Commercial Production of Shiitake (*Lentinula edodes*) Using Whole-log Chip of *Quercus*, *Lithocarpus*, and *Acer*

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ABSTRACT:

In pilot-scale trials of shiitake production using: three shiitake strains; eight media, composed of three woods (*Quercus*, *Lithocarpus*, and *Acer*); and three particle sizes (coarse and fine whole-log chip plus sawdust), all combinations produced mushrooms. Fine, whole-log chip of *Lithocarpus* had the highest yields.

1 INTRODUCTION

This paper describes experiments to test the use of whole-log chip of three hardwood tree species in commercial shiitake production.

Lentinula edodes (Berk.) Pegler, known as "xiangu" in China and "shiitake" in Japan and elsewhere, is a major mushroom crop in Asia. China and Japan are the largest producers; North America and Europe together produce only about 1% of the world crop (Chang 1987; Royse *et al*. 1985).

Traditionally, shiitake is grown on natural oak-log sections. However, expansion of production to non-traditional areas and depletion of suitable trees in traditional growing areas, has led to use of enriched, heat-treated sawdust in plastic containers (Przybylowicz and Donoghue 1988).

Qingyuan, a major mushroom producing province in China, is estimated to have produced 7,000 metric tons of dry "xiangu" in 1993 (56,000 tons fresh equivalent) on about 97 million "artificial logs", which required 810,000 m^3 of "sawdust" (Han 1994; Wu and Wu 1994). In contrast, the USA produced about 2,500 metric tons of fresh shiitake in 1995, mostly on sawdust blocks requiring by our estimate less than 25,000 m^3 of sawdust (USDA 1995).

In the USA, Europe and Japan the sawdust used is usually a sawmill residue from saws used to cut logs into boards. Consequently, it is relatively low in bark and sapwood. In China, on the other hand, most growers use "sawdust" produced by chipping and hammer-milling delimbed, whole trees of a variety of species. Their "sawdust" contains bark and more sapwood.

Oregon has an expanding shiitake industry, concerned about the sustained availability of substrate. Most production comes from sawdust blocks, and there may not be enough local hardwood sawmills to supply saw-kerf residue for future expansion of the industry. One goal of our work was to test the use of whole-log chip as a substitute for sawdust.

A second goal was to determine the optimum particle size of whole-log "sawdust", and how to produce it economically. Although Nisikado *et al*. (1941) and Ohga (1990) tested the effects of relatively fine (1-5 mm) particle size on growth and yield of shiitake, we found no studies of coarser material.

A third objective was to test which, if any, of three commercial mushroom strains will produce economically viable crops on these new substrates.

2 MATERIALS AND METHODS

2.1 *Culture strains*

We tested three shiitake strains maintained in our laboratory: CS-41, CS-53 and CS-287. All three strains are in commercial use. Each strain differs in its response to substrate and growing conditions (Donoghue and Denison 1995).

2.2 *Wood: species and treatment*

We used three indigenous tree species: Oregon white oak (*Quercus garryana)*, big leaf maple (*Acer macrophyllum*) and tanoak (*Lithocarpus densiflora*).

Fresh oak and maple sawdusts, from local sawmills, were used as controls. Tanoak sawdust was not available.

Freshly cut, whole, delimbed logs, 20-60 cm diam DBH, were chipped by mobile chipping units designed for commercial pulp chip production. The bark was not removed prior to chipping. Half of the resulting chip was further reduced to make coarse chip using a 500 Hp fixed-hammer hammermill with 75mm (2.5 inch) screens (West Salem Machinery Inc, USA). The remaining half was resized to make fine chip, using a 20 Hp chipper-shredder with 19mm (3/4 inch) screens (Crary Bearcat, USA). This machine has knife-edged swing-hammers. Both units had adequate output for commercial-scale shiitake production.

Table 1. Particle size distribution of oak wood used for shiitake media

	US Standard Screen Size			
	< 1/8" (< 3mm)	1/8" to 1/2" (3mm to 13mm)	1/2" to 1" (13mm to 25mm)	>1" (>25mm)
Primary chip	2%	17%	67%	15%
Coarse Chip	7%	61%	31%	1%
Fine Chip	22%	77%	1%	0%
Sawdust	84%	16%	0%	0%

Distribution given as percentage of wood by weight remaining between screens

Samples of all wood types were separated between different size US standard screens to determine particle size distribution. Table 1 shows the particle size distribution of the primary oak chip, oak sawdust, coarse oak chip and fine oak chip. Particle size distribution was similar for both tanoak and maple. The resized chip was not screened further prior to incorporating it in test media.

2.3 *Media preparation and inoculation*

The eight test media were each composed of the chip or sawdust of one of the woods, supplemented with millet grain and wheat bran in a ratio of 78% wood: 11% millet: 11% wheat bran (dry weight). Carbon/nitrogen (C/N) ratios were determined for each of the different chip and sawdust types, and the amendments using a C/N analyzer.

Each medium was thoroughly mixed, and hydrated to 58% moisture with tap water, then distributed into 12 polypropylene bags prior to sterilization. The bags (Unicorn Inc., USA), equipped with microporous filter patches, were each filled with 2.5 kg (wet weight) of medium, then autoclaved at 121^{o} C for 3.5 hrs.

After cooling, the bags were inoculated at a spawning rate of 3% (wet weight spawn/dry weight substrate) with rye grain spawn. The bags were sealed and the spawn mixed throughout the medium.

2.4 *Mushroom production: incubation, fruiting, and harvest*

Management during incubation, fruiting, and harvest duplicated methods commonly used in commercial farms in Oregon.

The inoculated media were incubated in bags for 94 days at 22^{o} C to 24^{o} C, with 12 hrs/day of light from cool white fluorescent lamps. The room CO_2 level remained below 0.16%.

Each week we measured CO_2 levels in the headspace of three bags per treatment, using a Riken RA-411A infrared CO_2 detector. Blocks were weighed periodically during incubation to determine losses in wet-weight due to respiration and evaporation. Visible signs of development were noted and recorded, specifically: completed colonization of the substrate; onset and extent of surface lumping and browning; and development of primordia or mushrooms.

After an incubation period of 94 days, the blocks were removed from their bags and placed in a fruiting room kept at 14^{o} C and 95% RH for 7 days, then at 18^{o} C and 90% RH for 14 days, during which time the first flush of mushrooms was harvested. Then, the blocks were kept for 7 more days at 22^{o} Cand 85% RH.

Light was provided throughout for 12 hrs/day with cool white florescent lamps and the room CO_2 level was maintained below 0.16% by ventilation.

After 28 days in the fruiting room the blocks were submerged in 15^{o} C tap water for 5 hrs to induce another flush of mushrooms, and then returned to the sequence of conditions described above. This was repeated twice more for a total of four fruitings per block in 112 days.

The occurrence and extent of contamination was noted between fruitings. Badly contaminated bags were removed.

Mushrooms were harvested daily when the cap margins were 80% opened. The mushrooms were sorted into two grades: firsts and seconds. Stipes were trimmed according to market standards. The number and weight of marketable mushrooms harvested from each block was recorded by market grade.

We chose to express yield as the percent of marketable mushroom (fresh weight) per dry weight of original medium, where marketable mushrooms consisted of grades one and two, minus stem trimmings and unsalable mushrooms. Yield differs from biological efficiency (BE), which includes discards. Yield is conservative, reflecting grower's interests, but less objective than BE, because it is affected by current market standards.

2.5 *Experimental design and analysis*

We tested 24 combinations of strain and medium; three strains tested on eight media. Each strain/medium combination was replicated as 12 bags during incubation, reduced to 10 bags for fruiting. During incubation and fruiting, bags were arranged in a randomized complete plot design. Harvest data were analyzed using the program Statview II. A 95% confidence level ($P<.05$) was used for ANOVA and Fisher's Least Significant Difference Test (FLSD) with a probability of $p=.0001$, or less, considered significant.

The economic implication of each test medium was determined by using harvest data and raw material costs as inputs into NMC economic farm models developed by Northwest Mycological Consultants, Inc. (Donoghue 1994; Hiromoto 1991).

Input values for labor and the costs of goods, services and materials were used that are typical for shiitake farms in Oregon. The cost per “unit”(1 unit =200 ft^3 = 5.83 m^3, enough for 1,000 bags) of sawdust was given at $75 for oak and $65 for maple. The resized chip was valued at $200 per unit, which competes with pulp chip prices in our area and includes the cost of resizing. An additional equipment expense of $5,000 was used when modeling chip media to cover the purchase of a chipper/shredder.

Profitability was modeled using the following assumptions: a weekly block production of 1,000 blocks; farm gate prices at $4.25/lb ($9.35/kg) for #1 mushrooms and $2.75/lb ($6.05/kg) for #2 mushrooms. We used dollars and pounds to reflect the US market.

3 RESULTS

3.1 *Incubation*

Media of all wood species and particle sizes supported normal growth and development of the three strains tested. However, there were differences in development attributable to strain, wood, or particle size.

More than 90% of the bags were fully colonized by the fourth week. No strain or particle size differences were seen in the rate of colonization. Maple was colonized slowest and tanoak colonized soonest; more than 90% of the tanoak bags were fully colonized by week three of incubation.

Between weeks four and seven, block surfaces began to show lumps of fungal tissue characteristic of shiitake. This lumpy “skin” is thought to affect mushroom production (Miller and Jong 1987). Strain CS-41 formed lumps soonest and produced larger lumps than the other strains. Coarse chip had smaller lumps than other media. Wood species had no effect on lumping.

Oxidative browning of the block surface typically follows lumping, given sufficient aeration of the bag (Donoghue and Denison 1995; Lelly and Feher 1994). Browning is often used as indication that blocks are ready to fruit. Strain CS-41 was quickest to brown regardless of medium; blocks were fully brown by the sixth or seventh week. More than 90% of all blocks were fully browned by the ninth week. In general, tanoak browned earliest of the woods, as did coarse chip among the particle sizes.

Beginning between weeks six and seven, CS-41 alone produced true pins that later formed into mushrooms prior to bag removal. By week eight, all treatments produced "star pins", i.e. primordia that emerge through the surface, producing star-shaped cracks.

Changes in CO_2 concentration within bags varied with strain, in agreement with our earlier work (Donoghue and Denison 1995). Particle size also affected the CO_2 profile. Media with larger particles had lower levels of CO_2. This trend is illustrated by the CO_2 profiles for the three strains on maple wood of different particle sizes (Fig. 1).

There were significant differences in wet-weight loss during incubation between strains, between wood types and between media of different particle sizes. Highest average weight loss for all media combined occurred with CS-41 (10.9%); the lowest with CS-287 (9.0%). All together, coarse chip had less weight loss than fine chip or sawdust, which were about equal (9.3%, 10.2, 9.9%, respectively). Of the wood types, tanoak lost the most weight followed by oak and maple (10.5%, 10.0%, 9.2%, respectively).

The initial C/N ratios of media ingredients varied. The supplements were lowest; millet at 30:1 and wheat bran at 16:1. Whole-log chip had lower ratios -- maple at 128:1, oak at 166:1, and tanoak at 176:1 -- than corresponding sawdusts -- maple 141:1, oak 186:1. Oak bark had a C/N ratio of 69:1, that was close to our target C/N ratio (60:1) for supplemented media.

Weeks of Incubation

Particle size: thick solid line, coarse chip; thin solid line, fine chip; dashed line, sawdust

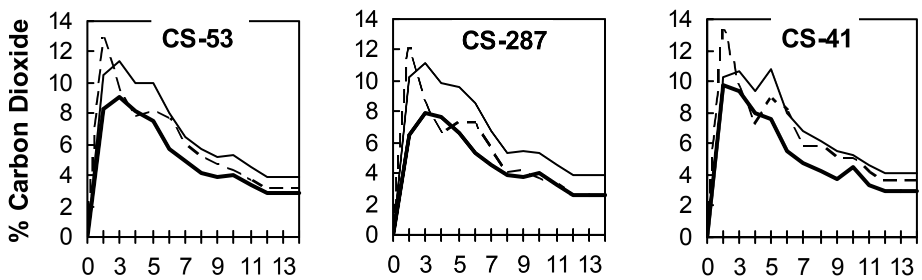


Fig. 1 Carbon Dioxide levels in the headspace of cultivation bags during incubation of three shiitake strains on maple wood of different particle size

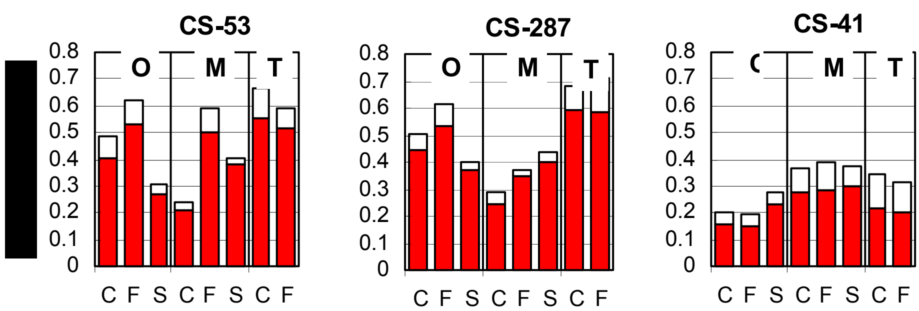
3.2 *Mushroom production*

All treatments produced mushrooms. Differences in yield could be attributed to strain, wood, or particle size. Figure 2 shows the relationship of yield to medium composition by shiitake strain.

Single factor ANOVA of all treatments showed the following trends. Strains CS-287 and CS-53 had a significantly higher yield that CS-41. Tanoak wood media had significantly higher yield than oak and maple wood media. Fine chip media had a significantly higher yield than coarse chip media and the sawdust-based control media.

Two-factor ANOVA by strain suggests that yield of the test strains was affected differently by wood type, particle size and the interaction of the two factors. The most significant difference in yield of CS-53 was attributed to particle size, with the fine chip media having a higher yield than both sawdust and coarse chip. Wood type had the most significant effect on yield of strain CS-287 with tanoak having higher yields than oak which had higher yields than maple. Only wood type significantly affected yield of strain CS-41 with maple out-producing the other two woods.

There were no significant differences in the percent of grade number two mushrooms produced (range: 5% to 37%) or in the amount of contamination of the blocks during fruiting attributable to wood type or particle size. However CS-41 produced a higher percentage of grade two mushrooms and had higher rates of contamination than the other two strains. Over all treatments, mushroom size was generally larger with lower yields.



Particle Size Total Yield FLSD=11% P<.05

Yield given as fresh weight marketable mushrooms/dry weight original substrate x 100. Dark bar is grade #1, white bar is grade #2. Wood species are: O, oak; M, maple; T, tanoak. Particle sizes are: C, coarse chip; F, fine chip; S, sawdust.

Fig. 2 Yield of three shiitake strains on media of different wood species and particle size

Table 2. Cost of production and profitability of modeled shiitake farming using fruiting trial data for different wood types

Treatment		Marketable	Yield	Percent	Income	Production	Pre-tax Profit	
Wood type	Strain	Yield %	lb /block	#2 grade	gross $ /lb	cost /lb	per lb	per year
Oak sawdust	CS-287	40%	0.87	8%	$4.13	$3.74	$0.39	$17,739
Maple sawdust	CS-287	44%	0.96	9%	$4.12	$3.46	$0.66	$32,745
Oak fine chip	CS-287	60%	1.31	12%	$4.07	$2.86	$1.21	$82,388
Maple fine chip	CS-53	59%	1.29	14%	$4.04	$2.90	$1.14	$76,467
Tanoak fine chip	CS-287	71%	1.55	17%	$4.00	$2.75	$1.25	$117,253

Cost of production and profit based on NMC economic model. Income based on trial yield and farm gate prices of: grade #1 at $4.25/lb, grade #2 at $2.75/lb. Yield % is fresh weight marketable mushrooms/dry weight substrate x 100.

3.3 *Economic Analysis*

Table 2 shows cost of production and profitability from different runs of the NMC economic model using yield data from the highest individual strain/medium combination for each of the two types of sawdust and the three species of chip. Fine chip media had the lowest costs of production and the highest profit per lb of mushroom produced, even with higher raw materials costs.

4 DISCUSSION

This work showed whole-log chip of all tree species tested could be used for shiitake production. This is the first report on the use of tanoak (*Lithocarpus densiflora*) for shiitake production. The tanoak chip media matured the quickest during incubation and gave higher yields than either of the more traditionally used woods and the sawdust controls.

Whole-log chip is a different resource than sawdust as it is not tied to the lumber industry. Tree species, like tanoak, that may be locally abundant but are not used for lumber production can be utilized for growing shiitake as whole-log chip. This greatly expands the resource base available for shiitake production.

Whole-log chip of small diameter trees contains more sapwood and bark than sawmill sawdust, making it chemically distinct. Lumber sawmills remove the bark and much of the sapwood before sawing boards from the remainder of the log.

Shiitake derives its nutrition mainly from the sapwood and bark of natural logs (Fukuda *et al.* 1987; Tsuneda 1988; Tsuneda 1991). Oak bark is used in France to supplement straw for shiitake production (Olivier and Delpech 1990).

We found the C/N ratio of *Q. garryana* sawdust to be nearly three times as high as oak bark, with bark having 0.53% nitrogen and sawdust having only 0.27% . This is in agreement with Okada *et al*. (1993) and Kawachi *et al*. (1992) who found that the levels of nitrogen and other minerals such as P, K and Mn were highest in the bark and outer sapwood and decreased toward the center of the tree. Whole-log chip of oak and maple had a C/N ratio that was 10% less than the respective sawdust, presumably due to higher levels of "nitrogen-rich" bark and sapwood in the whole-log chip. These nutritional differences make questionable any comparisons about the effects of particle size between sawdust and whole-log chip.

Particle size affected growth, development and yield of shiitake. The finer chip had greater weight loss, higher CO_2 levels during incubation and ultimately gave higher yields when compared to the coarse chip. This suggests that nutrients in the fine chip were more available to shiitake.

We identified acceptable particle-size distributions for chip used in shiitake production and found commercially available and affordable equipment that could be used to resize pulp chip to these specifications. The finer chip mixture gave higher yields than the coarse mixture, indicating that reducing the pulp-chip size was warranted. The particle size of the whole-log "sawdust" used in China is between our fine chip mixture and our sawdust (unpublished data). Additional tests will be needed to determine if the extra costs involved in further reducing the chip size would be offset by increases in yield.

Two of the three commercial strains tested produced economically viable crops on whole-log chip. The data points to the importance of matching wood type and particle size with shiitake strain. Prior research has shown strain specific responses to substrate composition, incubation time and other management practices such as pinning temperatures and gas concentrations (Donoghue and Denison 1995; Ohga 1992; Royse and Bahler 1986).

Strain CS-41 preformed poorly overall under the management regime of this work. The incubation time was much longer than is needed for this strain as evidenced by the production of fruit bodies in the bag during incubation. Gas levels in the cultivation bag were suboptimal for this strain which produces better given a more aerated incubation environment (Donoghue and Denison 1995). Optimal management for this strain may have given different yield results.

Resized pulp chip can be used economically even with the relatively higher costs associated with purchasing and re-sizing the raw chip. Only small increases in yield over sawdust based media are needed to offset the additional costs.

This is because sawdust is cheap, only accounting for 2% to 3% of the cost of production. Using whole-log chip only increases this percentage to 6% or 7%. However, the data show that choosing the appropriate combination of strain, medium and management is essential to profitability.

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