Natural-Log Cultivation of the Medicinal Mushroom *Ganoderma Lucidum* (reishi)

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ABSTRACT

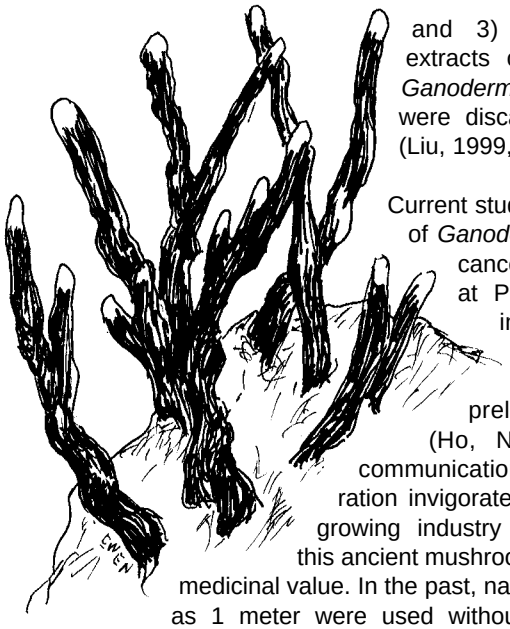
Recent biomedical reviews of *Ganoderma lucidum* and other medicinal species in the genus indicates that *Ganoderma* is a blood thinner. Active components in these species regulate and strengthen vital body functions. A wide range of effects have been reported, including anti-cancer and anti-tumor through enhancing TNF-a, IFN-g and IL-2; anti-HIV through inhibition of virus proliferation and anti-aging through increasing a-DNA poly-merase. Studies on the effect of *Ganoderma lucidum* on cancer cells at Purdue University in the United States provides encouraging preliminary results. Such exploration invigorates the mushroom growing industry in re-examining this ancient mushroom of exceptional medicinal value. In the past, natural logs as long as 1 meter were used without sterilization in growing *Ganoderma* species in China. Long incubation of 2-3 years was required to obtain mature fruiting bodies on such substrates. Since the late 1980s, new trends have been developed in using short logs. Today, almost all *Ganoderma* natural-log growers have adopted the time-saving short-log cultivation in China, Japan, the United States and elsewhere. This paper focuses on growing Ganoderma lucidum on short natural logs enclosed in ventilated synthetic bags during spawn run. This strategy shortens the production time and ensures the quality of fruiting bodies. Crucial factors and methodology controlling growth and fruiting are discussed here.

INTRODUCTION

Ganoderma lucidum, a mushroom primarily of tropical and subtropical climate that can also be found in temperate zones or regions, and a number of other *Ganoderma* species (*G. tsugae*, *G. sinensis*, *G. applanatum*, *G. capense* and *G. tenus)* have been used as medicinal mushrooms in China and Southeast Asia (Hseu, 1993, Chen and Chao, 1997). Recent pharmacological and clinical studies (Lin, 2000; Stamets, 1999; Chen and Chao, 1997; Chen and Miles, 1996a; Hobbs, 1995; Jong and Birmingham, 1992; Willard, 1990) show that *Ganoderma* is a blood thinner with high affinity to oxygen. Active components in *Ganoderma lucidum* and other medicinal species in the genus exhibit a wide range of effects, including anti-cancer/anti-tumor through enhancing TNF-a, IFN-g, and IL-2; anti-HIV through inhibition of virus proliferation, and anti-aging through increasing a-DNA polymerase. Of special interest at present, are 1) use of *Ganoderma* in integrative medicine, such as using *Ganoderma* in conjunction with chemotherapy or radiation therapy in treating cancer patients (Lin, 2000), 2) use of *Ganoderma* for late-stage cancer patients in recovery to a level which enables them to undergo surgery, chemotherapy or radiation therapy (Lin, 2000),

Table 1. Size and moisture content of short natural logs for cultivation of Ganoderma species.

Country	Log size		Moisture content	Reference
	Diameter	Length		
China	15 cm (5.9”) X	18-24 cm (7”- 9.5”)		Huang (ed.), 1993, p. 238
	6-15 cm (2.37 –5.9”) X	15 cm (5.9”)	36-38% (tight) 38-40% (loose)	Chen and Chao, 1997, p. 514
Japan	15 cm (5.9”) X	15 cm (5.9”)		Mayzumi, Okamoto and Mizuno, 1997 , p.365
USA	12.7 cm (5”) X	20.3” (8”)		Chen, 1999, p. 182



and 3) use of spore extracts or "shell-broken" *Ganoderma* spores which were discarded previously (Liu, 1999, 2001).

Current studies on the effect of *Ganoderma lucidum* on cancer cells initiated at Purdue University in the United States provides encouraging preliminary results (Ho, N2001, personal communication). Such exploration invigorates the mushroom growing industry in re-examining this ancient mushroom of exceptional medicinal value. In the past, natural logs as long as 1 meter were used without sterilization in growing *G. lucidum* and other species in China. Long incubation time of 2-3 years is required to harvest mature fruiting bodies on such substrates. Since the late 1980s, new and improved methods have been developed using short logs. Today, almost all *Ganoderma* natural-log growers adopt the time-saving short-log cultivation methods. This paper focuses on growing *G. lucidum* in Southeast Asia and its adaptation in the United States. Description of *Ganoderma* log-cultivation here, is based primarily on practices in China (Chen and Chao, 1997). Ventilated synthetic bags are used in enclosing the short logs during spawn run, a key strategy in shortening the production time and improving the fruiting quality. Addressed here will be crucial factors and methodology of controlling growth and fruiting.

PREPARATION OF LOGS

Tree species

Most broad-leaf hardwoods can be used to cultivate *Ganoderma lucidum* and other *Ganoderma* species. To be avoided are conifers and a few hardwoods which may contain harmful aromatic compounds, such as camphor-producing species. Most commonly used species include oak, pecan, elder, choke cherry, and plum etc. (Chen, 1999; Stamets, 2000; Chen and Chao, 1997).

Log size

The standard log size used in cultivation of *G. lucidum* is 15 cm in diameter or thinner, and 15-24 cm long (Table 1.). Commercial growers in Fujian province in China harvest logs from hardwood trees 25-30 years old. Moisture content in the log should be taken into consideration.

Harvesting the logs

Logs are cut from chosen hardwood species 15 to 20 days before spawning (Chen and Chao, 1997). Choose logs with intact bark and a diameter of 15cm or otherwise specified (see Table 1). Harvest the logs during dormant season of the tree prior to the formation of new buds when the tree trunks are full of sap and nutrients before they are consumed for germination of buds (Chen, 1999; You, 1987).

Air dry logs

Lightly air dry the logs for 15 to 20 days in a clean and well-ventilated place to obtain the desired moisture content in the log. For logs with tight and firm woody texture, a lower level of moisture is required compared to logs with looser texture (Table 1).

Cut into short logs and trimming

Cut into short logs, 15 cm or so in length. Retain the bark, but trim the periphery of the log by removing small side branches, spines, and any rough spots which may puncture a synthetic bag.

CHOICE OF BAG DESIGN, BAGGING AND STERILIZATION

Enclose logs singly in a bag, or two logs end to end in larger bags. Alternatively, logs can be bundled tightly into a bamboo loop and fit into a bag 40-60 cm in diameter and 40-50 cm long. Such massive loading, however, makes it difficult to sterilize effectively or to avoid contamination. Sterilize logs in bags at high pressure (1.5 kg/cm^2) for 1.5 hour or at normal air pressure and 100°C for 10 hour. Bag design is important. Heat-sealed polypropylene or polyethylene bags with microfilter windows can be used. Air exchange in these bags is regulated by the size, shape, number, location and nature of the microfilter on each bag, as well as air space primarily above the colonized substrate in the enclosed bag. Most, if not all, specialty mushroom growers in North America use Unicorn bags (see page 12).

PREPARATION OF SPAWN

A variety of spawns, such as pure culture liquid mycelial spawn (Moore mushroom lab.USA), grain spawn and sawdust–bran spawn can be used (Chen, 1999). Pure-culture liquid mycelial spawn can be grown in potato-dextrose broth or other formulation. For formulation of sawdust-bran substrate for spawn, see Table 2. Other formulations can be found in Chen (1999).

Table 2. Substrate formulation for sawdust-bran spawn (Chen, 1999).

Oak sawdust	80%	400 g
Wheat bran, coarse, unprocessed	18%	90 g
Sucrose	1%	5 g
$CaCO_3$	1%	5 g
Water	approximately	1 liter

THE CULTIVATION PROCESS

Spawning

Spawn can be prepared or purchased for use. Apply spawn evenly on the cut surface, 3-5 cm thick, usually 5-10 g 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 (Organotech in San Antonio, TX, USA), the same way as in shiitake log cultivation. Colonized wooden dowels can also be used.

Spawn Run

Mycelial penetration

Special attention should be given to ensure proper mycelial colonization 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, without mycelial penetration into the center of the log, is related to the log oxygen and moisture content. Lack of oxygen or poor aeration, sometimes due to water-logging, results in poor mycelial growth and slow growth rate. In contrast, in cultivation of shiitake synthetic logs, a mycelial coat on the surface of the colonized log is desirable. For proper management of growth parameters during spawn run, refer to the section on growth parameters. Spawn run tolerates fairly high CO^2 concentration, and is carried out in the absence of light.

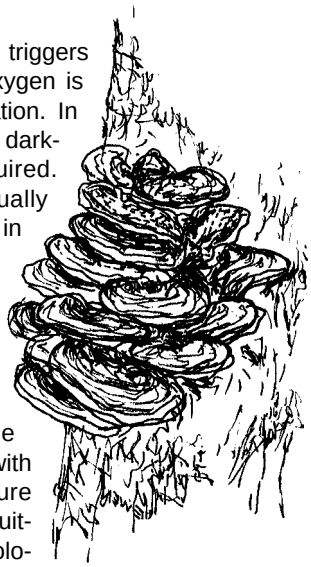
Remediation of low-oxygen in the bag

For heat-sealed, microfilter bags:

1. increase the size and number of microfilters through bag design.
2. Increase the air space in the bag, by using larger bags or smaller logs.
3. Puncture air pores (5-6 pores in each small bag or 8-10 pores in each larger bag) with needles just below the neck of the bag in bags with plugs. Spray the air with 1% calcium hypochlorite and wipe the bag surface with 75% ethylene alcohol before puncturing the pores. Then cover with clean paper, e. g. newspaper (Chen and Chao, 1997).

Primordia initiation

Brief exposure to very little light triggers *Ganoderma* primordia initiation. 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 log cultivation.



Embedding in soil

Embed the colonized logs directly in soil after primordia formation, leaving the primordia above the ground level. Then cover the soil with chopped straw to retain moisture (Chen and Chao, 1997). 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. Embed only 16-21 cm or 9/10 th of the log in soil, leaving well-formed primordia above ground (Chen and Chao, 1997). Mushroom yield from successful cultivation of soil-buried natural short logs has been reported to be superior than cultivation without soil. Log moisture can be better conserved by burying the log in soil. Embedding logs in soil also enable mushroom mycelia to absorb nutrients, particularly minerals and trace elements from soil (Huang, 1993, p. 238). Soil-buried log cultiva-

Table 3. *Ganoderma lucidum*: growth parameters for cultivation (Chen, 1999; Stamets, 2000).

	Humidity (R.H.)	Light (lux)	CO_2	O_2 Air	Temperature °C	Duration
Spawn run	60-70%	Nil	Tolerate high Conc. to 5%	0-1 exchange	25-30 or lower (20)	up to 2 months or as required
Primordia initiation	90-95%	100-200	0.1-0.5% or lower	O_2 a plus	25-30* or lower (20)	50-60 days after spawning
Stipe Development	70-80% or higher	150-200	0.1-1% high conc. branching	low	25-30* or lower (thicker)	10-14 days or as required
Pileus Differentiation	85-95%	150-200 12 hour. on/off	<0.1% low conc. cap formation	O_2 Air circulation	25-30* or lower (thicker)	25 days From primordia to harvest
Further growth in pileus	85%	Additional incubation of 7-10 days after maturation of the pileus				
Other practice	50-60%	Additional incubation after maturation of the pileus (caps)				

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

tion can be done in easily-constructed mushroom houses. Within the mushroom house, low loop frames with coves usually in two 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.

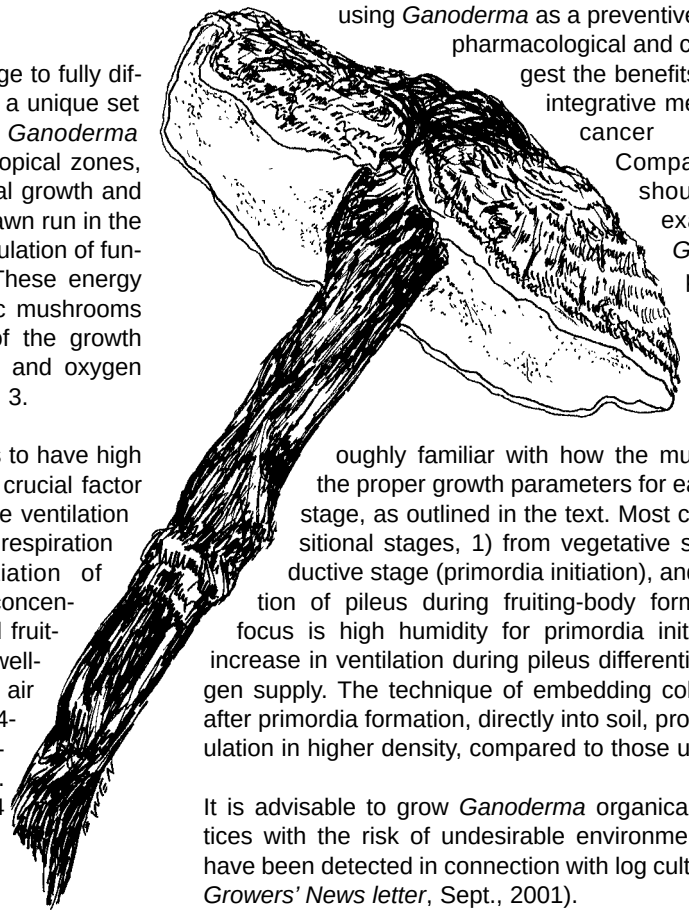
Short logs embedded in containers

Organotech in San Antonio, TX, USA (Chen, 1999) embeds the inoculated short logs in sawdust (or sand) in large plastic pots typically used by plant nurseries, one log in each container (The Texas Horticulturist, 1990). Soil casing is then applied on the top. Inoculation holes are drilled on the log periphery (see, spawning).

GROWTH PARAMETERS

As *Ganoderma* mushrooms grow from mycelial stage to fully differentiated and mature mushroom, each stage has a unique set of requirements in growth parameters. Since *Ganoderma lucidum* is a mushroom of temperate as well as tropical zones, high temperature near 30ºC supports rapid mycelial growth and shortens 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. For a full spectrum of the growth parameters, including temperature, humidity, light and oxygen supply at different developmental stages, see Table 3.

The most crucial factor during primordia initiation is to have high humidity, preferably 90-95% R. H., while the most crucial factor during pileus differentiation in fruiting, is to increase ventilation to reduce CO_2 build up from the drastic increase in respiration during that period of development. Differentiation of *Ganoderma* fruit bodies is highly sensitive to CO_2 concentration which determines whether an antler-shaped fruiting body (CO_2 >0.1%), or a fruiting body with a well-formed pileus (CO_2 < 0.1%) will be produced. Fresh air contains 0.03% CO_2. Aim for reducing CO_2 to 0.04-0.05%, as close to fresh air as possible, for production of pileated mushrooms (mushrooms with caps). Air humidity is provided by fine mist (1-2,or 3-4 times/day).



HARVEST THE MUSHROOMS

From primordia formation to fruiting-body for harvest, it takes approximately 25 days. Fruiting maturity is indicated by the disappearance of the undifferentiated white growth at the edge of the fruiting body. In other words, on the upper surface of the pileus, the pileate margin has similar color to the center, all reddish to reddish brown or yellowish to yellowish brown in *G. lucidum*. Continue cultivation at reduced air humidity of 85% R.H. for 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 2 cm 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 yield, especially the third flush.

POST HARVEST

Dry the harvested fruiting bodies immediately, air dry under the sun or with heat (60ºC). Complete drying within 2-3 days. When drying, place the fruiting bodies with the underside of the pileus facing down. During cloudy or rainy days, apply low heat (60ºC). Improper prolonged drying lowers the quality of the product by turning the underside pore surface into dark brown or becoming contaminated by molds.

DISCUSSION AND CONCLUSION

Growers should be well versed and continue updating their knowledge on the biomedical properties of *Ganoderma*. The main medicinal thrust of the fungus, regulating and strengthening body vital functions, clearly dictates the importance of using *Ganoderma* as a preventive measure. Recent pharmacological and clinical studies suggest the benefits of *Ganoderma* in integrative medicine, such as in cancer treatments. Comparative studies should be made to examine whether *Ganoderma* spore preparations have added value.

Ganoderma natural-log growers should be thoroughly familiar with how the mushroom grow and the proper growth parameters for each developmental stage, as outlined in the text. Most crucial are the transitional stages, 1) from vegetative stage to the reproductive stage (primordia initiation), and 2) the differentiation of pileus during fruiting-body formation. The major focus is high humidity for primordia initiation followed by increase in ventilation during pileus differentiation to allow oxygen supply. The technique of embedding colonized short logs, after primordia formation, directly into soil, produces fruiting population in higher density, compared to those using pots.

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' News letter*, Sept., 2001).

The question arises whether to use sawdust synthetic-log cultivation in bags 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 which command good price in markets in southeast Asia. However, the yield could be lower. The production time could also be a little longer (Chen and Chao, 1997). The major issue is conservation of natural resource, the forest, where the logs come from, a significant environmental concern. Selection of logging should be carefully done, such as choosing very old forest which does not have any significant environmental impact. Long-term planning of forestation should be coordinated with log cultivation.

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