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Effects of substrate moisture content, log weight and filter porosity on shiitake (*Lentinula edodes*) yield

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ABSTRACT

Production costs for shiitake (*Lentinula edodes*) are on the rise in the United States due to increasing expenses including materials, labor and energy. Increased yield and improved bioconversion of raw materials may improve grower profit margins and may help reduce the cost of shiitake to the consumer. Two crops (Crop 1 and 2) of shiitake were grown to evaluate effects of three substrate moisture contents (50%, 55% and 60%), two log weights (2.7 and 3.2 kg) and three porosities of bag filter (low, medium and high) on mushroom yield (g/log) and biological efficiency (BE). Yield data were collected under controlled environmental conditions for two breaks. The formulation with 55% substrate moisture gave the highest yield and BE. Higher mushroom yields were produced from heavier logs (3.2 kg), but BE was not significantly affected. Filter porosity significantly affected yield and BE in Crop 1 but not in Crop 2. Significant interactions were observed for log moisture content × filter porosity for both crops. There were no significant two-way interactions observed for filter porosity × log weight or three-way interactions observed for moisture content × filter porosity × log weight. Maximum yields were obtained from 3.2 kg logs with a substrate moisture content of ca. 55% using medium or low porosity-filtered bags. This study may provide growers with additional information to better optimize production practices and become more efficient and competitive.

1. Introduction

Production of shiitake (*Lentinula edodes*) worldwide increased more than 110-fold from 1936 (12,000 ton) to 1997 (1,321,600 ton; Chang, 1999, 2005). Most of this increase occurred in the 1990 s. Shiitake accounted for 26% of production of various mushroom varieties worldwide and ranked second after the button mushroom (*Agaricus bisporus*) in 1997 (Chang, 1999). China is the major shiitake producer, accounting for 85.1% of total world production in 1997 (1,125,000 ton). In the United States, production of shiitake increased nearly 6-fold from 1987/1988 (540 ton) to 2006/2007 (3122 ton) (USDA, 2007).

Most commercial production of shiitake is done on synthetic logs that contain hardwood sawdust, straw or corncobs as the basal ingredients and starch-based supplements (10–60% dry weight) such as wheat bran, rice bran, millet, rye, and maize. Sufficient water is added to adjust the moisture content of the mix to about 60% (Royse et al., 1990; Royse and Sanchez, 2007). For commercial production, the mix is weighed and filled into plastic bags automatically by machine so that a uniform amount (usually about 2.7 kg) is added to each bag. The bags are made of heat-resistant polypropylene and contain a special filter patch of laminated microporous plastic. The microporus patch provides a microbial barrier to contaminants and allows gas exchange with the outside environment during substrate colonization (Royse and Sanchez-Vazquez, 2001).

Growers, using two different methods, accomplish the substrate colonization phase either by completing initial spawn run and browning of the substrate inside the bag or by completing initial spawn run inside the bag and browning outside the bag. Browning is a term used by growers to describe the light-induced pigment that is formed by the leather-like mycelium (pellicle) on the surface of the log and is positively correlated with mushroom production (Matsumoto and Kitamoto, 1987). Browning in the bag generally requires a longer period of colonization, often 60–90 days before fruiting induction, while the second method requires only 42–49 days. Browning in the bag has the advantage of less handling and reduced management input while browning outside the bag requires special management techniques such as the control of carbon dioxide levels and humidity, as well as watering log surfaces to hasten browning. Browning outside the bag also allows for the use of higher levels of supplement and thus, potentially higher yields (Royse, 1997, 2001).

In the United States, commercially available sawdust logs grown with shiitake mycelium range from 2.5 to 2.7 kg (wet wt) and vary somewhat in shape. The shape of the plastic bag filled with

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supplemented sawdust determines the final shape of the log (Royse and Bahler, 1989). Larger logs (greater wet wt) are appealing to growers since they require less handling and fewer units per quantity of substrate as compared to small logs. Filling more substrate into the same bag by increasing the height of the fill, provides larger logs without increasing the size of a log's "footprint". Thus, larger logs may allow more substrate per production house because the logs' footprints are similar for both larger and smaller logs.

Donoghue and Denison (1995) found that oxygen and carbon dioxide levels inside the bags during 77-days of browning in the bag varied with the strain, metabolic activity, and stage of development of the fungus, as well as the size of the microporus filter patch ventilating the bag. They found that yield, mushroom size, number of mushrooms produced, and the severity of contamination during cropping were affected by filter patch aeration.

Optimum moisture content of synthetic logs during spawn run is not known. However, for natural logs, optimum moisture content for hyphal elongation is in the range of 55–70% (Akiyama et al., 1974). Major shiitake growers in Pennsylvania have target substrate moisture contents of 58–60% based on empirical information. Detailed investigations on the effects of moisture content of sawdust-based substrate during spawn run on yield and BE of shiitake have not been conducted.

The objective of this study was to investigate the interactions of log weight, filter porosity, and substrate moisture content on yield capacity and BE of shiitake logs with initial spawn run inside the bag and browning outside the bag.

2. Methods

2.1. Substrates and preparation

The general substrate formulation (oven-dry wt) consisted of 45% Pennsylvania red oak (*Quercus rubra* L.) sawdust, 20% white millet (*Panicum miliaceum* L.), 25% rye (*Secale cereale* L.), 10% wheat bran (*Triticum aestivum* L.) and 0.1% gypsum ($CaSO_4$). Three moisture contents (50%, 55% and 60%) of the substrates were evaluated. All ingredients were combined in a ribbon blender, and water was added to raise the moisture content of the mix to the desired level. Final substrate moisture contents were determined with an Infrared Moisture Determination Balance (Kett Electric Laboratory, Model FD-770). The majority of the moistened mix was filled into each bag with a filling machine and then adjusted by hand to reach the target weight. Two substrate weights (2.7 and 3.2 kg) were selected based on current formulations. The filled bags were stacked on racks, wheeled into an autoclave, sterilized for 2 h at 121 °C, cooled in a clean room, and inoculated with 20 g shiitake spawn/bag (spawn line S6 for both crops). The bags then were heat-sealed and the spawn was through-mixed into the substrate by shaking with hands (Royse and Shen, 2005).

2.2. Bags and filter porosities

Bags were obtained from Unicorn Import and Manufacturing (Garland, TX) and are manufactured from heat-resistant polypropylene fitted with a microporus filter patch to provide gas exchange during spawn run. Three types of filters with various air exchange capacities (described by the manufacturer as low, medium and high porosity) were selected for evaluation based on their current use in the industry for spawn and mushroom production. Low porosity filters (Type 14) require 12.7–20.3 cm water column pressure to start bubbling when the filter is under water. The pore size ranges from 0.3 to 0.5 μ nominal with percentage of pores/unit square <45%. Medium porosity (Type 3TN) filters require 5.1–7.6 cm of water column pressure, 0.3–0.5 μ pore size, percentage pores/unit square >70%. High porosity filters (Type 3BN) require 1.3–2.5 cm water column pressure, 1–5 μ pore size, percentage pores/unit square >90%.

2.3. Spawn run, browning and soaking

Spawn run, browning and production for both crops was conducted at 21 °C in the same standard Pennsylvania "double" mushroom house containing approximately 800 m^2 shelf space. Crop 1 (batch T123) was inoculated on 3rd May 2007 while Crop 2 (batch T301) was inoculated on 28th October 2007. Light was provided during spawn run by cool white fluorescent bulbs for 4 h/day. After 18–20 days, plastic bags were removed and logs moved to a "browning house". Log browning and soaking after first and second break were conducted as described by Royse and Sanchez-Vazquez (2001, 2003).

2.4. Harvesting and determination of BE and quality

Mushrooms were harvested from the substrate when they were mature and the margins of the pilei incurved. Harvesting normally occurred 7–11 days after soaking. At the end of the crop, mushroom yields for each log for two breaks were calculated and accumulated data was used to estimate the percentage biological efficiency (BE) ([weight of fresh mushrooms harvested/substrate dry matter content] × 100).

2.5. Experimental design

Two crops (1 and 2) were conducted in a 3 × 3 × 2 factorial design to evaluate the effects of three levels of substrate moisture (50%, 55% and 60%), three filter porosities (low, medium and high), and two log weights (2.7 kg and 3.2 kg) on BE and mushroom yield per log. Eighteen treatment combinations of substrate moisture contents, filter porosities and log weights were tested with 20 replicates per treatment. For Crop 1, the full complement of 360 experimental units were used in the final analysis while in Crop 2, 354 experimental units were used in final analysis due to breakage or contamination of six logs distributed among five treatments. The SAS program JMP (SAS Institute, 2006) was used to analyze data using a one-way analysis of variance (ANOVA) and a least squares analysis to examine factor interactions. The Tukey–Kramer Honestly Significant Difference (HSD) test was used to separate treatment means (SAS Institute, 2006).

3. Results

3.1. Sources of variation

Significant sources of variation for mushroom yield from the ANOVA for Crops 1 and 2 included moisture content, log weight, and moisture content × filter porosity (Table 1). Filter porosity

Table 1
Probabilities (P)[a] greater than Fisher's (F) test from analysis of variance for two crops (1 and 2) for (*Lentinula edodes*) yield and biological efficiency (BE%)

Source	df	Crop 1		Crop 2	
		Yield	BE (%)	Yield	BE (%)
Moisture content (MC)	2	<.0001	0.0007	<.0001	<.0001
Filter porosity (FP)	2	0.0085	0.0067	0.2613	0.1357
MC × FP	4	0.0005	0.0001	0.0105	0.0077
Log wet wt (LW)	1	<.0001	0.767	<.0001	0.8712
MC × LW	2	0.0761	0.0238	0.7166	0.5535
FP × LW	2	0.3697	0.385	0.1909	0.1564
MC × FP × LW	4	0.9237	0.9163	0.8037	0.793

[a] P values less than 0.05 were considered significant.

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was a significant source of variation in Crop 1 but not in Crop 2. Significant interactions were observed for log moisture content × filter porosity for both crops. There were no significant two-way interactions observed for filter porosity × log weight or three-way interactions observed for moisture content × filter porosity × log weight. BE was significantly affected by moisture content and moisture content × filter porosity in both crops. However, BE was only affected by filter porosity and moisture content × log weight in Crop 2. Log weight did not significantly affect BE in either crop (Table 1).

3.2. Yield and BE

In Crop 1, mushroom yields ranged from a high of 1022 g/log from 3.2 kg logs at 55% moisture with substrate incubated in bags containing a filter patch with medium filter porosity to a low of 615 g/log from 2.7 kg logs at 60.7% moisture incubated in bags containing high filter porosity (39.8% difference) (Table 2). For Crop 2, overall yields were generally lower than for Crop 1. Mushroom yields in Crop 2 ranged from a high of 794 g/log from 3.2 kg logs at 55.4% moisture incubated in bags containing a filter patch with low porosity to low of 509 g/log from 2.7 kg logs at 60.4% moisture incubated in bags containing medium filter porosity (35.9% difference) (Table 3).

Across all treatments, mean mushroom yields were significantly higher from logs weighing 3.2 kg compared to 2.7 kg logs (Table 4). Mean yields were 16.8% and 17.4% higher from heavier logs in Crops 1 and 2, respectively. There was no difference in mean BEs between the lighter and heavier logs in either crop (Table 4).

Means and groupings from the ANOVA for substrate moisture content for Crops 1 and 2 for mushroom yield and BE are presented in Table 5. Mushroom yields were significantly greater when moisture contents of 50% or 55% were used compared to 60% moisture. In Crop 1, yields were 16.1–16.7% greater on logs with 55% or 50% moisture contents compared to logs with 60% moisture content. In Crop 2, yields were 21.1–14.1% greater on logs containing 55% or 50% moisture compared to logs containing 60% moisture. BEs were greatest from logs containing 55% moisture in both crops.

Table 2
Yield and percentage BE (BE%) for *Lentinula edodes* produced on substrate at three moisture contents contained in plastic bags with three filter porosities and two log weights (Crop 1)

No.	Substrate moisture content (%)[a]	Filter porosity[b]	Log wet weight (kg)	Yield (g/log)[c]	BE (%)[c]
1	50.4	L	2.72	802.2 bcde	59.4 bc
2	50.4	M	2.72	871.9 abcd	64.5 abc
3	50.4	H	2.72	865.2 abcd	64.0 abc
4	50.4	L	3.18	881.7 abcd	55.9 c
5	50.4	M	3.18	960.2 ab	60.9 abc
6	50.4	H	3.18	928.9 abc	58.9 bc
7	55.0	L	2.72	738.5 def	60.2 bc
8	55.0	M	2.72	879.4 abcd	71.7 ab
9	55.0	H	2.72	808.9 bcde	66.0 abc
10	55.0	L	3.18	936.6 abc	65.5 abc
11	55.0	M	3.18	1021.9 a	71.5 ab
12	55.0	H	3.18	899.8 abcd	62.9 abc
13	60.7	L	2.72	723.7 def	67.6 abc
14	60.7	M	2.72	683.1 ef	63.8 abc
15	60.7	H	2.72	615.2 f	57.5 c
16	60.7	L	3.18	923.6 abc	73.9 a
17	60.7	M	3.18	845.2 abcde	67.7 abc
18	60.7	H	3.18	761.1 cdef	60.9 abc

[a] At time of spawning.
[b] L = low, M = medium, H = high.
[c] Means followed by the same letter within the same column are not significantly different according to Tukey's honestly significant difference (HSD) ($P = 0.05$).

Table 3
Yield and percentage BE (BE%) for *Lentinula edodes* produced on substrate at three moisture contents contained in plastic bags with three filter porosities and two log weights (Crop 2)

No.	Substrate moisture content (%)[a]	Filter porosity[b]	Log wet weight (kg)	Yield (g/log)[c]	BE (%)[c]
1	50.0	L	2.72	595.5 defg	43.7 d
2	50.0	M	2.72	611.5 cdefg	44.9 cd
3	50.0	H	2.72	665.9 abcdef	48.9 abcd
4	50.0	L	3.18	710.4 abcde	44.7 cd
5	50.0	M	3.18	763.6 ab	48.1 abcd
6	50.0	H	3.18	759.1 ab	47.8 abcd
7	55.4	L	2.72	707.6 abcde	58.2 a
8	55.4	M	2.72	658.0 abcdef	54.2 abcd
9	55.4	H	2.72	669.7 abcdef	55.1 abc
10	55.4	L	3.18	793.9 a	56.0 ab
11	55.4	M	3.18	777.7 a	54.9 abc
12	55.4	H	3.18	743.2 abc	52.4 abcd
13	60.4	L	2.72	575.9 efg	53.4 abcd
14	60.4	M	2.72	509.1 g	47.2 bcd
15	60.4	H	2.72	559.9 fg	51.9 abcd
16	60.4	L	3.18	723.5 abcd	57.5 ab
17	60.4	M	3.18	624.7 bcdefg	49.6 abcd
18	60.4	H	3.18	604.5 cdefg	48.0 abcd

[a] At time of spawning.
[b] L = low, M = medium, H = high.
[c] Means followed by the same letter within the same column are not significantly different according to Tukey's honestly significant difference (HSD) ($P = 0.05$).

Table 4
Means and groupings from analysis of variance for synthetic log weight for two crops (1 and 2) for (*Lentinula edodes*) yield and biological efficiency (% BE)

Log wt (kg)	No reps	Yield (g/log)[a]	BE (%)[a]
Crop 1			
2.73	180	776.4 b	63.9 a
3.18	180	906.5 a	64.2 a
Crop 2			
2.73	176	616.5 b	50.8 a
3.18	178	723.6 a	51.0 a

[a] Means followed by the same letter within the same column and crop are not significantly different according to Tukey's honestly significant difference (HSD) ($P = 0.05$).

Table 5
Means and groupings from analysis of variance for substrate moisture content for two crops (1 and 2) for (*Lentinula edodes*) yield and biological efficiency (% BE)

Moisture content (%)[a]	No reps	Yield (g/log)[b]			BE (%)[b]		
		Total	Break		Total	Break	
			1st	2nd		1st	2nd
Crop 1							
50.4	120	885.0 a	552.0 a	333.0 a	60.6 b	37.7 c	22.9 a
55.0	120	880.8 a	583.5 a	297.3 a	66.3 a	44.0 b	22.3 a
60.7	120	758.6 b	580.6 a	178.0 b	65.2 a	50.0 a	15.2 b
Crop 2							
50.0	120	684.3 a	393.3 b	291.0 a	46.3 c	26.6 c	19.7 b
55.4	117	726.5 a	434.4 a	292.1 a	55.1 a	33.0 b	22.1 a
60.4	117	599.8 b	445.1 a	154.7 b	51.3 b	38.1 a	13.2 c

[a] At time of spawning.
[b] Means followed by the same letter within the same column and crop are not significantly different according to Tukey's honestly significant difference (HSD) ($P = 0.05$).

Filter porosity significantly affected yield only for Crop 1, where medium filter porosity gave significantly greater yields than high filter porosity bags (Table 6). However, there was no significant difference in yields between medium and low filter porosities. A similar trend was noted for the effect of filter porosity on BEs.

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Table 6
Means and groupings from analysis of variance for filter porosity for two crops (1 and 2) for (*Lentinula edodes*) yield and biological efficiency (% BE)

Filter porosity	No reps	Yield (g/log)[a]	BE (%)[a]
Crop 1			
Low	120	834.3 ab	63.8 ab
Medium	120	876.9 a	66.7 a
High	120	813.2 b	61.7 b
Crop 2			
Low	120	684.4 a	52.3 a
Medium	119	669.0 a	49.8 a
High	115	657.4 a	50.7 a

[a] Means followed by the same letter within the same column and crop are not significantly different according to Tukey's honestly significant difference (HSD) ($P = 0.05$).

4. Discussion

With the trend toward the use of more synthetic medium for the production of shiitake worldwide, it is desirable to identify those variables that influence mushroom yield. In this study, we identified at least two variables that are important for increasing mushroom yield, i.e. substrate moisture content and log weight. Bag filter porosity had less impact on mushroom yield than the other two variables.

Optimum mycelial growth and mushroom production is dependent upon adequate moisture and gas exchange within the substrate. The moisture content of the substrate also changes the physical structure of the logs. In a high moisture log, less substrate dry weight is filled into the bags. In addition, there is less air space between substrate particles. Ohga (1990) demonstrated that air spaces saturated with a higher moisture content may slow the gas exchange from deep within the interior of the log to the surface. We observed that after spawn run was completed, higher moisture logs were softer, smaller and had a thicker, leather-like pellicle on the surface. These logs tended to have a heavier first break, but a much lower second break. Although the BE was relatively high, the yield/log was significantly lower. Growers are seeking greater yield/log, so a high moisture content log with lower dry substrate weight would not be desirable. Our observation on the driest logs (50%) was nearly opposite to that observed for greater moisture (60%) content logs. Yields were lower on first break but higher on second break. Yield distribution patterns for first and second break on logs with 50% substrate moisture were more evenly distributed compared to high- and medium-moisture content logs. In addition, BE was greatest on 55% moisture logs.

The most common log weight used by large shiitake producers in Pennsylvania for synthetic log production is 2.7 kg. In China and Thailand, 1.4–2 kg-logs are more common (Fan et al., 2005; Thevasingh et al., 2005). In this study, we found log weight had little effect on BE, but had a significant effect on mushroom yield/log. Adding additional dry wt to a bag by either decreasing the moisture content to 55% or increasing log size to 3.2 kg (or preferably both) would add little to the cost of production but would add substantially to the potential productivity of each log. Furthermore, increased shiitake production per m^2 of production surface could be realized. The cost per kg of mushrooms produced would be lowered due to the utilization of less production space, and a reduced requirement for labor, handling, bags and energy. For large commercial producers, the saving could be substantial.

Royse and Bahler (1989) examined the interactions of log diameter (log dry wt) and genotype on yield, BE and mushroom size. They evaluated four log sizes ranging in diameter from 15.4 cm to 32.4 cm (log dry wt ranging from 909 g to 4489 g) over a 70-day harvest period. They found that BE decreased with a log size of 29 cm × 27 ($D \times H$) (dry wt 3634 g) regardless of genotype although one genotype was much less affected than the other one. In the present study, the greatest dry wt per bag used was 1589 g/log and only one genotype was evaluated. It is not possible to make a direct comparison of our highest yields with those reported by Royse and Bahler (1989) because they harvested for 70 days while our harvest period was only ca. 28 days. In addition, higher yielding genotypes have supplanted those mentioned by Royse and Bahler (1989) so it may be worthwhile to examine other genotypes in combination with larger logs and lower moisture contents.

In recent years, polypropylene or polyethylene bags containing a microporous breather strip for gas exchange have become popular shiitake production containers. Although some growers still make holes in the bags for aeration, a filtered bag provides more consistent results and limits contamination. The cost of filters compensates for the cost of labor to punch holes in the bags. Our study showed a significant effect for filter porosity in Crop 1 but not in Crop 2. The reason for this difference remains unexplained, but we did observe that substrate incubated in bags containing a filter with low porosity tended to have thicker mycelial pellicles than bags with higher filter porosity. This slight difference in pellicle formation could be the reason for the non-consistent results observed between Crop 1 and 2. Crop 1 was produced in summer while Crop 2 was grown in winter so seasonal variation may have contributed to differences in pellicle formation. Virtually nothing is known about factors influencing pellicle formation on the surface of logs although growers are cognizant of the importance of this phenomenon on disease resistance, water uptake and log integrity.

We found that the combination of moisture content and filter porosity had a significant effect on yield and BE for both crops. Our empirical observations indicate that moisture (relative humidity) in the spawn run room has a pronounced effect on mycelium growth inside the bags. It is unclear to what extent filter porosity has on the moisture exchange between the environment inside and outside the bags. Donoghue and Denison (1995) speculated that different bag filter sizes might attribute to water vapor loss and affect yield. Further study is needed to determine the effect of substrate moisture content and room humidity on different stages of shiitake production. Donoghue and Denison (1995) conducted the most comprehensive study to date, but results apply only to growers who brown their substrate inside the bag. Since our substrate was kept inside the bag for a much shorter period (17–19 days vs. 77 days), it may be worthwhile to assay and monitor the carbon dioxide and moisture levels both inside and outside the bag during spawn run.

The cost of growing shiitake is on the rise in the United States due to increasing expenses including materials, labor and energy. In China, shiitake growers have developed many cultivation techniques to lower their production costs and stay competitive in both domestic and international markets (Shen et al., 2004). This study may provide growers in the United States with additional information to better optimize production practices and become more efficient and competitive.

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