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 indicate that *G. lucidum* is a blood thinner. Active components in these species regulate and strengthen vital body functions. Based on such a principle, the following wide range of effects have been reported: anti-cancer and anti-tumor, through enhancing TNF-α, IFN-γ and IL-2; anti-HIV, through inhibition of virus proliferation; and anti-aging, through increasing α-DNA polymerase. Studies on the effect of *G. lucidum* on cancer cells at Purdue University in the United States provide encouraging preliminary results. Such exploration has prompted the mushroom industry to re-examine this ancient mushroom's exceptional medicinal value. In the past, natural logs as long as one meter were used without sterilization in growing *Ganoderma* species in China. Long incubations (two to three years) were required to obtain mature fruiting bodies on such substrates. Since the late 1980s, new trends have been developed using short logs. Today, almost all *Ganoderma* spp natural-log growers adopt the time-saving short-log cultivation in China, Japan, the United States and elsewhere. This paper focuses on growing *G. lucidum* on short natural logs enclosed in ventilated synthetic bags during spawn run. Such a strategy shortens the production time and ensures the quality of fruiting bodies. Addressed here are crucial factors and methodology controlling growth and fruiting.

INTRODUCTION

Ganoderma lucidum, a mushroom of both temperate and tropical zones, 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 strains of this genus may act as a blood thinner with high affinity to oxygen. Active components in *G. lucidum* and other medicinal species in the genus exhibit anti-cancer/anti-tumor effects (through enhancing TNF-α, IFN-γ, and IL-2); anti-HIV (through inhibition of virus proliferation) and anti-aging (through increasing α-DNA polymerase). *Ganoderma* spp's fundamental working principle from a biomedical perspective remains constant - regulating and strengthening vital body functions – are continuing long-term priorities in health care. Of special interest at present, are 1) use of *G. lucidum* in integrative medicine, e.g. in conjunction with chemotherapy or radiation therapy in treating cancer patients (Lin 2000), 2) use of *G. lucidum* for late-stage cancer patients in recovery to a level which enables them to undergo surgery, chemotherapy or radiation therapy (Lin 2000), and 3) use of spore extracts or "shell-broken" *G. lucidum* spores which were discarded previously (Liu 1999, 2001).

Current studies on the effect of *G. lucidum* on cancer cells initiated at Purdue University in the United States provide encouraging preliminary results (Ho, 2001, pers. comm.). Such exploration has prompted the mushroom growing industry to re-examine this ancient mushroom's exceptional medicinal value. In the past, natural logs as long as one meter were used without sterilization in growing *G. lucidum* and other species in China. Long incubation time (two to three years) is required to harvest mature fruiting bodies on such substrates. Since the late 1980s, new and improved trends have been developed using short logs. Today, almost all *Ganoderma* spp natural-log growers adopt the time-saving short-log cultivation in China, Japan, the United States and elsewhere. This paper focuses on growing *G. lucidum* in Southeast Asia and its adaptation in the United States. *Ganoderma* spp log-cultivation is described in some detail here, 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 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 *G. 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 must be taken into consideration.

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

Timing log harvesting

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 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 are consumed for germination of buds (Chen 1999, You 1987).

Drying logs

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

Cutting and trimming logs

Cut into short logs, approximately 15 cm . 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 each bag, or place two logs end to end in a bag having a diameter slightly larger (> 15 cm in diameter), and a length of 25 cm to 50 cm. Alternatively, logs can be bundled tightly into a bamboo loop and fit into a larger 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 hr or at normal air pressure 100^oC for 10 hr. The bag design is important. 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 airspace primarily above the colonized substrate in the enclosed bag, Most, if not all, specialty mushroom growers in North America (Fungi Perfecti, Field and Forest Products and Penn State Mushroom Center are a few examples) use “Unicorn” bags.

PREPARATION OF SPAWNS

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 formulations. 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
CaCO3		1%	5 g
Water	approximately 67%		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 such a superficial leathery mycelial coat on the log surface only, without mycelial penetration into the center of the log is related to log oxygen and moisture content. Lack of oxygen or poor aeration, such as 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.

Instructions for remediation of low oxygen in the bag

1) For heat-sealed, microfilter bags, 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 hypochorite 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* spp primordia initiation. Oxygen is also conducive to primordia formation. In contrast, spawn run is carried out in darkness, and less oxygen is required. *Ganoderma* spp 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. The following is an example: 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 yields 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 the soil (Huang 1993). Soil-buried log cultivation 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.

Embedding in containers

Organotech in San Antonio, TX, USA (Chen 1999) is an example of embedding the inoculated short logs in sawdust (or sand) in the large plastic pots usually 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, “Sawning”).

GROWTH PARAMETERS

As *Ganoderma* spp grow from mycelial stage to fully differentiated and mature fruiting bodies, each stage has a unique set of requirements in growth parameters. Since *G. lucidum* is a mushroom of temperate as well as tropical zones, a high temperature near 30°C 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.

With proper perspective on over-all growth-parameters, 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 increase in ventilation to reduce CO_2 build-up from the drastic increase in respiration from *Ganoderma* spp fruiting. Differentiation of *Ganoderma* spp fruiting is highly sensitive to CO_2 concentration, which determines whether antler-shaped fruiting bodies (CO_2>0.1%), or fruiting bodies with a well-formed pileus (CO_2 < 0.1%) will be produced. CO_2 concentration at 0.04-0.05%, as close to fresh air as possible (0.03% CO_2), should be maintained for production of pileated mushrooms (mushrooms with caps). Air humidity can be supplied by a fine mist (1-2, or 3-4 times/day).

HARVESTING MUSHROOMS

From primordia formation to fruiting-body for harvest 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 TREATMENT

Dry the harvested fruiting bodies immediately, air dry under the sun or with heat (60°C). 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 (60°C). Improper prolonged drying lowers the quality of the product; the underside pore surface turn dark brown or become contaminated by molds.

DISCUSSION AND CONCLUSION

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

Ganoderma spp natural-log growers should be thoroughly familiar with how the mushroom grows and what the proper growth parameters for each developmental stage are, 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. Methodology 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* spp organically. Unsound practices, with accompanying risks of undesirable environmental contamination, have been reported in connection with cultivation on logs (Mushroom Growers' Newsletter, Sept., 2001).

Whether to use sawdust synthetic-log cultivation in bags or to use natural-log cultivation for *G. lucidum* is a continuing question. Successful natural-log cultivation produces *Ganoderma* spp fruiting bodies with superior quality. Thick and firm fruiting bodies are produced with desirable coloring and luster which command good prices in markets in Southeast Asia. However, the yield may be lower. Production time may also be a little longer (Chen and Chao 1997). The major issue is conservation of natural

forest resources a significant environmental concern. Selection of logging should be carefully done, choosing very old forests without any significant environmental impact. Long-term planning of forestation should be coordinated with log cultivation.

Table 3. *Ganoderma lucidum*: growth parameters for cultivation.

	Humidity (%)	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 mon. 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 hr. 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). Source: Chen 1999, Stamets 2000.

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