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Yield, mushroom size and time to production of *Pleurotus cornucopiae* (oyster mushroom) grown on switch grass substrate spawned and supplemented at various rates

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Abstract

To find a cost effective alternative substrate, *Pleurotus cornucopiae* 608 (yellow basidiomata) was grown on: (1) chopped, pasteurized switch grass (*Panicum virgatum*, 99%) with 1% ground limestone and (2) a mixture of pasteurized cottonseed hulls (75% dry wt.), 24% chopped wheat straw, and 1% ground limestone (all ingredients wt./wt.). The substrates were spawned at various levels (2.5%, 3.75% or 5% wet wt., crop I) and non-supplemented or supplemented with commercial delayed release nutrient (Campbell's S-41) at various levels (0%, 1.5%, 3%, 4.5%, 6%, 7.5% and 9% dry wt., crop II). Maximum yield (weight of fresh mushrooms harvested at maturity) was obtained on cottonseed hull/wheat straw substrate at a 3.75–5% spawn level and 6% S-41 supplement. On switch grass substrate, increasing spawn levels and supplement levels stimulated yields in a linear fashion. However, maximum yields were only 46% or less for those of similar treatments on cottonseed hull/wheat straw substrate. Yields were three times higher on switch grass that was harvested after the grass had senesced (winter; beige color) compared to material that was harvested when the grass was green (summer; time of flowering). Additional physical processing of the material, such as milling, may improve yield potential of this material.

1. Introduction

Increase in consumer demand for oyster mushrooms last year in the United States continued its rapid pace of the last six years. Annual production increases for *Pleurotus* spp. have averaged 14% (from 880 t in 1996 to 1940 t in 2002; USDA, 2002). International demand for oyster mushrooms has remained steady (at about 900,000 t annually) over the last decade. Production of *Pleurotus* spp. accounted for 14.2% of the total world output (6,161,000 t) of edible mushrooms in 1997, the most recent year statistics were available (Chang, 1999). The Peoples' Republic of China is the major producer and consumer of oyster mushrooms, accounting for nearly 90% of total world production.

Farmers in the United States received an average of \$4.40 (US) per kg for fresh oyster mushrooms while growers of *Agaricus bisporus* received an average of \$2.29 per kg (fresh product) in the 2001–2002 growing season. The higher price received for fresh oyster mushrooms reflects, in part, the less developed and less reliable technology available to growers for cultivating these species (Royse, 2002, 2003). Thus, growers need potentially higher incomes to help offset the increased risks and more demanding management required for producing *Pleurotus* spp.

In recent years, oyster mushrooms have been produced on a substrate containing cottonseed hulls, wheat or oat straw, sawdust or combinations of these ingredients (Royse and Schisler, 1987b; Royse and Bahler, 1988; Royse and Zaki, 1991; Royse, 1997). In some parts of the US, these raw materials may not be

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available or are available at relatively high prices. Thus, farmers are perpetually searching for alternative substrates that may be more readily available or cost effective, or that may provide higher yield and better mushroom quality. Switch grass (*Panicum virgatum*), a perennial native to all of the US except California and the Pacific Northwest, inhabits prairies, savannas and flood plains. In recent years, it has become a popular ornamental due to its fine-textured flowers and seed heads that produce a cloud like effect in urban landscapes. The C4 plant has a dense, upright growth habit that reaches a height of 1–2 m when it flowers in late summer in North America. The foliage is green to blue–green that takes on a beige color during the fall and winter. It is commonly used for ground cover for farm acres placed in the Conservation Reserve Enhancement Program of the federal government.

Recently, it was demonstrated that switch grass might be burned in power plants in combination with coal to produce electricity. In Europe, under the auspices of the European switch grass project, it was introduced from North America as seed and is being evaluated as an energy and fiber crop. The increasing availability of switch grass has prompted mushroom growers to consider use of this plant to produce mushrooms. Therefore, the purpose of this work was to investigate the suitability of switch grass as a raw material for oyster mushroom production. We sought to evaluate the effect of spawn rates and supplement levels in substrate on mushroom yield and time to production.

2. Methods

2.1. Substrates and preparation

Switch grass (*Panicum virgatum* L.) was harvested at two stages during the season. The first harvest (crop I) was completed when the grass had completed flowering and was still green in color (August, 2001). The second harvest (crop II) occurred in late winter (February, 2002) when the grass had turned a beige color. The grass was mowed, allowed to air dry for two days, and then baled in 500 kg round bales. The bales were transported to the Mushroom Test Demonstration Facility and stored under roof cover until used. For crop I, the baled grass was chopped to a 5–15 cm length with a bale chopper (WIC, Wickham, Quebec, Canada) and added to a scale-mounted Jalor® feed mixer (Orton, Ontario, Canada). Ground limestone (1% dry wt.) was dry mixed then sufficient water was added (by weight) to increase substrate moisture content to 68%. After a 5-min mix, the moistened substrate was filled into two galvanized metal boxes with perforated floors. For crop II, the grass was not chopped with a bale chopper prior to adding to the feed mixer. The grass was processed directly by the feed mixer that was equipped with cutter blades to process the raw material. Processing required about 15 min to adequately size the grass to the desired length (5–15 cm). The mixed substrates were pasteurized with aerated steam at 65 °C for 45 min by passing the air–steam mixture through the substrate from top to bottom (Royse, 2002, 2003). After pasteurization was complete, filtered air (HEPA filter; 99.9% efficiency) was passed through the substrate for cooling (1.5 h).

For standard (control) substrates for both crops I and II, wheat straw (*Triticum aestivum* L.) was chopped to a 5–15 cm length with a bale chopper. The straw (24% dry wt.) was added to cottonseed (*Gossypium hirsutum* L.) hulls (75% dry wt.) and ground limestone (1% dry wt.) contained in a feed mixer as outlined above. The addition of water and processing of raw materials was exactly as outlined for switch grass in crop I.

Dry substrate weights were determined by heating 100 g of freshly prepared substrate at 105 °C for 24 h. The calculated dry weight was subsequently used to determine the biological efficiency (BE) percentage ([weight fresh mushrooms harvested/dry substrate weight] $\times$ 100).

2.2. Spawn, supplements and spawn run

Isolate No. 608 (*P. cornucopiae*; yellow basidiomes; available from author) was selected for this study because it is used commercially in the United States. The isolate was maintained (Jodon and Royse, 1979) and spawn was prepared as outlined by Royse and Schisler (1987a). Depending on crop and treatment, various levels of spawn and commercial delayed release supplement (Campbell's S-41; Wu and Bretzloff, 1985) were added to each bag. Substrate for crop II was spawned with 3.75% spawn (based on wet substrate wt.). Each bag (black; $23 \times 15 \times 76$ cm, 0.003 mil; 48 pre-punched holes, 6 mm dia) contained 9.1 kg of moist substrate (68%) for both crops.

2.3. Experimental design

The experiments were designed as 2 (green switch grass versus CH + straw) $\times$ 2 (non-supplemented versus supplemented) $\times$ 3 (spawn rates of 2.5%, 3.75% and 5%); and 2 (senescent switch grass versus CH + straw) $\times$ 7 (supplement levels of 0%, 1.5%, 3%, 4.5%, 6%, 7.5% and 9%) factorials in completely randomized designs with six and five replicates per treatment (Steel and Torrie, 1980) for crops I and II, respectively. The SAS program JMP (SAS Institute, 1996) was used to analyze data. The standard least squares model procedure was used to perform a two-way analysis of variance with interaction. The Tukey–Kramer honestly significant difference

(HSD) was used to separate treatment means (SAS Institute, 1996).

2.4. Experimental conditions

After spawning the bags were moved to a production room where air temperature was maintained at 18 ± 2 °C. Relative humidity was maintained at 95–98% to minimize drying of the substrate surfaces. Only recirculated air was used for cooling and air distribution. The first 12 days of spawn run were completed without artificial lighting. At the end of this period, 4 h of light (necessary for proper basidiome development) were provided daily by eight 1.2 m cool white fluorescent bulbs. Light intensity, measured at various locations in the growing room, ranged from 50 to 300 lux and the lighting cycle was controlled automatically. At time of pinning, sufficient fresh air was introduced to lower CO_2 levels below 700 ppm.

2.5. Harvesting and determination of BE and mushroom size

Mushrooms were harvested from the substrate the same time each day when the in-rolled margins of the basidiomes began to flatten. The substrate clinging to the stipe was cut away and the mushrooms were counted and weighed. At the end of the 35-day harvest period, yield (fresh mushrooms harvested at maturity), BE and average mushroom size were calculated. BE is the ratio of kg fresh mushrooms harvested per kg dry substrate (including supplement) and expressed as a percentage. Mean mushroom size was determined as follows: total weight of fresh mushrooms harvested/total number of mushrooms harvested.

3. Results

3.1. Yield and BE

Significant sources of variation in the analysis of variance for yield for both crops I and II included substrate type (ST), supplement level (SL), and ST × SL (Table 1). Yields were at least 3-fold higher (crop I) for cottonseed hull/wheat straw substrate compared to all treatments containing switch grass (Table 2). Increased spawn and supplement levels in both substrates types stimulated yields. The highest yield with switch grass was obtained from substrate containing 6% supplement (Table 2). Overall yields (crop II) from switch grass substrate were >3-fold higher compared to crop I (Tables 3, 4). For example, treatments containing 6% supplement and spawned at 3.75% yielded 0.68 kg/bag (17.7% BE) in crop I (Trt 11) compared to 2.10 kg/bag (54.6% BE) in crop II (Trt 12).

On both substrate types, a positive correlation was observed between the amount of supplement/treatment and mushroom yield ($R^2 = 0.616$, 0.935; crops I and II, respectively on switch grass) ($R^2 = 0.694$, 0.456; crops I and II, respectively on cotton seed hull/wheat straw). As the supplement rate increased, the yield (BE) response increased (Table 5). Average yields ranged from 1.82 kg/9.1 kg (47.4% BE) for non-supplemented substrate to 2.37 kg/9.1 kg (61.7% BE) for 6% spawn rate (crop I, Table 5). Yields for crop II (Table 5) ranged from 1.95 kg/9.1 kg (53.6% BE) for non-supplemented substrate to 3.18 kg/9.1 kg (80.6% BE) for 9% supplement.

For crop I, increasing levels of spawn resulted in yield (BE) increases (Table 6). As the spawn rate doubled from 2.5% to 5%, yields increased from 1.93 kg/9.1 kg (50.2%) to 2.28 kg/9.1 kg (59.3%).

Table 1
Probabilities greater than Fisher's (F) test from analysis of variance for two crops (I, II) for (*Pleurotus cornucopiae*) yield, mushroom size and days to production (from time of spawning)

Source	Probability > F[a]			
	df	Yield	Size	Days to production
Crop I				
Substrate type (ST)	1	0.0001	0.0006	0.0001
Supplement level (SL)	1	0.0001	0.0062	0.0195
Spawn rate (SR)	2	0.0021	0.7837	0.0001
ST × SL	1	0.0005	0.0656	0.0496
ST × SR	2	0.2721	0.0958	0.0001
SL × SR	2	0.5975	0.0240	0.3320
ST × SL × SR	2	0.3754	0.0370	0.0895
Crop II				
Substrate type (ST)	1	0.0001	0.1146	0.0669
Supplement level (SL)	6	0.0001	0.0091	0.0001
ST × SL	6	0.0001	0.0182	0.0001

Mushrooms were grown on two substrate mixtures: (1) 75% cottonseed hulls, 24% wheat straw and 1% $CaCO_3$, or (2) 99% switch grass and 1% $CaCO_3$. Substrates were non-supplemented or supplemented with delayed release nutrient (Campbell's S-41) and spawned at various levels.
[a] Values of less than 0.05 were considered significant.

Table 2
Yield (kg), percentage BE (%BE), mushroom size (g/mushroom) and days to production (from time of spawning) for *Pleurotus cornucopiae* (crop I) spawned at three rates and grown on two substrates non-supplemented or supplemented with delayed release nutrient (Campbell's S-41)

No.	Substrate type	Supplement level (% dry wt.)[a]	Spawn rate (% wet wt.)[b]	Yield (kg)[c]	BE (%)[d]	Size (g)	Days to production
1	CH[e] + straw	0	5.00	3.42b[f]	93.9	11.5abc	24.7a
2	CH + straw	0	3.75	3.49b	95.9	12.3ab	25.3a
3	CH + straw	0	2.50	3.17c	87.3	12.4ab	25.7a
4	CH + straw	6	5.00	4.14a	107.7	11.8abc	23.3a
5	CH + straw	6	3.75	4.10a	106.7	11.2bcd	23.5a
6	CH + straw	6	2.50	3.99a	103.8	13.0a	25.2a
7	Switch grass[g]	0	5.00	0.58ef	15.8	10.8bcde	34.8bc
8	Switch grass	0	3.75	0.39fg	10.7	10.2cde	38.0cd
9	Switch grass	0	2.50	0.19g	5.3	9.2e	47.8f
10	Switch grass	6	5.00	0.97d	25.2	9.5de	34.3b
11	Switch grass	6	3.75	0.68e	17.7	10.9bcd	38.5d
12	Switch grass	6	2.50	0.37fg	9.6	12.1ab	42.2e

[a] Percentage of oven dry substrate weight (3.64 kg).
[b] Percentage of moist (68%) substrate weight.
[c] Yield = kg fresh mushrooms harvested at maturity/9.1 kg moist substrate weight.
[d] %BE = (kg fresh mushrooms/kg dry substrate) × 100 (includes supplement wt.).
[e] CH = Cottonseed hulls.
[f] Means followed by the same letter in the same column are not significantly different according to Tukey's honestly significant difference (HSD).
[g] Switch grass was harvested green and allowed to dry before use.

Table 3
Yield (kg), percentage BE (%BE), mushroom size (g/mushroom) and days to production (from time of spawning with 3.75% spawn, wet substrate wt.) for *Pleurotus cornucopiae* (crop II) grown on two substrate types non-supplemented or supplemented with seven levels of delayed release nutrient (Campbell's S-41)

No.	Substrate type	Supplement level (% dry wt.)[a]	Yield (kg)[b]	BE (%)[c]	Size (g)	Days to production
1	CH[d] + straw	0	3.29d[e]	90.5	9.2e	22.2cd
2	CH + straw	1.5	3.43cd	93.1	9.1e	21.6c
3	CH + straw	3	3.75b	100.3	9.1e	21.2c
4	CH + straw	4.5	3.89ab	102.6	9.9de	21.2c
5	CH + straw	6	3.69bc	96.1	9.1e	21.4c
6	CH + straw	7.5	3.90ab	100.0	10.1de	21.8c
7	CH + straw	9	4.03a	102.0	9.6de	21.8c
8	Switch grass[f]	0	0.61i	16.8	10.3cde	24.2bd
9	Switch grass	1.5	1.18h	31.9	10.7cd	24.4b
10	Switch grass	3	1.57g	41.9	11.4bc	24.4b
11	Switch grass	4.5	2.04f	53.8	10.8cd	24.2bd
12	Switch grass	6	2.10ef	54.6	13.1a	28.2a
13	Switch grass	7.5	2.16ef	55.4	12.1ab	29.6a
14	Switch grass	9	2.32e	58.8	13.0a	30.0a

[a] Percentage of oven dry substrate weight (3.64 kg).
[b] Yield = kg fresh mushrooms harvested at maturity/9.1 kg moist substrate weight.
[c] %BE = (kg fresh mushrooms/kg dry substrate) × 100.
[d] CH = Cottonseed hulls.
[e] Means followed by the same letter in the same column are not significantly different according to Tukey's honestly significant difference (HSD).
[f] Switch grass was harvested after senescence.

3.2. Size

Significant sources of variation for mushroom size (crop I) were found for substrate type (ST) supplement level (SL), SL × spawn rate (SR) and ST × SL × SR (Table 1). For crop II, significant sources of variation included SL, and ST × SL. Mushroom size was not significantly affected by spawn rate (crop I, Table 1). Mushroom size was significantly larger on cottonseed hulls/wheat straw substrate in crop I, but significantly smaller for crop II (Table 4).

3.3. Days to production

Significant sources of variation for days to production in crop I were observed for ST, SL and SR,

Table 4
Means and groupings from analysis of variance for two crops (I, II) for (*Pleurotus cornucopiae*) yield, mushroom size and days to production (from time of spawning)

Substrate type	No. of reps.	Yield (kg)	BE (%)[a]	Size (g)	Days to production
Crop I					
CH[b] + straw	36	3.72a[c]	99.5	12.0a	24.6a
Switch grass	33	0.53b	14.2	10.5b	38.7b
Crop II					
CH + straw	35	3.71a	96.5	9.4a	21.6a
Switch grass	35	1.71b	44.5	11.6b	26.4b

Mushrooms were grown on two pasteurized (65 °C/45 min) substrates non-supplemented or supplemented with various levels of Campbell's S-41 nutrient.
[a] BE (%) = Percentage biological efficiency including weight of supplement.
[b] CH = Cottonseed hulls.
[c] Means followed by the same letter within the same column and crop are not significantly different according to Tukey's honestly significant difference (HSD). Each bag (replication) contained 9.1 kg wet wt. (3.64 kg dry wt.) for crops I and II, respectively.

Table 5
Means and groupings from analysis of variance for two crops (I, II) for (*Pleurotus cornucopiae*) yield, mushroom size and days to production (from time of spawning)

Supplement level (%)[a]	No. of reps.	Yield (kg)	BE(%)[b]	Size (g)	Days to production
Crop I					
0	33	1.82b[c]	47.4	11.2 NS	32.0a
6	36	2.37a	61.7	11.4NS	30.9b
Crop II					
0	10	1.95b	53.6	9.8b	23.2ab
1.5	10	2.30ab	62.4	9.9ab	23.0ab
3	10	2.66ab	71.1	10.2ab	22.8b
4.5	10	2.97a	78.3	10.4ab	22.7b
6	10	2.90ab	75.4	11.1ab	24.8ab
7.5	10	3.03a	77.8	11.1ab	25.7a
9	10	3.18a	80.6	11.3a	25.9a

Mushrooms were grown on two pasteurized (65 °C/45 min) substrates non-supplemented or supplemented with various levels of Campbell's S-41 nutrient.
[a] Percentage of dry substrate weight.
[b] BE (%) = Percentage biological efficiency including weight of supplement.
[c] Means followed by the same letter within the same column and crop are not significantly different according to Tukey's honestly significant difference (HSD). Each bag (replication) contained 9.1 kg wet wt. (3.64 kg dry wt.).

Table 6
Means and groupings from analysis of variance for two crops (I, II) for (*Pleurotus cornucopiae*) yield, mushroom size and days to production (from time of spawning)

Spawn rate (%)[a]	No. of reps.	Yield (kg)	BE (%)[b]	Size (g)	Days to production
Crop I					
2.50	21	1.93a[c]	50.2	11.9a	33.7a
3.75	24	2.16ab	56.2	11.1ab	31.3b
5.00	24	2.28b	59.3	10.9b	29.3b
Crop II					
3.75	70	2.84	70.5	7.9	24.0

Mushrooms were grown on two pasteurized (65 °C/45 min) substrates non-supplemented or supplemented with various levels of Campbell's S-41 nutrient and spawned at various rates.
[a] Percentage of moist (68%) substrate weight.
[b] BE (%) = Percentage biological efficiency including weight of supplement.
[c] Means followed by the same letter within the same column and crop are not significantly different according to Tukey's honestly significant difference (HSD). Each bag (replication) contained 9.1 kg wet wt. (3.64 kg dry wt.).

ST × SL, ST × SR and ST × SL × SR (Table 1). For crop II, significant sources of variation included spawn level and ST × SL. In general, as supplement level increased up to 4.5%, days to production decreased (Table 4).

However, as supplement levels increased beyond 4.5%, days to production tended to increase (Table 4). Increased spawn levels resulted in decreased days to production (Table 6). At a 2.5% spawn rate, days to production were 33.7 while 29.3 was observed for a spawn rate of 5% (crop I, Table 6). Thus, doubling the spawn rate (from 2.5% to 5%) resulted in a decrease in time to production of 4.4 days.

4. Discussion

Time of harvest of switch grass is an important factor for production of oyster mushrooms. It is important that switch grass be harvested after the plant has senesced and turned a beige color. Yields, mushroom size and days to production were all positively affected when the grass was harvested later in its life cycle. However, substrates made from switch grass were overall inferior to cottonseed hull/wheat straw substrates. Even when switch grass was harvested at a later maturity, yields were only about 46% that of cottonseed hull/wheat straw substrates. Additional physical processing of the material, such as reducing particle size and crushing the stems and leaves, may improve yield potential of this material. A short composting cycle (3–4 days) may also help to soften the stems, degrade the waxy layer of the epidermis and increase water-holding capacity (Royse and Rhodes, unpublished).

Increasing spawn rate and supplementation with commercial nutrient were important factors in stimulating yield of *P. cornucopiae* on a pasteurized mixture of both substrate types. This is not unexpected, as other researchers have observed yield increases as spawn levels have increased (Laborde et al., 1985a,b, Royse, 2002). Yield increases may be due to several factors. First, the increased level of nutrient available in spawn at higher rates would provide more energy for mycelial growth and development. Second, more inoculum points, available from increased spawn levels, would provide faster substrate colonization and thus, more rapid completion of the production cycle. Finally, a more rapid spawn run would reduce the time non-colonized substrate is exposed to competitors such as weed molds and bacteria. The magnitudes of the yield responses to S-41 nutrient were significantly different on different substrate types. Highest BE (102.6%) was obtained on a 4.5% level of S-41 supplement for cottonseed hull/wheat straw substrate while the highest BE (58.8%) was obtained on 9% level of supplement on winter-harvested switch grass substrate. Supplement levels greater than 4.5% did not result in significantly higher yields on cottonseed hull/wheat straw substrate. On switch grass, however, increased yields were evident with increasing levels of supplement. This result was in line with earlier research on supplementation of wheat straw substrate with formaldehyde-treated ground soybean showing an increased yield response up to 12% of the substrate dry wt. (Royse et al., 1991). Thus, the type of substrate appears to have a major influence on the response of increasing levels of delayed release nutrient. The fibrous nature of switch grass may slow colonization and decay of cellular structures; thus, during early colonization, the fungus may obtain more nutrients from the S-41 supplement. On the other hand, nutrients from cottonseed hull/wheat straw raw material may be more readily available during early colonization so the fungus is ready to fruit sooner. There was a 14- and 5-day differential in fruiting on switch grass harvested summer or winter, respectively, compared to cottonseed hull/wheat straw substrate.

Cost effective production of oyster mushrooms depends on the reliability, availability and cost of substrate ingredients. Based on yield and net cost, it does not appear that switch grass is an economically viable alternative for growers currently using cottonseed hulls/wheat straw substrates. The possibility of using more physical processing, such a milling, grinding or composting, may increase the yield potential of switch grass. More rapid diversification of the US mushroom industry will ultimately depend on the use of more cost effective raw materials that are equivalent or better for production than the current industrial standard of cottonseed hulls/wheat straw. Development of alternative substrates with higher yield capacity and fewer days to production would lower the cost of production of *Pleurotus* spp. mushrooms and should ultimately lower the cost to consumers.

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