Cultivation of *Lentinula Edodes* on Synthetic Logs

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INTRODUCTION

Lentinula edodes (Berkeley) Pegler = *Lentinus edodes* (Berkeley) Singer is best known by its Japanese name (shiitake), Chinese name (xiangu), and French name (lentin). This is, by far, the most important specialty mushroom in North America (Chen, et al., 2000; Humble, 2001; Royse, 1997; Stamets, 2000). In China, where it was originated, xiangu means mushroom with great aroma (xian means aroma; gu means mushroom). Two highly prized forms of xiangu are dongu, the winter shiitake (dong means winter), and huagu, the flower shiitake (hua means flower). Both forms with thick meaty mushroom caps are produced at winter-like temperature. Huagu, the most sought-after shiitake and the most expensive on the global market, is a form of dongu with flower-like cracking pattern on the upper surface of the mushroom cap (Wang et al., 2000).

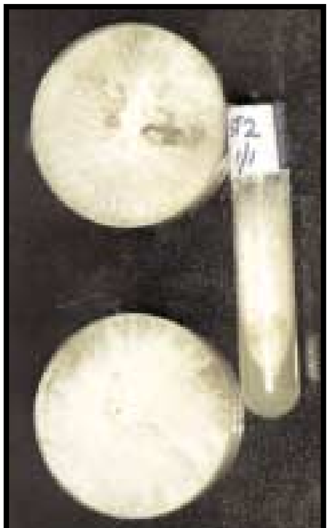

Figure 1: Stock culture in malt agar slant and colonies with whitish mycelia (Dr. Noel Arnold)

L. edodes, a mushroom primarily of temperate climate, is indigenous in the far east. No wild shiitake has been found in North America or Europe until recently. Ammirati (1997) and Desjardin (1998) reported siting of wild shiitake in the state of Washington and California. These mushroom inhabitants in natural habitats in the United States of America are likely wild transplants from domesticated cultivars, possibly runaway disposals.

Cultivation of shiitake on natural logs began in China almost a thousand years ago. Wu, Sang Kwuang in Zhejian Province in China was credited as the ingenious observer who figured out how to enhance fruitings in shiitake that grew wild in nature (Miles and Chang, 1989). Scientific methodology evolved much later, when Dr. Shozaburo Minura in Japan developed the technique of inoculating natural logs with pure shiitake mycelial culture in 1914 (Mimura, 1904, 1915 in Stamets, 1993).

During the early twentieth century, my father, Prof. Chang-Chich Hu, of Jing-Ling University (now Nanjing University) in Nanjing, China, was one of the pioneers trying to improve shiitake cultivation practice in China, after he returned from advanced studies at Tokyo University in Japan (Huang ed., 1987). Only about two decades ago, in 1979, after a dozen years or so in research, China succeeded in large-scale shiitake synthetic-log cultivation on substrate blocks in bags, a much faster production compared to cultivation on natural logs (Huang ed, 1987). Today, China remains one of the largest producers, consumers and exporters of shiitake. In 2000, imports of shiitake to Japan rose 33% to 42, 057 tons. These mushrooms had a value of $93.65 million. Almost all of the shiitake imported to Japan came from China (*The Mushroom Growers' Newsletter*, June, 2001).

In the United States of America, shiitake cultivation began to take off between 1986-1996, following the lifting of a ban on importing life cultures of *L. edodes* by USDA in 1972 (Royse, 1997). Now, fresh shiitake, cultivated by American growers is, no doubt, the leading specialty mushroom in supermarkets across the country. Dry shiitake has a long-standing history as a mushroom treasure in Oriental grocery stores, particularly in China towns and other Oriental communities.

Table 1
Strain Classification by Fruiting Temperature

Low temperature	10ºC
mid temperature	10-18ºC
high temperature	20ºC and above
wide-range temperature	5-35ºC
(e.g. strain, China-Stamets-2)	

Today, shiitake cultivation, using synthetic-log cultivation, is widely practiced, not only in southeast Asia (China, Taiwan, Japan, Korea, Singapore, the Philippines, Sri Lanka and Thailand) but also in North America (The United States and Canada), Europe (with France leading, Germany, the Netherlands, Spain, Italy, England, Switzerland, Belgium, Finland, Sweden), Australia and New Zealand (Oei, 1996; Romanens, 2001). Shiitake cultivation is, indeed, a global industry.

For medicinal benefits, refer to Hobbs, 1995; mizuno, 1999; Stamets, 1999, 2000. This paper focuses on North American shiitake synthetic-log cultivation, using Chinese methodology and other studies as references.

STRAIN SELECTION

Shiitake strains vary widely, particularly in fruiting temperature and mycelial maturation (early or late; shorter or longer production time). Substrate selectivity, growth rate (some fast strains may produce pre-mature fruiting), quality (shape, size, thickness, color, flavor and aroma, etc.), yield and ecological adaptability to extreme temperature (usually cold tolerance) are also strain-related.

Based primarily on the Chinese system, strains are classified into 4 categories according to their fruiting temperatures:
Faced with massive imports, the Japanese developed a number of new shiitake strains with large and thick basidiocarps (Watanabe, 2001). Performance and stability of superior strains are both important. Experienced growers know the potential problems of strain attenuation. For example repeated subcultures and prolonged storage of the stock culture may result in smaller fruiting bodies and lower yield (Huub Habets, Forest Products, Schimmert, the Netherlands).

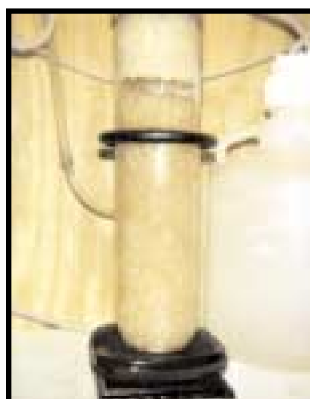

Figure 2: Liquid Spawn (Moore Mushroom Farm Labs, Glenmont, Ohio)

SUBSTRATE SELECTION

Aged broadleaf sawdust has been used in China for shiitake cultivation (Huang ed., 1989;1993).

Without aging, fresh sawdust can be used for production of shiitake only if it is from high quality tree species, such as those graded 4, excellent by FAO (Oei 1996). Oak, chinkapin, hornbean, sweetgum, poplar, alder, ironwood, beech, birch, willow are examples of commonly used hardwoods in the U.S. Sawdust from tree species of lower quality, however, has to be aged by fermentation (Oei 1996, Ting 1998, Wu et al. 1995). Growers typically select the best and least expensive, locally available substrate materials. Fermented Eucalyptus sawdust, for example, is used in Australia. Some growers prefer to use aged sawdust regardless of tree species.

Table 2

Sawdust-based Substrate Formulae for Shiitake

A. Wu (1993)		
	sawdust	100 kg
	wheat or rice bran	23.25 kg
	gypsum	2.5 kg
	calcium superphosphate	0.5 kg
	sucrose	1-1.5 kg
	water	10-140 kg
B. Stamets 1993, p.162,; 2000		
	sawdust	100 lb (or 64 gal.)
	woodchips	50 lb (or 32 gal)
	rice or rye bran	40 lb (or 8 gal)
	gypsum (calcium sulfate)	5-7 lb (or 1 gal)
	water	60%
C. The Forestry Research institute of New Zealand		
	pine	6 parts (monterey pine-Pinus radiata)
	Hardwood	3parts (beech or poplar)
	Grain	1 part (barley)
D. Straw-based substrate(Oei, 1996, p. 198)		
	rice straw	50 kg
	wheat straw	20 kg
	sawdust	20 kg
	sucrose	1.3 kg
	CaCO3	1.5 kg
	citric acid	0.2 kg
	CaSO4	0.5 kg

SUBSTRATE FORMULATION

Both substrate nutrients and physical textural property in aeration are important. Sawdust should not be smaller than 0.85 mm. (Royse, D. J. and Sanchez-Vazquez, J. E. 2000).

For commonly used hardwood, sawdust-based formulations, see Table 2. Many growers use a simple substrate with sawdust, bran and 1% $CaCO_3$ (Oei, 1996, p. 194). 1% sucrose is also frequently added. In addition to hardwood, use of pine is a subject of great interest, since pine is a readily available resource in some areas. Supplemented pine-hardwood substrate (Table 2. Formula C) was used as partial substitute for basal ingredient by the Forestry Research Institute of New Zealand for shiitake production with satisfactory results. Agricultural wastes, such as cottonseed hulls, corn cobs, bagasse, and straw can also be used as alternative basal ingredients. For additional formulae, see Hsu, 1997; Miles and Chang (1989) and Oei (1996, pp.198, 200).

SUBSTRATE STERILIZATION

Sterilization depends on the nature of the bags (polypropylene or polyethylene), bag size and, nature and amount of the substrate. For 2-3 kg. Sawdust-based substrate in polypropylene bags, sterilize in an autoclave for 2 hr. at 121ºC. Adjust this rule-of-thumb as necessary.

SPAWNING

In general, "through spawning" (spawn thoroughly mixed with the entire substrate) in larger bags is used in the U.S., while top or localized spawning (spawn is left on the substrate surface or the inoculation hole) in smaller bags is used in China and Australia. Through spawning gives a much faster growth rate. Heat-sealed larger bags with microporous breathing filters, partly filled with the substrates, allow mixing spawn with the substrate by shaking mechanically or manually. Smaller bags with ring necks and plugs, however, when fully loaded without leaving any air space in bags do not lend themselves to through spawning.

MANAGEMENT OF GROWTH PARAMETERS

Stamets (1993, 2000) summarized the growth parameters for shiitake cultivation presented in Table 3.

Table 3

Growth Parameter Management

Stamets,(1993, 2000)

	Spawn Run	Induce Primordia	Fruiting
Temperature	21-27ºC 10-16ºC* (70-80ºF) for all strains	16-21ºC** (50-60ºF) (60-70ºF) temperature fluctuation	16-18ºC* 21-27ºC** (50-70ºF) (60-80ºF)
Humidity	95-100%R.H.	95-100%R.H.	60-80%R.H.
Incubation (Strain-dependent)	ca. 1-2 mo.	5-7 days	5-8 days
CO2	>10,000 ppm	<1000 ppm	<1000 ppm
Ventilation (air exchanges)	0-1/hr	4-7/hr.	4-8/hr.
Lighting	50-100 lux	500-2000 lux	500-2000 lux

(green to uv At 370-420 nm; <500 lux = long stem)

*cold temperature strains, ** warm temperature strains

HOW SHIITAKE GROW

Production of shiitake involves both a vegetative phase of mycelial growth and maturation, and a reproductive phase of fruiting-body formation. It is imperative for growers to grasp the concept and observe closely this continuous process with intricate physiological changes and morphogenesis, focusing on the transition from the vegetative phase to the reproductive phase.

SPAWN RUN

The "spawn run" phase is the period of mycelial growth and maturation. Fresh and vigorous spawn of appropriate age should be used for spawn run. This intricate vegetative phase includes 5 stages. All shiitake strains show optimal mycelial growth at 25ºC.

The duration of spawn run is usually 1-4 months, depending on strains and methodology. No light is necessary during spawn run, however, some light in the day/night cycle towards the end of the spawn run is conducive to induction of primordia. Different approaches can be used, such as short exposure to light (e.g. 4 hr/day-night cycle (Royse, 1997), or use a low level of light, 50-100 lux (Stamets, 2000), throughout spawn run. The dramatic change from vegetative mycelial growth to produce macroscopic fruiting bodies in the reproductive phase requires enormous amount of energy reserves. A vigorous spawn run is of ultimate importance. It should be noted that strains vary greatly in duration for mycelial maturation. For one strain, 60 days is sufficient to mature, whereas this would be insufficient time for another strain (Miles and Chang, 1989).

Stage 1. Mycelial Growth

Immediately following inoculation, whitish shiitake mycelia begin to grow on the supplemented substrate, until colonization is completed. This is an active assimilation phase with high fungal metabolic rate. Enzymes are activated to break down complex substrate components (e.g. cellulose, hemicellulose and lignin) into simpler molecules which can be absorbed by mycelia as nutrients for growth and propagation.

In special cultivation practices, colonized mycelial blocks are subjected to higher temperature toward the end of spawn run: 1) exposing colonized blocks to 25-27ºC for a week before fruiting in Japan (Watanabe, 2001); 2) incubating colonized mycelial blocks at the upper limit, 27ºC-30ºC for a period of time in China (Miles and Chang, 1989). The rational for this methodology is based on the conjuncture that higher temperatures that do not promote mycelial growth may facilitate the degradation of sawdust. It is not clear whether these conjectures have been supported by independent studies. Some growers spray water on colonized mycelial blocks without bags to promote mycelial maturation and browning (Watanabe, 2001). Be aware these effects may vary by strain.

Figure 3: A heat-sealed cultivation bag with a micro-filter breathing window (Unicorn Imp. & Mfg. Corp., Commerce, Texas)

It helps to keep in mind that some fast-growing strains may produce unexpected pre-mature fruiting before mycelial maturation (not desirable). Care should be taken to move the blocks as little as possible during spawn run as moving (physical shock) may trigger pre-mature fruiting in these strains. Fast-growing and shorter production time may not be the best choice. The resulting mushrooms may not have the meaty texture desired in Asian markets, but could be acceptable in newer markets elsewhere. Usually, slow growth at winter-like temperature produces high quality dong-gu, the winter shiitake or huagu, the flower shiitake, the most expensive and sought-after shiitake, formed at cold and dry temperature with diurnal fluctuations.

Stage 2. Mycelial Coat Formation

Two to four weeks after spawning, toward the later stages of the spawn run, a thick mycelial coat forms on the outer surface of the colonized substrate block. Initially the coat is white in color. At high CO2, a very thick mycelial coat could be formed.

Stage 3. Bump Formation (blister stage, or popcorn stage)

Clumps of mycelia appear as blister- or popcorn-like bumps of various sizes on the surface of the mycelial coat in most strains. This usually begins when colonization of white mycelia covers the entire substrate in the bag, or sometimes earlier. Primordia are produced at the tips of some of these bumps. However, most bumps are aborted and never develop into fruiting bodies. Time of bump formation varies with strains, substrate and temperature. Usually bumps form 10 days faster at 25ºC than at 15ºC (Miles and Chang, 1989). Fluctuation of temperature and high CO_2 concentration encourage bump formation. Lower the CO_2 in the bag, when bumps become too numerous by cutting slits on the bag. In any case, some aeration should be provided when bumps are formed.

Figure 4: Cultivation bags with supplemented substrate (top); colonized substrate blocks with whitish mycelia in cultivation bags (bottom).

Stages 4. and 5. Browning and bark-formation

There are two different approaches, browning outside of the bag vs browning inside of the bag. Some growers remove the entire bag when browning covers 1/3-1/2 of the mycelial coat in the bag (Oei, 1996). Royse (1997) recommends removing the bags before pigmentation, thus allowing browning to occur outside of the bag.

Figure 5: Browning (pigment formation) initiation and completion in bags.

Timing of bag removal is crucial. Yield can be effected if bag removal is too early or too late. Maintain 60-70-% R.H. to avoid contamination after bag removal. Air enhances browning. Mycelia turn reddish brown at the surface in exposure to air and eventually forms a dark brown protective, dry, and hardened surface which functions like a tree bark. The inner substrate becomes soft and moist as a consequence of fungal metabolism. The inner moisture content can be as high as 80% (Oei, 1996), ideal for fruiting formation.

Fruiting Induction (Oei 1996)

Apply fruiting induction when spawn reaches physiological maturity and after browning and bark formation. Water soaking is commonly used for fruiting induction after browning and bark forming. Table 4 lists the factors that promote fruiting.

Table 4
Factors in Primordia Initiation
(Oei, 1996; Watanabe, 2001)

- water soaking (Royse 1997: 2-4 hr. at 12oC; Stamets 1993: 24-48 hr.)
- temperature fluctuation
- high humidity
- removal of CO2, or increase of oxygen supply
- physical shocks (agitation, disturbance), stab (with a long metal needle) and inject (with water), turn the blocks up-side-down (half-way during spawn run: Watanabe, 2001), beating (e.g. natural logs), electric stimulation

Basidiocarp Formation

- primordia formation at the tip of the bump (blister)
- formation of the young mushroom button (dark brown)
- elongation of the stipe (stalk) as the button increases in size
- expanding (opening) of the mushroom cap; color becomes lighter

For a description of shiitake mushrooms, refer to Huang ed., 1987).

Harvest, Post Harvest and Subsequent Flushes

Lower the humidity to 60% R. H. for 6-12 hr. before harvesting for better shelf life. Harvest when the edge of the mushroom cap is still in-rolled, or when the mushroom cap is only partly extended (60-70%). This is the form desired by the Asian markets. Hand pick the mushroom by holding the mushroom stalk and gently twisting it from the substrate block. Trim the end of the stalk after harvest when necessary. Cut off residual stalk stubs from the substrate. Lower the humidity to 30-50% R.H. at 21ºC, for 7-10 days of dormancy (Stamets, 1993), then soak the substrate block for up to 12 hours for the second flush, and up to 18 hours for the third flush (Royse 1997). Adjust as necessary. Larger bags with more substrate produce more flushes.

Table 5
Features of American Synthetic-log Cultivation

- Use heat-sealed larger bags with microfilter windows
- Use larger amount of supplemented substrates
- Through spawning
- Mechanization
- Growing indoors under controlled parameters
- Growing mushrooms organically

In China and Japan, shiitake quality is determined by shape (rounded with downward in-rolled edge before the cap is fully extended and central stalk), texture (thick and tight context, the meaty part), size, color, flavor (enhanced by cooking especially the fresh shiitake) and aroma (enhanced by drying). Freshness and freedom from pests and impurities are also critical factors for high quality mushrooms.

Shiitake Cultivation in the U.S.

In contrast to growers in southeast Asia, heat-sealed larger bags with microfilter breathing windows are used in general by growers in North America. These bags, each filled with 2-3 kg, or more (5.5 kg) substrate, produce more flushes of mushrooms in shorter production time by using through spawning. This is less labor intensive, less time-consuming, and results in less contamination.

Table 6
National Organic Standards for Crops and Wild Harvests

1. No pesticide. 3% tolerance of pesticide residue.
2. Growing field pesticide free for three years.
3. No hormones.
4. No sewage sludge fertilizer (reprocessed wastes).
5. No ionizing irradiation.
6. No genetic engineering.

Mushrooms are produced under indoor controlled growth parameters. Mechanization is used by more sophisticated growers. There is a tendency for North American growers to use faster growing strains, especially the new growers, to gain confidence. Shiitake mushrooms produced by growers here are sold fresh. The markets of specialty gourmet mushrooms in North America are fairly new with, perhaps, some less sophisticated customers.

U.S. NATIONAL ORGANIC STANDARDS (www.ams.usda.gov/nop; *The Mushroom Growers' Newsletter*, Feb. 1998; personal communication, 2001)

In recent years, pollutants such as heavy metals and others, have been found in mushroom products. In rare cases, higher concentration of heavy metal was detected in fruiting bodies than in the substrate and casing soil used (Li et al., 1998). The potential capability for fungi to absorb and accumulate heavy metals in mycelia and fruiting bodies has been demonstrated (Huang et al., 1998). To cultivate mushrooms organically is an effective way to safeguard the quality of mushroom produce and products. Organic farming was a $60 billion market in the United States in 1999. It is advisable to adopt the process of growing mushrooms organically regardless of the specific methodology a grower chooses to use.

NOP (the National Organic Program) under the Agricultural Marketing Service of the USDA published a proposed rule for a national organic program (7 CFR 205) for public comment. On March 7, 2000, they released a new proposal for uniform and consistent national standards for organic food. Certification is required for farmers and wild-crop harvesters who wish to use the organic label on their products. A specific rule for organic standards for mushrooms will be announced in the near future. In general, for crops and wild harvests to meet the national organic standards, the standards listed in Table 6 apply.

Production of Huagu, the Flower Shiitake

Introduction

Huagu, the flower shiitake, occurs spontaneously in nature when mushroom spores are deposited under cold and dry winter months. Huagu is not a genetically inherent trait. On the contrary, huagu, the shiitake mushroom with a unique morphological flower-like cracking pattern on the cap, is produced through manipulation of growth parameters. Success in cultivation of huagu can bring growers considerable extra income in China for domestic and foreign consumption. Model huagu production systems can be found in Gutien County, Fujian Province and Quingyuan County, Zhejiang Province, in China. As a dry produce, huagu varies in quality. White huagu with deep and wide cracking grain and thick context (mushroom meaty part) tops the grading scale, while dark tea-colored with less pronounced cracking grain are inferior.

The principle of huagu formation

Growth parameters are manipulated during the formation of shiitake basid-

Figure 6: Young shiitake mushrooms with dark caps formed after soaking in water (Dr. Noel Arnold)

iocarps (fruiting bodies), under winter or winter-like conditions. When the young mushroom buttons (not primordia) reach 2-3 cm in diameter, dry air and cold temperature force the pilial (cap) surface into dormancy. Under such adverse environment with drastic diurnal fluctuation of temperature and humidity, a protective dry surface is formed on the young mushroom cap. However, the inner context continues to grow at a slow pace with water available solely from the substrate. When favorable growth conditions return, the surface grows at a retarded rate, while the inner context develops at a normal pace. Under these conditions, shiitake mushroom buttons grow with the alternation of dormancy and growth,

Figure 8: Shiitake cultivation in the U.S.

Figure 7: Growth of young shiitake mushrooms: the color of the caps become lighter and the size larger as the stipes elongate.

and considerable differential growth rate between the surface and the inner context. In time, the rapid growth of the inner context ruptures the mushroom surface, producing a flower-like cracking pattern on the cap surface, thus the name, huagu.

Selection of strains for huagu production

Use low temperature, high quality and easily adaptable strains for huagu production. Strains towards the lower temperature margin in mid-temperature range can also be used. Examples of desirable huagu strains are: L-241-1, Jean-Yin #1, Yee-You #5, 7402, N-06. Strain characteristics should be thoroughly studied before cultivation. For fruiting outdoors, time of spawning should be

Figure 9: Shiitake cultivation in the U.S.

coordinated with the maturation characteristics in order to benefit from the winter stimulation. For example, strain 7402, N-06, late maturing strains, should be inoculated early during March and April, while 9018, Le 204, early to mid-maturing strains, should be inoculated in May-June in Bi-Yang, China (Yu 1998). Adjust for your local weather.

Forcing of Huagu

Initiate huagu forcing when the mushroom buttons reach 2-3 cm (diameter). If huagu forcing is applied too early when the buttons are smaller than 1.5 cm (diameter), the fragile young buttons may die of drought or freezing. If the technique is applied too late ,when the mushroom already reach 3.5 cm (diameter) or larger, the mushrooms do not respond readily.

Figure 10: Shiitake cultivation in the U.S.

Huagu Forcing Technology
(Ting, 1994, Xu, 1998, Huang, 2000)

There are three stages of huagu formation (Yu, 1998):

1. Pre-conditioning by low temperature. Subject shiitake buttons of the proper developmental stage (from primordia to 2cm in diameter) to:
   Temperature 8-12ºC
   Humidity 85-90% R. H. (remains high)
2. Forcing Huagu as follows when buttons reach 2-2.5 cm (diameter).
   Temperature 15ºC +- 1ºC (8-18ºC)
   Humidity 50-67% R.H.
   apply misting of water when <47% R.H.
   Substrate moisture 50-55%
   Diurnal fluctuation: temperature/humidity
3. Enhancing huagu formation when caps reach 3.5 cm in diameter to maturity:
   Temperature 15-25ºC
   Humidity 55-65% R. H.

Conclusion

Shiitake cultivation, including shiitake synthetic-log cultivation, is a global industry. In the United States, *Lentinula edodes*, no doubt, is the most important specialty mushroom. Despite increasing interest in growing this species, successful cultivation is a challenge. Appropriate strains must be used for a given methodology, particularly in fruiting temperature and mycelial maturation. Strains vary greatly not only in fruiting temperature and the time required for spawn maturation but also other traits. Experienced growers know that strain attenuation (degeneration) can be a problem. Close attention should be given to crucial stages during transition from intricate vegetative phase to reproductive phase. Techniques for forcing huagu, the most expensive form of shiitake in the global market provide inspiration for aspiring growers. American shiitake growers using 2-3 times or more substrate volume per bag than Asian growers have the advantage of using through-spawning. Through-spawning produces more flushes of mushrooms in shorter time, saves labor, save time, and results in less contamination. In the fierce competition with inexpensive imports, production of organic shiitake with reliable high quality, is the right direction to follow to meet the increasing interest in shiitake as a culinary delight and beneficial medicinal in the 21st century!

Figure 11: Haugu (flower shiitake) cultivation in Tibet

Table 7
Huagu Forcing

Subject shiitake buttons, 2-3 cm in diameter, to the following conditions.

Dry air	65% R.H. no misting or spraying of water
Cold temperature	8-12ºC Optimum 5-15ºC
Diurnal fluctuation	10ºC in difference, desirable. exaggerate the difference by using covers during the day only.
Substrate moisture	55% for controlled slow growth inject water into the substrate

When too dry:
- Shorten exposure to winter sunshine with 70% shading
- Improve drainage to maintain low humidity
- Line the ground with coarse sand

Already formed cracks on the mushroom cap can be re-sealed by new growth during Rainy, cloudy or misty weather.

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Figure 12: Huagu (flower shiitake) cultivation in China.

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