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Influence of wood chip particle size used in substrate on biological efficiency and post-soak log weights of shiitake

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ABSTRACT: Wood chips from seven commercial sawdust sources were profiled by sieving materials through ten US standard sieve sizes (4 to 0.21 mm). The majority (mean=70.7%) of wood chips passed through US standard sieve size 16 (<1.18 mm). Sawdust of particle size 0.5-0.85 mm accounted for the single largest particle size class (mean=32.2 %). The next single largest class had a particle size distribution of 0.85-1.18 mm (mean=17.8%). Shiitake was grown in three crops to determine the effect of four particle size classes (1= 2.8-4.0 mm; 2= 1.7-2.8 mm; 3= 0.85-1.7 mm and 4= <0.85 mm + 2 controls) on mushroom yield. In addition, logs from two crops were weighed after each soak (3) to determine the effect of particle size on water up-take. Yield from substrate prepared with wood chip particle size class 4 (extra fine; <0.85 mm) was significantly less than yields from the other particle size classes and the controls. Yield from particle size class 3 (0.85-1.7 mm) was highest among the four classes. Water up-take was greater in synthetic logs made with extra fine wood chips (<0.85 mm). Profiling of wood chips at the source may help growers optimize their production media and reduce production costs.

1 INTRODUCTION

Shiitake production worldwide has increased >7-fold from 1983 (207,000 t) to 1997 (1,573000 t; Chang 1999). Most of the increase during this 14-yr period occurred in China where shiitake is widely consumed and exported (Zhang 1993, Chiu *et al.* 1999). China produced 88% of the total world output in 1997 (Chang 1999).

In the United States, both consumers and producers have enjoyed the increasing popularity of this crop over the last few years. For the 10-yr period 1990 to 1999, production of shiitake in the United States more than tripled from 1,123 t to 3,9411, respectively (USDA 1990, 1999). This production increase roughly parallels the increase in the amount of controlled-environment production space devoted to growing shiitake on synthetic logs from 1990 to 1999 (74,200 m^2 to 212,400 m^2, respectively).

Sawdust is the most popular basal ingredient used in synthetic formulations of substrate for producing shiitake in the United States (Miller & Jong 1987; Royse 1997a). Starch-based supplements (20-60 % dry wt) such as wheat bran, rice bran, millet, rye and maize may be added to the mix. These supplements serve as nutrients to provide a more optimum growth medium (Royse 1996, 1997b).

Most sawdusts used for production of shiitake originate from sawmills were band saws are used for cutting logs to make lumber. In recent years, band saw blades have decreased in band thickness due to the increasing availability of harder and stronger steel used for blade manufacture. Today, high strength steel blades may have a band thickness of only 0.55 mm. Thus, wood chips generated by band saws have become finer as blade thickness has decreased. In addition, number of teeth per cm of band also may affect wood chip size. The availability of thinner bands combined with a high number of teeth/cm may profoundly affect the distribution of wood chip sizes found in any single commercial source of sawdust.

In general, shiitake growers believe that finer sawdust is more desirable than course material because finer chips may a allow faster breakdown of the wood by the developing mycelium. The purpose of this work was to: 1) profile commercial sawdust piles for particle size distribution, 2) determine the effect of particle size classes on mushroom yield (BE), and 3) examine the effect of particle size on water up-take during log soak.

2 MATERIALS AND METHODS

2.1 *Particle size distribution of commercial sources of sawdust*

Samples (400 g) were collected from seven commercial sources of sawdust. Four random samples from each pile were collected at a site approximately 25 cm under the surface layer of each pile. Samples were pooled for each pile and then dried in an oven at 80 C for 48 hr. Samples then were screened with ten US standard sieves and percentages of wood chips remaining between screens were determined.

2.2 *Substrates and preparation*

Six substrates (two controls and four sawdust particle size classes) were prepared from mixed hardwood sawdust collected from a local sawmill in Centre County, Pennsylvania. The predominant species found in the sawdust was the Northern red oak *(Quercus rubra* L.). The sawdust was collected in the fall of 1998 and was stored in an enclosed building until it was used. The moisture content of the fresh sawdust was approximately 37% by weight. Four sawdust particle size ranges (1= 2.8-4.0 mm; 2= 1.7-2.8 mm; 3= 0.85-1.7 mm and 4= <0.85 mm) were obtained by sieving sawdust through the appropriate US standard sieves. The general substrate formulation (all ingredients based on oven dry substrate wt) consisted of 45% sawdust, 30% millet, 15% wheat bran and 10% rye.

Mixed substrate ingredients were pasteurized, cooled, inoculated and bagged with a 0.283 m3 paddle mixer. Injecting live steam into the mixer and allowing the substrate to heat to 111°C pasteurized the substrate. This temperature was maintained for 20 min with continuous agitation to insure uniform substrate heating. Passing cold tap water through a jacket fitted to the mixer rapidly cooled the substrate. Sterility of the mixture was maintained by injecting filtered air into the mixer during cool down to create a positive airflow. When the substrate had cooled to below 27°C, the substrate was spawned with 210 g rye grain spawn contained in a 500 ml Erlenmeyer flask. When the spawn was thoroughly mixed with the substrate, the resulting mixture was bagged in unused virgin polyethylene bags (20.3 cm x 12.7 cm x 50.8 cm) and closed with a twist-tie. The amount of substrate was weighed at time of filling with a digital scale placed under the bagging port of the mixer. Each bag contained 2.5 kg spawned substrate at 59% moisture (1.03 kg oven dry weight). Dry substrate weight was determined by drying 100 g of the processed substrates in an oven for 48 h at 80°C. Dry weight subsequently was used to determine the percentage biological efficiency (BE; ratio of fresh mushrooms harvested per dry substrate weight and expressed as a percentage).

2.3 *Experimental design and statistical treatment*

The experiments were a completely randomized design with 12 to 14 replicates per treatment. The general linear models procedure was used to perform an analysis of variance (SAS Institute 1998). The Waller-Duncan K-ratio t test was used to separate treatment means (Steel *et al.* 1997). The experiments were repeated three times and designated Crops I, II, and III.

2.4 *Isolate and spawn*

Isolate R26 was selected because it is a commercially used cultivar. The isolate was maintained on potato-dextrose yeast-extract agar as outlined previously by Jodon & Royse (1979). Spawn of R26 was prepared as outlined previously by Royse & Bahler (1986).

2.5 *Spawn run, log browning and soaking*

After a spawn run of 7 days, 20 slits (5 mm each) were made in the top of each bag with a sharp scalpel to provide gas exchange. At the end of 22 days incubation at 22±1°C, the plastic bags were removed and the synthetic logs moved to a "browning room". In the browning room (93-98% relative humidity, 18±1°C air temperature), synthetic logs were hand-watered lightly with a 600-hole roseface nozzle each day. At the end of 14 days in the browning room all logs were rotated 180° to provide a more uniform browning of the logs' surface. Three h of light was provided daily by cool-white fluorescent bulbs. Sufficient air changes were maintained to hold CC>2 levels below 1,900 ppm (1,200 µl/liter). At the end of 28 days in the browning room environment, the logs were soaked in cool water (13± 2°C) until each weighed approximately 2.3 kg. After each flush of mushrooms was harvested, logs were re-soaked to increase log weight to 2.3 kg. For two crops (Crops I & II), logs were weighed after each soak and data were used to calculate mean weight for each treatment.

2.6 *Harvesting and determination of BE*

Mushrooms were harvested from the substrate at the same time each day, when the veil had broken and the gills were fully exposed. The mushrooms were then counted and weighed. At the end of the harvest period (63 days), the accumulated data were used to calculate the BE. The substrate dry weights were used to calculate the percentage of BE ([weight of fresh mushrooms harvested/substrate dry matter content] x 100).

3 RESULTS

3.1 *Wood chip particle size distribution*

Particle size distributions of seven commercial sawdust sources are shown in Fig. 1. Considerable variation in wood chip size was found among the commercial sources. Approximately 98% of all sawdust particles was smaller than 4 mm while 87-99% of particles were larger than 0.21 mm. The majority (mean=70.7%) of wood chips passed through US standard sieve size 16 (<1.18 mm). However, this value ranged from 15 to 58% among commercial sources (Fig. 1).

3.2 *Biological efficiencies*

BE for Crop I was significantly lower for a wood chip particle size of <0.85 mm (79.6%; Fig. 2). While mushroom yield was highest for the substrate containing a particle size distribution of 0.85-1.7 mm (110.5%), there was no significant difference in any of the other treatments and the control treatments (Fig. 2).

Results for Crop II were similar to Crop I where mushroom BE for the substrate containing particle sizes of <0.85 mm gave the lowest yield (Fig. 2). BEs for the control treatments and the treatment containing particles of 0.85-1.7 mm were highest and not significantly different from each other. Crop II had the highest overall BE for the three crops. BEs ranged from 107.2% for the <0.85 mm particle size to 121.8% for the 0.85-1.7 mm particle size.

Overall BEs for Crop III were the lowest of the three crops (Fig. 2). However, results were similar to Crops I and II where a substrate containing wood chips of <0.85 mm gave the lowest BEs. A substrate containing wood chips of 2.8-4 mm gave the highest BE but BE was not sig-

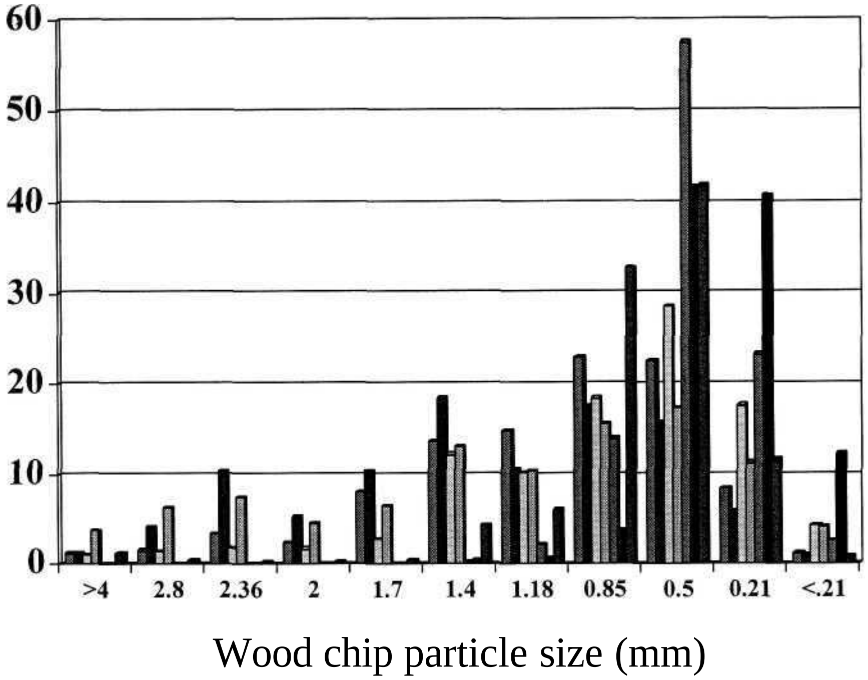

Figure 1. Particle size distribution for seven (7) commercial wood chip sources. Values are for percentage wood chips remaining between screens of US standard sieve size.

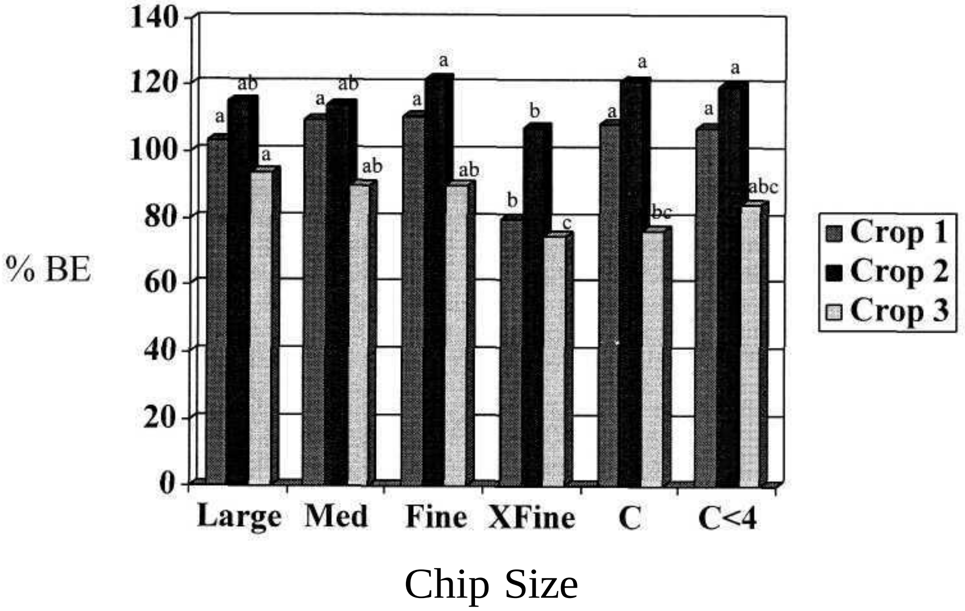

Figure 2. Biological efficiencies for four classes (Large= 2.8-4 mm; Medium=l.7-2.8 mm; Fine=0.85-1.7 mm; and XFine=<0.85 mm) of wood chips and two controls for three crops of shiitake. Means within a crop with the same letter are not significantly different (P=O.05).

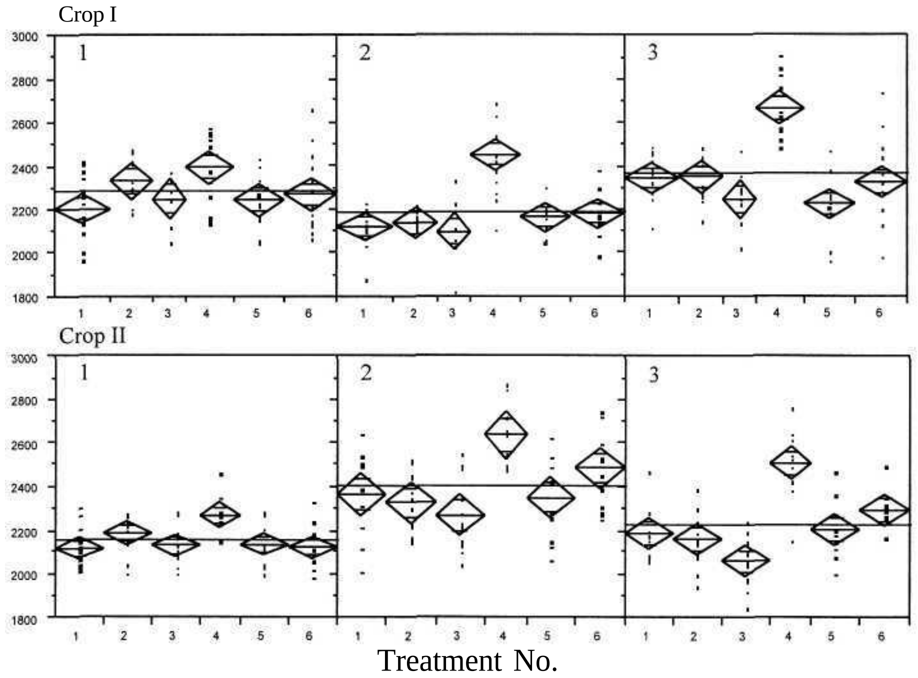

Figure 3. Post-soak weights of synthetic logs for three breaks of shiitake (two crops). Dotted line across middle indicates grand mean of treatments. Treatments are for four chip size classes (1= 2.8-4 mm; 2=1.7-2.8 mm; 3=0.85-1.7 mm; and 4=<0.85 mm) and two controls. Middle line in diamond is the response group mean for the treatment. Vertical end points of the diamonds form the 95% confidence interval for the mean.

nificantly different than one of the control treatments (control <4.0 mm). BEs ranged from 74.6% for the <0.85 mm particle size to 93.7% for the 2.8-4.0 mm particle size.

3.3 *Log weights after soak*

Synthetic log weights after soaking for three flushes (Crops I & II) are given in Fig. 3. For Crop I, grand mean of treatments for soak weights (shown by dotted line) ranged from a low of 2.2 kg for 2nd soak to a high of 2.37 kg for 3rd soak (Fig. 3). In all three soaks, treatment 4 (sawdust class 4, <0.85 mm) had significantly (P=O.05) higher soak weights. Mean soak weights for these (class 4) logs ranged from 2.4 kg to 2.65 kg per log for 1st and 3rd soaks, respectively. Differences in post-soak log weights became more pronounced as the crop progressed from 1st to 3rd soaks.

Synthetic log weights after soaking for Crop II followed a different pattern than for Crop 1 (Fig. 3). In this crop, grand mean (line) post soak log weights were higher for the 2nd flush than the 1st and 3rd flushes. Grand mean log weights for all treatments ranged from a low of 2.18 kg for the 1st flush to 2.41 kg for the 2nd flush. In all three soaks, group treatment 4 (sawdust class 4, <0.85 mm) had significantly (P=O.05) higher soak weights. Group mean soak weights for these (class 4) logs ranged from 2.29 kg to 2.62 kg per log for 1st and 2nd soaks, respectively. As observed for Crop I, differences in post-soak log weights became more pronounced as the crop progressed from 1st to 3rd soaks.

4 SUMMARY AND CONCLUSIONS

Particle size distribution for commercial sawdust varied by as much as 600% within a sieve size. For example, US standard sieve size 70 retained 41.4% of particles from one source while only 5.8% was retained by sieve size 70 for another source. Thus, substantial variation is present in various commercial sources of sawdust. Considering the potential impact of fine particles in substrate, growers may want to consider profiling their sawdust at the source prior to purchase. Such profiling could help reduce production dips when sub-optimal sawdust is used for production.

Our finding that a particle size of class of <0.85 mm may negatively impact yield was unexpected. Nisikado *et al.* (1941) found that vegetative mycelial growth of shiitake was greater on large (2-3 mm) and medium (1.5-2 mm) sized particles than on small (<1.5 mm) particles. Factors other than particle size, *per se,* however, may have influenced yield. For example, Ohga (1990) demonstrated that the vegetative growth rate of shiitake mycelium was different at the surface compared to the interior of substrate prepared with various wood chip particle sizes. As particle size decreased, the radial mycelial extension rate on the substrate surface increased while mycelial biomass decreased. Ohga (1990) suggested that oxygen (O2) depletion was the cause of reduced mycelial biomass development in substrate containing smaller particle size. Donoghue & Dennison (1995, 1996) also demonstrated that O2 and CO2 levels in the airspace above incubating substrate were correlated with subsequent mushroom yields. Thus, gas exchange restriction in substrates prepared with smaller particle sizes (<0.85 mm) may be responsible for sub-optimum yields observed in this study. Small air spaces present in small particle size media may slow gas exchange from deep within the interior of the synthetic log to the surface.

More rapid water gain during soaking for chip size class 4 (<0.85mm) consistently was observed in this study. The reason(s) for this phenomenon are not known but may be related to a lesser vegetative growth within the interior of the log as observed by Ohga (1990). Reduced vegetative growth would allow water to penetrate the interior of the log more rapidly reducing the time required to meet the desired log weight.

This study demonstrated that a substrate containing only wood chips of <0.85 mm is not optimum for production of shiitake. Alternatively, removal of all particles O.85 mm from substrates resulted in a mean (three crops) yield increase of about 5% over controls. Assuming an average commercial yield of about 800g per 2.5 kg (wet wt) synthetic log, an increase of 5% would amount to approximately 40 g per log. At 1999 prices (U.S $6.15/kg; USDA 1999), a 40 g yield increase would produce an additional $0.28 per log in revenue. It remains to be seen if a 5% yield increase at 1999 prices would justify the expense of removing wood chips <0.85 mm from commercial sawdust piles. And, because we did not test a wood chip particle size class in the range of 0.5-0.85 mm, it is not known if these particle sizes would negatively impact yield. Such a test may reveal that this particle size range is benign in regard to influence on yield. If this were the case, growers would only have to concern themselves with sieving out particles <0.5 mm (a substantial saving in raw materials). As shiitake production becomes more competitive, however, and profit margins are squeezed, growers willing to optimize their production media may have an advantage in the marketplace.

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