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ABSTRACT: Two experiments were conducted to determine the effects of supplementing wood chips and cottonseed hulls with brewer's grain and SpawnMate IISE® (SMIISE) on yield and size of two strains of *P. eryngii*. On oak wood chips, biological efficiency (BE) ranged from 30.7% (5% brewer's grain supplementation; strain WC827) to 54.1% (15% brewer's grain supplementation; strain WC515). For strain 515, increasing brewer's grain supplementation of oak chips from 5% to 15% increased yields by 41%, whereas for strain 827 the increase was only 19%. On cottonseed hulls, yields and BEs were highest for 45% brewer's grain supplementation. Yield increases were not linear, however. As supplementation levels for brewer's grain increased from 15 to 30%, yields increased 39%, whereas yields only increased an additional 15% when brewer's grain in the cottonseed hulls substrate was increased from 30 to 45%. A similar trend was observed for SMIISE supplementation; i.e., as SMIISE was increased from 3 to 6% of the dry substrate weight, yield increased 90%, whereas an increase from 6 to 9% supplementation resulted in an additional 84% increase. Thus, the magnitude of the yield response was much greater for SMIISE® than for brewer's grain supplementation.

1 INTRODUCTION

World-wide production of *Pleurotus* spp. mushrooms increased from 169,000 t in 1986 to 797,000 t in 1994 (372%; Chang and Miles 1991, Chang 1996). In 1986 and 1994, *Pleurotus* spp. production accounted for approximately 7% and 16%, respectively, of the total world production of edible mushrooms. Most of the increase in *Pleurotus* spp. production occurred in China, where output increased from 100,000 t in 1986 to about 654,000 t in 1994. In the United States, *Pleurotus* spp. production has increased from approximately 218 t in the 1987-88 growing season to about 909 t in the 1997-98 growing season (317%; Royse 1992, United States Department of Agriculture 1998).

In the United States (and in some Asian countries), farmers produce *Pleurotus* spp. on basal ingredients of either chopped straw (wheat or oat), cottonseed hulls or sawdust (hardwood) substrates. These basal ingredients are supplemented with commercially-available delayed release nutrients or various grains, meals, or bran (Royse 1997).

Two species of *Pleurotus* (*P. ostreatus* and *P. pulmonarius*) account for the majority of oyster mushrooms produced in the United States. These two species have a relatively limited shelf-life when compared to *A. bisporus* (button mushroom) or *Lentinula edodes* (shiitake). Development of improved varieties to extend shelf-life in oyster mushrooms may allow increased consumer demand for these varieties.

Pleurotus eryngii is renowned for its large basidiomes. Chefs in the United States who have tried the king oyster mushroom have enthusiastically welcomed it as a valuable addition to their menus. Market demand for *P. eryngii* has increased steadily in the last two years and, some growers are receiving a relatively high farm gate price ($8.80 to $11 per kg) for their product.

Little information on cultivation of *P. eryngii* is available to growers. The purpose of this paper is to report on two experiments designed to evaluate the effect of brewer's grain and SpawnMate IISE (SMIISE) supplementation of cottonseed hulls and hardwood sawdust on yield and and size of two strains of *P. eryngii*.

2 MATERIALS AND METHODS

Substrates and preparation

Two basal ingredients were used for substrate preparation. For Crop I, mixed hardwood sawdust, collected from a local sawmill in Centre County, Pennsylvania was used. The exact composition of hardwood species comprising the sawdust was not determined, but the predominant species was Northern Red Oak (*Quercus rubra* L.). The sawdust was collected in summer, 1997 and stored in an enclosed building until used (approximately five months; 35% moisture). For Crop II, cottonseed hulls (12% moisture content) were used as the main substrate ingredient.

Crop I formulation contained 15% white millet, 10% soft winter wheat bran and 1% calcium carbonate. Crop II formulation contained cottonseed hulls only supplemented with various levels of brewer's grain (15, 30 and 45% dry substrate wt) and SpawnMate (SM) IISE® (3, 6, and 9% dry substrate wt). For both Crops I and II, substrate ingredients were processed with a 0.283 m^3 (10 ft^3) paddle mixer (Royse 1997). The substrate was pasteurized by injecting live steam into the mixer and allowing the substrate to heat to 100°C. This temperature was maintained for 20 min with continuous agitation to insure uniform substrate heating. After pasteurization, the substrate was rapidly cooled by passing cold tap water through a jacket fitted to the mixer. Positive pressure airflow was maintained on the substrate by injecting filtered air into the mixer during cool down. When the substrate had cooled to below 27°C, it was spawned with 210 g rye grain spawn per 40 kg moist substrate. When the spawn was thoroughly mixed with the substrate, the 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 2.65 kg spawned substrate at 59% moisture (1.09 kg oven dry weight). Dry substrate weight was determined by drying 100 g of the processed substrates in an oven for 24 h at 105°C.

Isolates and spawn

Isolates of *P. eryngii* (515 and 827) were from The Pennsylvania State University Mushroom Culture Collection. These cultures were selected for Crop I to evaluate possible strain differences in yield and size. Isolate 827 was used for Crop II because it produced a larger mushroom in Crop I. This isolate was originally obtained from the Soviet Union in Fall, 1997 and maintained on potato-dextrose yeast-extract agar as described by Jodon and Royse (1979). Spawns of both 515 and 827 were prepared as described by Royse and Bahler (1986).

Spawn run

After a spawn run of 5 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 30-days incubation at 22±1°C, the tops of the plastic bags were removed and the bags were moved to a production room. In the production room (85-90% relative humidity, 18±1°C air temperature), three h of light was provided daily by cool-white fluorescent bulbs. Sufficient air changes were maintained to hold CO_2 levels below 800 ppm (800 µl/liter).

Harvesting and determination of BE and basidiome size

Mushrooms were harvested from the substrate at the same time each day, when the mushroom cap surfaces were flat to slightly up-rolled at the cap margins. The mushrooms then were counted and weighed. At the end of the harvest period (35 days), accumulated yield data were used to calculate the BE and mushroom size. Substrate dry weights were used to calculate percentage BE ([weight of fresh mushrooms harvested/substrate dry matter content] x 100). Basidiome size was determined by: total weight of fresh mushrooms harvested (yield)/total number of mushrooms harvested.

Experimental design

Both Crops I and II were completely randomized designs with 28 and 16 replicates, respectively, 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 and Torrie 1980).

3 RESULTS

Mushroom yield and biological efficiency

For Crop I (Table 1), yields ranged from 326 g/bag (5% brewer's grain supplementation; strain 827) to 573 g/bag (15% brewer's grain supplementation; strain 515). There were no significant differences in yield between strains within individual supplementation levels of 5 and 10%. However, strain 515 produced significantly greater yields at 15% brewer's grain supplementation than strain 827. For strain 515, increasing brewer's grain supplementation from 5% to 15% increased yields by 41%, whereas for strain 827 the

increase was only 19%. Biological efficiencies for Crop I ranged from 30.7% to 54.1% (Table 1).

For Crop II, yields and BEs were highest for cottonseed hulls supplemented with 45% brewer's grain (Table 2). Yield increases for brewer's grain supplementation were not linear, however. As supplementation levels for brewer's grain increased from 15 to 30%, yields increased 39%, whereas yields only increased an additional 15% when brewer's grain in the substrate was increased from 30 to 45%. A similar trend was observed for SMIISE® supplementation; i.e., as SMIISE® was increased from 3 to 6% of the dry substrate weight, yield increased 90%, whereas an increase from 6 to 9% supplementation resulted in an additional 84% increase. Thus, the magnitude of the yield response was much greater for SMIISE® than for brewer's grain supplementation.

Table 1. Effect of brewer's grain supplementation on yield and size of *Pleurotus eryngii* (Crop I; stains 827 and 515) produced on a mixture of oak sawdust, white millet (15% dry wt) and wheat bran (10% dry wt) for 28 days.

	Yield[x]		BE[y]		Size[z]	
Brewer's Grain	Strain		Strain		Strain	
Supplementation	827	515	827	515	827	515
--- % dry wt ---	------- g -------		------ % ------		------- g -------	
5	326c	338c	30.7	31.9	27.2a	15.8c
10	403b	430b	38.0	40.7	21.5b	15.8c
15	404b	573a	38.1	54.1	22.9b	17.5c

[x]Yield expressed as g mushrooms harvested per 2.65 kg wet (59%) substrate. Means followed by the same letter are not significantly different according to the Waller-Duncan k-ratio t-test.
[y]BE; biological efficiency (ratio of g fresh mushrooms harvested per g dry substrate weight expressed as a percentage).
[z]Average weight per mushroom. Means followed by the same letter are not significantly different according to the Waller-Duncan k-ratio t-test.

Table 2. Effect of SpawnMate IISE® and brewer's grain supplementation on yield and size of *Pleurotus eryngii* (Crop II; strain 827) produced on cottonseed hulls for 28 days.

Supplement	Amount	Yield[x]	BE[y]	Size[z]
	- % dry wt -	- g -	- % -	-- g --
SpawnMate IISE®	3	80d	7.3	43.5a
SpawnMate IISE®	6	152d	14.0	37.9a
SpawnMate IISE®	9	279c	25.7	35.7ab
Brewer's grain	15	327c	30.1	35.3ab
Brewer's grain	30	455b	41.9	22.0c
Brewer's grain	45	538a	49.5	27.6bc

[x]Yield expressed as g mushrooms harvested per 2.65 kg wet (59%) substrate. Means followed by the same letter are not significantly different according to the Waller-Duncan k-ratio t-test.
[y]BE; biological efficiency (ratio of g fresh mushrooms harvested per g dry substrate weight expressed as a percentage).

[z]Average weight per mushroom. Means followed by the same letter are not significantly different according to the Waller-Duncan k-ratio t-test.

Basidiome size

For Crop I, mushroom size was significantly higher for strain 827 when compared to strain 515 (Table 1). Basidiome size ranged from 27.2 to 21.5g/mushroom for treatments containing strain 827 while mushroom size ranged from 17.5 to 15.8g/mushroom for strain 515. Mushroom size decreased for strain 827 as brewer's grain supplementation increased from 5 to 15% of dry substrate weight. There was no significant difference in mushroom size for strain 515 for any of the treatments.

For Crop II, there was a general tendency for mushroom size of strain 827 to decrease as the level of supplementation increased for both SIISE® and brewer's grain (Table 2). Basidiome size ranged from 43.5 g/mushroom for 3% SMIISE® to 22 g/mushroom for 30% brewer's grain supplementation.

4 DISCUSSION

Both sawdust-based and cottonseed hull-based substrates produced basidiomes of similar quality. Highest yields and BE's were recorded for strain 515 grown on a sawdust-millet-wheat bran-based medium supplemented with 15% brewer's grain. Upadhyay and Vijay (1991) reported BE's as high as 76% using chopped wheat straw supplemented with 20% brewer's grain. In contrast, our highest yields (54% BE) were observed on wood chips supplemented with white millet (15%) wheat bran (10%) and 15% brewer's grain.

Our data show that an addition of 9% SM to pasteurized cottonseed hulls, stimulated mushroom yield 3-fold over substrates containing only 3% SM. This finding would make it feasible to grow the king oyster mushroom on pasteurized substrates using technology similar to that currently used to grow other species of *Pleurotus*. However, growers should take into account the tendency for size to decrease as yields increase. In some markets, a smaller basidiome size would result in a decrease in the value of the mushroom crop. In other markets, reduced size may not be a consideration.

Testing of other commercial supplements either singly or in combination, may lead to higher yielding substrates for *Pleurotus* production (see Royse and Zaki, 1991). In addition, the development of controlled release brewer's grain may also result in higher yielding substrates for the king oyster.

At present, it appears that *P. eryngii* would hold the greatest future potential for production increases of oyster mushrooms. The relatively long shelf life of this species compared to the other species of *Pleurotus*, make it a valuable addition to the specialty fare available to consumers.

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