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Effects of nutrient supplements on biological efficiency, quality and crop cycle time of maitake (*Grifola frondosa*)

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Abstract The effects of various combinations of wheat bran, rye and millet (at 20% and 30% of total dry substrate wt) on crop cycle time, biological efficiency (BE) and mushroom quality were evaluated for a commercially used isolate of Grifola frondosa (maitake). Supplements were combined with a basal ingredient of mixed oak (primarily red oak) sawdust, and the resulting mixture was pasteurized, cooled, inoculated and bagged with an autoclaving mixer. Times to mushroom primordial formation and mushroom harvest were recorded, and mushroom quality was rated on a scale of 1–4, where 1 was the highest quality and 4 was the lowest quality. The combinations of 10% wheat bran, 10% millet and 10% rye (BE 47.1%, quality 1.8 and crop cycle 12 weeks) and 10% wheat bran plus 20% rye (BE 44%, quality 1.7 and crop cycle 10 weeks) gave the most consistent yields and best basidiome quality over time.

Introduction

Strong consumer demand has stimulated increased world-wide production of maitake (*Grifola frondosa*). The increased demand for maitake is due to the unique culinary and medicinal properties associated with this choice mushroom. Annual commercial production has increased 41-fold (to 33,100 t in 1997; Chang 1999) since 1981, the year when commercial production of maitake first began in Japan (Takama et al. 1981). Maitake production and consumption is also increasing rapidly in the United States (up 38% in 1999–2000; USDA 2000). Presently, most maitake is marketed as food. Powdered basidiomes also are used in the production of many health foods such as maitake tea, whole powder, granules, drinks, and tablets (Royse 1997; Mizuno 1999).

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Commercial production of most maitake is on synthetic substrate contained in polypropylene bags. A common substrate used for commercial production of maitake is supplemented sawdust. Oak (Lee 1994; D.J. Royse, unpublished data) is the most popular choice in the United States and Japan, while beech (Kirchhoff 1996; Yoshizawa et al. 1997) and larch (Stamets 2000) are also preferred to a lesser extent in Japan. In China, cottonseed hulls have been successfully used as a substitute for sawdust (Zhao et al. 1983). Brans, derived from cereal grains, such as rice (Takama et al. 1981), wheat (Mayuzumi and Mizuno 1997), oats and corn, are widely used as nutrient supplements. Other nutrient supplements used for maitake cultivation include millet (D.J. Royse, unpublished data), corn meal (Kirchhoff 1996), and soybean cake (Mizuno and Zhuang 1995).

There are not many reference texts available for use in producing maitake. The techniques currently used to grow maitake are mostly adapted from those used to produce other specialty mushrooms, such as shiitake. Extensive research has been carried out on the most efficient methods, genotypes and nutritional formulation of specialty mushrooms other than maitake (Diehle and Royse 1986; Royse and Bahler 1988; Royse et al. 1990; Stamets 2000). The rapid increase of maitake production in Japan and the United States has focused the need to develop more efficient substrate formulas to improve yield and quality and to shorten the crop cycle. In this study, two experiments were conducted to determine the effects of selected nutrient supplements at various levels on maitake crop cycle time, biological efficiency (BE), yield and quality. Significant differences among different formulations were found and the best combinations of nutrient supplements among those tested were identified. For continued growth of the commercial industry, efforts directed toward improving BE, yield, quality, and reduced time to primordium formation and harvest are desirable.

Materials and methods

Substrates and preparation

The major substrate ingredient – mixed oak sawdust (mostly *Quercus rubra* L.) with approximately 30% moisture – was obtained from a local sawmill in Centre County, Pennsylvania. The general substrate formulation consisted of sawdust, nutrient supplements and 0.2% gypsum (CaSO₄). The nutrient supplements used in the study included white millet (*Panicum miliaceum* L.), wheat bran (*Triticum aestivum* L.) and rye (*Secale cereale* L). Moisture contents of the substrates were adjusted to 55–58% of the fresh weight. All ingredients were combined, mixed, pasteurized (20 min at 111°C), cooled, inoculated, and bagged with an autoclaving paddle mixer as previously described by Royse (1985). 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.

Spawn, spawn run, primordial development and basidiome development

Isolate WC828 (apparently of Asian decent and available from the authors) was selected for this study because it is a commerciallyused cultivar in the United States and is also an isolate that consistently produces high yield and quality at the Mushroom Research Center (MRC), The Pennsylvania State University. Spawn was prepared in 500 ml flasks with a spawn formula as follows: 100 ml beaker level full of Hesco (Watertown, S.D.) mushroom rye grain, 50 ml beaker of hardwood sawdust, one-half teaspoon CaSO₄, and 120 ml of warm tap water. After inoculation with spawn, virgin polyethylene bags were used to contain moist (55–58%) substrates (2,650 g per bag) for incubation. Spawn run temperatures were maintained at 20 ± 1 °C. The bags were sealed with a twist tie and, after the spawned substrate was incubated for 1 week, 20 slits (5 mm long) were made at the top of each bag with a sharp scalpel to allow for gas exchange. Spawn run is defined as the period from the beginning of inoculation to primordia formation. After primordia formation, two holes were cut in the polyethylene bags, exposing the developing primordia. The top of the bag was folded over, exposing only the developing primordia to the fruiting environment. Taped bags then were moved to a production room for basidiome development (Fig. 1). The period of basidiome development was initiated when the primordia began to grow and differentiate to form small pilei and stipes. A crop cycle of 12 weeks or less was considered short, both on the basis of our experience and compared to the 15-week crop cycle reported by Stamets (2000).

Harvesting and determination of BE and quality

Mushrooms were harvested from the substrate when the caps were fully mature. The substrate clinging to the main stipe was removed and the clusters of mushrooms were weighed. BE was determined as the ratio of the weight of fresh mushrooms harvested per kg dry substrate, and was expressed as a percentage (Royse 1992). The shape and color of the basidiome was used to evaluate the quality of maitake [rated 1–4 (Table 1) based on the description of Kunitomo (1992) and our observations].

Table 1 Rating scale (1–4) for evaluating basidiome quality for *Grifola frondosa* (maitake) grown on sawdust substrate supplemented with various combinations and levels of nutrients

Rating Description The best quality mushrooms with black to dark gray color, uniform and no misshapen pilei Mushrooms with gray to light gray color and mostly uniform shape Mushrooms with more than one half of the pilei misshapen Mushrooms with misshapen, immature and undeveloped pilei

Experimental design

Two experiments (two crops per experiment; four crops in total) were conducted to evaluate the effects of two levels of total nutrient supplements (20% and 30%) on BE, mushroom quality and crop cycle time. Ten combinations (simplex lattice mixture design; SAS Institute 1996) of wheat bran, millet and rye were tested for each of the two experiments. Experiments were conducted as completely randomized designs (10 replicates per treatment) and carried out at the MRC. Environmental conditions were as described by Royse (1985). Briefly, relative humidity (90 to 95%) was maintained by water atomizers placed in air handling ducts, 4 h of light were provided daily by eight (1.22 m, 40 W) cool-white fluorescent bulbs, and temperatures were maintained at $17\pm2^{\circ}$ C. Sufficient air changes were maintained to hold CO₂ concentrations below 700 ppm (μ I/I). The SAS program JMP (SAS Institute 1996) was used to analyze data. The general linear models procedure was used to

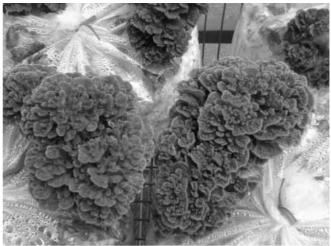
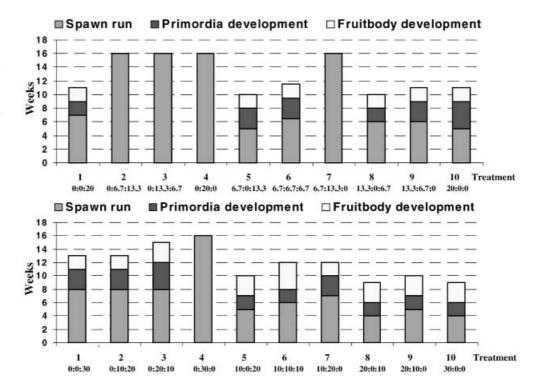




Fig. 1 Maitake (*Grifola frondosa*; WC828) fruiting from oak sawdust supplemented with wheat bran (10%) and rye (20%) 63 days (*top*) and 68 days (*bottom*) after inoculation.

Fig. 2 Graphic summary (10 treatments) of crop cycle time of *Grifola frondosa* (WC828) as influenced by 20% (top) and 30% (below) levels of wheat bran, millet and rye used alone or in various combinations. Ratios shown below each treatment number indicate percentages of wheat bran: millet: rye



analysis of variance. Treatments with zero values were excluded from the data analysis. Tukey-Kramer Honestly Significant Difference (HSD) was used to separate treatment means (SAS Institute 1996). To test for uniformity between crops within the same experiment, a two-way analysis of variance (ANOVA) was used with crop as a source of variation. If significant differences were found between the two crops for BE and quality, then both crops within an experiment were analyzed separately. If no significant difference was found for the *F*-test for crops as a source of variation within an experiment, then data was combined for the two crops for the ANOVA.

Results

Effects on crop cycle time

Total nutrient levels (20% and 30% of total dry substrate weight) and various combinations of wheat bran, millet and rye significantly influenced mushroom crop cycle time (Fig. 2). All treatments resulted in completion of the crop cycle, except treatment 4 (millet only) at both levels, and treatments 2, 3 and 7 at the 20% level. At the 20% level, crop cycle times for treatments 8 [wheat bran (13.3%): millet (0%): rye (6.7%)] and 5 [wheat bran (6.7%): millet (0%): rye (13.3%)]were the shortest (10 weeks). At the 30% level, 9 weeks was the shortest crop cycle achieved (treatments 8 and 10).

Effects on BE and quality

Twenty percent level of supplements

The BEs and quality of two crops for the 20% level of combined wheat bran, millet and rye are shown in Table 2. BE ranged from 42% (treatment 8) to zero

(treatments 2, 3, 4 and 7). Treatments 8 (13.3% wheat bran and 6.7% rye), 6 (6.7% each of wheat bran, millet and rye), 5 (6.7% wheat bran and 13.3% rye) and 9 (13.3% wheat bran and 6.7% millet) had the highest BEs. A significant difference for BE was found between crops I and II. However, the results from the two crops were not in conflict. Results for crop II were similar to crop I except the overall values for BE and quality were lower. Total means for BE for crop I and II were 33.9% and 31.2%, respectively. There was no significant difference in mushroom quality for any of the treatments in both crops I and II. When wheat bran and rye were used, both combinations of 6.7%:13.3% (treatment 5) and 13.3%:6.7% (treatment 8) produced high BEs. When wheat bran and millet were used, only the combination of 13.3%:6.7% (treatment 9) produced high BEs. This suggests that a higher level of wheat bran might provide additional yield increases. The combination of wheat bran, millet and rye was also effective in stimulating mushroom yield.

Thirty percent (30%) level of supplements

The BEs and quality for two crops for the 30% level of wheat bran, millet and rye are shown in Table 3. Significant differences in BEs and quality were found in both crops. In crop I, BEs ranged from 48.9% (treatment 6) to zero (treatment 4), and quality ranged from 1.6 (treatment 3) to 2.5 (treatment 10). The BEs for treatments 6 (10% each of wheat bran, millet and rye) (48.9%) and 5 (10% wheat bran and 20% rye) (44.1%) were significantly higher than the other treatments. Treatment 10 (30% wheat bran only) resulted in lower mushroom

Table 2 Percentage biological efficiency (%BE) and quality rating for *Grifola frondosa* (WC828) grown on substrate supplemented with various combinations of selected nutrients (wheat bran, millet and rye at 20% total)

Treatment	Selected nutrient supplements (%)			Crop I		Crop II		Mean (Crop I and II)	
	Wheat Bran	Millet	Rye	BE (%)	Quality ^a	BE (%)	Quality	BE (%)	Quality
1	0	0	20	29.2 b	1.8 NS ^d	25.3 b	1.9 NS	27.3	1.9
2	0	6.7	13.3	0 c	_	0	_	0	_
3	0	13.3	6.7	0	_	0	_	0	_
4	0	20	0	0	_	0	_	0	_
5	6.7	0	13.3	36.1 a	1.6 NS	36.6 a	1.7 NS	36.4	1.7
6	6.7	6.7	6.7	38.5 a	1.4 NS	36.1 a	1.4 NS	37.3	1.4
7	6.7	13.3	0	0	_	0	_	0	_
8	13.3	0	6.7	42.0 a	1.3 NS	38.3 a	1.2 NS	40.1	1.3
9	13.3	6.7	0	32.9 ab	1.8 NS	30.2 ab	1.7 NS	31.5	1.8
10	20	0	0	24.4 b	1.6 NS	20.7 b	1.9 NS	22.6	1.8
Crop Means	S			33.9		31.2		32.5	

^a Quality rating based on scale of 1–4 where 1 is highest quality ^b Means in the same experiment in the same column followed by the same letter are not significantly different at the *P*=0.05 level according to Tukey-Kramer HSD

Table 3 Percentage biological efficiency (%BE) and quality rating for *Grifola frondosa* (WC828) grown on substrate supplemented with various combinations of selected nutrients (wheat bran, millet and rye at 30% total)

Treatment	Selected nutrient supplements (%)			Crop I		Crop II		Mean (Crop I and II)	
	Wheat Bran	Millet	Rye	BE (%)	Quality ^a	BE (%)	Quality	BE (%)	Quality
1	0	0	30	39.0 b b	2.0 ab	30.8 bc	2.0 ab	34.9	2.0 a
2	0	10	20	31.6 b	1.9 ab	28.5 bc	1.6 ab	30.1	1.8 a
3	0	20	10	33.4 b	1.6 a	27.9 bc	1.4 a	30.7	1.5 a
4	0	30	0	0 c	_	0	_	0	_
5	10	0	20	44.1 ab	1.7 a	43.8 a	1.6 ab	44.0	1.7 a
6	10	10	10	48.9 a	1.7 a	45.2 a	1.8 ab	47.1	1.8 a
7	10	20	0	34.1 b	2.0 ab	30.9 bc	1.9 ab	32.5	2.0 a
8	20	0	10	39.2 b	1.7 a	34.2 ab	1.8 ab	36.7	1.8 a
9	20	10	0	36.9 b	2.0 ab	36.1 ab	1.8 ab	36.5	1.9 a
10	30	0	0	22.2 c	2.5 b	22.8 c	2.3 b	22.5	2.4 b
Crop means				36.6		33.4		35.0	

^a Quality rating based on scale of 1–4 where 1 is highest quality ^b Means in the same experiment in the same column followed by the same letter are not significantly different at the *P*=0.05 level according to Tukey-Kramer HSD

quality. There was no significant difference in quality for the other treatments. In crop II, BEs ranged from 45.2% (treatment 6) to zero (treatment 4), and quality ranged from 1.4 (treatment 3) to 2.3 (treatment 10). Similarly to crop I, a combination of 10% each of wheat bran, millet and rye (treatment 6) and 10% wheat bran plus 20% rye (treatment 5) had the highest BEs. In this crop, combinations of 20% wheat bran plus 10% rye (treatment 8) and 20% wheat bran plus 10% millet (treatment 9) also were significantly higher. Quality results for crop II were the same as crop I with treatment 10 (30% wheat bran only) significantly lower than the others. Significant differences for BE were found between crops I and II. The overall BE for crop I (36.6%) was higher than that for crop II (33.4%). There was no significant difference in quality between the two crops. Overall results for combined data were the same as that for each individual crop.

Comparison of the 20% and 30% levels of nutrient supplement showed that, in general, increasing the nutrient level increased yield, although this was not always the case. For example, BEs for treatments 7, 8 and 10 were higher for the 20% level than for the 30% level. In addition, some treatments did not produce mushrooms at the 20% level of supplementation, while at the 30% level, relatively high yields were obtained (treatments 2, 3 and 7). The BEs of treatments 6 (wheat bran: millet: rye = 10%:10%:10%) and 5 (wheat bran: rye = 10%:20%) were significantly higher than the other treatments at the 30% and 20% levels.

Discussion

Knowledge is currently very limited as to how various nutrient types and levels influence maitake crop cycle

^c Treatments where no fruiting occurred (0.0) were eliminated from the analysis of variance

d Not significant

^c Treatments where no fruiting occurred (0) were eliminated from the analysis of variance

time, mushroom yield and quality due to the short history of its commercial cultivation. Our results clearly indicate that type and quality of nutrient supplements influence crop cycle time, yield and basidiome quality. Wheat bran is one of the most important factors for reducing crop cycle time. Formulations with only rye produced mushrooms, but were significantly lower in BEs than formulations with combinations of wheat bran and rye. Comparison of the supplement levels of 20% and 30% showed that, in most cases, as the nutrient levels increased BEs also increased. In fact, no mushrooms were produced when total nutrient levels were at a 10% level, regardless of nutrient combination (Shen and Royse, unpublished data). The combination of 10% wheat bran, 10% millet and 10% rye and the combination of 10% wheat bran plus 20% rye were the best overall formulations for isolate WC828.

We found that better quality mushrooms and more consistent yields were produced from a more nutritionally balanced substrate. Combinations of two or three nutrients selected from wheat bran, rye or millet were the most desirable formulations found. For example, higher levels of wheat bran alone significantly shortened the crop cycle, but produced poorer quality mushrooms and lowered BEs. On the other hand, increasing wheat bran levels in sawdust substrates containing millet and rye, or both, increased productivity and often improved mushroom quality. Millet also resulted in poor yield and quality when used alone. However, when it was used together with wheat bran and rye, significantly higher BEs and quality were achieved. Royse (1985) noted similar trends in nutritional work on shiitake (Lentinula edodes). Additional work evaluating the effects of other types and quantities of nutrients on mushroom BE and quality might reveal more productive combinations than we found in this study. We also suggest that additional nutritional investigations be coupled with the use of two or more strains of diverse genetic origin. This would help minimize the potential independent effect of germplasm on crop cycle time, yield and mushroom quality.

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