

Oyster Mushroom Cultivation

Part III. Mushrooms Worldwide

Chapter 11

Mushrooms for the Tropics

GROWING GANODERMA MUSHROOMS

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Why Choose to Grow *Ganoderma* Mushrooms?

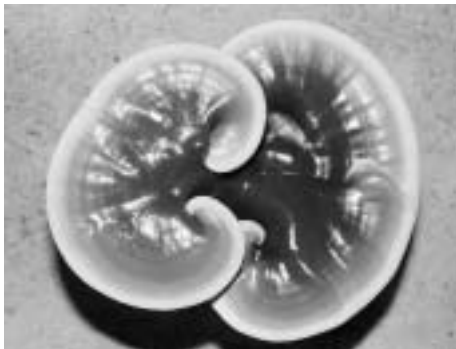


Figure 1. *Ganoderma lucidum* in a kidney shape (Photo courtesy of Henk Voogt)

Ganoderma lucidum, the most famous species in this group is a legendary mushroom in China with a long fascinating history dating back over two thousand years. Not only it is a sparkling beautiful woody mushroom, but more importantly, *G. lucidum* is known as the mushroom of immortality and is the number one medicinal mushroom in China. Dr. Andrew Weil, a most popular authority in the West on Eastern medicine, recently advised readers of his daily health tip to consume Reishi to prevent cancer. Reishi is the Japanese name for *G. lucidum*, while Ling Zhi is the Chinese name. Dr. B.K. Kim, a world leader in research on *Ganoderma* in Korea showed that *G. lucidum* has an anti-AIDS property. AIDS is a worldwide problem, particularly in Africa and Asia.

Best known as an immune system enhancer and modulator with health benefits, *G. lucidum* is generally safe for long-term use. The LD 50 (lethal dose to kill 50% of the study subject) for a single intraperitoneal injection dose of *Ganoderma* extract in rodents was as high as 38g/kg. The LD 50 of a water-soluble polysaccharide fraction of *G. lucidum* in rodent was higher than 5g/kg. Since the toxic/lethal doses in rodent are quite high relative to conventional human dosages, they do not indicate significant limitations for clinical dosages of *Ganoderma* (Chang, 1995).



Figure 2. *Ganoderma* bonsai

The following section will discuss the methods for cultivation of *G. lucidum* although other medicinal species in this group include *G. tsugae*, *G. sinense*, *G. applanatum*, and *G. capense*, whose cultivation methods are similar to those for *G. lucidum*. To bring this much-worshipped mushroom alive to aspiring mushroom growers, the author will build up readers' interest and knowledge as she describes why to choose this mushroom, how many ways there are to grow them, and how *Ganoderma* mushrooms grow. Such questions as what are the crucial stages in *Ganoderma* cultivation, how to speed up the spawn run, how to control the

environmental factors, and how to produce *Ganoderma* mushrooms with caps will be addressed. Growers will benefit by concentrating on the step-by-step instructions on how to cultivate this widely distributed species.

It is essential for growers to learn from successful cultivation examples of *G. lucidum* first, and then adapt them to their own local needs. *G. lucidum* is fascinating to look at, beneficial to health, and has the possibility to generate income for growers. Based on toxicity studies, it has the reputation of being safe for long-term consumption with a large safety margin.

Selection of *Ganoderma lucidum* Strains

It is crucial for both new and experienced growers to understand the features and qualities of the best strains. This knowledge gives a grower a good head start. The choice of a proper strain can determine success or failure. For growing *G. lucidum* to be used for their medicinal benefits, there are good strains from Japan, China, Korea and North America.



Figure 3. Reddish strain with a distinct white margin on synthetic log



Figure 4. Yellowish brown strain with a growing edge on its cap margin

Table 1. Strain selection for *Ganoderma lucidum*

- A. Superior genetic make up.
- B. Stability of the strains.
- C. Strains producing prime fruiting bodies
 - Well formed caps, broad kidney shape, with stalks attached laterally.
 - Choose reddish strains or yellowish strains.
 - Produce highly glossy lacquered surface.
 - Yellow underside at harvest, indicating high triterpenoid content.
 - Thick fertile hymenium layer with long spore producing tubes, indicates high yield of triterpenoids and spores.
 - Size of basidiocarps (fruiting bodies), 9-12cm (width) or above.
 - Weight of basidiocarps, 15-30g or above.
 - High contents of bioactive polysaccharides and triterpenoids, etc.
- D. Vigorous and fast growth rate.
- E. High mushroom yield.
- F. Resistance to weed molds (unwanted mold contamination).

Synthetic Log Cultivation

Preparation of substrates

Substrate formulations

Table 2. Formulation for supplemented sawdust-bran substrate for *Ganoderma* bag cultivation

Oak sawdust	80%	400g
Wheat bran, coarse, unprocessed	18%	90g
Sucrose	1%	5g
CaCO ₃	1%	5g
Water	approximately 67%	1L

(Source: Chen and Miles, 1966b; Chen, 1999)

Scale up according to your need. This formulation was developed on a laboratory scale first, and successfully duplicated in scale-up operations by mushroom growers in US and Canada. In large-scale cultivation, well water can be used. Be sure that the water is not contaminated with undesirable pollutants.

Table 3. Formulations of commonly used *Ganoderma* fruiting substrates

Sawdust	Bran	Supplement	CaCO ₃	H ₂ O	References
80%	18%	Sucrose	1%	1%	67% Chen and Miles, 1996b
80%	20%	-	a little	70%	Hseu, 1993
78%	20%	-	2%	*	Liu <i>et al.</i> , 1990
75%	25%	-	-	*	Lu and Chang, 1975
					Quimio, 1986
87%	10%	-	3%	*	Tong and Chen, 1990
93.5%	5%	Mg SO ₄ 0.2%	-	*	Triratana <i>et al.</i> , 1991
sawdust (alder) : wood chip (oak) = 1 : 1 (5lb /bag wet wt.)					
wood chips soaked and fermented in molasses-enriched water (50mL of molasses/5 gal of water)					Stamets, 2000

*Appropriate amount of water.

(Source: Chen, 1999)

Making the synthetic logs in bag cultivation

Synthetic logs are made by filling the bags (heat resistant polypropylene or polyethylene) with the chosen substrate. For *G. lucidum*, a sawdust-bran substrate with supplements is generally used. Check the formulation of recommended substrates in Table 2 and 3. Using heat-sealed bags with microfilter windows, substrate is usually fills two thirds of the bags to leave air space for ventilation. The substrate is sterilized



Figure 5. Heat-sealed bags with microfilter windows



Figure 6. Cylindrical bags with plugs

right after bagging, if possible, to avoid contamination. To sterilize the substrate is to ensure that it is germ free for you to grow your chosen mushroom. Cylindrical bags with plugs can also be used.

Substrate sterilization

An autoclave is the standard equipment for sterilization. New growers or small growers with no autoclave may contact the mushroom training center or university to obtain already sterilized synthetic logs in bags.

Substrate media is usually autoclaved by standardized sterilization at 121 °C (15 psi) for 15 minutes. Adjust the time according to the amount of substrate to be sterilized. A greater amount of time is required for sterilization of sawdust-based substrates. Do not sterilize for a longer than necessary time, to avoid a possible breakdown of substrate components.

In a home laboratory, a pressure cooker can be used. For example, Stamets (1990) recommends sterilization of 17.50 × 8.25 × 4.75” bags of heat-resistant polypropylene, filled with 3 lbs wet weight of a sawdust/wood-chip substrate, at 15 psi for two hours. On the other hand, 2-lb polyethylene bags, which are not heat resistant, should be sterilized at a lower temperature of 85 °C for 72 hours. Again, depending on the nature and the bulk of the substrate, sterilization of the woody substrate may need to be adjusted (Stamets, 2000).

The cultivation process

Spawn and spawning



Figure 10. Spawning

Spawn is the seed inoculum used to inoculate the sterilized substrate after cooling. Many growers use grain spawn, while some prefer liquid spawn. There are also sawdust-bran spawn, dowels, skewers and grooved woody plug spawn. Choose spawn from the best strain with the most desirable qualities, including good genetic traits, stability, production of quality

fruiting bodies of high health benefits, and high yield. Use vigorous spawn of the right age. Use fresh spawn that has not been stored, or with the least amount of storage time possible.

Methods of spawning (inoculation)

It is smart to follow up inoculation right after sterilization and cooling of the substrate to avoid uninvited contamination (Chen and Moy, 2004). New growers may not be aware that the air is full of contaminants such as uninvited fungal and bacterial spores. Inoculate under a hood in a clean place. There are two ways for spawning: “through spawning” and “localized spawning.” Through spawning involves mixing the spawn throughout the entire sterile substrate, by shaking for instance, while localized spawning involves depositing the spawn on top or on the sides of the substrate block. The choice of spawning procedures should be based on the following desirable characteristics. Growers should seek the lowest possibility of contamination by uninvited microorganisms, the highest speed for inoculation, the greatest ease for handling, the least amount of labor, the most cost-effective methods, the grower’s preference, and a fast spawn run.



Figure 11. Spawn run

How should the bags be arranged?

After inoculation, how should the bags be arranged? In some growers in Taiwan, China, and the U.S.A., bags are arranged vertically on shelves. In some growers in Thailand and the U.S.A., bags are arranged horizontally. Use durable, strong and mold-resistant material for making the shelves. The bottom of each shelf should allow air circulation. In North America, open lattice designs are usually chosen. Treated wood, bamboo, stainless steel and high quality synthetic materials have been used by growers for shelves.

How to control the environmental factors?

The environmental factors, such as humidity, light, and oxygen supply, and temperature are usually known as growth parameters. As *Ganoderma* mushrooms grow from the mycelial stage to fully differentiated and mature mushrooms, each stage has a unique set of requirements for the growth parameters. Since *G. lucidum* is a subtropical mushroom that can also be found in temperate climates, a high temperature near 30°C supports rapid mycelial growth and shortens the time required for spawn run. It has been suggested that spawn run in the absence of light promotes the formation and accumulation of fungal food reserves such as glycogen and lipids. These energy reserves are essential for producing macroscopic mushrooms from microscopic mycelia.

What are the crucial stages in Ganoderma cultivation?

Growers should pay special attention to transitional stages, such as the stage from vegetative phase to reproductive phase when primordia (the initial stage for fruiting body formation) are beginning to form. The most crucial factor during primordia initiation is high relative humidity, preferably 90-95%. Oxygen supply, exposure to diffused dim light and inclusion of calcium in the fruiting substrate are also important.

The most crucial management practice during pileus differentiation (the specialized growth for mushroom cap development) is to increase ventilation to reduce CO₂ concentration, along with high humidity and diffused dim light. When the temperature is too high, the fruiting bodies produced are very thin and of poor quality. Differentiation of *Ganoderma* fruiting is highly sensitive to CO₂ concentrations as these will determine whether antler-shaped fruiting bodies (CO₂ > 0.1%), or fruiting bodies with well-formed caps (CO₂ < 0.1%) will be produced. Fresh air contains 0.03% CO₂. Aim for reducing CO₂ to 0.04-0.05%, as close to fresh air as possible, for the production of mushrooms with caps (Table 5). Humidity is provided by fine mist 1-2 or 3-4 times per day.



Figure 7. Antler-shaped fruit bodies

Table 4. *Ganoderma* growth parameters

Stage	Duration	Humidity (%, R.H.)	Light (lux)	CO ₂ (%)	O ₂ (Ventilation)	Temperature (°C)
Spawn run	up to 2 months	60 -70%	Nil	Tolerate/ high conc.	0-1 exchange	25-30*or lower (20)
Primordia initiation	50-60 days after spawning	90-95%	100-200	0.1-0.5% or lower	O ₂ a plus	25-30*or lower (20)
Stipe (stalk) formation	10-14 days in development	70-80% or higher	150-200	0.1-1% high conc. (branching)	low	25-30*or lower (20): thicker

Pileus (cap) differ-entiation	25 days or longer from primordia to harvest	85-95% (on/off)	150-200 12hr circulation	< 0.1% thicker	low conc. air	25-30*or lower (20):
For further	7-10 days	85% 50-60%	Additional incubation of after cap maturation		growth	

*Set temperature at 28°C, the actual temperature may become 2-3°C higher (heat generated by massive mycelial respiration).

(Source: Chen, 1999; Stamets, 2000)

Table 5. Control of CO₂ concentration to produce *Ganoderma* with caps

Fresh air	0.03%
<i>Ganoderma</i> with caps	< 0.1% (Optimum: 0.04-0.05%)
<i>Ganoderma</i> with antler fruiting body	> 0.1%

How *Ganoderma* mushroom grow

Vegetative phase (spawn run)

Spawn run simply refers to the growth and propagation of the mushroom mycelia in the colonized substrate bags during the vegetative stage before the formation of mushrooms. *Ganoderma* spawn should be incubated in the absence of light at 25-30°C or lower, even as low as 20°C. Almost all strains of *G. lucidum*, regardless of the geological location where the strain was isolated, have an optimal spawn run temperature of around 30°C. Avoid drying. When the substrate in the bag is fully covered by the whitish growing mycelia, it is time for fruiting. Liquid droplets and color may occur. Spawn run can be speeded up.

How to speed up the spawn run

- Select a vigorous, fast-growing fruiting strain with a superior genome.
- Choose the best substrate formula. Provide an appropriate level of substrate moisture. Avoid the substrate's being waterlogged. Make sure that the substrate provides sufficient aeration. Check also for good substrate texture and particle contact for excellent nutrient and water transport. Use the right amount of substrate and leave ample air space in the bag.
- Choose a properly functioning bag system of the right size. When heat-sealed bags with microfilter windows are used, bags of large diameters (not long and narrow) with ample air space above the substrate will facilitate oxygen supply and air exchange.
- Use a generous amount of fresh, pure, vigorous and high quality spawn.
Avoid spawn being too old or too immature. Avoid long spawn storage, if any.
- Use through spawning by distributing the spawn throughout the substrate.
- Light inhibits mycelial growth. No light is necessary for spawn run.
- Set the highest optimal incubation temperature for spawn run (usually 30°C).
- Keep the inoculated bags free from contamination.

Trigger primordia formation

Primordia formation is triggered by exposure to light (100-200 lux), oxygen and high relative humidity (85-95% R.H.). Cold shock is not necessary to trigger the formation of *Ganoderma* primordia. However, when occasionally

a few colonized blocks fail to respond to the standard triggering treatment, they can be transferred to a freezer over night. The remedial cold shock can be applied to unresponsive vegetative blocks more than once. Brief exposure to low levels of light is sufficient to initiate *Ganoderma* primordia.

Fruiting differentiation and development

Towards the end of the vegetative spawn run, whitish mature mycelia begin to form tighter growth in knots in response to environmental stimulation. These centers of tighter mycelial growth gradually develop into primordia and rise above the surface of the substrate as whitish rounded mounds, much bigger than the pin heads of white *Agaricus* button mushrooms. Amorphous primordia mass may ooze out first.

These primordia elongate vertically in the air into whitish finger-like young stalks. Growth of the stalks takes place by elongation and an increase in diameter. Under favorable conditions, the tips of mature stalks with color and shine on their lower parts begin to enlarge and give rise to young mushroom caps that are laterally attached to the tips of stalks. The young mushroom caps continue to grow and develop into typically broad kidney-shaped caps, increasing in size at the cap margins. Meanwhile as the cap matures, beautiful yellowish brown or reddish and then reddish brown coloration appears, depending on the strains. Under the diffused dim light, the mushrooms transform into sparkling specimens with lacquer-like shining upper surfaces and glossy dark brownish stalks.

Less visible to our eyes, but more important, is the differentiation of a fertile layer called the hymenium on the underside of the mushroom cap. The hymenium contains long fertile tubes in which basidiospores are produced. With our naked eyes we are only able to see the ends of these tubes as pores when we turn the cap upside down. That is why *Ganoderma* mushrooms are woody polypores, quite different from the fleshy shiitake mushrooms with gills and a centrally attached stalk. This knowledge is important because a biomedically important component called triterpenoid is produced in this region. The thicker the hymenium layer and the longer the tubes, the better, provided the physiologically active triterpenoids are produced. This determines the medicinal value of the *Ganoderma* mushroom strain and whether it is worthwhile to grow it.

Natural Log Cultivation

In the past, natural logs as long as 1m were used without sterilization in growing *Ganoderma* species in China. A long incubation of two to three years was required to obtain mature fruiting bodies on such substrates. Since late 1980s, new trends have been developed that use short logs. Today, almost all *Ganoderma* natural-log growers adopt the time-saving short-log cultivation. This is true in China, Japan, the United States and elsewhere. Here we focus on growing *G. lucidum* on short natural logs enclosed in air-permeable synthetic bags during spawn run. Such a strategy shortens the production time and ensures mushroom quality. Addressed here are the crucial factors and methodology controlling growth and fruiting.

Preparation of logs

Tree species and log size

Most broad-leaf hardwoods can be used to cultivate *Ganoderma lucidum* and other *Ganoderma* species. Commonly used species include oak, pecan, elder, choke cherry, and plum etc. (Chen, 1999; Stamets, 2000; Chen and Chao, 1997). To be avoided are conifers and hardwoods containing harmful aromatic compounds, such as camphor-producing species, although these tree species can be used after fermentation. The standard log size used in cultivation of *G. lucidum* is 15cm in diameter or thinner, and 15-24cm long (Table 6). Commercial growers in Fujian province in China harvest logs from about 30-year old hardwood trees. Moisture content in the log should be taken into consideration.

Table 6. Size and moisture content of *Ganoderma* short natural logs

Country	Log size (diameter × length)	Moisture content	Reference
China	15 × 18-24cm	36-38% (tight)	Huang (ed.), 1993, p. 238
	6-15 × 15cm	38-40% (loose)	Chen and Chao, 1997, p. 514
Japan	15 × 15cm		Mayzumi, Okamoto/ Mizuno, 1997, p.365
U.S.A.	12.7 × 20.3cm		Chen, 1999, p. 182

The correct time for harvesting the logs, air-drying and cutting into short logs

Cut logs from chosen hardwood species 15-20 days before spawning (Chen and Chao, 1997). Choose logs with intact bark and a diameter of 15cm unless otherwise specified (Table 6). Harvest the logs during the dormant season of the tree prior to the formation of new buds, when the tree trunks are full of sap and nutrients and before these nutrients are consumed during the germination of buds (Chen, 1999; You, 1987). Lightly air dry the logs for 15-20 days in a clean and well-ventilated place to obtain the desirable moisture content in the logs. For logs with a tight and firm woody texture, a lower level of moisture is required compared to logs with a looser texture (Table 6). Cut into short logs, 15cm or so in length. Retain the bark, but trim the periphery of the logs by removing small side branches, thorns, and any rough spots that may puncture a synthetic bag.

Choice of bag design, bagging and sterilization

Enclose logs singly in each bag, or two logs end to end in a bag having a diameter slightly larger (> 15cm in diameter), and a length of 25-50cm. Sterilize logs in bags at high pressure (1.5kg/cm²) for 1.5 hours or at normal air pressure, or 100°C for 10 hours. Heat-sealed polypropylene or polyethylene bags with microfilter windows can be used. Permeation of air exchange of such bags is regulated by the size, shape, number, locality and nature of the microfilter on each bag, as well as air space above the colonized substrate in the enclosed bag.



Figure 9. Short log in the bag before sterilization

Preparation of spawns

A variety of spawns, such as pure culture liquid mycelial spawn, grain spawn and sawdust-bran spawn can be used. Pure-culture liquid mycelial spawn can be grown in potato-dextrose broth. For information on the sawdust-bran substrate used for spawn (Table 2 , 3).

The cultivation process***Spawning***

Apply spawn evenly on the cut surface, 3-5cm thick, usually 5-10g spawn for each log. When using freshly cut logs, instead of sterilized logs, as in traditional log-cultivation in Japan, inoculation is applied immediately, or soon after log cutting to avoid contamination, based on the fact that the interior of a healthy tree is sterile. Alternatively using an inoculation gun, liquid mycelial spawn can be dispensed into the drilled holes on the periphery of the log. Colonized dowels can also be used.

Spawn run (mycelial penetration)

Special attention should be given to ensure proper mycelial growth in the log. Efforts should be made to encourage

mycelial growth throughout the interior of the log. Avoid having superficial mycelial growth on the log surface only as a tough leathery mycelial coat (layer). The formation of superficial leathery mycelial coat on the log surface only without mycelial penetration into the center of the log is related to the log oxygen and moisture content. Lack of oxygen or poor aeration, such as water-logged, results in poor and slow growth. This is the opposite to Shiitake synthetic logs cultivation, for Shiitake, a mycelial coat on the surface of the colonized log is desirable. For proper management of the environmental factors during spawn run, refer to growth parameters. Log spawn run also tolerates fairly high CO₂ concentration, and is carried out in the absence of light.

Primordia initiation

Same as Bag synthetic log cultivation, brief exposure to very little light triggers *Ganoderma* primordia. Oxygen is also conducive to primordia formation. In contrast, spawn run is carried out in darkness, and less oxygen is required. *Ganoderma* primordia are usually formed 50-60 days after spawning in natural log cultivation.

Embedding in soil

Embed the colonized logs directly in soil after primordia formation, leaving the primordia above ground. Then cover the soil with chopped straw to retain moisture. During fruiting, at the primordia stage, the colonized logs become resistant to microbial contamination in the non-sterile soil (Chen, 1996b). Embed the short logs vertically, with the cut surface where spawning is applied facing upwards. Soil with good drainage, such as sandy soil, should be used. Following is an example: embed only 16-21cm or 9/10th of the log in soil, leaving well-formed primordia above ground (Chen and Chao, 1997). Log moisture can be better conserved by burying the logs in soil. Embedding logs in soil also enables mushroom mycelia to absorb nutrients, particularly minerals and trace elements from the soil. Soil-buried log cultivation can be done in easily-constructed mushroom houses. Within the mushroom house, low loop frames with covers usually in 2 rows, are routinely set up. Alternatively, soil-buried log cultivation of *Ganoderma* species can also be carried out in the open air in the wild.



Figure 12. Tips of young mushrooms about to form caps



Figure 13. short logs embedded with top soil in nursery pots



Figure 14. *Ganoderma* mushroom growing on Short logs



Figure 15. *Ganoderma* with further cap growth

Harvest the Mushrooms

From primordia formation to fruiting bodies ready for harvest, takes approximately 25 days under favorable conditions. Disappearance of the white growing margin at the edge of the yellowish brown or reddish brown mushroom is a sign for harvest. Continue cultivation at reduced air humidity of 85% R.H. for an additional 7-10 days to encourage further growth in pileate thickness and firmness (50-60% R.H. in another practice). Harvest by cutting the stipe (stalk). Keep only 2cm of the stipe with the pileus. If so desired, continue cultivation under the optimal growth parameters for second and third flushes, although the subsequent flushes have lower yields, especially the third flush.

Post Harvest

After harvest it is essential to avoid storage of the dried mushrooms under humid, damp, warm and soiled unsanitary conditions. If not properly cared for, you could be shocked to find that the beautiful mushrooms you grew have been deteriorated into powders by the infestation of miniature beetles called ‘cecids.’

Air dry harvested fruiting bodies under the sun or with heat (60°C) immediately. Complete drying within 2-3 days. Place the fruiting bodies with the underside of the mushroom cap facing down. During cloudy or rainy days, apply low heat (60°C). Improper prolonged drying lowers the quality of the product by turning the underside pore surface dark brown or becoming contaminated by molds.

Discussion and Conclusion

Whether to use bag cultivation or natural log cultivation, growers should be thoroughly familiar with how the mushrooms grow and what the proper environmental factors are for each developmental stage. The major focus is high humidity for primordia initiation followed by an increase in ventilation during pileus differentiation to allow an increase in the oxygen supply.

It is advisable to grow *Ganoderma* organically. Unsound practices with the risk of undesirable environmental contamination have been detected in connection with log cultivation (Mushroom Growers’ Newsletter, Sept., 2001). The question arises as whether to use bag synthetic log cultivation or to use natural log cultivation for *G. lucidum*. Successful natural log cultivation produces *Ganoderma* mushrooms with superior quality. Thick and firm fruiting bodies are produced with desirable coloring and luster that command good prices in the markets of Southeast Asia. However, the yield could be lower, and the production time could be a little longer. The major issue is conservation of the natural resource of the forest where the logs come from, which is a significant environmental concern.

Selection of logging should be carefully done, such as choosing very old forests within which some logging does not have any significant environmental impact. Long-term planning of forestation should be coordinated with log cultivation.

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