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Enhanced Yield of Shiitake by Saccharide Amendment of the Synthetic Substrate†

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Three experiments were performed to determine the effect of selected saccharides on mushroom yield and basidiome size of shiitake (*Lentinula edodes*) when grown on a synthetic substrate. Substrate formulations of sawdust, wheat bran, and millet were nonamended or amended with sucrose, fructose, or glucose. Addition of sucrose (0.6 to 1.2% [dry weight]) to the substrate stimulated mushroom yield by 11 to 20% or more. Addition of fructose at 1.2% and glucose at 0.6% resulted in similar yield increases. Most of the yield increase occurred on the first break. The substrate amended with 1.2% sucrose tended to have a more synchronous maturation for the second break resulting in fewer days when mushrooms were harvested.

Production of the shiitake mushroom (*Lentinula edodes* (Berk.) Pegler) in the United States and other Western countries has increased at an accelerated rate during the last 5 years (4, 6). This increase in production is in response to increasing consumer demand and to the relatively high prices farmers receive for the product. According to the United States Department of Agriculture (14), farmers received an average of $5.34 (U.S.) per lb (1 lb. = 453.592 g) for fresh shiitake in the 1987 to 1988 growing season. Producers of *Agaricus bisporus*, on the other hand, received an average of $0.95 per lb for their fresh product. Thus, production of shiitake is potentially a lucrative commercial enterprise; however, the grower must be able to provide the specialized management that this crop requires.

In the United States, most commercial production of shiitake is on synthetic logs. Royse (5, 6) and Royse et al. (9) have pointed out the advantages and disadvantages of producing shiitake on synthetic logs. The major advantages of producing shiitake on synthetic logs compared with producing shiitake on natural logs are as follows: consistent market supply through year-round production, increased yields, and decreased time required to complete a crop cycle. These advantages far outweigh the major disadvantage of a relatively high initial investment cost to start a synthetic log manufacturing and production facility.

In order to further enhance the productivity and reliability of shiitake production on synthetic logs, our laboratory has concentrated on identifying genetic and environmental factors that influence efficient production (1, 7, 8). Environmental factors such as spawn run time and substrate formulation have already been identified as important variables in the efficient production of shiitake on supplemented sawdust (2, 6). In our earlier work (6), we reported on the effects of starch-based (wheat bran) supplements on yield and size of selected lines of shiitake. However, little is known about the effect of less complex carbohydrate supplements on yield and size of shiitake, even though some researchers have advocated the use of sucrose in the substrate formulation (13). This study examines the effects of selected saccharide amendments on the biological efficiency (BE) and basidiome size of shiitake produced on synthetic logs.

MATERIALS AND METHODS

Substrates and preparation. Mixed hardwood sawdust, collected from a local sawmill in Centre County, Pennsylvania, was used as the main substrate ingredient. The predominant species found in the sawdust was the Northern red oak (*Quercus rubra* L.). The sawdust was collected in the summer of 1987 and was stored in an enclosed building until it was used. The moisture content of the fresh sawdust was approximately 40% by weight. The general substrate formulation consisted of 14,528 g of sawdust (24% moisture), 1,342 g of white millet (*Panicum miliaceum* L.) (12% moisture), and 1,342 g of wheat bran (*Triticum aestivum* L.) (12% moisture). Saccharides used as amendments to the basic substrate were sucrose (J. T. Baker Chemical Co.), glucose (Difco Laboratories), and fructose (Sigma Chemical Co.). Saccharides were added to the appropriate treatments in dry powder or crystalline form in the following amounts (percent weight of oven-dried ingredients): 80 g (0.6%), 160 g (1.2%), or 320 g (2.4%). These levels of saccharides were initially chosen because of a previously published report (13) and because preliminary experiments indicated a stimulation of yield at levels between 0.6 and 1.2%.

All ingredients were combined, mixed, pasteurized, cooled, inoculated, and bagged with an apparatus described previously by Royse (5). The substrate was pasteurized by having live steam injected into the mixer and by being allowed to reach 100°C. This temperature was maintained for 20 min with periodic (10 s each 5 min) agitation to ensure uniform substrate heating. After the substrate was pasteurized, it was rapidly cooled by cold tap water being passed through a jacket fitted to the mixer. When the substrate had cooled to below 30°C, the substrate was inoculated and bagged as outlined previously by Royse (5). Dry matter contents of the processed substrates were determined by drying 100 g of the processed substrates in an oven for 24 h at 105°C.

Isolates, spawn, and spawn run. Three lines (defined as maintenance of an isolate over time) of *L. edodes* (R3, R6, and 305) were selected because they are commercially used cultivars. The lines were maintained on potato-dextrose-yeast extract agar as outlined previously by Jodon and Royse (3). The lines used for each of the three crops were as

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follows: crop I (305 and R3), crop II (305 only), and crop III (305 and R6). Spawns of the lines were prepared as outlined previously by Royse and Bahler (6).

After the substrate was inoculated, bagged, and sealed with a twist tie, each bag was weighed and moved to an incubation room where temperatures were maintained at 22 ± 2°C. After the substrate was incubated for 7 days, 40 slits were made in the top of each bag with a sharp scalpel to provide gas exchange.

Experimental design. The experiments (crops I, II, and III) were designed as randomized complete blocks with factorial treatment arrangements of five replicates each (11). The factors considered for each crop were as follows: crop I, sucrose (0, 0.6, 1.2, and 2.4%) and line (305 and R3); crop II, sucrose (0, 0.6, 1.2, and 2.4%) only; crop III, line (305 and R6), saccharide (sucrose, fructose, and glucose), and saccharide rate (0.6 and 1.2%). The general linear models procedure was used to perform an analysis of variance (10). Fisher's protected least squares difference test was used to separate treatment means (11).

Experimental conditions. After the completion of spawn run (90 days), the plastic bags were removed and the synthetic logs were placed in a production room. Misting was provided for 3 h daily by overhead nozzles controlled automatically (5). Relative humidity was maintained at 95 to 98% during periods when nozzles were not in operation. Three hours of light were provided daily by cool-white fluorescent bulbs. The temperature was maintained at 17 ± 2°C throughout the experiments. Sufficient air changes were maintained to hold CO_2 levels below 1,200 ppm (1,200 μl/liter).

Harvesting and determination of BE and basidiome size. 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 (60 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] × 100). Basidiome size was determined as follows: total weight of fresh mushrooms harvested/total number of mushrooms harvested.

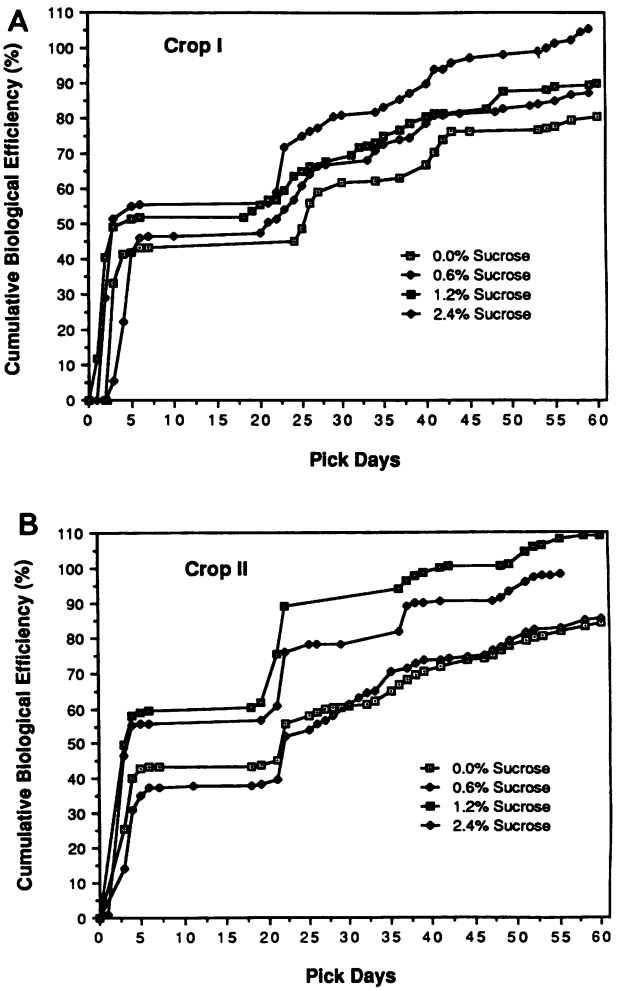

FIG. 1. Cumulative BEs (60 pick days) for crop I (A) and crop II (B) of *L. edodes* (305) grown on synthetic substrate nonamended or amended with three levels (0.6, 1.2, or 2.4%) of sucrose. The synthetic substrate contained sawdust:wheat bran:millet in an 8:1:1 ratio.

RESULTS AND DISCUSSION

Crop I. Sucrose addition (0.6%) at spawning significantly increased the BE for line 305 by approximately 22% when compared with that for the substrate containing no sucrose amendment. As levels of sucrose increased above 0.6%, however, no significant increase in BE was observed. Thus, within the ranges we examined, it appears that a threshold level of about 0.6 to 1.2% sucrose addition may be optimal. Most of the increase in BE occurred on the first break (Fig. 1).

The basidiome size of line 305 was not significantly affected by the addition of sucrose at any level. Mushrooms from line R3 were substantially larger than those from line 305. This difference could have been due to the relatively low productivity of line R3 relative to line 305. Frequently, there is an inverse relationship observed between mushroom yield and size. In general, the lower the BE, the larger the mushrooms tend to be.

Within line R3, mushrooms were larger from substrate not amended with sucrose (Fig. 2). However, since this genotype performed poorly under the environmental management practices used for our experiments, this result may not be confirmed when R3 is managed under different conditions. Line R3 currently is being used by at least one large commercial shiitake farm in Pennsylvania. At this farm, a spawn run period of only 3 weeks is used prior to placement of the logs in a production environment. Since line R3 appeared to be noncompatible with our management system, we chose not to use line R3 in subsequent crops.

Crop II. The addition of sucrose at 0.6 and 1.2% significantly increased the BE compared with that of nonamended and 2.4% amended substrate. A similar trend was observed for crop II compared with crop I, i.e., most of the yield increase occurred on the first break (Fig. 1). In addition, mushrooms emerging from substrate supplemented with 0.6 and 1.2% sucrose tended to have less-scattered pick cycles. For example, mushrooms actually matured and were picked only 22 days out of the 60-day pick on 1.2% sucrose compared with 34 days of pick on nonamended substrate (Fig. 1). The reasons for this phenomenon are unknown but are probably related to the maturation pattern of the second break of mushrooms. The substrate supplemented with 1.2% sucrose tended to have more synchronous maturation for the

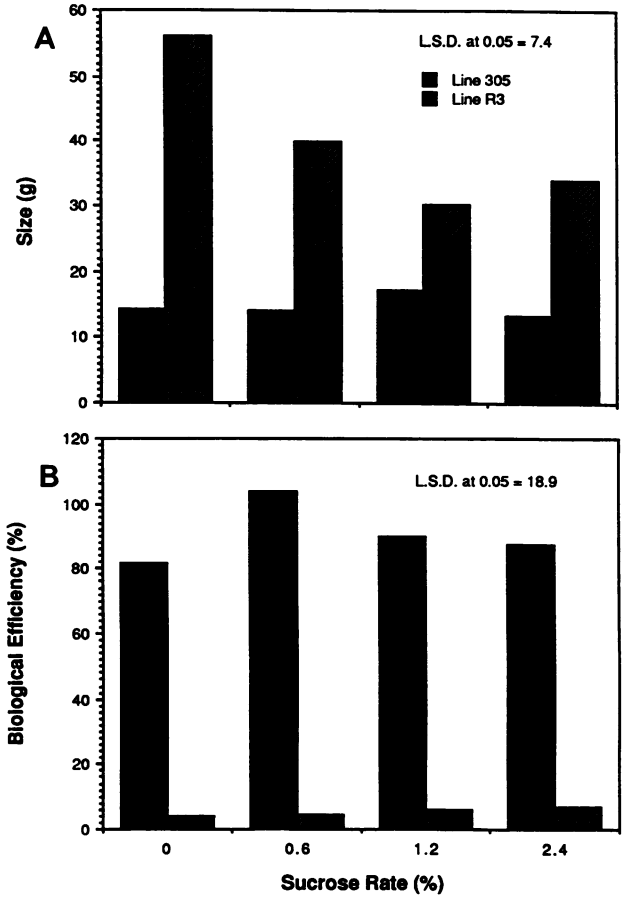

FIG. 2. Basidiome size (A) and BEs (B) of two lines of *L. edodes* grown on synthetic substrate nonamended or amended with three levels (0.6, 1.2, or 2.4%) of sucrose. The synthetic substrate contained sawdust:wheat bran:millet in an 8:1:1 ratio. Abbreviation: L.S.D., Least squares difference.

second break. This phenomenon was observed in crop I but was particularly evident in crop II.

Crop III. Four sugar types were tested for crop III. Probabilities greater than *F* from analysis of variance for three factors for BE and size of shiitake mushrooms on synthetic logs are presented in Table 1. Significant sources of variation for BE and size included line (L) and saccharide (S) × saccharide rate (SR).

TABLE 1. Probabilities greater than *F* from analysis of variance for three factors tested for BE and size of shiitake mushrooms on synthetic logs (crop III)[a]

Source	*df*	Probability > *F*[b]	
		BE	Size
Blocks	4	0.2926	0.5123
Line (L)	1	0.0001	0.0001
Saccharide type (S)	2	0.7882	0.3543
Saccharide rate (SR)	1	0.1028	0.9790
L × S	2	0.6431	0.3801
L × SR	1	0.9770	0.3647
S × SR	2	0.0157	0.0166
L × S × SR	2	0.5240	0.1355

[a] Error, *df*, 44; coefficient of variation, 29.08 and 32.27 for BE and size, respectively.
[b] Values of 0.05 or less were considered significant.

TABLE 2. BE and basidiome size of shiitake mushrooms as influenced by the synthetic substrate, nonamended or amended with one of three saccharide types used at one of two rates

Saccharide type	Saccharide rate (%)	BE (%)[a]	Size (g)[a]
Sucrose	1.2	65.4 a	19.6 ab
	0.6	49.3 bcd	14.3 cd
Fructose	1.2	61.8 ab	19.9 ab
	0.6	46.2 cd	19.5 abc
Glucose	1.2	49.3 bcd	15.4 bcd
	0.6	60.2 abc	21.3 a
Check	0	41.2 d	12.1 d

[a] BE and basidiome size values are means of two *L. edodes* lines (R6 and 305). Means followed by the same letter are not significantly different according to Fisher's protected least squares difference test (11).

Data for BE and mushroom size for the amendment of the synthetic substrate with four saccharide types at two rates are presented in Table 2. The values presented in Table 2 for both BE and basidiome size are means for two *L. edodes* lines. Sucrose and fructose added to the substrate at the rate of 1.2% resulted in significant increases over the 0.6% rate for these two saccharides. On the other hand, substrate supplemented with glucose at a rate of 0.6% resulted in a higher BE than substrate amended with 1.2% glucose, although this difference was not statistically significant.

Mushrooms produced from sucrose-amended substrate were significantly larger from substrate with a 1.2% amendment compared with substrate with a 0.6% amendment (Table 2). Mushrooms were significantly larger from substrate amended with 0.6% glucose as compared with mushrooms from substrate amended with 1.2% glucose.

The addition of sucrose to shiitake substrate has been reported previously (12, 13). However, conflicting results on the effect of sucrose addition to the substrate are apparent. For example, Tokimoto and Kawai (13) reported yield increases of *L. edodes* at rates of up to 8%. On the other hand, Tan and Chang (12) concluded that 1 and 3% sucrose addition to substrates did not result in a yield increase. Tan and Chang (12) did observe, however, that both a 1 and a 3% sucrose addition to sawdust substrate significantly increased linear mycelial growth of the mushroom. They reported that linear mycelial growth for strain L38, measured at day 35, was 15 and 21% greater for 1 and 3% sucrose additions, respectively. For strain L13, measured at day 35, the difference in linear mycelial growth between nonamended and sucrose-amended substrate was even greater than that for strain L38.

Our findings partially support the findings of Tokimoto and Kawai (13) where some levels of sucrose stimulate yield of *L. edodes*. With our production management system, however, we found that optimum sucrose addition may be in the range of 0.6 to 1.2%. Our substrate formulation is substantially different than that used by Tan and Chang (12). Our formulation contained twice as much wheat bran (10%) than the amount of wheat bran (5%) used in their studies. In addition, our formulation contained 10% white millet, which would have provided additional nutrients and which may contain a fruiting stimulator(s) (Royse, unpublished observations).

It was interesting to note that glucose at a 0.6% amendment rate gave nearly an identical BE as sucrose at a 1.2% amendment rate. Since sucrose is a disaccharide made up of

equal portions of glucose and fructose, it is tempting to speculate that the glucose portion of the sucrose molecule may be responsible for the yield increase. Sucrose at a 1.2% level would contain the equivalent of glucose at a 0.6% level. It was obvious, however, that fructose also can stimulate yields. Fructose amendment of substrate at 1.2% resulted in yields equivalent to sucrose amendment at 1.2% and glucose amendment at 0.6%. Perhaps *L. edodes* may prefer glucose over fructose or sucrose, but additional research is required to elucidate this possibility. Sucrose, however, may be more readily available and less costly for commercial production, even though twice as much sucrose as glucose would be required to achieve optimal yield stimulation.

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