

**CROP CYCLE TIME, YIELD AND QUALITY OF
MAITAKE *GRIFOLA FRONDOSA*
AS INFLUENCED BY NUTRIENT SUPPLEMENTS**

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ABSTRACT

Wheat bran, rye, millet and corn meal supplements used alone or in various combinations with mixed oak sawdust significantly influenced crop cycle time, biological efficiency (BE) and basidiome quality of maitake (*Grifola frondosa*). The combination of 10% wheat bran, 10% millet and 10% rye and the combination of 10% wheat bran plus 20% rye gave the highest and most consistent yields. The shortest crop cycle time achieved (nine wk) was with 20% wheat bran and 10% rye and 30% wheat bran only, while the best quality mushrooms were produced with 20% millet and 10% rye. Considering the desirable characteristics of yield, short crop cycle time and best basidiome quality, an equal mixture of wheat bran (10%), millet (10%) and rye (10%) proved the best formulas for the most widely used commercial isolate (WC828) in the United States.

INTRODUCTION

Strong consumer demand has stimulated increased production of maitake (*Grifola frondosa*) world wide. The annual commercial production has increased 41-fold (Chang 1999) since 1981, the year when commercial production of maitake began (Takama *et al.* 1981). Maitake production and consumption also is increasing rapidly in the United States (up 38% 1999-2000). Presently, most maitake is marketed as food. Powdered fruitbodies are also used in the production of many health foods such as maitake tea, whole powder, granules, drinks, and tablets (Royse 1997).

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, Royse 1997) 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 were used as a substitute for sawdust with acceptable yields (Zhao *et al.* 1983). Bran derived from cereal grains, such as rice bran (Takama *et al.* 1981), wheat bran (Mayuzumi and Mizuno 1997), oat bran and corn bran, are widely used as nutrient supplements. Other nutrient supplements used for maitake cultivation include millet (Royse 1997), corn meal (Kirchhoff 1996), and soybean cake (Mizuno and Zhuang 1995).

There still are limited reference texts available for producing maitake. Presently, the techniques used to grow maitake are mostly adopted from other specialty mushroom cultivation, such as shiitake. Extensive research has been done on efficient methods, genotypes and nutritional formulation of specialty mushrooms other than maitake (Douglas and Royse 1986, Royse and Bahler 1988, Royse *et al.* 1990). The rapid growth of maitake production has focused the need for a desire 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 and their levels on maitake crop cycle time, 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 biological efficiency, 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.) was obtained from a local sawmill in Centre County, Pennsylvania with approximately 30% moisture. The general substrate formulation consisted of mixed oak sawdust, nutrient supplements and 0.2% gypsum (CaSO₄). The nutrient supplements used in the study include white millet (*Panicum miliaceum* L.), wheat bran (*Triticum aestivum* L.), rye and corn meal. Moisture contents of the substrates were adjusted to 59% of the fresh

weight. All ingredients were combined, mixed, pasteurized, cooled, inoculated, and bagged with an autoclaving paddle mixer described previously by Royse (1985). Dry matter contents of the processed substrates were determined by drying 100g of the processed substrates in an oven for 24 hours at 105°C.

Spawn, spawn run, primordial development and fruitbody development

Isolate WC828 was selected for this study because it is a commercial cultivar used in the United States and also is an isolate consistently producing high yield and quality in our culture collection (unpublished data). Spawn was prepared in 500ml flasks following a spawn formula (100 ml beaker level full of Stanford mushroom rye grain, 50 ml beaker of hardwood sawdust, one-half teaspoon CaSO_4 , and 120 ml of warm tap water, D.J. Royse, unpublished). After inoculation with spawn, polyethylene bags were used to contain moist (55-58%) substrates (2650 grams per bag) for incubation. Spawn run temperature was maintained at $20 \pm 1^\circ\text{C}$. The bags were sealed with a twist tie and, after the spawned substrate was incubated for one week, 20 slits (5mm long) were made at the top of each bag with a sharp scalpel to provide for gas exchange. Spawn run is 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 fruiting. The period of fruitbody 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 based on 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. The biological efficiency (BE) was determined as the ratio of kg fresh mushrooms harvested per kg dry substrate and expressed as a percentage (Royse 1992). The quality of maitake was evaluated by the shape and color of the basidiome and rated as 1 to 4 (Table 1) based on Kunitomo (1992)'s description and our observations.

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

| Rating | Description |
|--------|---|
| 1 | The best quality mushrooms with black to dark gray color, uniform and no misshapen pilei. |
| 2 | Mushrooms with gray to light gray color and mostly uniform shape. |
| 3 | Mushrooms with more than half of the pilei misshapen. |
| 4 | Mushrooms with misshapen, immature and undeveloped pilei. |

Experimental design

An experiment was designed to determine the influence of selected nutrient supplements on mushroom yield with 10 replicates for each treatment. Two crops were grown and 30% total supplements were used. Selected nutrient supplements included wheat bran, millet and rye. Different combinations of these supplements were tested by adding them to a basal substrate of sawdust which included 69.8% mixed red and white oak plus 0.2% CaSO₄. Table 2 shows the treatments with proportions of nutrient supplements in a basal substrate according to a simplex centroid mixture design (SAS Institute, 1996).

Table 2. Percentage biological efficiency (%BE) and quality for *Grifola frondosa* (WC828) grown on substrate supplemented with various levels of selected nutrients (wheat bran, millet and rye at 30% total) at the Mushroom Research Center.

| Treatment | A | | | B | | | |
|-----------|-----------------------------------|--------|-----|---------------------|----------------------|---------|---------|
| | Selected nutrient supplements (%) | | | Crop I | | Crop II | |
| | Wheat Bran | Millet | Rye | BE (%) | Quality ^a | BE (%) | Quality |
| 1 | 0 | 0 | 30 | 39.0 b ^b | 2.0 Ab | 30.8 bc | 2.0 ab |
| 2 | 0 | 10 | 20 | 31.6 b | 1.9 Ab | 28.5 bc | 1.6 ab |
| 3 | 0 | 20 | 10 | 33.4 b | 1.6 A | 27.9 bc | 1.4 a |
| 4 | 0 | 30 | 0 | 0 ^c | - | 0 | - |
| 5 | 10 | 0 | 20 | 44.1 ab | 1.7 A | 43.8 a | 1.6 ab |
| 6 | 10 | 10 | 10 | 48.9 a | 1.7 A | 45.2 a | 1.8 ab |
| 7 | 10 | 20 | 0 | 34.1 b | 2.0 Ab | 30.9 bc | 1.9 ab |
| 8 | 20 | 0 | 10 | 39.2 b | 1.7 A | 34.2 ab | 1.8 ab |
| 9 | 20 | 10 | 0 | 36.9 b | 2.0 Ab | 36.1 ab | 1.8 ab |
| 10 | 30 | 0 | 0 | 22.2 c | 2.5 B | 22.8 c | 2.3 b |

Total mean

36.6

33.4

^a Quality rating based on scale of 1-4 when 1 is highest quality and 4 is lowest 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.

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

The experiments were conducted as completely randomized designs and carried out at the Mushroom Research Center of the Pennsylvania State University under the same conditions. Environmental conditions were as described by Royse (1985). Relative humidity (90 to 95%) was maintained by water atomizers placed in air handling ducts, 4 hours of light were provided daily by cool-white fluorescent bulbs, and temperature was maintained at 17±2°C. Sufficient air changes were maintained to hold CO₂ concentrations below 700 ppm (µl/l). SAS program JMP (SAS Institute 1996) was used to analyze data. The general linear models procedure was used to perform an 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).

RESULTS

Effects on crop cycle time

Isolate WC828 performed differently on sawdust supplemented with different combinations of nutrients. The results are shown in Figure 1. All treatments resulted in completion of the crop cycle, except treatment 4 (millet only). The crop cycle was the shortest (9 weeks) when combinations of 20% wheat bran plus 10% rye and 30% wheat bran only were used (treatments 8 and 10, respectively). These treatments also had the shortest spawn run (4 weeks). Combinations of wheat bran and rye appeared to allow shortest spawn run time and shortest crop cycle time.

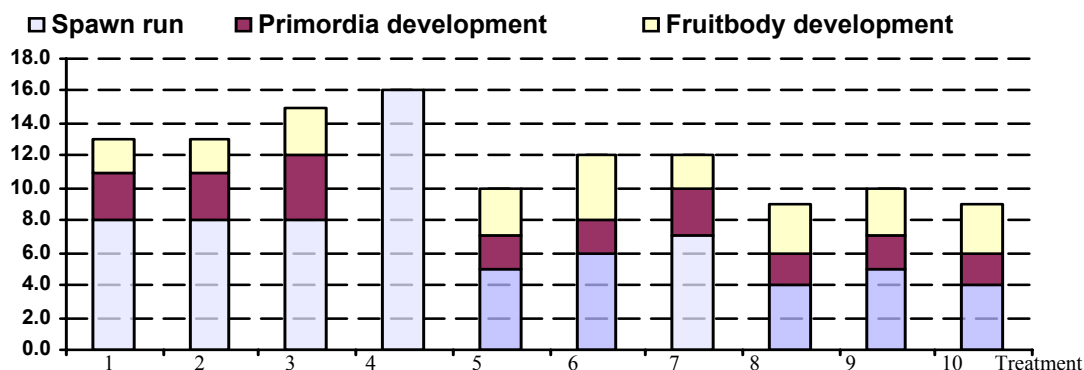


Figure 1. Graphic summary (10 treatments) of crop cycle time of *Grifola frondosa* (WC828) as influenced by 30% level of wheat bran, millet and rye used alone or in various combinations.

Ratios shown below each treatment number indicate percentages of wheat bran : millet : rye.

Effects on biological efficiency (BE) and quality

Significant differences in yield and quality were found among 10 treatments in crops I and II. In crop I, BEs ranged from 48.9% (treatment 6) to zero (treatment 4). Quality ranged from 1.5 (treatment 3) to 2.4 (treatment 10). A combination of 10% wheat bran, 10% millet plus 10% rye (treatment 6) added to substrate produced the highest BE and best quality. However, there was no significant difference in BE between treatments 1-9. Among the treatments that produced basidiomes, the treatment with 30% wheat bran only gave the lowest BE.

In Crop II, BEs ranged from 45.2% (treatment 6) to zero (treatments 4). Quality ranged from 1.4 (treatment 3) to 2.3 (treatment 10). The combination of 10% wheat bran, 10% millet plus 10% rye (treatment 6), along with treatment 5, 8, and 9 had the highest BEs with no significant difference. The highest quality mushrooms were produced by substrates from treatments 1-9. Mean BEs and qualities are presented in Fig. 2.

Comparison of all substrate formulations evaluated in this experiment revealed that for isolate WC828, wheat bran, rye and millet produced the overall best fruiting and yields. In general, combinations of more than one type of nutrient were better than only one nutrient. Combinations of two or three nutrients selected from wheat bran, rye or millet was the most desirable formulation with shortest crop cycle, best quality and highest BE.

DISCUSSION

Knowledge currently is very limited as to how different nutrient types and levels influence the crop cycle, and mushroom yield and quality due to the short history of maitake (*Grifola frondosa*) commercial cultivation. Our results clearly indicate that type and combination of nutrient supplements influence crop cycle, yield and mushroom quality. Combinations of two or three nutrients selected from wheat bran, rye or millet were the most desirable formulations found to date. 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.

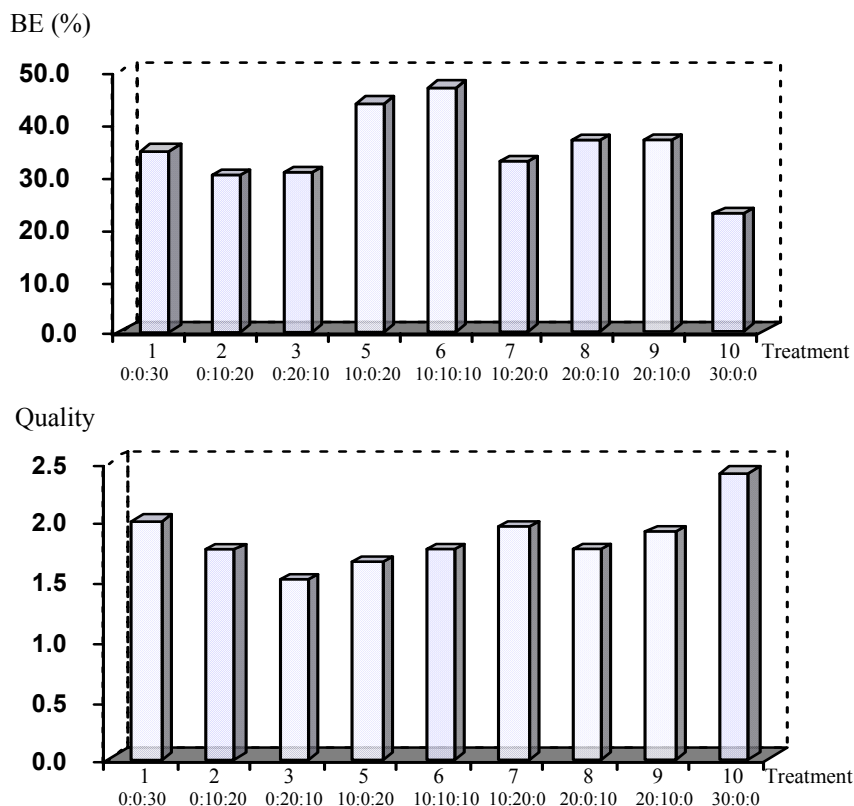


Figure 2. Graphic summary (9 out of 10 total treatments) of means of BEs (top) and quality (below) of two crops of *Grifola frondosa* (WC828) grown on substrate containing 30% wheat bran, millet and rye used alone or in various combinations. Quality was rated 1-4 with 1 being the highest quality while 4 was the lowest quality rating. See Table 2 for significance values for individual crops (I and II). Ratios shown below each treatment number indicate percentage of wheat bran : millet : rye.

We found that better quality mushrooms and more consistent yields were produced from a more nutritionally balanced substrate. For example, higher levels of wheat bran 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 times, improved mushroom quality. The use of millet in substrate is another good example of poor yield and quality when millet is used alone. However, when it was used together with wheat bran and rye, significantly higher BEs and quality were achieved. Additional work evaluating the effects of other types and quantities of nutrients on mushroom BE and quality may reveal more productive combinations than we found in this study. We also would suggest that additional nutritional investigations be completed with two or more

strains of diverse genetic origin. This would help minimize the potential independent effect of germplasm on crop cycle time and mushroom yield and quality.

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