impacts and when no accurate model is available. Emissions can also be assessed by measurements, e.g. mineral nitrogen in soil before winter, nitrate concentration below roots measured by ceramic cups. The drawbacks of these indicators are that their costs can be high and that they cannot be used to trace cause–effect relationship with a satisfactory level of accuracy (Merkle and Kaupenjohann 2000). For example a given level of mineral nitrogen in soil can be explained by the soil and climate, the crop yield, the nitrogen management. Hence it is not easy to derive directly advices for farmers.

3 Methodological Issues for Designing Agri-Environmental Indicators

A part of this section concerns the three groups of indicators presented above, but some specific points only concern the category "calculated indicators" and, more specifically, indicators derived from models. This section treats the development of single indicators as well as sets of indicators.

3.1 Preliminary Choices and Assumptions

The identification of the end-users and the definition of the practical objectives of the indicator, were pointed out as an essential step by several authors (Mitchell et al. 1995; Crabtree and Brouwer 1999; Girardin et al. 1999; Yli-Viikari et al. 2007). This preliminary step will serve as a basis to design the indicator and to evaluate its quality. Different users group can be identified like, for example, scientists, advisors, farmers, decision maker, or consumers. The group of people doing the calculations and the group of people using the results should be differentiated. In general, a given indicator will be adapted to one group of stakeholders only due to the variability of the users' requirements.

An indicator can be developed for various objectives like *ex ante* evaluation of actions, in a planning phase (Sadok et al. 2007), *ex post* evaluation of an action at the end or during its implementation, monitoring purpose with an alert role, decision support in real time to drive the system, communication. After clarifications of those general items the developer should focus on the issues of concern covered by the indicators, e.g. the environmental compartment or impact.

Those are generally translated into a list or even more into a framework which is more or less explicit and elaborated. This list of issues can be set up in interactions with different users-groups or experts, by consultation, e.g. with Delphi techniques (Hess et al. 1999). Girardin et al. (1999) proposed to synthesize them in a matrix crossing issues of concern and element of the system to assess. Actually two groups of approaches can be distinguished according to two conception of sustainability (Hansen 1996). The former one is a goal-oriented, based on a set of themes or objectives (von Wirén-Lehr 2001). Those can address the main abiotic (air, soil, water) and biotic (species, ecosystems) environmental components, ecological functions or environmental impacts like in Life Cycle Analysis (Brentrup et al. 2004). The latter is property-oriented, based on systemic properties e.g. adaptability, security. A synthesis of attributes of properties can be found in López-Ridaura et al. (2005). Bossel (1999) showed that sustainability can be assessed by means of a set of seven generic systemic properties, such as existence, effectiveness, freedom of action, security, adaptability, coexistence, psychological needs. This systemic approach is an alternative to the goal-oriented approach which Bossel (1999) describes as based on the "intuition" of experts and contingencies. It should also help to reduce the number of indicators. However, the concept of systemic property appears to be abstract to non-initiated users. Efforts to make it operational are still needed to help the user to select relevant indicators for each property.

Last, the definition of the system boundaries is another important step (Van Cauwenbergh et al. 2007). It includes the calculation scales, spatial and temporal which will be influenced by the users' needs, the issues of concerns, etc. Again these choices will guide the type of indicators and the required qualities. For example, indicators calculated on data obtained at regional or national levels should show good statistical qualities. In Life Cycle Analysis approaches, users are forced to define the system boundaries. It can be the product, the farm including or not upstream such as production of inputs and off-stream activities such as waste management. In other assessment methods quoted in the introduction, this definition seems to be neither explicit nor unified between indicators. Regarding spatial and time scales one should paid attention to the resolution of calculation, the level at which basic calculations are carried out. Farm and year are typical resolution for environmental indicators. This should not be confused with the extent, i.e. the whole area, e.g. the region, or time span, e.g. period, crop rotation, covered by the indicators calculation (Purtauf et al. 2005).

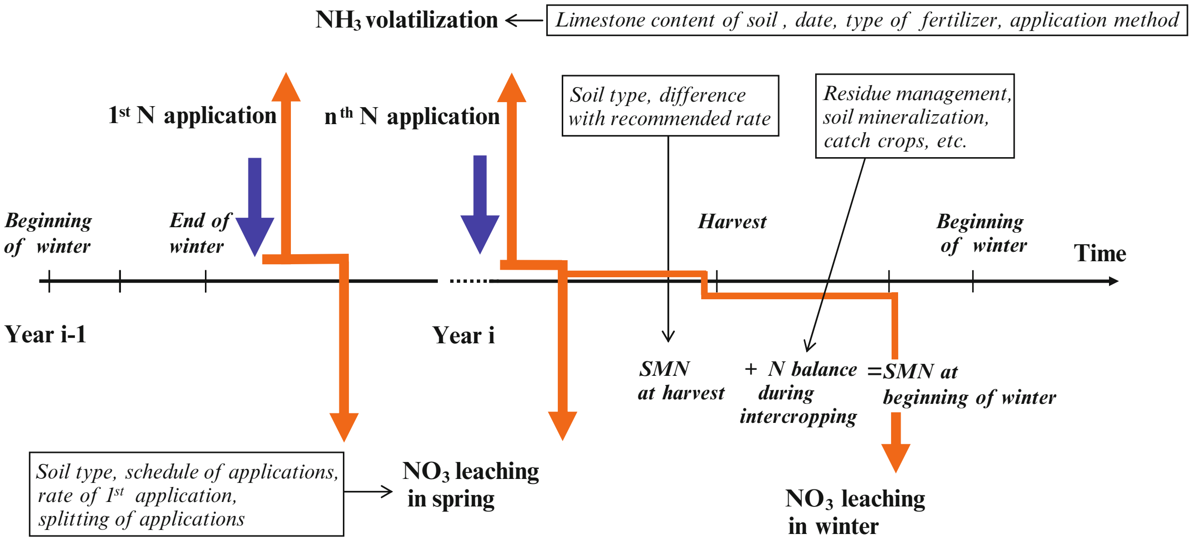
3.2 Indicator Design

3.2.1 Nature of the Indicator Outputs

As shown in Fig. 1 indicator outputs may represent the result of a measurement, the result of a simple calculation based on a combination of data, or the result of a simulation derived from a complex model. Such outputs can be transformed into a score which expresses (1) a risk or an impact, ranging from 0 (low) to 1 (high) (van der Werf and Zimmer 1998). Other authors used a scale between 1 and 10 (Eckert et al. 2000) or 1 and 5 (see Fig. 3), (2) an environmental performance ranging between 0 (low), and 10 as in Bockstaller et al. (1997). (3) Scales between a negative value and a positive one, e.g. −3 to +3 as in Rigby et al. (2001), expressing a negative and positive effect respectively are also used. The choices of the scale, of the scoring function and of the range of value are subjective, will depend on practical considerations, and can be subject to discussion (Andreoli et al. 1999). They have an importance for communication. In any case, these choices should be explicit and transparent.

3.2.2 Model-Based Indicators

A model output can be used to calculate an indicator. This option is attractive by the potentialities of modelling, but may lead to some practical problems due to the complexity of many models. A solution is to create a matrix of simulations and to derive an indicator from this matrix. The interest of this approach is that the model is run for a limited number of situations. For example Brown et al. (2003) used the model MACRO to build a table of concentrations of pesticides in groundwater from 2,280 model simulations. Another approach is to derive a metamodel which can be then used to calculate an indicator (Garcet et al. 2006). Such a tool can be elaborated with a learning machine (Shan et al. 2006). Finally, another solution is to develop a simplified model based on a reduced number of input variables which are easily accessible. The latter approach is illustrated by the nitrogen indicator presented below (Fig. 2). Such an operational modelling can be characterized by the statement of Durand et al. (2002): "… to compare the effect of different agricultural practices on nitrogen pollution in a catchment, it may not be necessary to simulate quantitatively the whole nitrogen and water cycle to calculate nitrate concentration in the river at each time step".



INO3, INH3, IN2O : resulting from the transformation of N losses into a score between 0 and 10 (no losses) with 7 acceptable losses (Ex : leaching with 50 mg NO3.L-1)
IN = minimum (INO3, INH3, IN2O)

Fig. 2 Overview of the nitrogen indicator IN assessing the risk of nitrogen losses, nitrate, ammoniac, nitrous oxide. The main input variables involved in the different nitrogen losses are given in the boxes. *Black arrays* and *grey arrays* represent respectively the nitrogen inputs and the evolution of nitrogen in the agrosystem, i.e. gaseous emission, plant uptake, and leaching to groundwater (*SMN* soil mineral nitrogen)

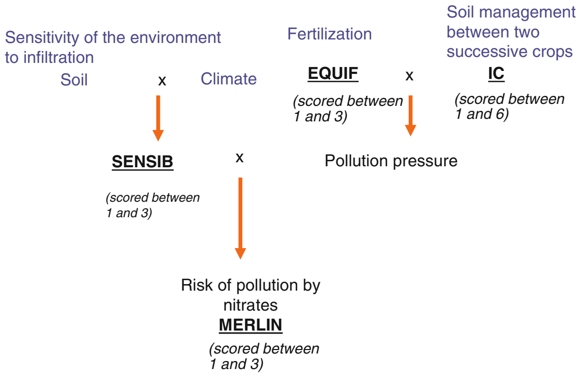


Fig. 3 Overview of the indicator MERLIN, combining three subindicators (EQUIF, IC and SENSIB) assessing the risk of nitrate leaching

3.2.3 Qualitative Approach

An indicator can also be qualitative. It can represent a decision rule which can be expressed as a "if then" rule, or as a contingency table. This will be illustrated by the example of MERLIN presented in Sect. 3.4. Fuzzy logic can be used to account for the uncertainty in the indicator outputs by avoiding the effect of knife-edge limit of a given class like the ones used in MERLIN (Fig. 3), (Silvert 2000; Enea and Salemi 2001). It is applied in a growing number of examples, e.g. van der Werf and Zimmer (1998); Prato (2005). Ranking method like SIRIS based on scoring was also proposed to derive qualitative indicators (Aurousseau 2004).

3.3 The Setting of a Reference Value

According to Riley (2001c), indicators are defined as "observations relative to their respective reference point". This reference value helps the user to interpret the raw value of the variable, the calculation or the measurement, for instance to assess whether "an action A is environmental friendly or not". The reference value may be implicit. For example, the reference value for nitrogen balance indicators is zero for many users, assuming that the system has reached a steady state. But such an implicit reference is often subject to criticisms in terms of environmental impact due to a lack of scientific arguments (Oenema et al. 2005). The reference may be a threshold, e.g. critical load for soil pollutant (Skeffington 2006), a norm, e.g. water quality guidelines for nitrate, pesticides in the E.U., or a target, expressed in an absolute or in a relative way (von Wirén-Lehr 2001; Van Cauwenbergh et al. 2007). In many cases, the definition of a reference value is not studied by scientists and is determined by the stakeholders. To our opinion it should result from the interaction between scientists and policy makers.

The subjectivity behind the choice of an absolute value is an issue and leads some authors to use references based on relative values (e.g. average of the raw values for a sample, initial value of the indicator). Such reference may enable the user to conclude that "the indicator is showing that A is better or worse than B" which does not automatically mean that "A is good, e.g. environmental friendly".

3.4 Two Examples of Indicators to Assess Nitrogen Losses

The following two indicators were developed to assess the importance of nitrogen losses in agrosystems. They belong to the second group of indicators presented in Sect. 2. The first indicator is the nitrogen indicator IN (Bockstaller and Girardin 2001; Pervanchon et al. 2005) included in the INDIGO method (Bockstaller et al. 1997). It is based on a simple model simulating nitrate leaching and nitrogen gaseous emissions, NH_3 and N_2O, in a quantitative way. The model outputs are transformed into scores (Fig. 2). Concept of operational model refers here to the choice of input variable based on the availability of data. Several complex inputs such as the wind speed which is a relevant variable for NH_3 volatilisation are not included into the nitrogen indicator IN.

The second indicator is MERLIN (Aimon-Marié et al. 2001). This indicator can be used to assess nitrate leaching under cropping system in a qualitative way, in form of risk classes, and consists in the aggregation of three components (Fig. 3): EQUIF, an equation calculating the difference between nitrogen supply and crop requirement and assessing the risk of increase of soil mineral nitrogen at harvest due to overfertilization, IC assessing the risk due to the management between two crops, based on the nitrogen uptake by crops and catch crops, and SENSIB assessing the leaching sensitivity of the field that results from climatic and soil conditions. SENSIB and IC components are based on contingency tables.

4 Evaluation of an Indicator

4.1 Sensitivity Analysis

A sensitivity analysis presents two major interests. First it aims at testing whether the indicator outputs are sensitive to the input variables which are known to have a strong effect, or whether these outputs are different for actions, e.g. cropping systems, which were found to produce different results in past studies. An example is the amount of active ingredient currently used to assess the risk of pesticide use (Levitan 2000). This indicator is insensitive to the pesticide properties and does not differentiate two active ingredients applied at the same rate. It can not be used to assess the choice of pesticides made by farmers.

Second, sensitivity analysis allows one to analyse the effect of several input variables on the outputs of a given indicator. The results of such an analysis can be used to identify the inputs with a strong effect and those with a minor effect. The users could then decide to invest more effort on the inputs showing a strong effect on the indicator outputs. An example can be found in Pervanchon et al. (2005) for the nitrogen indicator IN for grassland.

4.2 Evaluation of the Quality of an Indicator

A classical approach is to evaluate the accuracy of model predictions by comparing the predicted value with observed or measured data. Some authors considered that this approach is difficult to apply to simplified indicators (Rigby et al. 2001; Reus et al. 2002). As a consequence, the accuracy of many indicators is not evaluated (Devillers et al. 2005). Bockstaller and Girardin (2003) proposed a methodological framework with three steps for the evaluation of environmental indicators which will be detailed below. This evaluation does not concern the quality of prediction only. It was recently completed by a social validation (Cloquell-Ballester et al. 2006).

4.2.1 Evaluation of the Indicator Design

This procedure consists in an evaluation of the design of an indicator by a panel of experts or by peer reviewed article. Whereas most of the publications on indicators came from the grey literature in the 1990s (Levitan 2000), the number of scientific papers on indicators is now increasing rapidly. Such procedure allows one to check whether the design of an indicator is based on scientific knowledge and may generate outputs with a good level of accuracy. It can indicate the need of an improvement. The nitrogen indicator IN for grassland (Pervanchon et al. 2005) was strongly modified following the advices of reviewers who had rejected a first version.

4.2.2 Evaluation of the Indicator Output

This step is based on the comparison of the indicator output with measured data. If those are not available, Bockstaller and Girardin (2003) propose alternative procedures consisting in comparison with model output or other indicator output, but this approach must be applied with caution. If the indicator is directly based on a simulation model, the model itself can be evaluated based on experimental data. For simplified indicator whose goal is not to give an accurate prediction but only some information about an environmental risk, specific approaches have been recently proposed. Bockstaller and Girardin (2003) developed a probability test which was implemented to assess the nitrogen indicator IN for grassland (Pervanchon et al. 2005) and the EQUIF sub-indicator of the indicator MERLIN (see Fig. 3). The test consists in assessing the proportion of cases in which the difference predicted value – observed value falls within a probability or acceptance area defined in function of the expected performance of the indicator and the precision of the measurements. In the example shown in Fig. 4, the authors considered that the risk of leaching is low when the EQUIF output is lower than 50 kg N ha^{-1} and the soil mineral nitrogen at harvest does not exceed 50 kg ha^{-1}. Above those

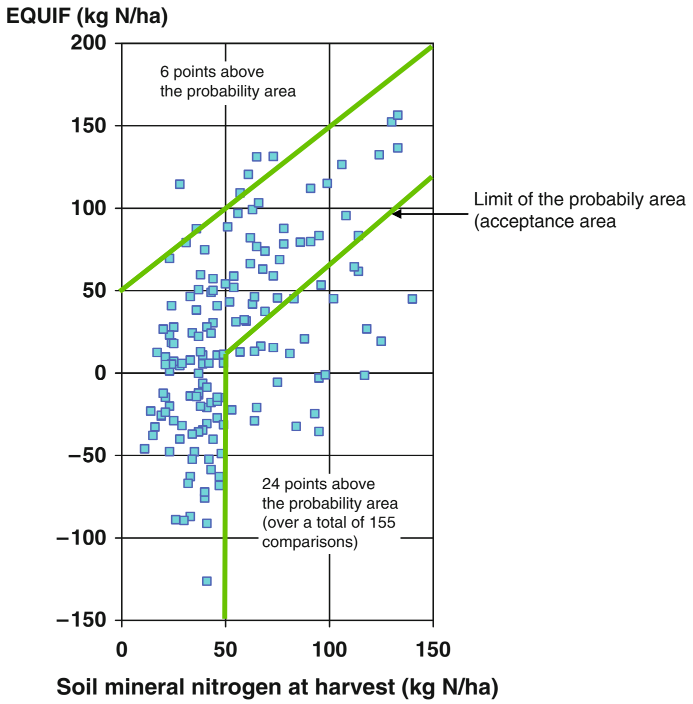


Fig. 4 Probability test of the EQUIF component of the MERLIN indicator (see Fig. 3). Each point corresponds to an agricultural plot where the value of EQUIF was compared to a measurement of soil mineral nitrogen at harvest. The test consists in assessing the number of points being within a probability or acceptance area defined in function of the expected performance of the indicator and the precision of the measurements

thresholds, the risk increases as shown by the shape of the probability area. The test showed that a proportion of 80% of the experimental plots are in the area (Rousseau 2003).

Makowski et al. (2005) and Primot et al. (2006) used the Receiver Operating Characteristic (ROC) methodology (Swets 1988) to assess the ability of an indicator to discriminate between plots with high environmental risks and plots with low environmental risks. This approach was adapted to measure the accuracy of agri-environmental indicators with experimental data. It consists in estimating two criteria named sensitivity and specificity. The sensitivity measures the proportion of agricultural plots with high risk correctly predicted by the indicator, the specificity measures the proportion of agricultural plots with low risk correctly predicted by the indicator. Levels of risk are determined from a measured gold standard variable and from an injury threshold. The plot of Sensitivity vs. $(1 - \text{Specificity})$ is called a "ROC curve" (see Fig. 5). The area under the ROC curve, named "AUC" for "area under curve", is equal to the probability that the indicator values for a randomly selected pairs of agricultural plots with high and low risks will be correctly ordered. The area AUC is within the range 0–1. For a given injury threshold, perfect indicators are characterised by an AUC value equal to 1 whereas the AUC value of an useless indicator is equal to or lower than 0.5. The ROC curve can also be used to determine a decision threshold leading to a good compromise of sensitivity and specificity. Table 1 shows the AUC values estimated from 89 experimental plots located in the basin of Bruyère in France for seven nitrogen indicators. The values of seven indicators were computed and the mineral nitrogen at harvest was measured in each plot. Mineral nitrogen at harvest was considered as the gold standard and three injury thresholds were used successively. Figure 5 shows two examples of ROC curves. The results of the ROC analysis show that the EQUIF indicator (I_6) and the Surface nitrogen balance CORPEN (I_7) are slightly more accurate than those based on an amount of applied fertilizer (I_1 to I_5).

Table 1 Values of area under curve (AUC) for the seven indicators and the three different injury thresholds of soil mineral nitrogen at harvest Y_t. The AUC under the receiver operating characteristic (ROC) curve is equal to the probability that the indicator values for a randomly selected pairs of agricultural plots with high and low risks will be correctly ordered. The value of AUC shows the ability of an indicator to discriminate between two contrasted situations. Useless indicators are characterised by an AUC value equal to or lower than 0.5. The AUC values of accurate indicators are close to 1

	Area under the curve (AUC) estimated for the indicator for given injury thresholds Y_t (kg N ha^{-1})		
Indicator	30	40	50
I_1 = amount of applied nitrogen	0.58	0.57	0.55
I_2 = applied nitrogen + soil mineral nitrogen at winter	0.59	0.58	0.59
I_3 = applied nitrogen − recommended fertilizer dose	0.58	0.54	0.56
I_4 = applied nitrogen / grain yield	0.62	0.62	0.62
I_5 = (applied nitrogen + soil mineral nitrogen at winter) / grain yield	0.65	0.66	0.64
I_6 = soil nitrogen + apparent recovery × applied nitrogen − nitrogen requirement × grain yield (EQUIF[a])	0.64	0.64	0.64
I_7 = applied nitrogen − nitrogen content in grain × grain yield (surface nitrogen balance CORPEN[b])	0.64	0.63	0.65

[a] Sub-indicator of the MERLIN indicator (see Sect. 3.4 and Fig. 3)
[b] Department of the French ministry of Ecology

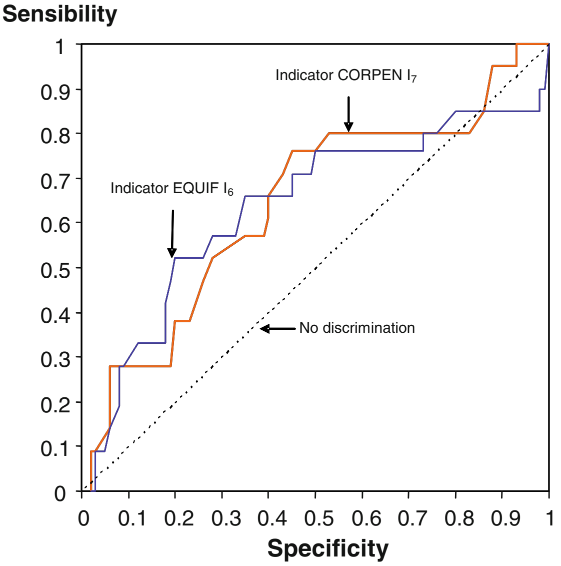


Fig. 5 Receiver operating characteristic (ROC) curves obtained for two indicators, the EQUIF I_6 (see Sect. 3.4 and Fig. 3) and the surface nitrogen balance of the CORPEN I_7 and for a threshold $Y_t = 50$ kg ha^{-1} of soil mineral nitrogen at harvest. The *dotted line* shows an area under the curve (AUC) equal to 0.5 which characterised a non discriminating indicator. Accurate indicators are characterised by high AUC values

4.2.3 Evaluation by End-Users

This is the last step of the framework presented by Bockstaller and Girardin (2003). The purpose is to see whether a given indicator is used and how it is used. This step is important to identify the situations where an indicator is non applicable. It also stimulates exchanges between the developer of the indicator and its potential users. At this step, the developer can collect feed-backs from users, i.e. suggestions for improvement, problem in implementation, misunderstanding, etc. This step was implemented in the area of evaluation research to analyse how indicators are used by policy makers (Gudmundsson 2003). Few applications were also published in cropping and farming system assessment. For example, Douguet et al. (1999) collected and analysed the reactions of farmers to indicators developed by Bockstaller et al. (1997). For the indicator MERLIN (see Sect. 3.4.), several quantitative criteria (Fig. 6) were quantified and qualitative feed-back recorded to seven users. The results showed a good level of satisfaction for the indicators MERLIN and for EQUIF.

5 Discussion

Different types of indicators were presented in this article. We showed that a great diversity of indicators is available and we discussed their advantages and limitations in details. The typology presented in Fig. 1 is based on the cause effect chain which was also used for the framework Pressure/ State/Response of the Organisation for Economic Cooperation and Development

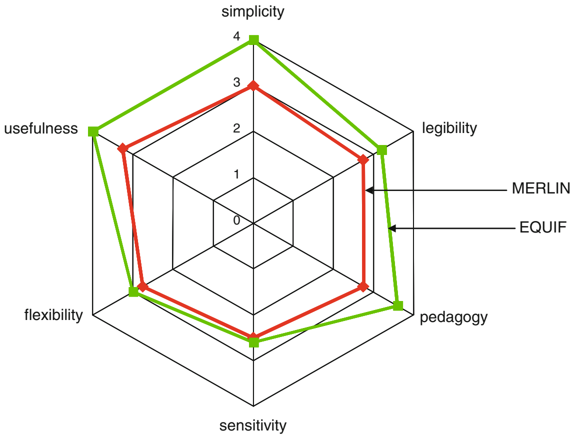


Fig. 6 Satisfaction level of users about MERLIN and EQUIF for several criteria on a scale between 1 (not satisfied) and 4 (very satisfied). Each value is an average of seven responses

(OECD) and for its improved version the Driving-force/Pressure/State/Impact/Response (DPSIR) of the Environmental European Agency (EEA 2005). Payraudeau and van der Werf (2005). Braband et al. (2003) qualified respectively the first group of indicators addressing only farmers practices as "means-based" indicator and "action-oriented" indicators whereas the other kinds on the cause–effect chain are "effect-based" indicator or "result-oriented" indicators (Fig. 1). The first group of indicators which are classified as pressure indicator failed to provide in many situations a clear link between pressure and state as it was advocated by Crabtree and Brouwer (1999). Thus, "effect-based" or "results-oriented indicators" are preferred by the authors. Of course, users have always forced to find a compromise between scientific soundness and feasibility constraints (Girardin et al. 1999), especially at the national level (Crabtree and Brouwer 1999; Yli-Viikari et al. 2007). The cost of implementation is an important issue for many users (Romstad 1999).

In the second part of the article, several methodological issues were reviewed; preliminary choices before the indicator development, indicator design, and indicator evaluation. All those steps imply choices and assumptions which cannot always be justified from quantitative data, but should always be transparent (von Wirén-Lehr 2001). Interactions between scientists and stakeholders should play an important role during the whole process. Regarding the design of indicators and their evaluation, the article highlights some significant progresses. The possibility of using models to design or to derive indicators goes beyond the opposition between model and indicator discussed by Bockstaller et al. (1997). For the evaluation of indicators by comparison with measured data, two methods have been proposed which can be complementary. A probability test can be used to provide information about the relation between indicators and measurements. The ROC method can be used to assess the ability of an indicator to discriminate between situations with high and low environmental risks. It can also be used to define decision thresholds from experimental data in function of sensitivity and specificity target values.

Some issues were not addressed in this article but will deserve more attention in the next few years. The choice of the scale for calculating indicator outputs is an important issue and should be discussed in relation with the type of impact, the status of the indicator on the cause–effect chain. For example, water quality indicators should be used at the scale of the water catchments or for a landscape. Emissions can be assessed at lower scale of the cropping and farming systems. For indicator assessing emissions, results can be upscaled by aggregation of results obtained by calculation of an average value at higher scale weighted by the size or the number of entity at lower scale. Such aggregation at higher scales like a nation is not relevant for local impact, e.g. water quality, erosion, whereas it is possible for global impact, e.g. greenhouse gases. Upscaling requires some statistical skills for data management but must also integrate new processes (Stein et al. 2001) and new environmental components (e.g non cropped area).

A second issue concerns the evaluation of the indicators, and more precisely the uncertainty linked to the indicator. Authors working on Life Cycle Analysis approaches are concerned by this issue (Basset-Mens et al. 2006). Some addressed it using fuzzy logic approach (Ardente et al. 2004). Uncertainty was analysed for nitrogen balances by using fuzzy logic (Mertens and Huwe 2002) or Monte-Carlo approaches (Oenema et al. 2003). Finally, a third issue concerns the interpretation and use of the indicator outputs (Yli-Viikari et al. 2007). Recommendations about the significance of the scores, about the uncertainty of the results, the relevance of the reference level should be given to the users to help them to interpret the results.

To facilitate the interpretation of set of results, aggregation is often used. This is true for the two types of aggregation regarding upscaling procedures (see above) and composite or multi-criteria aggregation where indicators related to different themes are combined. The relevance of aggregation is often discussed because of the loss of information but also due to the methodological problems it raises. A major problem is "adding apple and pear" in the case of composite indicators which can appear in scoring method (Rigby et al. 2001). Several method are available to avoid this problem like the normalization technique in monetary unit or physic unit, the multivariate approach (Nardo et al. 2005), or the decision trees using fuzzy logic (van der Werf and Zimmer 1998; Phillis and Andriantiatsaholiniaina 2001). An alternative consists in using the multi-criteria methods based on an outranking procedure (Arondel and Girardin 2000; Hayashi 1998). One shortcoming of this last family of methods is the principle of outranking based on relative comparisons and not on an absolute assessment. The use of weightings procedure in aggregation as well as in multi-criteria methods is also often criticized due to its subjectivity. This cannot be totally avoided but should be transparent. Andreoli et al. (1999) proposed guidelines for this problem. Sensitivity analysis may be useful to assess the effects of weighting. Such analysis will also help to cope with compensation and trade-off between sub-indicators in a composite indicator (Nardo et al. 2005). We advise to use both aggregated and individual indicators.

Several issues mentioned in this article require interactions with users, from the elaboration and selection of indicators, to the interpretation of results. The users can be involved at the beginning of the elaboration of an indicator or a set of indicators in a procedure of participative research. In this case, the characteristics and required qualities of indicators are defined together by end-users and scientists. At the end of the development, an evaluation by end-users may bring new information from end-users to scientist to improve the indicator. With the growing number of indicators and methods available for the end-users, the question will shift from "how to elaborate an indicator?" to "which indicators?", so that they need comparative information as in Reus et al. (2002), Devillers et al. (2005), or methodological help to compare indicators. More methodological research is needed on this issue of indicator comparison.

6 Conclusion

Many indicators are available to help agronomists and stakeholders working on the assessment of sustainability of farming and cropping systems. This article presents a typology of environmental indicators and discusses their advantages and limitations. In many cases, only few data are available, especially at regional or higher levels. Only simple indicators based on farmers' practices can be used in such cases. These indicators generally present a low quality of prediction. They can be combined in order to improve their accuracy, but the use of multiple indicators is often complicate in practice. Efficient methods for integrating various processes are still needed. When input data on soil and farmers' practices are available, indicators based on operational model like those presented in this article for nitrogen losses can be useful to analyse the effects of various factors related to soil, climate, and cropping systems. Such indicators are still lacking in several areas, notably to assess the impact of agriculture on biodiversity.

In cropping system experiments, measured indicators and model-based indicators should be both used. Model-based indicators are often required in this context because all the variables of interest cannot always be measured like, for example, gaseous emissions or pesticide losses. In any case, we advised agronomists and environmentalists to use the methodological framework proposed in this paper to design indicators. Issues like scales and upscaling procedures, uncertainty analysis, interpretation of the results, interaction with indicator users and comparison of indicators should be the object of more research work during the next few years.

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Methodological Progress in On-Farm Regional Agronomic Diagnosis: A Review

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Abstract The development of sustainable cropping systems is a key priority for agronomists and crop scientists. A first step involves understanding the relationship between cropping system performance and farmers' practices. To complete this step, a methodological framework entitled Regional Agronomic Diagnosis (RAD) has been developed. During the last 10 years, the scope of the regional agronomic diagnosis has been enlarged to include several factors describing crop quality and the environmental impact of cropping systems. Regional agronomic diagnosis has led to several major advances such as (1) the assessment of the effect of preceding crop and soil structure on malting barley quality in France and (2) the assessment of the effects of ploughing, nematicide use and fertilisers on soil properties in intensive banana plantations in the West Indies. Improvements have also been gained in methodology, particularly by the selection of indicators for assessing the effects of crop management, soil and weather conditions, and data analysis. Finally, regional agronomic diagnosis has been integrated into more general approaches of agricultural development. We review here this methodological progress.

Keywords Agronomic performance • Banana • Barley • Cropping system • Diagnosis • Indicator • Nematicide • On-farm research • Soil • Yield gap analysis

1 Introduction

An understanding of the effects of cropping systems on soil characteristics, plant growth and development, and biocoenosis is essential for the improvement of farming practices. Improvement may increase crop yield and quality and reduce the environmental impact of cropping systems, thereby contributing, to various extents, to sustainable development. Many studies on this topic have been carried out at research stations, in trials in which different factors are fixed and combined, to evaluate the effects of different experimental treatments on crop performance, quality or environmental value. However, on-farm research studies are also carried out in farmers' fields. Some of this on-farm research aims to assess the value of innovative cropping systems, as shown in case studies by Dejoux et al. (2003), Jackson et al. (2004), Blaise et al. (2005), Esilaba et al. (2005), Hasegawa et al. (2005). This evaluation is the final stage in a process starting with identification of the main factors limiting crop production. Other on-farm studies try to identify and to rank the cropping practices responsible, in interaction with the environment, for a large proportion of the total variability in crop production, crop quality and environmental impact in a region. Such studies are not based on experimental trials. Instead, they are based on monitoring and series of measurements in a network of fields cultivated by farmers using current cropping practices. These on-farm studies are used for diagnostic purposes (Fig. 1), their results being used to define innovative cropping systems at the next step, the design step. These innovative cropping systems are then evaluated through on-farm trials or experiments

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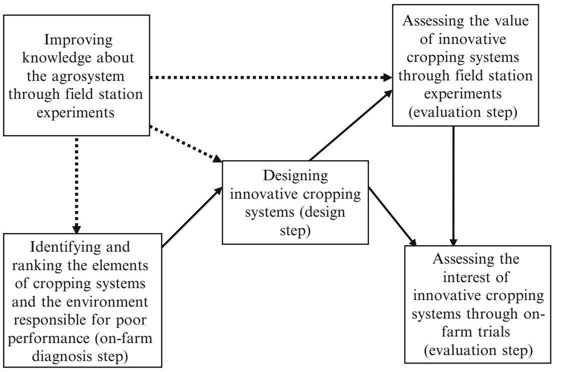

Fig. 1 Relationships between the diagnosis step and the other steps of a general framework for cropping system improvement (*continuous lines*). The *dashed lines* indicate the use of analytical experiments carried out at research stations. *Continuous lines* indicate flows of information between steps

at field stations, before being passed on to farmers. As pointed out by Lobell and Ortiz-Monasterio (2006) for crop yield, "the identification of strategies to reduce the yield gap requires an understanding of its causes", hence the need for diagnosis.

Doré et al. (1997) proposed a methodological framework for carrying out such an agronomic diagnosis: regional agronomic diagnosis (RAD). Case studies based on RAD were reviewed by Doré et al. in 1997. These case studies demonstrated the relevance of RAD for identifying and ranking limiting factors for crop yield on the regional scale. Nitrogen deficiencies linked to soil compaction affecting pea yield in France, crop establishment affecting wheat yield in Tunisia, and plant losses due to rapid submersion and/or rat and crab damage and paddy water level affecting rice yield in Thailand provide examples of major limiting factors identified by RAD. Several aspects of RAD have been improved over the last 10 years. In the past, diagnosis was generally applied to a single variable: crop yield. In several recent studies, this method has been applied to other variables relating to crop quality and environmental impact, with methodological consequences. New methods have also been proposed and used to analyse causal relationships between farmers' cropping systems and their agronomic or environmental performances. Finally, RAD has been better integrated into action-oriented agricultural projects for the dissemination of new knowledge to farmers. We will first summarise the main features of RAD and will then review these recent developments.

2 Overview of the Regional Agronomic Diagnosis Approach

2.1 Explaining the Variability in Cropping System Performances on the Regional Scale

RAD aims to determine why some fields in an agricultural region do not achieve the expected level of performance. This approach involves determining and accounting for variability in production (or production gaps) or environmental damage within a set of farmers' fields. Agricultural production depends on soil and climate characteristics, which vary between sites. In certain cases, this variability in production may be desirable. For instance, a spread of harvesting dates or a range of different size grades is often required for fresh vegetable production. However, variability in agricultural production may also limit performance on the regional scale (Le Bail and Meynard 2003). So, whether we try to exploit it or to avoid it, many agronomic studies aim to understand the causes of this variability so that we can find the solutions best satisfying the target objectives.

Unsatisfactory situations occur on various scales. Yield may vary even within a single field, as shown by the numerous within-field yield maps now available. On a national or large regional scale, yield variation is just as frequent, and can largely be accounted for by differences in physical (soil type, weather) or socio-economic conditions, resulting in different local attainable and potential yields. Yield differences are also found on the intermediate scale of a common agricultural region (see the references cited in Doré et al. 1997; Wopereis et al. 1999; Van Keer 2003). Regional agronomic diagnosis is applied to such small, homogeneous agricultural regions, defined on the basis of common climatic and soil characteristics and socio-economic features, including agri-food and food chains. Residual variability in soil and climatic characteristics in this agricultural region may account for some variation in performance (e.g. crop yield), but many studies have demonstrated that agricultural practices play a critical role (Boiffin et al. 1981; Aubry et al. 1994; Leterme et al. 1994; Affholder et al. 2003; Le Bail and Meynard 2003; David et al. 2005a; Lobell and Ortiz-Monasterio 2006).

2.2 Applying a Functional Analysis to a Set of Farmers' Fields

Different elements of the cropping system, such as previous crop, genotype, crop protection management, date and mode of soil tillage, sowing date and density, and fertilisation strategy interact with the environment to determine crop yield. The identification by RAD of the element or combination of elements potentially responsible for the range of variation in crop yield observed on a regional scale requires the disentangling of complex relationships, and this must be tackled in the real situation of farmers' fields. Indeed, it would be difficult to reproduce the full range of diversity of combinations of physical environment and farming practices existing within a region in experimental trials (Sebillotte 1974). It would also be impossible to choose the relevant factors for study in these experimental trials, as this identification of the most important factors is the intended result of the diagnosis step. For this reason, RAD studies are carried out mostly on a network of farm fields.

However, in practice, to achieve the objectives of RAD on a set of fields requires special attention to a major problem: different techniques are associated in various ways in an agricultural field, and farmers' practices may differ in several ways between fields. It is therefore difficult to determine, by simple comparisons of yield and farmers' practices, which techniques are responsible for yield variations. RAD involves functional analysis (see Doré et al. 1997) based on (1) an analysis of the relationships between yield variability and crop and/or environment characteristics during the growing period, and (2) an analysis of relationships between the characteristics of the soil-plant system and farmers' practices.

2.3 Designing the Field Network

The performance of RAD depends on the quality of the farmers' field network (Boiffin et al. 1981; Doré et al. 1997). The field network must represent the diversity of existing systems and environments (soil and climatic types) in the studied area. Some authors also include in this representative network rare or contrasting situations very different from the most common ones, or even innovative situations tested in experimental trials (see, for example, Sebillotte et al. 1978; Clermont-Dauphin et al. 2004b). These plots are useful for demonstrating the relationships between the variables to be explained (e.g. yield) and crop and environment characteristics during the crop cycle. However, caution is required when quantifying the effect of the different elements of cropping systems, as these additional contrasted fields may result in overestimation of the effect of some factors. Finally, some authors (Becker and Johnson 1999, 2001; Becker et al. 2003) have excluded hypothetical and evident yield-limiting parameters in the network *via* superimposed and researcher-managed subplots in farmers' fields. Studies of the subplot network make it possible to assign part of the differences in yield to factors other than these evident parameters.

2.4 Characterising Crop and Environment Status

The variables recorded for each field characterise the cropping system, in terms of the timing and rate of fertilisation, pest and disease control, sowing density and date and nature of the preceding crop, for example. They also include indicators providing information about the environment, such as temperature data, soil moisture content or soil-available nutrient content, and crop status, such as insect damage, vegetative biomass, rooting depth or leaf water potential. Some indicators are easy to access and reflect the entire growth cycle rather than just giving an instantaneous picture. These indicators include yield components, such as numbers of ears and grains, and mean grain weight in cereals. Indeed, the value of each yield component depends on the previously formed components and environmental factors during the formation of the yield component. The value of a given yield component can thus provide information about agronomic, edaphic and climatic conditions during its formation phase (see Fleury 1991; Meynard and David 1992; and Sect. 3.2.). In some cases, as in indeterminate legumes, it may be more appropriate and convenient to adopt a yield analysis based on measurements of vegetative growth. As suggested by Doré et al. (1997), many indicators used for RAD have been shown to be effective tools for analysing the functioning of agricultural fields (see, for example, Davidson and Ramsey 2000; Clermont-Dauphin et al. 2004a; Haefele et al. 2006).

2.5 *Analysing the Data*

Data are analysed in two steps: on a field scale and on a regional scale. On a field scale, the objective is to explain crop performance as a function of edaphic, climatic and agronomic factors. For example, Le Bail and Meynard (2003) observed alternating areas of tall and short plants in barley fields. The shorter zones corresponded to the drill lines situated behind the wheels of the tractor that sowed the crop. The simultaneous identification in the short-plant zones of (1) a compacted soil structure, (2) crop nitrogen deficiency, and (3) a small number of ears/m^2 suggested a nitrogen nutrition problem caused by soil compaction, due to soil tillage management in wet conditions in this field. The shift to the scale of a whole network of fields is of importance for two reasons. It makes it possible (a) to rank the various limiting factors according to their impact and frequency in a region, and (b) to validate hypotheses based on the analyses of individual fields. Indeed, if an environmental condition has been identified as responsible for the low yield of one field, all fields with an environment at least as unfavourable should have similarly low yields, unless an interaction with another variable can be implicated. This reasoning back-and-forth between the two scales – a major feature of this analysis – often requires data for more than one cropping season before the desired precision for the final diagnosis is reached (Meynard et al. 1981; Doré et al. 1998; Clermont-Dauphin et al. 2003, 2004a; David et al. 2005a). Finally, if the diagnosis identifies elements of the cropping system with effects highly dependent on an interaction with weather conditions, a specific study of the frequency of these interactions may be useful, although such studies are rarely carried out (see Boiffin and Meynard 1982).

3 Methodological Improvements

3.1 *New Variables of Agronomic Interest as Subjects for Regional Agronomic Diagnosis*

Food production has traditionally been the main function of agro-ecosystems (Costanza et al. 1997). However, cropping systems are now increasingly evaluated not simply on their production capacity, but also on their role in and impact on regional (e.g. surface- or ground-water quality) or global (e.g. participation in climate change via carbon storage or greenhouse gas emission) ecosystems. As a result, RAD has been applied to an increasing number of agronomic and environmental variables. These new applications led to several methodological developments, summarised below.

3.1.1 Productive Function

In the past, the main variable studied in RAD was crop yield, defined as the quantity of useful biomass harvested annually per hectare. Recently, RAD has also been applied to variables characterising the quality of the harvested product, such as the grain protein content of malting barley (Le Bail and Meynard 2003). This approach can also be extended to other quality criteria, such as the size or visual appearance of the harvested organs, or undesirable compound (pesticide residues, mycotoxins, heavy metals, etc.) content. These different objectives clearly require the development of diagnostic indicators complementary to those used for yield. In grapevine, for example, the quality of the grapes for wine-making is strictly dependent on water stress, and an indicator has been developed to evaluate the moisture regime of the vineyard (Pellegrino et al. 2006).

The effects of pests on crop product quality have led to specific studies, such as those on fungal diseases of bananas (Chillet et al. 2000) and pineapple (Marie et al. 2000). RAD may be used to evaluate the impact of certain pests or pest profiles (a combination of pathogens, herbivores and weeds) on crop performance (Valantin-Morison et al. 2007). Establishing the damage function, describing and quantifying the loss of yield or quality due to pests may indeed be an objective in itself.

3.1.2 Non-productive Functions

Environmental concerns have increased in importance over the last 20 years, resulting in a need for new methods for measuring and evaluating relationships between agriculture and the environment (Boiffin et al. 2001). RAD is particularly suitable for empirical analysis of the impact of cropping systems on the environment in a given region. Nutrient balance (see, for example, Corre-Hellou and Crozat 2005) and biological regulation (e.g. allelopathy, as in Sène et al. 2001) have already been the subject of specific regional diagnoses based on the same methodological framework as used for analyses of productive functions. Analyses of the variability in fertiliser efficiency on a maize/bean intercrop with low inputs in a small upland region of Haiti (Clermont-Dauphin et al. 2003) provides an example of the use of RAD to improve both production and soil fertility management. Clermont-Dauphin et al. (2004b) analysed the relationships between intensive management practices in a banana plantation and soil fertility characteristics, such as the organic matter content of soils, microbial respiration, nematode populations and earthworm biomass, in a field network in the French West Indies. Corbeels et al. (2006) evaluated soil carbon storage potential for different cropping systems with and without mulch in the Brazilian *cerrados*.

3.1.3 Methodological Consequences

The RAD approach to evaluating new productive and non-productive functions appears to be very similar to that described above for yield. However, some differences should be emphasised. Firstly, available knowledge concerning environmental processes is often less complete or detailed than that for the mechanisms involved in yield formation. Measurement of the environmental variable at field level often constitutes a major obstacle. For example, measurements of the emissions of greenhouse gases, such as nitrous oxide (N_2O) or methane (CH_4) in the field remain difficult and costly, and improvements would be required before RAD could be attempted. A lack of knowledge concerning the processes involved also causes problems with analysis of the observed variation, particularly as environmental functions require a change of scale for the analysis. In some cases, it is difficult to distinguish clearly between the effects of crop management and the effects of landscape structure. Finally, the layout of fields in the area studied constitutes another methodological difficulty, as the impact of cropping systems on many environmental variables depends on the spatial location of the field in the territory considered. This is the case for erosion (role of slope and of the position of the field within the catchment area) and biodiversity (border effects, mosaic effects, etc.). In this context, the classical methodology of RAD, which does not take into account the position of the field in the landscape, requires adaptation. Valantin-Morison et al. (2007), in their study of the effect of cropping systems on insect populations and damage in organic oilseed rape, showed that explanatory variables characterising the spatial environment of the fields should be incorporated into the analysis.

Another methodological difficulty stems from the regional distribution of the values taken by the studied variable and sometimes from the existence of a threshold splitting the data into two subgroups, potentially calling into question the very definition of the aim of diagnosis. In most situations, the statistical distribution of the yield values obtained for a network of farm fields is approximately normal. However, in some cases, the distribution of yield or quality attributes may follow a log-normal law, as reported by de Bie (2004) for mango yields in northern Thailand, and by Champeil (2004, Fig. 2) for the *Fusarium* mycotoxin content of organic wheat grains. In cases of log-normal distributions, which are probably more frequently observed if quality criteria are considered, there are large numbers of fields with low or null values. These fields are of little use for establishing a hierarchy of characteristics of cropping systems determining variability. This is particularly true if, as is often the case for quality criteria, there is a standard threshold separating the sample of studied fields into subgroups. In one analysis of mycotoxin contamination in wheat (Champeil 2004), the threshold concerned the maximal value of contamination, above which the crop becomes difficult to sell. Given the

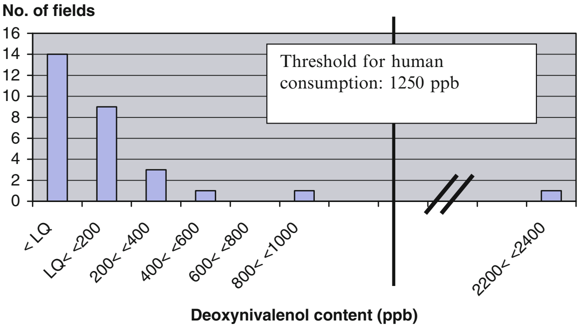

Fig. 2 Example of a log-normal distribution of deoxynivalenol content in organic wheat grain in an area in France (LQ = limit of quantification). From Champeil (2004)

observed distribution (Fig. 2), the aim of the diagnosis becomes identifying the main effects of cropping systems accounting for the very high levels of contamination of certain fields. The products of these fields, when mixed in silos with those from fields with lower levels of contamination, are likely to increase considerably the toxin concentration of the average batch, making it unsaleable.

3.2 Data Analysis Methods

It is not easy to interpret data from field networks. Some progress has been made in this area in recent years, in three main areas: the estimation of potential yields, the choice of indicators and the establishment of relationships between yield and limiting factors. These methodological developments mostly concern diagnosis for yield variations. Nevertheless, the second and third aspects are also relevant to diagnosis for crop quality and environmental impacts.

3.2.1 Estimation of Potential Yield and Potential Yield Components

Estimates of potential yields (limited by solar radiation and temperature only) or potential yield components can be used as yardsticks for assessing the results obtained in field networks (Meynard and David 1992). They make "yield-gap analysis" possible within the RAD, just as this analysis of the difference between actual and potential yields is carried out in different environments and on different scales in various ways: on-farm, at research stations, or sometimes exclusively through modelling (e.g. Van Ittersum and Rabbinge 1997; Mussgnug et al. 2006). Various criteria, such as yield shortfall in t ha^{-1} (Becker and Johnson 1999), yield shortfall in % (David et al. 2005a) or indices calculated from two successive yield components obtained at different growth stages (Wey et al. 1998), can be used to quantify the effect of limiting factors.

Potential yield values are frequently estimated from crop models (Van Ittersum et al. 2003). For example, ORYZAS has been used for diagnosis on irrigated rice systems in the Sahel and savannah regions of West Africa (Wopereis et al. 1999), and LINTUL-POTATO has been used for potato production in Argentina (Caldiz et al. 2002). The drawbacks of this approach are that (1) crop models require a large number of input variables and (2) crop model predictions may be inaccurate. It is therefore necessary to assess the accuracy of crop models before using them in practice, and adaptation of the model to the area studied may even be required. Another approach involves estimating potential yield (or yield component) values from experimental data (Mercau et al. 2001). One way to do this is to use the most extreme yield value within the dataset. Brancourt-Hulmel et al. (1999) used the mean of the most extreme values obtained for n subsamples of the dataset, using a bootstrap procedure. However, this approach does not take measurement error into account and is therefore likely to overestimate the true potential yield. The variance of this estimator may also be very high due to sampling variability. A second approach involves defining a boundary line for a particular quantile and using this boundary line to determine potential yield values for different environments (Johnson et al. 2003). Makowski et al. (2007) suggested defining a quantile value as a function of the probability distributions for measurement error and limiting factor effect.

3.2.2 Choice of Relevant Indicators

It remains important to improve the precision and specificity of indicators, as defined above, without complicating their collection or affecting their robustness. Ideal indicators of limiting factors should generally display (1) high specificity (varying only with the limiting factor considered), (2) "memory" (making it possible to diagnose past deficiencies), (3) simple monitoring and (4) robustness (large validity domain) (Meynard et al. 1997). Several studies in the last decade have aimed to improve indicator quality, and some such studies are still underway.

It is often useful to express the characteristics of the crop and the environment in the form of differences with respect to standard values. The standards may be (1) taken from previous publications (e.g. the critical curve for nitrogen content in aerial biomass published by Lemaire and Gastal 1997), (2) defined during trials in the same environment (e.g. the rice yield potential used by Van Asten 2003), or (3) simulated using existing models (see Meynard and David 1992; Affholder and Scopel 2001). In certain cases, in which the characteristics of the plants or environment are difficult to measure, models can be used to evaluate them. For example, Poussin et al. (2003) used the RIDEV model to estimate spikelet sterility for rice farmers' fields.

Some indicators based on crop attributes are not very robust. For instance, the delay in flowering proposed by Homma et al. (2004) as an indicator of water stress for a local rice cultivar in north-east Thailand, although more sensitive to soil moisture variations than the more commonly used "number of days of submersion" (Sharma Pradeep et al. 1995; Savary et al. 2005; Haefele et al. 2006), is only valid for the genotype studied. A similar problem applies to yield components. Thus, if several cultivars are used within the RAD network of farmers' fields, it is better to compare the differences between actual and potential yield for a given component rather than actual yield components (David et al. 2005a). In this case, the availability of genotype references for the potential values of yield components is essential for the analysis.

In the specific case of intercropping systems, the yield of each plant is highly dependent on the climatic environment created by the neighbouring plants, which varies according to the plants considered. For instance, Lamanda et al. (2006) showed that, in coconut plantations intercropped with annual crops, the relative density of the two species varies both between fields and within a given field. A similar situation applies to monospecific systems with a heterogeneous within-field phenology, such as perennial banana systems (Tixier et al. 2004). These structural heterogeneities complicate diagnosis in such scenarios, as the choice of relevant indicators of crop status is not straightforward. It is therefore advisable, in such cases, to use models taking these heterogeneities into account (such as that of Lamanda et al. 2006 and Tixier et al. 2004).

3.2.3 Methods for Establishing Quantitative Relationships Between Limiting Factors, Indicators and Yield in a Field Network

Identifying and ranking limiting factors is often based on the definition of a model relating growth, yield or yield components to limiting factors. In this approach, it is necessary (1) to define the mathematical function relating growth, yield or yield components to the limiting factors, (2) to estimate the parameters of the model equations from data, and (3) to choose a procedure for selecting the most influential limiting factors and excluding those with no significant effect. Various statistical methods can be used to achieve this end. The most popular involves the use of a linear model to estimate the parameters by least squares methods and to select the limiting factors by stepwise regression: see David et al. (2005a), Le Bail and Meynard (2003), and Mercau et al. (2001) for recent examples. The main advantage of stepwise regression is that the various limiting factors are ranked according to their contribution to overall yield variability. However, this method can account for only a fraction of the complexity of the cropping system: few, if any, interactions between limiting factors are considered and the limiting factors are assumed to be linearly related to growth or yield components and to be additive, which is not always the case, as shown by Lecomte (2005) through an agronomic diagnosis on a trial network.

Other statistical methods can be used. Principal component analysis with instrumental variables (PCAIV, Lebreton et al. 1991) was used by Van Keer (2003) to identify limiting factors for upland rice yields in farmers' fields in northern Thailand. PCAIV allows the simultaneous analysis of two multivariate data matrices (for upland rice yield components in Van Keer's study) and an independent matrix including all the measured crop environmental and management variables. Finally, Cade et al. (1999) suggested using quantile regression techniques to analyse the relationships between plant characteristics and one or several limiting factors, but this method has not yet been used in RAD. Whatever the statistical method used, one of the major concerns is that the final estimation and interpretation of parameter values and model predictions are generally based only on the selected model. Uncertainty in the selection of the model and in parameter estimation is basically ignored once a final set of variables has been identified (Draper 1995; Chatfield 1995). However, this selection process may result in highly unstable non-robust results. More attention should be paid to this problem in the future.

Affholder et al. (2003) proposed an alternative method based on the use of a crop model to identify the causes of differences between potential and actual yields for maize production in central Brazil.

This method involved generating a virtual experiment for each field situation, to estimate the extent to which yield is affected by a given constraint considered by the model. According to Lecomte (2005), if a limiting factor is present in all the fields of a network and published studies provide no threshold value above which yield is affected, the only way to identify and quantify the effect of that factor is to use a model. However, Affholder et al. (2003) pointed out that the main problem is building a model that can take into account the exhaustive list of limiting factors likely to occur in the study area.

3.3 Connecting RAD to Other Research and Development (R&D) Actions

3.3.1 Implications for the RAD Framework

The major aim of RAD is to identify and rank the elements of cropping systems responsible for variations in crop performance. It is often the first step in a research and development (R&D) project aiming to improve cropping systems or farming systems on the regional scale. In such cases, RAD must be coupled with methods developed to provide an understanding of farm variability on the regional scale (see, for example, Rapey et al. 2001; Maton et al. 2005). This approach makes it possible to target technical options and to adapt dissemination to diverse farming systems. For example, David et al. (2005a) have been running a research and development project in south-eastern France since 1998, to improve agronomic conditions in organic cereal farming systems. Indeed, they selected organic wheat fields for RAD based on criteria designed to represent the diversity of organic farm types and of agronomic, edaphic and climatic conditions. The farm network was selected on the following criteria: (1) the main characteristics of the farming system: mixed vs. arable, (2) the significance (% area and % profit) of the organic cereal sector within the farm, from 5% to 80%, and (3) the time period over which the farm had been managed organically. The field network was selected from the farm network on the following criteria: (1) crop rotation and preceding crop, and (2) soil type. RAD identified the most limiting factors and facilitated adaptation for further experimental trials, followed by recommendations for the various farming systems. Another example is provided by the work of Trébuil et al. (1997), who took into account differences in land use between farmers when choosing the fields for their network.

3.3.2 Value of Combining RAD with Additional Research Work

The recommendations resulting from RAD are only relevant if the improvements suggested by agronomists are compatible with the way in which the farmers make their technical decisions (Cerf and Sebillotte 1988; Aubry et al. 1998; McCown 2002). It is therefore useful to combine RAD with a good knowledge of farmers' decision rules before defining recommendations. For instance, Meynard (1985, 1986) identified nitrogen deficiencies at the beginning of stem elongation due to delays in fertiliser application as a major factor limiting wheat yield in northern France. He analysed the work schedules and showed that these delays were due to competition with sugar beet drilling. Changes in work organisation therefore provided the solution to the problem of N deficiency in wheat (Meynard 1986). Mathieu (2005) recently carried out a similar combined analysis for sorghum transplanted in the dry season in northern Cameroon. RAD on sorghum yield identified stem borer attack, weed infestation and water stress as major factors affecting yield. A simultaneous analysis of farmers' decision rules for crop planting and weed control made it possible to produce references not only for alternative techniques to exclude limiting factors or to reduce their effect, but also concerning the compatibility of these techniques with farm management. This work therefore led to the construction of analytical tables defining adapted crop management, which can be used by agricultural advisors considering individual farmers' situations. This work could be extended to the linking of RAD results and social modelling through multi-agent models, making scenario testing, prognosis and extrapolations possible.

The rapid identification by RAD of major limiting factors makes it possible to initiate additional studies enlarging the impact of RAD, even before the RAD has been entirely completed. An example is provided by the work of David (2004), analysing low yields and protein contents in organic wheat. RAD rapidly showed that nitrogen deficiency largely accounted for poor performance. Researchers have developed solutions for improving fertiliser efficiency in the springtime (i.e. delaying and fractioning applications to improve the synchronisation of wheat nitrogen requirements with organic fertiliser mineralisation, and adapting N application to soil water availability) through the use of a crop model parameterised and evaluated in parallel with the RAD (David et al. 2005b). This model makes it possible to adjust the recommended nitrogen fertilisation strategies according to recent weather events, the characteristics of the field linked to its soil and cropping system, and the incidence of other limiting factors, such as weeds, pests and diseases. Clermont-Dauphin et al. (2003) also highlighted the value of combining RAD with agronomic model-building for decision-making tools: the diagnosis ranks the limiting factors included in the model, making it possible to suggest innovative cropping methods (design step). This may make it necessary to modify the diagnostic process, including some technical variants for each field in the network so as to provide references for modelling. For example, modelling of the response of field bean crops to fertiliser in Haiti led to double diagnosis on farmers' fields, which were typically unfertilised, in the presence and absence of fertiliser (Clermont-Dauphin et al. 2003, 2004a).

3.3.3 Exchanges with Farmers in RAD

The use of RAD in R&D operations leads to exchanges with farmers and their advisors. It is important for researchers to integrate the farmers' knowledge into their hypotheses. During sampling of the field network, knowledge about cropping history and farm constraints is very useful. During data collection in the field, farmers' observations have, in some cases, led to the measurement of additional variables, which turned out to be valuable. Finally, when the researchers present the results to farmers, the observations of the farmers concerning their fields may assist researchers in their interpretation of the data. However, the many exchanges between researchers and farmers during RAD may give rise to unexpected complications or valuable results. If, during the study, the RAD is accompanied by frequent consultation with the farmers, then the farmers may rapidly make use of some or all of the results, altering their practices in real time without waiting for the end of the RAD. As a result, the diagnosis is made on constantly changing cropping systems. This hinders global analysis of the pluriannual network, but transforms diagnosis for the last few years into an evaluation of technical proposals based on the first years' diagnosis. This process was observed in the study conducted by Le Bail and Meynard (2003) on the variation in yield and protein content in malting barley, in which the frequency of fields with a low yield and/or very high protein content fell markedly from the first year to the third year of study. This effect was attributed to a sharp reduction in the average amount of nitrogen fertiliser applied and to a change in the choice of the crop preceding barley in the rotation, which previous results of diagnosis had shown to be determining factors. In this case, solutions based on the results of the RAD were implemented by farmers before completion of the RAD.

4 Discussion

The RAD method, as presented by Doré et al. (1997), and its extensions, as reviewed here, must be compared with other means of identifying and interpreting variations in yield (or other agronomic variables) on a regional scale – i.e. other diagnosis methods. The two most common alternative approaches are compared with RAD in Table 1. The first ("oral diagnosis") involves asking farmers directly for their opinions concerning the reasons for these variations. This participative method involves a system-based diagnosis of the farmers' problems (Singh 2004) and was used in the studies by Ingle et al. (2000) and Kataki et al. (2000). The major advantage of this method is its rapidity, because no measurements are required. After interviewing each farmer for just a few hours, this method can be used to attribute yield variations to specific effects of climate, soil and cropping systems. The main drawback of this system is that it depends on the farmers' expertise concerning agronomic processes. However, farmers may not always have sufficient technical knowledge to support their hypotheses, particularly if the cropping system is frequently modified. Yield losses due to soil compaction or soil-borne disease are, for example, commonly underestimated by farmers.

The second method ("correlative diagnosis") involves analysing correlations, in a large sample of fields, between the yield or yield-gap and cropping system, soil permanent characteristics and weather

Table 1 A comparison of different on-farm diagnosis methods used to identify and rank the cropping practices responsible, in interaction with the environment, for the variability in crop production, crop quality and environmental impacts in a region

	Regional agronomic diagnosis	Oral diagnosis	Correlative diagnosis
Analysis of the relationships between farmers' practices and agronomic or environmental variables	Systemic functional analysis of the interactions between cropping practices, the crop and its environment	Farmers' expertise	Statistical correlations or factorial analysis on yield and crop management
Criteria for designing field networks	Representing the diversity of existing systems and environments	No specific network of fields	Representing the diversity of existing systems and environments
Data to be recorded on each field	On each field of the network, yield, farmers' practices, indicators providing information about the environment and crop status	Farmers' opinions about limiting factors and agronomic problems	On each field of the network, yields and farmers' practices
Cost in time and money	High	Low	Low

features. This method has been used in many studies involving various statistical tools. In some of these studies, such as those by Naidu and Hunsigi (2003) on sugarcane, parallels were observed between variations in yield and crop management practices (fertilisation practices in this case). Other studies, such as that by Hussain et al. (2003), compared different linear and non-linear models, whereas others, such as that by Casanova et al. (1999), compared different procedures (simple correlation, stepwise regression and the boundary line method). This diagnostic method, which, unlike RAD, does not use data on soil and crop status, is also very cheap and not particularly time-consuming, as the data required may be readily obtained from each farm by interview or mail (unless complete soil data are collected, as in the study by Casanova et al. 1999). The main drawback of this method is that significant correlations between two or more variables do not always reflect causal relationships. As the different aspects of cropping systems are closely associated, two variables are often found to be linked solely because both are linked to a third variable. Thus, even strong statistical correlations often reflect coincidence rather than a true functional relationship. Different solutions to this problem have been tested. Calviño and Sadras (2002) applied the method to wheat yield in Argentina. They did not consider actual yield, focusing instead on the difference between actual yield and the yield simulated with a water-stress model. This approach makes it possible to identify limiting factors other than those taken into account by the model. Lobell and Ortiz-Monasterio (2006) recently investigated the value of data concerning the spatial distribution of yields for analyses of the causes of yield variability at the landscape level. This promising approach combined stochastic crop models for translating assumed spatial patterns of soil and management conditions into spatial patterns of yield and Monte Carlo simulation, repeating the process for many different sets of conditions. It resulted in a modelled relationship between yield patterns and the relative importance of soil and management yield constraints. Based on this relationship, it was then possible to infer from observed yield patterns the proportion of yield variability accounted for by soil and management. However, this method depends on the quality of the crop model used and the availability of precise soil and climate data for a large number of fields. Nevertheless, this approach, making use, through the crop model, of information about the functional relationships between crop, soil and crop management, converges with that of RAD, which is becoming increasingly reliant on the use of models.

RAD seems to have an advantage over oral and correlative diagnosis in terms of the robustness of the relationships it reveals between cropping systems and agronomic variables, as the analysis is based on soil and crop status data from farmers' fields. It is not possible to exclude confounding factors entirely, but this method does at least reduce the risk of their occurrence. The major drawback of RAD remains its higher cost in terms of both time and money. RAD facilitates more effective use of current progress in agronomy than oral diagnosis, as illustrated by the frequent use of updated agronomic models at various stages of RAD.

RAD is based on an analysis of the functioning of the agro-ecosystem, making it a particularly powerful investigative tool. Agronomic diagnosis can also be performed on networks of field experiments, increasing understanding of the bases of the experimental results observed and improving assessments of the validity of the results (Meynard 1985; Dejoux et al. 2003; Barbottin et al. 2005). For example, Barbottin et al. (2005), whilst characterising the performance of wheat cultivars in different environments, carried out a diagnosis on cultivar trials to analyse variability among genotypes in nitrogen remobilisation to grains. Separate analyses of typified environments with no limiting factors and environments subject to major abiotic or biotic limiting factors demonstrated the absence of a genotypic effect on remobilisation in favourable growing conditions, and a marked genotypic effect in the presence of airborne diseases.

Whatever the method used and the variable studied (yield, quality or any other variable), diagnosis work often makes it possible to define new research priorities when it reveals influences of cropping systems on little-studied variables of agronomic interest. Thus, at the International Rice Research Institute, diagnosis has made it possible not only to identify and to rank problems responsible for poor yields in peasant rainfed rice systems in Laos and Cambodia, but also to open up possibilities for initiating new research (Fujisaka 1991; Fujisaka et al. 1994). Other examples can be found elsewhere in the world (Castella et al. 1997; Caldiz et al. 2002; Kudadjie et al. 2004). Diagnosis often helps to increase our knowledge of the agro-ecosystem and understanding of the diversity of the cropping systems present within a region, although these are not the primary objectives of this approach. Generally, diagnostic work demonstrates the heuristic value of carrying out part of the agronomic research directly in farmers' fields. This type of approach also efficiently increases the skills of the agronomist. In learning this approach, agronomists become familiar with an approach to the complex systems typical of real agriculture, the study of which cannot always be reduced to the "all other things being equal" comparisons of factorial experimentation. Moreover, diagnosis is based on an inductive method of reasoning, in which the aim is to work back to the causes of the observed results. This is at least as much a part of the duty of agronomists involved in R&D as the more usual hypothetical and deductive reasoning.

5 Conclusion

Over the past 10 years, the scope of RAD has been enlarged and its methods improved. Although time-consuming, RAD appears to be a useful complementary approach to research station experiments. Together with other on-farm programmes, RAD makes use of the data gathered in agricultural situations, whereas analytical experiments serve as an essential source of knowledge about the agrosystem. Efforts are continuing to improve certain aspects of the methods. Two questions in particular require additional detailed research. These questions concern the rules to be used for optimising the number and choice of fields for RAD and the possible use of remote-sensing data to reduce the cost of RAD and improve its efficacy.

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Ex ante Assessment of the Sustainability of Alternative Cropping Systems: Implications for Using Multi-criteria Decision-Aid Methods – A Review

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Abstract Sustainability is a holistic and complex multi-dimensional concept encompassing economic, social and environmental issues, and its assessment is a key step in the implementation of sustainable agricultural systems. Realistic assessments of sustainability require: (1) the integration of diverse information concerning economic, social and environmental objectives; and (2) the handling of conflicting aspects of these objectives as a function of the views and opinions of the individuals involved in the assessment process. The assessment of sustainability is therefore increasingly regarded as a typical decision-making problem that could be handled by multi-criteria decision-aid (MCDA) methods. However, the number and variability of MCDA methods are continually increasing, and these methods are not all equally relevant for sustainability assessment. The demands for such approaches are also rapidly changing, and faster ex ante assessment approaches are required, to address scales currently insufficiently dealt with, such as cropping system level. Researchers regularly carry out comparative analyses of MCDA methods and propose guidelines for the selection of a priori relevant methods for the assessment problem considered. However, many of the selection criteria used are based on technical/operational assumptions that have little to do with the specificities of ex ante sustainability assessment of alternative cropping systems. We attempt here to provide a reasoned comparative review of the main groups of MCDA methods, based on considerations related to those specificities. The following main guidelines emerge from our discussion of these methods: (1) decision rule-based and outranking qualitative MCDA methods should be preferred; (2) different MCDA tools should be used simultaneously, making it possible to evaluate and compare the results obtained; and (3) a relevantly structured group of decision-makers should be established for the selection of tool variants of the chosen MCDA methods, the design/choice of sustainability criteria, and the analysis and interpretation of the evaluation results.

Keywords Cropping system • Decision rules • Multi-criteria decision aid • Outranking qualitative methods • Qualitative information • Sustainability assessment

1 Introduction

The precise meaning of *Sustainable Agriculture* is far from clear (e.g. Hansen 1996; Smith and McDonald 1998; Pannell and Schilizzi 1999; Rigby and Caceres 2001), but efforts have been made to produce an integrated definition of this term. According to Ikerd (1993), *Sustainable Agriculture* should be capable of maintaining its productivity and usefulness to society in the long term. This implies that it must be environmentally sound, resource-conserving, economically viable and socially supportive. Based on this definition, economic, environmental and social objectives should be analyzed as the principal dimensions of sustainability when sustainable practices are implemented in a given agricultural system (Schaller 1993;

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Vereijken 1997; den Biggelaar and Suvedi 2000; Gafsi et al. 2006). If these objectives are to be considered together, then knowledge and research from relevant disciplines must be integrated while handling a mixture of multiple long-, short-term, interacting and potentially conflicting goals, depending on the scale on which sustainability is considered (farm, landscape, region, nation, group of nations or global; Kruseman et al. 1996; Meyer-Aurich 2005).

Assessing the sustainability of agricultural systems is a key issue for the implementation of policies and practices aiming at revealing sustainable forms of land use (Neher 1992; Sulser et al. 2001; Pacini et al. 2003). However, if they are to be realistic and effective, such assessments must handle the complexity of the concept of "sustainability", as described above, whilst taking personal and subjective views concerning the relative importance of priorities into account (Dent et al. 1995; Park and Seaton 1996; Andreoli and Tellarini 2000). The assessment of sustainability is therefore increasingly regarded as a typical decision-making problem, leading to the development, by some researchers, of sustainability assessment decision-aid methods. Most of these approaches are based on multi-criteria decision-aid (or making) methods (MCDA or MCDM), and some have resulted in prototype sustainable solutions in the field (Rossing et al. 1997; Zander and Kächele 1999; Loyce et al. 2002a,b; Dogliotti et al. 2005).

However, in practice, such assessments are confronted with two major problems. Firstly, the number of MCDA methods and tools available is continually increasing (Bouyssou et al. 1993, 2000, 2006), and studies aiming to assess the sustainability of agricultural systems rarely justify clearly their choice of one MCDA method over another. Only a few studies have presented a comparative, or at least exploratory, evaluation of the principal MCDA approaches available, in terms of the relevance to the purposes of the assessment. In contrast, many authors have concluded that, in typical decision-aid problems, there is rarely one ideal method and a group of MCDA methods should therefore be applied (Salminen et al. 1998; Zanakis et al. 1998; Macharis et al. 2004; Wang and Triantaphyllou 2006). Moreover, the guidelines emerging from comparative studies are generally developed within the operational research community, based on technically oriented arguments and criteria from this field of research (see Guitouni and Martel 1998 for review) without considering constraints related to the application domain. It should also be noticed that although some general guidelines, concerning specific features of sustainability assessment in most cases, have been developed (Rehman and Romero 1993; Munda et al. 1994, 1995; Munda 2005), they are still rarely followed explicitly in real-case contexts.

Secondly, demand is increasing among farmers' groups and policy-makers for more innovative sustainability assessment, highlighting a need for (1) faster ex ante assessment approaches for rapidly identifying alternative systems without assessing the entire initial systems in the field (European Commission 2005; Van Ittersum et al. 2008), and (2) the expansion of sustainability assessment to scales rarely studied at the moment, such as the cropping system scale. Indeed, most published studies have been carried out on a plot scale or on an even larger scale: farm, landscape, state or nation (Bontkes and van Keulen 2003; Meyer-Aurich 2005). A cropping system consists of a set of management procedures applied to a given, uniformly treated agricultural area, which may be a field, part of a field or a group of fields (Sebillotte 1990). A given farming system may therefore be composed of a group of cropping systems, the sustainability assessment of which is potentially relevant, as they represent different, uniformly treated units. However, few published studies have described sustainability assessment explicitly at the level of the cropping system, with a given MCDA method (Mazzetto and Bonera 2003), and those dealing with assessments at this level carried out no initial comparative assessment of MCDA methods.

The major aim of the paper is to provide a comparative review of the main families of MCDA methods, based on criteria related to the specificities of the sustainability assessment, for the a priori selection of groups of candidate MCDA methods for ex ante assessment of the sustainability of alternative cropping systems. Fine-tuning the selection process to the scale of individual methods is beyond the scope of our review, as there are dozens of algorithms/tools available in the literature and probably as many selection criteria which are set in a more technical background. A second aim of the study is thus to provide suggestions regarding the participatory process to be followed by the decision-makers, starting from the final MCDA tool choice to the analysis/interpretation of the ex ante assessment results.

2 Overview and Taxonomy of Multiple Criteria Decision-Aid Methods

Multiple-criteria decision aid (MCDA) is a research area within the field of decision analysis (DA), which aims to develop methods and tools to assist with decision-making, particularly in terms of the choice, ranking or sorting of options (alternatives, solutions, courses of action, etc.), in the presence of multiple, and often conflicting criteria (Zanakis et al. 1998; Figueira et al. 2005). MCDA methodology can be seen as a nonlinear recursive process including four main steps: (1) structuring the decision problem, (2) articulating and modeling the preferences, (3) aggregating the alternative evaluations (preferences), and (4) making recommendations (Roy 1985; Maystre et al. 1994).

MCDA methods have developed considerably over the last 30 years, resulting in a large number of methods and tools (Figueira et al. 2005). This has resulted in a need for the comparison of MCDA methods, to identify the most appropriate methods for the decisional problem considered (Zanakis et al. 1998; Brunner and Starkl 2004). Many authors have stressed the need for a taxonomy of MCDA methods, as a starting point for the selection process (MacCrimmon 1973; Hwang and Yoon 1981). Dozens of taxonomies are currently available, based on a number of criteria, including:

- The number of alternatives considered: discrete vs. continuous distribution of alternatives (Schärlig 1985; Maystre et al. 1994).
- Information measurement level of criteria – qualitative vs. quantitative, and the level of uncertainty (Munda et al. 1994, 1995).
- The methods used to construct the preference model: mathematical decision analysis approach vs. artificial intelligence approach (Nijkamp and Vindigni 1998; Figueira et al. 2005).
- The criteria aggregation mode: complete, partial or local aggregation (Schärlig 1985; Maystre et al. 1994; Vincke 1989).
- The degree of compensation between the criteria (Hayashi 2000).
- The descriptive, prescriptive, constructive or normative nature of decision-making (Bouyssou et al. 2006).

One of the most integrative taxonomies was established by Hwang and Yoon (1981). This taxonomy distinguished between multiple-objective decision-making (MODM) and multiple-attribute decision-making (MADM) methods, within the MCDA area. MODM methods can be used in cases in which there are an infinite (continuous) or large number of alternatives. They are based on multiple-objective mathematical programing models, in which a set of conflicting objectives is optimized and subjected to a set of mathematically defined constraints, for selection of the "best" alternative. MADM methods are used in cases of discrete, limited numbers of alternatives, characterized by multiple conflicting attributes (criteria). They are based on (1) the aggregation of judgments for each criterion and alternative, and (2) the ranking of the alternatives according to the aggregation rules. MCDA, as used in many published studies, generally refers only to MADM, mainly because of the great number of methods of this type available. Indeed, a review of the literature spanning the last 25 years revealed an increasing number of new and hybrid MADM methods, leading to a great variability in taxonomies (Schärlig 1985; Roy 1985; Vincke 1989; Nijkamp et al. 1990; Roy and Bouyssou 1993; Maystre et al. 1994; Bouyssou et al. 2000, 2006; Figueira et al. 2005). Nevertheless, a synthesis of these taxonomies revealed that a majority of the most used MADM methods can fall into one of the following three categories: (1) multi-attribute utility methods, (2) outranking methods, and (3) mixed methods. The boundaries of the latter remain fuzzy in the reviewed literature to a point that led us to provide our own understanding of the term (see Sect. 3.1. for discussion).

2.1 Multi-attribute Utility Methods

These methods are essentially based on multi-attribute utility theory (MAUT, Keeney and Raiffa 1976), which emerges from the philosophical doctrine of Utilitarism. If the decision is made in conditions in which the attributes are known with certainty (deterministic approach), the term "utility" is replaced by "value" (MAVT). The term "utility" is preferred to indicate that the preferences of stakeholders against risk are formally included in the analytical procedure. The MAUT method has three major steps: (1) normalization and evaluation of the performance of each alternative in terms of its utility, (2) identification of the weights statistically representing the decision-maker's priorities for each criterion, and (3) aggregation (based on additive, multiplicative, or other distributional formalisms, Guitouni and Martel 1998) and ranking of the various alternatives.

The analytic hierarchy process (AHP) is another major approach first developed by Saaty (1980), based on the same aggregation principles as MAUT, but differs from the latter with respect to the way the decisional problem is handled. The AHP comprises four major steps:

1. Disaggregating a complex problem into a hierarchy, in which each level consists of specific elements. The overall objective of the decision lies at the top of the hierarchy, and the criteria, sub-criteria and decision alternatives are placed at descending levels in this hierarchy.
2. Pair-wise comparisons between all elements at the same level, based on a method converting verbal and subjective assessments into a set of overall scores or weights. The conversion depends on the decision-maker's answers to questions of the general form: "How important is criterion A relative to criterion B?" A verbal scale is then used to translate the response into a score from 1 to 9. All pair-wise comparisons between single objects are used to constitute a pair-wise comparison matrix.
3. Checking the consistency of the matrix and deriving priorities from it.
4. Aggregation of criteria, with the help of a given additive or multiplicative utility function.

2.2 Outranking Methods

Outranking methods are based on social choice theory. These methods lack the axiomatic basis of multi-attribute utility methods, but are useful in practice (Guitouni and Martel 1998). "Outranking" is a concept originally developed by Roy (1985). It involves comparisons between every possible pair of options considered, to define binary relationships, such as "alternative *a* is at least as good as alternative *b*". Procedures based on outranking have two phases. Decision-makers first provide information about their preferences for individual criteria, in the form of indifference and preference thresholds. Partial binary relationships are then calculated for all criteria, taking into account the inter-criterion preferences expressed in terms of weightings denoting relative importance. These weightings do not represent a trade-off between criteria scores (as in MAUT-based methods), as they are used to combine preference relationships rather than scores of alternatives. The ELECTRE method (*Élimination et choix traduisant la réalité*; Roy 1968) was the first to use an outranking approach. It was followed by many others, including different versions of ELECTRE (II, III, IV, IS and TRI; Maystre et al. 1994) and the PROMETHEE methods (preference ranking organization method for enrichment evaluations; Brans 1982). These methods are based on different preference structures.

2.3 Mixed Methods

Many approaches other than the MADM methods described above have been proposed. Some have been referred to as "non-classical" or mixed. There seems to be no common definition of these terms within the MCDA community (see Munda et al. 1994; Maystre et al. 1994; Guitouni and Martel 1998; Figueira et al. 2005 for comparative review), but we understand these terms to correspond to a group of MADM methods (1) able to handle mixed qualitative-quantitative or qualitative criteria information explicitly, and/or (2) with a preference model different from those classically used for multi-attribute utility and outranking methods.

A first major group of mixed MADM methods consists of outranking approaches handling qualitative or mixed information (Munda et al. 1994; Guitouni and Martel 1998). There are many variants among this group, such as the REGIME methods (Nijkamp et al. 1990), QUALIFLEX (Paelinck 1978), ORESTE (Roubens 1982), EVAMIX (Voogd 1983), MELCHIOR (Leclerc 1984) and ARGUS (de Keyser and Peters 1994).

A second group consists of decision rule-based approaches, which are often generically referred to as "expert systems" (Kim et al. 1990). These methods were initially developed for the assessment of complex situations that cannot be handled through preference models based on conventional mathematical tools (means, sums, simple weighting and complex models;

Tixier et al. 2007). In these methods, the preference model can be constructed through learning from examples. The global preference is defined by sorting the objects of analysis into predefined categories (e.g. acceptance, rejection) through a set of logical statements, typically representing "if/then" decision rules, which are often organized in the form of decision trees or decision tables. These decision rules are formulated on the basis of expert factual-heuristic knowledge (derived from interviews and literature) and/or with the help of data-mining and knowledge discovery tools (Kim et al. 1990; Pawlak 1991; Zupan et al. 1999).

3 Selection of Multiple Criteria Decision-Aid Methods for Ex ante Assessment of the Sustainability of Cropping Systems

3.1 Relevance of MODM Methods

Most of the decision-aid approaches developed for assessing the sustainability of agricultural systems have classically been based on multiple-objective decision-making methods (MODM) (Meyer-Aurich 2005). These methods are often implemented within some "systems approach" frameworks consisting of (1) systematic and quantitative analysis of agricultural systems for the mathematical definition of objectives and constraints, and (2) the synthesis of optimal "solutions", using optimization techniques (Rossing et al. 1997; Zander and Kächele 1999; Ten Berge et al. 2000; Kropff et al. 2001; Hengsdijk and van Ittersum 2002; Bontkes and van Keulen 2003; Dogliotti et al. 2005). The ex ante evaluation of innovative cropping system sustainability poses two major problems for MODM methods based on optimization techniques:

1. These methods are known to be sensitive to missing, inconsistent or mixed (quantitative and qualitative) data (Dent et al. 1995; Weersink et al. 2002; Dogliotti et al. 2005). In typical ex ante assessments of sustainability – particularly on the cropping system scale, the assessment of which has not been extensively documented – there is unlikely to be sufficient scientific and/or expert quantitative knowledge available. Furthermore, as innovative demands cannot generally be systematically translated into scientific and/or quantitative data, the use of qualitative information in the assessment process is likely to be necessary. In addition, the use of qualitative data is increasingly considered a rule rather than an exception for the realistic assessment of the holistic environmental and socioeconomic issues underlying sustainability (Maystre et al. 1994; Munda et al. 1995).
2. These methods are mostly required in cases in which "infinite" alternatives must be assessed to identify the "optimal" option (Steuer 1986; Zhou et al. 2006). In our case, we are more likely to be assessing a finite number of alternative cropping systems, ranking them in terms of their potential sustainability. Such rankings allow a more extensive comparative analysis of the outputs of different assessment methods, potentially identifying promising alternatives not initially highly ranked.

Both these issues highlight the need for more appropriate and realistic approaches to the ex ante assessment of sustainability on the scale of the cropping system. The specificity of the sustainability assessment problem, with the implied need for MCDA approaches other than classical MODM methods, has already been highlighted by many authors (Voogd 1983; Nijkamp et al. 1990; Munda et al. 1994; Nijkamp and Vindigni 1998). Below, we will define selection criteria for identifying the most relevant of these methods for our purpose.

3.2 Criteria for Selecting Relevant MADM Methods

We consider here two groups of criteria for identifying relevant approaches from the many MADM methods, based on the recommendations of Munda et al. (1994, 1995): (1) the ability of these methods to handle the typical multi-dimensional characteristics of sustainability assessment, and (2) their ability to handle mixed measurement levels of criteria.

The need for methods to handle multi-dimensional characteristics translates operationally into three requirements: (1) incommensurability – an absence of the need for a common measure aggregating several dimensions, (2) non-compensation – an advantage in one dimension of the evaluation is not totally offset by a disadvantage, and (3) incomparability – the method does not offer a single comparative term by which all alternatives could be ranked (Schärlig 1985; Maystre et al. 1994; Stewart and Losa 2003). In realistic evaluations of the sustainability of agricultural systems, tackling environmental, social and economic dimensions, translates then into the fact that strong assumptions about the commensurability, compensation and comparability of values may not be relevant, as the criteria for different sustainability dimensions may have different units with low levels of trade-off (O'Neill 1997; Martinez-Alier et al. 1998). The notions of commensurability, compensation and comparability are interconnected, in that strong commensurability implies full compensation of criteria and a high level of comparability of the actions considered. They are therefore not considered here to be independent selection criteria.

The second recommendation concerns the ability of these methods to handle heterogeneous measurement levels of criteria information (i.e. quantitative vs. qualitative) and their uncertainty (Munda et al. 1994, 1995). We will not consider the ability of MADM methods to handle uncertainty in this review. This criterion does not seem to be discriminatory, as almost all MADM and MODM methods can be linked to a procedure handling fuzzy or stochastic uncertainty (Chen and Hwang 1992; Munda et al. 1995; Ertugrul Karsak 2004). The suitability of each of the MADM methods considered will therefore be assessed for the interconnected characteristics of incommensurability, non-compensation and incomparability, together with their ability to handle qualitative or mixed criteria explicitly. Based on these criteria, we discuss in detail below the assessment process for each of the three groups of considered MADM methods identified in Sect. 1. The results are summarized in Fig. 1.

3.2.1 Multi-attribute Utility Methods

Classical MAUT/MAVT methods are based on (1) a totally compensatory aggregation of criteria, and (2) commensurable judgments, resulting in high levels of trade-off between criteria. Consequently, it is difficult, with most of these methods, to take into account the incommensurable and partly compensatory criteria that often underlie the dimensions of sustainability in agricultural systems (Rehman and Romero 1993). Furthermore, these methods do not take qualitative or mixed (qualitative and quantitative) criteria into account efficiently and explicitly (Munda et al. 1994). Consequently, although some authors have reported the use of MAUT methods for some agricultural and environmental assessments (Salminen et al. 1998;

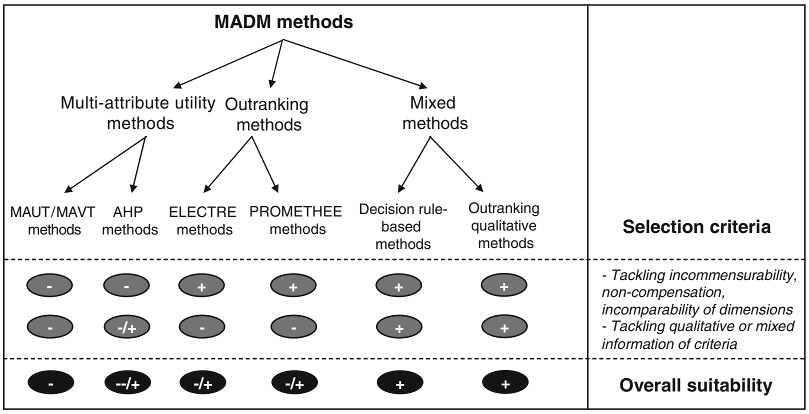

Fig. 1 Taxonomy of multiple-attribute decision-making (MADM) methods and selection criteria used for the identification of suitable approaches for ex ante assessment of the sustainability of alternative cropping systems
Gray symbols are given (–; - -/+; / –/+; +) to indicate the a priori irrelevance, partial relevance or relevance, respectively, of each group of methods based on the selection criteria considered. Overall assessments are given for each group of methods, in the form of dark symbols (–; –/+; –/+; +) indicating the overall level of suitability (ranging from non-suitable to suitable) for ex ante assessment of the sustainability of alternative cropping systems. *MAUT* multi-attribute utility theory, *MAVT* multi-attribute value theory, *AHP* analytic hierarchy process, *ELECTRE* élimination et choix traduisant la réalité, *PROMETHEE* preference ranking organization method for enrichment evaluations

Hayashi 2000), these models may be considered inappropriate for assessment of the sustainability of alternative cropping systems, according to our objectives (Fig. 1).

AHP methods offer alternative advantages compared with classical multi-attribute utility methods, consisting of (1) the hierarchical decomposition of the decisional problem, and (2) the use of subjective and verbal expressions to define the relative importance of the criteria (Macharis et al. 2004). Some authors consider most AHP models to be able to handle missing quantitative data and a lack of precision, based on the judgment and experience of decision-makers, making it possible to prioritize information to improve decisions (Alphonce 1997). This may explain why AHP is the most used MAUT-based method for solving agro-environmental decisional problems, mainly ex ante. Indeed, some authors have used AHP models to choose crops so as to determine the best allocation of resources (Alphonce 1997), to evaluate soil productivity (Zhang et al. 2004) and to assess the environmental, economic and social factors relating to the adoption of silvopasture techniques in south-central Florida (Shrestha et al. 2004). Other authors have used this method to rank alternatives for preserving or increasing social benefits from the sustainable use of natural resources, such as forestry management (Schmoldt 2001), wetland management (Herath 2004) and land preservation (Duke and Aull-Hyde 2002).

The main drawbacks of the AHP method are potential internal inconsistency and the questionable theoretical basis of the rigid 1–9 scale, together with the possibility of rank reversal following the introduction of a new alternative (French 1988; Goodwin and Wright 1998; Macharis et al. 2004). Alternative methods, such as the MACBETH method (Bana e Costa and Vansnick 1997) have been developed to overcome some of these objections. However, one of the most often-cited objections to AHP methods is the totally compensatory aggregation procedure, resulting in a high level of trade-offs between criteria (Roy and Bouyssou 1993; Macharis et al. 2004). Thus, as for other MAUT approaches, the use of an AHP method may limit, to some extent, the possibility of taking into account incommensurable and partly compensatory criteria, which often underlie the concept of "sustainability", as applied to agricultural systems. Moreover, although the method uses verbal scales, it is not considered truly qualitative (Munda et al. 1994). Instead, it is described as semi-qualitative (Ayalew et al. 2005) or purely quantitative (Moffett and Sarkar 2006). Consequently, attention must be paid to these advantages and disadvantages if an AHP method is used alone for ex ante evaluation of the multi-dimensional sustainability of alternative cropping systems (Fig. 1).

3.2.2 Outranking Methods

The key feature of outranking methods, due to the vague determination of preferences, is that they give low levels of comparability between options (i.e. performance not measured on the same cardinal scale), making it possible to deal with incommensurability (Maystre et al. 1994). This advantage may account for their widespread use in agricultural-environmental sustainability evaluation frameworks, on different scales, for dealing with problems of choice between alternatives. At policy level, the ELECTRE and PROMETHEE methods have been used to rank different projects for environmental conservation and the sustainable use of resources for several specific problems: irrigation management planning (Pillai and Raju 1996), the conservation of multifunctional forests (Kangas et al. 2001), waste management (Salminen et al. 1998) and environmental quality (Rogers and Bruen 1998). On the farm and cropping system scales, outranking methods have been used successfully more frequently than other MADM methodologies for various assessments. Van Huylenbroeck and Damasco-Tagarino (1998) used the PROMETHEE method to choose the best crops for the ideal cropping timetable for the farmer. Arondel and Girardin (2000) used an ELECTRE method to sort cropping systems on the basis of their impact on groundwater quality. Loyce et al. (2002a,b) developed an ELECTRE-based method (the BETHA system) for assessing winter wheat management plans with respect to a set of conflicting economic, environmental and technological requirements. Mazzetto and Bonera (2003) developed a multi-criteria software package derived from the ELECTRE method (MEACROS), with the aim of identifying alternative cropping systems meeting a set of technical, economic and environmental criteria. These examples highlight the possibility of using outranking methods for ex ante evaluation of the sustainability of cropping systems, based on their ability to tackle the incommensurability, non-compensation and incomparability of the sustainability dimensions more efficiently than classical multi-attribute utility methods. However, it has never been clearly stated that these outranking methods – in their strictest definition – are compatible with the use of explicitly qualitative or mixed (qualitative/quantitative) criteria. Consequently, this possible limitation should be borne in mind when selecting an appropriate outranking MADM method for the ex ante assessment of alternative cropping systems (Fig. 1).

3.2.3 Mixed Methods

Outranking Qualitative Methods

Given the ability of these methods to tackle incommensurability, non-compensation and incomparability of the sustainability dimensions while handling qualitative criteria, they should be considered potentially relevant for ex ante assessment of the sustainability of cropping systems. However, it is noteworthy that though many of these methods, and especially the REGIME approach (Nijkamp et al. 1990), are regularly used for environmental planning and management purposes, such approaches are rarely applied in the agricultural sector. Nevertheless, the successful use of a REGIME method in a real case for evaluation of the sustainability of agricultural land use in terms of environmental, economic and social objectives reported by Hermanides and Nijkamp (1997) is another argument in favor of its use for ex ante assessment of the sustainability of cropping systems.

Decision Rule-Based MADM Methods

These non-classical methods are considered potentially relevant for the solution of various agricultural decisional problems (Dent et al. 1995). Indeed, the decision rules approach (1) is intelligible and uses the language of the decision-maker, through symbolic qualitative variables, (2) is based on transparently expressed preference information based on the observations, views and opinions of the decision-maker, and (3) offers the possibility of handling inconsistencies in preferential information resulting from hesitation on the part of the decision-maker (Bontkes and van Keulen 2003; Greco and Matarazzo 2005). Moreover, decision rule-based methods can be used for the explicit handling of totally non-compensatory decision processes (Ma 2006), making it easier to tackle incomparability and incommensurability (O'Neill 1997; Martinez-Alier et al. 1998; Stewart and Losa 2003). This makes the decision rule approach more flexible for the modeling of the decision process, as it takes into account a large diversity of considerations much more general than those taken into account by all other existing classical decision models used within the MCDA area (Figueira et al. 2005). However, according to Ma (2006), one of the main limitations of this approach is that, in some complex real-life situations, too many decision rules may be required to represent the decisional problem, making this technique cumbersome. Conversely, others would argue that in many real-life situations, particularly those concerning the decisions facing farm households, such approaches are far more realistic and practical than other classical MCDA methods (Dent et al. 1995). In any case, the level of complexity of the decision rules probably depends more on the specific features of the decisional problem considered than on the approach itself.

In agricultural contexts, decision rules have been used for the development of agri-environmental indicators for assessing the sustainability of cropping systems in terms of pesticide impact (van der Werf and Zimmer 1998; Ferraro et al. 2003; Tixier et al. 2007). Those authors combined their expert decision rules with fuzzy logic to cope with uncertainty and to avoiding the effect of a knife-edge limit for a given attribute. On the landscape scale, "classical" expert methods have been used to assess soil erosion risks (Cerdan et al. 2002) and biodiversity (Crist et al 2000). Phillis and Andriantiatsaholiniaina (2001) have developed a more integrative conceptual methodology based on fuzzy expert decision rules for evaluating the sustainability of agricultural systems, according to their economic, ecological and social goals. However, this method did not focus explicitly on the cropping system scale, as it evaluated farming systems on regional and national scales. Agronomy researchers have recently begun to make use of expert tools initially designed for non-agricultural assessment purposes for assessing sustainability-related issues. For example, Bohanec et al. (2004) tested and established the a priori usefulness of the expert tool DEXi for evaluating the ecological and economic sustainability of cropping systems based on genetically modified maize (*Bt*-corn). Such expert tools may thus be relevant for ex ante evaluation of the sustainability of cropping systems (Fig. 1).

As summarized in Fig. 1, this review revealed that the most suitable MADM methodologies for ex ante assessment of the sustainability of alternative cropping systems are of the "mixed" type (qualitative outranking and decision rule-based methods), followed by outranking methods, and then AHP methods, based on criteria presented at the beginning of Sect. 3.2.

4 General Discussion

4.1 Bibliographic Survey and Selection of MCDA Methods: Difficulties Encountered

In this work, we have discussed the pre-selection of families of MCDA methods according to their relevance for the ex ante assessment of the sustainability of alternative cropping systems. We had an idea concerning the strategy to be followed – identification of a relevant taxonomy of methods and of an appropriate set of selection criteria – but the selection process was nonetheless laborious. The laboriousness of pre-selection may explain why so few real-case studies include a comparative or explorative evaluation of the main groups of MCDA approaches available for the specific purposes of the assessment (Zanakis et al. 1998; Hayashi 2000).

The main difficulty in this process was the identification of a relevant taxonomy of MCDA methods. A review of the literature published on MCDA over the last 25 years revealed that this research field has increased in diversity and complexity, leading to an increasing number of new and hybrid methods, resulting in turn in a large number of taxonomies (see Roy 1985 and Figueira et al. 2005 for review). The result was that in work aiming at selecting relevant MCDA methods, the considered taxonomy was often found not to be independent of the views of the authors and the specific purposes of the assessment. This was also the case for more "conceptual" studies proposing formalized typological tree or expert system approaches for the selection of relevant MCDA methods, while initially based on a specifically established taxonomy and thus not appropriate for systematic generalization (Jelassi and Ozemoy 1988; Guitouni and Martel 1998). Another difficulty was that some of these taxonomies were conflicting and even, in some cases, contradictory. This was particularly true for the "mixed" category, the characteristics of which were highly variable, according to the authors' own understanding of this term. For instance, some authors considered the REGIME methods to be mixed (Munda et al. 1995), whereas others considered them to be simple classical outranking methods (Brunner and Starkl 2004). Some authors consider the EVAMIX approach to be a mixed outranking method (Munda et al. 1994), whereas others consider this approach to be neither of the outranking nor of the mixed type (Guitouni and Martel 1998). Similar discrepancies have also been observed regarding AHP methods, which are considered by some authors to be qualitative (Alphonce 1997), whereas others explicitly consider them to be quantitative (Moffett and Sarkar 2006). In each of these situations, our classification is based on the predominant view expressed in published studies, with particular weight given to classifications relating to agro-environmental or environmental sustainability assessment problems (Munda et al. 1994, 1995; Nijkamp and Vindigni 1998).

4.2 Relevance of the Considered MCDA Taxonomy and Selection Criteria

As recommended by Zanakis et al. (1998), our selection was based on a taxonomy serving more as a tool for elimination than for selection of "the right method". Moreover, rather than using selection criteria based exclusively on technical/operational assumptions, we based our criteria on assumptions derived from more realistic situations, reflecting the specific features of the sustainability assessment, as recommended by Munda et al. (1994, 1995). These criteria were then translated into more technical criteria (incommensurability, incomparability, non-compensation; mixed information). Though the considered taxonomy and selection criteria are linked to the specific purpose of our assessment, these two initial steps might serve as guidelines for similar cases.

4.2.1 MADM Vs. MODM

In our process for selecting potentially relevant MCDA methods for ex ante assessment of the sustainability of alternative cropping systems, we first considered one of the most integrative taxonomies within the MCDA area (MODM vs. MADM), to exclude the largest possible group of methods (see Sect. 3.1).

At that stage of MCDA method selection, we were confronted with two opposite approaches within the agricultural sustainability research community. Users of MODM methods claim that only such quantitative methods can disentangle the complex relationships between agricultural production, environment and economy, thereby increasing the transparency of choices regarding sustainability (Hengsdijk and van Ittersum 2002). Similarly, others even consider that the use of expert rules and semi-quantitative indicators in such studies is cause for concern as it is difficult to evaluate such rules, rendering the results of local relevance at best, whereas MODM methods are more effective (Dogliotti et al. 2005). Conversely, some authors consider that realistic assessment of the holistic and uncertain issues of sustainability requires a method capable of handling qualitative information (Munda et al. 1994, 1995; Hermanides and Nijkamp 1997; Phillis and Andriantiatsaholiniaina 2001). Based on these elements and the particular features of ex ante assessment of the sustainability of alternative cropping systems, as stated in Sect. 2.1, we have therefore discarded MODM methods in the selection process.

However, it should be pointed out that, although rejected in this study, some MODM methods have been used for ex ante assessments of alternative farming systems with respect to sustainability-related objectives (e.g. Dogliotti et al. 2004, 2005; Tré and Lowenberg-Deboer 2005). In these cases, the innovative aspect of these systems consisted of the design of sustainable production activities, based on the optimization of an innovative combination of a limited set of quantitatively measurable criteria representing inputs and outputs. This is quite different from considering innovative sustainability issues and objectives directly translated into innovative criteria, some of which cannot be measured quantitatively. For instance, this would be the case for criteria related to (1) holistic issues such as biodiversity, or (2) subjective considerations such as social wellbeing, which are not taken into account in those quoted studies using a MODM method. However, this does not mean that optimization approaches are necessarily unsuitable for purposes similar to ours. Indeed, within the mathematical programing area, optimization algorithms able to handle qualitative criteria have already been developed (Brewka 2006). With new developments continually occurring in the MCDA field, these algorithms are likely to be integrated into MODM methods in the near future, making it possible for these methods to handle qualitative data. Our decision to reject MODM methods regarding our aims and the present state of the art should therefore not be regarded as definitive. It will be reconsidered regularly, based on surveys of future developments within the MCDA area.

4.2.2 Selection from MADM Methods

In this study, we considered an integrative taxonomy of MADM methods, so many methods' variants did not find their way into this review. Indeed, our purpose was to discuss general guidelines for the selection of a relevant MADM method for ex ante assessment of the sustainability of alternative cropping systems, rather than a complete and exhaustive survey of the existing methods and their evaluation for this purpose. In our case, a more detailed comparative review of algorithm variants within each method group would extend far beyond the scope of this paper, as it would require more technical background information and fewer sustainability assessment-related considerations.

We did not consider here selection criteria based on the ability of the methods reviewed to tackle information uncertainty through fuzzy (and/or stochastic) procedures, for two main reasons. Firstly, the uncertainty criterion is not discriminatory, as all the reviewed methods could be coupled to such procedures. Secondly, some authors have argued that (1) there is a lack of convincing evidence that the imprecision captured through fuzzy sets could match the real fuzziness of perceptions that humans typically display with respect to the components of decision problems, and (2) means of calibrating and manipulating fuzzy functions with a transparent rationale from the point of view of non-specialists are lacking (UK DTLR 2001).

Some conceptual selection criteria used in some taxonomies were not considered in this work. For instance, we did not consider the mode of decision-making, which distinguishes between normative (postulation), descriptive (observation), prescriptive (unveiling) or constructive (reaching a consensus) methods (Bouyssou et al. 2006). Indeed, some authors have expressed the view that this classification is not really discriminatory, as in practice, methods initially considered normative may be used in a constructive, descriptive or prescriptive manner, depending on the context in which they are applied (Dias and Tsoukiàs 2003). Nevertheless, to some extent, we have considered (besides criteria specific to ex ante sustainability assessment requirements) a decision-making mode-based selection approach when discarding MODM methods in favor of MADM ones. Indeed, the former aim at reaching one optimal (normative) "solution" (i.e. a cropping system) whereas the latter allow for relative ranking of different ones (see Sect. 2.1), which fitted our objectives much more.

Other reported selection criteria based on operational assumptions, such as transparency, ease of use, profile of the decision-maker and number of decision-makers, were not taken into account in our review, as we consider that (1) some of these considerations depend more on the control and reporting capabilities of the corresponding software/tool than on the method itself, and (2) these considerations are only loosely connected to the specific features of sustainability assessment (as described in Sect. 2.2). Such an assessment would – at least theoretically – address all levels of decision-makers, from stakeholders to policymakers. However, this does not mean that these aspects are of secondary importance in the process of ex ante evaluation of the sustainability of cropping systems. They are simply more relevant to consider in the steps following the pre-selection of relevant MADM methods (see section below).

4.3 Recommended Next Steps

The proposed ranking of candidate MADM methods (Fig. 1) should be considered as a starting point for effective ex ante assessment of the sustainability of alternative cropping systems in order to identify the ones that could be implemented in the field.

Ideally, the next sequence of steps should be closely monitored by a relevant group of decision-makers which should include researchers and other stakeholders who interact following a participatory/cooperative approach. This is essential to avoid a classical researcher-driven process toward a pre-determined direction, which is risky especially for sustainability assessments as those address intrinsically holistic and subjective issues (Brunner and Starkl 2004). This group of decision-makers should then work on the basis of a three-axis framework (Fig. 2), consisting of the following steps:

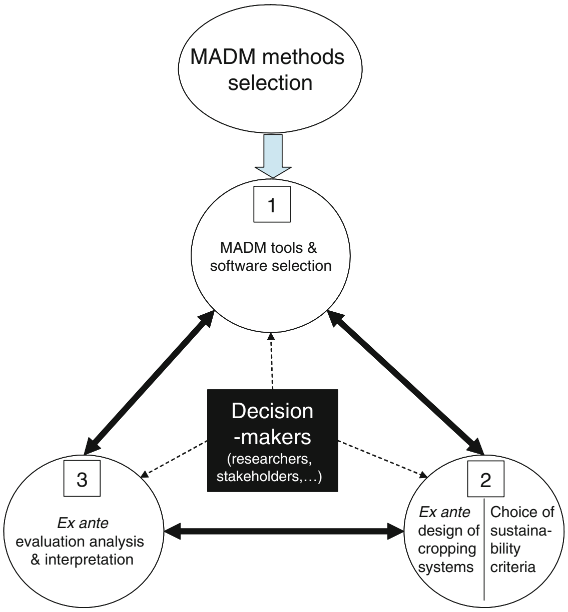

Fig. 2 Suggested structure of the framework to be set up following the multi-criteria decision-aid (MADM) method selection process, in order to carry out ex ante assessment of the sustainability of cropping systems

1. The collective selection of given decision-aid tools/software from the most suitable MADM method categories. Each MADM group being composed of numerous method variants and dozens of corresponding tools, a comparative (even restricted) assessment of these methods and tools might be necessary before selection. In order for the tool to be used by a large variety of decision-makers, the comparison should be based on operational and practical criteria, such as (1) the availability of the tool and its documentation, (2) the time and manpower resources required for the analysis, and (3) the ease of use, transparency and reporting capabilities of the tool (UK DTLR 2001).

2. The collective ex ante design of the options (i.e. alternative cropping systems) to be evaluated based on vectors of sustainability criteria, with respect to the specific features of the MADM tool considered

(Fig. 2). In this key step, it is essential that the knowledge and expertise of the decision-makers encompasses the considered sustainability issues, in order to design (quantitatively and/or qualitatively) relevant sustainability criteria (e.g. groundwater pollution, erosion and compaction risks, impact on biodiversity, energy consumption, gross margin, health risks). In order for the group to promote the discovery/design of alternative sustainability criteria not obvious or apparent at first sight, a work strategy based on brainstorming tools such as lateral thinking, affinity diagrams and interrelationship diagraphs can be of importance (Baker et al. 2002).

3. The ex ante assessment of the designed cropping systems and the analysis/interpretation of the results by the decision-makers based on the considered sustainability criteria and the characteristics of the applied MADM tools. Consistent with the recommendations of Zanakis et al. (1998), Macharis et al. (2004) and Wang and Triantaphyllou (2006), this multi-tool-based analysis may reveal alternative cropping systems that would be considered a priori sustainable, independently of the method applied. However, before the final selection of the options, it is recommendable to perform sensitivity and explanation analysis of the evaluation results obtained via each considered tool.

During all these steps, it is necessary to maintain a regular feedback with the operational research community, in order to ensure a cohesive operational framework.

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Comparison of Methods to Assess the Sustainability of Agricultural Systems: A Review

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Abstract Since the 1990s, numerous agri-environmental indicators and indicator-based methods have been developed to assess the adverse effects of cropping and farming systems such as water pollution by nitrates and pesticides, and gaseous emissions due to nitrogen inputs. This wealth of environmental indicators and assessment methods based on indicators raises issues on the quality of the methods and of the indicators, and on the relevancy of results. Evaluation and comparative studies are therefore needed to answer such issues. Here, we present four recent comparative studies selected for their illustrative value, first, to analyse the methodologies used for comparison of methods, and second, to highlight the main results of the four comparisons. The first study involves 23 indicators to address nitrate leaching. The second study involves 43 indicators to address pesticide risk. The third and fourth studies compare environmental assessment methods based on a set of indicators used in French and Upper Rhine plains (France, Germany and Switzerland). Both studies also compare the outputs of the methods and highlight the low degree of convergence between them. The approach proposed in the last study is the most elaborate among the four case studies. It could be used to develop a generic evaluation and comparison methodology. The review of those four case studies shows the need to formalise the methodology underlying any comparison work of indicators or evaluation methods.

Keywords Environmental assessment • Indicators • Nitrate • Nitrogen • Pesticide

1 Introduction

During the 1990s, there was a growing concern for environmental issues in agriculture, e.g. water pollution by nitrates, pesticides, erosion, or more recently, greenhouse gas emissions and biodiversity losses (Kirchmann and Thorvaldsson 2000). This led to the demand for operational assessment tools considered as a prerequisite to the development of new farming or cropping systems (Bockstaller et al. 1997). This was favoured by the popularisation of the concept of environmental management approaches like the ISO-14000 which rests on the four steps of the "quality spiral of continuous process improvement": to plan, to do, to check, to act (Meynard et al. 2002). The step "check" requires an assessment method of environmental impacts. The use of indicators has appeared as an alternative to direct impact measurement (Mitchell et al. 1995; Bockstaller et al. 2008), and is linked to methodological difficulties (impossibility of measurement, complexity of the system) or practical reasons (time, costs) for carrying out direct measurements. Another reason is the use of such tools for prospective goals (development of new agricultural strategies, prevention of environmental damage) in an *ex ante* assessment for which it is per definition not possible to perform measurements.

An "indicator explosion" (Riley 2001a) has occurred for the last two decades with the development of numerous indicator-based methods which are aimed

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at assessing environmental impacts of agricultural activities, or the whole sustainability of agricultural systems (Rosnoblet et al. 2006). Among the works on indicators, one can distinguish those on a specific theme, on one hand, like the environmental risk due to pesticide application (Maud et al. 2001; Reus et al. 2002; Devillers et al. 2005) and multi-criteria approaches based on a set of indicators addressing different environmental issues, on the other. Examples at regional (Payraudeau and van der Werf 2005), farm (Eckert et al. 2000; van der Werf and Petit 2002; Hülsbergen 2003; Meyer-Aurich 2005), and cropping system levels (Bockstaller et al. 1997; López-Ridaura et al. 2005; Nemecek et al. 2005) can be given for both.

This multiplicity and variety of indicators and methods raise questions. Riley (2001b) pointed out that it is a source of confusion which is increased by the fact that many methods are not evaluated for their scientific relevance and feasibility. The potential user, either a researcher working on innovative cropping systems, or an adviser working with farmers or a stakeholder involved in an environmental debate, will have questions about the selection of a given method adapted to his needs and how to make this selection. A second group of questions deals with the stability of the outputs of the different methods: do they provide the same conclusions? Answers to such questions require an evaluation and comparison study which provides information, not only about the strengths and drawbacks of each method, its field of use and validity, but also about the comparison of the conclusions derived from the outputs of the methods. Some authors (Meynard et al. 2002; Bockstaller et al. 2008) have already pointed out the requirement of a comparative analysis and validation of the various indicators available. To answer this concern of potential users, different kinds of comparative works have been undertaken. Comparison works of assessment methods based on a set of indicators, such as those at farm level (van der Werf and Petit 2002; Halberg et al. 2005) or regional level (Payraudeau and van der Werf 2005) are based on a descriptive approach. In other comparative studies on impact assessment (Thompson 1990; Hertwich et al. 1997) or more specific to the agricultural sector (Gebauer and Bäuerle 2000; Thomassen and de Boer 2005), authors use a set of qualitative or semi-quantitative evaluation criteria to compare the methods. No information is given on the comparison of the outputs or conclusions of the methods by all those authors, except by Thomassen and de Boer 2005. They also study correlation between results of comparable indicators belonging to the input–output accounting approach and Life Cycle Analysis for a dataset obtained on eight dairy farms.

This short review of the literature points out the diversity of approaches and a lack of formalised comparison methodology. The first goal of this article is to analyse the methodologies used in four comparative studies (CORPEN 2006; Devillers et al. 2005; Galan et al. 2007; Bockstaller et al. 2006), selected to derive some methodological principles for potential users who need to undertake such a comparison. Second, the main results of the four comparisons will be highlighted to guide potential users of indicators or an evaluation method in their choice. Attention is paid to agronomists working on the design of innovative cropping systems and to environmental impact due to pesticides and nitrogen issues, for which many indicators are available. The four case studies structuring the article were selected for their diversity and illustrative value. The type of indicators and methods covered by the case studies and their target users, agronomists assessing and designing cropping systems, was another reason for their selection.

2 Presentation of the Four Case Studies: Context and Method of Comparison

2.1 Comparison of Indicators Assessing Nitrogen Losses

2.1.1 Context of the Work

The work was initiated by the CORPEN organisation, which depends on the French Ministry for Ecology and Sustainable Development and has the mission to bring together experts and stakeholders involved in the issue of water quality and agriculture in order to deliver recommendations (CORPEN 2006). The objective was to help users to choose and to implement indicators depending on the question and the scale of study. It was carried out by a group of experts on nitrogen fertilization and losses, from research and technical institutes.

The expert group listed 23 indicators currently used by agricultural advisors, farmers or even public policy agents to assess nitrogen losses in France, and especially nitrate leaching at farm and regional levels. For the sake of concision, we will restrict the presentation of this work to the field and farm scale since our article addresses the evaluation of cropping and farming systems.

2.1.2 Method of Comparison

A descriptive sheet was filled in for each indicator with a list of descriptors: reference values, calculation method, time and spatial scale, periodicity of calculation, time for implementation, recommendations for interpretation and similar indicators, etc. In the report of the project, a synthetic table was added to present the assessment of two evaluation criteria for 15 indicators: (1) the feasibility, i.e. easiness of implementation due to accessibility of data and cost of implementation expressed on a qualitative scale between 1 (low) and 4 (high), and (2) the relevance assessed by experts on a four-class scale, from 1 (indicator not to be implemented alone) to 4 (indicator "advised"). Indicator sheets as well as the two evaluation criteria were filled in by members of the group of experts and validated by the group of experts. A selection of descriptors and the assessment of the two criteria are presented in Table 3 in Sect. 3.1 of the "results" chapter.

2.2 *Comparison of 43 Pesticide Risk Indicators*

2.2.1 Context of the Work

This work followed the studies of Maud et al. (2001) and Reus et al. (2002) who compared, respectively, six and eight pesticide risk indicators. The study was ordered by the French Ministry for Ecology and Sustainable Development and was expected to be as exhaustive as possible to make the review available to a large panel of users and to help the ministry to choose the "best" indicators for the assessment of its policy (Devillers et al. 2005).

2.2.2 Method of Comparison

Each indicator was presented in a descriptive sheet, with a list of 25 criteria, a short presentation of the calculation, and the list of the parameters and variables used for calculation (Devillers et al. 2005). The following criteria were used (1) some general descriptors on the use, users and planned use, (2) others on the spatial scale, the environmental compartments taken into account and the calculation method, (3) some information useful for assessing the qualities of the indicators, the calculation time, and the existence of a scientific validation procedure according to the framework of Bockstaller and Girardin (2003), and (4) finally, four evaluation criteria expressed on a qualitative four-level scale: −, −−, +, ++ covering the readability, the feasibility, the reproducibility and the relevance for the end-users. All the indicator sheets as well as the evaluation criteria were filled in by the same person and validated by a group of experts. Information sources were the references from grey and scientific literature. No implementation test was presented in this book. For the sake of concision, the number of indicators presented in this article was reduced to a selection of indicators chosen for their illustrative value or because they are already implemented (see Table 4 in Sect. 3.2 of the "results" chapter).

2.3 *Comparison of Five Assessment Methods of Sustainability in France*

2.3.1 Context of the Work

This work was launched by a regional organisation, Agro-Transfert, at the request of the agricultural sector's representatives to develop a quality management and environmental management approach in the Picardie region, North of France (Galan et al. 2007). The first step was to develop a regional benchmark for good farming practice "Quali'terre" (Aubry et al. 2005). The second step (developed as an extension to the "Quali'terre" programme) is the development of an environmental management system which is relevant and user-friendly. In order to have a state of the art of the existing tools and to choose the best fitted tool, Agro-Transfert performed a comparison of the five methods used most frequently in France to evaluate the environmental impacts of agriculture. Those methods are all based on a set of indicators addressing different environmental themes, whereas the first method below also includes the economic and social dimensions of sustainability. The five methods are:

(1) IDEA ("Indicateur de Durabilité des Exploitations Agricoles"), which was developed by a working group under the patronage of the French Ministry of Agriculture (Vilain et al. 2008).
(2) DIAGE ("DIAgnostic Global d'Exploitation"), which was developed by the Regional Federation of Agricultural Cooperatives (FRCA) in the French "Centre" administrative region, in partnership with agricultural technical institutes (FRCA-Centre 2002).
(3) DIALECTE ("DIAgnostic Liant Environnement et CTE"), which was developed by the Solagro association (Solagro 2000) as well as the next method.
(4) DIALOGUE ("Diagnostic agri-environnemental global d'exploitation"), which addressed more themes than DIALECTE at field level (Solagro 2001).
(5) INDIGO® ("indicateurs de diagnostic global à la parcelle"), which was developed by the INRA's Sustainable Agriculture Research Unit in Colmar (Bockstaller et al. 1997).

2.3.2 Method of Comparison

As for previous work, a set of criteria was selected by the authors to compare the methods: (1) general criteria: type of agricultural production evaluated, spatial scales, implementation time, target users, spreading and developers; (2) environmental themes and impacts, (3) main activities, crop rotation, nitrogen fertilization, etc., (4) aggregation levels, calculation method, rating scores and thresholds, and (5) type of data required (field data, management at farm level, sensitivity of the environment). Unlike the second case study on 43 pesticide indicators where each indicator was described and evaluated in a separate sheet, the methods are here compared directly in tables.

To get some of those data, e.g. implementation time, the authors tested the five methods on a set of 15 farms in Picardie (all with cereals and sugar beet, +450 ewes for 1 farm, +50 beef for 1 farm, + potatoes for 3 farms, + vegetables for 1 farm, size ranging between 93 and 460 ha). The results obtained with each method on the 15 farms were compared in two ways:

- For a single impact, the results for all 15 farms were compared with each of the five tools. The effect of crop protection on water quality was selected.
- For each method, the individual result for four different activities (management of inert waste, nitrogen fertilization, crop protection and energy management) within the "water pollution" theme were compared on one particular farm.

The results of the indicators were normalised by expressing them as a percentage of the maximum possible rating for the indicator, so that they can be compared (Nardo et al. 2005).

2.4 Comparison of Four Farm Management Tools in the Upper Rhine Plain (COMETE Project)

2.4.1 Context of the Work

The last work was initiated in a transregional context, in the upper Rhine plain by French, German and Swiss partners in 2003. The French and Swiss methods were compared with two German tools widely used in Germany. As in the previous study, the four selected methods based on a set of environmental indicators are:

1. INDIGO®, also compared in the previous project (see Sect. 2.3.2).
2. SALCA ("Swiss Agricultural Life Cycle Assessment), developed at the Agroscope ART Reckenholz in Zurich (Switzerland), (Rossier and Gaillard 2004).
3. KUL/USL (Criteria and Standards for Sustainable Agriculture), developed at the state agricultural institute of Thuringe in Iena (Germany), (Eckert et al. 2000).
4. REPRO, developed at the University of Halle (Germany), (Hülsbergen 2003).

The tools were assessed according to the version valid in mid-2004. For REPRO, only a subset of the whole indicator set with high relevance for environmental items was analysed.

2.4.2 Method of Comparison

Since no adapted methodological framework for comparison was found in the literature, the working group of the COMETE project developed its own approach, which consists of two stages:

- First, a comparative evaluation using a list of criteria which were grouped into three domains ("scientific soundness", "feasibility" and "utility") (Table 1).
- Second, the test of the implementation of the methods in a set of 13 farms. For the first step, for each criterion, a score between 1 (the lowest) and 5 (the highest) was defined by a set of decision rules. An example is given in Table 2, the details being available in Bockstaller et al. (2006). The criteria addressing the users' needs and the whole list were discussed during a workshop with the three identified user groups: farmers, advisers and agents of administration.

The four methods were evaluated by the authors themselves for INDIGO® and SALCA and validated by the whole working group. For the German methods the authors did not take part in the project, so the group decided to send the evaluation carried out by the German partner to the developers of the two methods. The feedback of the latter was validated by the working group. The previous evaluation was completed by a test of the methods on a group of 13 farms (three in Switzerland, five in France and five in Germany) for two years. The type of production was various, arable farms (maize monoculture, cereals), arable farms with special crops and mixed farms (arable crops and cattle or milk).

Following the evaluation with a set of criteria, the results obtained on the group of farms were compared in two ways. First, an aggregated indicator was calculated by means of an average value which was weighted for SALCA according to the experience gained by sensitivity analysis by the authors, without a weighting procedure for INDIGO® and KUL, and a sum of scores for REPRO. The ranking of farms obtained with each aggregated indicator was compared by means of Spearman's correlation coefficient.

Table 1 List of evaluation criteria used in the COMETE project (Bockstaller et al. 2006)

Scientific soundness	Feasibility	Utility
Coverage of environmental issues	Accessibility of data[a]	Coverage of needs[a]
Coverage of agricultural production branches	Qualification of user	Clearness of conclusion from results
Coverage of production factor	Need for external support	Quality of communication of results
Indicator type,[b] depth of environmental analysis	User-friendliness	
Avoidance of incorrect conclusions	Integration with existing farming software	
Transparency	Time requirement	

[a] For three user groups: farmers, advisers, administration

[b] Based on the driving-force, pressure, state, impact, response framework (EEA 2005)

Table 2 Example of assessment for the criterion: "avoidance of incorrect conclusions"

Decision rules for the assessment of the criterion "avoidance of incorrect conclusions"	Score (1–5)
Lack of data on evaluation of the indicator and and criteria "indicator type" = 1	1
Indicator based on a non-validated model	1
No agreement of indicator value with observed data	1
Indicator criticised in a peer-reviewed article	2
Indicator based on a partially validated model	2
Lack of data on evaluation of the indicator and and criteria "indicator type" = 2 to 5	2
Medium agreement of indicator value with observed data	3
Calculation method recommended by experts	3
Scientific peer-reviewed article on the indicator	4
Indicator based on a validated model	4
Good agreement of indicator value with observed data	5

Second, the conformity of recommendation derived from the indicators was compared by means of a newly developed index of conformity (I_K) ranging between 0 (no conformity between the methods) and 1 (total conformity between the methods):

$$I_K = 1 - \left[\sum_{p=1-n} \sum_{q=1-b} \sum_{r=1-vk} \left| i_{pqr} - j_{pqr} \right| / (2nb) \right]$$

with:

i_{pqr}: degree of achievement of recommendation r for the production factor q for farm p for method 1;
j_{pqr}: degree of achievement of recommendation r for the production factor q for farm p for method 2;
n, b, vk: respectively, number of farms, production factors and recommendations per production factor.

For example, the production factor "nitrogen management" was decomposed into recommendations like "reduce the amount of fertilizer"; "increase the amount of fertilizer", "change the type of fertilizer" and "change the date, method of fertilization". If a method gives the recommendation "reduce the amount of fertilizer", the degree of achievement will be 1 for this recommendation and 0 for the other recommendation. It should be noticed that a value inferior to 1 can be given if more than one recommendation is given.

3 Main Results of the Four Case Studies

3.1 *Comparison of Indicators Assessing Nitrogen Losses*

Several groups of indicators can be distinguished in Table 3: (1) a first group of simple indicators (Bockstaller et al. 2008) focusing on nitrogen input management, mainly mineral/organic fertilization, but

Table 3 Comparison of "nitrogen indicators" (CORPEN 2006)

Indicator	Spatial scale	Threshold value	Time for interpretation	Agronomic relevance	Feasibility
Fertilization					
Amount of applied nitrogen	Field/farm/region	Local per crop	Year	1	4
Amount of available nitrogen	Field/farm/region	Local per crop	Year	1	3
Number of nitrogen applications (organic and mineral)	Field/farm/region	Local per crop	Year	1	3
Deviation from the recommendation of nitrogen rate	Field	Zero	Year	2	3
Period of application	Field/farm/region	Local	Year	1	3
Number of grazing days	Field/farm/region	Local	Year	3	2
Soil cover					
Area with bare soil during drainage period	Farm/region	Local	3–4 years	2	4
Area with catch crops	Farm/region	Local	3–4 years	2	4
Assessment of surpluses or losses					
Input/output budget (CORPEN)	Field/farm/region	Local per cropping system	⩾5years	2	3
N supply / requirement budget (EQUIF)	Field/farm/region	Local: close to zero	Year	3	2
Soil mineral nitrogen at harvest[a]	Field	Local per soil type	Year	3	2
Soil mineral nitrogen at beginning winter[a]	Field	Local per soil type	Year	4	2
Model predicting N losses: I_N INDIGO®	Field/farm/region	7 (matching a concentration below roots of 50 NO_3 mg L^{-1})	Year	4	3[b]
Model predicting nitrate lixiviation: DEAC	Field/farm/region	Local per cropping system	Year	4	3[b]

[a] Measured, or assessed by a model
[b] When the parametrization has been achieved

also organic input due to grazing. They are considered as descriptors of practices; (2) a second group addressing soil cover in winter, assessing nitrogen uptake during the period after harvest until winter, and (3) a third group resulting from the combination of variables such as nitrogen balance or model-based. Some of them are based on calculation of the input–output balance to estimate surplus. Others include nitrogen cycle processes to estimate fluxes/emission of nitrogen. Among them, the nitrogen indicator from the INDIGO® method (I_N), based on an operational model, provides the amount of nitrogen lost to water and air (Bockstaller et al. 2008), whereas DEAC focuses on nitrate leaching in winter (Cariolle 2002). The evaluation of the relevance and feasibility shows a relative discrepancy between the feasibility and relevance for the first and the last group in Table 3. Indicators from the first group are straightforward to calculate (high feasibility) but not really relevant if they are used alone. In contrast, indicators including in their equation nitrogen cycle processes gain in relevancy to the detriment of feasibility. In the description sheet of each indicator, recommendations are given to the users about interpretation of results and the domain of validity, and propositions of complementary indicators are given to improve the relevance of the first group. An example can be given for indicators based on the calculation of a balance (input minus output) used by several authors and institutions as an indicator for nitrogen losses (e.g. Goodlass et al. 2003; EEA 2005). However, several authors (Lord et al. 2002; Oenema et al. 2005; ten Berge et al. 2007) pointed out by comparison with measurements of nitrate leaching that such nitrogen balances are bad estimators of nitrate leaching risk, if they are used on an annual basis (Laurent et al. 2000). Thus, the report recommended an interpretation based on pluriannual calculation.

3.2 Comparison of 43 Pesticide Risk Indicators

The output of the work was a book describing the 43 indicators, 24 in a detailed way and 19 in a simplified way. Several groups of indicators can be distinguished: (1) indicators resulting from transformation of variables into scores and summed up or aggregated in an empirical way, among them EIQ, one of the first indicators published (Table 4); (2) a second group of indicators uses outputs from model calculation. 14 indicators among the 43 are based on the risk ratio approach which is used in registration of pesticides (Vercruysse and Steurbaut 2002): it is the quotient of the estimated human exposure or predicted concentration and toxicological reference value used for different environmental compartments, e.g. EPRIP, POCER. (3) The third group contains specific approaches such as the qualitative one based on decision rules associated with fuzzy logic (e.g. I-Phy) or based on a multicriteria ranking method (Vaillant et al. 1995; Aurousseau 2004).

Other trends which can be pointed out through this comparison is the lack of indicators which were validated by comparison with experimental data (12 among the 43), only one (EYP) being validated by end-users (Bockstaller and Girardin 2003). Most of the indicators are calculated on the field scale and only 3 among 43 on the watershed scale, which is relevant for assessment of surface water quality. The implementation of the indicator requires in general less than 1 h per calculation, except for EPRIP and EYP, which need more time because of the high number of data for calculation. Only 8 among 43 propose reference values which help users in the interpretation of the outputs. No specific focus was put on the use of the indicators.

3.3 Comparison of Five Assessment Methods of Sustainability in France

The first part of the work is descriptive. A synthesis of the results is given in Table 5. Besides general information, Galan et al. (2007) assess on a qualitative scale the degree of coverage of environmental themes and farm activities (practices) at field as well at farm level. For the first item, "water quality (sporadic pollution)", "air quality" and "social environment (noise, odours)" are not covered by a majority of methods, whereas for the second item, most of the methods neglect or poorly integrate the activities "construction/modification of buildings or storage", "production of renewable energy" and "management of inert waste". Additional information is given on the type of data needed for which INDIGO® differentiates from the others by

Table 4 Examples from the comparison of 43 pesticide risk indicators (Devillers et al. 2005)

Indicator	Developer/ reference	Target user[b]	Spatialscale	Environ. compartment addressed[a]	Calculation method	Validation (D, O, U)[c]	Time for data (for calculation)	Readability[d]	Feasibility[d]	Reproducibility[d]	Relevance for user
ADSCOR	OCDE group	DM	Field/region/ country	Sw	Sum of scores	D, O	<1h (<1h)	–	+	++	+
EIQ	Kovach et al., 1992	DM,R,T, F	Field	Sw, Gw, Bio, Hf, Hp	Some/ product of scores	D, O	<1h (<1h)	–	+	++	+
EPRIP	Trevisan et al. in Reus et al.,1999	DM,R,T, F	Field	Sw, Gw, Air, Soil	Risk ratio	D, O	>1h (<1h)	+	+	+	+
EYP	Reus and Leenderste, 2000	DM,F, other	Field/farm/ region/country	Sw, Gw, Soil	Risk ratio	D, U	<1h (<1h)	+	+	++	++
I-Phy	Van der Werf and Zimmer, 1998	DM,R,T, F	Field/farm	Sw, Gw, Air,	decision tree + fuzzy logic	D, O	<1h (<1h)	++	+	++	+
p-EMA	Hartet al, 2003	D, T, F	Field/farm	Sw, Gw, Soil, Bio, Hf, Hp	Risk ratio	D	>1h (<1h)	++	++	+	++
PERI	Nilsson 1999 in Reus et al., 1999	T, F	Field/farm	Sw, Gw, Air, Soil	Some/ product of scores	–	<1h (<1h)	+	++	++	+
POCER	Vercruysse and Steurbaut, 2002	DM, R, F	Field/farm	Sw, Gw, Soil, Bio, Hf, Hp	Risk ratio	D	<1h (<1h)	++	+	+	+
REXTOX	OCDEgroup	DM	Field/farm/ region/country	Sw	Risk ratio	D	<1h (<1h)	–	+	++	+
SIRIS	Vaillant et al., 1995	DM,R,T	Region/country	Sw	Ranking method	D	<1h (<1h)	–	+	++	+
SYNOPS	Gütscheand Rossberg in Reuset al., 1999	DM, T, F	Field/farm/ region/country	Sw, Soil	Risk ratio	D	<1h (<1h)	–	+	++	+
SyPEP	Pussemier in Reuset al., 2002	DM,R, F	Water catchment	Sw, Gw	Risk ratio	–	<1h (<1h)	+	+	++	++

[a] *Sw* surface water, *Gw* groundwater, *Bio* biodiversity, *Hf* health of farmer, *Hp* health of population, *other* other compartment

[b] *DM* decision-maker, *R* researchers, *T* technicians, *F* farmers

[c] *D* validation of the design (peer-reviewed article or by experts), *O* validation of output with experimental data, *U* end-user validation

[d] On a four-class scale: – (low), ––, +, ++ (high).

Table 5 Comparison of five assessment methods of environmental sustainability in France (Galan et al. 2007)

Method	Type of production	Spatial scale	Impact not or poorly addressed[a]	Activity not or poorly tackled[b]	Type of data	Indicator type	Aggregation method	Implementation time	Target users
IDEA	Crops, Animal husbandry, Market gardening	Farm	Social environment	P, K fertilization Buildings or storage	Field practices (mean data) Site practices	Simple, composite, system indicators.	Sum	1day	Farmer, Student, Technician, Teacher.
DIAGE	Crops, Animal husbandry, viticulture	Farm	Air Quality Natural Resources Use of non-renewable resources	Cropping pattern Crop rotation Renewable energy Management of inert waste	Field practices (mean data) Site practices Sensitivity to the environment	Composite indicators	Product-sum	1.5 days	Farmer, Technician
DIALECTE	Crops, Animal husbandry,	Farm	Water Quality (sporadic pollution) Soil Chemical quality Air Quality Social environment	Crop protection Nitrogen fertilization P, K fertilization Organic manure Water management, Buildings or storage, Renewable energy Management of inert waste	Field practices (mean data) Site practices	Simple, composite, system indicators	Sum,	1–1.5 days	Farmer, Technician
DIALOGUE	Crops, Animal husbandry,	Field, farm	Water Quality (sporadic pollution) Soil Chemical quality Air Quality Social environment	N fertilization P, K fertilization Organic manure Renewable energy, Management of inert waste	Field practices (per field) Site practices	Simple, composite, system indicators	Sum	2 days	Farmer, Technician
INDIGO®	Crops, (grassland viticulture, fruit production)	Field, farm	Water Quality (sporadic pollution) Landscape Social environment	Installation of "non-productive" elements Buildings or storage Renewable energy Management of inert waste	Field practices (per field) Sensitivity to the environment	Composite indicators	Expert system, model	2 days	Farmer, Technician, Researcher

[a] In Galan et al. (2007) positive list with: water quality (diffuse pollution), water quality (sporadic pollution), soil physical quality (structure, loss), soil chemical quality (chemical pollution), air quality, landscape fit with the landscape natural milieus domestic and wild biodiversity, natural resources use of non-renewable resources, natural resources use of water reserves, social environment (noise pollution, odours, etc.)

[b] In Galan et al. (2007) positive list with: crop protection, N fertilization, P K fertilization, organic manure, soil management, servicing and maintenance of machinery, cropping pattern, crop rotation, installation of "non-productive" elements, water management, construction/modification of buildings or storage, production of renewable energy, management of inert waste

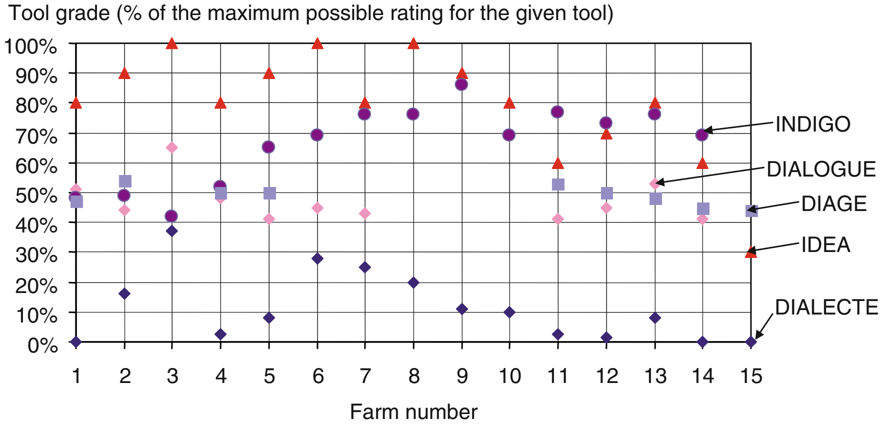

Fig. 1 Comparison of the output of pesticide indicators for water quality from five assessment methods. Indicators are calculated on 15 farms and their outputs are normalised as a percentage of the maximum value (Galan et al. 2007)

using detailed field practice data and data on the sensitivity of the environment, soil and climate, but no site data such as maintenance of the storage tank or sprayer, or building management. About the aggregation of the information, most of the methods use a simple method based on the sum of scores, and product (for DIAGE), whereas indicators in INDIGO® are based on models and expert systems (Bockstaller et al. 2008).

The authors go a step further by comparing the five assessment methods for water quality. They compare the impact of pesticide use on 15 farms. The normalised values of the pesticide indicators are represented in Fig. 1. All the methods except DIAGE, and DIALOGUE to a lesser extent, show significant variations between farms. IDEA yields in general higher results, showing less impact on water quality, than the other methods. In any case, no correlation between methods appears on the sample of farms, which means that the recommendations for pesticide management will not be the same between methods for a given farm. This can be explained by the difference between methods in: (1) the integration of aspects of sporadic pollution (point source), as it is the case for IDEA and DIAGE, (2) type of data used, pesticide properties (INDIGO® and DIALOGUE), and soil and environment sensitivity (INDIGO® and DIAGE), and (3) the aggregation method. Similar discrepancies between the five methods are found for one particular farm when they are compared on four different activities (management of inert waste, nitrogen fertilization, crop protection and energy management).

3.4 Comparison of Four Farm Management Tools in the Upper Rhine Plain (COMETE Project)

Based on the versions available in mid-2004 for the four methods and on a subset of indicators for REPRO, the results yielded by each method for the 15 criteria are shown in Fig. 2. For the domain "scientific soundness", SALCA presents the best environmental scores, but none of the methods was able to cover all relevant environmental issues, especially regarding biodiversity. The low scores of INDIGO® for the criteria "coverage of agricultural production" and "consideration of production factors" result from its specialisation in plant production. However, this method allows a detailed analysis of a cropping system, enabling the user to trace the cause of an environmental risk to the management, e.g. risk analysis of each pesticide application, taking into account the field conditions, tillage, spraying techniques and active ingredient properties. The "depth of environmental analysis" is low for REPRO due to the fact that this method considers for each environmental issue all types of indicators without priority despite the risk of redundancy between them; and for KUL, due to the type of indicator (mainly only driving forces). Those take into account only farmers' practices and not emissions or impacts. The low score of KUL/USL for the criterion "transparency" reflects the non-accessibility of the software, which is balanced by the score in the domain "feasibility" for which KUL/USL receives the best score as a

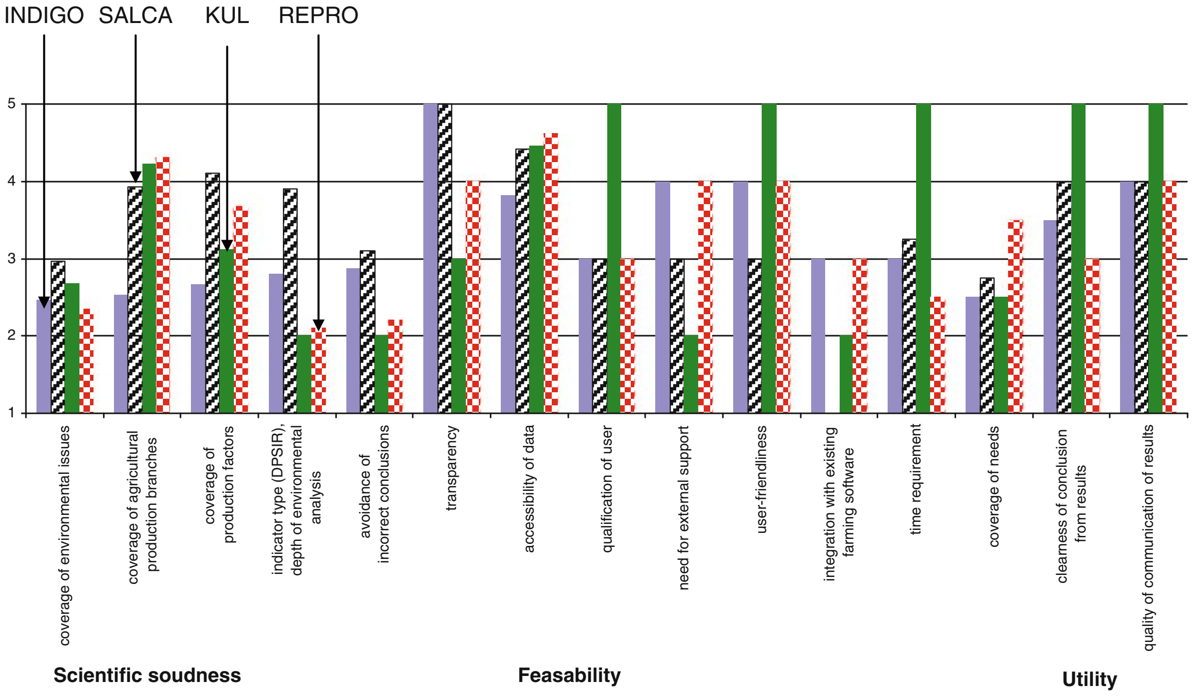

Fig. 2 Comparison of four farm management tools in the upper Rhine plain with the help of 15 criteria (see Table 1) in the frame of the COMETE project (Bockstaller et al. 2006)

result of its cleverly devised organisation form. On the contrary, SALCA's electronic entry data form was not user-friendly. The evaluation with REPRO is comparatively more time-consuming. For the domain "utility", no great differences were observed between the four methods. The better score of KUL/USL is due to the criterion "communicability" thanks to the possibility of labelling, which is compensated for by the lack of specific recommendations at field level.

There was a high correlation between SALCA, REPRO and INDIGO® (not enough farms for KUL/USL) regarding the environmental ranking of the analysed farms (Spearman coefficients range between 0.72 and 0.88, see Fig. 3a). In other words, for the four methods, there is no reason to fear that the choice of the environmental management tool determines whether a farm performs well or badly from an environmental point of view. On the other hand, the conformity index shows a low convergence between the recommendations for the four methods (index range between 0.48 and 0.64, see Fig. 3b).

These discrepancies are explained by major conceptual differences between the investigated methods, namely: (1) in the different environmental issues considered. This can be illustrated by the phosphorus management: INDIGO® addresses soil fertility issues which can lead to a recommendation "increase the amount of fertilizer", whereas SALCA focuses on eutrophication (of soil and water) and environmental soil quality aspects (here linked to heavy metals present in some fertilizers). Provided that a minimal yield is reached, SALCA does not recommend from an environmental point of view to increase the amount of fertilizer, whereas INDIGO can do it to maintain soil fertility. (2) In the production factors which are used for the calculations of indicators dealing with similar issues. INDIGO® and SALCA take into account amount of nitrogen, crop management, e.g. soil cover in winter, and soil mineralisation to assess nitrate leaching, whereas KUL and REPRO, for the indicator considered in the study, only take into account nitrogen input and output, and (3) to a lesser extent in the benchmark used to derive a recommendation for some similar indicators.

Besides the evaluation with criteria and the comparison of outputs, some general qualitative aspects were pointed out through the experience gained by implementing the method on farms. Two deserve more

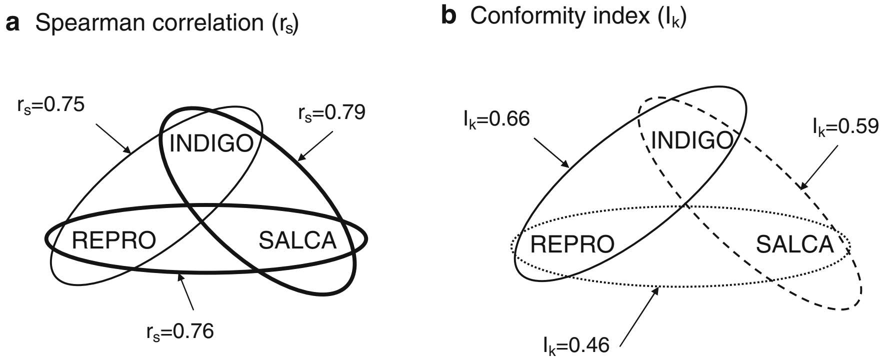

Fig. 3 Comparison of outputs based on (**a**) the ranking of farms by means of the Spearman correlation coefficient (r_s), (**b**) the conformity of recommendations by means of the conformity index (I_k), (Bockstaller et al. 2006). A value 1 indicates a perfect correlation for r_s and conformity for I_k. Both comparisons are made with a sample of 13 farms with the data of 2002 (KUL not included in the comparison because it was implemented on three farms only)

attention. The implementation of a method outside the country where it was developed raises several problems such as the accessibility of data or different description schemes for the same issue (especially for soil description) and bugs in the software due to national parametrization. On the other side of the chain, the user stands alone for the interpretation of results and is not provided by any methods with an interpretation system in the software to interpret the results except for KUL. In this case, the user receives a written report with the interpretation and recommendations to improve the system. However, the user does not have access to the calculations and has to pay for those recommendations.

4 Discussion

In this discussion we will not discuss the results obtained for each method but focus on the methodology used to compare and evaluate assessment methods or thematic indicators. First, it should be noticed that if such a study is in many cases user-oriented, it can also help indicator or method developers to improve their methods. For example, the work on pesticide risk indicators was followed by a second project on indicator validation (Girardin et al. 2007) and on the improvement of two of them, e.g. introduction of a risk component on biodiversity. The developers of SALCA took into account the poor assessment of their method according to the criteria "integration with existing farming software" and "user-friendliness" (see Fig. 2) for the SALCA version of mid-2004. They integrated the use of commercial farm management software for the data collection and the implementation of a new software program for data validation and preparation before calculation, for the last two years. The comparison of the five assessment methods in the third case study led the authors to develop a new method more fitted to the need of the local users.

In Table 6 we synthesise the main features of the comparison and evaluation approaches used for the four case studies of this article. It highlights the variability between the approaches, explainable by a lack of a generic methodology. The criteria and their organisation vary between the case studies. Criteria on feasibility and relevance (or soundness) can be found in the four cases. This can be compared with previous studies. Hertwich et al. (1997) proposed only three criteria: "information requirement", "tolerance for imperfect information" and "potential for undesirable outcome". Other authors such as Gebauer and Bäuerle (2000) or Thomassen and de Boer (2005) developed a longer list organised, respectively, into different groups: "implementation" and "utility", and, "relevance" for user, "quality" and "availability of data". Other comparative studies remained mainly descriptive, including information on the time needed for data collection and recommendations on the type of indicators and linked issues, e.g. choice of threshold, scale of result expression (van der Werf and Petit 2002; Halberg

Table 6 An overview of approaches used to compare indicators and assessment methods in the four case studies

Authors	CORPEN (2006)	Deviller et al. (2005)	Galan et al. (2007)	Bockstaller et al. (2006)
Number of indicators/ methods compared	23 indicators	43 indicators	5 assessment methods (French)	4 assessment methods (2 German, 1 Swiss, 1 French)
Evaluation criteria	feasibility agronomic relevance	readability feasibility, reproducibility relevance for user	time for implementation environmental themes and activities tackled by the method	15 criteria grouped into : scientific soundness feasibility utility (see Tab. I)
Scale of evaluation	Semi-quantitative scoring between 1 and 4	Qualitative scale (– –; –; +; ++)	Quantitative (days) Qualitative scale (–; +; ++; +++)	Semi-quantitative scoring between 1 and 5 with explicit decision rules
Implementation of the evaluation	Working group	1 person for the 43 indicators and validation by the group	Authors of the article	Authors of the method with cross-validation and feedback of method developer
Comparison table	Yes	No	Yes	Yes
Direct comparison of outputs	No	No	For a sample of 15 farms for one indicator, For one farm, and several indicators	Ranking of 13 farms for an aggregated indicator The conformity of recommendations for the sample of farms

et al. 2005; Payraudeau and van der Werf 2005). Such descriptive comparison studies allow the users to know the construction methods better, and to appropriate the tools and complete the evaluation step which highlights strong and weak points of each method.

It should be noticed that the cost of implementation is not used in the four case studies or by all the authors previously quoted, although it is an important criterion (Romstad 1999). This can be explained by the fact the studied methods were at an experimental stage, and that most costs are internalised by the method developers so that no realistic assessment of this criterion could be achieved.

From the list of criteria presented in Table 6 or used by other authors, it appears that the meaning of the word can in some cases vary between authors. With regard to the feasibility, Hertwich et al. (1997), like Thomassen and de Boer (2005), linked it mainly to the availability of data, whereas it covers more aspects in the fourth case study (COMETE project), like in the work of Gebauer and Bäuerle (2000). Even within a working group like this of the CORPEN, the assessment of the criterion "relevance" was not so easy. It refers to a synthesis or even compromise of criteria such as sensitivity, representativeness, legibility and robustness, which are not so easy to specify. This explains the reason why the group of the COMETE project prefers to increase the number of criteria with the risk of providing too much information to the user. A solution to this inflation of criteria would be to synthesise the outcome of the evaluation with a multi-criteria analysis, as was proposed for social validation of indicators (Cloquell-Ballester et al. 2006).

The objective of the CORPEN group (CORPEN 2006) was to guide the users in the selection of indicators addressing the nitrogen leaching issue in order to avoid misuse outside the domain of use, or misinterpretation. In the study of the CORPEN group, an evaluation of indicators is briefly presented in the main text but no criteria are given in the descriptive sheet, whereas a synthesis in the form of text but no comparative tables are given in the book of Devillers et al. (2005). A database with queries to help to choose a pesticide indicator is in development (Girardin, personal communication). The third comparative case study (Galan et al. 2007) provides several tables comparing the French assessment methods for their technical features regarding their calculation method, the domain of use, etc., which could be used for an evaluation work. The time for implementation is quantified but not valued like in the last case study, the COMETE project (Bockstaller et al. 2006). The last case study, the COMETE project, clearly differentiates description and evaluation and proposes a method based on a set of criteria with decision rules to assess them (see Table 2). This should increase the transparency. However, a degree of subjectivity may remain in the criteria of the COMETE project as some criteria are the results of a scoring procedure without decision rules, e.g. coverage of an environmental issue. The cross-validation which was done in the project could help to reduce the subjectivity. Another point to notice is the effort to make the evaluation more precise by differentiating different user groups. The authors of the COMETE project (Bockstaller et al. 2006) identify three groups (farmers, advisers and employers of administration) which are differentiated for the evaluation of two criteria, accessibility of data and coverage of needs. This was also done by Thomassen and de Boer (2005), who added a fourth group of scientists to the three groups for one criterion, "comprehensibility". A criterion such as accessibility of data also has to be adapted to the context of use. Some data, such as those describing soils, vary a lot between countries or even regions (Bockstaller et al. 2006).

An interesting output of the third case study (Galan et al. 2007) is the comparison of the outputs of the methods, which is rarely done according to our knowledge. Examples can be found in the literature on comparison of outputs for pesticide risk indicators (Maud et al. 2001; Reus et al. 2002). However, those authors compared the ranking of pesticides but did not take into account the absolute value of the indicator, so that the actual difference between the results of two indicators is not assessed. In the work of Galan et al., assessment methods based on different sets of indicators are compared. Consequently, Galan et al. (2007) restricted the analysis to comparisons farm by farm or indicator by indicator. In the COMETE project, results of the individual indicators are aggregated although the developers (except for REPRO) do not propose it for users because of methodological problems due, for example, to the addition of scores (Schärlig 1985). The second approach based on a conformity index is original and avoids this problem. However, it requires an effort of formalisation of the potential recommendations for each indicator within an evaluation method. Comparisons of outputs in Galan et al. (2007), like the comparison of recommendations in COMETE, yielded poor convergence between the compared methods, which can be explained by the ground difference in assumptions and choices in the calculation methods. The potential users should be aware of this, which is only possible if those assumptions are transparent.

5 Conclusion

This article highlights through the four case studies the variability in approaches used to compare indicators or assessment methods. The first two studies focus on, respectively, 23 and 43 indicators addressing the nitrate leaching issue and pesticide risk, respectively. Those studies provide a lot of descriptive information about the indicators summarised in the article. Few evaluation criteria are used to point out strong and weak points of those indicators. The third and fourth studies compare environmental assessment methods based on indicators, respectively, five used in France and four tested in the upper Rhine plain (France, Germany and Switzerland, COMETE project). Both studies also compare the outputs of the methods and highlight a low degree of convergence among them. The approach developed in the COMETE project appears to be the most elaborate. It should be tested in other comparative studies like the third case study. An adaptation to the comparison of pesticide risk indicators is ongoing in the Endure network (Kägi et al. 2008).

Our study can contribute to developing a "meta-method" which should help with the selection of indicators or of assessment methods. Such a "meta-method" could rest on a list of criteria like those of COMETE which would require local adaptation: which criteria are relevant for a given context, but also how they should be assessed, e.g. availability of soil data, which can change between countries or even regions. It should include descriptive information, evaluation criteria based not only on theoretical information but also on a test in practice. Basic assumptions, the potentialities of the methods, e.g. environmental issues covered, factors addressed, should in any case be stated clearly because they strongly influence the final results and explain the divergence between methods in terms of recommendations. Further work is needed to help users to cope with those potential discrepancies between indicators for the same issue, or between assessment methods.

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Soil-Erosion and Runoff Prevention by Plant Covers: A Review

Víctor Hugo Durán Zuazo and Carmen Rocío Rodríguez Pleguezuelo

Abstract Soil erosion is a critical environmental problem throughout the world's terrestrial ecosystems. Erosion inflicts multiple, serious damages in managed ecosystems such as crops, pastures, or forests as well as in natural ecosystems. In particular, erosion reduces the water-holding capacity because of rapid water runoff, and reduces soil organic matter. As a result, nutrients and valuable soil biota are transported. At the same time, species diversity of plants, animals, and microbes is significantly reduced. One of the most effective measures for erosion control and regeneration the degraded former soil is the establishment of plant covers. Indeed, achieving future of safe environment depends on conserving soil, water, energy, and biological resources. Soil erosion can be controlled through a process of assessment at regional scales for the development and restoration of the plant cover, and the introduction of conservation measures in the areas at greatest risk. Thus, conservation of these vital resources needs to receive high priority to ensure the effective protection of managed and natural ecosystems. This review article highlights three majors topics: (1) the impact of erosion of soil productivity with particular focus on climate and soil erosion; soil seal and crust development; and C losses from soils; (2) land use and soil erosion with particular focus on soil loss in agricultural lands; shrub and forest lands; and the impact of erosion in the Mediterranean terraced lands; and (3) the impact of plant covers on soil erosion with particular focus on Mediterranean factors affecting vegetation; plant roots and erosion control; and plant cover and biodiversity.

Keywords Biomass • C • Climate • Global warming • Soil degradation • Soil erosion • Soil productivity • Sustainability

1 Introduction

Worldwide, agricultural production occupies about 50% of the terrestrial environment. Soil degradation is as old as agriculture itself, its impact on human food production and the environment becoming more serious than ever before because of its extent and intensity. Soil erosion exacerbates the loss of soil nutrients and water, pollutes surface waterways, constitutes the prime cause of deforestation, contributes to global change, and reduces agricultural and environmental productivity. Each year, about 75 billion tons of soil is eroded from the world's terrestrial ecosystems, most from agricultural land at rates ranging from 13 to 40 $\mathrm{Mg\,ha^{-1}\,year^{-1}}$ (Pimentel and Kounang 1998). According to Lal (1990) and Wen and Pimentel (1998) about 6.6 billion tons of soil per year is lost in India and 5.5 billion tons are lost annually in China, while in the USA, soil loss is more than 4 billion tons per year. Because soil is formed very slowly, this means that soil is being lost 13–40 times faster than the rate of renewal and sustainability. Rainfall energy is the prime cause of erosion from tilled or bare land, occurring when the soil lacks protective vegetative cover.

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According to Naylor et al. (2002) the effects of vegetation on soil can be divided into two major related categories: bioprotection and bioconstruction. Plant cover protects soil against erosion by reducing water runoff (Rey 2003; Puigdefábregas 2005, Durán et al. 2006a; 2008) and by increasing water infiltration into the soil matrix (Ziegler and Giambelluca 1998; Wainwright et al. 2002).

Plants shelter and fix the soil with their roots (Gyssels et al. 2005; de Baets et al. 2007a,b) reduce the energy of raindrops with their canopy (Bochet et al. 1998; Durán et al. 2008). Also, vegetation can act as a physical barrier, altering sediment flow at the soil surface (Van Dijk et al. 1996; Lee et al. 2000; Martínez et al. 2006). The way the vegetation is spatially distributed along the slopes is an important factor for decreasing the sediment runoff (Lavee et al. 1998; Calvo et al. 2003; Francia et al. 2006). This barrier effect can lead to the formation of structures called phytogenic mounds. Such structures are found on the upslope side of large strips of grass disposed perpendicular to the slope (Meyer et al. 1995; Van Dijk et al. 1996; Abu-Zreig et al. 2004). Several mechanisms are involved in mound formation: the differential erosion rates in the closed environment of the plant (Rostagno and del Valle Puerto 1988), or the deposition of sediment resulting from a decrease in overland water flow (Sanchez and Puigdefabregas 1994; Bochet et al. 2000). On the other hand, Van Dijk et al. (1996) pointed out the interest in the relationships between plant morphology and the effects on soil erosion, showing that plant length and a complete canopy are key features for sediment trapping.

The importance of plant cover in controlling water erosion is widely accepted. In the short term, vegetation influences erosion mainly by intercepting rainfall and protecting the soil surface against the impact of rainfall drops, and by intercepting runoff. In the long term, vegetation influences the fluxes of water and sediments by increasing the soil-aggregate stability and cohesion as well as by improving water infiltration. This complex relationship has usually been reported as a negative exponential curve between vegetation cover and erosion rates for a wide range of environmental conditions. Concerning soil loss, this relationship can be defined by the following equation:

$$\mathrm{SL_r} = \mathrm{e}^{-\mathrm{bC}}, \tag{1}$$

where $\mathrm{SL_r}$ = relative soil loss (or soil loss under a specific vegetation cover compared to the soil loss on a bare surface), C = vegetation cover (%) and b = a constant which varies between 0.0235 and 0.0816 according to the type of vegetation and experimental conditions (Gyssels et al. 2005). Regarding to the runoff (R_r) for a wide range of vegetation types:

$$\mathrm{R_r} = \mathrm{e}^{-\mathrm{bC}}, \tag{2}$$

where b values ranging from 0.0103 to 0.0843 according to the experimental conditions (Fig. 1).

In some cases, however, a linear decline in runoff volume has been described as vegetation cover increases (Branson and Owen 1970; Kainz 1989; Greene et al. 1994). Some variations in the classical negative trend of the cover-erosion function have also been reported by de Ploey et al. (1976), Morgan (1996) and Rogers and Schumm (1991) under different specific experimental conditions, showing greater soil-loss rates as vegetation cover thickens, at least partially for a given range of covers.

The impact of herbaceous and woody crop production on soil erosion is crucial. Perennial grasses provide year-round soil cover, limiting erosion sometimes even with continued biomass harvest. Vigorous perennial herbaceous stands reduce water runoff and sediment loss and favour soil-development processes by improving soil organic matter, soil structure and soil

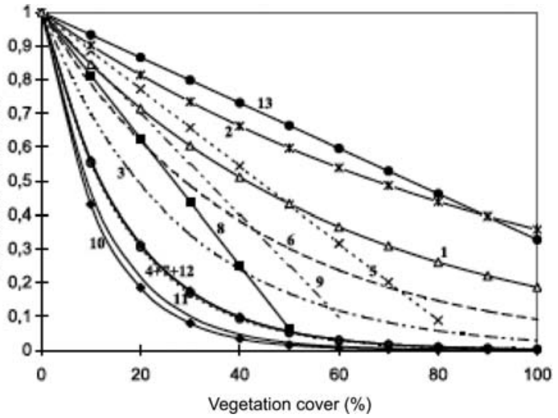

Fig. 1 Relationship between plant cover and relative runoff. 1, 2, Packer (1951); 3, 4, Marston (1952); 5, Branson and Owen (1970); 6, Elwell and Stocking (1976); 7, Lang (1979); 8, 9, Kainz (1989); 10, 11, Francis and Thornes (1990); 12, Lang (1990); 13, Greene et al. (1994)

water and nutrient-holding capacity. Minimum tillage management of row crops reduces erosion compared with systems involving more frequent or more extensive tillage. Woody crops reduce water erosion by improving water infiltration, reducing impacts by water droplets, intercepting rain and snow and physically stabilizing soil by their roots and leaf litter. Harvesting of woody plants may be followed by increased erosion. Forestry clear cutting, especially on steep slopes, often results in a large increase in water erosion.

In the semi-arid Mediterranean region, most experimental studies on the influence of the native vegetation on erosion have quantified soil loss and runoff under woodlands or shrublands comprising a mixture of plant species (Francis and Thornes 1990; Romero et al. 1999; Durán et al. 2006a). All of these studies have concluded that typical Mediterranean shrubland vegetation is efficient in reducing water erosion, even under extreme torrential simulated rainfalls (González et al. 2004). In this context, Bochet et al. (2006) studied the influence of plant morphology and rainfall intensity on soil loss and runoff at the plant scale for three representative species: *Rosmarinus officinalis*, *Anthyllis cytisoides* and *Stipa tenacissima* of a semi-arid patchy shrubland vegetation in relation to bare soil in eastern Spain. The results indicate that the individual plants were valuable in interrill erosion control at the microscale, and the different plant morphologies and plant components explained the different erosive responses of these three species. Canopy cover played was key in reducing runoff and soil loss, and the litter cover beneath of plants was fundamental for erosion control during intense rainfall. In assessing the great potential of plant covers, it is therefore essential to consider its impact on soil protection.

2 Impact of Erosion on Soil Productivity

The loss of soil from land surfaces by erosion is widespread globally and adversely impacts the productivity of all natural, agricultural, forest, and rangeland ecosystems, seriously decreasing water availability, energy, and biodiversity throughout the world. Future world populations will require ever-increasing food supplies, considering that more than 99.7% of human food comes from the land (FAO 1998), while less than 0.3% comes from the oceans and other aquatic ecosystems. Maintaining and augmenting the world food supply depends basically on the productivity and quality of all soils. Soil erosion and runoff reduce the soil productivity decreasing rainfall water infiltration, and water-storage capacity. In this sense, the effect of plant cover on soil represents a sustainable measure for improving productivity, given their many environmental benefits (Fig. 2). Since water is the prime limiting factor of productivity in all terrestrial ecosystems, when soil-water availability for agriculture is reduced, productivity is depressed. Particularly, in semiarid areas vegetation suffers longer periods of water deficit, determining the vegetation structure and complexity, and thus soil protection and water conservation. During precipitation, some water is intercepted by the plant covers, and a new spatial distribution of rainfall takes place due to the throughfall and stem-flow pathways (Bellot and Escarré 1998). In this context, the type of plant community buffers the kinetic energy of rainfall before the water reaches the soil (Brandt and Thornes 1987; Durán et al. 2004a).

2.1 Climate and Soil Erosion

Recent studies suggest that climatic variability will increase as a consequence of global warming, resulting in greater frequency and intensity of extreme weather events, which will inevitably intensify erosion (Nunes and Seixas 2003; Nearing et al. 2005). This trend could be especially threatening in Mediterranean areas highly susceptible to soil erosion, where precipitation is characterized by scarcity, torrential storms and extreme variability in space and time (Romero et al. 1998). Flash storms are common throughout the Mediterranean area, and they have very short return periods (de Luis 2000). Several researchers have pointed out that this irregularity of precipitation is the main cause for temporal irregularity of erosion rates in Mediterranean landscapes (Zanchi 1988; Renschler et al. 1999; Renschler and Harbor 2002). Also, no exact relation has been found between extreme rainfall and extreme fluvial discharge (Osterkamp and Friedman 2000; Nunes et al. 2005), and largest rainfall events do not necessarily produce the maximum soil erosion (González et al. 2004; Romero et al. 1999). On the other hand, according to Marqués et al. (2007),

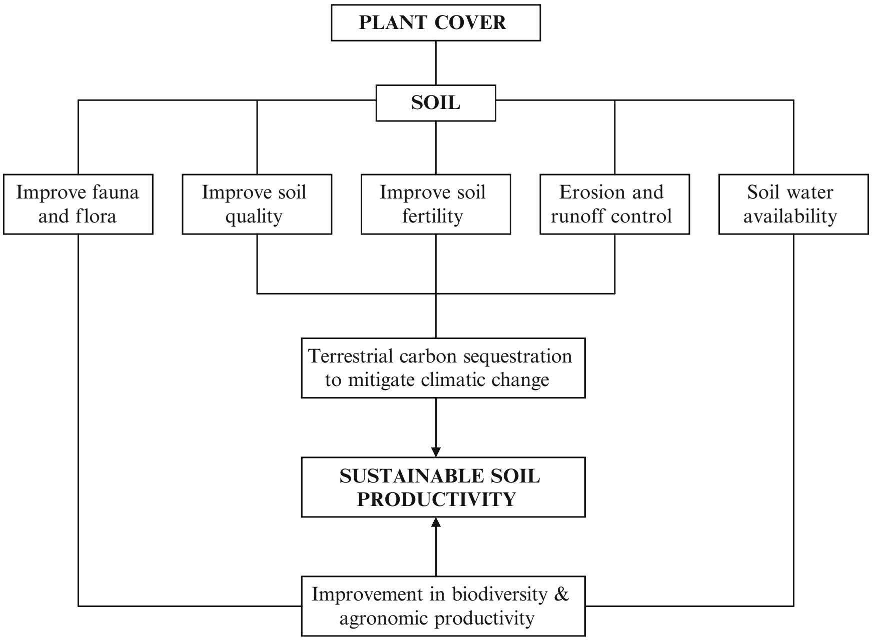

Fig. 2 Soil protection by plant cover for sustainable soil productivity

the erosive power of a single light rainfall event of 20.8 mm h^{-1} with kinetic energy of 13.5 J m^{-2} mm^{-1} is negligible when plots are covered with natural vegetation. Moreover, in addition to the Mediterranean rainfall being highly variable in space, soil and plant cover is extremely diverse and, as a consequence, erosion rates display great spatial variation. In any case, quantification of magnitudes of daily soil eroded can be affected by field methods. All these factors explain the extreme disparity of erosion amounts reported at different times and in different places and highlight the difficulty presented by extrapolating data obtained from experimental plots (Roels 1985; Stroosnijder 2005).

Although soil erosion varies from site to site, and from year to year, the annual amount of soil eroded depends on a few daily erosive events. Each year scattered daily erosive events represent more than 50% of annual soil eroded, regardless of the total amount.

2.2 Soil Seal and Crust Development

Surface crusts and seals can form from a variety of processes, both physical and biological, and have the potential to alter runoff and erosion, especially in regions with low plant covers. Despite the obvious links between seals and crusts, these features have rarely been considered together. Many soils, especially those in semiarid regions, develop compacted surface layers that are denser and have lower porosities than the materials immediately below them (Valentin and Bresson 1992). These layers, known as “seals” when wet and “crusts” when dry, are generally no more than a few millimeters thick and form through the interaction of several, often interrelated, processes (Bradford et al. 1987; Singer and Shainberg 2004). Most commonly, crusts and seals are described as having a physico-chemical origin in which soil aggregates are initially broken down by raindrop impacts and/or slaking processes. The dispersed particles are subsequently deposited within and clog soil pore spaces, creating a low-permeability layer at the surface (Assouline 2004). As the seals dry to form crusts, clays can also act to bind particles together, reinforcing the persistence of the crusted layer (Shainberg 1992). However, crusts and seals can form in a variety of other ways, including through the compaction of soils by raindrop impacts, from the erosion of coarse surface layers by runoff, through the deposition of fine particles brought in by overland flows, from clay swelling at the soil surface (Valentin 1991), and from biological organisms (such as fungal hyphae) binding soil particles together (Greene et al. 1990).

Despite the large array of possible formative processes, physical (or nonbiological) crusts and seals are commonly classified as either structural or depositional features (West et al. 1992). Structural seals and crusts form in association with rain falling directly on soils and typically require raindrop impact to develop (Fox et al. 2004). Depositional seals and crusts, however, result from the lateral redistribution of sediment by runoff, and do not require soils to be directly exposed to rainfall (Assouline 2004). Once formed, sealed soils generally have lower hydraulic conductivities and infiltration rates and have higher shear strengths than unsealed soils although this very much depends on the type of seal in place. These conditions combine to increase runoff and influence local erosion processes (McIntyre 1958; Assouline 2004).

Almost all of the existing research into seal and crust formation has been undertaken on soils that have been extracted, physically and theoretically, from their surrounding environments (Diekkruger and Bork 1994; Fox and Bissonnais 1998; Mills and Fey 2004). Therefore, the loss of vegetation covers from soil increases the development of surface crusts and seals, and consequently increases soil erosion and runoff.

2.3 Carbon Losses from Soils

Soil degradation is one of the greatest environmental problems in the world. In semiarid Mediterranean areas the dry climate leads to a low level of plant cover which, in turn, leads to very scarce organic-matter input, and, consequently, to a poor soil-structure development (Díaz et al. 1994). Under these conditions the role of plant covers in protecting soil against erosion is crucial, since the removal of vegetation strongly increases surface runoff and sediment yield and, as a result, soil quality deteriorates (Kaihura et al. 1999).

Vegetation removal is normally followed by a period in which the soil has sufficient organic matter to maintain its physical–chemical properties, enabling it to recover from the damage, according to the concept of soil resilience (Castillo et al. 1997; Durán et al. 2006a). Soils rich in organic matter, such as those of many rainy regions, are more resilient than soils with low organic-matter content, such as those which predominate in arid and semiarid areas. In the latter case, when the surface layer, which contains fresh plant remains, is eroded, the subsurface material is exposed and the capacity of this material to hold nutrients becomes crucial (Gregorich et al. 1998).

Although there is general agreement with regard to the role of erosion in soil organic carbon losses, some controversy persists with respect to the intensity of soil organic carbon losses caused by mineralization. According to Martínez-Mena et al. (2002), the mineralization process was found to be much more influential than erosion in the soil organic carbon losses recorded during the 9 years following vegetation removal in a semiarid Mediterranean soil. In the first 6 years, rapid mineralization was the main cause of the soil organic carbon decreases measured; while in the next 3 years the soil organic carbon losses were due mainly to erosion. Vegetation removal led to a progressive enrichment of the sediments in organic carbon and nitrogen with time. These results reflect the importance of preserving the plant cover in semiarid areas, where it is crucial for maintaining the soil organic-carbon stock. In this sense, Yaalon (1990) indicated that mineralization would lead to a reduction in the soil organic-matter content within 50 years in the Mediterranean area. On the contrary, Squires et al. (1998) pointed out that the carbon stored in dryland soils is a very substantial deposit, since it has been stabilized over a period of hundreds to thousands of years. Scharpenseel and Pfeiffer et al. (1998) indicated that these areas may be very sensitive to climatic change due to inadequate reserves of water and soil nutrients. Therefore, the vulnerability of Mediterranean arid and semiarid lands to human-induced changes in soil use means that the effects of climate change upon these environments will be exacerbated.

Reduced precipitation or increased temperature accelerates land degradation through the loss of plant cover, biomass turnover, nutrient cycling and soil organic-carbon storage, accompanied by higher greenhouse emissions (Ojima et al. 1995). An understanding of the dynamics of soil organic carbon is required to appreciate fully the ability of soils to stabilize carbon and its implications for global change (Bajracharya et al. 1998).

It is well known that water erosion selectively removes the fine organic particles from the soil, leaving behind large particles and stones. Fertile soils frequently contain about 100 tons of organic matter per hectare (or 4% of the total soil weight) (Young 1990). Because most of the organic matter is close to the soil surface in the form of decaying leaves and stems, erosion of the topsoil significantly decreases soil organic matter. Several studies have demonstrated that the soil removed by either wind or water erosion is 1.3–5.0 times richer in organic matter than the soil left behind (Barrows and Kilmer 1963).

Soil organic matter facilitates the formation of soil aggregates and increases soil porosity. In this way, it improves soil structure, which in turn facilitates water infiltration and ultimately the overall productivity of the soil (Chaney and Swift 1984; Langdale et al. 1992). In addition, organic matter aids cation exchange, enhances root growth, and stimulates the increase of important soil biota. About 95% of the soil nitrogen and 25–50% of the phosphorus are contained in the organic matter.

Once the organic matter layer is depleted, the productivity of the ecosystem, as measured by crop-plant yields, declines both because of the degraded soil structure and the depletion of nutrients contained in the organic matter. Soils that suffer severe erosion may produce 15–30% lower crop yields than un-eroded soils (Schertz et al. 1989; Langdale et al. 1992).

The main losses of C from soil is in the form of CO_2 from OM mineralisation although fires cause direct C emissions to the atmosphere and changes species composition of the vegetation (Harden et al. 2000), altering the dynamics of terrestrial C stores for subsequent decades. The gaseous C efflux from soils depends initially on the rate of CO_2 (or CH_4) production within the soil-plant root system, and subsequently on the rate of gaseous diffusion and mass flow from soil waters to the atmosphere; a function of soil moisture and textural properties (Skiba and Cresser 1991).

Increased C sequestration in soils, as a way to reduce atmospheric CO_2 concentrations, was first proposed in 1977 (Dixon et al. 1994). One appropriate option is to restore a proportion of the C historically lost from soils that have previously been depleted in C, such as agricultural and degraded soils (Smith et al. 2001a,b), e.g. revegetation of abandoned arable land may increase soil C by $0.3–0.6 \times 10^3$ kg C ha^{-1} year^{-1}. In order to maximize C sequestration, knowledge of factors such as erosion and the translocation of soil across the landscape also need to be considered (Van Oost et al. 2005) particularly regarding agricultural land, where tillage and erosion are strongly related. At present, most grasslands are believed to be C sinks, with an estimated $0.03–1.1 \times 10^3$ kg C ha^{-1} year^{-1} as C sequestration is strongly influenced by the productivity and management of the ecosystems (Soussana et al. 2004) although grassland-derived soils do tend to have higher base saturation, enhancing aggregation and increased capability to sequester C (Collins et al. 2000).

The sheet erosion is flow over vegetated surfaces while channel erosion is limited to where soils lack plant cover. This overland flow occurs, removing topsoil and hence substantial OM translocation, when runoff is greater than the soil-infiltration capacity. Carbon and nutrients from water-eroded soil is relocated downslope from one area to another or transported to surface waters (Stallard 1998; Smith et al. 2001b; Liu et al. 2003; Rodríguez et al. 2007a). The amount of C mobilized by erosion processes has been estimated at $0.20–0.76 \times 10^{12}$ g C year^{-1}, of which $0.08–0.29 \times 10^{12}$ g C year^{-1} was re-deposited and $0.12–0.46 \times 10^{12}$ g C year^{-1} was transported to surface waters (Quinton et al. 2006). Rodríguez et al. (2007a), in south-eastern Spain reported the SOC losses about 12.2 g C m^{-2} from the taluses of orchard terraces without plant covers.

3 Land Use and Soil Erosion

The main problems for soils in the European Union are irreversible losses due to increasing soil sealing and soil erosion, and continuing deterioration due to local and diffuse contamination. It is envisaged that Europe's soil resource will continue to deteriorate, as a result of changes in climate, land use and other human activities.

Soil erosion, in particular, is regarded as one of the major and most widespread forms of land degradation, and, as such, poses severe limitations to sustainable agricultural land use. Erosion reduces on-farm soil productivity and contributes to water-quality problems from the accumulation of sediments and agrochemicals in waterways.

Prolonged erosion causes irreversible soil loss over time, reducing the ecological functions of soil: mainly biomass production, crop yields due to removal of nutrients for plant growth, and reduction in soil-filtering

Table 1 Extend of human-induced soil degradation by erosion in Europe (million hectares)[a]

	Erosion type	Light	Moderate	High	Extreme	Total
Accession countries	Water erosion	4.5	29.2	14.7	0.0	48.4
	Wind erosion	0.0	0.0	0.0	0.0	0.0
	AC total	4.5	29.2	14.7	0.0	48.4
EFTA countries	Water erosion	0.8	1.5	0.0	0.0	2.3
	Wind erosion	0.6	1.3	0.0	0.0	1.9
	EF total	1.3	2.9	0.0	0.0	4.2
Rest of Europe	Water erosion	0.8	19.3	6.5	1.0	27.7
	Wind erosion	0.0	5.8	0.0	0.7	6.5
	ER total	0.8	25.1	6.5	1.7	34.2
European Union	Water erosion	12.8	11.9	1.4	0.0	26.2
	Wind erosion	1.0	0.1	0.0	0.0	1.1
	EU total	13.8	12.0	1.4	0.0	27.3
Europe (excl. the Russian Federation)	Water erosion	18.9	62.0	22.6	1.1	104.6
	Wind erosion	1.6	7.2	0.0	0.7	9.5
	AC total	20.5	69.2	22.6	1.8	114.1[b]

[a] Gobin et al. (2003)

[b] 17.4% of total land area

Note: Any mismatch between totals and disaggregated figures is due to the rounding process

Source: EEA (Oldeman et al. 1991; Van Lynden 1995; data: Glasod, UNEP and ISRIC-UNEP/GRID)

capacity due to disturbance of the hydrological cycle (from precipitation to runoff).

Soil losses are high in southern Europe, but soil erosion due to water is becoming an increasing problem in other parts of Europe. Table 1 shows some of the findings regarding to the area affected by soil degradation in Europe (Gobin et al. 2003).

The Mediterranean region is considered to be particularly prone to erosion. This is because it is subject to long dry periods followed by heavy bursts of intensive rainfall, falling on steep slopes with fragile soils and low plant cover. According to the EEA (2001), soil erosion in north-western Europe is considered to be slight because rain is falling mainly on gentle slopes, is evenly distributed throughout the year and events are less intensive. Consequently, the area affected by erosion in northern Europe is much more restricted in its extent than in southern Europe.

In parts of the Mediterranean region, erosion has reached a stage of irreversibility and in some places erosion has practically ceased because there is no more soil left. In the most extreme cases, soil erosion leads to desertification. With a very slow rate of soil formation, any soil loss of more than $1\,\mathrm{Mg\,ha^{-1}\,year^{-1}}$ can be considered as irreversible within a time span of 50–100 years.

Losses of $20\text{–}40\,\mathrm{Mg\,ha^{-1}}$ in individual storms, which may happen once every two or three years, are measured regularly in Europe with losses of more than $100\,\mathrm{Mg\,ha^{-1}}$ in extreme events (Morgan 1992).

Attention is focused mainly on rill- and interrill erosion because this type of erosion affects the largest area. Other forms of erosion are also important – for example, gully erosion, landslides and, to a lesser extent, wind erosion.

The rate of soil degradation is depends upon the rate of land-cover degradation, which in turn is influenced by both adverse climatic conditions and land-use management changes. Plant cover, type of land use, and intensity of land use are clearly key factors controlling the intensity and the frequency of overland flow and surface erosion. Vegetation cover may be altered radically by human activity within a short time, but physical and biological changes within the soil, affecting erosion rates, may take longer periods. The type of land use and land-use intensity is affected by various environmental and socio-economic factors; therefore indicators for soil erosion-risk assessment should be related to these factors.

3.1 *Soil Loss in Agricultural Lands*

Approximately 50% of the earth's land surface is devoted to agriculture; of this, about one-third is planted with crops and two-thirds dedicated to grazing lands (WRI 1997; USDA 2001). Cropland is more susceptible to erosion because of frequent cultivation of the soils and the vegetation is often removed before crops are planted. In addition, cropland is often left without vegetation between plantings, intensifying erosion on agricultural land, which is greater than erosion in natural forest areas.

According to Pimentel et al. (1995), worldwide erosion on agricultural lands averages about $30\,\mathrm{Mg\,ha^{-1}\,year^{-1}}$ and ranges from 0.5 to $400\,\mathrm{Mg\,ha^{-1}\,year^{-1}}$. As a result of soil erosion, during the last 40 years about 30% of the world's arable land has become unproductive and, much of that has been abandoned for agricultural use (Kendall and Pimentel 1994). Each year an estimated 10 million ha of cropland worldwide are abandoned due to lack of productivity caused by soil erosion (Faeth and Crosson 1994). On the other hand, extensive Mediterranean areas cultivated with rainfed crops are mainly restricted to hilly lands with shallow soils, very sensitive to erosion. In this context, Extensive areas cultivated with rainfed crops (i.e. vines, almonds and olives) are mainly confined to hilly lands with shallow soils which are very prone to erosion under traditional soil-management systems (Francia et al. 2006; Martínez et al. 2006; Durán et al. 2008) but erosion can be significantly reduced by the use of plant strips running across the hillslope (Table 2) especially with aromatic and medicinal plants (Fig. 3). Garcia et al. (1995) pointed out that the cereal cultivation in steep slopes encourages soil erosion, especially under non-conservative systems, and the change of cereals into meadows represents an improvement of the hydrological functioning, which reaches its most positive values with colonization by a dense shrub cover. Otherwise, these areas become vulnerable to soil erosion because of the decreased protection by vegetation cover in reducing effective rainfall intensity at the ground surface. Almonds and vines require frequent removal of perennial vegetation using herbicides or by tillage. In fact, soils under these crops remain almost bare during the whole year, creating favourable conditions for overland flow and soil erosion. Erosion data measured along the northern Mediterranean region and the Atlantic coastline located in Portugal, Spain, France, Italy and Greece in a variety of landscapes and under a number of land uses representative of the Mediterranean region (rainfed cereals, vines, olives, *Eucalyptus* groves, shrubland) showed that the greatest rates of runoff and sediment loss were measured in hilly areas under vines, i.e. in south-eastern France $34\,\mathrm{Mg\,ha^{-1}}$ (Wainwright 1996), in Spain $282\,\mathrm{Mg\,ha^{-1}}$ (Martínez-Casasnovas et al. 2005). Areas cultivated with wheat are sensitive to erosion, especially during winter, generating intermediate amounts of runoff and sediment loss especially under rainfalls higher than 380 mm per year. Olives grown under semi-natural conditions, particularly where there is an understorey of annual plants greatly restrict soil loss to negligible values. Erosion in shrublands increased with decreasing annual rainfall to values in the range of 280–300 mm, and then decreased as rainfall decreased further.

Rainfall amount and distribution are the major determinants of cereal biomass production (Kosmas et al. 1993). These areas become vulnerable to erosion because of the decreased protection by vegetation cover in reducing effective rainfall intensity at the ground surface (Faulkner 1990), the reduction of infiltration rate due to compaction from farm machinery, and the formation of a soil surface crust (Morin and Benyamini 1977).

Land-use changes affecting many mountains in the world have serious consequences on runoff and

Table 2 Average soil-erosion and runoff prevention by plant strips in semiarid slopes with olive and almond orchards under 30% and 35% slope, respectively

	Olive orchards		Almond orchards	
Soil-management system	Erosion ($\mathrm{Mg\,ha^{-1}\,year^{-1}}$)	Runoff ($\mathrm{mm\,year^{-1}}$)	Erosion ($\mathrm{Mg\,ha^{-1}\,year^{-1}}$)	Runoff ($\mathrm{mm\,year^{-1}}$)
NT	25.6	39.0	n.a.	n.a.
CT	5.70	10.9	10.5	58.1
BS	2.10	19.8	1.66	23.8
NVS	7.1	8.6	n.a.	n.a.
LS	n.a.	n.a.	5.18	47.8
TS	n.a.	n.a.	0.50	26.1
SS	n.a.	n.a.	2.10	31.5
RS	n.a.	n.a.	0.60	23.2

Abbreviations: *NT* non-tillage without plant strips, *CT* conventional tillage, *BS* non-tillage with barley strips, *NVS* non-tillage with native vegetation strips, *LS* lentil strips, *TS* thyme strips, *SS* salvia strips, *RS* rosemary strips, *n.a.* not available

Fig. 3 Thyme strips used for erosion control in semiarid slopes under almond orchards and plots used for study the harvest intensity of biomass from cultivated sage (*Salvia lavandulifolia V.*), oregano (*Origanum bastetanum* L.), santolina (*Santolina rosmarinifolia* L.), and lavender (*Lavandula lanata* L.)

sediment yield and are probably the most important factor in controlling soil conservation and sustainability. For instance, the traditional system of cereal cultivation (i.e. shifting agriculture) was very extensive in past centuries on steep sunny hillslopes. Nowadays the hillslopes cultivated in the past by means of shifting agriculture are characterized by an open submediterreanean shrub on a very thin and stony soil, testimony of intense soil loss. The consequences of fertilizing the cereal fields can be observed several years later not only by the solute outputs by runoff, but also by the quick plant colonization after farmland abandonment.

3.2 Shrub and Forest Lands

The Mediterranean basin has seen the development of some of the world's oldest civilizations, spreading agriculture and livestock while using trees for building and fuel being these areas has long been exploited and as a result the tree cover is drastically reduced in Mediterranean countries (Le Houerou 1981; Thirgood 1981; Blondel and Aronson 1999). About the 9–10% of the Mediterranean area is currently forested, and in the Iberian Peninsula only 0.2% can be considered natural or seminatural forests (Marchand 1990). Simultaneously, the surface area covered by shrublands has increased, representing stages of degradation of mature forests as well as stages of vegetation recovery in abandoned agricultural lands (di Castri 1981; Grove and Rackham 2001). In both cases, local and regional characteristics, such as resource availability or the lack of tree propagules, act as barriers to succession (Pickett et al. 2001) and result in self-perpetuating systems that hardly return to the structure and complexity of the original mature community (Blondel and Aronson 1999).

Several hilly areas under natural forests around the Mediterranean region have been reforested with exotic species such as *Eucalyptus*. Such soils are undergoing intense erosion as compared with soils left under natural vegetation. However, the measured rates of erosion under *Eucalyptus* are relatively lower than those measured under vines, almonds and cereals.

Soil-erosion data measured from various types of vegetation and certain physiographic conditions showed that the best protection from erosion was measured in areas with a dominant vegetation of evergreen oaks, pines and olive trees under semi-natural condition.

Pines have a lower ability to protect the soils in southern aspects due to the higher rate of litter decomposition and the restricted growth of understorey vegetation. Deciduous oak trees offer relatively low protection from erosion in cases where the falling leaves do not cover the whole soil surface.

The main factors affecting the evolution of the Mediterranean vegetation, in the long term, are related to the irregular and often inadequate water supply, the long period of the dry season, and in some cases fire and overgrazing. According to the types of leaf generation, the following two major groups of vegetation can be distinguished: (a) deciduous: drought avoiding with a large photosynthetic capacity but no resistance to desiccation; and (b) evergreen (*sclerophyllous*): drought enduring with low rates of photosynthesis. The main response of the plants to increased aridity is the reduction in leaf-area index. Severe droughts that cause a reduction in leaf-area index may be beneficial in the short term as plant transpiration is reduced, but such drought will increase the probability of enhanced soil erosion when rain eventually falls, as protective vegetation cover is reduced.

The various ecosystems present in the Mediterranean region have a great capacity of adaptation and resistance to aridity, as have most of the species, to survive under Mediterranean climatic conditions. For many months, plants may have to endure soil-moisture contents below the theoretical wilting point. Most probably the expected changes in the vegetation performance, resulting from a gradual precipitation decrease, would only be noticed after a critical minimum number of years.

In stable forest ecosystems, where soil is protected by vegetation, erosion rates are relatively low, ranging from only 0.004–0.05 Mg ha^{-1} year^{-1} (Roose 1988). Tree leaves and branches intercept and diminish rain and wind energy, while the leaves and branches cover the soil under the trees to protect the soil further. However, this changes dramatically when forests are cleared for crop production or pasture.

Vacca et al. (2000) has estimated runoff coefficients of 0.65–1.59%, and erosion rates between 0.03 and 0.05 Mg ha^{-1} in plots of 20 m^2 covered by herbaceous plants and shrubs, while in *Eucalyptus* sp. plots (15 years old and 25% vegetation cover) the estimated rates were 2.01% and 0.19 Mg ha^{-1}, respectively. Romero et al. (1988) calculated annual soil losses of 0.08–2.55 Mg ha^{-1} year^{-1} in a catchment with 35% of vegetation cover. In a microcatchment with 60% of vegetation cover, Albadalejo and Stocking (1989) determined rates between 0.5 and 1.2 Mg ha^{-1} year^{-1}, and López et al. (1991) reported annual losses of 0.1 Mg ha^{-1} year^{-1} in plots with 80% shrub cover. Areas with reduced plant cover (lower than 50%) caused by human interference or affected by wildfires can increase soil loss in the first years after disturbance (Soto and Díaz 1997). According to Durán et al. (2004a), on a hillslope with 35.5% of slope under *Rosmarinus officinalis* cover runoff ranged from 7.9 to 1.3 mm year^{-1} and erosion from 0.16 to 0.002 Mg ha^{-1} year^{-1}, while under native vegetation, runoff ranged 4.4–0.9 mm year^{-1} and erosion from 0.32 to 0.002 Mg ha^{-1} year^{-1}. Chirino et al. (2006) measured the erosion rates with different plant cover types: dry grassland formations with dwarf scrubs (*Brachypodium retusum*, *Anthyllis cytisoides* L., *Helianthemum syriacum*, and *Thymus vulgaris* L.) with 0.049 Mg ha^{-1} year^{-1}; under landscape patches composed of scattered thorn and sclerophyllous shrublands (*Quercus coccifera* L., *Pistacia lentiscus* L., *Erica multiflora* L., *Rhamnus lyciodes* L. and *Rosmarinus officinalis* L.) 0.042 Mg ha^{-1} year^{-1}; afforested dry grasslands 0.035 Mg ha^{-1} year^{-1}, and finally afforested thorn shrublands of 0.019 Mg ha^{-1} year^{-1}. By contrast, the rate of bare soil had a runoff coefficient and soil loss of 4.42% and 1.90 Mg ha^{-1} year^{-1}, respectively (Chirino et al. 2006). In this context, for hilly areas with 13% of slope in SE Spain and bare soil the runoff ranged from 154 to 210 mm and erosion from 4.5 to 7.8 Mg ha^{-1} year^{-1}, differing significantly from those protected with plant covers of aromatic and medicinal plants (Fig. 4) (Durán et al. 2006a).

The inappropriate wild harvest of aromatic plants by uprooting in mountainous areas endangers the soil

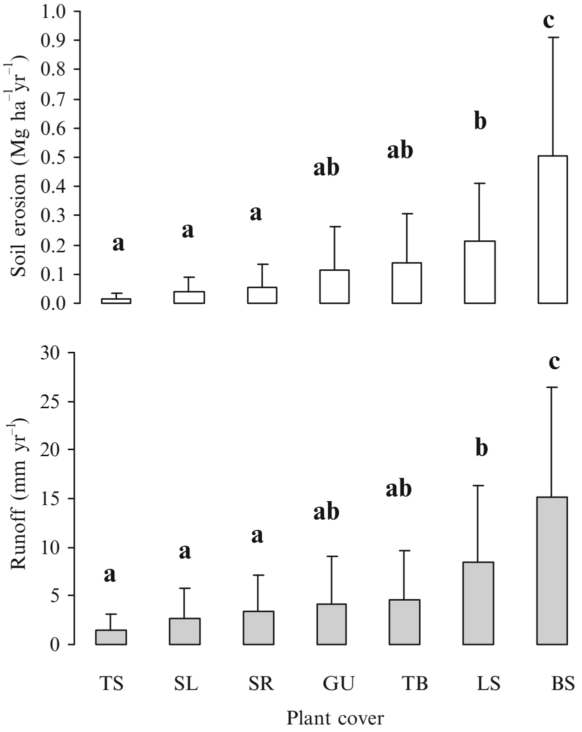

Fig. 4 Mean soil erosion and runoff for each plant cover and bare soil. Columns with different letters are statistically different al level 0.01 (LSD). TS, *Thymus serpylloides*; SR, *Santolina rosmarinifolia*; SL, *Salvia lavandulifolia*; GU, *Genista umbellate*; TB, *Thymus baeticus*; LS, *Lavandula stoechas*. *Vertical bars* represent Standard deviation (n = 24)

conservation, and there is an urgent need to implement appropriate land management. Over a four-year period, soil erosion and runoff were monitored in erosion plots in Lanjarón (Granada, SE Spain) on the southern flank of the Sierra Nevada Mountains, comparing four harvest intensities of four aromatic shrubs (*Lavandula lanata* L., *Santolina rosmarinifolia* L. *Origanum bastetanum,* and *Salvia lavandulifolia V.*): 0% (HI-0), 25% (HI-25), 50% (HI-50), and 75% (HI-75). The average soil loss for HI-0, HI-25, HI-50, and HI-75 during the study period was 144.6, 187.2, 256.0, and 356.0 kg ha^{-1}, respectively, and runoff 2.6, 3.2, 3.4, and 4.7 mm, respectively (Fig. 5). Since no significant differences were found between HI-25 and HI-50 for soil erosion and runoff, and harvest and distillation of wild aromatic plants currently persists as an important economic activity in mountainous areas of the study zone, this study demonstrated that the cultivation of aromatic shrubby plants (even when removing 50% of the above ground biomass) protected the soil from rain erosivity and produced reasonable essential-oil yields Consequently, the rational harvest of cultivated aromatic and medicinal herbs in semiarid slopes not only protect the soil against erosion and improved soil quality but also made sustainable agriculture possible in mountain areas.

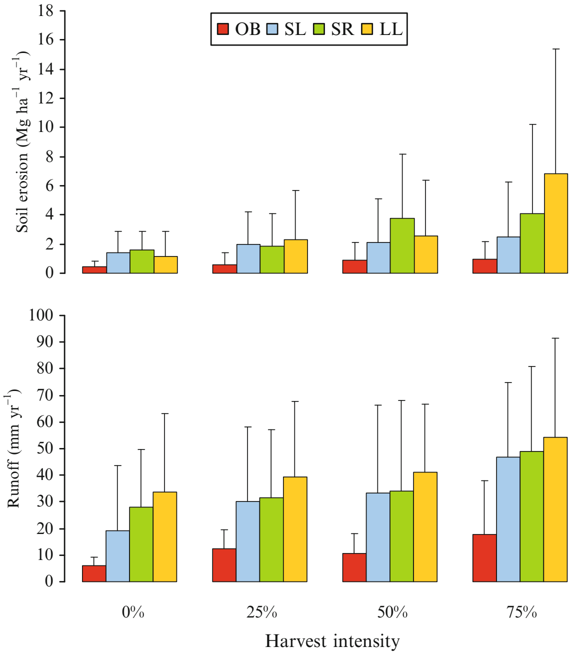

Fig. 5 Mean annual soil erosion and runoff for each harvest intensity and plant cover. OB, *Origanum bastetanum*; SL, *Salvia lavandulifolia*; SR, *Santolina rosmarinifolia*; LL, *Lavandula lanata. Vertical bars* represent Standard deviation (n = 4)

3.3 Impact of Erosion in the Mediterranean Terraced Lands

The need for terracing as a soil-conservation technique on sloping land has been emphasized. In much of the steeply sloping lands of Mediterranean basin, terracing was introduced in a bid to control soil erosion (Durán et al. 2005; Abu Hammad et al. 2006). Most of the terraces commonly develop a systematic variation in crop production showing a low yield on the upper part, which progressively increases down the lower sections of terrace. This uneven terrace productivity, which is observed for all crops, is hypothesised to be mainly a result of hoeing down the slope perpendicular to the contour, which is ergonomical but gradually causes scouring of the topsoil on the upper parts of the terrace which is then deposited in the lower parts.

An important land use change recorded in the Mediterranean basin comprises the abandonment of agricultural lands due to economic and social changes, which is followed by significant impacts on soil erosion. Observed land abandonment may have positive or negative impacts on soil protection from erosion because fundamental ecosystem processes are influenced by changes in agricultural practices and soil-resource management. Olive and almond orchards comprise typical examples of traditional, extensive cultivation, which is abandoned. The olive groves are spread on marginal areas and located mainly on sloping terraced lands with low-productivity soils.

In these areas with high erosion risk, land abandonment is followed by natural vegetation regeneration, resulting in decreased soil erosion (Grove and Rackham 2001). According to theory as shrub vegetation is filling in, protection of soil resources is increasing while soil erosion is decreasing (Elwell and Stocking 1976; Morgan 1996). Also, after abandonment, soil properties such as organic-matter content, soil structure, and infiltration rate improve, resulting to more effective soil protection to erosion (Trimble 1990; Kosmas et al. 2000). However, simultaneous stopping of traditional land management practices results in soil erosion increase (Morgan and Rickson 1990). Specifically, on sloping lands, an important abandonment of conservation practices, which are applied on traditional drystone terraces, is recorded. According to Koulouri and Giourga (2006) the abandonment of traditional extensive cultivation in the Mediterranean basin has different impacts on soil erosion which closely related to slope gradient. That is, when the slope is steep (25%), soil erosion increases significantly because the dense protective cover of annual plants decrease and shrubs' vegetation cover increases, and if the slope gradient is very steep (40%), soil erosion remains at the same high levels after cultivation abandonment. And the drystone terraces play an important role by supporting soil material and collapse from runoff water.

On the other hand, the study was carried out in Almuñécar (SE Spain) addressing the impact of erosion in the taluses of orchard terraces. The farmers in this zone construct bench terraces primarily to use the steeply sloping lands for agriculture, and to reduce soil erosion (Fig. 6). Today, on these steep terraces, intensive irrigated agriculture has established subtropical crops, including avocado (*Persea americana* Mill.), mango (*Mangifera indica* L.), loquat (*Eriobotrya japonica* L.), custard apple (*Annona cherimola* Mill.), litchi (*Litchi chinensis* Sonn.) and others (Durán et al. 2003, 2006b). However, severe soil erosion occurs frequently on the bare taluses of bench terraces, especially those with sunny southern orientations (Durán et al. 2005). The detached soil from the talus accumulates on the platform of the terrace below, hindering manual fruit harvesting and orchard maintenance. The use of native (mostly weeds) and aromatic and medicinal plants (AMP) to control soil and nutrient losses were also investigated using erosion plots 16 m^2 (4 m ×4 m) in area, and located in the taluses of orchard terraces (Durán et al. 2004b). The severity of soil erosion is thought to vary according to the structure of the bench terrace and the ground cover conditions. Rills are the primary form of erosion on the taluses of orchard terraces with extreme and heavy storms, some of which develop into gullies that can run from the upper terrace down to the lower terrace (Fig. 7). Nevertheless, rills and gullies are rarely found on plant-covered

Fig. 6 Orchard terraces for subtropical farming species in Granada coast (SE Spin)

Fig. 7 Rills and gullies in the taluses of orchard terraces

Fig. 8 Gullies in the taluses of orchard terraces and plots used for monitoring the erosion control by plant covers

taluses. The plant covers of thyme and sage in relation to bare soil reduced erosion by 63%, and 30%, and decreased runoff and by 54% and 40%, respectively (Durán et al. 2002) (Fig. 8). Also, the loss of nutrients (NPK) from taluses of orchard terraces was controlled by plant covers (Durán et al. 2004b). Terrace pollution and erosion (even destruction) were prevented by planting the taluses with covers of plants having aromatic, medicinal, and mellipherous properties. This increased the feasibility of making agricultural use of soils on steep slopes. Moreover, an ecological balance was at least partially restored, reducing pollution that is injurious to the environment as well as to humans.

4 Impact of Plant Covers on Soil Erosion

Runoff is a fundamental process in land degradation, causing soil erosion and influencing the soil water balance and hydrology of the catchments. Many authors have discussed the runoff behaviour of different land-use types and the effects of land-use change on runoff production (Kosmas et al. 1997; Narain et al. 1998; Cammeraat and Imeson 1999; Bellot et al. 2001; McDonald et al. 2002; Pardini et al. 2003). In the context of afforestation/reforestation or vegetation restoration, it is commonly concluded that runoff rates and peak flows are reduced (Mapa 1995; Zhou et al. 2002; Zhang et al. 2004, Marqués et al. 2005), but also base flows may decrease as a result of increased evapotranspiration (Bruijnzeel 2004). Pilgrim et al. (1988) stress the importance of an increased knowledge concerning the impact of vegetation, land management and grazing practices on runoff production to support decision making in land-use planning in arid and semi-arid regions. A significant number of studies have been conducted on runoff processes in relation to vegetation and other variables in semi-arid regions, but the majority of them focus on the Mediterranean environment (Sala and Calvo 1990; Sorriso et al. 1995; Nicolau et al. 1996; Castillo et al. 1997; Puigdefábregas et al. 1999; Lasanta et al. 2000; Archer et al. 2002; Calvo et al. 2003). More studies refer to runoff characteristics in arable land than to natural vegetation and rangeland areas (Mapa 1995; Descroix et al. 2001; Archer et al. 2002). Studies on runoff processes in rangelands have been conducted mainly in North America (Wilcox and Wood 1988, 1989). Gutierrez and Hernandez (1996) further indicate the great uncertainty regarding the amount of vegetation cover needed to counteract runoff in semi-arid rangelands.

From these studies it is clear that for a successful soil and water conservation strategy is urgent in order to combat runoff by vegetation restoration. The resulting higher infiltration benefits plant growth and biomass production and can also lead to groundwater recharge, thus replenishing deeper-lying water resources. Another important advantage of the decreased in runoff is that lower-lying croplands become less subject to damaging floods from the formerly degraded steep hillslopes.

Many authors have demonstrated that in a wide range of environments both runoff and sediment loss will decrease exponentially as the percentage of vegetation cover increases (Table 3). Semi-arid landscapes by definition are water-limited and therefore are potentially sensitive to environmental change (Schlesinger et al. 1990) and its effect on biomass production. However, hilly areas in the Mediterranean with sclerophyllous vegetation are not necessarily of low biomass production, especially those with annual

Table 3 Relationship between vegetation cover and soil loss by sheet and rill erosion

Vegetation type	Equation erosion relative (Er)	Original equation	Reference
Rangelands: grass, bushes and trees	$\mathrm{Er} = \mathrm{e}^{-0.0235\mathrm{C}}$	$\mathrm{E}\ (\mathrm{cm\ year}^{-1}) = 0.0668\mathrm{e}^{-0.0235\mathrm{C}}$ $\mathrm{R}^2 = 0.89$	Dunne et al. (1978)
	$\mathrm{Er} = \mathrm{e}^{-0.0168\mathrm{C}}$	$\mathrm{E} = 0.9258\mathrm{e}^{-0.0168\mathrm{C}}$	Rickson and Morgan (1988)
Grasses	$\mathrm{Er} = 0.0996 + 0.9004\mathrm{e}^{-0.0370\mathrm{C}}$	$\mathrm{E} = 433.43 + 3920.44\mathrm{e}^{-0.037\mathrm{C}}$ $\mathrm{R}^2 = 0.56$	Dadkhah and Gifford (1980)
Rangelands: grasses	$\mathrm{Er} = \mathrm{e}^{-0.0300\mathrm{C}}$	$\mathrm{E}\ (\mathrm{g\ m}^{-2}) = 10.4856\mathrm{e}^{-0.0300\mathrm{C}}$ rain 30 min, $\mathrm{R}^2 = 0.25$ $\mathrm{E}\ (\mathrm{g\ m}^{-2}) = 34.1240\mathrm{e}^{-0300\mathrm{C}}$ rain during 60 min, $\mathrm{R}^2 = 0.37$	Snelder and Bryan (1995)
Mediterranean matorral	$\mathrm{Er} = \mathrm{e}^{-0.0411\mathrm{C}}$ $\mathrm{I} = 100.7\ \mathrm{mm\ h}^{-1}$	$\mathrm{E}\ (\mathrm{g\ L}^{-1}) = 5.4172\mathrm{e}^{-0.0411\mathrm{C}}$ $\mathrm{R}^2 = 0.99$	Francis and Thornes (1990)
Pasture	$\mathrm{Er} = \mathrm{e}^{-0.0435\mathrm{C}}$	$\mathrm{E} = 0.6667\mathrm{e}^{-0.0435\mathrm{C}}$	Elwell and Stocking (1976)
Rangeland: grasses	$\mathrm{Er} = \mathrm{e}^{-0.0455\mathrm{C}}$	$\mathrm{E}\ (\mathrm{g\ m}^{-2}) = 653.27\mathrm{e}^{-0.0455\mathrm{C}}$ $\mathrm{R}^2 = 0.62$	Moore et al. (1979)
	$\mathrm{Er} = \mathrm{e}^{-0.0477\mathrm{C}}$	$\mathrm{E}\ (\mathrm{t\ ha}^{-1}) = 64.4240\mathrm{e}^{-0.0477\mathrm{C}}$ $\mathrm{R}^2 = 0.99$	Lang (1990)
Pasture	$\mathrm{Er} = \mathrm{e}^{-0.0527\mathrm{C}}$	$\mathrm{E} = 0.9559\mathrm{e}^{-0.0527\mathrm{C}}$	Elwell (1980); Elwell and Stocking (1974)
Pasture: grasses	$\mathrm{Er} = \mathrm{e}^{-0.0593\mathrm{C}}$	$\mathrm{E}\ (\mathrm{t\ ha}^{-1}) = 16.857\mathrm{e}^{-0.0593\mathrm{C}}$ $\mathrm{R}^2 = 0.96$	Lang (1990)
Pasture: grasses	$\mathrm{Er} = \mathrm{e}^{-0.0694\mathrm{C}}$	$\mathrm{E}\ (\mathrm{t\ ha}^{-1}) = 335.38\mathrm{e}^{-0.0694\mathrm{C}}$ $\mathrm{R}^2 = 0.98$	Lang (1990)
Cultivated land: sugar beet + mulch	$\mathrm{Er} = \mathrm{e}^{-0.0790\mathrm{C}}$ $\mathrm{R}^2 = 0.86$	$\mathrm{E} = 136\mathrm{e}^{-0.0790\mathrm{C}}$	Kainz (1989)
Mediterranean matorral	$\mathrm{Er} = \mathrm{e}^{-0.0816\mathrm{C}}$ $\mathrm{I} = 25.8\ \mathrm{mm\ h}^{-1}$	$\mathrm{E}\ (\mathrm{g\ L}^{-1}) = 5.5669\mathrm{e}^{-0.0816\mathrm{C}}$ $\mathrm{R}^2 = 0.99$	Francis and Thornes (1990)

The equations reflect the combined effect of both above-ground (stems and leaves) and below-ground (roots) biomass. *C* vegetation cover (%); *Er* erosion, relative to erosion of a bare soil; *E* erosion; *I* rainfall intensity

rainfall of 400 mm or more, in which biomass production ranges from 170 to 350 t ha^{-1} (Bazivilinch et al. 1971; Whittaker and Likens 1973).

4.1 Mediterranean Characteristics Affecting Vegetation

The Mediterranean climate has, in effect, three different definitions: (1) climate of the Mediterranean Sea and bordering land areas; (2) climate that favours broad-leaved, evergreen, sclerophyllous shrubs and trees; (3) winter-wet, summer-dry climate. However, portions of the Mediterranean region do not have winter-wet, summer-dry climate, while parts that do may not have evergreen sclerophylls. Places situated away from the Mediterranean Sea have more Mediterranean climate than anywhere around the sea under the third definition. Broad-leaved evergreen sclerophylls dominate some regions with non-Mediterranean climates, typically with summer precipitation maximum as well as winter rain, and short droughts in spring and fall. Thus, such plants may be said to characterize subtropical semi-arid regions. On the other hand, where summer drought is most severe, i.e. the most Mediterranean climate under definition 3, broad-leaved evergreen sclerophylls are rare to absent. Rather than correlating with sclerophyll dominance, regions of extreme winter-wet, summer-dry climate characteristically support a predominance of annuals, the life form best adapted to seasonal rainfall regimes. Therefore, the characteristics of the climate of an area that can affect vegetation growth and vegetation cover and therefore soil erosion are rainfall, both amount and intensity, and aridity.

Erosion data collected in various sites along the Mediterranean region show that the amount of rainfall has a crucial effect on soil erosion. Generally, there is a tendency towards increasing runoff and sediment loss with decreasing rainfall in hilly Mediterranean shrublands, especially in the region where rainfall is greater than 300 mm year^{-1}. Below the 300 mm annual rainfall limit, runoff and sediment loss diminish with decreasing rainfall. Rainfall amount and distribution are the major determinants of biomass production on hilly lands. Lower amounts of rainfall combined with high rates of evapotranspiration drastically reduce the soil moisture content available for plant growth. In areas with annual precipitation of less than 300 mm and high evapotranspiration rates, the soil water available to the plants is severely reduced.

Aridity is a critical environmental factor in determining the evolution of natural vegetation by considering the water stress, which may occur and cause reduced plant cover. In the Mediterranean region, vegetation presents a great capacity of adaptation and resistance to dry conditions, and numerous species can survive many months through prolonged droughts with soil-moisture content below the theoretical wilting point. Aridity can greatly affect plant growth and vegetation cover, particularly annual plants. Under dry climatic conditions in areas cultivated with rainfed cereals, the soil remains bare, favouring high erosion rates under heavy rainfalls following a long dry period. Closely related to climatic characteristics is the topographic attribute, slope orientation, which is considered an important factor for land-degradation processes. In the Mediterranean region, slopes with southern and western facing orientations are warmer, and have higher evaporation rates and lower water-storage capacity than northern and eastern orientations. Therefore, a slower recovery of vegetation and higher erosion rates are expected in southern and western than in northern and eastern orientations. As a consequence, southern exposed slopes usually have a persistently lower vegetation cover than northern exposed slopes. The degree of erosion measured along south-facing hill slopes is usually much higher (even two-fold) than in the north-facing slopes under various types of vegetation cover.

Indicators of soil erosion related to the existing vegetation can be considered in relation to: (a) fire risk and ability to recover, (b) erosion protection offered to the soil, and (c) percentage of plant cover. Forest fires are one of the most important causes of land degradation in hilly areas of the Mediterranean region. During recent decades, fires have become very frequent especially in the pine-dominated forests, with dramatic consequences in soil erosion rates and biodiversity losses. Also, Mediterranean pastures are frequently subjected to human-induced fires in order to renew the biomass production. The Mediterranean vegetation type is highly inflammable and combustible due to the existence of species with a high content of resins or essential oils. Conversely, it is known that vegetation has a high ability to recover after fire, and the environmental problems related to fire normally last for only a limited number of years after the fire.

Human interference, such as livestock grazing or change in the land-use pattern, may irreversibly damage the recovering vegetation. Particularly, in hilly areas the indiscriminate uprooting of aromatic and medicinal plants could promote the soil erosion (Durán et al. 2004a, 2006a).

Vegetation and land use are clearly important factors controlling the intensity and the frequency of overland flow and surface wash erosion. Among the prevailing perennial agricultural crops in the Mediterranean, olive trees present a particularly high adaptation and resistance to long-term droughts and support a remarkable diversity of flora and fauna in the undergrowth. This undergrowth is even higher than for some natural ecosystems.

Under these conditions, annual vegetation and plant remains form a satisfactory soil-surface cover can prevent surface sealing, minimising the velocity of the overland water. In the case where the land is intensively cultivated, higher erosion rates are expected. Many studies have shown that the variation in runoff and sediment yields in drainage basins can be attributed to changes in the vegetation cover and land-use management. A value of 40% vegetative cover is considered critical, below which accelerated erosion dominates in a sloping landscape. This threshold may be shifted for different types of vegetation, rain intensity, and land attributes. It shows, however, that degradation begins only when a substantial portion of the land's surface is denuded; then it proceeds at an accelerated rate that cannot be arrested by land resistance alone. Deep soils on unconsolidated parent materials show slow rates of degradation and loss of their biomass production potential. By contrast, shallow soils with lithic contact on steep slopes have low productivity, and low erosion tolerance if they are not protected by vegetation.

4.2 Plant Roots and Erosion Control

Many soil-erosion studies focus on the effects of plant cover, whereas much less attention has been paid to the effects of plant roots on water erosion processes (Gyssels et al. 2005; de Baets et al. 2006, 2007a; Reubens et al. 2007). The impact of roots on water erosion rates might become critical when the above ground biomass disappears because of grazing or surface fire and when concentrated flow occurs. Especially in semi-arid environments, where plant covers can be restricted and shoots can temporally disappear, roots can play a crucial role. Bui and Box (1993) showed that roots had no stabilizing effect during interrill soil erosion, but Ghidey and Alberts (1997) found that interrill erodibility decreased as dead root mass and dead root length increased. The decline in soil loss is even more pronounced in the case of rill and ephemeral gully erosion. Studies on the effects of roots on concentrated flow erosion rates (Li et al. 1991; Zhou and Shangguan 2005; Gyssels et al. 2006; de Baets et al. 2006) used several root parameters to describe the root effect (root density, root length-density, root dry weight, root surface area density and root area ratio). Most studies use root density or root-length density to predict the effects of roots on soil erosion rates by concentrated flow. Few studies report an effect of root diameter on the erosion resistance of the topsoil to concentrated flow erosion. Many authors reported an exponential decline of rill erodibility and soil detachment rates with increasing root-length densities or root densities (Mamo and Bubenzer 2001a,b; Gyssels et al. 2006; de Baets et al. 2006). Li et al. (1991) reported that soil-erosion resistance increased exponentially with greater root density and that the ability of plant roots to bolster soil-erosion resistance depends mainly on the distribution of roots and on the number of fibrous roots less than 1 mm in diameter. Zhou and Shangguan (2005) observed a similar relation but with root surface-area density as the root variable. According to Gyssels et al. (2005) fine roots (<3 mm in diameter) are considered more important to soil fixation than coarse roots. Decades ago, Wischmeier (1975) and Dissmeyer and Foster (1985) pointed out that species with contrasting root architectures have a different erosion-reducing effect, and recently de Baets et al. (2007b) and Reubens et al. (2007). In general, the distinction between the root systems, consists mainly in whether the first root keeps on growing and performs as a thick primary root with few or many laterals (gymnosperms and dicotyledons) or disappears (monocotyledons). In the monocotyledons, the first root commonly lives a short time and the root system is formed by adventitious roots sprouting from that shoot, often in connection with buds.

The decrease in water-erosion rates with increasing vegetation cover is exponential, as pointed out above. According to Gyssels et al. (2005), the decline water erosion rates with expanding root mass is also exponential, as reflected in the following equation:

$$\mathrm{SEP} = \mathrm{e}^{-\mathrm{bRP}}, \tag{3}$$

where SEP is the soil-erosion parameters (interrill or rill erosion rates of bare top soils without roots), RP is a root parameter (root density or root-length density) and b is a constant that indicates the effectiveness of the plant roots in reducing soil-erosion rates.

For splash erosion, b is zero, for interrill erosion the b-value is 0.1195 when root density ($\mathrm{kg\,m^{-3}}$) is used as root parameter, and 0.0022 when root-length density ($\mathrm{km\,m^{-3}}$) is used. For rill erosion these average b-values are 0.5930 and 0.0460, respectively. The similarity of this equation for root effects with the equation for vegetation cover effects is striking (Table 4). Moreover, all the studies on the impact of the vegetation cover attribute soil-loss reduction to the aboveground biomass only, whereas in reality this reduction results from the combined effects of roots and canopy cover (Gyssels and Poesen 2003).

It is well-know (as mentioned above) that plants reduce soil erosion by intercepting raindrops, enhancing infiltration, transpiring soil water and by providing additional surface roughness by adding organic substances to the soil (Styczen and Morgan 1995). Plant roots have a mechanical effect on soil strength. By penetrating the soil mass, roots reinforce the soil and increase the soil shear strength (Styczen and Morgan 1995). Since roots bind soil particles at the soil surface and increase surface roughness, they reduce

Table 4 Relationship between a soil erosion and a plant root parameter for different erosion processes (excluding the effects of above-ground biomass)

Vegetation type	Root parameter	Root parameter range	Root sampling depth (cm)	Erosion process	Equation	Reference
Alfalfa, Canada bluegrass, corn, soybeans	Dead RD ($kg\,m^{-2}$) and dead RLD ($km\,m^{-2}$)	0.092–0495 2.363–12.289	15	Splash	No root effect	Ghidey and Alberts (1997)
Alfalfa, Canada bluegrass, corn, soybeans	Dead RD ($kg\,m^{-2}$) and dead RLD ($km\,m^{-2}$)	0.092–0.495 2.363–12.289	15	Interrill	$Ki = 3.55\exp(-0.71\ RD)$ $Ki = 3.55\exp(-0.029\ RLD)$	Ghidey and Alberts (1997)
Weeds, grasses	Root network in topsoil, relative to meadow (%)	n.a.	n.a.	Interrill + rill	n.a.	Wischmeier (1975)
Corn	RLD ($cm\,cm^{-3}$)	0.75–5.10	5	Interrill	No root effect	Bui and Box (1993)
Matorral	Root depth (cm)	50–100	>100	Interrill + rill	n.a.	Guerrero (1998)
Lateral-taprooted forest trees	Percentage of bare soil with fine roots (%)	0–100	2.5–5	Interrill + rill	n.a.	Dissmayer and Foster (1985)
Cropland	Living and dead RD ($lb\,acre^{-1}\,in^{-1}$)	n.a.	10	Interrill + rill	PLU-subfactor SR-subfactor	Renard et al. (1997) (RUSLE)
Cropland	Living and dead RD ($kg\,m^{-2}$)	n.a.	15	Interrill + rill	Cropland: interrill $\Delta Ki_{dr} = \exp(-0.56\ dr)$ $\Delta Ki_{dr} = \exp(-0.56\ dr)$ Cropland: rill $\Delta Ki_{lr} = \exp(-2.2\ dr)$ $\Delta Ki_{lr} = \exp(-0.35\ Ir)$	Flanagan and Nearing (1995) (WEPP)
Rangeland	Living and dead RD ($kg\,m^{-2}$)	n.a.	10	Rill	Rangeland: rill $Ki = 0.0017 + 0.0042\ clay - 0.0088\ OM - 0.00088\ \rho b/100 - 0.00048\ RD$	Flanagan and Nearing (1995) (WEPP)
Exp I: rye grass 8, 12 and 16 w Exp II: rye grass 6, 8 and 10 w	RLD ($cm\,cm^{-3}$)	Exp I: 0.3–2.2 Exp II: 4–23	15.2	Rill	Exp I: $Kr = 9.85\exp(-0.020\ RLD)$ Exp II: $Kr = 43.66\exp(-0.323\ RLD)$	Mamo and Bubenzer (2001b) (laboratory experiment)
Corn and soybean	RLD ($cm\,cm^{-3}$)	Corn: 1–4 Soybean: 0.15–0.7	7.62	Rill	Corn: $Kr = 0.64\exp(-0.345\ RLD)$ Soybean: $Kr = 0.552\exp(-1.142\ RLD)$	Mamo and Bubenzer (2001a) (field experiment)
Pinus tabulaeformis, *Hippophae rhamnoides*, grass	NR [number of roots $(10\ cm)^{-2}$]	0–700	Every 10 up to 200	Rill	Sediment reduction = $KNR^b/(A + NR^b)$ with K, A and b plant specific constants	Li et al. (1991)
Toendra	RD ($kg\ m^{-3}$)	0–45	5	Rill	$Vcrit = 2.25\ sqrt(d + 0.18Cr^{2.25})$	Sidorchuk and Grigorev (1998)
Cereals and grasses	RD ($kg\ m^{-3}$)	0–30	10	Rill and gully	n.a.	Gyssels and Poesen (2003)

Abbreviations: *RD* root density ($kg\,m^{-2}$ or $kg\,m^{-3}$ or $lb\,acre^{-1}\,in^{-1}$), *RLD* root length density ($km\,m^{-2}$ or $cm\,cm^{-3}$), *NR* number of roots per unit soil surface [number of roots $(10\,cm)^{-2}$], *w* weeks, *Ki* interrill erodibility ($kg\,s\,m^{-4}$), *Kr* rill erodibility ($s\,m^{-1}$), *dr* dead root mass ($kg\,m^{-2}$), *Ir* living root mass ($kg\,m^{-2}$), *clay* soil clay content (0–1), *OM* soil organic matter content (0–1), *ρb* dry soil bulk density ($kg\,m^{-3}$), *Vcrit* critical flow velocity for soil detachment ($m\,s^{-1}$), *d* mean diameter of soil aggregates (m), *Cr* cohesion of root permeated soil (10^5 Pa), *n.a.* not available the susceptibility of the soil to rill and gully erosion. Roots also have hydrological effects by increasing surface roughness and soil permeability, roots increase soil infiltration capacity. While the aboveground shoots bend over and cover the surface or reduce flow velocity when concentrated flow occurs, roots physically restrain or hold soil particles in place (Gray and Sotir 1996). Prosser et al. (1995) showed that the critical flow-shear stress decreased by clipping off the above ground vegetation, but that the dense root network prevented the surface from significant scour and sediment transport.

Most of the existing root studies deal with agricultural crops, i.e. the effect of maize (*Zea mays* L.) roots on interrill erosion rates was studied by Bui and Box (1993). Mamo and Bubenzer (2001a), studied the effect of soybean (*Glycine max* L.) and maize (*Zea mays* L.) roots on rill erodibility and found significant differences in channel erodibility and soil detachment rates between root-permeated and fallow soils.

A few studies report the effects of roots of natural vegetation on erosion processes. Li et al. (1991) examined the effect of roots of *Pinus tabulaeformis* and *Hippophae rhamnoides* on rill erodibility. Sidorchuk and Grigorev (1998) reported the effect of the root density of tundra vegetation on the critical shear velocity for different soil types. Meanwhile, de Baets et al. (2007a) described the root characteristics of Mediterranean plant species and their erosion-reducing potential during concentrated runoff, showing the implications for ecological restoration and management of erosion-prone slopes. Tengbeh (1993) investigated the effects of grass-root density on the shear strength increase with decreasing soil moisture content. In this context, both soil type and soil-moisture conditions control root architecture (Schenk and Jackson 2002) and soil-erosion rates (Govers et al. 1990). It is important to understand the effects of soil type and soil moisture on the erosion-reducing potential of plant roots. Sheridan et al. (2000) found low rill erodibilities for clay and silt soils, and high erodibilities for soils with particle sizes larger than silt but <10 mm, reflecting different levels of cohesion. The resistance of the soil to concentrated flow erosion increases with growing initial soil-moisture content (Govers et al. 1990).

Li et al. (1991) and Mamo and Bubenzer (2001b) reported the effects of grass roots (ryegrass) on concentrated flow erosion. So far, it is not clear to what extent grass roots contribute to the erodibility of topsoil during concentrated flow, because different relationships were reported. Moreover, grasses grow in many different environments which can be threatened by concentrated overland flow, for instance after surface fire or overgrazing. Once the above ground biomass has disappeared, only roots can offer resistance to concentrated flow erosion. Kort et al. (1998) indicated that post-burning erosion on a naturally vegetated rangeland dominated by grass species did not differ for simulated rainfall intensities. This indicates that the network of fibrous roots in the soil surface layers contributes to erosion control. These authors state that grasses provide perennial protection and minimal soil erosion. Moreover, grasses have proven to be the most effective for erosion control in most areas, because they germinate quickly, providing a complete ground cover (Brindle 2003) and a dense root network that reinforces the soil by adding extra cohesion (Gray and Leiser 1982). Additionally, Li et al. (1991) reported that the effect of roots in increasing soil resistance is highly dependent on the presence of effective roots (fibrils <1 mm). Also Gyssels and Poesen (2003) indicate that cross-sectional areas of gullies under grassy field parcels were much smaller than under agricultural cropland for the same flow intensity.

4.2.1 The Effect of Roots on Soil Properties

The shear strength of a soil has been recognized as a determinant of its resistance to erosion. From the start of slope-stability research it was clear that plant roots were vital for soil reinforcement. The shear strength of a soil is a measure of its cohesiveness and resistance to shearing forces exerted by gravity, moving fluids and mechanical loads. Soil is strong in compression, but weak in tension. Plant roots are weak in compression, but strong in tension. When combined, the soil-root matrix produces a type of reinforced earth which is much stronger than the soil or the roots separately (Simon and Collison 2001). Thus, roots reinforce the soil (Anderson and Richards 1987). This conclusion was found independently by different researchers (Gray and Leiser 1982), showing that soil erodibility is inversely proportional to the resistance of the soil to erosion. In this context, the intrinsic properties of the soil such as aggregate stability, infiltration capacity, soil bulk density, soil texture, organic and chemical content and shear strength are the most important determinants.

According to Amezketa (1999) the positive impact of plant roots and root hairs on soil aggregation and stability consist of many effects, such as:

1. Enmeshing fine particles into stable macroaggregates by root secretions, even when the root is dead
2. Drying the soil environment around the roots, reorienting clay particles parallel to the axis of the roots and drawing soil particles together
3. Supplying decomposable organic residues to the soil
4. Supporting a large microbial population in the rhizosphere
5. Providing food for soil animals
6. Releasing polyvalent cations and increasing concentrations of ions in solution

Field observations in southeast Asia reported by Ryan (1995) and Turkelboom et al. (1997) show that soil loss in newly prepared fields is generally very slight in the first year after clearing, as the roots of the fallow vegetation create stable aggregates, but losses augment rapidly afterwards as the roots decay and aggregates break down. The effect of living roots on soil-structure stability depends on the plant species. Monocotyledonous plants are superior to dicotyledonous plants and grasses are better than cereals in stabilizing aggregates, because the former contain a much larger root biomass with exudates (Glinski and Lipiec 1990; Amezketa 1999). Maize and tomato, on the other hand, can decrease soil aggregate stability by chelating iron and aluminium, thus destroying chemical bonds with organic matter (Reid and Goss 1987).

Plant roots penetrating the soil leave macropores that improve water movement and gaseous diffusion. They contribute to the system of continuous pores in the soil and enhance the infiltration capacity of the soil (Glinski and Lipiec 1990). Li et al. (1992) indicates that soil infiltration increases because plant roots improve the noncapillary porosity of the soil and promote the formation of water-stable aggregates of 2–5 mm, and >5 mm in diameter. A higher soil infiltration capacity reduces the runoff volume and consequently soil erosion.

Roots growing in the soil occupy space that was previously occupied by soil pore space and soil particles. Since root diameter is usually larger than soil pores, soil particles are pushed aside and the bulk density of the soil up to 8 mm near the root increases (Glinski and Lipiec 1990). However, fine roots less than 1 mm in diameter can significantly decrease the bulk density of the soil and increase the soil porosity (Li et al. 1992, 1993). This effect depends on the root diameter and the nature of the soil, and erosion resistance presumably derives from the large number of roots in the topsoil.

Texture, organic content, and chemical composition of a soil are important because of their influence on soil-aggregate stability (Morgan 1996). According to Sakkar et al. (1979), modifications in particle-size distribution and composition of the clay fraction was found within the rhizosphere around French bean roots. These researchers attributed the changes in texture and mineralogy to an intensified weathering of the soil materials around the plant root. Preferential uptake of ions or water by roots leads to depletion or accumulation profiles of ions. Examples of this are depletion zones of phosphorus and potassium or the accumulation of sodium and chlorine (Glinski and Lipiec 1990; Pojasok and Kay 1990). Finally, roots also have a positive effect on soil aggregation by supplying decomposable organic residues to the soil, supporting a large microbial population in the rhizosphere and providing food for soil animals (Tisdall and Oades 1982; Amezketa 1999).

4.3 Plant Cover and Biodiversity

The biological diversity existing in any natural ecosystem is directly related to the amount of living and nonliving organic matter present in the ecosystem (Wright 1990). By diminishing soil organic matter and overall soil quality, erosion reduces biomass productivity in ecosystems. Plants, animals, and microbes are vital components of the soil, as mentioned above, and constitute a large measure of the soil biomass. One square meter of soil may support about 200,000 arthropods and enchytraeids and billions of microbes (Wood 1989; Lee and Foster 1991). A hectare of productive soil may have a microbial and invertebrate biomass weighing nearly 10,000 kg ha^{-1}. In this context, Anderson (1978) reported that a forest soil with abundant organic matter supports up to 1,000 species of animals per square meter, including arthropods, nematodes, and protozoa.

Erosion rates that are 10–20 times higher than the sustainability rate (less than 0.5–1 Mg ha^{-1} year^{-1}) decrease the diversity and abundance of soil organisms (Atlavinyte 1964), whereas agricultural practices that maintain adequate soil organic-matter content favour the proliferation of soil biota (Reid 1985).

Macrofauna (mostly arthropods) species diversity more than doubled when organic manure was added to grassland plots in Japan (Kitazawa and Kitazawa 1980). Rodríguez et al. (2007b) in south-eastern Spain pointed out the proliferation of arthropod species under plant covers in comparison to uncovered bare soils in the taluses of orchard terraces.

Because increased biomass is generally correlated with increased biodiversity, greater biomass of arthropods and microbes implies an increase in biodiversity (Pimentel et al. 1992).

The effects of erosion may be responsible for the loss of a keystone species, an absence that may have a cascading effect on a wide array of species within the agroecosystem. Species that act as keystone species include plant types that maintain the productivity and integrity of the ecosystem; predators and parasites that control the feeding pressure of some organisms on vital plants; pollinators of various vital plants in the ecosystem; seed dispersers; and the plants and animals that provide a habitat required by other essential species, such as biological nitrogen fixers (Heywood 1995).

Soil biota performs many beneficial activities that improve soil quality and productivity. For example, soil biota recycles basic nutrients required by plants for growth (Pimentel et al. 1980). In addition, the tunnelling and burrowing of earthworms and other organisms enhance productivity by increasing water infiltration into the soil.

This churning and mixing of the upper soil redistributes nutrients, aerates the soil, exposes matter to the climate for soil formation, and increases infiltration rates, thus enhancing conditions for soil formation and plant productivity. Controlling erosion not only conserves the quality of soils but enhances vegetative growth and increases total biodiversity.

5 Conclusion

Soil erosion is a natural process which has been greatly accelerated by human action. A reduction in plant cover can intensify erosion processes that diminish soil quality. In arid and semi-arid areas with sparse vegetation cover, it is urgent protect the soil by understanding degradation processes and establishing adequate management measures. Moreover, the proven efficiency of the plant covers for the restoration of degraded environments should be considered more widely. Research needs to concentrate future efforts on developing ecological successions and revegetation methods which promote a substantial and sustainable canopy cover.

Some of the basic reflections of this review include:

1. Plant covers maintain crucial interrelationship with soil properties, enhancing biodiversity for steeply sloped areas that have highly erodible soils. Erosion is likely to be more affected by changes in rainfall and plant cover than runoff, though both are influenced.

2. Changes in plant cover have a greater impact on both runoff and erosion than changes in canopy cover alone. Insights into soil-erosion processes and the renewed hydrological situation encouraged by plant covers can provide a valuable design for new strategies of erosion management and ecosystems restoration.

3. The inappropriate removal of plant cover and the intense farming systems of mountain areas endanger land conservation, raising an urgent need to implement appropriate land management which has a large-scale perspective but acts at the local level.

4. Erosion can be mitigated through a process of assessment at regional scales to set broad targets, for development and restoration of the plant cover, and the introduction of conservation measures within the areas at greatest risk.

Therefore, at both regional and local scales, the plant cover deserves careful assessment for the sustainable management of soil resources, in order to avoid catastrophic degradation. This will help adapt to land-use change and, in terms of conservation, it will aid in establishing an equilibrium between economic and environmental interests.

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Integration of Soil Structure Variations with Time and Space into Models for Crop Management: A Review

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Abstract Soil structure plays a major role in the design of new crop management systems. For instance, the transition from conventional to no-tillage changes soil structure, which, in turn, has implications on crop yield greenhouse gas emissions, and pesticide and nitrate leaching. Modelling soil structure at field scale faces two main issues: (1) the spatial variability and (2) the temporal variability. Here, we review how spatial variability of soil structure is taken into account in water transfer models at field scale. We discuss the effects of soil structure on hydraulic properties. We present options to model soil structure effects using pedotransfer functions or calculations based on pore network geometry. Then we review studies on water transfer. Here, we show the utility of one-dimensional (1-D) and 2-D models, and the range of soil profile partitions. In the second part, we study a mean to model the temporal variation of soil structure. We propose an indicator of soil structure dynamics based on the proportion of compacted clods in the tilled layer. This indicator was measured from the observation face of soil pits. We studied this indicator in a long-term field experiment involving various risks of compaction. The results showed that this indicator gave a more precise description of the time course changes in soil structure than the mean soil bulk density measured on the same experimental plots. Lastly, we discuss the principles of a model that predicts the evolution of this indicator under different soil tillage and climatic conditions. This model can be used to evaluate the effects of different crop management systems on soil structure and soil water transfer.

Keywords Crop management • Model • Soil structure • Tillage • Water transfer

1 Introduction

For many years, conventional tillage involving mouldboard ploughing was used in agriculture to control the development of weeds, to incorporate crop residues into soil, to recycle leached nutrients in surface soil and to prepare a favourable tilth before seeding (Dexter et al. 1983). However, for multiple reasons such as cost reduction, increase in work productivity, prevention of soil erosion and protection of soil fauna, ploughless or reduced soil tillage systems have been introduced, and co-exist with conventional systems in many parts of the world. In some regions such as Brazil and Argentina the non-ploughed areas are increasing exponentially, together with the use of herbicides and genetically-modified (GM) crops. These changes happen very quickly and they profoundly modify the environmental conditions of crop production. In many cases, particularly in Western Europe, their sustainability has still to be ascertained (Carter 1994). This diversification of tillage management systems induces variation in soil structure within a given territory, where, more often, different tillage systems co-exist. It is therefore necessary to coordinate the design of new crop management systems at the catchment level when addressing problems such as erosion control, or on the regional scale

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if the mitigation of greenhouse gas emissions is concerned (Ball et al. 1999). This territorial aspect of the design of crop management systems will not be discussed in this paper, where we will consider soil structure only on the field scale.

Soil structure varies considerably within a field (Roger-Estrade et al. 2000). Indeed, cultivated soils are subjected to mechanical stresses which are not applied homogeneously to the soil. For instance, compaction during traffic affects only the soil volume beneath the wheel tracks and soil fragmentation depends on tillage depth. Moreover, soil strength depends greatly on soil water pressure which also varies, especially with depth. Consequently, the spatial variation in soil structure is very important, both in the direction perpendicular to the traffic direction and in depth. This variability has consequences on the soil functioning, e.g. water transfers (Stenitzer and Murer 2003), nitrous oxide emission (Röver et al. 1999), mineralisation (Guérif et al. 2001), seedling emergence or root establishment. It therefore has an impact on the performance, productive as well as environmental, of the crop management systems, and must be addressed in models used for their design.

The temporal dimension of soil structure variation is also a key point to consider. Indeed, new conditions for soil structure dynamics are created by the diversification of tillage management and the widening range of soil moisture at cultivation due to the increase in the area cultivated *per capita*. Consequently, changes with time in several biological, chemical and physical soil properties are also modified. For instance, changes with time in the internal structure of fragments of tilled soils were quantified on sections cut on 400-mm blocks of resin-impregnated soil by Dexter (1976), Ojeniyi and Dexter (1983), and (Dexter et al. 1983). It was observed that there is a change in the topology of the soil structure with time. Immediately after tillage, the sections show “islands” of aggregates in a “sea” of pore space. With time, the aggregates become joined together so that the observed structures change to be “islands” of pore space surrounded by a sea of soil matrix, formed from modified aggregates. Aspects of these changes in soil internal structure have been modelled by Or et al. (2000) and by Leij et al. (2002). Other studies have shown that the remaining inter-aggregate pore spaces become cut off from each other when the macroporosity is reduced to about 10% (Davis et al. 1973). Such cutting off and isolation of macropores can cause sudden and dramatic reductions in saturated hydraulic conductivity and air permeability.

In conventional agriculture, deep tillage was an efficient way to recover damaged soil structure, even if this operation could also damage the soil, when it is performed while soil water content is excessive, for instance. With no or only shallow tillage, soil structure recovery generally takes far more time (Horn et al. 1995) than with deep tillage, depending of course on soil type, intensity of weathering or biological activity. Therefore, periods in the crop cycle during which soil structure is suboptimal become more and more frequent, and this must be taken into account when designing new crop management systems.

Generally, crop models do not consider changes with time in the soil structure and the associated soil properties. Moreover, crop models are mainly one-dimensional, using a constant bulk density as a descriptor of soil structure, e.g. APSIM (McCown et al. 1996), CERES (Ritchie et al. 1998) and STICS (Brisson et al. 1998). Therefore, an improvement of these models would be the incorporation of a sub-model that describes the time variation in soil structure. This would enable simulation of the effects of agricultural practices during a whole crop rotation.

This article comprises two main sections: a review of how spatial variation in soil structure is taken into account. To illustrate the consequences of this, we chose the specific subject of water transfer modelling. This particular aspect of the consequence of soil structure was chosen because of the importance of water transfer in the environmental impact of cropping systems. The second section deals with the changes with time in soil structure, as a function of crop system management.

2 Integrating Spatial Variation in Soil Structure into Water Transfer Models

Knowledge of the hydraulic properties of cultivated soils on the field scale is essential for the understanding and prediction of the main processes in the water cycle: infiltration, runoff, evaporation and redistribution, which in turn affect crop performance, e.g. germination and water uptake by roots, and aspects of environmental quality, e.g. erosion, nitrate and pesticide leaching, and N_2O emissions. Hydraulic properties depend on the geometry of the pore network which is determined by soil texture as well as its structure, i.e. the size of the soil particles and the way they are packed together (Green et al. 2003). In naturally-formed aggregates, hydraulic intra-aggregate properties are affected by the initial formation of a dense particle arrangement with fine pores through repeated wetting and drying. Such aggregates have a continuous pore system which results in a high water availability. On the macroscale, elementary soil volumes retain more water near saturation when they are more porous or when soil fragments are large (with large macropores between them). Therefore, the saturated hydraulic conductivity (Ks) increases when the soil bulk density decreases, or when the soil fragments are large. When water is not located in the pore space between soil fragments an increase in bulk density leads directly to an increase in volumetric water content, by definition (Reicosky et al. 1981). When the soil water content is characterised by the gravimetric water content or the soil water ratio rather than the volumetric soil water content, it appears that an increase in bulk density, i.e. soil compaction, sometimes leads to an increase in water content (Richard et al. 2001) and sometimes not (Reicosky et al. 1981; Sillon et al. 2003). Which effect predominates depends on whether the compaction affects only the macroporosity or both macro- and microporosity. Hydraulic conductivity depends on the continuity of the small pores between soil fragments under these moisture conditions. Hydraulic conductivity at low water potentials, i.e. at large negative water potentials, is greater for a dense tilled layer than for a loose tilled layer (Sillon et al. 2003), or for an aggregated tilled layer composed of small aggregates than one made up of large ones (Hadas 1997). In both cases, it can be presumed that the area of contact between soil fragments, i.e. the water-filled pore continuity, is greater in a compacted tilled layer than in a soil layer composed of small aggregates. However, hydraulic conductivity generally varies by a factor less than 10 between the two situations.

To predict the effect of soil structure on soil hydraulic properties, retention and hydraulic conductivity curves are used (Millán and González-Posada 2005; Sain et al. 2006). They can be described with several functions: van Genuchten, Brooks and Corey, Mualem, or Burdine. Changes with soil structure in the values of the coefficients can be assessed by indirect or direct methods. Indirect methods consist of mathematical relationships, e.g. pedotransfer functions, between these coefficients and several characteristics of soil structure, most often soil bulk density and soil texture: percentages of clay, silt, sand and organic carbon. With direct methods the soil hydraulic properties are calculated directly from the geometry of the pore network. The pore size distribution of the dried soil can be obtained from mercury intrusion measurements or of the moist soil by tomography. It can also be calculated from the particle and aggregate size distribution (Arya and Paris 1981).

Models of water flux in soils are based on Richards' equation. This equation describes water transfer from the generalised law of Darcy on a macroscopic scale for which the soil, which is a polyphasic porous media, can be considered as homogeneous and without any discontinuity. When there are macropores in the soil, i.e. voids from tillage, cracks from climate, earthworm channels and root channels, water flux is not uniform because water is transferred much more quickly in the macropores than in the soil matrix, and consequently, preferential flows can occur. Darcy's law, when applied to a soil volume with macropores, cannot fully describe water flux. In that case, the soil pore volume may be divided into at least two sub-volumes: the microporosity, or intra-aggregate porosity, and the macroposity, or inter-aggregate porosity. Water flux is then described for each type of porosity with different soil properties and/or physical laws, and between the two types of porosity. Within the microporosity, water flow is still described by Darcy's law. Within the macroporosity, water flux can be calculated with several laws: Darcy, Poiseuille, Green and Ampt, or kinematic waves (Simunek et al. 2003).

Numerous models of soil water flow have been described in the literature. They can be 1-D, i.e. as a function of soil depth, 2-D, i.e. within a soil profile, or 3-D models. They can integrate (1) only Darcy's law, (2) Darcy's law and a specific law for preferential flows, or (3) only a specific law for preferential flows. Three-dimensional models have mainly been applied to laboratory soil columns where water is transferred only in the macroporosity using Poiseuille's law (Delerue and Perrier 1999).

The various approaches to integrating the soil structure on the field scale concern 1-D and 2-D models based on Darcy's laws. The first approach consists of applying a 1-D model to soil layers which differ in soil structure due to tillage or compaction. Linden (1982) was one of the first authors to analyse the effect of a change in bulk density of the tilled layer on evaporation with model simulations. He analysed the theoretical effect of a change in bulk density on hydraulic properties and showed that tillage reduced evaporation (from 18–12 mm after 10 days). Richard et al. (2001) and Sillon et al. (2003) have used such models to estimate the change in hydraulic conductivity for soil layers with contrasted bulk density using an inverse method. Hydraulic conductivity was calculated for the whole range of soil water content (even for the dry state) from field measurements of change in soil water content under natural climate. They showed that soil layers with a high bulk density remained wetter near the soil surface than soil layers with a low bulk density during a dry period.

It should be noted that values of hydraulic conductivity estimated by inverse methods are, in reality, *effective* hydraulic conductivities. They do not distinguish between water flow through the soil matrix to the soil surface and transport of water in the vapour phase through the macropore space caused by convective air movements resulting from fluctuations of atmospheric pressure or temperature (Farrell et al. 1966; Kimball and Lemon 1971). These air movements can become significant when inter-aggregate macropores are larger than about 4 mm diameter (Ojeniyi and Dexter 1984).

De Tourdonnet (1998) has proposed distributing 1-D numerical models to take into account the spatial variation in soil structure. Studying water transfer in a greenhouse (plastic tunnel), he combined the heterogeneity of soil structure (two levels of compaction, i.e. under or outside wheel tracks) with that of irrigation (between 54% and 107% of the mean water supply). He defined seven zones from the centre to the border of the tunnel and studied, in particular, the effect of this heterogeneity of greenhouse conditions on nitrate leaching.

The possible influence of lateral water transfer has led several authors to propose 2-D models of water transfer. Benjamin et al. (1990) have proposed a 2-D water and heat simulation model to compare water and heat flow from a flat or a ridge soil surface (with tall or short ridges). They took into account heterogeneity of the physical properties within a soil profile distinguishing three zones: the plant row, the untracked interrow and the wheel-tracked interrow. Benjamin et al. (1990) showed that water potential at the top of the wheel-tracked interrow remained less negative than at the top of the untracked one during a dry period. However, as for Sillon et al. (2003), only dry periods were simulated. Lamandé (2003) and Ndiaye et al. (2007) have proposed a soil profile partition similar to that of Benjamin et al. (1990) to simulate (using HYDRUS-2D) the effect of the heterogeneity of the soil structure under a maize crop on water infiltration after a rainfall event. For instance, Lamandé (2003) distinguished four zones under a maize crop: non-compacted, interrow (untracked), compacted interrow (wheel-tracks) and plough pan. He showed that the water flux at the bottom of the plough pan was determined mainly by the hydraulic properties of the non-compacted interrow, where water could infiltrate, rather than that of the compacted interrow, where water could not infiltrate.

The studies of Benjamin et al. (1990), Lamandé (2003) and Ndiaye et al. (2007) showed that 2-D models can be used to evaluate the effect of heterogeneity of soil structure both on evaporation during dry periods and on infiltration during rainy periods. However, the geometry of the zones of the soil profile defined by these authors was quite simple. All the limits were either vertical or horizontal, while more complicated geometry can be created by tillage. Coutadeur et al. (2002) used the same partitioning as Lamandé (2003) and identified, within the untracked soil compartment, two soil zones: compacted and porous zones. Compacted zones in the untracked compartment resulted from the action of the mouldboard plough, i.e. the fragmentation and displacement of previously-compacted soil areas. The contour of the compacted zones was accounted for using the adaptative mesh system of HYDRUS-2D (more than 10,000 triangles and 6,000 nodes were needed). Coutadeur et al. (2002) could then evaluate the effect of the position and shape of the compacted zones on water infiltration.

However, none of the water flow models presently in use considers changes with time in the soil structure and the associated hydraulic properties, and this is also true for the crop models. Therefore, these models could be improved by linking the water flow models with the models that describe the change in soil structure with time (e.g. Roger-Estrade et al. 2000). This should enable simulation of the effect of agricultural practices on hydraulic properties during a whole crop cycle or during a crop sequence.

3 Taking into Account the Temporal Variation in Soil Structure

3.1 An Indicator of Soil Structure Dynamics

Until now, most studies on soil structure dynamics have focused on only one of the processes involved; displacement, compaction or fragmentation (Dexter and Birkás 2004; Keller et al. 2007). Moreover, they generally involve only a small volume of soil, such as that immediately under a tyre or in front of a tine. Their results could perhaps be extended to a heterogeneous field whose tilled layer is composed of many such volumes, each having a specific density and S value (Dexter 2004). It should be noted that pedo-transfer functions are available for prediction of values of S. However, the use of these results to forecast structure dynamics remains difficult because temporal changes are due to a complex succession of different processes. Aspects of this subject need to be further developed to take structure dynamics into account. Until that has been done, crop management design requires the use of a global model such as the one we present in this paper, even if the processes involved in structure dynamics are represented in a rather simplified way.

We have developed a field method based on a morphological description of soil structure, which is described in detail in Roger-Estrade et al. (2004). One of our major concerns was to describe and analyse not only the spatial variation in soil structure but also its dynamics. This led us to propose an indicator of the effect of the crop management system on soil structure. This indicator (Roger-Estrade et al. 2000) is based on the evaluation of the proportion of severely-compacted clods and zones in the tilled layer (or the equivalent soil volume, i.e. roughly the first thirty centimetres of soil in non-tilled situations, the exact value depending on the depth of the last ploughing). This evaluation is made on the vertical observation face of a pit, dug far from the edges of the field and chosen to be large enough to take into account the pattern of wheel tracks created by the successive field operations. The location and width of the pit is decided depending on the geometry (tyre size, working width) and the location of the passes of the tillage tools, tractor and harvesting wheel tracks. It is also slightly deeper than the greatest working depth of the tillage tools. Once the pit is dug, the observation face is prepared so that one can distinguish the zones and clods in the soil resulting from severe compaction, i.e. without any visible structural porosity. These zones, located under the recent wheel tracks, and clods, resulting from the fragmentation by the plough of existing compacted zones, are called Δ clods and zones (see Roger-Estrade et al. (2004) for the precise definition and properties of Δ structure). Digital photographs of the observation face were taken in the field, and transferred onto a computer where the limits of the Δ zones and clods were drawn and digitised for image analysis. The areas and the locations of the Δ zones were determined, as well as the thickness of the tilled layer. The percentage of Δ areas was given by the ratio of the cumulated Δ surface area to the total surface area of the tilled layer. This percentage was measured just after crop establishment. Examples of two observation faces are shown in Fig. 1.

3.2 Time Course Changes in Soil Structure

Bulk density and the above-mentioned indicator were simultaneously measured to evaluate soil structure dynamics in a field experiment where two crop management systems were compared. In the first crop management system, the crop sequence was pea (*Pisum sativum* L.) – winter wheat (*Triticum aestivum*) – flax (*Linum usitatissimum* L.) – winter wheat. The wheat was sown in early autumn. The pea and flax were sown in early spring. This crop management system, in which compaction risk is low, was labelled LCR for Low Compaction Risk. In LCR plots, two types of tillage were performed from 1999: (1) deep (0.3 m) ploughing with a mouldboard plough (LCR-P treatment) and (2) reduced tillage, in which only a superficial (0.07 m depth) chiselling was performed to prepare the soil before sowing (LCR-NP treatment). In the second crop management system, the crop sequence was maize (*Zea mays* L.) – winter wheat – sugar beet (*Beta vulgaris* L.) – winter wheat. In this system, sugar beet and maize were sown as soon as the seed bed was warm enough for germination, whatever the soil moisture conditions, and were harvested as late as possible, generally at the end of autumn, whatever the soil moisture conditions. This crop management system was labelled HCR (for High Compaction Risk).

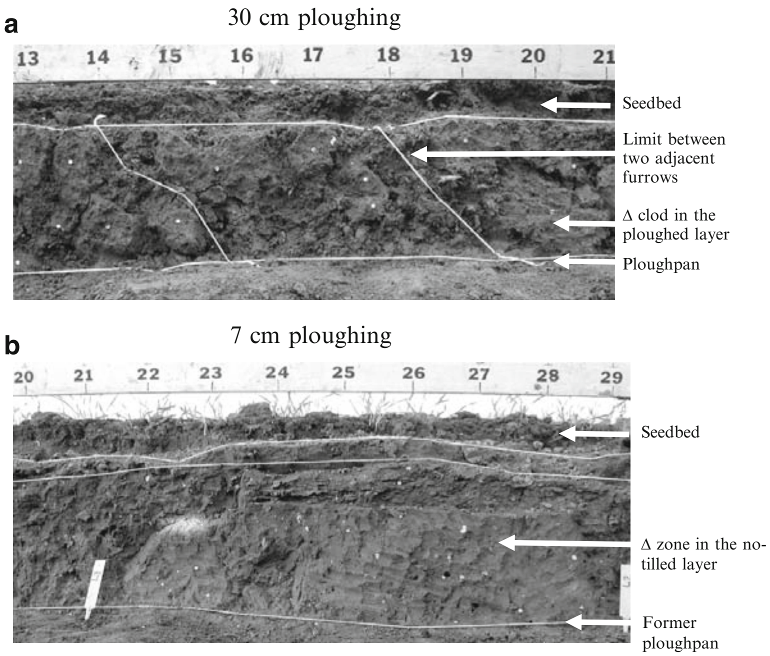

Fig. 1 Photographs of the observation faces of two soil profiles (**a**) in the LCR-P plot, (**b**) in the HCR-NP plot of the long-term field experiment in Mons. LCR-P: deep ploughing (0.3 m depth). LCR-NP reduced tillage (0.07 m depth). The distance between two successive numbers is 10 cm

As for the other system, the same two tillage systems were compared from 1999, giving the two following treatments: HCR-P and HCR-NP.

In all the experimental trials, the plots were large enough to allow use of the farmers' equipment and crop management was performed classically for this French area for industrial crop production (details of the cultivation operations are given in Boizard et al. 2002). In four plots of this experiment, we made measurements of the mean bulk density and the percentage of Δ clods and zones in the 5–30 cm soil layer at seven dates between 1999 (just after ploughing was interrupted on half of the plots) and 2006. The measurements of the Δ zones were made just after sowing of the crops, in the layer between the bottom of the seedbed and the present or most recent plough pan. The mean dry bulk density was measured after each sowing on unwheeled zones with a transmission ray probe (10 replicates) up to a depth of 0.35 m at every 3.5 cm depth. The results are plotted in Fig. 2.

After two years, the ploughed and non-ploughed parts of the experiment exhibited significant and constant differences in mean bulk density (Fig. 1a), whatever the crop sequence. This result is classically explained considering that undisturbed soil is denser than regularly-ploughed soil (Guérif 1994; Hamza and Anderson 2005). Conversely, change in bulk density over time was more similar between the HCR and the LCR plots than between the P and NP treatments. There was no significant difference between HCR and LCR for the ploughed treatments. In reduced tillage, bulk density depended on the cropping system and year. In LCR, values of bulk density fluctuated greatly around a mean value (ca. 1.45 g cm^{-3}). These variations were probably due to the changes from one year to another in the moisture conditions at sowing and harvesting. In HCR, the bulk density sharply increased in 2002. The sugar beet harvesting in 2001 caused particularly high compaction in these plots, because of the high soil water content in late autumn and the high proportion of the soil surface affected by traffic. The bulk density remained very high, between 1.52 and 1.58 g cm^{-3}, during the following years, though there was no further severe compaction during field operations during the three years.

Figure 2b shows that the ranking of the plots is different when the percentage of Δ clods is considered. At the beginning of the period, if bulk density was equivalent between the plots, the percentage of Δ clods was different. During the period, the percentage of Δ zones was higher in HCR plots, whatever the tillage mode, except in 2004, when the Δ percentage decreased sharply in the HCR ploughed plot and became similar to that of the LCR plots.

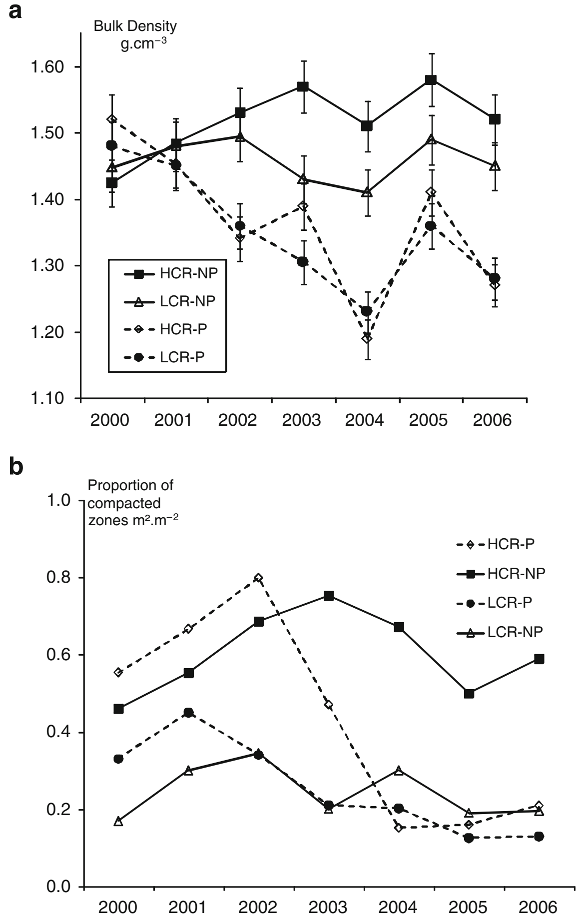

Fig. 2 Change with time in (**a**) bulk density ($\mathrm{g\,cm^{-3}}$) and (**b**) the proportion of compacted zones (Δ areas, $\mathrm{m\,m^{-2}}$) in four experimental plots of the long-term field experiment in Mons (vertical bars: confidence interval, $P = 0.05$). *HCR* cropping system with high compaction risk, *LCR* cropping system with low compaction risk, *NP* unploughed treatments, *P* ploughed treatments

This dynamics can be explained by the fragmentation action of the plough. This tool creates mixed structures, with highly-fragmented zones separated by highly-compacted blocks (fragmented ancient Δ zones). This spatial variation on the layer scale is averaged out by the gamma ray probe, which gave higher bulk density values in the homogeneous NP treatments. The decrease in 2003 and 2004 can also be explained by the inverting action of the plough, which brought up compacted soil volumes towards the soil surface, causing their destruction by the intense fragmentation action of the secondary tillage tools. This effect was, in 2000, 2001 and 2002 annihilated by compaction during sowing, subsequent to the ploughing and/or harvesting. In the NP plots, compacted zones disappeared much more slowly in this loamy soil, where the climate action on soil structure is not very intense. These results are coherent with those of Watts and Dexter (1994), showing that the cloddiness of soil after tillage depends more on the soil water content at the time of the previous harvest than on the water content at the time of tillage, because of its effect on the compaction produced by the heavy equipment during harvest. This example shows that the soil structure dynamics results from a complex balance between fragmentation actions (reducing Δ volumes) and compaction ones (creating Δ zones). The information given by bulk density and the morphological approach are complementary. Moreover, the level of compaction risk associated with the crop management system is not sufficient to predict completely the structure dynamics.

3.3 Modelling Temporal Changes in Soil Structure

These considerations led us to propose a model, called SISOL, of soil structure dynamics, on the field scale, predicting the changes with time in the percentage of Δ zones within the tilled layer of cultivated fields.

3.3.1 Principles of the Model

It is based on the following assumptions about the changes with time in the percentage of Δ zones (Roger-Estrade et al. 2000). The Δ zones are created only under the wheels, as a function of soil water content and equipment characteristics (Richard et al. 1999). Soil surface crusting is not considered significant because it affects only a small volume of soil.

The Δ zones are destroyed within the layer affected by superficial tillage (seed bed preparation and stubble disking). Here, weather reinforces the fragmentation produced by tillage: drying and wetting and/or freezing and thawing increase the sensitivity of Δ zones to fragmentation by subsequent tillage, or directly transform them into fine aggregates and individual particles. Consequently, all the Δ zones within the layer disturbed by superficial tillage are eliminated, whatever the moisture conditions. The action of weather is considered insufficient to fragment the Δ zones in the horizon located below the superficial layer. We also consider fragmentation caused by the plough to be insufficient to eliminate Δ zones (Coulomb et al. 1993). The destruction of Δ zones by soil macrofauna is also considered negligible. This is consistent with observations in fields that are tilled several times a year. The Δ zones are broken into smaller units (but not totally disintegrated into fragments <2 cm) during ploughing by the combined action of the share and the coulter of the plough. All causes of soil displacement other than ploughing (such as the formation of ruts) are neglected.

The modelled system corresponds to a 2-D soil layer, the depth of which is that of the thickness of the tilled layer. The profile width is chosen so that the pattern of wheel tracks created by the successive field operations is taken into account. The tilled layer is represented in the model as a set of 1 cm × 1 cm pixels, located regularly on a square grid. Each pixel is defined by its co-ordinates and a specific structure, Δ or non-Δ. The pixel co-ordinates are modified during ploughing, for which the model calculates the lateral and vertical displacement of the soil. The structure of any individual pixel is changed depending on the soil condition and the kind of operation. The number of Δgixels is computed by the program after each operation. The percentage of Δ areas is calculated as the ratio of the Δ pixels to the total number of pixels representing the tilled layer.

The initial locations of the Δ zones in the tilled layer are read by the program as an array of pixels. Each step of the program corresponds to a single cultivation operation. At each step, the equipment characteristics (axle load, tyre width, working depth) and the operation conditions (location of the wheel tracks, soil water content) are read from parameter files. The compaction procedure calculates the area of the Δ zones created under the wheels for each operation. The new co-ordinates of the pixels are recalculated as explained above when the operation is mouldboard ploughing. The pixels of the upper part of the soil profile are assigned a non-Δ structure for secondary tillage to a depth corresponding to the working depth of the tool. The program then calculates the ratio of the pixels with a Δ structure to the total number of pixels and draws a structural map of the tilled layer. This gives the percentages of Δ zones at each step. Simultaneously, the program draws structural maps, showing the location of the Δ pixels after each cultivation operation (Fig. 3).

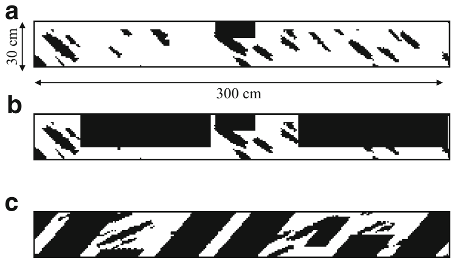

Fig. 3 Examples of structural maps drawn by the simulation program after three cultivation operations. Each map corresponds to 9,000 pixels. Δ pixels appear in black. (**a**) After sugar beet sowing; (**b**) after sugar beet harvesting; (**c**) after ploughing

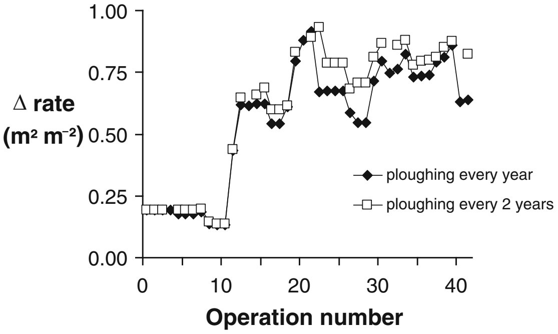

Fig. 4 Simulation of the change in soil structure with two ploughing frequencies, i.e. ploughing every year or ploughing every two years, using the SISOL model

3.3.2 Evaluation and Use of the Model for Designing Crop Management Systems

The SISOL model was evaluated on some ploughed plots belonging to the above-described field trial (Roger-Estrade et al. 2000). We verified that the general trend of the change over time in the percentage of Δ zones was correctly simulated by the model, which also predicted accurately the order of magnitude of the measured percentages of Δ areas. However, the program underestimated the decrease in the percentage of Δ zones. Indeed, the decrease could also be due to fragmentation occurring beneath the seed bed as a result of weather or fauna, which are not taken into account at that depth in the model.

Despite this limitation, SISOL can be used to forecast the global effects of a cropping system on the changes over space and time in soil structure, on the field scale. An example of the use of SISOL is given in Fig. 4. SISOL was used to predict the changes with time in soil structure in conditions similar to those of the above-presented field trial (HCR plots). The first scenario supposed that the plot was ploughed every year; in a second scenario, the plot was ploughed only every two years, before the sowing of wheat. The results showed that when the frequency of ploughing is decreased, the Δ percentage tends to increase, but in a rather small proportion. In these conditions, reduction in the frequency of ploughing to once every two years, saving fossil fuel, seems rather beneficial to the farmer.

4 Conclusion

This review outlines the three main following points. (1) Various models of water transfer are now available but the use of these models on real soil structure is still difficult for two main reasons: (a) the lack of measurements of the changes in hydraulic conductivity with soil structure, both near saturation and in dry conditions, and (b) the limit of the Mualem–van Genuchten equations to formulate mathematically (with a single equation) the change in retention and hydraulic conductivity curves with water potential. (2) The morphological description of soil structure described in this paper allows not only a precise analysis of the spatial variability in soil structure within the tilled horizons, but also 2-D modelling of the dynamics of soil structure in the field. (3) Preliminary investigations (Coutadeur et al. 2002; Ndiaye et al. 2007) suggest that the coupling of a 2-D model of soil structure and 2-D models of water transfer in soil could be a fruitful approach to modelling cropping system effects on water transfers in soil.

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Management of Grazing Systems: From Decision and Biophysical Models to Principles for Action

Michel Duru and Bernard Hubert

Abstract Changes in forage systems taking into account new environmental issues often lead to a reappraisal of the agricultural practices by farmers. These changes also raise new issues about the relevancy of traditional practices of grassland management. We therefore propose an analytical scheme to describe a production plan compatible with land resources (sown or native grasslands). To that end, we present a conceptual model that can be used to design a set of grazing management practices suited to a diversity of specifications. It involves of a combination of defoliation and fertilizer practices allowing different targets to be achieved in terms of herbage yield, composition, and grassland biodiversity. Finally, we suggest several management principles underlying these changes in forage systems. These management principles allow to take decisions on a monthly to yearly basis, to assess the conformity between farm resources and farmers' objectives, and to coordinate different combinations of herd and land at different periods of the year.

Keywords Decision • Extensification • Fertilization • Grazing• Model

Résumé – Gestion des systèmes de pâturage: des modèles biophysiques et de décision aux principes pour l'action. Les transformations des systèmes fourragers nécessaires à la prise en compte de nouvelles préoccupations (environnement, qualité des produits) se traduisent souvent par une désintensification. Il s'agit d'abord de reconsidérer les entités gérées par les éleveurs. Nous proposons un cadre d'analyse permettant de décliner un projet d'ensemble en objectifs cohérents attachés à chacune de ces entités et compatibles avec les ressources en surfaces, en fourrages cultivés ou spontanés. Ces choix remettent aussi en cause les références habituelles sur la conduite des prairies. C'est pourquoi nous présentons ensuite un modèle conceptuel qui permet de définir des itinéraires techniques adaptés à une diversité de cahiers des charges, d'une part en caractérisant les modes de conduite à partir des pratiques de défoliation et de fertilisation (efficience d'utilisation des ressources, flexibilité), d'autre part en rendant compte de leurs effets sur la production de biomasse et sur la diversité spécifique et fonctionnelle des végétations pâturées. Enfin, nous énonçons quelques principes comme autant de préalables à la création d'outils permettant d'accompagner de telles transformations. Ces principes permettent de raisonner les décisions pluriannuelles (cohérences entre les ressources et les objectifs) et annuelles (coordination entre les différentes "saisons-pratiques").

Mots clés Décision • Extensification • Fertilization• Modèle • Pâturage

1 Introduction

Livestock farming systems are questioned by citizens concerns about the quality of the environment and of agricultural products. On one hand consumers put pressure on the authorities through the media and their choice of products; on the other hand scientists

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alert the political authorities as to the environmental damage caused by intensive agriculture. Furthermore, authorities are forced to find solutions to overproduction in industrialised countries. They encourage de-intensification as a way for farmers to reduce their production and expenses, to adapt to new practices, e.g. organic farming, and to simplify their work (Dedieu et al., 1997). In this paper we describe the potential consequences of these changes on grazing systems, which are called for de-intensify through reducing animal stocking rates.

The concepts of intensification and extensification apply to several production factors: land, work and capital. In Western Europe land is becoming the least restrictive factor (Peeters, 1993). These concepts are all relative because the evaluation of the de-intensification processes depends on the initial level of intensification. Therefore the goal is less to define a precise limit after which a system is considered as extensive, than to propose an approach for reasoning the coherence between the different decisions to make in a process of de-intensification. These leads to the creation of new concepts and methods to manage the resources more in regard of these new aims than in term of stocking rate or available biomass as it was mainly prescribed in the classical grazing systems recommendations. For example, de-intensification in grazing systems could involve either decreasing nutrient fertilizers or increasing the contribution of grazing in animal diet by enhancing the available surface area, either by starting grazing earlier during periods of slow herbage growth, or by practising deferred grazing to extend the period of grazing when the growth of grass slows down due to climatic factors (Wilkins, 1995). In this paper, we focus our purpose on plant resources and not on herd management.

With regards the agro-ecosystem sustainability, the question of de-intensification of grazing systems is raised as soon as there is an imbalance that disrupts the replacement of resources for plants. This may concern the vegetation itself when biodiversity or soil fertility are considered. This first type of situation, observed in most of the grass or range ecosystems throughout the world, generally stems from modalities of herbage off-take (rates and levels) which do not allow the characteristics of the vegetation to be maintained in the medium and long term. Low agro-ecosystem sustainability may also be due to excessive inputs, nitrogen and phosphorus for grass swards or intensive fodder crops such as maize, and pesticides for fodder crops. It is the result of complex phenomena concerning the functioning of ecosystems on a larger organisational scale and over longer periods. This second type of situation is common in Western Europe where the main incentives for de-intensification are ecological and economic (Leaver, 1985). But experiments under way in these countries also show the limits not to be exceeded in countries where, by contrast, there is a trend towards intensification. Thus, the raising question is no more to match a one way improvement model but to know how to fit locally with the most appropriate level in regard of allowed means, production goals and present legislations.

Whatever situation, the technical solution to such imbalances is not limited to a reduction of inputs or herbage off-take. De-intensification of grazing systems cannot be understood without reference to the livestock system. It must most often be accompanied by a change in feeding systems, in land use (nature of sward used to feed livestock), in the choice of animal genetic material and, more broadly, in the logics in which the organisation of technical production systems are grounded. Thus, in countries with marked seasons, where vegetation virtually stops growing for several months because of temperatures or lack of water, the storage and use of forage is essential. In most cases, grass and maize silage are used, but industrial by-products can also be used (beet, sugar cane, rice, etc.). When produced on the farm, this forage is often intensified (nitrogen fertilizer and even irrigation). The use of such energy-rich fodder furthermore requires large protein supplements that are often purchased (e.g. Soya). Grazing animals' food is then based essentially on bought inputs – seed, fertilizer, supplements – and thus includes little of the renewable resource grazed grass. In such breeding systems de-intensification implies a reduction in the use of conserved forage and a consequent increase in grazing, in so far as the cost of production of the former (mainly the cost to yield the crop) depends only a little on the crop mass per ha (Clark and Jans, 1995). In other situations, where surface areas are large enough, herds are led on reserved areas where standing plants are still available for grazing, as stockpiled grass. This brief analysis shows that de-intensification cannot be reduced to an increase in the surface area allocated per cow to offset a lower herbage mass resulting from reduced quantities of nitrogen.

Land intensification increased the animal output per hectare, but it looks also as an insurance policy, based on systematic correction of restrictive factors in the environment (mineral elements, water) and high livestock production targets. Yield mechanization, which most often accompanied land intensification, allows the creation of stored forage stocks which make food systems more reliable by planning for sufficient quantities to compensate for production variability. De-intensification means not necessarily aiming for the production potential allowed by the climate, and therefore accepting lower livestock performance always per hectare or even sometimes per animal.

The planning in grasslands management is done in so far as the aim is to have permanent vegetation in the long run in order to avoid sward deterioration and systematic reseeding. Adaptation of feeding system to herbage growth variations (variability in the growth rate from year to years, and irregularity within the period) is based on grass sward or plant community properties: respectively to vary the interval between defoliations in some extent and possibility to under graze plant community at a particular season without irreversible after-effects on its agronomical value. It suppose to allocate enough surface, leading to a lower consumption rates in the course of years whenever herbage growth is better. Thus, grassland set of questions meet rangeland issues.

In the three following parts of this paper, we detailed three main standpoints that were previously summarized (Duru and Hubert, 2001). Part 2 presents concepts and tools to recognize the entities and objects managed by livestock farmers in order to clearly answer – when de-intensification of grazing systems occurred – to the questions: which goals grazing systems are aiming? And how they should be designed to take into account their relationships with ecological and societal issues. Such analysis ensures that meaningful questions are addressed in research projects concerning grazing systems. It is required to identify specific objectives assigned to each of these entities before to determine suitable grazing management. Then, in part 3 we argue that most often, when grazing management should be reconsidered and diversified it leads to study the underlying processes in plants and their interactions with grazing animals. Finally, in part 4 we point out that de-intensification needs to rethink principles for system designing, planning and steering at different space and time scales. The two last parts are illustrated by examples chosen among different livestock systems and different situations of transition towards de-intensification. To illustrate our purpose, the three examples of grazing management that were given in the insert were mobilized at different places of the paper.

2 Re-thinking and Diversifying Production Systems in Grazing Management

In this section we present an action-oriented farmer behaviour model to understand how grazing systems are working. The conception of production systems is actually revised in order to meet new objectives through a diversity in grazing management. Research has to build concepts and methods to make understandable how grazing systems are working, so as to produce new scientific knowledge that could be integrated to improve these new grazing management systems, and that is relevant to do it. Revision of such conceptions usually starts with territorial reorganisation on which farmers base their schedules for the grazing year – schedules that can be adapted, from season to season, depending on climatic events or any other fact which affect their herds' or flocks' lives. To advise and think ahead these transformations, we need to model the situations in which livestock farmers design their technical systems, and then apply these models to decision-making situations.

2.1 A Model to Render Decision-Making Processes Intelligible

To design and manage their production system, livestock farmers must apply their knowledge – of the farm, of the livestock management and the animals' food needs, of the management of the different resources they want to use, of their own skills, etc. – to meet their objectives as regards not only on production but also on the constitution of a heritage and social recognition in their professional community and family (Darré, 1985). Researcher analysis of the coherence between these different components of production system management requires the construction of a functional representation of the system.

The concept of an "action model" was suggested by Sébillotte and Soler (1990) to depict the farmer's decision-making process, seen as part of a permanent recursive, adaptive process in which "the actor readjusts his ultimate goals and his action (on reality) at the same time". In order to do this, he develops a veritable "guide for action" of which a representation can be made through three main points (overall objectives/forecast programme/set of rules), that more or less characterise what we call the "farmer's behaviour model".

Development of an action-oriented farmer behaviour model requires the construction of a two-tier representation of decision-making processes (discussion with the farmer) and technical operations (field observations) (Fig. 1):

- The farmer's objective and plan: in a livestock production system facing a diversity of goals, overall objectives may be diverse but they always consider animal production to be obtained from a biotechnical programme that achieves its production goals through proper herd feed (including resource renewal) and reproduction management. The programme includes a biological cycle based on a calculated mating schedule which conditions the sequence of events throughout the annual plan, viz. parturition dates, early growth period, marketing forecasts per product type, etc. consistent with grasslands states.
- The rules for action that are used to make a combination of elemental decisions constitute the set of rules referred to by the farmer in running his production project. There are *general rules* connected to the organisation of the production system and *circumstantial rules* (Hubert et al., 1993). The former stem from the systems broken down into technical operations independent of the events of the moment; they are vital to project satisfaction. The latter, conversely, are activated as a result of information on the state of some elements in the system; they conditionally trigger actions of various kinds that can be accelerated or delayed, depending on the conditions of the moment.

Formalisation of these rules provides insight into the *information system* that farmers use in a process of self-diagnosis preceding technical operations, and constitutes an unique opportunity for scientist-farmer dialogue. A such framework was used too by scientists to design their experiment (Example 2, Brelurut et al., 1998). It is therefore essential at this stage to build a representation that is compatible with the scientist's knowledge of livestock and grazing management (Darré and Hubert, 1993), and to link both knowledge sets. It would then be feasible to articulate integrated biotechnical sub-models on livestock husbandry or, for instance, on the effects of management on vegetation characteristics and dynamics (as developed in Part 3), to decision-making models on farmer's decisions. New

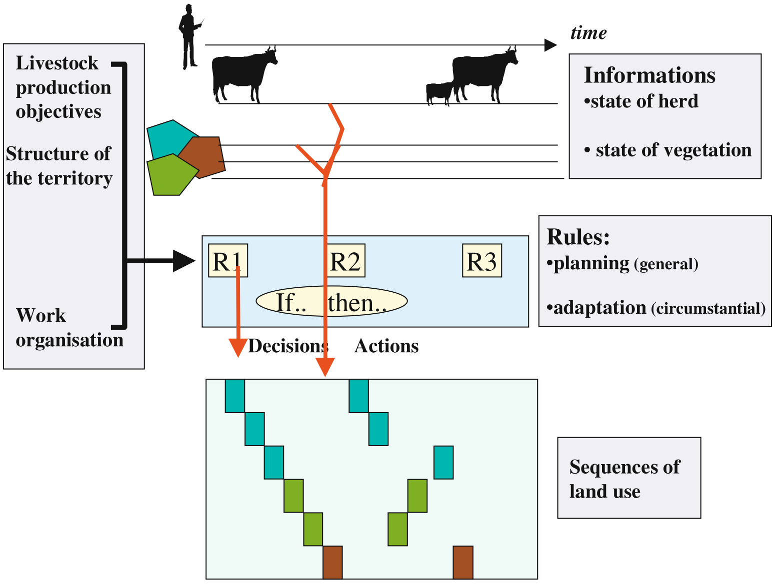

Fig. 1 Theoretical scheme of the action model of farmer behaviour for a grazing system (Adapted from Girard and Hubert, 1997)

goals leading to de-intensification, such as environmental issues or labour organisation, could change the standards applied for resource management, fertilisation practices, farmland structure, batch duration, etc. Thus, it is possible to identify (Hubert et al., 1993):

- Significant decisions which mark the annual plan management: they may be of various types, e. g. decisions on the herds themselves, assignment of animal groups to certain plots and their removal, the distribution of feed supplement or mowing of grazing plots, etc.
- Goal-oriented phases (subsequent to the significant decision-making phase) during which herd management can be considered stable (early lactation, dry cattle, young animal growth, ...) if measured according to criteria selected for their relevance to identifiable final goals. These phases determine the timing and chronology of the annual plan and the use of farm lands; they support the achievement of the different functions that have to be meet to bring off the farmer's aims.

Phases that are closely connected, in pursuit of the same ultimate goal, can be grouped into "sequences" or, in other words, form intermediary elements of the timeframe sequence. Considering land use in terms of "functions" is most meaningful from the viewpoint of overall organisation. It is this understanding of how the farmer does act on the biological processes who allows us to reconsider the models which are used to explain sward functioning in order they fit better with what farmers are actually doing and what they take into consideration, as we develop it in Part 3: which elemental processes are actually questioned?

2.2 Choosing and Combining Different Grazing Management Practices on Different Time-Scales

Configuring a territory for use of heterogeneous pastures by livestock, combining widely diverse types of forage resources, foreseeing the effects of practices on future resources, and planning peak periods are some of the new challenges that these frameworks have to help farmers to meet.

Moreover, to study reorganisations and their implementation, agreement has to be reached on the different time scales of decisions and actions; in other words, it is necessary to define "a time-based decision structure" as Sheath and Clark (1996) suggest:

- On annual and pluri-annual scales, the decisions concern feed profiling which is the setting of long-term policy, such as stocking rate, timing of parturition and general stock buying and selling policy. A key indicator of success is the profile of average farm pasture cover throughout the year. Condition score and live-weight of stock should show planned seasonal variation. This strategic planning results in the allocation of areas to a particular use in a given period: a set of animals and plots combinations per period within the year.
- On a seasonal scale, for a given sequence combining a set of animals and plots, grazing plans seek to meet production (sward state) targets, and include decisions on rotation length, daily supplement fed and date to move stock; key indicators are daily lactation, pasture residual sward mass, plant phenology and its sensitivity to grazing.

To fit their goals at different scales, farmers have to combine strategic planning, action plans and daily management. Strategic planning concerns the configuration of the territory of the farm, the areas to reserve for mowing, with or without topping, the spreading of regrowth after mowing, and key grazing dates from turnout to grass until return to shed – all in relation to the herds/flocks or batches of animals to feed. A forecast is essential for planning the organisation of means of production, allocating areas for mowing and grazing, and setting production targets. It should go even further than the context of an annual plan, by fitting in with the farm's strategic goals and taking into account their evolution. It consists of a programme for the production year, which has to be completed by adjustments for each period, depending on the development of the annual plan, climatic and economic uncertainties, health hazards, tactical changes or adjustments in production targets and in the organisation of labour. This is a management based on alternatives, regulations, resources substitution, etc. It needs knowledge on how does the vegetation react to management practices as fertilisation or defoliation through grazing and how this behaviour panel can be used to plan the grazing agenda.

Girard and Hubert (1997) have underlined two main approaches to conceiving decision aids: the first simulates the consequences of different technical or organisational options (in order to investigate the "what happens if?" aspect) and the second is focused on the farmer's project (to understand "what the farmer acts for?"). The above proposal – that could easily be modelled by using Knowledge-Based Systems (Girard and Hubert, 1997) – relates to the "what for?" approach. Our experience of such models enables us to highlight the consistency between the different decisions farmers makes with regard to their overall project. They help to make this project understandable to outsiders such as agricultural advisers and scientists. But prioritising decisional components poses problems in connecting the model with the biological components of the system. In Part 3 we suggest shifting from an approach dealing with the productivity and quality of grass, to the management of resource dynamics in terms of changes in grassland states and ways of triggering, now and later, the desired states for satisfactory grazing throughout the year. Such data can then easily be integrated in a "what if" approach, which, usually, carries out simulations to investigate the consequences of different options and help choosing better ones. But this shift is particularly relevant to a "what for?" approach, which throws the "decision context" as an effective frame to identify which issue is important for the manager and to characterise the objectives he aims to achieve. This new standpoint on the biotechnical components allows then to forecast the efficiency of the system by managing the resources dynamics, stemmed from interactions between fertilisation practices and grazing management and their effects on grass growth and sward composition. In this perspective, decision aid is not to be viewed as supplying generic technological solutions, but as a learning process induced by investigation of alternatives between the farmer and the advisor (Walker, 2002). Such models are then used as mediation tool to support dialogue between farmers and advisors and not as a strict recommendation to be prescribed and followed.

The above representation of livestock farmer practices – through a farmer behaviour model – is useful in positioning each of the territorial units in the grazing system as a whole and in understanding reasons for the mode of utilisation and the overtopping constraints. By dividing the land into space frames, territorial units can be identified as a result of observation of farmers' practices: arable plots, paddocks, sectors within land allotments or simply portions of space the herders feel are basic in their land use practices. The usual procedure prioritises plant canopy characteristics while here the main knowledge comes from the analysis of utilisation practices and their effects on plant dynamics (growth, leave/stems, species competition, etc.). In the following chapter, we introduce some new concepts to diversify grazing management rules.

3 Some Teaching from Applied Ecology to Rethinking Grazing Management

Studies on grazing have most often been conceived in a perspective of optimisation in order to maximize efficiency of fertilizer use or herbage utilisation, each of variables being considered alone. Agronomists have tried above all to optimise the use fertilizer inputs from the point of view of production, by calculating the N recovery. Establishing a critical mineral content in relation to the quantity of accumulated herbage mass (Lemaire and Gastal, 1997) has been used to perform diagnoses independently of sites and years by defining, for example, a quantity of nitrogen necessary to maximise the production of herbage mass, and by taking into account losses that could have been generated by leaching or volatilisation (Jarvis et al., 1996, 1998). On the other hand, researchers working on the grass-animal interface have tried to optimise livestock production by defining appropriate stocking levels (Béranger, 1985) and, since some decades, by defining optimum states of sward which allow high grazing efficiency level (ratio of herbage intake to herbage growth) (Hodgson, 1985; Parsons, 1988). To define optimum management, constructed indicators (herbage nitrogen index, height of sward) have a far more general value than the definition of norms (quantity of nitrogen, stocking rate) which depend on local situations. Yet these references have always been conceived in a perspective of technical optimisation of the use of inputs (herbage mass per fertiliser unit) or of herbage growth (animal ouput per unit of herbage growth or of standing herbage mass).

Different forms of grazing management must, furthermore, be characterised in terms of flexibility of management and of the means required for their implementation. For de-intensified grazing systems, it is needed to know the effects of a large range of nitrogen rates and of the modalities of defoliation on the characteristics of the vegetation. Models should be used in this way and not inevitably to determine an optimum management for one criteria. Furthermore, we need integrated models which take into account the properties of regulation of agro-ecosystems resulting from strong interactions between the structure of the sward, herbage off-take by animals, herbage growth and composition of the herbage mass.

3.1 Integrated Models of the Effects of Fertilisation and Defoliation on the Characteristics of Vegetation

3.1.1 Grazing Pressure Increases the Grazing Efficiency but Decreases Nutrient Use Efficiency

By combining the two main action variables, fertilisation and utilisation, it is possible to define diverse management modes whose effects can be evaluated on the net production of herbage mass and its composition, and on the efficiency of the harvesting and use of nutrients (Table 1). Intensive grassland management allows for high grazing efficiency, at least as long as the value of the residual leaf index does not hinder growth, whereas de-intensification, by reducing either inputs or intensity of use, enhances the nutrient use efficiency. We present below the state of the available knowledge about the biological mechanisms that can be steered by these two levers to manage grazing in different chosen ways.

The proportion of nitrogen application in the form of fertilisers found in the aboveground herbage mass decreases with the increasing quantity applied. The result is an increase in risks of loss by leaching and volatilisation (Jarvis, 1998). Furthermore, the use efficiency of mineral elements (production of herbage mass by unit of nitrogen or phosphorus absorbed) increases when the dose applied decreases and when the growth time increases. These variations in efficiency stem from allometric relations between the nitrogen content and the accumulation of herbage mass (Lemaire and Gastal, 1997). The minimum quantity of nitrogen absorbed (kg/ha) that allows maximum growth (t of DM accumulated by ha) differs among species in C3 and C4 but hardly varies among species in the same metabolic group: $N_{\text{uptake}} = a(W)^{0.68}$ ($a = 48$ for C3 or 36 for C4) (Lemaire and Gastal, 1997). The more favourable the growth conditions (radiation and temperature), the faster this dilution and the higher the quantities of nitrogen needed to obtain a given level will be. This critical quantity allows us to calculate the efficiency variation for a canopy, when N is non-limiting for growth and when the accumulated herbage mass increases due to longer growth time: $\text{NUE} = W/48(W)^{0.68} = 20.8(W)^{0.32}$ for species in C3. The ratio between quantity of nitrogen absorbed and the reference quantity calculated for the same herbage mass enables us to estimate the nitrogen nutrition level of the sward: $N_{\text{uptake}}/48(W)^{0.68}$. It is linearly correlated with the rate of growth (Bélanger et al., 1992; Duru et al., 1995). Thus, the target of a non-limiting nitrogen nutrition level of the grassland necessarily leads to an accumulation in the soil of unconsumed nitrogen, likely to subsequently be lost. Low grazing frequency and small fertiliser inputs are therefore two factors favouring the efficient use of nutrients.

For a given grassland species, an optimum defoliation regime to maximize grazing efficiency has been defined. When the height is too low, growth is reduced due to a leaf area index that is too low for capture of most of the incident radiation, but beyond this threshold, reducing the intensity of use, either by lengthening the interval between defoliation or grazing, or by increasing the residual height after grazing, results in greater losses by senescence (Davies, 1988), in other

Table 1 Qualitative ranking of the effects of nitrogen rate and grazing pressure on grass swards characteristics and management efficiency, from low (−−) to high (++)

Nitrogen application	Grazing pressure[a]	Net herbage growth	Nitrogen use efficiency	Grazing efficiency
Low	Low	−−	++	−−
	High	−	+	−
High	Low	+	−	+
	High	++	−−	++

[a] Over a minimal threshold of residual sward height and interval between two defoliation events which does not reduce herbage growth rate (Adapted from Duru and Delaby, 2003)

words reduced grazing efficiency (Lemaire, 1999) and hence of stocking density (Hodgson, 1985; Parsons, 1988). This type of farming is also concomitant with a reduction in the quality of the grass offered, either due to lignification of the tissue related to longer growth time, or due to a less favourable anatomic composition (Wilson, 1976) related to the length of the sheath estimated through the height of the grass (Duru et al., 1999). Thus, variations in the intensity of use, either above or below the optimum, reduce grazing efficiency. Reduced nitrogen fertilisation also reduces grazing efficiency in so far as the rate of senescence remains the same whereas the production of herbage mass is reduced.

3.1.2 Flexibility in Grazing Management Depends on N Fertilizer Supply Related to Animal Performance and N Excretion Targets

Flexibility in grazing management was defined as the possibility to be able to vary the defoliation interval while keeping given thresholds of offered herbage mass and herbage N content for animal performances and N excretion.

The production of grazing animals depends on the quantities ingested and the nutritive value of herbage intake. For homogeneous mono-specific swards, the quantities ingested increase with the intake per bite (Peyraud and Gonzalez, 2000) which is a function of the herbage offered per animal (herbage allowance) or the height of the sward on which it depends and, more precisely, on the proportion of leaf blades (McCown et al., 2002). For a given stocking rate, the quantity of grass offered per animal is a function of the available herbage mass. A minimum quantity of blades, depending on the animal species, is required if the quantities ingested on a daily scale are not to be penalised (Penning et al., 1996). The nitrogen content of the vegetation informs on both the crude protein content offered (leaf blades or aboveground herbage mass) and the potential level of N animal urine excretion. The protein content is lower for nitrogen-free treatments, especially for short re-growth time. The same nitrogen content can therefore be attained soon after defoliation in the case of reduced nitrogen fertilisation, or later, in the case of a higher nitrogen application. A minimum protein content, likely to reduce animal production performance, is reached sooner when nitrogen applications are reduced. A minimum threshold of crude protein content, in relation to the dairy production objective, is required. On the other hand, to limit the nitrogen content of animal excretion, a reduction in the nitrogen content of forage can be obtained either by reducing nitrogen fertilisation or by increasing the time of re-growth. Both alternatives have the same effect on urine excretions (Peyraud, 2000). Yet the nutritional consequences are different since in the latter case, in addition to the reduced protein content, there is reduced digestibility and quantities of nitrogen absorbed (Peyraud, 2000). The comparison of intervals between defoliations with which several criteria can be met when high livestock production performance is required (minimum quantity of blades, minima and maxima nitrogen contents), shows that the reduction of nitrogen application makes it possible to reduce the risk of N animal excretion. However, it delays the starting date of possible grazing to attain a minimum quantity of blades, and brings forward the closing date of possible use so as not to descend lower than a minimum protein content. The reduction in the level of nitrogen nutrition therefore reduces the interval between two defoliations, compatible with these different constraints, and thus reduces the flexibility of grazing management. However, the range of solutions decreases to a less extent when the objective of livestock production performance decreases. Increasing the intervals between two defoliations, without necessarily reducing N the input, also reducing risks of N losses from animal excretion, but leads to a loss of flexibility, since it reduces the minimum time between utilisations.

The proposed framework, made up from a set of figures, is meant to help the choice of consistent N fertilizer rates to meet both animal and environmental targets while leaving room for flexibility, as illustrated on Table 2.

3.1.3 Biodiversity of Natural or Semi-Natural Grasslands Depends on the Intensity of Defoliation and the Availability of Mineral Nutrients

Apart from its importance as a natural heritage (Nösberger and Rodriguez, 1998), biodiversity can have a functional role in livestock production. For one,

Table 2 Assessement of grazing management flexibility for two N fertilizer treatments (N+, N−). Management flexibility is defined as the difference between late and early dates of a defoliation event (number of days) permitting to meet targets for herbage mass (W) and crude protein content (CP) (An example adapted from Duru et al., 1995)

Sward state thresholds	N+	N−
	Number of days of regrowth to reach herbage mass and CP thresholds	
Minimum lamina herbage mass (1,200 kg ha^{-1})[a]; date: $\mathrm{Wl}_{\min}$	20	40
Maximum lamina herbage mass (3,000 kg ha^{-1})[a]; date: $\mathrm{Wl}_{\max}$	55	70
Minimum CP content (120 g 1,000g^{-1})[b]; date: $\mathrm{CP}_{\min}$	70	50
Maximum CP content (240 g 1,000g^{-1})[c]; date: $\mathrm{CP}_{\max}$	25	20
	Flexibility (days): $[\mathrm{Min}(\mathrm{Wl}_{\max}, \mathrm{CP}_{\min}) - \mathrm{Max}(\mathrm{Wl}_{\min}, \mathrm{CP}_{\max})]$	
	30	10

[a] From Peyraud and Gonzalez (2000)
[b] From Delaby and Peyraud (1998)
[c] From Peyraud (2000)

specific diversity within a plant community gives it an advantage regarding its use. At field plot level this may consist of grassland management no longer has the aim only of herbage off-take but also of maintaining or changing the botanical composition (Stuth et al., 1993). These changes can be brought about by the introduction of new species or regression in the quantities of existing species. Agricultural practices (fertilisation, defoliation) have a direct effect on survival rates of seedlings of species likely to grow there, on fertility rates of species already present, and indirectly on competitive relations. On a larger space scale, biodiversity is also a way of preserving those species which enable the botanical composition to evolve through the creation of different types of pasture with different characteristics in terms of the production levels of herbage mass and its composition.

Both competition and herbivory can affect plant abundance and distribution (Lavorel and Garnier, 2002). That is why natural grasslands can be classified in relation to the intensity of farming and, more precisely, to the level of fertilisation and the intensity of use (Switzerland: Jeangros et al. [1994]; Massif Central: Loiseau et al. [1998]; French Alps: Jeannin et al. [1991], Pyrenees: Balent [1991]). It is therefore the same two factors which govern the characteristics (canopy height, bulk density, leaf stem ratio) of natural grasslands and of mono-specific sward (cf. 3.1.1). These models allow a rough classification of grazed vegetation and hay meadows, depending on the diversity of species within the pastures (Duru et al., 1998).

Functional ecology enables us to go from a descriptive approach of the vegetation, based on the identification of species, to an approach based on the morphological or ecophysiological characteristics of the species, in order to group together those having the same function in the ecosystem (Weiher et al., 1999). Knowing which plant strategies are suited to which combination of factors, it is then possible to deduce the agronomic properties of communities without necessarily referring to the list of species, since plant strategies are independent of the situations (landscape, soil, etc.), unlike species (Grime, 2001).

Intensive natural grasslands have a reduced number of species due to the fierce competition between them, especially for light, except if they are intensively defoliated. It is the species with the best light resource capture abilities that dominate. One of the main trait of these species is both high specific leaf area and leaf area ratio (Berendse et al., 1992; Van Der Werf et al., 1993). By contrast, when the nutrient availability is low, a larger number of species can cohabit, provided they have the capacity to preserve resources (Table 3). That is why these species develop adaptations such as a longer aboveground nutrient mean residence time (N, P) in leaves (Aerts and Van der Peijil, 1993). Species which preserve resources (slow relative growth) have higher lignin and hemicellulose levels (Poorter and Bergkotte, 1992). They also have a richer secondary metabolite content. The effects of the defoliation regime on the number of species can also be interpreted by the features of the species. In case of frequent defoliation, the specific diversity decreases because many species reach the limits of their phenotypic plasticity. This is because these species are no longer able to place their growth zone below the off-take level. The only species that

Table 3 A framework to study the effect of management (nutrient, defoliation) on vegetation characteristics for rich-species grasslands, using plant leaf traits: + and − means respectively positive and negative relationships

Leaf traits	Leaf plant traits response when there is an increase in		Vegetation characteristics	Main leaf trait which is positively correlated
	Nutrient availability	Grazing pressure		
Specific leaf area	+[a]	+[b]	Herbage mass at ceiling yield	Leaf lifespan[e]
Leaf lifespan	−[c]	−[d]	Herbage digestibility	Specific leaf area[d]

[a] Knops and Reinhart (2000)
[b] Westoby (1999)
[c] Ryser (1996)
[d] Duru (1997)
[e] Cruz et al. (unpublished data)

remain are those which develop strategies of avoidance of or tolerance to frequent defoliation (Briske, 1996). The height of the mature plant can be an indicator of its adaptation to defoliation (Westoby, 1998). On the other hand, when the intensity of use decreases, species with a tissue accumulation strategy develop (Duru et al., 1998). The species corresponding to this type of strategy are characterised by longer leaf lifespan and later phenology (Table 3). When the intensity of herbage use decreases sharply, there is a risk of invasion by so-called undesirable species likely to accomplish their complete demographic cycle (production and dissemination of seeds) and thus to dominate the pasture. This risk is greater when the availability of mineral resources is high. In this case, there is a reduction in the specific species diversity.

When the number of species is small, they generally present a high level of similarity in their plant traits, that is, they can be related to the same functional group (Lavorel and McIntyre, 1999). The agronomic characteristics of the pasture can then be deduced from those of the dominant functional group. By contrast, when there is less competition for light, several functional groups can coexist (Lavorel and McIntyre, 1999). The level of available mineral resources therefore has an effect on the production of herbage mass and its composition due to the strategies of species facilitated by that habitat. Similarly, the defoliation regime introduces changes of species composition either directly (mortality following meristem intake) or indirectly (change in relative competitive ability), which will result in differences in the dynamics of accumulation of herbage mass during regrowth (when leading to change in average date of flowering or leaf livespan), or even in the mineral needs per unit of herbage growth. In the case of pastures that are exclusively grazed, this phenomenon can result in substantial heterogeneity of the vegetation, mainly in set-stocking grazing. Properly adapted under-stocking thus favours several types of vegetation, each with species belonging to a different functional group. This type of result has been obtained in Brazil during long-term experimentation in continuous grazing where the stocking rate is regularly adapted to the available herbage mass (Nasbinger et al., 1999).

The extent of these dynamics depends a great deal on the botanical potential of the environment (above-ground vegetation, seed bank) (Alexandre, 1989), but also on the seed vectors (manure, animals, etc.). Each grassland community characterized by a given botanical composition can be linked to an agronomical value to meet a given function (as identified in Part 2), in terms of adapted defoliation regime, level of production, and possible herbage off-take. The different types of vegetation then constitute a range that can be combined in a forage system, as we saw it in Part 2. That is why biodiversity plays a functional role on the scale of a small region, by way of the possibilities it affords to move from one type of vegetation to another, and thus to change its use.

3.2 Definition of Different Modes of Grazing Management

3.2.1 Decreasing Fertilizer Input and Defoliation Regime: Two Ways to De-intensify Grasslands

The knowledge presented above enables us to define different forms of grazing management in terms of combinations of levels of fertilizer supply and defoliation regimes (frequency of defoliation and grazing pressure, i.e. number of animal of a specified class per unit weight of herbage) adapted to objectives and to the available resources at farm level (labour, land. . .).

Starting from a high grazing pressure, this is called “intensive set” management (quasi-synchronism between growth and utilisation), de-intensification may concern only a reduction in the application of nitrogen and other fertilisers (Example 2), without changing the species planted and the frequency or the height of defoliation, which nevertheless reduces the grazing efficiency. That is why the increase in surface area must do more than compensate for reduced growth. This type of management necessitates a low residual sward height, short intervals between defoliation or early harvesting of fodder silage. In planted pastures, species with short life-span leaves are well-suited to this type of farming (e.g. perennial ray grass). In natural or semi-natural grasslands, these species grow better with this mode of farming, and more or less rapidly, depending on the richness of the seed bank and the extent of seed rain. It is important to ensure that changes in agronomic characteristics (digestibility, height) concomitant with changes in the botanical composition remain compatible with the intended use of these grasslands.

When de-intensification also involves a reduction in the period of distribution of stored feed, the grazing season has to be extended (Example 1). It is therefore necessary to decrease the grazing frequency. As a result, over a threshold which depends on the leaf livespan, the efficiency of the grazing and of the food value of the grass offered decreases. But these options can remain compatible with the feeding of the herd or flock, especially if they are adopted when the demands of livestock production are smaller. The choice of species with long-life leaves or whose nutritive value declines slowly (white clover) facilitates the implementation of this type of management. In natural grasslands this type of species is favoured by such management (Example 3). The consequences in terms of intake quantity and quality and sward structure can be quantified to identify whether such an option remains compatible with the livestock production objectives at the time it is adopted. This deferred or lenient grazing management consists of longer intervals between production and use of grass, and to a greater residual height, which makes it particularly flexible. With deferred grazing management it is possible to: (a) guarantee grazing by creating and maintaining a quantity of grass ahead of needs so that the livestock can be fed even in cases of temporarily slower growth, then by contrast, in set management it is important to vary the surface area offered by adding or removing paddocks; (b) reduce production costs by prolonging the grazing season at the end of winter and during periods when growth declines (early summer or late autumn), by extending the interval between two periods of use, following the introduction of new fields. However, limits have to be defined for these modalities of defoliation in cases of natural or semi-natural grasslands, to avoid deterioration of the sward. In cases of considerable under-stocking and when risks exist of introduction of species that are hardly eaten or not at all, set management is necessary to limit the survival of seedlings or young plants during periods when they are still sensitive to grazing, so that deferred management can be practised the rest of the time (Magda et al., 2003).

Most often, de-intensification combines both a reduction in quantities of fertilisers leading to a decrease in stocking rate, and changes in the modalities of defoliation, some of them stressing more the decrease in stocking rate. It is possible to vary modalities of defoliation during the year in the same field (Example 1) or, on the contrary, to specialise fields by type of management (Example 2).

These types of management differ in terms of resource needs at farm level. Intensive set management allows high stocking rates and consequently requires less surface area than deferred management. However, large reserves of stored fodder are necessary since the grazing season is shorter. Moreover, this model requires the utmost vigilance and attentiveness to anticipate variations in the growth of grass and mobilise buffer areas, used for cutting or grazing in order to regulate the whole grazing system as developed in Part 2, depending on the state of the grazed pastures and the stored fodder reserves (Coléno, 1999). Paddocks with mixed use must however be identified in advance, at the end of winter, although they will be allotted during the spring grazing only (Coléno and Duru, 1999). With deferred management, stocking rates are lower due to increased losses, and often to reduced nitrogen application. In those cases vegetation acts as a buffer as regards variations in herbage growth (Duru, 2000; Duru et al., 1999). The stock of standing herbage, owing to its variations, makes it possible to reduce the effects of fluctuations in herbage growth. These two models are archetypes in so far as intermediate or hybrid models, depending on the time of the year, are frequently observed (Coleno and Duru, 1998).

3.2.2 Managing Defoliation for Its Immediate and Deferred Effects

Apart from their function of immediate herbage offtake to feed herbivores whose quantitative and qualitative needs vary, grazing also serves to prepare resources for later use. Thus dual function differs, however, depending on the intensification of the pasture.

For intensified systems, this preparation is intended to create a sward structure favourable to intake in the subsequent grazing period, most often between 1 and 5 weeks following utilisation. Either the grazing is continuous or rotations are short. This preparation is carried out preventively by opting for intense grazing so that grass shafts remain short and thus favourable to quality re-growth (Coleno and Duru, 1998) and so that herbage rejection is limited. Sward height is an indicator used to decide on variations in the stocking rate or changing of paddock numbers. It applies to the height of the vegetation in continuous grazing (Le Du et al., 1981) or the residual height compared to the height before a rotational grazing (Le Du et al., 1979; Mayne et al., 1987). This indicator, which is usually sufficient, has constant values or generally has increasing values during the grazing season. The hay or silage harvest is needed periodically to regulate the supply so as to attain these grassland states despite variations in herbage growth. If this regulatory function is not performed, curative mechanical means (cutting ungrazed grass) are usually necessary to maintain an appropriate sward structure in the paddocks.

Following de-intensification, this preparation is no longer based on a single and stable criterion throughout the grazing period. A pasture managed conventionally in early spring may subsequently be managed by deferred grazing from the period of high production to a period of feed shortage (Example 1). The hay or silage harvest has not only the function of storing forage resources but also the function of initiating series of re-growth for planned use 5–7 weeks later. Moreover, in this logic the interval between the time of the action and the time the effects are expected to increase, which requires the manager to have greater foresight. On the other hand, the same precision is not sought in the grassland states to achieve.

When de-intensification is on a bigger scale of space, the function of controlling the vegetation can play a preponderant part (Example 3). Thus, for natural grasslands grazed by animals with low energy requirement, the aim may also be to control the morphology of a given species or its abundance if it is undesirable. The aim of the grazing system may be to eliminate the spikes in spring by a targeted high stocked grazing period, in order to cut the apexes so as to avoid a too much decrease in herbage quality or a deterioration in sward structure. It may be also to favour mortality of young shoots of undesirable species, whether herbaceous or woody, at a time when they are palatable, by densely stocking high-risk fields (Theau et al., 2000). These aims can have the result, more than in the preceding case, of modifying the rules of batching which are then no longer set exclusively in terms of livestock production objectives. Batching of herds or flocks is a way of organising animal assignment to target plots in relation to their diverse food needs, in order to fulfil the different functions of grazing, as it has been developed in Part 2.

Achieving such objectives, with more complex interactions to deal with and such an organisation to steer in space and time, needs to renew usual frameworks and criteria to manage grazing.

4 Approaches to Conceiving Decision Aid at Farm Level

The development of new livestock production systems is an iterative process based partly on observed situations designed by farmers in different situations and formalised knowledge produced by research and extension services, based on prototypes in experimental farms and partly on more analytical research results and modelling since a few decades (McCown et al., 2002). Prototypes aims to experiment a limited number of scenarios corresponding to different operational logics and are then compared to real situations or evaluated in farmlet experiments. In this part, we only gave some principles according to two main management levels (designing and planning on one hand, steering on the other hand) in order to show that new ways for grazing management can be achieved by using new models to produce a diversity of resources from grasslands (see Part 3) in respect of farmers decisional frameworks (see Part 2). It's not DSS but a perequisite to build them.

4.1 Principles for System Design and Planning the Agricultural Year

Once a herd/flock reproduction management system has been defined, different logics can be described *ex ante* in terms of rules for land use (type of grasslands, grazing, mowing, fertilisation) and livestock feeding, to draw up grazing and feeding schedules (Examples 1–3). These schedules are defined by the nature and extent of the planned adjustments to environmental variations: extension of transition periods, planned grazing area and conserved forage provisions (Cros, et al., 2003). Thus the whole grazing and mowing area is organized in the best way to plan for the year long, including on course choices to regulate unexpected events, some identified plots being allocated to a range of potential uses (Hubert, 1994). For grazing as such, the nature, order of operations and sequences in time are also specified. The *season-practice* concept proposed by Bellon et al. (1999) enables us to represent these functional grazing entities by integrating both their expected period of use, the grazing modalities (number and type of animals, supplemental feeding, if any, duration of grazing period), adapted to the type of resource required at that time of the year and to the expected effect on the dynamics of these resources and their respective subsequent use. The annual grazing plan can thus be represented as a planed sequence of season-practices based on the simultaneous or combined use of several paddocks by different batches of animals formed by the farmer, through generic models build through a top down standpoint by the use of Knowledge-based systems (Girard and Hubert, 1999). This representation, which shows the farmer's scheduling of the immediate and delayed effects of defoliation produced each time animals graze in a paddock, as developed in Section "Managing Defoliation for its Immediate and Deferred Effects", as well for set or deferred management, could be implemented using mathematic tool for making DSS based on combination of a biophysical model and a decisional model from a bottom up point of view (Cros, et al., 2003). In situations of de-intensification, this planning is particularly important since it conditions the success of the grazing year which no longer depends only – as in the case of more intensive systems – on the immediate adjustment of the growth of plant biomass and animal uptake, but also on the control of defoliation regimes (Examples 2 and 3).

The different de-intensification options involve various difficulties in implementation (Table 4). They could be taking into account when building DSS, designing relevant decision rules (Cros, et al., in press). In the intensive logic, grazing management is hardly flexible when grazed grass is the alone feed resources. It is more so in de-intensification and is facilitated by rotational grazing in so far as it is possible to vary the intervals between two uses, without necessarily creating refusal (Maxwell and Milne, 1995). The spatial heterogeneity of vegetation is greater and production levels more variable from year to year, since they are not corrected by high nitrogen applications. The organisation must fully incorporate these difficulties rather than trying to avoid or ignore them, so that it can take advantage of them to enhance flexibility and reliability (Example 3).

Table 4 Some key differences in de-intensified grazing systems compared with intensive ones. (After Maxwell and Milne, 1995; Thompson, 1997)

Issues	Intensive system	De-intensified system
Input	High inputs to overcome limitations of natural soil fertility	Low inputs and outputs per ha
Grazing and winter forage conservation	Limited grazing period through conserved feed	Extended grazing period
Adaptation to grass growth variation	Conserved feed	Low stocking rate; both pasture plants and grazing animals have to adjust to the effects of any imbalance in forage supply and demand
Information availability/functional complexity	Relatively good, both on ecological processes and resource base	Poor, leading to flat optimising surfaces; scenario
Grazing flexibility	Low	High, prefer rotational grazing to continuous stocking
Spatial heterogeneity	Between management units	Within management units
Temporal variability	Reasonable annual replicability	Wider year to year variability

Table 5 Classifying attitudes to uncertainty in sheep farming in Southern France (From Girard and Hubert, 1997)

Attitudes to uncertainty	Designing a livestock farming system	Steering a livestock farming system
Avoid hazards	Crops to secure resources in periods with unavoidable hazards	
	Allocate sufficient areas for growing winter feed supplies to control indoor feeding	
Rule out hazards	Oversize the farm territory	Set absolute decision rules
Diversify to reduce the effects of hazards	Decentralise livestock equipment to increase the number of grazing sectors	Organise grazing circuits
	Grow forage resources within the rangeland to create a mosaic of fields	Divide flock into batches, reserving the best resources for part of flock only
React to effects of hazards		Divide flock into batches to seize opportunities
		Supplement the flock to complete pasture feeding

Applying Knowledge-based systems on a regional scale, a study by Girard and Hubert (Girard and Hubert, 1997) on farmers' strategies and responses to uncertainty in sheep farming systems in a valley in Southern France confirms the subjective nature of the notion of "risk". In a nutshell: farmers set up strategies to cope with risk and in so doing they display contrasting attitudes to uncertainty resulting from risk (climatic, in a mountainous Mediterranean area, and economic, in a very competitive market). The scale of farmers' preferences, their ways of doing things and, finally, the representations they have of their environment –almost a from one to another- vary considerably. The information processed, the indicators used, and the perception of seasonal time patterns and important events are not the same throughout this category of farmers, as summarised in Table 5. Thus, sheep farms, which are largely divided between intensified systems, de-intensified and never-intensified ones (from the top to the bottom of the table) illustrates that, among these categories, those which are the most intensified give greater importance to designing than steering; inversely, de-intensification increases the need for steering without giving up designing.

System designing and planning is an iterative process requiring alternatively framework in which decisions are made and actions carried out (part 2), and biotechnical models (part 3).

4.2 Consequences on Decision Support With or Without Formal DSS

It is in the respect of decision aid that grazing management models presented above can be applied. Considering that choices have already been made, certain decisions taken, resources and feeding methods decided on, and types of forage resources graded, it is therefore necessary, for a period of the year and a set of paddocks, to define rules concerning dates and conditions of intervention, e.g. date of turnout to grass after wintering (Example 1), duration of the transition, number of paddocks allocated and evolution during the period, as well as the rules governing the sequencing of interventions (e.g. intervals between two uses). Rules for adjustments to variations in the environment (hazards) or in biological systems make it possible to modify the nature, intensity or order of interventions.

Thus, in management of the system, the role of the information system is critical in regard of decision making: decision are taken according to the knowledge available to the farmer. It consists to provide access to the relevant data concerning the biophysical system and the external environment. What is relevant is highly subjective and is actually part of the decision-making behaviour adopted. Decision support systems aim to improve knowledge on the system working and its environment; obviously it triggers directly the farmer's information system. Let's remind that this information system has two functions:

- Interpreting and storing some decision-relevant data about the biophysical system and external environment, and communicating the results to the decision system.
- Monitoring some expected events in the biophysical system or external environment and notifying their occurrence to the decision system that uses them as decision-making temporal landmarks.

De-intensification issues thus helps to enhance livestock farmers' information system through new knowledge, that is, new indicators to observe and new means to guarantee control of defoliation regimes as developed in Part 3. Deferred grazing management, for example, is not based on the same functional signals of the grazing system as traditional rotational grazing. The view of defoliation levels and proportions of leaves and sheaths, the perception of heterogeneity of uptake in a paddock (Example 3), and the evolution of grassland flora (Example 2) will not be interpreted in the same way and will not lead to the same corrective actions in terms of fertilisation, mowing or changes in the modalities of grazing. It is thus new technical references that are expected and have to be produced in situ, either on the basis of experiments or on that of observations of livestock farms, followed by research-development in the context of either research programmes or technical-economic networks, as the current examples show.

Providing models and producing strictly biotechnical references does not suffice to deal with the problems posed in types of organisation that differ and to support decision that are relevant. A similar modelling effort is needed to understand these organisations which form the framework in which decisions are made and actions carried out. They therefore require differentiated advice and interventions from researchers and agricultural advisors, leading to a new generation of DSS less prescriptive and more interactive.

5 Conclusion: An Approach to Functional Integrity

The movement towards de-intensification is often seen in terms of more sustainable agricultural systems. Available knowledge does not prove that such systems are more sustainable, especially since the movement is still recent and few reliable data exist to evaluate changes that can be analysed only in the long term. It is, moreover, difficult to establish the range of evaluation criteria in the economic, ecological and social domains, if only because the effects and consequences of farming systems mostly need to be measured at levels of organisation other than only those of the farms concerned. This would require complex analytical tools and efficient models, most of which are currently a subject of controversy among the scientists who use them. We can nevertheless contribute towards current conceptual and methodological reflection and debate on the subject of approaches to sustainable agriculture, as follows.

For instance, one of the difficulties is to stay within the limits where this change in the intensity of use is compatible with livestock production performance targets and does not affect the sustainability of resources for plant as well as plant resources for animal. De-intensification implies sufficient land resources. Less fertiliser application, when it results in a reduction in the herbage mass produced, or a less intense defoliation regime, always imply the need for a larger surface area if the number of animals is constant. But an increase in the required surface area will depend to a large extent on the maintenance or not of the duration of the grazing period. We find here two options that we developed above: when the proportion of stored forage is reduced, the required surface area for grazing increases more than if the change concerned only fertilisation, since the feeding modalities have not changed. This condition is not possible in all regions, because access to land is limited or too expensive, nor in all production systems. Furthermore, additional constraints concern the spatial organisation of grazed fields. For example, with dairy animals, which are milked, grazed areas must be close to the shed. When de-intensification requires changes to the grassland species sown (or the presence of different dominant species in permanent grasslands), certain conditions have to be met regarding soil and landscape. Likewise, the extension of grazing periods can cause smaller areas to be reserved for animals with smaller needs and for use in a period when risks of damage to the grassland are low, and can therefore cause paddock use allocation to be revised.

Thompson (1997), considering the varieties of sustainability in livestock farming, identified two different ways to use this concept, taking into account that it is more difficult to separate fact and values with respect to sustainability than in other domains of scientific research:

- The notion of *resource sufficiency* is based on the assessment of a practice by measuring the rate at which resources are being consumed and then multiplying that rate by the time frame over which the practice is to be sustained. It directs attention to potential sources of total resource scarcity, taking into account future generation needs and potential substitutions between resources. These two last points are very controversial, from the standpoint of scientific data as well as ethics.

- The idea of *functional integrity* presupposes an account of a system having crucial interactions between elements reproduced over time in a manner or at a rate that depends upon previous system states. Thus, it looks at weak links in a system's ability to reproduce its essential elements. The main questions are how to find the correct dimensions for the relevant system (including time steps) and whether human activities are part of the system.

Unlike most research on grazing, involved in evaluating resources through carrying capacities or in modelling resource management rules, our proposal is part of the *functional integrity* approach, focused on interaction between the grazing herd and pasture dynamics and its management as the relevant system, assessed at the farmland level. For us the functioning of the grazing system is the key element in sustainability of the production system on which the farmer's project is based. The critical interaction, upon which the ability of the system to fit new issues – i.e. its sustainability – relies, relates to the management of grazing and fertilisation, some of plots having distinct use goals, and having to be steered on a flexible way in order to be grazed or mowed according to a given year economic and climatic conditions. These interactions put at stake the farmer's ability to meet a range of sward heights relying on a range of species with different leaf characteristics at different stages of growth. The fertilisation practices and the frequency and duration of grazing are the two key levers according to a given land configuration and some options in animal production.

Thus, although we do not take into account the local social system, human activities, i.e. herd (reproduction planning, making batches, marketing) and resource (grazing planning, balance between mowing and grazing, fertilisation) management practices are definitely part of the system. The originality of our approach is based on the focus on characterisation of an efficient grazing system, and not only on grazing efficiency at the plot level. It is broader in scope, including issues related to environment, labour or economics on larger scales, such as the farm and its surrounding landscape. We think that from this standpoint the functional integrity approach highlights the sustainability of the system at a higher level than what we identified as its core, the grazing system. This amply illustrates how, in this field, new trends induce the need for new decision support systems and not only a revision of strictly agronomic recipes. In this meaning, knowledge produced in several situations doesn't fit a universal value, only arguments and principles become general.

Examples of implementation of de-intensification

The examples came to three experiments: one conducted on dairy farms in Brittany (prototypes of forage systems based on herd monitoring); the second on suckled ewes in the Massif Central; and, lastly, a stocking experimental system in Brazil.

Example 1: De-intensification by increasing grazing period length: Prototypes of forage systems on dairy farms (Brittany, West of France [Thébault et al., 1998])

Like in many livestock producing regions, feeding systems in Brittany are characterised by their diversity. Different "menus" have been defined, based on livestock producing networks. Five models for grazing dairy cows have thus been proposed after observation in the field (Thébault et al., 1998). Depending on the expected herbage yield and the grazing surface accessible per cow, prototypes are proposed, characterised globally by the number of grazing days per year. Each of these models also specifies elements of decision-making for key dates: turnout to grass, night and day grazing, closing and opening of the silo, end of grazing. Calving is in autumn so that milk production is lowest when growth slows down in summer. Similar results are aimed for from an economic point of view.

We summarised here the decision-making logics defined for the two extreme strategies: "maize all year round: M" and "all grass: H". The target is a grazing contribution of 25% and 60% of the basic ration, respectively. To achieve that percentage, it is necessary to have a grazeable area of 0.20 and 0.70 ha per cow, respectively. The former strategy is reserved for farms with a high dairy production quota per ha. The share and the management of stored forage is different in the two strategies. In M, maize silage is given in unlimited quantities throughout the year, except in spring. In H, the proportion of stored forage (grass silage) is limited to 2t DM per head. Climatic variations are offset by the distribution of maize in M, whereas in H their effects are reduced by the large surface area allocated to grazing. In M grazed pastures are covered primarily with English rye-grass while in H there is a white clover mixture. Lastly, nitrogen fertilisation can be as much as 250 kg in M while in H it is limited to liquid manure and possibly to 50 kg of mineral nitrogen. Grazing areas required in H are larger if fertilisation is reduced.

Consequently, grazing practices differ. For example, in H turnout to grass is one month earlier. This change is possible because the allocated area is more than three times bigger. Given the early turnout to grass in H, wide variations in grazing time per day are acceptable. The first grazing cycle in H ends in late March, approximately one month earlier than in M. Provision is made for hay in grazed fields, if necessary. The residual height after grazing rises from 4 cm (early spring) to 6 cm (summer) in H. It is about 1 cm higher in M. It is possible to reduce the residual sward height in H to slow down growth in case of an excess of herbage. In summer the silage ration increases to compensate for the reduced herbage growth rate in M, while in H stocks of standing herbage are constituted for summer feed whenever possible. This strategy requires a stock of standing herbage on 1 July equivalent to between 25 and 50 days' grazing. The target age of aftermaths is roughly 50 days for a ray-grass/white clover mixture. The indicator proposed to decide whether to add or remove paddocks is primarily the grass height measured in a paddock or the level of all the paddocks in the grazed area (Duru, 2000; Duru et al., 1999).

Example 2: De-intensification by increasing the surface area and reducing the stocking rate: Prototypes of forage systems in suckled ewe farming (mountain area, centre of France [Brelurut et al., 1998; Thériez et al., 1997])

Faced with the prospect of expanding farms in grassland areas, trial systems have been set up to conceive variations in livestock management and surface areas, so that new areas can be included whereas flock sizes stay the same. In comparison with a pilot system (T), an enlarged system (A) was designed. The aim was to maintain livestock production performance and economic results, but also to avoid deterioration of the vegetation in this system of lower stocking rates. These two models were designed by researchers, based on their technical knowledge and on observations on farms, and tested experimentally.

Advantage was taken of the reduction in the stocking rate, from 1.2 (T) to 0.85 UGB/ha (A), to reduce inputs of fertilisers and concentrates. Livestock production and economic performance targets were similar in both systems. They were maintained de facto despite a reduction of close to 30% in the consumption of concentrated feed and of 50% in forage production costs (Thériez et al., 1997).

Below we mention some of the changes effected in decision-making logic. In system T, grass silage and nitrogen fertilisation (roughly 100 kg/ha) allowed for a high stocking rate. In system A, stocks were hay-based and grazing was given priority (on average + 22%). In A, breeding management was revised so as to have a class of animals of animals with low needs in early spring and thus to reduce the survival rate of young bushes by immediately implementing a high stocking rate. Reduced quality of available herbage resulting from the reduced stocking rate was also avoided in the lamb fattening period by timely mowing of the pastures to be used for that purpose.

Example 3: De-intensification by maintaining and developing heterogeneity of grassland vegetation: South Brazil (Nasbinger et al., 1999)

This case concerns large-scale cattle farms. Cattle graze natural unfertilised grasslands continuously throughout the year, despite substantial variations in herbage growth. High stocking rates tend to deteriorate these ecosystems, causing high-productivity species to be replaced by low-productivity ones, and increase in soil cover by creeping species. As a consequence of less soil cover there is an increase in superficial leaking, leading to erosion. On the other hand, excessively low stocking rates produce high herbage patches with a dominance of cespitous graminae of low nutritional value, as well as bushes and other undesirable species mainly from the genera *Baccharis* and *Eryngeum*.

Four grazing management systems based on offered herbage (4, 8, 12 and 16 kg of herbage dry matter per 100 kg^{-1} live-weight) were compared. The maximum herbage dry matter production and animal live-weight gain occurred with the 12% treatment (Nasbinger et al., 1999).

The four per cent offer corresponds to excessively high grazing pressure leading to low radiation capture and high population with almost no grass. The greater the forage availability, the better harvest and forage selection by the grazing animal, leading to two types of patch: one with palatable species eaten over the summer (short sward), and the other composed of less palatable species, mainly although not exclusively eaten though the winter. As seen below, these species have the ability to accumulate tissues with low senescence rates through long leaf lifespan and stem. In these grazing systems, the three main decisions are the animal reproductive schedule, the stocking rate in terms of herbage allowance per animal live weight, and the size of the paddock.

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Cadmium in Soils and Cereal Grains After Sewage-Sludge Application on French Soils: A Review

Denis Baize

Abstract Recycling sewage sludges as fertilisers on soils for crop production has several potential benefits such as providing large amounts of phosphorous and organic matter. However, the spreading of urban sewage sludge is a constant cause of controversy because it is known to introduce potentially toxic trace metals into the soil, particularly cadmium. In order to clarify this debate, this review article presents a synthesis of the results of several studies carried out in France on the impact of sewage-sludge spreading. This article reports mainly Cd results but also some results on other trace metals such as Cr, Cu, Hg, Ni, Pb and Zn. Two kinds of data are presented: (1) soil data including total metal contents and data from partial extraction to evaluate the phytoavailable fractions, and (2) plant data including metal content of wheat, a major agricultural crop. The field experiments involved very different amounts of applied sewage sludges and Cd. Indeed, three categories of experiments stand out, the first and second involving high amounts of applied Cd, and the third involving low amounts of applied Cd: (1) during the 1970s and 1980s, sludges with a high trace metal content, especially Cd, were spread at the INRA trials at Couhins experimental farm on sandy soils and in the Vexin area on silty topsoils. The quantities of applied Cd were very high, ranging from 3,600 to 641,000 g/ha. Here, the results show a notable impact on total Cd contents of topsoil and cereal grains. (2) Sludges containing high levels of industrial cadmium were spread on acid soils in the Limousin region for more than 20 years up to 1998. Topsoil Cd contents were measured in fields where the cadmium input was highest, of 300–600 g Cd/ha. Here, a clear increase in the Cd content of cereal grains was found. (3) During the 1990s and 2000s, numerous experiments with sewage-sludge applications compatible with the new French regulations of 1998 were implemented. The amounts of applied Cd were therefore much lower, from 0.6 to 270 g/ha. Here, no impact was detected on the composition of cereal grains. This review article concludes that the application of huge quantities of sewage sludges in the 1970s and 1980s had a clear and long-lasting effect on both soil and grain Cd compositions. Nonetheless, spreading sewage sludge in accordance with the new French regulations had no significant impact on soil and cereal-grain Cd concentrations.

Keywords Cadmium • Cereal grains • Phytoavailability • Sewage sludge • Soil • Total content

1 Introduction

Trace metals – cadmium, chromium, copper, mercury, nickel, lead, zinc, etc. – are unwanted though often unavoidable constituents of urban sewage sludge. As some of these metals, such as Cd, Pb and Hg, are potentially toxic and have no agronomic interest (Wani et al. 2007; Wahid et al. 2008), their presence generates a certain worry in France as well as in all of Europe (Cattani et al. 2008). This is perfectly understandable as some sludge is spread over soil destined for growing food. However, the term "sludge" covers

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a multitude of different compositions, depending on whether such residue comes from a small, rural, water-treatment plant or from a huge plant in an industrial conurbation. This illustrates the importance of knowing the exact origin of the sludge and its trace metal concentrations.

Going far beyond the European directive 86/278/CEE, the new French regulation of 1998 firmly urges the sludge producers to improve the quality of their by-product. The two main points of this new ordinance were (1) the lowering of the maximum metal contents in the sludge to be spread: for instance, the maximum cadmium content of sludge was 20 mg/kg DM until 01/01/2001, then 15 mg/kg until 01/01/2004 and only 10 mg/kg since this date, and (2) the limitation of the metal fluxes applied on farmlands over a 10-year period (see Table 4).

Fortunately, the efforts of the past 30 years in this field have borne fruit, as all total trace metal contents in sludge have steadily decreased over time, particularly for cadmium (Fig. 1). Such efforts should continue without respite, so as to reduce the input of metals into agricultural land as much as possible.

Other questions are: what happens to these trace metals once they have been spread on soil? How much do the chemical forms in which these metals occur – and their carrier phases – change over time, when comparing newly spread sludge with 5- to 10-year-old residues (McGrath et al. 2000; Bergkvist et al. 2003)? Which constituents of the receiving soils, such as iron and manganese oxides, clay minerals and organic matter, will immobilise such metals? And, in that case, will the indefinite progressive accumulation of such metals not have a harmful effect in the short to medium term, i.e. 20–100 years, such as the transfer of metals into plants, i.e. into our food chain (phytoavailability)? Or will, on the contrary, such metals migrate rapidly toward groundwater, because of their high mobility, or toward surface water through runoff and transport of solid particles?

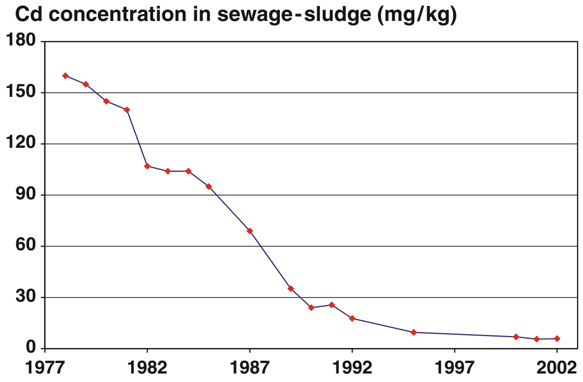

Fig. 1 Mean annual cadmium concentrations from 1978 to 2002 in sludge from the treatment plant of waste-waters of Greater Paris (in Achères). This concentration has decreased strongly over time from 160 to 5.8 mg/kg DM

Finally, must we fear a "time bomb" effect in the medium to long term, i.e. the release of initially immobilised metals under the influence of a major environmental change, such as the progressive acidification of soil abandoned by agriculture?

Hereafter, the results are presented of several experiments, most of which focused on soft wheat. They derive from studies carried out in France by various research institutions and/or Agricultural Chambers. Their common objective was to evaluate the impact of the spreading of sludge from urban waste-water treatment plants under different conditions of composition and volume.

An exhaustive paper on this subject was published in the "Courrier de l'Environnement de l'INRA" (Baize et al. 2006), presenting the protocols and main results of numerous French experiments, in particular the most recent ones, studying the impact of urban sewage sludge.

In the present paper, we refer especially to the older and better known studies that are most often mentioned. In addition, we discuss in particular cadmium (Cd), as this is one of the most toxic trace metals, as well as being among the most mobile and phytoavailable ones. Like mercury and lead, the presence of cadmium is highly dependent upon human agricultural and industrial activities. Except for some well-known and well-localised anomalies, its natural pedogeochemical concentrations generally are very low, around 0.10–0.15 mg/kg in surface horizons, though human input from various sources can easily triple or quadruple such values (Baize et al. 1999).

2 Indicators for Impact Assessment

In order to estimate the impact of sewage-sludge application, three kinds of rather simple tools are often available:

(1) The total metal concentrations determined in topsoil samples.
(2) The easily phytoavailable quantities as assessed by "partial" (also known as "single") extractions applied to the same topsoil samples.
(3) The metal composition of plants cultivated on these soils, especially cereal grains (maize, barley and wheat), the latter being a major product in the human food chain that is of vital importance for the French economy.

2.1 Total Trace Element Contents in Soil and Soil–Plant Transfer

In order to be representative of a truly "total" content, an analytical process must be able to extract all chemical forms of the element to be assayed, including those in the silicate lattices. It is thus necessary to use X-ray fluorescence or very strong dissolution methods, such as that using hydrofluoric acid (AFNOR 2001) or alkaline fusion.

Total contents are measurements of stocks at a given time. The repetition of such measurements over different periods at the same point monitors the content and identifies any changes. In contrast with partial or sequential extractions, total contents today are routinely and easily determined and pose no problems of interpretation, regardless of the soil sample properties such as *p*H, particle size, presence of carbonates and of iron and manganese oxides, etc. (e.g. Nirel and Morel 1990; Tack and Verloo 1995; McBride et al. 2006). Unfortunately, total-content methods do not distinguish the chemical species, which means that such analyses provide no information about the mobility of the element in the soil, nor of its availability or toxicity toward living organisms (e.g. Rieuwerts et al. 1998a).

As a general rule, there is no direct relationship between the total content of a trace element in the soil and the quantity of this element absorbed by a plant (e.g. Rieuwerts et al. 1998b; Meers et al. 2005). In addition to the chemical forms in which each trace metal occurs, plant absorption is determined by certain soil properties, such as the *p*H or the abundance of constituents that can easily retain metals, as well as by the physiological and genetic specifics of the plants. Once absorbed by roots, trace metals can accumulate in the roots or move to other parts of the plant, e.g. leaves, stems or grains, where they are stocked. Each plant species has its proper strategy concerning such trace metal absorption and transfer phenomena.

2.2 Trace Element Determination in Plant Organs and "Partial" Extraction

In order to know whether urban-sludge spreading has an impact on the composition of plant organs that are consumed, it is necessary to assay trace metal contents directly in these organs, such as wheat or maize grains, spinach leaves, potatoes, etc. In addition, it is needful to know the concentrations of such metals in the same crops under "normal" agriculture, i.e. without sludge spreading. To obtain such references, the AGREDE-QUASAR[1] (Baize et al. 2003) and GESSOL-La Châtre[2] (Baize and Tomassone 2003) research programmes were set up. These studies on the composition of agricultural products are indispensable for obtaining reliable answers to these questions and they must consider not only the plant species and the cultivar, but also past agricultural practices as well as soil types, which are highly varied in France.

The simplest method for defining trace metal species in soil uses chemical reagents that dissolve part of the metals in an air-dried soil sample sieved at 2 mm. After this, the metals thus extracted are assayed. In the case of such "partial", also called "selective", extraction, a single reagent is used and the searched-for speciation is essentially "functional" (Ure 1991; Bermond 1999). The aim is to extract only the trace metal forms of interest, i.e. the potentially "mobile" or "phytoavailable" ones, through a judicious choice of the reagent and the operating conditions.

However, this approach of assessing phytoavailability by using partial extraction faces major theoretical objections. It is somewhat presumptuous to try

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[1] INRA Programme "Agriculture et épandage de déchets urbains et agro-industriels. QUAlité des Sols Agricoles et des Récoltes (*Agriculture and spreading of urban and agrobusiness sludge. Quality of agricultural soil and crops*)".

[2] Programme of the French Ministry for the Environment entitled "Fonctions environnementales des sols et gestion du patrimoine sol (*Environmental functioning of soil and managing the soil heritage*)".

to simulate absorption by means of an instantaneous chemical reaction, in vitro, between a reagent and a dried and sieved soil sample. Absorption is a biochemical reaction that occurs at the interface between the soil solution and the roots of a plant, over a period that can take several months while the plant grows. Another problem is that several methods exist for such assaying work, the protocols (and thus the results) of which are difficult to compare. No method seems to be universal, i.e. valid for all soil types, all elements and all plants.

3 Spreading Huge Volumes of Sludge with High Trace Element Contents During the 1970s and 1980s

3.1 Sludge from the Achères Plant Spread in the Vexin Area

Sludge with a high trace metal content from a large waste-water treatment plant in Achères (see Fig. 1) was spread on nearby agricultural land because it could be an interesting source of fertilisers.[3] At the time – 1975 to 1986 – no surveillance or regulations existed for this type of practice, when quite large tonnages were spread in some cases. This sludge had such a good reputation as a fertiliser and humus-rich amendment that it was then sold to farmers. Twenty-two non-experimental fields of the Vexin area were studied by Bernardon (1993), who calculated that during that period one to four sludge-spreading events contributed as much as 0.22–4.3 kg of Cd/ha, 6.5–40 kg of Pb/ha, 28–189 kg of Zn/ha, and 8–61 kg of Cu/ha.

Such an input obviously had a clear impact on the total trace metal contents of the receiving soils, especially on a particular soil series (luvisols developed in loess – Baize 1997). Figure 2 shows that the three surface horizons of soil with a large volume of spread sludge have a much higher cadmium content than that usually observed in surface horizons of this soil series (1.55–2.16 vs. 0.15–0.40 mg/kg). The rare studies of wheat-grain quality, however, did not show a significant "before and after" difference in cadmium content (Bauvois et al. 1985; Tercé et al. 2002).

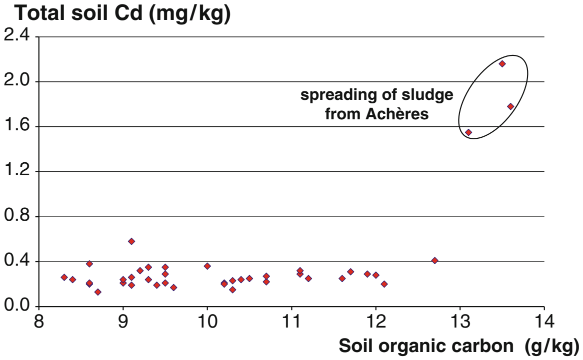

Fig. 2 Cd vs. soil organic carbon content for 39 surface-tilled horizons of a particular soil series (Luvisols developed in lœss in the Vexin area). There is no relation between the two variables, but the three sites where sludge was spread in the 1970s show a clear cadmium contamination and carbon enrichment

3.2 The Experiment in Bézu-le-Guéry

Another example is the experiment run by the Agricultural Chamber of Aisne during the 1970s, when sludge from the Achères plant was spread over very hydromorphic silty soil near Bézu-le-Guéry in the Haute Brie area (Bauvois et al. 1985). In all, 208 t/ha of sludge, or 118 t of dry matter (DM) per hectare, was applied in two double-dose applications in 1974 and 1977. Knowing that the cadmium content of the sludge was 117 mg Cd/kg DM, the quantity of cadmium thus spread can be estimated at 13.8 kg/ha, corresponding to an increase in the concentration of about +3.5 mg/kg of the tilled surface horizon.

Such experimental conditions that applied huge quantities of metals had a major impact on the total cadmium contents of the surface horizon (and also on the other metals – Table 1). The average cadmium content of winter barley grains, grown on four plots with spread sludge and harvested in 1983 (six years after the last spreading), was 0.79 mg/kg, whereas that of four control plots without spread sludge was only 0.33 mg/kg (Bauvois et al. 1985).

3.3 First Trials at the La Bouzule Experimental Farm (Lorraine)

The experiment started in 1974 on 10 m^2 plots (Morel and Guckert 1984), where sludge from the city of

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[3] At the time, the major waste-water treatment plant in Achères processed 90% of the waste-water from Greater Paris.

Table 1 Bézu-le-Guéry field trial: Total trace metal contents of the surface horizons, measured in 1983 and 1993 (Bauvois et al. 1985; Ducaroir and Cambier 1994)

	Four plots with sludge (118 t/ha DM)			Four control plots without sludge		
	1983		1993	1983		1993
	Min.	Max.	Mean	Min.	Max.	Mean
Cd	2.47	3.38	3.06	0.09	0.21	0.12
Cr	56.6	67.7	70.4	27.2	37.6	27.0
Cu	42.0	52.5	49.2	7.0	9.2	7.3
Hg	0.29	0.35		0.04	0.06	
Ni	16.0	20.4	19.8	11.5	15.1	11.1
Pb	43.9	48.5	57.2	18.8	23.6	23.4
Zn	143	169	181	33	46	38

Nancy (average cadmium content of 27 mg/kg) was spread in 1974–1975 and 1979 at doses between 30 and 340 t/ha DM, or cumulative quantities of cadmium varying from 0.81 to 9.18 kg/ha. The soil is silty-clayey over about the first 40 cm, becoming more clayey and less permeable farther down.

In 1981, the sludge application had caused a significant increase in the total Zn, Cu, Cd and Pb contents of the tilled horizon. However, analysis of this surface horizon found only half of the total trace metal input. The presumably easily assimilated metal fractions were assayed by means of partial extraction with DTPA,[4] on soil samples taken on six dates between March 1979 and October 1981. The quantity of cadmium extracted with DTPA showed a linear relationship with the initially applied dose, as well as a regular decrease in the total contents over time. According to Morel and Guckert (1984), this would indicate that the metals evolve over time toward forms that are less easily mobilised (ageing).

3.4 *Experiments at the Couhins Experimental Farm (INRA, Bordeaux)*

This well-known French experimental site was set up in 1974. It resulted in many publications, the main ones being Juste and Solda 1977; Legret et al. 1988; Gomez et al. 1992; Juste and Mench 1992; and Bermond et al. 1998. The trial plots measured only 6 m by 3 m, and every treatment was repeated in four plots. The initial objective was to compare control plots that received the following: (1) only mineral fertiliser; (2) farm manure (with a Cd content of 0.70 mg/kg DM); (3) sewage sludge from the plant in Ambarès; and (4) sewage sludge from the "Louis Fargue" plant in Bordeaux.

The Ambarès (a small town of 9,000 inhabitants) sludge represented "standard" (at the time) urban sludge. It contained 60 mg of Cd/kg and was spread at a rate of 10 and 100 t/ha DM every two years from 1974 to 1988. The Louis Fargue sludge was selected for its extraordinary cadmium (and nickel) content because of the presence of a battery factory upstream from the treatment plant. This plant, in fact, processed more industrial than simple urban waste-water. This sludge was spread three times, in 1976, 1978 and 1980 at rates of 10 and 100 t/ha DM. According to Legret et al. (1988), the maximum cadmium content was 2,672 mg Cd/kg for an average of 1,830 mg Cd/kg. Gomez et al. (1992) reported the main features and results of this experiment. They calculated the cumulative cadmium quantities thus spread as:

Ambarès – 100 t/ha × 8 applications → 27 kg of Cd/ha

Louis Fargue – 100 t/ha × 3 applications → 641 kg of Cd/ha

The soil of the experimental plots is highly particular. Over at least 1 m depth, it contains only 4% clay and >80% sand. Its initial *p*H was 5.3. Such sandy-gravelly soils are locally known as "grave" (Arenosols according to the WRB). The tested crop was irrigated maize. Soil properties and plant composition at different growth stages were regularly monitored.

Under these conditions, major impacts were observed in the plots that received a total of 300 t/ha of Louis Fargue sludge. Firstly, after the third application in 1980, a strong phytotoxic effect was observed on the maize yield, which decreased by half. Secondly, the cadmium concentration of the surface horizon determined in 1989 was 94.9 mg/kg DM vs. <0.50 before the launching of the trial. However, Gomez et al. (1992) calculated that about half of the cadmium input was no longer present in the first metre of soil! Part of this missing cadmium may have been evacuated laterally, linked with particles transported by runoff. The trial plots are indeed quite small and those serving as controls contained on average 1.3 mg Cd/kg in 1989, which is the evidence of a lateral transfer. Massive

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[4] DTPA = diethylene triamine pentaacetic acid.

vertical losses through the soil should not be excluded either, in view of the sandy, quick filtering and acid character of this soil.

In 1976, in the plots that received two Louis Fargue sludge applications (i.e. 200 t/ha DM), the mature maize kernels contained 0.50 mg Cd/kg vs. 0.17 mg Cd/kg in the control plots and those with manure application (Juste and Solda 1977).

These experiments supplied extravagant quantities of cadmium (up to 641 kg Cd/ha) on an "extreme", very sandy and rather acid soil without any constituents that might fix the supplied trace metals. The impact of such spreading on this soil and the plants cultivated on it is thus not surprising. In the 1990s and more recently, the INRA researchers from Bordeaux have carried out many more experiments on the same plots, studying the best procedures for remediating such polluted soils by means of in situ immobilisation (e.g. Boisson et al. 1998; Mench et al. 2000, 2002). These old experimental plots today constitute a fascinating research laboratory, even though they do not represent today's reality of the spreading of sewage sludge on agricultural soil.

4 Spreading Cadmium-Rich Sewage Sludge in the Limousin Region

In the Limousin region, crop rotation commonly consists of 4–5 years of temporary grassland, followed by maize for silage fodder and a cereal with straw. The soils in this region commonly being acid, a risk exists of transferring trace metals from topsoils to plants.

In the past, the sludge from some waste-water treatment plants had high cadmium content, e.g. 20.7 mg/kg DM on average for one of them before 1998. The cadmium came mainly from industrial waste related to china manufacturing and decoration. Although since late 1999 such industrial effluents no longer affect sludge quality, the Agricultural Chamber of Haute-Vienne has still carried out a study of soil and soft-wheat-grain quality according to the protocol of the AGREDE-QUASAR programme (Courbe et al. 2002). A total of 36 sites was studied, corresponding to soils developed in metamorphic rocks (leptynite, migmatite and gneiss), and to soils derived from diorite.

Four types of sites were distinguished in terms of cadmium quantities supplied by sludge spreading over the preceding 10-year period: 22 control sites without spreading; 2 sites that received less than 100 g Cd/ha, designated below as "small quantities"; 4 sites on diorite that received between 100 and 300 g Cd/ha, designated below as "moderate quantities"; and 8 sites that received between 500 and 600 g Cd/ha, designated below as "large quantities", i.e. much more than the cumulative flow now authorised by the French regulation of January 1998 (150 g/ha over 10 years). In fact, these fields probably received three times more cadmium over the past 30 years.

In this rural area of little intensive agriculture, any total cadmium content of soil >0.40 mg/kg results probably from human-induced contamination. Of the 36 analysed soil samples, 11 values exceeded this threshold of 0.40 mg/kg and three contained between 1 and 2 mg/kg (not shown). Eight correspond to plots that received "large quantities" and two others to soils over diorite that received "moderate quantities".

In the wheat grains, 12 values are $\geqslant 0.11$ mg/kg DM, the maximum concentration for cadmium in wheat grains as recommended by the Higher French Council for Public Health (CSHPF). Eight of these were measured in "large quantities" sites and three in "moderate quantities" sites, but this cadmium abundance in the wheat grains seemed to have no relationship with the soil *p*H.

The soil samples were subjected to partial extraction with DTPA. The quantities of DTPA-extracted cadmium correlate well with the Cd contents measured in the grains (Fig. 3 – correlation coefficient $R^2 = 0.71$). Beyond the threshold of 0.10 mg/kg extracted with DTPA, there is a high probability that the Cd concentration in the wheat grains will be equal to or higher than the recommended CSHPF value of 0.11 mg Cd/kg DM.

The four soils developed from diorite which received moderate quantities of Cd have rather low cadmium contents at 0.22–0.47 mg/kg, but Cd concentrations in the grains that are as high as those of the eight "large quantities" sites over metamorphic rocks (0.08, 0.13, 0.14 and 0.18 mg/kg). This means that the exogenous cadmium brought to the diorite sites is phytoavailable for the wheat, notwithstanding a lower total content in the soil.

In conclusion, the study carried out in the Limousin shows that moderate spreading of sewage sludge

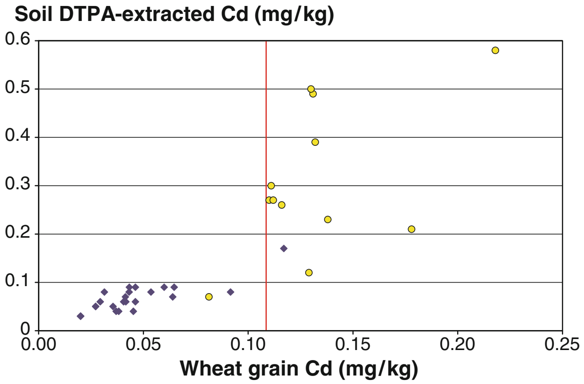

Fig. 3 Soils of the Limousin region (36 tilled horizons). Relationship between cadmium extracted with DTPA from soil samples and cadmium assayed in wheat grains. "Moderate and large quantities" sites are shown by *circles*; *diamonds* represent the control sites and the two "small quantities" sites (Courbe et al. 2002). *The red line* corresponds to the French recommended maximal value

containing cadmium quantities compatible with the new regulation (small quantities sites) does not show up in either the soil analyses or the wheat-grain analyses. However, any higher and continuous input of cadmium (moderate quantities sites over diorite and large quantities sites) clearly stands out in both soil and wheat-grain analyses.

The reader will have noted that this quite particular case belongs to the past. Though it is of scientific interest, it questions neither the rules laid down by French regulations, nor the reasonable spreading of urban sludge over agricultural land.

5 Spreading of Sewage Sludge Over Farmland Complying with French Regulations

5.1 AGREDE-QUASAR Research Programme

The AGREDE-QUASAR study by INRA in collaboration with several Agricultural Chambers (Baize et al. 2003) took place over two periods. First, soil series were studied that had not received sewage sludge, or other urban waste. Located in rural areas, these soils had steadily received phosphate fertilisers and they were not sheltered from the possible little atmospheric deposition. Only two cultivars of soft wheat were studied (Trémie and Soissons), and the sampling strategy was soil series-oriented. The mature wheat ears were harvested over 1 m^2 and over a widely separated dozen fields for each soil series, after which the trace metal contents of the grains were determined. On each grain-sampling site, the surface horizon of the soil was taken and characterised by in-depth analyses, including agro-pedological characterisation, total trace metal contents and partial extraction of the metals with three different reagents ($CaCl_2$ 0.01 M – $NaNO_3$ 0.1 M and NH_4NO_3 1 M). This provided references for various soil series with "sludge-free" agriculture.

During the second period, in 1999, new sites covering the same soil series as before were studied following the same protocol, but which had received sewage sludge at rates in accordance with the current regulations at the time (Cd content of the sludge <3.5 mg/kg DM, most often <1.5).

In all, 163 sites were studied, 33 of which were "with sludge", belonging to 11 contrasting soil types; these were soils developed over a large range of sedimentary rocks, including Quaternary silt, river terraces and marine sediments such as Cretaceous chalk and Jurassic limestone. The sampled fields were located in nine departments of the northern half of France.

In the framework of this programme, the composition of wheat grains could be compared with the analytical characteristics of the soil (surface horizon) where the wheat grows. In the rare other French studies, the analysed grain batches were selected by "production region", and nothing is known about the type and properties of the soils in which the wheat was grown.

The quantities of sludge spread over the trial plots as part of this programme were entirely reasonable and respected the regulations (Table 4). Cadmium input was estimated to be between 0.8 and 15 g/ha for one or two spreading events.

Partial extraction with neutral salts was carried out on the soil samples, after which the extracted Cd, Cu, Pb and Zn were assayed.

The "with sludge" soil samples did not show any difference from the "sludge-free" ones. The *p*H was the main factor for explaining the quantities of extracted cadmium and zinc, the highest quantities being obtained for the lowest *p*H (<6.5). The reverse was true for copper, where the highest extracted quantities were obtained from certain samples with a *p*H of 8.0 or more. Most of the "with sludge" soil samples had a *p*H >8.0, but other soils that had the same range of *p*H values, but were "sludge-free", gave similar results (Baize et al. 2003).

During this study it was thus never possible to show a significant impact of sludge spreading on soil composition (based on partial extraction), or on that of the wheat grains. In the case of soil types with a natural tendency toward acidity (soils developed in sands or loess-like deposits), the highest cadmium contents of wheat grains occurred on soil with the lowest *p*H, <6.5, as could be expected, because *p*H is the most important soil factor controlling cadmium uptake (Kirkham 2006).

5.2 Difficulties of Soil Monitoring: The Barneau and Bouy Experiments (SEDE (1999–2003))

Identical protocols were adopted on two experimental sites in Barneau (Brie Plateau, thick luvisol), and in Bouy ("Champagne crayeuse", hyper-calcareous chalk-derived soil). Unlimed sewage sludge from the Valenton plant was spread over the trial sites in September 1997 and the autumn of 1999. The sludge contained on average 5.4 mg Cd/kg DM. Soft wheat was harvested in 1999 and 2001.

Each trial site comprised 12 plots of 40 by 9 m subjected to four repetitions (coded R1 to R4) of three applications:

(1) Control plot fertilised with superphosphate 45.
(2) D1 = Normal sludge dose of 6 t DM/ha).
(3) D3 = Triple dose of 18 t DM/ha.
(4) A composite sample of 10 samples was taken on each plot before sludge application. After application, the samples consisted of 15 composites, systematically taken at depths of 0–30, 30–60 and 60–90 cm.

This experiment provided a good opportunity for highlighting the difficulties of this type of diachronic monitoring of trace metal contents in soil, in this case between the "initial state" measurements made in 1997 and, after two sludge applications, in August 2001.

Table 2 Cadmium and lead concentrations measured in the 12 elementary plots in Barneau before (1997) and after (2001) two sewage sludge applications

	Cadmium		Lead	
Plot	Before spreading (1997)	After spreading (2001)	Before spreading (1997)	After spreading (2001)
Control R1	0.279	0.300	88.6	47.9
Control R2	0.267	0.280	45.9	100.1
Control R3	0.269	0.280	39.7	65.5
Control R4	0.300	0.300	53.7	70.4
20 t/ha R1	0.359	0.320	32.0	38.7
20 t/ha R2	0.271	0.320	47.4	63.7
20 t/ha R3	0.305	0.360	43.4	83.1
20 t/ha R4	0.321	0.300	53.1	52.1
60 t/ha R1	0.277	0.340	36.1	43.4
60 t/ha R2	0.286	0.350	67.4	55.4
60 t/ha R3	0.319	0.370	52.1	120.7
60 t/ha R4	0.327	0.340	48.4	55.6

The cadmium quantities supplied by the two applications were evaluated at 64–112 g/ha for the D1 applications and 228–268 g/ha for the D3 ones, theoretically corresponding to an increase in the concentration of 0.016–0.068 mg/kg (calculated for a 30-cm-thick tilled horizon with an apparent density of 1.3, weighing 3,900 t/ha). Such a low theoretical increase remains within the order of magnitude of analytical uncertainty and of the spatial variability earlier introduced by different agricultural practices.

Moreover, in Barneau the experimental terrain did not have homogeneous initial TM values. When starting the trial in 1997, the total lead contents of the 12 plots varied between 32 and 89 mg/kg (Table 2).

Another point is that there were incoherencies between the soil analyses of the individual plots before and after sludge application. For instance, in some cases the Cd and Pb concentrations measured in Bouy and the Pb contents in Barneau were lower in 2001 than the 1997 values. Moreover, the strong Pb-content increases in the Barneau control plots between 1997 and 2001 are inexplicable as no sewage sludge was spread over these plots (Table 2).

This discrepancy might be due to the systematic sampling over a constant thickness of 30 cm, which, because of the variable compactness of soil over time, might not always correspond to the true thickness of the tilled surface horizon. In 2001, the 0–30 cm layer may well have corresponded to 28 cm of tilled horizon +2 cm of underlying soil, much less enriched in cadmium, lead and other metals, thus explaining the lower values than those of 1997. In Bouy, this might explain why the average $CaCO_3$ content of soil measured in 2001 was 10% higher than the 1997 value (76% instead of 66% for the control plots). By sampling to 30 cm depth in 2001, the top of a more chalky horizon than the tilled one may have been included.

It is thus clear that, when sampling, one should respect the lower limit of the tilled horizon, rather than sampling at systematic increments that commonly do not correspond to field reality.

The average compositions of wheat grains harvested in 1999 and 2001 from the three types of trial plots on both sites show no significant differences in Barneau (all Cd values <0.05) or in Bouy (all Cd values <0.04), regardless of the year and even after triple sludge doses (D3). However, significant differences occur for zinc (Table 3).

Table 3 Average zinc contents in wheat grains in Barneau and Bouy (in mg/kg)

	Barneau		Bouy	
	1999	2001	1999	2001
Control	22.9	23.1	17.5	20.2
D1	23.9	25.8	19.6	23.5
D3	25.1	30.6	21.2	27.7
CV %	7.4	8.1	7.1	5.4
Signif. level	NS	HS	S	HS

5.3 Other Experiments

A national inventory lists 136 agronomic experiments in France that tested the interest and impact of various residual organic and mineral substances. Not all covered trace metals and some did not concern large-scale farming. Baize et al. (2006) succinctly described eight experiments, all implemented after 1995, some elements of which are shown in Table 4. The soils tested

Table 4 Estimated cadmium quantities reported from experiments presented or mentioned in Baize et al. (2006) (expressed as g/ha). Comparison with regulation fluxes and amounts supplied by phosphate fertilisation

			Waste-water	Number of	Cd quantities (g/ha)	
	Study	Years	treatment plant	spreading events	Minimum	Maximum
Before 1990						
Experiments	Couhins – sludge from Louis Fargue	1970s	Bordeaux	3		641,000
	Couhins – sludge from Ambarès	1970–1980s	Ambarès	8		27,000
	La Bouzule (1974–1981)	1970–1980s	Nancy	2	810	9,180
	Bézu-le-Guéry	1970s	Achères	2		13,800
Field reality	Vexin (Bernardon 1993)	1970–1980s	Achères	1–4	220	4,320
	Vexin – Vélannes (Tercé et al. 2002)	1970–1990s	Achères	4		3,624
After 1990						
Limousin	Limousin small quantities	1990s	Limousin	Over 10 years	<100	<100
	Limousin moderate quantities	1990s	Limousin	Over 10 years	100	300
	Limousin large quantities	1990s	Limousin	Over 10 years	500	600
Others	Barneau & Bouy – simple rate	1990s	Valenton	2	64	112
	Barneau & Bouy – triple rate	1990s	Valenton	2	228	268
	QUASAR programme (1999)	1990s	Rural areas	1–3	0.8	15
	Burgundy & Franche-Comté	1990s	Regional	1–4	0.57	21
	Agricultural Chamber of Somme	1990–2000s	Somme	4 in 10 years	7	27
	Ensisheim (maize) (Schaub 2004)	1990s	Local	5 in 10 years	25	36
	Colmar	2000s	Local	3 in 5 years	11	23
	La Bouzule (after 1996)	1990–2000	Local	4 in 7 years	50	88
	Poucharramet (wheat)	2000s	Toulouse	3	2	18
	City of Mayenne	2000s	Mayenne	3 in 5 years		17.6
Legal thresholds	U.S. EPA – field crops			Cumulative		18,400
	European regulation 86/278/CEE 1986			Over 10 years		1,500
	French regulation – *until 01/01/2001*	1990–2000s		Over 10 years		300
	French regulation – *from 01/01/2001 on*	2000s		Over 10 years		150
P fertilisers	Amounts supplied by phosphate fertilisers			Over 10 years	3	60

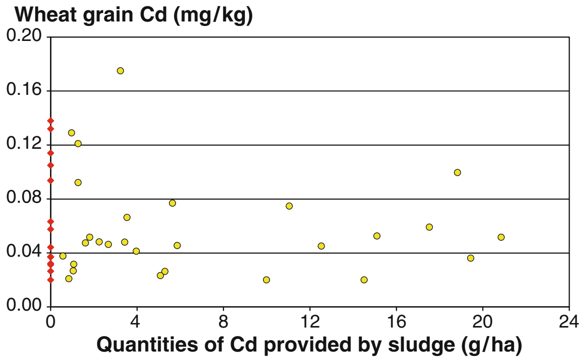

Fig. 4 Trials in Burgundy and Franche-Comté Deléan and Kockmann 2003). Relationship between cadmium content in wheat grains and Cd quantities provided by sewage-sludge application ($R^2 = -0.016$). Cadmium input in 15 control plots is nil

are of different types and the experimental design can be rather simple or quite complex and thorough. The cadmium input varied strongly between trials and even within a single trial, though always remaining between 0.5 and 88 g/ha. None of the experiments showed a noticeable effect of sludge application on crop quality. Figure 4 provides a good example. Deléan and Kockmann (2003) studied a set of experimental fields in Burgundy and Franche-Comté belonging to various soil types: 15 parcels having never received sludge before were subjected to sludge application on half of their surface, the other half being used as a control plot. Thirteen parcels spread with one or several spreadings during the previous five years were also investigated. Analyses of topsoils and wheat grains were carried out ($n = 43$).

Obviously, no link exists between the cadmium content of grains and the Cd quantity provided via sewage-sludge application. Differences observed in grains result much more from the soil properties, such as pH, texture, or Fe and Mn oxyhydroxide abundance, and from the cultivar grown.

6 Conclusion

First, a major point should be highlighted: the phrase “sewage-sludge application on farmland” does not do justice to the possible colossal differences in metal-quantity input. Such differences depend upon different circumstances, such as the cumulative tonnage that was applied and the sludge composition, which has shown large variations over time and space. Table 4 shows the input of estimated cadmium quantities for the various trials described in this paper. Such quantities go from <1 g Cd/ha (sludge from small plants with minor trace metal content, spread in little quantities), to 4,320 g Cd/ha (four applications of sludge from the Achères plant in the 1970s and 1980s). Worse, in the case of the experiment in Bézu-le-Guéry, two “double-dose” applications supplied 13.8 kg Cd/ha, and the maximum quantity was reached in Couhins Louis Fargue with 641 kg Cd/ha!

This illustrates the importance of always evaluating as precisely as possible the real input of trace metal quantities from sludge applications, and to compare this with figures from other types of input, such as phosphate fertiliser, compost or manure.

In this respect, it is instructive to note that the U.S. Environmental Protection Agency limits to 18.4 kg/ha the cumulative quantity of cadmium that can be applied to field crops, whereby the cadmium content of the sludge cannot exceed 85 mg/kg, or 39 mg/kg to be considered as a “high-quality” biosolid (US EPA 1993). It is clear that the French and European authorities are much more severe than the U.S. Agency.

It is commonly very difficult to measure the impact of sludge applications on soil. Today, in France, the input of cadmium is very low to minute, which means that any Cd increases in the surface horizon often are smaller than analytical uncertainty. In this respect, it is useful to remember that:

– To increase a trace metal concentration by 1 mg/kg in a tilled horizon weighing between 3,600 and 3,900 t/ha, the input of this trace metal must be 3.6–3.9 kg/ha.

– Therefore, the input of 100 g Cd/ha theoretically will increase the average Cd content of the receiving tilled horizon by only about 0.028 mg/kg, which is obviously of the same order as analytical uncertainty.

Quantifying the impact of sludge spreading on the chemical composition of consumed organs of plants is not easy either. This kind of analysis is difficult to perform and only a few laboratories are able to carry out such analyses with good reliability.

Another, commonly ignored, difficulty is that many experimental fields are pedologically heterogeneous, at least as far as their initial trace metal content is concerned. This is often discovered afterwards. Other problems, equally difficult to identify, such as those related to sampling, sample processing, or analytical work, can again generate absurd post-sludge-application analytical results when compared with the pre-application ones. Generally, such results are clearly unrelated, being far too high or much too low, compared with the real input of trace metal quantities.

Where trace metal input quantities were enormous (Couhins, Bézu-le-Guéry, first trials in La Bouzule, and Vexin in the 1970s and 1980s), quite clear impacts are visible. These appear in the total metal contents of soils, in the quantities of metals recovered by partial extraction, and in the composition of certain plant organs. Where cadmium input is small and compatible with the requirements of French legislation, it is impossible to demonstrate even the slightest impact on soil or crops. In fact, this is quite reassuring, at least in the short term. The main merit of 1998 French regulation of sewage-sludge application has thus been to greatly decrease the trace metal input into soils. For the most part, this aim was reached.

However, several difficulties remain. First of all, not enough time has passed to have a long-term view of the impact of sewage-sludge application at a reasonable rate. It is clearly difficult to extrapolate the results from short-term experiments – 4 to 15 years as a maximum – to evaluate the impact of such spreading in the long term. In addition, much more work has to be done on other food crops such as spinach, cabbage and salads, which accumulate trace metals more easily than wheat grains.

The best way to evaluate the possible transfer from soil to plants is to carry out direct analyses of specific organs of the harvested plants. However, such analyses are expensive and delicate, posing problems for many analytical laboratories. This is the main interest of partial extraction with neutral salts or complexing reagents (EDTA, DTPA) on soil samples, in order to make a best-possible estimate of phytoavailability or mobility of trace metals (Baize and Tomassone 2003, 2005).

Finally, it should be stressed that the *p*H of the receiving soil is a major factor influencing the risk of trace metal transfer and especially of cadmium in soil toward plants; the lower the *p*H, the higher the risk of phytoavailability and mobility. Fortunately, farmers can easily control this parameter in the medium term by regular application of alkaline calcic amendments.

In fact, limed sludge carries its own antidote against contained trace metals, by increasing the *p*H of the receiving soils and thus decreasing the potential for mobility and phytoavailability of potentially dangerous metals, at least in the short term. However, the problem is what happens when naturally acid agricultural land, amended with sewage sludge, is abandoned. A progressive re-acidification might lead to desorption and liberation of the metals.

As a last point, we should stress the role played by certain natural soil constituents as powerful trace metal adsorbers. These include organic matter, clay minerals, and especially iron and manganese oxyhydroxides. Different soil series do not present the same capacity of trace metal retention, according to the relative abundance of such constituents in all their horizons, not only in the topsoil. Their vulnerability is thus highly variable and merits being taken into account in order to plan an optimal location of sewage-sludge spreading.

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Mobility, Turnover and Storage of Pollutants in Soils, Sediments and Waters: Achievements and Results of the EU Project AquaTerra – A Review

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Abstract AquaTerra is one of the first environmental projects within the 6th Framework program by the European Commission. It began in June 2004 with a multidisciplinary team of 45 partner organizations from 13 EU countries, Switzerland, Serbia, Romania and Montenegro. Results from sampling and modeling in four large river basins (Ebro, Danube, Elbe and Meuse) and one catchment of the Brévilles Spring in France led to new evaluations of diffuse and hotspot input of persistent organic and metal pollutants including dynamics of pesticides and polycyclic aromatic hydrocarbons, as well as metal turnover and accumulation. While degradation of selected organic compounds could be demonstrated under controlled conditions in the laboratory, turnover of most persistent pollutants in the field seems to range from decades to centuries. First investigations of long-term cumulative and degradation effects, particularly in the context of climate change, have shown that it is also necessary to consider the predictions of more than one climate model when trying to assess future impacts. This is largely controlled by uncertainties in climate model responses. It is becoming evident, however, that changes to the climate will have important impacts on the diffusion and degradation of pollutants in space and time that are just at the start of their exploration.

Keywords Acetochlor • Alkyphenol • Atrazine • Biodegradation • Br diphenyl ethers • Chlortoluron • Climate change • Contaminants • Drugs • European river basins • Ground- and surface water • Heavy metals • Inorganic • Isoproturon • Organic • Organochlorine • Pesticides • Review • Sediment • Soil • Sorption • ^{87}Sr • TOF mass spectrometry

1 Introduction

Europe has historically been a hotspot of environmental pressures and continues to be so because of its demographic and industrial developments. In order to better understand pollutant behaviour in a comprehensive way and meet the challenges of environmental impacts, the EU integrated Project AquaTerra was established. The full title of the project is "Integrated modeling of the river–sediment–soil–groundwater system; advanced tools for the management of catchment areas and river basins in the context of global change". The primary objective of this project is to lay foundations for a better understanding of the behavior of environmental pollutants and their fluxes with respect to climate and land use changes. Environmental topics cover a wide range of disciplines form about 250 researchers across Europe and the study areas are the catchments of the Ebro, Meuse, Elbe and Danube Rivers and the Brévilles Spring (Fig. 1).

The scales of investigation range from the laboratory bench to river basins and research results bear the potential to provide enhanced soil and groundwater monitoring as well as early identification and

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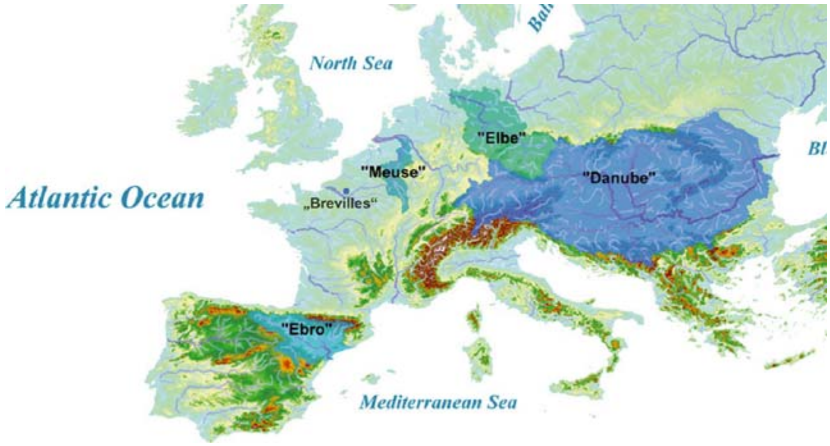

Fig. 1 Basins and catchments of work within the AquaTerra project. Map by D. Kuntz

forecasting of impacts on water quantity and quality, and with that, improved river basin management. Within this context, the project performs research as well as modeling and quantifies deposition, sorption and turnover rates that lead to developments of numerical models. These include fluxes and trends in soil water and sediment functioning.

AquaTerra was one of the first environmental projects within the 6th Framework programme by the European Commission and counts among the largest environmental research projects worldwide. Work began in June 2004 and brought together a multidisciplinary team of 45 partner organizations from 13 EU countries, Switzerland, Serbia and Montenegro. The project has already made significant impacts to global environmental research within the first three years of its existence. For instance, close to 2,000 environmental samples have been collected so far across Europe and, together with historical results and new environmental concepts, have led to close to 400 deliverable reports with novel environmental information. These reports make up about 9,000 pages containing new information about pollutant behaviour in times of rapid environmental change. In part, these deliverable reports are made publicly available on the AquaTerra website that also provides constantly updated information at http://www.eu-aquaterra.de/. These together with the non-public deliverables are used as a basis for further scientific publications and high quality environmental databases. Publication activities are mostly documented by the so far 104 finalised peer-reviewed articles in internationally high ranking journals, 10 book chapters, and a large amount of public press appearances including TV, radio and newspapers (cf. reference list on AquaTerra website).

The AquaTerra team also just completed a special issue in the journal *Environmental Pollution* with the title "AquaTerra: pollutant behavior in the soil, sediment, ground, and surface water system" (Baborowski et al. 2007; Baran et al. 2007; Barth et al. 2007a; Bleeker and van Gestel 2007; Bürger et al. 2007; Graf et al. 2007; Hsu et al. 2007; Joubert et al. 2007; Kalbus et al. 2007; Klaver et al. 2007; Kolditz et al. 2007; Labandeira et al. 2007; Morasch et al. 2007; Petelet-Giraud et al. 2007; Poot et al. 2007; Rozemeijer and Broers 2007; Slob et al. 2007; Vanbroekhoven et al. 2007; Vijver et al. 2007; Vink and Meeussen 2007; Visser et al. 2007). This special issue covers topics including pollutant transfer from groundwater to surface waters, storage and turnover of heavy metals and persistent organic pollutants in soils and aquifers, recharge and climate change impacts. The global impact of the project is also maintained through special sessions at leading conferences. For instance the AquaTerra special session at the 9th International ConSoil conference in Bordeaux in October 2005 was heralded as a significant success and set trends for research and management directions in Europe. Another newly planned special workshop "Contaminant dynamics in periodically flooded soils" in the international Workshop at the EUROSOIL Congress in Vienna (August, 2008) is expected to have a similar impact on the environmental research community.

The large amount and diversity of information produced by AquaTerra, with its new results and investigation techniques, is worth a review in its own right. Even though often conducted at a local scale, results and techniques presented here are particularly important because new information can be transferred to other case studies elsewhere. Overall, AquaTerra is a good example of international collaboration, with scientific approaches extending across boundaries. In addition, the AquaTerra project is one of the few environmental projects to include socio-economic issues. This includes plans to link scientific results to stakeholder needs and policy makers. One of the central outcomes of the project is that environmental issues need to be evaluated for the system as a whole within interdisciplinary approaches.

2 Consortium and Project Structure and Their Organisation

The challenge of managing a large integrated project such as AquaTerra is met through the organisational structure of the project and its division into 11 sub-projects (Barth and Fowler 2005; Gerzabek et al. 2007). The latter are again divided into work packages. For instance, the sub-project BASIN constitutes a research platform, which provides logistics for fieldwork and, in part, access to historical data from the Ebro, Elbe, Danube and Meuse Basins, and the Brévilles Catchment. Two other sub-projects, HYDRO and MONITOR, mainly provide new inputs of hydrological and chemical data through analyses of hydrological databases and models and chemical analyses and methods. These inputs are then taken up by the sub-projects BIOGEOCHEM, FLUX, TREND and COMPUTE for laboratory tests and further evaluation of input, storage, turnover and exchange of pollutants between compartments as well as modelling of specific cases for water and contaminant transport. Potential global changes are then introduced into this systems modelling approach by the first work package in HYDRO, which produces scenarios of climate change so that their catchment-scale impacts can be better understood. Finally, socio-economic evaluations of such new results are then developed through conceptual models and recommendations in the sub-projects INTEGRATOR and EUPOL.

In order to maintain internal and external discussions of the project and to disseminate results, a separate subproject (KNOWMAN) has organised various courses covering topics on modelling, the fate of pollutants, socio-economic and legal aspects and environmental trends. This subproject also provides information on intellectual property rights as well as maintaining the AquaTerra public website. Between February 2006 and January 2007, 2,311 MB of data were uploaded to the website and user statistics show the broad and international perception of AquaTerra with users from New Zealand, Pakistan and Vietnam. A parallel internal web-based information forum (the AquaTerra Intranet) stores all relevant data and activities, which are continuously updated for availability to project participants.

To allow good functioning of the entire project, the subproject PROMAN is responsible for the management of AquaTerra, including reviews of reports, gender action plans and measures for integration. It acts as the interface between the European Commission and the project consortium in all financial and administrative issues and arranges the transferral of information and revised documents. This includes individual approaches to the partners as well as consultation by phone and e-mail. In addition, the logistics of regular AquaTerra meetings are organised by the PROMAN team and include the scientific technical realization of the work plan, reports and publications. This includes maintenance of a calendar for forthcoming and past events as well as a “Who is who” database that constitutes a useful overview of addresses and activities of project participants.

Overall, this structure is highly efficient for the conduct of environmental research that extends across boundaries and environmental compartments. It enables an innovative and more thorough global understanding of the soil, sediment, ground and surface water system. Furthermore, the project structure provides excellent opportunities for all partners to gain high quality experience of state-of-the-art environmental methods in other laboratories across Europe. This is particularly exploited by young researchers, who on several occasions received scholarships to work in other counties based on their AquaTerra work.

3 Objectives of the Work and General Achievements

With the fundamental mission of gaining improved understanding of the river–sediment–soil–groundwater system, participants focus on identifying relevant processes and quantifying their associated parameters. The study areas within Europe all have their own characteristics in terms of environmental concerns, climatological and demographic conditions. Within this context, the goal is to achieve a better understanding of pollutant dynamics in river basins as a whole to achieve potential transferral of results and techniques. This allows us to quantify the interaction and pollutant transfer between the various compartments (atmosphere, soils, sediments ground- and surface waters). New scientific results can then lead to improved environmental management tools and conceptual models for environmental planning on larger scales.

Selected achievements include:

- The establishment of 15 new databases so far with hundreds of environmental parameters about specific environmental pollutants.
- Active links to other environmental projects. This is achieved through several avenues such as participation at other EU project meetings (RISKBASE, SWIFT, NORMAN, Harmoni-CA, Modelkey) and national activities as well as presenting AquaTerra at international conferences.
- International courses on unifying themes such as modeling and software demonstration, trends in the Elbe Basin, socioeconomic and legal issues and biogeochemical themes including sorption of organic pollutants vs. biodegradation.
- Documents such as the AquaTerra Glossary (that explains more than 2,000 specialist terms) to allow further integration within the project.

In the following section the key achievements will be listed grouped by sub-project. In all cases direct beneficiaries include the global environmental research community who receives new environmental monitoring and laboratory techniques together with highly relevant results and conclusions. For instance, AquaTerra has further developed high-resolution monitoring techniques such as passive samplers for ultra-low concentration measurements. Through these techniques it has become increasingly evident that most pollutants are ubiquitously present in the environment and, in many cases, stored for long time periods that range from decades to millennia. Other findings have confirmed that pollutants are often buffered by soils, while their mobility is mainly controlled by the aqueous phase such as ground- and/or surface water.

3.1 Diffuse Pollution and Hotspots, Logistics for Fieldwork, Provision of Data in Collaboration with Other Subprojects Through the Subproject BASIN

Research in the Brévilles catchment focuses mainly on pesticides and their turnover in the subsurface (Baran et al. 2007; Morvan et al. 2006; Roulier et al. 2006). Work has led to an extensive database with chemical data, information on land use as well as a detailed description of the aquifer and hydrological setting. The Brévilles catchment belongs to a wider aquifer system of about 12 km^2 that constitutes a closed system. The area has been investigated by questioning farmers about pesticide and fertilizer use, the installation of a total of 20 piezometers, tracer tests, microbiological investigations and the development of numerical models simulating water and material transport. The local spring, previously an important source for local water supply, was disconnected from the distribution network in August 2001 because pesticide and nitrate concentrations exceeded water quality limits. Continued research on this system shows that pesticides such as atrazine with an application ban in 1999 (4 years before the official ban in France) can be found with its metabolites in groundwater years after their application and in the spring without any significant decrease (Fig. 2). This suggests persistence and slow transport of this molecule and its related compounds in the subsurface. Scaling up such pollution problems to catchment and basin scales would likely cause even longer time periods for pesticide turnover in subsurface environments.

In the Ebro Basin, several detailed monitoring campaigns have been carried out since 2004 for ground- and surface water as well as sediment and soil samples (Eljarrat et al. 2004, 2005b; Lacorte et al. 2006; Lucas et al. 2006). This has led to analyses of a total of 70 contaminants. Focus was put on the determination of potential contamination sources including a textile industry, a tannery and several other production factories. The Flix risk area near Zaragoza initiated specific work for the study of novel dioxins and dioxin-like compounds in fish (Eljarrat et al. 2005a), while the contamination by new brominated flame retardants was monitored at the Cinca risk area. A comprehensive survey of pharmaceutical residues in the Ebro

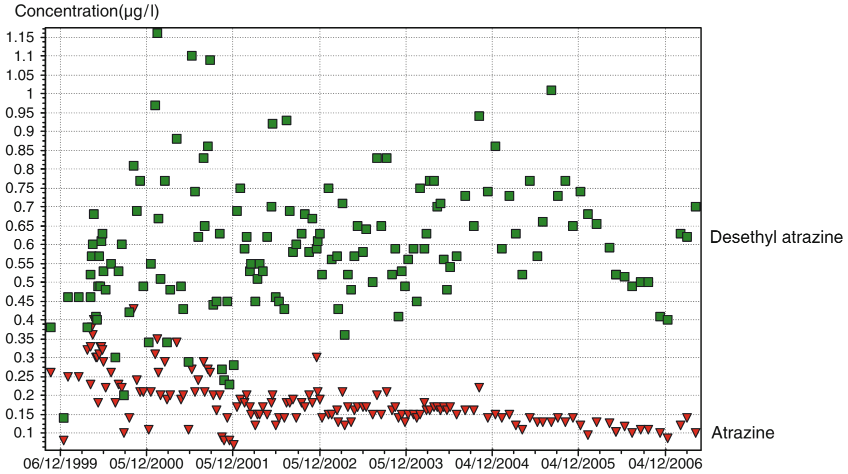

Fig. 2 Atrazine and its metabolites at the Brévilles spring since end of spreading in 1999: desethyl atrazine does not show any significant decreasing trend. Monitoring from October 27, 1999 to April 12, 2007

Basin included the monitoring of several possible point sources (wastewater treatment plants in Zarggosa, Logrõno, Pamplona, Lleida, Vitoria, Miranda, Tudela) (Gros et al. 2007). Another specific study was conducted by evaluating chemical and biological effects, including data interpretation of environmental hazards caused by pesticides on the water flea at the risk zone of the Ebro Delta (Barata et al. 2007). Work also included high-resolution statistical assessment with geographic information systems and led to several new publications about basin-wide pollutant evaluations (Navarro et al. 2006; Terrado et al. 2006, 2007a,b).

In the Meuse Basin, new case studies applied a model predicting pore water composition in flood plain soils. The model includes an ecotoxicological module that models invertebrate and plant uptake. These studies revealed that risks depend mainly on the type of land use, habitat and key plant species (Bernhard et al. 2005; Vink and Meeussen 2007). On the other hand, modern hydrogeological experiments at a pollution hotspot of a former cokery near Liège consisted of hydraulic- and tracer tests to quantify hydrodynamic and dispersive properties of the local alluvial gravel aquifer to evaluate groundwater fluxes at the aquifer–river interface. Together with results of bioassays and chemical analyses on sediments and suspended solids of sampling locations along the Belgian Meuse, this helped to estimate biogenic capacity and toxic effects (Morasch et al. 2007; Vanbroekhoven et al. 2007). Further groundbreaking work on the Geer sub-basin of the Meuse led to the prediction of mobility of pollutants and fertilisers over an entire catchment (Brouyère 2006).

Work in the Elbe Basin has investigated the floodplains in the Czech Republic next to Les Kralovstvi Reservoir with re-suspended load that was polluted by mineral oil. Further downstream, near Magdeburg, sediments of floods were collected with novel trap mats to be further investigated together with soil samples. This revealed important new dynamics of pollutant transgression of contaminants such as cyclo-β-hexachlorohexane, a derivative of the herbicide lindane. At the same site, depth-specific soil water samples were collected for the determination of pollutant flux and transformations of organic and inorganic pollutants on the passage from soils via groundwater to surface water (Baborowski et al. 2007; Graf et al. 2007; Hsu et al. 2007). In the Bitterfeld region, other specific investigations of pollutant transfer on the passage from ground- to surface water were combined with piezometric and temperature measurements and an integral pumping test as well as isotope analyses to reveal only minor pollutant input through groundwater (Petelet-Giraud et al. 2007; Schmidt et al. 2006). This is an unexpected but important result as an example for ground-surface water interaction that is central for the Water Framework Directive.

In the Danube Basin, the AquaTerra team completed a successful river sampling campaign on the main river in August 2004. This included the collection of sediments, water, fish and benthic organisms from 30 stations in six different countries along a 1,150 km stretch of the river between Vienna and the Iron Gate Reservoir in Romania. This is not only a prime example of excellent European collaboration but also led to a highly complex database containing thousands of environmental results and has already led to a publication about sediment transport (Klaver et al. 2007). The work laid the foundations for further monitoring campaigns on the Danube. Further work in the Danube Basin has focused on atmospheric deposition of persistent organic pollutants (POP) and comparative soil investigations (Graf et al. 2007).

3.2 Climatic Variability and Change, Water Balances, Hydrological Input Data and Their Processing: The HYDRO Sub-project

The HYDRO team of AquaTerra have developed a framework for probabilistic scenarios of climate change for impact assessment and the reproduction of climate variable statistics using downscaling techniques (Fowler et al. 2007a). The methodology uses an assessment of climate model simulation of mean climate and extremes, such as droughts (Blenkinsop and Fowler 2007a; Fowler et al. 2007a,c) and heavy rainfall (Fowler et al. 2007b) to weight model predictions of future change. In addition, rainfall modelling techniques have been further developed to integrate into the downscaling framework (Burton et al. 2008) and sophisticated methods have been produced to downscale in time from daily data, important for impact studies (Botter et al. 2007; Fowler et al. 2007a; Marani and Zanetti 2007) This methodological framework is now being applied to the AquaTerra catchments in collaboration with numerical modellers in COMPUTE and TREND (Bürger et al. 2007). This involves links to selected impact studies (Fowler et al. 2007a) such as changes in drought frequencies in water supply regions of the UK (Blenkinsop and Fowler 2007b). Key rainfall modelling deficiencies in large catchments have also been identified and improvements to optimization schemes have been made for models that were applied to the Gallego Catchment in the Ebro Basin (Bürger et al. 2007).

A more local climatic focus was placed on the Brévilles Catchment by assessing water inputs and monthly water levels in 20 established piezometers. One interesting feature of the climatic series is the progressive increase in rainfall from September 1995 to August 2000 and a subsequent decrease after 2000, which was also observed in the aquifer and the spring with a delay of two to three years. Further high-resolution geophysical investigations, tracer tests and determination of material properties were also carried out in the catchment and are currently being evaluated to constrain the subsurface hydrodynamics of this densely instrumented catchment.

3.3 Novel Analytical Methods and Their Application with Focus on Emerging- and Priority Pollutants

Several analyses were carried out on target priority and emerging contaminants for selected soil/sediment and water samples at temporal and spatial distributions relevant to the river basin scale and water as well as soil quality (Van Beusekom et al. 2006). They included priority compounds from the directives 2006/11/CE and 2455/2001/EC and comprised 20 organochlorine compounds, 8 polyaromatic hydrocarbons, 2 alkylphenols and 40 polybrominated diphenyl ethers congeners as well as various pesticides and metabolites and approximately 30 pharmaceutically active compounds (Eljarrat and Barcelo 2006; Eljarrat et al. 2004; Lacorte et al. 2006; Ratola et al. 2006; Gros et al. 2007). Specific analyses of selected pesticides with emphasis on compounds used in rice fields in the delta region of the Ebro and further pesticides applied in the Brévilles Catchment (atrazine, desethyl- and deisopropyl-AT, isoproturon, chlortoluron, acetochlor, acetochlor ethanesulfonic and oxanilic acid) in water and soil were also developed (Barata et al. 2007; Eljarrat et al. 2005a; Hildebrandt et al. 2007).

Further new analytical methods have included a multi-residue method with liquid chromatography triple quadrupole and quadrupole time of flight mass spectrometry for the determination of a wide range of pharmaceutical compounds in the environment (Gros et al. 2007; Peschka et al. 2007). Analyses have included anti-inflammatory drugs, antibiotics, β-blockers, lipid regulating agents, anti-histaminic and psychiatric drugs. In addition, new brominated flame-retardants (hexabromocyclododecane, decabromodiphenylethane) were detected in environmental samples of the Ebro Basin and led to the advanced modelling of these compounds in the food chain (Eljarrat et al. 2004, 2005a,b; Lacorte et al. 2006; Van Beusekom et al. 2006).

Further new analyses of persistent organic pollutants with a focus on polycyclic aromatic hydrocarbons (PAHs) from field deposition samplers and from sorption experiments were conducted (Barth et al. 2007c; Gocht et al. 2007a,b; Turner et al. 2006). A first calibration dataset for validation of passive adsorption cartridges as a new method for time-integrated surface water sampling of organic compounds was also developed. This can become highly important for river water sampling where low concentrations of compounds in surface waters still need to be analysed for flux considerations over longer time periods. Passive samplers were also further developed for additional compound groups and environmental compartments. This work produced new data about the performance of passive air sampler designs for validation of, for instance, brominated flame retardants (Harner et al. 2006).

3.4 Transport, Storage and Turnover of Organic and Metal Pollutants: A Summary from the Subproject BIOGEOCHEM

Part of the team demonstrated the importance of colloids, dissolved organic matter and the role of micro organisms for contaminant mobility in floodplains (Abelmann et al. 2005; Weber et al. 2007; Voegelin et al. 2007). They applied a combined approach of field lysimeters, laboratory microcosm and flow-through experiments. Flooding enabled the direct field sampling of contaminants including copper, cadmium, lead and polyaromatic hydrocarbons. The group further developed protocols for the sampling of water-dispersible particles under reducing conditions and their characterization using TEM and X-ray absorption spectroscopy (Weber et al. 2007).

Furthermore, sorption properties of selected field samples yielded an extensive list of solute descriptors for a wide range of organic compounds to enable rapid prediction of soil-water distribution coefficients. Laboratory methods for sorption dynamics of polar compounds were also extended and led to the determination of sorption isotherms of 21 polar and non-polar compounds. This is crucial to determine how rapidly pollutants can be released into the water and helps to quantify their mobility and availability for biodegradation. Another column experiment to evaluate movement of pesticides through rocks was performed and further coal petrography microscopy of carbon phases in soil and sediment samples helped to define the phases in soils and sediments that are responsible for sorption.

Statistical analyses further determined the impacts of temperature, *p*H, gas phase and microbial activity on inorganic pollutants in the soil- and groundwater zone. The resulting equilibrium constants served to characterise soils and aquifer material of the Dommel, Flémalle and Gallego sites as examples for successful applications. Further studies of bacterial biodiversity were performed with statistical analyses for soil incubations and provided new relationships between the bacterial community structure and, for instance, arsenic turnover while combined lysimeter experiments under vegetation showed preferential flow of lead via particle transport (Joubert et al. 2007; Vanbroekhoven et al. 2007).

Microbiological work revealed new functional activities and composition of microbial communities throughout the Brévilles, Danube, Ebro and Elbe areas by applying new molecular markers that respond to system perturbations such as changes in redox conditions, water saturation and pollution. Laboratory model systems including microcosms and sediment columns are currently applied to simulate various perturbations under environmental conditions such as changing temperature, redox, water saturation and influx of pollutants as typical environmental changes.

One important new finding of the microbiological team is that the sum of all nonylphenol (NP) isomers can serve as an indicator of estrogen pollution in sediments to characterise estrogenic activity response (de Weert et al. 2008). They studied the bioavailability and biodegradability of NP over time in contaminated river sediment of a tributary of the Ebro River. The bioavailable fraction was collected with Tenax TA® beads, and biodegradation was determined in aerobic batch experiments. The presence of NP was analyzed chemically using GC-MS and indirectly as estrogenic potency using an in vitro reporter gene assay (ERα-luc assay). Our study revealed that 95% of the total extractable NP in the sediment desorbed quickly into the water phase. By aerobic biodegradation, the total extractable NP concentration and the estrogenic activity were reduced with respectively 97±0.5% and 94 ± 2% (cf. Table 1). This shows that the easily biodegradable

Table 1 Residual microwave assisted extractable nonylphenol (NP) or estrogenic activity in estradiol-equivalents (EEQ) after biodegradation as fraction of the total microwave assisted extractable amount in the original sediments

	NP (%)	EEQ (%)
Original sediment	100 ± 6	100 ± 43
After biodegradation	3 ± 0.5	6 ± 2

fraction equals the potential bioavailable fraction. Furthermore, 43% of the estrogenic activity in the total extractable fraction, as detected in the ERα-luc assay, could be explained by the present NP concentration. This indicates that other estrogenic compounds must be present and that their bioavailability and aerobic degradation should be similar to that of NP. With this, the use of NP as an indicator compound to monitor estrogenic activity in Ebro sediment was proposed.

The microbiological team of AquaTerra also quantified degradation of a variety of organic compounds including atrazine, nonylphenol, DDT, vinylchloride, 1,2-dichloroethane, chlorinated benzenes, brominated flame retardants, polycyclic aromatic compounds (PAH), atrazine, isoproturon, acetochlor under laboratory conditions. Biodegradation rates capacity may work differently in the field, for which a novel compilation of changes in compound specific stable isotope ratios was introduced as a suitable monitoring tool to quantify degradation rates of organic compounds (Barth et al. 2007c; Morasch et al. 2007). In the field dechlorinating bacteria were detected and quantified. Especially *Dehalococcoides* were detected in the presence of bioorganic pollutants. This suggests that a high abundance of these bacteria can be used as an indication of contamination.

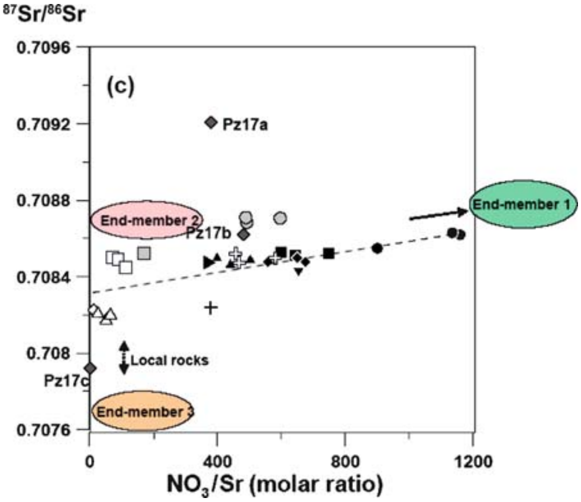

Fig. 3 Sr isotopes ($^{87}Sr/^{86}Sr$) vs. the NO_3/Sr molar ratio of all piezometers and springs of the Brévilles Catchment. Samples plot between two natural sources (end-members 1 and 2) and the anthropogenic source (fertilizers, end-member 1)

3.5 Pollutant Input, Fluxes and Exchanges Between Compartments

On local scales such as for the Brévilles Catchment interviews with local farmers helped to assess present and past pesticide inputs, while groundwaters were analysed for the same compounds on a monthly basis (Baran et al. 2007). Furthermore, interaction between water bodies in the Brévilles catchment could associate Sr from anthropogenic sources via unique isotope ratios and demonstrated the capacity of Sr isotopes to trace fertilizer contributions vs. natural sources (Fig. 3).

Another local study characterised temperature- and integral pumping tests for pollutant transfer determinations in the Mulde Area in the Elbe Basin (Kalbus et al. 2007; Schmidt et al. 2006). This led to definitions of spatial heterogeneity of ground- and stream water interactions that were also confirmed by isotope tracing with strontium and water isotopes (Petelet-Giraud et al. 2007).

On larger scales, sampling campaigns for major ions, trace elements and isotopes in the Ebro River, combined with historical data, led to new quantifications of long-term suspended and dissolved matter (Négrel et al. 2007). This work is best summarised in Fig. 4 that evaluates suspended matter concentrations vs. total dissolved solids to outline influences of dams.

The Meuse River basin was investigated through a new lake sediment core, recording the major flood events, and the study of present suspended matter exportations in order to compare the present and past SPM fluxes at the scale of the Meuse Basin. A subcatchment of the Meuse, the Dommel, was also studied for its surface- and groundwater dynamics with novel methods including strontium, boron, lithium, cadmium, zinc and lead isotopes. This helped to decipher the anthropogenic sources (urban, industrial) and their behaviour at the basin scale. Work within the FLUX

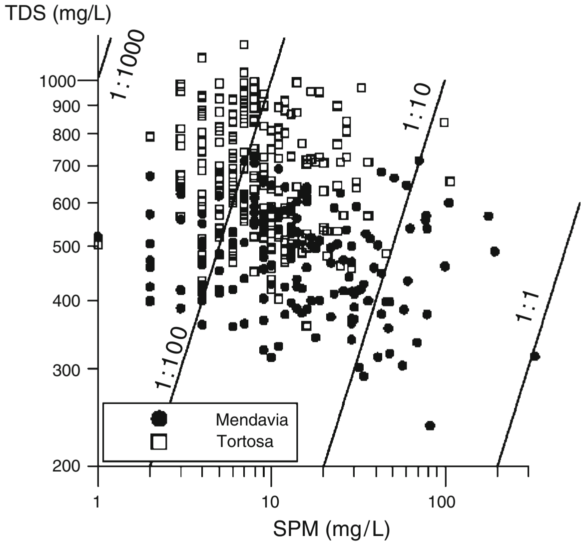

Fig. 4 Plot of the total dissolved solids (TDS) and suspended particulate matter (SPM), all in mg L^{-1} for the Ebro River at Mendavia (n = 172) and Tortosa (n = 217). This reflects the influence of dams and their hampering of suspended matter exportations in Tortosa at the outlet of the basin, where dissolved exportations largely dominate

subproject was further accompanied by monitoring of suspended matter transport by novel acoustic methods. A new prototype near-bed hydrophone array provided high-resolution data during in-situ experimentation under high flux conditions (Hermand and Holland 2005; Hermand et al. 2006). Further tests are now considered at selected AquaTerra sites and are expected to develop into a fully-coupled sediment transport model that is leading for sediment transport in the field.

For comparison between basins, passive air and deposition data were also collected for the areas of Brévilles, Danube, Ebro, Elbe and Meuse catchments to cover a total period of 24 months (Barth et al. 2007c). Sampling of soil and water was finalized for the same areas and samples were prepared for polyaromatic hydrocarbon analyses.

3.6 Temporal Spatial Soil and Groundwater Developments and Their Numerical Tracing

Sediment, water and soil samples were sampled in the above-mentioned river basins and studied by several new techniques in order to identify trends in the fate and risk potential of contaminants. Sorption studies with floodplain soils pointed to an increasing sorption capacity with progressing soil formation (e.g. Graf et al. 2007). Retention of naphthalene and heavy metals was found to be mainly influenced by soil *p*H and soil organic matter (Lair et al. 2007a,b). Trends for metal behavior in soils are presented in Fig. 5.

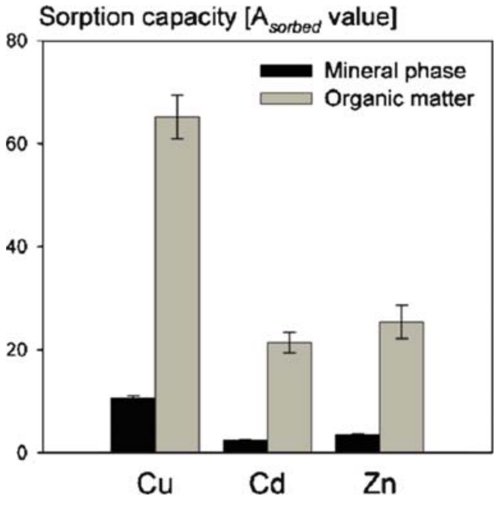

Fig. 5 Sorption capacities of soil phases for selected heavy metals in a Cambisol (37% clay, 41% silt, 2.6% organic C, soil *p*H 5.7). $A_{sorbed}[mmol^2\ kg^{-1}\ L^{-1}]$ represents a calculated parameter from sorption isotherms

Other novel live biological monitoring approaches involved earthworms and revealed differences between selected floodplain soils in terms of feeding activity and weight loss (Bleeker and van Gestel 2007; Vijver et al. 2007). On the other hand, breakdown of leaves from the Dommel Area (Meuse Basin) showed differences between sampling sites that are probably linked to macro fauna abundance and site properties. Other new bioassay studies with larvae and other sediment habitat in the Elbe System also found decreases of contaminant bioavailability through ageing in sediment-water systems, which may be counteracted by increasing temperatures, thus giving important indications for impact assessment under changing climate (Hsu et al. 2007).

In this context physical models were developed for deterministic trend analysis in groundwater and associated reactive transport models (Barth et al. 2007b; Visser et al. 2007). Several tools for trend detection and forecasting for groundwater quality were developed. These included tools based on backscaling of time series using the recharge time, which was determined by $^3H/^3He$ age dating.

Field campaigns in the Brévilles Catchment initiated parameterisation of pesticide leaching to simulate atrazine and water fluxes in the unsaturated zone and in the aquifer (Morvan et al. 2006). On the other hand, the Geer Basin in the Meuse comprised a groundwater flow and solute transport model from input of groundwater sampling for dating techniques including the tritium method to relate the nitrate concentration in the aquifer to water ages (Brouyère 2006). This model was further developed for nitrate transport as an example of highly soluble diffuse inputs (Batlle Aguilar et al. 2007; Brouyère et al. 2007; Orban et al. 2005).

In addition to this, a several new theories in the field of machine learning (Schölkopf and Smola 2002; Tipping 2001) and artificial neural networks were investigated and applied to rainfall-runoff modelling in the Gallego Catchment in the Ebro Basin (Bürger et al. 2007). Further interpolation studies on environmental screening data was carried out using diverse network types and new work was also initiated for pattern recognition and clustering of a new soil parameter data set across Europe.

3.7 Modeling Hydrological and Pollutant Transport and Software Development

Modeling efforts in AquaTerra addressed relevant flow and transport processes at scales ranging from centimetres to hundreds of kilometres. At the smallest scale, one-dimensional models of preferential transport have been developed that account for the mobility of small soil particles and contaminants in macro-pores with initial focus on cadmium transport (Dohnal et al. 2006; Dusek et al. 2006). At the local scale the MARTHE 3D code was applied to model preferential flow and mass transport in the Brévilles Catchment (Thiery and Amraoui 2001; Vanderborght et al. 2005). This complemented a finite differences model with inputs from the HYDRO and FLUX subprojects. In addition, the time resolution of the soil-atmosphere boundary fluxes was found to be a significant factor when modeling the mobility of dissolved contaminants in soils.

At the hillslope scale, a conceptual framework was developed and tested for modeling subsurface flow and transport and in the Meuse-Dommel area were data collection and models comparisons were completed. Furthermore, a new database for the Meuse-Geer Basin was established including data preparation and geometrical set-up of data. Analysis of hillslope-scale processes in small, snow dominated, catchments was accomplished by using the GEOtop model (Zanotti et al. 2004), together with a comprehensive study on soil moisture dynamics and travel time distribution (Botter et al. 2007).

On larger scales a comprehensive modeling framework for solute transport was developed and applied to the Dese catchment in Italy (Botter et al. 2005, 2006; Rinaldo et al. 2006a,b). Based on this theoretical framework the GEOTRANSF model was developed and applied to the Brenta and Gallego catchments. The first is located in north-eastern Italy, while the second is a tributary of the Ebro River in Spain. This work improves our understanding of anthropogenic effects, such as reservoir operation and withdrawals for agricultural purposes, on the hydrological system and enables to distinguish between the effects of climate change and water use. Developments of new modules considering nitrogen cycling and a specific salinisation mechanism were also started (Botter et al. 2006). The Ebro Basin is now planned as a target for an entire basin model and the salinisation issue will be addressed within the Gallego catchment in collaboration with INTEGRATOR and FLUX teams.

The modelling effort was supplemented by the development of unified simulation tools for coupled surface/subsurface flow models that uses different approaches and constitutes a software toolbox for environmental modelling (Beinhorn et al. 2005; Kalbacher et al. 2005; Kolditz and Bauer 2004; Kolditz et al. 2005, 2007). An application linking soil and groundwater compartments was finalised in the Beerze-Reusel area, a catchment of about 300 km^2 in the Meuse Basin (Kolditz et al. 2007). This provides a regional hydrologic soil model for groundwater recharge patterns using various infiltration scenarios. Such work provides new toolboxes to help linking environmental compartments in modelling through implemented codes (Geo-Sys/RockFlow). For the Meuse-Geer subcatchment, a concept for multiscale modelling was also started and the team developed a new interface for coupling surface and subsurface flow. With this step, extremely fine resolutions of soils (5 cm vertical) can now be modeled for several hundreds of km^2 by parallel computation techniques to address highly resolved regional groundwater recharge patterns in heterogeneous soils.

3.8 Integrating Socio-economic Outcomes and Policy Interactions

The INTEGRATOR team identified key environmental issues through stakeholder feedback from the four main river basins studied within AquaTerra (Ebro, Meuse, Elbe, Danube). A first economic analysis was performed on selected case studies derived from this work, leading to new initial conceptual models. The new approach proposed to determine how best to address the challenge to assist decision making for a range of end-users at various levels with reference to work from the INTEGRATOR and EUPOL teams. In a common work effort by the INTEGRATOR team all deliverables produced in AquaTerra up to February 2007 were broadly reviewed and assessed with respect to their environmental, social and economic characteristics (basin location, scale, key pressures in the selected areas, type of contaminant, for example). In order to enhance the delivery of AquaTerra key findings and achievements and to bring them to potential end-users, a delivery/integration methodology was developed. This consisted of interviewing subproject- and work package leaders by phone, using simultaneously questionnaires to provide a structure to the interview. This helped to determine which key findings were produced by AquaTerra to date, to understand how these key results could address river basin management issues and identify potential end-user types who could benefit from these results. The process helped to define the Knowledge & Data and Tools produced by AquaTerra and their applicability with respect to relevant issues in river basin management.

A further key objective of the INTEGRATOR project was the identification of the inter-relationships between AquaTerra and other scientific projects. Interviews with AT work package leaders, along with internet searches and conference workshops, have identified where potential and real collaborations exists. It is important to note that AquaTerra does not exist in isolation and that there is an overall awareness of where and how interactions take place. While at a European scale such work is truly the role of a Co-ordination Action, the identification of interaction between projects has been a valuable exercise and has helped to support the proposals for an interlinked web-based resource. This is realised through a website that will present the project information in a format of value to target end users (technical, management and policy users connected with the implementation of the Water Framework Directive to 2015 and beyond). It will also provide a means of interconnecting with other project websites in the field of water management with the aim to create a wider resource of added value to end-users, projects and scientists.

The EUPOL scientific framework was a first attempt to link outputs of AquaTerra to the needs of policy through a series of generic policy questions identified through brainstorm meetings with policy makers from across Europe. Since this work was produced, the EUPOL team has summarised their ongoing work in a review that deals with the challenges of linking scientific knowledge to river basin management policy (Slob et al. 2007).

4 Conclusion

The AquaTerra work shows that the understanding of organic and inorganic pollutant turnover, storage and transport in soils, sediments, ground- and surface water needs interdisciplinary and international approaches that enable the combination of techniques and transgression of compartments. The main challenge remains the improvement of understanding of the large-scale behaviour of pollutants with the complexity and heterogeneity of the systems involved. Particularly, links between compartments such as the atmosphere, soils, ground- and surface water, as well as sediments, have interfaces where the steepest biogeochemical gradients can be expected. For instance, within basins, floodplains are perhaps the most interesting sites for dynamic biogeochemical research as they largely control pollutant storage and release. They also link the atmosphere, soils, ground- and surface waters and further research and exchange of knowledge needs to focus on such exemplatory dynamic areas in order to evaluate how pollutants are turned over and under which water level and associated redox, pH and temperature conditions they may be mobilised or immobilised.

For catchment- and basin-wide results the detailed pesticide study in the Brévilles area has shown that application of diffuse pollutants even in small areas leads to highly complex responses in ground- and surface water systems. Turnover, storage and degradation times appear much longer than expected and may affect even small systems such as the Brévilles catchment for decades to centuries. When scaling up to larger areas, even longer transport times can be anticipated because of the greater distances and generally slower groundwater transport. It becomes clear that response times of the soil, ground- and surface water system can be much longer than measures taken to stop pollutant and nutrient loadings. They often range between decades and millennia and therefore comprise much longer time spans than policy instruments such as the Water Framework or the Groundwater Directives currently take into account. Such instruments must therefore adapt to these time frames in order to efficiently implement environmental protection.

On the other hand, long time frames may also offer advantages. For instance, microbiological turnover in the subsurface may have more time to remove pollutants before they are transported to vulnerable receptors such as drinking water aquifers or organisms in ecosystems. Further research is necessary to determine which metabolites would be expected under such a scenario and whether they are harmful and in which time frames they develop and disappear.

The MONITOR subproject has demonstrated that we are able to determine the status quo of environmental systems with ever increasing accuracy and detail of analytical techniques. This allows better estimation of distributional patterns and the evaluation of sources and fluxes of pollutants. On the other hand, when process-relevant studies are based in the laboratory alone, they yield crucial first information on the environmental behaviour of pollutants. Nonetheless, these laboratory results need to either adapt to the field (which remains a major challenge) or demand evaluation with great care when transferring results to field sites. Some first field-based techniques, such as stable isotope quantifications of turnover of organic compounds in the field, are beginning to show the extent to which processes such as natural attenuation actually take place in natural environments.

For field investigations, our understanding of pollutant dynamics is often still limited by too sparse sample densities in space and time. While individual sampling campaigns often yield highly localised information and snapshots of long-term processes, new integral monitoring techniques might offer future solutions and trends in this respect. With this, our initial results of AquaTerra showed hotspot showed and diffuse pollution patterns that the evaluation of large-scale and long-term pollutant behaviour needs to be further resolved with temperature changes and mixtures of pollutants, changing geochemical and microbiological conditions. More field studies under real conditions are necessary to feed results into reactive transport models. For the above, networks of passive samplers may hold the key for taking laboratory results to the field.

In terms of potential climate change impacts, not explicitly considered by either the Water Framework or Groundwater Directives, results from the HYDRO sub-project have shown that it is necessary to consider the predictions of more than one climate model when trying to assess future impacts due to the uncertainties in model response. Initial results from impact studies suggest that in Europe we can expect increases in heavy rainfall, particularly in winter months, with a potential knock-on effect on flooding, and increases in long term (southern Europe) and short-term (northern Europe) drought frequencies and intensities. These changes to climate will have important impacts on the diffusion and degradation of pollutants in space and time that are just at the start of their exploration. AquaTerra is providing one of the first attempts to link climate change impacts on the soil, sediment, ground- and surface water system at the catchment scale to management and policy decisions. However, more research is needed in this area to provide guidance for management of the effects of climate change on already stressed river basins in Europe.

Overall, work within AquaTerra leads to recommendations that crop controls and fertilisers need to be applied with care and under consideration of the consequences for receiving water systems that often serve as drinking water supplies. We developed models to quantify export of nutrients by the hyporheic zone and further studies are underway in order to better understand the impact of the river network structure on cycling of nutrients, and at the smaller scale the interplay between riparian and hyporheic areas along the river system.

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Effect of Metal Toxicity on Plant Growth and Metabolism: I. Zinc

Gyana Ranjan Rout and Premananda Das

Abstract Zinc toxicity and problems with regard to tolerance and ecological significance are briefly discussed. Differential tolerance of plant genotypes exposed to zinc toxicity is a promising approach to enrich our understanding of zinc tolerance in plants. Knowledge concerning the physiology and biochemistry with regard to phytotoxicity, uptake and transport of zinc and tolerance and its characterization are also discussed. The cytotoxic effects of zinc on plants are elucidated. The major change was seen in the nucleus of the root tip cells due to zinc toxicity. The chromatin material was highly condensed and some of the cortical cells showed disruption and dilation of nuclear membrane in presence of 7.5 mM zinc. The cytoplasm became structureless, disintegration of cell organelles and the development of vacuoles were also observed. The number of nucleoli also increased in response to zinc resulting in the synthesis of new protein involved in heavy metal tolerance. This review may help in interdisciplinary studies to assess the ecological significance of metal stress.

Keywords Accumulation • Phytotoxicity • Tolerance • Transport • Uptake • Zinc

Résumé **Effet de la toxicité des métaux sur la croissance et le métabolisme des plantes: I. Zinc**. La toxicité du zinc et les problèmes de tolérance ou de conséquence écologique liés sont rarement discutés. L'approche en terme de tolérance différentielle des génotypes de plantes exposées à la toxicité du zinc est prometteuse pour l'enrichissement de notre compréhension de la tolérance des plantes au zinc. Les connaissances de la physiologie et la biochimie face à la phytotoxicité, à l'absorption et au transport du zinc, ainsi que la tolérance et sa caractérisation sont aussi discutées dans ce papier. Les effets cytotoxiques du zinc sur les plantes sont maintenant élucidés. La modification majeure concerne la noyau des cellules de l'extrémité des racines. La chromatine est fortement condensée et certaines des cellules corticales montrent la rupture et la dilatation de leur membrane nucléaire en présence de 7.5 mM de zinc. De plus, le cytoplasme perd sa structure, la désintégration d'organites et le développement de vacuoles sont aussi observés. Enfin, le nombre de nucléoles augmente en réponse au zinc. Ils résultent de la synthèse d'une nouvelle protéine impliquée dans la tolérance aux métaux lourds. Cette synthèse bibliographique pourra aider les études interdisciplinaires à évaluer les conséquences écologiques des stress dus aux métaux.

Mots clés Accumulation • Phytotoxicité • Tolérance • Transport • Absorption • Zinc

1 Introduction

The occurrence of heavy metals in soils may be beneficial or toxic to the environment. Excess of metals may produce some common effects of individual metals on different plants (i.e. both macro- and microflora). The biota may require some of these elements in trace quantities but higher concentrations there may be toxicity problems. Metal toxicity in plants have been

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reported by various authors (Bollard and Butler, 1966; Brown et al., 1972; Brown and Jones, 1975; Foy et al., 1978; Gerloff, 1963). Generally, zinc is an essential element which belongs to Group-II of the periodic table. It acts as a plant nutrient (Shier, 1994; Welch, 1995) but at higher concentrations, it is toxic. Since it is assimilated early by plants, it can be highly phytotoxic. Growth inhibition is a general phenomenon associated with zinc toxicity (Collins, 1981). Zinc is also a constitute of metalloenzyme or a cofactor for several enzymes such as anhydrases, dehydrogenases, oxidases and peroxidases (Hewitt, 1983) and plays an important role in regulating the nitrogen metabolism, cell multiplication, photosynthesis and auxin synthesis in plants (Shier, 1994). It also plays an important role in the synthesis of nucleic acid and proteins and helps in the utilization of phosphorous and nitrogen during seed formation. Hyper-accumulation of zinc has been observed in many plant species (Baker and Walker, 1990; Verkleij and Schat, 1990), accumulations of 1% of the dry weight being common among plants growing on zinc minewastes (Rascio, 1977). The toxic effects of zinc on plants have been reported by many researchers (Beckett and Davis, 1977, 1978; Davis and Parker, 1993; Taylor et al., 1991; Webber, 1981; Wheeler and Power, 1995; White et al., 1974). Genotoxicity of zinc in micro- and macroflora were also elucidated by various researchers (Mukherjee and Sharma, 1985; Subhadra and Panda, 1994). In addition, the toxic effects of the metals on photosynthesis in plants were elaborated (Clijsters and VanAssche, 1985; Garty et al., 1992; Stiborova et al., 1986; Van Assche and Clijsters, 1986; Van Assche et al., 1979, 1980). Recent progress in the study of toxic metals and their interactions with essential elements has greatly increased our understanding of the mechanism of toxicity at the biochemical level (Abdulla et al., 1985). In this communication, the salient features of zinc toxicity, the effects on plant metabolism and its interaction with other essential elements are discussed; their possible implications in the plant ecosystem are highlighted.

2 Zinc Toxicity

Zinc toxicity depends on pH, which controls the concentration of zinc in solution. High concentrations of zinc can cause toxicity in plants (Daviscarter and Shuman, 1993). The general symptoms are stunting of shoot, curling and rolling of young leaves, death of leaf tips and chlorosis. Ye et al. (1997) reported that the seedlings of *Typha latifolia* were chlorotic in the presence of $\sim 80\,\mu$M zinc. Foliar chlorosis appeared on *Thlaspi ochroleucum* seedlings treated with 1 mM Zn after 4 days of culture as reported by Shen et al. (1997).

2.1 Effect on Germination

Zinc is an essential nutrient for plant growth, although elevated concentrations resulted in growth inhibition and toxicity symptoms. It does not affect seed germination but helps in plumule and radicle development. Baker (1978) reported that the seeds of *Silene maritima* were germinated better and rapidly on calcium nitrate solutions containing different concentrations of zinc.

2.2 Effect on Root

Zinc though an essential element for plant growth, showed toxicity symptoms at higher concentrations inhibit root growth (Baker, 1978; Bradshaw and McNeilly, 1981). Zinc toxicity was marked in root system particularly in root blunt, thickening and caused restraint on both cell division and cell elongation (Barcelo and Poschenrieder, 1990; Wainwright and Woolhouse, 1976). Doyar and Van Hai Tang (1980) reported that the nitrogen and phosphorous increased with the increase in zinc content in the roots. The concentration of zinc in the roots decreased with plant age as reported by Pearson and Rengel (1995). Sresty and Madhava Rao (1999) based on transmission electron microscopy concluded that radicle elongation was more adversely affected than the plumule extension. The root elongation of *Cajanus cajan* cv. ICPL 87 was completely inhibited after 24 h treatment with 7.5 mM zinc. They also reported that root cortical cells were extensively damaged and major changes took place in the nucleus of the root tip cells treated with 7.5 mM zinc.

2.3 Effect on Reproductive Growth

Metal sensitive plants have great difficulties in reaching the reproductive phase when exposed to metals.

The seeds of most plant species growing on heavy metal enriched soils have very low metal concentrations (Ernst, 1974; Lepp and Dickison, 1994). Zinc-resistant genotypes of *Silene vulgaris* were stimulated by increased levels of zinc, not only in vegetative growth but also in seed production (Ernst, 1998). Surpassing a critical zinc concentration resulted in reduced growth the no flower production.

2.4 Effect on Plant Physiology and Morphology

The physiology of metal toxicity in plants was mainly concerned with metal movement from soil to root and metal absorption and translocation. Plant availability of a metal in the soil depends on soil adsorption strength as well as plant effectors such as root exudates for metal chelation or reduction. Lindsay (1972) and others developed models for the chemical activity of metal ions in soil. The chemical activity was usually dependent on soil pH, CO_2 concentration and redox potential of the soil (Hodgson 1963, 1969; Lindsay, 1972, 1974). The concept of metal equilibrium in soil, and of metal movement from soil to root were well documented (Foy et al., 1978). Studies on the physiological and biochemical metal toxicity were almost unknown. The activity of a metal in the cytoplasm was affected by chelating carboxylic and aminoacids and the pH. Zinc inhibited Fe translocation in some cases (Ambler et al., 1970) and the young chlorotic leaves showing Zn^{2+} toxicity generally contained more than 100 ppm Fe; Fe deficiency generally occurred at concentrations lower than 40 ppm Fe in the young leaves. The metal toxicity resulted with the increased metal supplied to the root which affect the disintegration of cell organelles, disruption of membranes and condensation of chromatin material and increase in number of nucleoli were major events during zinc toxicity (Sresty and Madhava Rao, 1999). Ernst (1998) elaborated details on mechanism of heavy metal toxicity in plants at the cellular and organism level. He also reported that the plasma membrane was the cell compartment which regulated metal entry into the cell, in addition its proteins, especially the SH groups might be affected in their activity causing damage to membrane stability due to zinc toxicity. As soon as heavy metals passed through the plasma membrane, they could immediately interact with all metabolic processes in the cytosol. Godbold and Huttermann (1985) reported that increasing zinc levels in culture solution decreased the shoot to root ratios and translocation of Zn, Fe, Mg, K, P and Ca and caused accumulation of these nutrients in the root. Pearson and Rengel (1995) indicated that higher concentration of zinc affected the leaf and the root morphology. They suggested that the zinc supply from the roots into the leaves of different ages might be determined by the relative transpiration rate of the leaves. Further, Malea et al. (1995) tested the effect of zinc on leaf cell mortality of *Halophila stipulacea*. The mortality of the cells increased along with the increase of zinc concentrations (10^{-4} to 10^{-7} M) and the increase of incubation time (2nd–12th days). Necrosis was evident in the epidermal cells at all concentrations on the 12th day of culture. Necrosis was also detectable in the mesophyll cells at the highest concentration (10^{-4} M) after the eighth day. Zinc toxicity was primarily associated with alteration of root physiology, thereby inhibiting the root elongation (Woolhouse, 1983).

3 Differential Zinc Tolerance in Plants

3.1 Differential Tolerance In vitro and In vivo

Zinc toxicity and differential zinc tolerance in various plant groups were reported (Ambler et al., 1970; Brown and Jones, 1975; Earley, 1943; Polson and Adams, 1970; Symeonidis et al., 1985; White et al., 1974). Earley (1943) reported that different soybean cultivars responded differentially to toxic Zn in sand culture. White (1976) studied the differential varietal tolerance in soybean and found that they were associated with differences in susceptibility to zinc-induced Fe deficiency, Zn uptake and translocation, and susceptibility to toxicity unrelated to Fe deficiency. Gregory and Bradshaw (1965) developed Zn-tolerant efficiency in bentgrasses. Further, Ernst (1977) and Mathys (1973) accepted the compartmentalization hypothesis and suggested that malate might chelate zinc in the vacuoles of tolerant plants (Mathys, 1977a). The fact that high malate, Zn-tolerant ecotypes were not tolerant to Cu and was difficult to understand since malate would be expected to bind Cu much more strongly than it does in Zn. Mathys (1977b) and Ernst (1977) conducted detailed studies on metal tolerant enzymes in tolerant and non-tolerant ecotypes. However, in in-vitro studies, the activities of several enzymes remained at normal levels in tolerant ecotypes as metal supply increased, but activities fell markedly in nontolerant ecotypes. They also confirmed that tolerant plants were able to maintain the normal metabolic processes when subjected to metal stress which severely interfered with the metabolism of nontolerant plants. Hertstein and Jager (1986) studied the tolerances of different populations of three grass species (*Agrostis tenuis*, *Festuca rubra* and *Anthoxanthum odoratum*) to zinc and other metals. They observed that all populations originating from metal contaminated habitats possessed multiple-metal-tolerance. Genecological and physiological aspects of tolerance were studied extensively with regard to zinc and other metals tolerance (Antonovics et al., 1971; Woolhouse, 1983). Baker (1978) selected zinc tolerant *Silene martitima* populations in water-culture experiments. Subsequently, Amado-Filho et al. (1997) screened six seaweed species for zinc tolerance for a period of 21 says using rapid hydroponic methods. They reported that all species died at 76 μM of zinc, two species (*Ulva lactuca* and *Enteromorpha flexuosa*) died at 152 μM and one, *Hypnea musciformis* died at 0.152×10^{-2} mM. Ye et al. (1997) tested four populations of *Typha latifolia* collected from metal-contaminated and uncontaminated sites grown in nutrient solution containing 1.0–5.0 μg/ml Zn under controlled environmental conditions. They also reported that seedlings from metal contaminated populations showed three times more tolerance to zinc as compared to the uncontaminated population. Shen et al. (1997) compared the growth of hyperaccumulator species *Thlaspi caerulescens* and non-hyperacumulator species *Thlaspi ochroleucum* in nutrient culture experiment with zinc. They reported that *Thlaspi caerulescens* was able to tolerate 0.5 mM Zn in solution without growth reduction, and up to 1 mM Zn without showing visible toxic symptoms but with a 25% decrease in dry matter yield. Sresty and Madhava Rao (1999) and Madhava Rao and Sresty (2000) tested two genotypes of pigeonpea (*Cajanus cajan*) in nutrient solution containing zinc (2.5, 5.0 and 7.5 mM) to assess Zn tolerance in term of root and shoot tolerance index, metal uptake and dry mass population. Rout et al. (1999) selected Zn tolerant callus lines of *Brassica* species using in vitro techniques. They assessed various growth parameters such as fresh and dry weight of callus, growth tolerance index and metal uptake to determine the tolerant line. The tolerant calli had the maximum peroxidase and catalase activity and higher rate of metal uptake as compared to non-tolerant calli. Subsequently, Samantaray et al. (2000) developed zinc tolerant calli and regenerated of plantlets from tolerant calli of *Setaria italica* using in vitro technique. The tolerant plants showed luxuriant growth in zinc rich minespoil. Rengel (2000) observed that the ecotypes of *Holcus lanatus* tolerant to zinc toxicity also tolerated Zn-deficiency better than the Zn-sensitive ecotypes because of their greater ability to taking up zinc from Zn-deficient soil.

4 Effect of Zinc on Nuclear Activity

Growth of plants was mainly dependent on cell division. Fujii (1954) found that zinc played an important role in mitosis. Gebhart (1984) observed higher frequency of chromosome aberrations in presence of heavy metals. Heavy metals, which constituted one of the important groups of environmental pollutants, were mostly genotoxic (Sharma and Talukdar, 1987). Further study indicated that the heavy metals like nickel, cadmium and zinc had genotoxic activity through oxidative pathways involving free radicals (Michaelis et al., 1986; Ochi et al., 1983). Sresty and Madhava Rao (1999) observed a major change in the nucleus of the root tip cells in response to zinc. The chromatin material was highly condensed and some of the cortical cells showed disruption and dilation of nuclear membrane in presence of 7.5 mM zinc. The cytoplasm became structureless; disintegration of cell organelles and the development of vacuoles were also observed. They also noted that the number of nucleoli increased in response to zinc resulting in the synthesis of new proteins involved in heavy metal tolerance. Further, the information of SH-rich phytochelatins appears to play a role in heavy metal detoxification in different organisms (Grill et al., 1987; Rauser, 1995; Robinson et al., 1988; Tomsett and Thurman, 1998). Ernst (1998) compared the nuclear activity and cell division in Zn-sensitive and Zn-resistant ecotypes of the perennial grass *Festuca rubra* in presence of different level of zinc. He observed that the nuclear volume decreased by 30% and doubled the length of the cell cycle when Zn-sensitive ecotypes were exposed to 3 μM Zn for 4 days (Powell et al., 1986); the nuclear volume increased by 50% in Zn-resistant ecotypes exposed to same Zn level.

5 Effect of Zinc on Metabolism

In general, a number of plant species were resistant to certain amounts of metals. This was probably achieved through trapping of these metals with in the metal-binding proteins. Zinc was known to be a constituent of many enzymes which stimulated various metabolic activities such as nucleic acid metabolism, protein synthesis, photosynthesis, respiration and carbohydrate metabolism (Shkolnik, 1984). Zinc was easily assimilated by plants, and could also be strongly phytotoxic; growth inhibition was a general phenomenon to zinc toxicity (Collins, 1981). Many authors observed inhibition of photosynthesis by heavy metal ions but the mechanism of action was not known in details (Stiborova et al., 1986; Tripathy and Mohanty, 1980). Van Assche (1973) reported that the high concentration of zinc inhibited metabolic activity. In some cases, the concentration ranged from 0.4–1.0 mM inhibited photosystem (Baker et al., 1982; Hampp et al., 1976; Tripathy and Mohanty, 1980; Van Assche and Clijsters, 1986). Lorimer (1981) and Lorimer and Miziorko (1981) reported that bivalent cations (Zn^{2+}) played a major role in the activation of the key enzyme of the Calvin cycle and in the equilibrium between CO_2 and O_2 binding by protein and inhibition of photosynthesis reactions localized in the thylakoid membranes of chloroplasts (Van Assche and Clijsters, 1984). Interference of zinc in photochemical reactions of chloroplast was intensively studied (Van Assche and Clijsters, 1986). Garty et al. (1992) studied the effects of low pH and Zn on chlorophyll degradation in lichen (*Ramalina duriaei* Bagl.). They found that Zn decreased photosynthesis in lichens having chlorophycean photobionts under pH 2.0. Verkleij and Schat (1990) and Meharg (1993) reported that the plasma membrane was the compartment of the cell which might at least partially regulate the entry of a heavy metal ion into the cell. This direct exposure might have consequences for the adaptation and selection of plant species and genotypes. Marschner (1986) indicated that cation and anion uptake by the cell could be actively regulated by electrogenic proton pumps (H^+ – ATPase), transmembrane redox pumps (NAD (P) oxidase), and ion channels (Marschner, 1995). Davis et al. (1995) indicated that the plants exposed to higher concentrations of zinc disturbed the mitochondrial structure and reduces the energy.

6 Zinc Uptake and Transport

Zinc an essential element for the normal growth and metabolism of plants played very important role in enzyme activation and was also involved in the biosynthesis of some enzymes and growth hormones (Devlin, 1967; Nanson and McElroy, 1963). The transport and distribution of zinc and other metals in plants were reported by various researchers (Longnecker and Robson, 1993; Marschner, 1986; Wahbeh, 1984). The accumulation of zinc in the roots in the tolerance races were reported (Peterson, 1969; Turner and Marshall, 1972a, b). Baker (1978) reported that the populations of *Silene maritima* accumulated zinc to a high degree in the roots relative to the shoots. Turner and Marshall (1972a, b) detected a correlation between the uptake of ^{65}Zn by cell wall and mitochondrial fractions from the roots of a range of *Agrostis tenuis* populations and the indices of zinc tolerance. Wahbeh (1984) reported that the distribution and abundance of zinc in various vegetative tissues of the sea grasses *Halophila ovalis*, *Halophila stipulacea* and *Halodule uninervis*. He also indicated that both the root and the leaf absorbed metals, and that translocation was low (Lyngby et al., 1982). Availability of heavy metals in soil and their uptake by plants not only was dependant on the total metal content in the soil but also upon other factors i.e. soil organic matter and cation exchange capacity (Bjerre and Schierup, 1985; MacLean, 1976; Miner et al., 1997; Strickland et al., 1979). Hinesly et al. (1984) found that the uptake of heavy metals (Zn and Cd) by *Zea mays* generally decreased as the soil pH increased. They also assumed that the increased solubility of Zn in the alkaline pH range was due to the dispersion of organic matter containing complexed zinc form. Adriano (1970) demonstrated that the distribution of metals in the fractions depended on the soil properties, and, for most mineral soils, the largest amounts of zinc were present in the residual fraction.

Whitehead (1987) found that the accumulations of zinc (Zn^{+2}) varied in different plant species. The ratio between the accumulation of root and the shoot was always greater than 1.0 in ryegrass and clover. McKenna et al. (1993) found a complex interaction between Zn^{+2} and Cd^{+2} on the accumulation in the root and leaf of lettuce and spinach; the interactions were dependent on the relative concentration of those metals in the growth medium. Zinc accumulation was higher in the younger than the older leaves. Shetty et al. (1994) reported that the application of mycorrhizal fungi helped in higher accumulation of zinc in the roots than in the shoots. The influence of arbuscular mycorrhizae (AM) on plant growth and zinc uptake by *Lygeum spartum* and *Anthyllis cytisoides* was studied in soils with different levels of heavy metals (Diaz et al., 1996). Rout et al. (1999) and Samantaray et al. (2000) conducted in vitro studies on zinc accumulation in tolerant and non-tolerant calli of *Setaria italica* and *Echinochloa colona* respectively and observed that the uptake and accumulation was more in tolerant calli than the non-tolerant ones. Bert et al. (2000) investigated zinc tolerance in five populations of *Arbidopsis halleri* raised from seed collected from contaminated and *Arbidopsis thaliana* from non-contaminated sites. They observed that the populations of *Arbidopsis thaliana* from non-contaminated area accumulated zinc in shoots and roots more quickly than the populations from the contaminated sites. Frey et al. (2000) measured zinc concentration in shoot which was higher and reached a maximum value of 83 mM kg^{-1} dry mass whereas total concentration of zinc in roots were lower upto 13 mM kg^{-1}. They observed that the distribution of zinc in *Thlaspi caerulescens*; Zn got mainly accumulated in the vacuoles of epidermal leaf cells and was totally absent from the vacuoles of the cells from the stomatal complex, thereby protecting the guard and subsidiary cells from high zinc concentrations. They concluded that zinc also got accumulated in high concentrations in both the cell walls of epidermal cells and in the mesophyll cells, indicating that apoplastic compartmentation was another important mechanism involved in zinc tolerance in the leaves of *T. caerulescens*. Chardonnens et al. (1975) demonstrated that the tonoplast vesicles derived from Zn-tolerant ecotype of *Silene vulgaris* accumulated more Zn than the Zn-sensitive ecotype. They also characterized the tonoplast-transport system that caused this difference in the uptake and demonstrated its genetic correlation. The most prominent differences being its insensitivity to protonophores and ortho-vanadate and its stimulation by Mg-CTP. They concluded that in both Zn-tolerant and Zn-sensitive ecotypes, Zn was actively transported across the tonoplast (temperature coefficient >1.6), most likely as a free ion, since citrate did not accumulate in vesicles. Lasat et al. (2000) reported the molecular physiology and zinc transport in the Zn-hyperaccumulator *Thlaspi caerulescens* and a non-accumulator. They reported that Zn transport was stimulated at a number of sites in *T. caerulesans* contributing to the hyperaccumulation trait. The transport processes that were stimulated included Zn influx into both root and leaf cells, and Zn loading into the xylem. They also hypothesized that the stimulation of Zn influx was 4–5 fold into the root because of an increased abundance of Zn transporters in *Thlaspi caerulescens* root cells. Zinc was sequestered in the vacuoles of non accumulator *T. arvense* root cells which retarded Zn translocation to the shoot. Subsequently, they characterized the Zn transport genes in *T. caerulescens* and reported that ZNT1 (Zn transporter) gene was highly expressed in roots and shoots of *T. caerulescens*. But in *T. arvense*, ZNT1 was expressed at far lower levels and this expression was stimulated by imposition of Zn deficiency. Further, Whiting et al. (2000) indicated the positive responses to Zn and Cd by roots of the Zn and Cd hyperaccumulator *Thlaspi caerulescens*. Hacisalihoglu et al. (2001) reported the high and low-affinity zinc transport systems and their possible role in zinc efficiency in wheat (*Triticum aestivum*). The low-affinity system showed apparent Km values similar to those previously reported for wheat (2–5 mM). High-affinity Zn transport system with apparent Km values were found in the range of 0.6–2 nM. Because it functioned in the low range of the available Zn level found in most soils, this novel high affinity uptake system was likely to be the predominant Zn^{2+} uptake system. Zn^{2+} uptake was similar for cv. Dagdas and cv. BDME-10 over both the high- and low-affinity Zn^{2+} activity ranges.

6.1 Mechanisms Involved in Zn Tolerance

Mechanism of metal tolerance by plants has been studied by various authors (Baker, 1987; Cunnigham et al., 1975; Foy et al., 1978). At first, exclusion of metals from roots seemed a likely mechanism because root cell walls could bind metals; the extent of binding by the cell wall was related to the degree of tolerance to a specific metal (Turner, 1970; Turner and Marshall, 1972a, b). Jones et al. (1971) found that Zn complex was formed in roots of Zn-tolerant bentgrass after digesting the roots with cellulase. Although binding of metals to root cell walls might contribute to metal tolerance, it was not adequate enough to enable the plants to prevent metal transport to their leaves (Wainwright and Woolhouse, 1977). Woolhouse (1983) felt that there existed different specific mechanisms of tolerance within each of the populations which were related to the contamination of their original habitat. The mechanism of tolerance depended possibly on three major factors i.e. an exclusion of heavy metals from uptake, i.e. an "avoidance" (Levitt, 1980) or translocation to the shoots (Baker, 1978), a protection of sensitive structures in the cytoplasm either by immobilization of metals in the cell wall (Turner and Marshall, 1972a, b) and metal complexion with soluble compounds, e.g. organic acids and aminoacids (Hertstein and Jager, 1986) or by binding to specific proteins (Rauser, 1984). Cumming and Taylor (1990) described the signal transduction and acclimation mechanisms on metal tolerance in plants. They also indicated that the mechanism of metal tolerance were due to exclusion and external metal detoxification mechanism, internal metal detoxification mechanisms, and multiple mechanisms and co-tolerances. The production of metal-chelating ligands high in thiol groups might also render co-tolerance to Zn^{2+}. Further, Neumann et al. (1997) found by electron microscopy that most of the heavy metals were tightly bound to the cell wall in tolerant plant populations as compared to non-tolerant ones. Frey et al. (2000) elucidated tolerance mechanism at the cellular and subcellular level for the detoxification of the accumulated zinc within the leaf of hyperaccumulator *Thlaspi caerulescens*. They also observed that there was apoplastic compartmentation in the leaves of *T. caerulescens*. Hall (2002) reported the range of potential cellular mechanisms that may be involved in the detoxification of heavy metals and thus tolerance to metal stress. These includes the binding to cell wall and extracellular exudates, reduced uptake or efflux pumping of metals at the plasma membrane, chelation of metals in the cytosol by peptides such as phytochelatins, repair of stress-damaged proteins and the compartmentation of metals in the vacuole by tonoplast located transporters.

7 Phytotoxicity

To evaluate meaningful physiological and biochemical effects of toxicity, one must know the metals which are phytotoxic in nature and interactions with other metals (Cunnigham et al., 1975). Before starting a phytotoxicity experiment one should be fully aware of the movement of the metal including its absorption and translocation in the plant system. Availability of metal in the soil depends on soil adsorption strength as well as plant effectors such as root exudates for metal chelation or reduction. Metal phytotoxicity can result only if metals can move from the soil to root systems (Foy et al., 1978). Phytotoxicity levels of zinc in different crop plants were reported by many workers (Chardonnens et al., 1999; Staker and Cummings, 1941). The most significant phytotoxicity symptoms were stunting of growth, chlorosis and reduction in biomass yield. The phytotoxicity caused by a wide variety of metals has been well documented; however, models designed to quantify the relationship between exposure to metal ions and progressive yield losses are lacking (Taylor et al., 1991).

7.1 Phytotoxicity and Its Interaction with Other Nutrients

Ambler et al. (1970) indicated that zinc induced inhibition of Fe translocation from root to tops which causes chlorosis in plants. Zinc causes phytotoxicity like chlorosis at low pH level as reported by Chaney et al. (Chavan and Banerjee, 1980). Chavan and Banerjee (Chaudhry et al., 1977) reported that Zn toxicity appear to be due to Fe deficiency. Cayton et al. (1985) reported that the absorption and translocation of plant nutrients like Fe, Mg, K, P and Ca depended on Zn concentration in soil. They reported that zinc was an antagonist to Cu at the primary absorption site, in contrast with its action on Fe, P, Mg, K and Ca. Zinc interfered at the loading site of the roots and decreased the rate of translocation or absorption of essential nutrients to plants or caused mineral imbalances (Brown, 1979; Chaney, 1975; Chaudhry et al., 1977; Warnock, 1970; Watanabe et al., 1965). White et al. (1976) observed that increased levels of Zn in soil greatly increased translocation of Mn to tops which indicate the appearance of chlorosis. They hypothesized that the Zn and Mn interfere with Fe utilization in the leaves for chlorophyll synthesis. Accumulation of zinc in the roots or shoots was generally accompanied by accumulation of calcium (Baker, 1978). He also compared the interaction of zinc and calcium with regard to uptake by zinc-tolerant and non-tolerant population of *Silene maritima* by conducting solution culture experiment. The result indicated that the total zinc uptake was not affected by calcium level in the tolerant population but was decreased significantly in the non-tolerant population. The stimulation by calcium of zinc uptake in the tolerant plants may reflect an involvement of calcium in the zinc tolerance mechanism sited in the roots. Davis and Parker (1993) reported that zinc toxicity were highly correlated with Ca: Zn ratio and reduced stem biomass. Shetty et al. (1994) pointed out that growth inhibition was due to interference of zinc with phosphorous uptake by plants. They also reported that the application of vesicular-arbuscular mycorrhizae (VAM) fungi at zinc contaminated sites increased plant biomass even at elevated levels of zinc in the soil. Synergism was observed between boron and zinc when both were in excess together as excess accelerated the effect of high zinc by lowering the biomass, economic yield and carbonic anhydrase activity in mustard (Sinha et al., 2000). Gianquinto et al. (2000) concluded that the Zn concentration in leaf of *Phaseolus vulgaris* cv. Borlotto nano was reduced by the addition of phosphorous to plants grown at low Zn supply. Further, Rengel and Romheld (2000) reported that the zinc deficiency depressed the rate of Fe transport to shoots at early stages of *Triticum aestivum*. Kaya and Higgs (2001) tested the effectiveness of phosphorus and iron supplemented in nutrient solution on growth of tomato plant at high zinc (77 μM) concentration. They also reported that the application of supplementary P and Fe in the nutrient solution resulted in increase of both dry weight and chlorophyll concentration in *Lycopersicon esculentum* cvs. Blizzard, Liberto, Calypso and also decreased zinc concentration in the leaves and roots of plants grown at high Zn treatment.

7.2 Phytotoxicity and Its Interaction with Other Heavy Metals

Zinc and cadmium have many physical and chemical similarities as they both belong to Group II of the Periodic Table. They are usually found together in the ores and compete with each other for various ligands. Thus interaction between Zn and Cd in the biological system is likely to be similar. The fact that cadmium is a toxic heavy metal and zinc is an essential element which makes this association interesting as it raises the possibility that the toxic effects of cadmium may be preventable or treatable by zinc (Chowdhury and Chandra, 1987). Hinsely et al. (1984) suggested that both cadmium and zinc uptake by plants were dependent on the pH of the growing media. The basic mechanisms of Cd–Zn in the tissues induced the synthesis of different types of metallothionein, binding characteristics of metallothionein, alteration in absorption and tissue distribution of one metal by another, and competition at the level of zinc containing metallozymes known to be involved in the interactions. McKenna et al. (1993) reported the interactions between zinc and cadmium and the concentration and tissue distribution in spinach and lettuce. They observed that the cadmium concentration in young leaves of lettuce and spinach decreased exponentially in the solution containing Zn at low (0.0316 μM) but not at high (0.316 μM) concentration of Cd. The Zn:Cd concentration ratios in young leaves of lettuce and spinach grown at 0.316 μM Cd was greater as the concentration of Zn increased. Cayton et al. (1985) indicated that Zn enhanced translocation of Mn and Cu but decreased Cu absorption by the root.

8 Conclusion

This review mostly concerns with the role of zinc toxicity in micro- and macroflora. Zinc acts as a plant nutrient (Shen et al., 1997; Watanabe et al., 1965);

but at higher concentrations it is toxic. Zinc toxicity in plants is clearly visible with the inhibition of growth and decrease in biomass production; severe toxicity can also be fatal. Zn-toxicity might be the result of complex interactions of the major toxic ions i.e. Cd^{+2}, Cu^{+2}, Pb^{+2} with Ca, Mg, Fe and P and other environmental factors. Zinc toxicity was almost certainly involved with metabolism through competition for uptake, inactivation of enzymes, displacement of essential elements from functional sites. Generally, Zn toxicity caused chlorosis and inhibited Fe translocation in some cases (Ambler et al., 1970). The physiology and biochemistry of zinc toxicity have been less studied in intact plants.

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Phytoremediation of Organic Pollutants Using Mycorrhizal Plants: A New Aspect of Rhizosphere Interactions

Erik Jautris Joner and Corinne Leyval

Abstract Phytoremediation as a means of cleaning up polluted soils has gained popularity during the last decade due to its convenience and low costs of installation and maintenance. When the target pollutant is biodegradable, this technology exploits the stimulating effect that roots have on microbial processes and physical/chemical modifications in the rhizosphere. Among the microorganisms that affect rhizosphere processes, symbiotic fungi forming mycorrhizas induce a series of changes in plant physiology, nutrient availability and microbial composition that may determine the outcome of a phytoremediation attempt. Beyond the rhizosphere, mycorrhizal hyphae act as the roots of the roots, and may thus extend the rhizosphere into the bulk soil by creating a new interface of soil-plant interactions; the hyphosphere. We here discuss some of the recent results on phytoremediation of organic pollutants with emphasis on processes in the mycorrhizosphere, and highlight future research priorities.

Keywords Arbuscular Mycorrhiza • Bioremediation • Polycyclic aromatic hydrocarbons • Root exudation

Résumé La phytoremédiation de polluants organiques utilisant des plantes mycorhizées; un nouveau champ d'étude pour les interactions de la rhizosphère La phytoremédiation est une technique de traitement des sols pollués qui attire beaucoup d'attention depuis une dizaine d'années en raison de la facilité et du faible coût d'installation et de maintenance. Quand le polluant ciblé est biodégradable, cette méthode utilise les racines pour stimuler l'activité microbienne et modifier les propriétés physico-chimiques dans la rhizosphère. Parmi les microorganismes qui influencent les processus rhizosphériques, les champignons mycorhiziens formant des mycorhizes induisent une certain nombre de changements dans la physiologie des plantes, la disponibilité des nutriments, la composition des communautés microbiennes, qui peuvent aussi influencer la phytoremédiation. Au-delà de la rhizosphère, les hyphes de champignons mycorhiziens agissent comme les racines des plantes, et prolongent ainsi l'étendue de la rhizosphère en créant une nouvelle interface entre la plante et le sol: l'hyphosphère. Des résultats récents sur la phytoremédiation de polluants organiques sont discutés ici, et des hypothèses sur les mécanismes impliqués et les priorités pour les recherches futures dans ce domaine sont présentées.

Mots clés Bioremédiation • Exudation racinaire • Hydrocarbones polycycliques aromatiques • Mycorhize arbusculaire

1 Introduction

1.1 Phytoremediation

Phytoremediation refers to the use of plants to clean up contaminated soils. In the case of non-degradable pollutants like heavy metals and metalloids, the precise

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terms covering the involved aspects of phytoremediation are rhizofiltration (metals in water), phytoextraction (metals in soil), phytovolatilization (metals that may be volatilized; e.g. Se and Hg) and phytostabilization (control of spread by erosion or leaching). When organic, biodegradable pollutants are the target, phytoremediation may comprise rhizodegradation (microbial degradation in the rhizosphere), phytodegradation (degradation of compounds absorbed by the plant), and hydraulic control (limiting the spread of a plume in soil by plant evapotranspiration) (EPA, 2000; Flathman and Lanza, 1998). Here we will mainly consider different aspects of rhizodegradation.

1.2 Organic Pollutants

Apart from being more or less degradable, organic pollutants (Fig. 1) have other important characteristics that vary greatly between compounds, notably electrochemical charge and solubility. Non-polar compounds, like hydrocarbons, are thus poorly soluble in water and sorb readily to hydrophobic soil constituents like soil organic matter. Polar compounds, like some halogenated organics, many pesticides and explosives are more soluble, but not necessarily more mobile in soil due to adsorption to various charged surfaces (Marschner, 1999).

The manner by which organic pollutants are introduced to soil may vary form diffuse atmospheric depositions to intended application and spills. The resulting concentrations may thus vary greatly, a factor that together with contact time has a strong influence on bioavailability and toxicity (Reid et al., 2000; Stucki and Alexander, 1987). A particular effect of the contact time between soil and a range of organic pollutants is that many compounds are rendered more strongly sequestered and less bioavailable with time. This phenomenon, known as aging, strongly affects the feasibility and success of soil remediation (Hatzinger and Alexander, 1995).

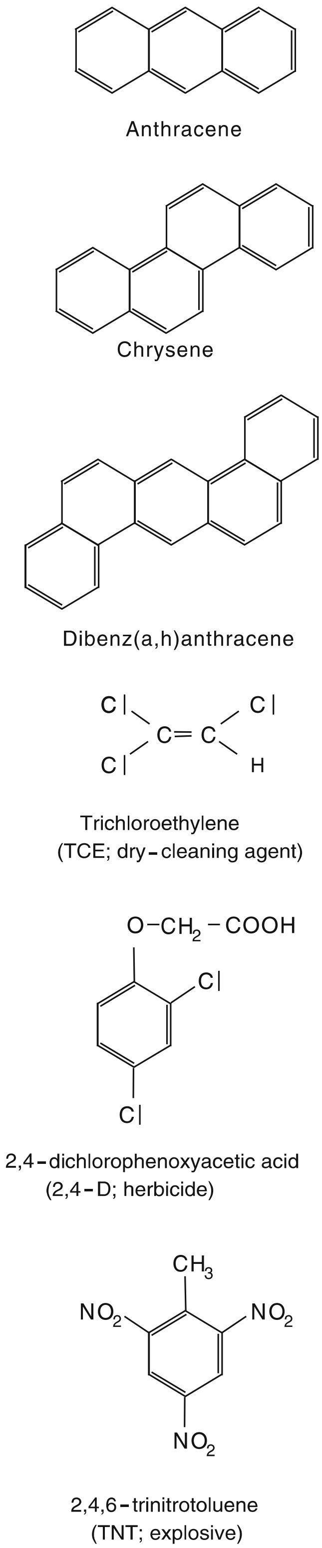

Fig. 1 Examples of some organic pollutants for which the feasibility of phytoremediation has been verified

1.3 Mycorrhizas

Mycorrhizas are ubiquitous root-fungus symbioses that comprise three major groups; ectomycorrhizas

(ECM; formed mainly by forest trees), ericoid mycorrhizas (formed by heather plants like the *Ericaceae*) and arbuscular mycorrhizas (AM; formed mainly by herbaceous plants) (Smith and Read, 1997). The two latter groups are endomycorrhizas, as they enter into root cells during colonization, as opposed to ectomycorrhizas in which the fungi merely envelop the epidermis and partially the cortex cells of the root.

The major function of mycorrhizas is nutrient transport. Extra-radical hyphae anchored in the root thus exploit soil outside the root where it absorbs mineral nutrients (mainly N, P and micronutrients), translocate them back to the root, and transfer them to the host plant in exchange for phytosynthetically fixed C in the form of sugars. The fact that these hyphae are fed with C and energy from the host plant gives them an advantage over other microorganisms with respect to growth and active metabolism in nutrient poor substrates. In a biodegradation context, it is important to note that the three groups of mycorrhiza have very different saprophytic capacities. The ericoid mycorrhizal fungi are potent degraders, ECM fungi are moderately capable, while AM fungi are obligate symbionts with little or no capacity for degradation of organic materials (Michelsen et al., 1996, 1998). All groups of mycorrhiza do however interact with and modify the microbial communities that the hyphae encounter in soil (see below), and in this manner they may all affect microbial degradation processes indirectly.

2 Experimental Evidence

2.1 Rhizosphere Effects

Successful phytoremediation (rhizodegradation) of organic pollutants has been demonstrated for a wide range of compounds or compound mixtures, like aliphatic hydrocarbons (Chang and Corapcioglu, 1998; Günther et al., 1996), fuel oil and other mixed petroleum hydrocarbons (Chaineau et al., 2000; Nicolotti and Egli, 1998; Suominen et al., 2000), polycyclic aromatic hydrocarbons (PAHs) (Pradhan et al., 1998; Schwab and Banks, 1994), explosives (Siciliano and Greer, 2000; Thompson et al., 1998), pesticides (Hsu and Bartha, 1979) and chlorinated organics (Anderson et al., 1993; Siciliano and Germida, 1999).

The mechanisms behind enhanced degradation in the rhizosphere are not known, and mechanistic explanations are probably as complex as the environment in which they take place. Putative explanations include direct effects of root-derived enzymes (Gramss and Rudeschko, 1998), and indirect effects of enhanced aeration due to root burrowing and water consumption, enhanced microbial activity and modified microbial composition due to C input from root exudates, priming or triggering effects of metabolic precursors exuded by roots (e.g. phenolics) that induce enzymatic activity/metabolic pathways that may attack the pollutant, and unspecific effects of changes in pH, osmotic potential, red-ox potential, partial pressures of O_2/CO_2, etc. (Curl and Truelove, 1986). Enhanced microbial activity is probably a key component in rhizodegradation. However, the modified conditions for microbial growth in the rhizosphere do not proportionally increase the biomass of all its organisms, but rather change the community composition (Marschner et al., 2001; Steer and Harris, 2000), thus altering the rhizosphere with respect to its metabolic capacities (Duineveld et al., 1998). Similar changes are observed when soil is contaminated with organic pollutants (Thompson et al., 1999), and typically the proportion of compatible degraders increases after a certain lag period, the length of which depends on the complexity and recalcitrance of the introduced pollutant (MacNaughton et al., 1999). Changes in microbial populations of a polluted soil following input of root exudates has in some cases selectively increased the proportion of pollutant degraders in the sense that the number of organisms that can grow on the pollutant as sole source of C and energy is enhanced (Nichols et al., 1997). It is however more commonly observed that the proportion of pollutant degraders remain unchanged in polluted soil receiving root exudates, even when degradation is enhanced (Fang et al., 2001; Joner et al., 2002). The addition of mineral nutrients may on the other hand specifically favor pollutant degraders, particularly in nutrient poor soil where the pollutant is degradable by direct microbial metabolism and present in large amounts, thus contributing to increased soil C/N and C/P ratios (Lindstrom et al., 1991; Margesin et al., 2000).

In many cases degradation is not mediated by direct metabolism, but rather by co-metabolism (Horvath, 1972), partially rendering enumeration of pollutant degraders irrelevant. Good methods for distinguishing co-metabolism from diauxic growth and direct metabolism in soil are lacking, but for certain compounds, the former is the only way of degradation, as no organisms are known that may degrade them directly. However, the exploitation of co-metabolic processes may also be a pitfall, as the input of readily degradable C can in some cases reduce the mineralization of the organic pollutant, due to the fact that degrader organisms prefer the more readily degradable substrate rather than the pollutant (Abdelhafid et al., 2000; Carmichael and Pfaender, 1997; Swindoll et al., 1988).

The rhizosphere is not only enriched with readily degradable C, it is also frequently deficient in mineral nutrients due to root absorption and slow diffusion of certain nutrients like NH_4 and PO_4 towards the root surface (Jungk and Claassen, 1986; Nye and Tinker, 1977). Many soils polluted with organics contain organic matter with high C/N ratios, which partially explains why biodegradation responds positively to N additions (Breedveld and Karlsen, 2000; Swindoll et al., 1988; Walworth et al., 1997). As plants compete successfully for soil inorganic N (Kaye and Hart, 1997; Wang and Bakken, 1997), microbial degradation in rhizosphere soil will to some extent suffer from a lack of mineral nutrients. Yet, enhanced degradation rates may be observed, possibly in defined zones where the combination of chemical parameters is optimal: Carbon and energy from root deposits are available, but the level of inorganic nutrients is not decreased to levels that impede microbial activity. In a recent study with PAH rhizodegradation in two industrially polluted soils planted with clover and ryegrass (Joner and Leyval, 2003), the highest pollutant dissipation[1] was observed in the soil in the inner rhizosphere (<0.2 mm from the root surface), with little or no effect remaining at a distance of >1 mm (Fig. 2). As this experiment lasted for 6 months, effects of irreversible sorption on senescent roots and cell debris may have been equally important for PAH dissipation as degradation, particularly for soil in the inner rhizosphere (Fig. 3a). At a distance of ca. 0.3–0.6 mm from the surface of the roots, we observed no additional PAH dissipation compared to unplanted soil. This may be due to a lower amount of root debris involved in adsorption and a severe depletion of mineral nutrients at this location (Fig. 3b). Further away from the surface of the roots (ca. 0.6–1.5 mm), PAH levels were again reduced. Here, degradation may have been enhanced, as both mineral nutrients and probably O_2 concentrations were higher than closer to the roots, while root exudation still provided an input of C.

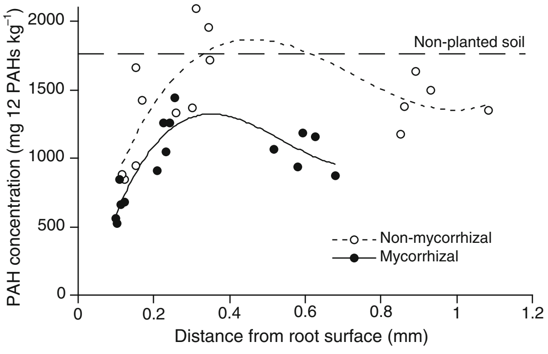

Fig. 2 Rhizosphere gradients of PAH dissipation (sum of the concentrations of 12 priority PAHs) measured in an industrially polluted soil in the presence and absence of arbuscular mycorrhiza (redrawn from Joner and Leyval (2003, with permission)

The soils used in this experiment were either highly organic (38% organic matter) or rich in clay and carbonate (30% and 23%, respectively). Less extreme soils with coarser texture may of course have wider rhizodegradation zones and behave differently with respect to irreversible PAH adsorption. In sand, the effect of ryegrass roots on microbial growth and phenanthrene (a three-ring PAH) degradation was observed up to 9 mm from the surface of roots (Corgié et al., submitted).

2.2 *Mycorrhizal Effects – Plant Growth*

Plant colonization by symbiotic soil microorganisms (N_2-fixing bacteria and mycorrhizal fungi) has obvious beneficial effects on host plant nutrition and

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[1] The term 'dissipation' is more correct than 'degradation' when the mechanism responsible for reduced concentration of the parent compound is unknown. Dissipation comprises one or more of the following processes: Complete or partial biodegradation (i.e. mineralization or biotransformation), sorption/polymerization, volatilization, photo-oxidation, and transport loss by dispersion, leaching, plant uptake etc. Under controlled conditions where only non-volatile compounds are considered and no chemical oxidizing agents (e.g. O_3) or thermal treatments are imposed, degradation and sorption are the only significant components of dissipation. For easily degradable compounds (e.g. low molecular weight PAHs), degradation is largely dominant, while for more recalcitrant molecules (e.g. high molecular weight PAHs), sorption may contribute significantly.

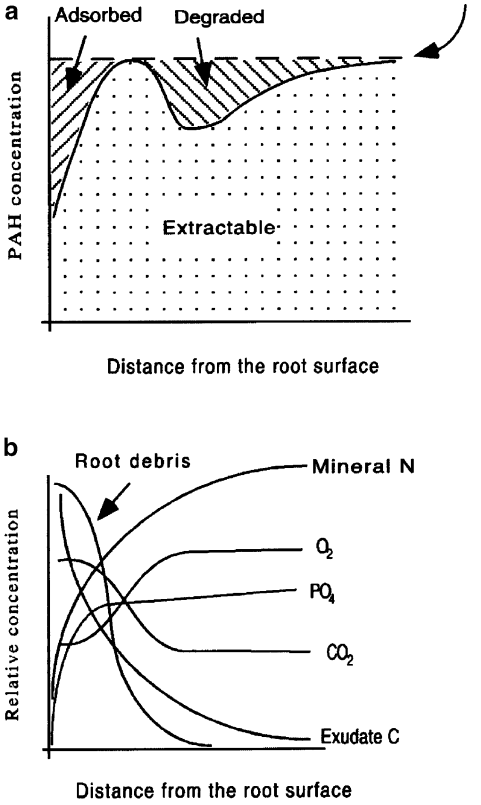

Fig. 3 Schematic diagram of PAH dissipation in the rhizosphere (**a**), proposing a division of PAH dissipation into a non-extractable adsorbed fraction and degraded fraction. The zone of PAH adsorption corresponds to a zone where the root surface, root debris and root hairs are influential (**b**), and the zone of degradation corresponds to a zone where both O_2, mineral nutrients (N and P) and root exudates are present in ample amounts

physiology. One aspect of these effects is related to stress tolerance of plants, most commonly direct or indirect stress induced by nutrient deficiency. In the case of mycorrhiza, enhanced plant tolerance against other types of stress (toxic metals, salinity, drought, pathogens, etc.) is also a well established phenomenon (Gianinazzi and Schüepp, 1994). The role of mycorrhizas in metal-polluted soils has been investigated for more than two decades (Bradley et al., 1981; Leyval et al., 1997), but only recently has their corresponding role in organically polluted soils been addressed. The first reports on this aspect concerned mycorrhizal impact on plant establishment and growth on polluted soil. Here, improved toxicity tolerance may be difficult to distinguish from effects on improved plant nutrition, but results consistently indicate a favorable effect of mycorrhiza (Heinonsalo et al., 2000; Joner and Leyval, 2001; Leyval and Binet, 1998; Olexa et al., 2000).

Toxicity effects of organic pollutants on mycorrhizal fungi have also been described. The toxicity of single compounds in soil is usually limited, unless they are known as acute toxins. Single PAHs do for example not always affect plant growth or mycorrhiza formation (Joner and Leyval, 2001; Leyval and Binet, 1998; Olexa et al., 2000), due to their low solubility and low acute toxicity. Some plant species do however seem more susceptible than others, e.g. clover more than ryegrass, and in the susceptible plant even AM colonization is affected (Joner and Leyval, 2001). PAHs do however rarely occur as single pollutants in soil, but rather as a complex mixture of >200 different molecules. In concert, these may be additive with respect to toxicity and alter soil characteristics towards a hydrophobic matrix where water availability may pose additional constraints on aerobic biological activity like root growth. Assessment of toxicity has thus commonly been made with complex pollution, either by diluting polluted environmental samples with non-polluted soil, or by adding a mixture of compounds to non-polluted soil. In one case where increased concentrations of crude oil (0–50 g/kg) was added to a non-polluted soil, formation of arbuscular mycorrhizas was more sensitive than ectomycorrhizal formation (Nicolotti and Egli, 1998). The authors did however observe a change in dominant ectomycorrhizal morphotypes, and large differences between fungal species grown in pure culture at increasing hydrocarbon concentrations, indicating that ectomycorrhizal fungi differ widely in their tolerance towards organics. In contrast, Leyval and Binet (1998) observed reduced growth of ryegrass, but no reduction in arbuscular mycorrhizal colonization using a single non-adapted fungus and ryegrass grown in soil amended with up to 5% of a heavily PAH-polluted soil (8.1 g PAHs kg^{-1}).

2.3 Mycorrhizal Effects – Degradation

Few reports have addressed phytoremediation of organic pollutants as affected by mycorrhiza, in spite of repeated calls for such studies during the last decade (e.g. Anderson et al., 1993; Cunningham et al., 1996). Most of these concern ectomycorrhizal fungi in pure culture, rather than in symbiosis with plants. It has thus been demonstrated that ECM fungi may degrade several recalcitrant compounds like 2,4-dichlorophenol (Meharg et al., 1997), 2,4,6-trinitrotoluene (Scheibner et al., 1997), atrazine (Donnelly et al., 1993), polychlorinated biphenyls (Donnelly and Fletcher, 1995) and some 3–5 ring PAHs (Braun-Lüllemann et al., 1999). In a symbiotic state, with mycorrhiza-associated bacteria present, degradation capacities may be enhanced and extended to other compounds like toluene and xylene (Dittmann et al., 2002; Sarand et al., 1999), though degradation rates may be influenced negatively when the pollutants are contained in soil or a soil-like material rather than in liquid media (Meharg et al., 1997). These rather diverse results concerning ectomycorrhizas are contrasted by a much lower number of reports on arbuscular mycorrhizas. A series of studies in our laboratory have focused on AM and its impact on degradation of PAHs. Starting out with spiking experiments and AM fungi with no history of contact with anthropogenically introduced PAHs (PAHs are also produced naturally during fire), we did not observe any differences in degradation of either anthracene (ANT) or a mixture of eight PAHs between soil planted with mycorrhizal or non-mycorrhizal ryegrass after a growth period of 40 days (Binet et al., 2000). A longer experiment with the same AM fungus, but using a mixed ryegrass/clover sward, three of the former eight PAHs and sequential harvesting (56 and 112 days) did however show a positive effect of AM inoculation on degradation of two of the three PAHs (Joner et al., 2001): Initially added ANT was degraded almost completely in all treatments (including unplanted soil) within the first harvest. The more recalcitrant compounds, chrysene (CHY) and dibenz[a,h]anthracene (DBA), disappeared faster in planted soil than in unplanted soil, but reached final concentrations that were similar, except for the mycorrhizal treatment that had lower concentrations for both CHY and DBA after 112 days (34% and 58% of initially added CHY and DBA remained in the mycorrhizal treatment, vs. 44% and 80%, respectively, in both unplanted and planted treatments without AM).

Mechanistic explanations of arbuscular mycorrhizal effects on PAH degradation do not relate to fungal catabolism or co-oxidation acting on the pollutant, as the involved fungi have very limited saprophytic capacities. Thus, we must seek explanations involving indirect effects of AM on the degradation activity of other rhizosphere microorganisms. Indeed, the time-course spiking experiment did demonstrate important qualitative differences in microbial community structure (based on phospholipid fatty acid analyses) of rhizosphere soil as affected by AM (Joner et al., 2001). Differences in microbial community structure, assessed as bacterial carbon utilization patterns using Biolog plates, were also observed by Heinonsalo et al. (2000) when comparing the rhizospheres of non-mycorrhizal and ectomycorrhizal treatments in an experiment with petroleum hydrocarbon-spiked soil. Enhanced degradation could in this case not be attributed to the ectomycorrhizal fungi or the associated bacteria, as they coexisted and could not be introduced individually. Both ectomycorrhizal fungi and their associated bacteria are able to degrade simple aromatic compounds (Sarand et al., 1998). Other mechanistic explanations include mycorrhizal effects on the activity of oxidative enzymes in roots and rhizosphere soil. Such enzymes are usually involved e.g. in the initial ring cleavage of PAHs, and both peroxidase activity and increased co-factor concentrations (hydrogen peroxide) have been enhanced by arbuscular mycorrhizal root colonization (Criquet et al., 2000; Salzer et al., 1999).

Spiking experiments have several advantages: One knows how much of each compound that is added to the soil, and degradation metabolites are normally not present initially, so that degradation pathways may be deducted when these appear. Further, the added compounds have a high bioavailability, and toxicity effects on plants and soil organisms may be followed using an identical, non-spiked soil as a negative control. The corresponding disadvantages include that recently introduced pollutants behave differently relative to aged ones, with large differences in bioavailability, and thus degradation rates and toxicity. Also, most bioremediation treatments will have an advantage if they exploit the indigenous microorganisms that have persisted after the pollution event, some of which would surely have the ability to attack the pollutant after long-term adaptation and selection. Finally, real polluted soils usually contain a wide range of recalcitrant compounds that must be dealt with simultaneously by any organism involved in bioremediating the soil. This complexity, and concurrent unfavorable soil characteristics (lack of proper soil structure, hydrophobicity, etc.) can not be mimicked by spiking, and makes it important to verify results obtained from spiking experiments by applying similar treatments to polluted soils.

Experimental evidence for the impact of mycorrhizas on degradation of organic pollutants using industrially polluted soils is scarce (Meharg and Cairney,

Table 1 Residual PAH concentrations in two industrially polluted soils as affected by the presence of plants, their growth period and mycorrhizal status (from Joner and Leyval, 2003, with permission)

Harvest	Soil 1		Soil 2	
Treatment	$\sum$ 12 PAH (mg kg^{-1})		$\sum$ 12 PAH (mg kg^{-1})	
Start	405	b	2,030	a
13 weeks				
No plants	348	c	1,494	c
Non-mycorrhizal	315	c	1,577	bc
Mycorrhizal	311	c	1,539	bc
26 weeks				
No plants	460	a	1,763	b
Non-mycorrhizal	477	a	1,382	c
Mycorrhizal	435	ab	1,042	d

2000). Enhanced degradation of non-polar petroleum hydrocarbons has been reported in treatments with mycorrhizal versus non-mycorrhizal pine (Heinonsalo et al., 2000), and mycorrhiza was assumed to play a role for the efficiency of TCE rhizodegradation in an experiment with *Pinus taeda* grown on soil from a solvent disposal site (Anderson and Walton, 1995). For arbuscular mycorrhiza, we are only aware of our own degradation studies using two PAH polluted soils. Here we have observed enhanced dissipation of several PAHs in a time-course experiment favored by mycorrhization of a mixed ryegrass-clover sward (Joner and Leyval, 2003). The least polluted (400 mg PAH kg^{-1}) and highly organic soil was proportionally less affected by mycorrhiza (but also by the presence of plants) than the severely polluted (2,000 mg PAH kg^{-1}) soil where the presence of AM led to an additional 20% reduction in PAH concentration (Table 1). The same two soils grown with the same plants in the presence and absence of mycorrhiza and/or rhizobia did however not differ in residual PAH concentration in two experiments lasting up to 10 months (Joner, Johnson, McGrath and Leyval, unpublished results). The reasons for these contrasting results remain uncertain, but may be related to differences in mineral nutrient availability, pot sizes, magnitude of plant growth or other uncontrolled factors.

2.4 Mycorrhizal Extension of the Rhizosphere

Extraradical mycorrhizal hyphae are known as the roots of the roots, as they extend the sphere of root influence beyond the traditional rhizosphere, leading to e.g. nutrient depletion and enhanced activity of enzymes in soil further away from the roots than in non-mycorrhizal plants (Joner et al., 1995; Tarafdar and Marschner, 1994). The question if this zone of influence (the “hyphosphere”, *sensu* [Li et al., 1991]) may also be a priviliged site for degradation of organic pollutants has so far not been addressed. The degradation potential in the hyphosphere is however significant, as hyphae allocate relatively importants amounts of carbon to this soil compartment (Schreiner and Bethlenfalvay, 1995), both as exuded glycoproteins and through subsequent hyphal decay. One may thus observe elevated microbial activity (Van Aarle, 2002) and identify highly specific bacterial populations in this soil compartment (Mansfeld-Giese et al., 2002) which may potentially enhance the degradation of organics.

3 Conclusions

The data available on mycorrhiza and degradation of organic pollutants are scarce. But when coupled with the well known effects of enhanced stress tolerance in plants harboring mycorrhizal endophytes, they become a strong incentive to include these symbioses in future experiments or in situ attempts on phytoremediation.

Considering mycorrhizas brings a complicating factor into the study of rhizosphere processes. When studying the rhizosphere, we are already placed, somewhat uncomfortably, across several areas of science as different as soil science, plant sciences, microbiology and chemistry. Adding “symbiology” to this package may be to ask too much. Commercially available inoculum is now commonly used in forestry and vegetation restoration, and the costs of mycorrhizal inoculation are no longer prohibitive. In the other end, new methodology for detection of both ectomycorrhizas (Bååth, 2001; Erland, 1995) and arbuscular mycorrhizas (Olsson et al., 1995; 1999) facilitate detection, identification and quantification of the involved fungi, so that spotting and characterizing mycorrhizas based on morphology (a know-how that takes long to acquire) is no longer a necessity.

Phytoremediation is becoming a major application for rhizosphere technology. Still, we are only starting to learn about the detailed processes that takes place in the rhizosphere, with even less knowledge existing for polluted soils where toxicity adds a complicating factor. Including mycorrhizas is another complicating consideration, but one that seems mandatory if rhizosphere technology should realize its potential in phytoremediation.

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Index