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Effects of Genotype, Spawn Run Time, and Substrate Formulation on Biological Efficiency of Shiitake†

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Substrate formulations of sawdust, wheat bran, and millet were inoculated with spawns from one hybrid and two parental genotypes of *Lentinula edodes*. Biological efficiency (BE) and size data on mushrooms harvested from two substrate formulations with spawn run times of 60, 90, and 120 days were analyzed. A significant genotype-spawn run time-substrate formulation interaction was observed for BE. The longer spawn runs resulted in greater BE than the shorter spawn runs. This study points to the need for more work to determine the underlying variability within genotypes.

A sustainable market for the shiitake mushroom (*Lentinula edodes* (Berk.) Pegler) has recently developed in the United States (10). There are several reasons for the increase in market demand for this and mushroom species other than the common cultivated mushroom, *Agaricus brunnescens* Peck. First, unique flavor-enhancing compounds (e.g., lenthionine) are present in *L. edodes* that are not present in other species (5, 13). Second, consumers are becoming more aware of additional possibilities in food selection and are interested in experimenting with exotic mushrooms in their cuisine both in restaurants and at home.

The increased consumer demand for the shiitake mushroom has focused the need to develop more efficient and cost-effective methods of mushroom production. Shiitake has been produced for hundreds of years in the Orient primarily by growth on hardwood logs (3, 10, 11). Relatively recent developments, however, have provided growers with the option of producing shiitake on supplemented sawdust (1, 10; M. Chu-Chou, Mushroom Newsl. Trop. 5:8–10, 1984). The advantages of producing shiitake on sawdust as opposed to natural logs include less time required to complete a growing cycle and greater biological efficiency (BE). The major disadvantage, however, is the relatively high initial investment costs.

Work in our laboratory has concentrated on increasing the economic viability of production by improving the efficiency of shiitake production on supplemented sawdust. Environmental factors such as spawn run time and substrate formulation have already been identified as important variables in the efficient production of shiitake on sawdust (7). Relatively little is known, however, concerning the performance of selected genotypes with different management practices. It has been observed that considerable genetic variation exists within *L. edodes* (8, 9), but few attempts have been made to exploit this genetic variability for improved production on sawdust.

The present study examines the effect of selected genotypes on BE and mushroom size as influenced by spawn run time and substrate formulation.

Substrates and preparation. Mixed hardwood sawdust, collected from a local sawmill in Centre County, Pa., was used as the main substrate ingredient. Although the exact ratio of hardwood species comprising the sawdust could not be determined, the predominant species was Northern Red Oak (*Quercus rubra* L.). The moisture content of the fresh sawdust was approximately 30% by weight. The two substrate formulations consisted of (i) 6,350 g of sawdust, 680 g of white millet (*Panicum miliaceum* L.), and 680 g of wheat (*Triticum aestivum* L.) bran and (ii) 6,350 g of sawdust, 680 g of white millet, and 1,360 g of wheat bran. Ingredients were combined, mixed, and processed as outlined by Royse (7). Dry matter contents of the processed substrates were determined by drying 100 g of processed substrate in an oven for 24 h at 105°C. The dry weights were subsequently used to determine the percent BE ([weight of fresh mushroom(s) harvested/substrate dry matter content] × 100).

Hybrid and parental lines. Two parental lines (WC377 and WC380) were selected because of their commercially desirable characteristics. Hybrid Le12 is a cross between single-spore isolates of WC305 and WC380. This hybrid line was evaluated in earlier trials and was determined to have desirable commercial characteristics (unpublished data).

The lines were maintained on potato-dextrose-yeast extract agar as outlined by Jodon and Royse (2). Spawns of the lines were prepared in 500-ml flasks containing rye grain (80 g), sawdust (6 g), $CaCO_3$ (2 g), and tap water (105 ml). The ingredients were autoclaved for 45 min at 121°C and allowed to cool for 24 h. The ingredients were then inoculated, and the mycelium was allowed to grow for 2 weeks. The spawn samples were shaken twice weekly to prevent clumping caused by mycelial knitting of the ingredients.

Spawn run. After inoculation and bagging as outlined by Royse (7), the substrates were weighed and moved to an incubation room where temperatures were maintained at 22 ± 3°C. To determine the effect of spawn run time on mushroom yield and size, three treatments (60, 90, and 120 days) were selected. At the end of the respective periods, the plastic bags were removed and the colonized substrates were moved to the production room.

Experimental design and environmental conditions. The experiment was designed as a randomized complete block with a 3 × 3 × 2 factorial treatment design (12). Genotypes, spawn run time, and substrate formulation were the three factors considered. The general linear models procedure was

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TABLE 1. Probabilities greater than F from analysis of variance for three factors for BE and size of shiitake mushrooms on synthetic logs

Source[a]	df	Probability > F^b	
		Size	BE
Blocks	7	0.9908	0.7306
Substrate formulation (SF)	1	0.2233	0.1592
Genotype (G)	2	0.0605	0.0001
SF × G	2	0.1993	0.8872
Spawn run time (ST)	2	0.5052	0.0001
SF × ST	2	0.2241	0.2588
G × ST	4	0.0843	0.0001
SF × G × ST	4	0.1828	0.0411

[a] Error, df = 119; CV = 69.53 and 42.90% for size and BE, respectively.
[b] Values of 0.05 or less were considered significant.

used to perform an analysis of variance (6). Tukey's studentized range test was used to separate treatment means. An analysis of variance by genotype for BE also was performed to examine individual genotype response to the two management factors.

Misting was provided for 3.5 h daily by using overhead nozzles controlled automatically. Relative humidity was maintained at 95 to 98% during periods when nozzles were not in operation. Light was provided for 4 h daily by cool white fluorescent bulbs. The lighting was controlled automatically. Temperatures were maintained at 17 ± 2°C throughout the experiment. Sufficient air changes were maintained to hold CO_2 levels below 1,200 ppm.

Harvesting and determination of BE. Mushrooms were harvested (picked 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 (64 days), the data were used to calculate BE. The BE was determined as the ratio of fresh mushrooms harvested per dry substrate (both measured in kilograms) and expressed as a percentage. For example, a 70% BE would indicate that 0.7 kg of fresh mushrooms were harvested from each kilogram of dry substrate.

BE. The BE was significantly affected by the interaction between genotype, spawn run time, and substrate formulation (Table 1). On substrate formulated with extra wheat bran (W), as spawn run time increased the BE also increased (Fig. 1). An incubation period of 120 days significantly increased BE for lines WC380 and Le12 (Fig. 1). Line WC377 increased with incubation time but not significantly. On standard substrate formulation (S) (i.e., without extra bran), line Le12 showed an increase in BE with an incubation time of 120 days (Fig. 1). This difference was significantly greater than that for either the 60- or 90-day spawn run times. Line WC377 did not show a significant difference in BE between the three spawn run times. Line WC380 showed a higher BE after a 120-day spawn run than after the 60- or 90-day spawn run time when the S substrate was used.

Comparison of the W and S substrate formulations revealed that for WC380 at a 120-day spawn run time, formulation W showed a significantly higher BE than did formulation S. For lines WC377 and Le12 there was no significant difference in BE for the two substrates for a 120-day spawn run.

Size. There were no significant differences in size for any of the factors examined.

CVs. The genotypes clearly had an effect on the amount of

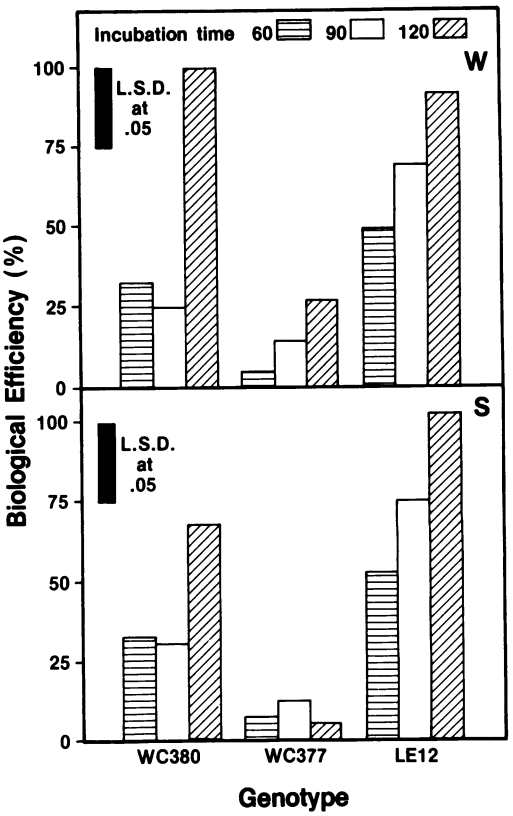

FIG. 1. BEs of three genotypes of *L. edodes* grown on synthetic logs of two different formulations and three different spawn run times. Formulation S contained sawdust-millet-wheat bran in an 8:1:1 ratio. Formulation W contained sawdust-millet-wheat bran in an 8:1:2 ratio.

variation in the data (Table 2). Line WC377, with a coefficient of variation (CV) of 126.11, is too variable to reveal BE or size differences if they truly exist. Line Le12 had the lowest amount of variation with a CV of 44.88 (Table 2).

An incubation period of 120 days resulted in the highest BE. These findings support an earlier report that longer spawn runs result in higher rates of production (7). Work by Leatham (4) with supplemented wood medium has shown that with strain ATCC 48085, sporadic and limited fruiting occurs at about 60 days after inoculation, whereas prolonged, more-reproducible fruiting occurs 105 to 150 days after inoculation. Leatham suggested that two marked changes in nutrient availability occur that permit fruiting. The first change occurs at 40 to 45 days of vegetative growth when the starch is depleted, while the second change occurs

TABLE 2. Probabilities greater than F for analysis of variance by genotype for BE

Source[a]	df	Probability > F^b		
		WC380	WC377	Le12
Blocks	7	0.8453[a]	0.8810	0.4649
Substrate formulation (SF)	1	0.2735	0.1228	0.7336
Spawn run time (ST)	2	0.0001	0.1704	0.0001
SF × ST	2	0.0590	0.1023	0.2722

[a] Error, df = 35; CV = 51.71, 126.11, and 44.88 for WC380, WC377, and Le12, respectively.
[b] Values of 0.05 or less were considered significant.

at 105 to 150 days of vegetative growth when utilizable protein is depleted (4). A grower could expect greater economic return if consistent and high BE could be obtained after 40 to 45 days of spawn run. Additional work is needed to develop more consistent and reliable production after 40 to 45 days of spawn run.

The three-way interaction observed in this study indicates the importance of the factors selected. If the objective of a shiitake research program is to reduce the time required for spawn run and to increase BE, then both genotype and nutritional factors should receive attention. For example, the CV for line WC377 indicates that this line should be eliminated from the breeding program. Additional breeding and selection would be warranted for lines WC380 and Le12. Perhaps, CVs, for the first flush of mushrooms harvested from synthetic substrate after a 40- to 45-day spawn run, could be used as one of the selection criteria in a shiitake breeding program. Given the CVs observed for genotypes used in this and other studies (7, 10), it is apparent that additional work is needed to determine the underlying variability within genotypes.

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