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Improvement of yield of *Pleurotus eryngii* var. *eryngii* by substrate supplementation and use of a casing overlay

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ABSTRACT

Improved yield and biological efficiency (BE) of *Pleurotus eryngii* var. *eryngii* were achieved by supplementation of substrate with a commercial delayed-release nutrient and use of a casing overlay. Yield increases of 14% were achieved from cased substrates that were supplemented at time of casing with delayed-release nutrient (Remo's). Use of a casing layer enhanced yield by 141% over non-cased substrates. When casing and substrate supplementation were combined, yield increased 179% over non-cased/non-supplemented substrates. Mushrooms harvested from cased substrates were darker in color and solids contents were lower compared to non-cased substrates. An additional break of mushrooms was harvested from non-cased "spent" substrate by fragmenting and re-supplementing the substrate prior to the application of a casing overlay. Three production methods were compared for their effect on mushroom yield: "standard", "casing" and "casing after first break". Casing of the substrate before first break ("casing" production method) resulted in the highest yield and biological efficiency.

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1. Introduction

The crop cycle of many cultivated edible mushrooms consists of more than one break or flush. For example, growers typically harvest three breaks in single crops of *Agaricus bisporus*, *Lentinula edodes*, and *Pleurotus ostreatus* before the substrate is discarded (Velazquez-Cedeno et al., 2002; Royse, 2001; Royse et al., 2008). However, growers that produce *Pleurotus eryngii* var. *eryngii* in bottles or bags typically harvest only one break before cleanout. On farms where environmental controls are rudimentary or lacking or where production is outdoors, growers may use a casing overlay that minimizes the loss of substrate moisture content and allows more than one break (Oei, 2006; Rodriguez Estrada and Royse, 2008; Tan et al., 2005).

Biological efficiencies (BE), obtained from a single break of *P. eryngii* grown on substrate in bottles or bags, may average 50–80% (Rodriguez Estrada, 2008). Although these values are relatively high, discarding substrate that produced only one break is inefficient, especially since mushroom growers are experiencing shortages of cost-efficient raw materials. One strategy to improve yield and BE may include supplementation of substrate at early or later stages of the production cycle (Curvetto et al., 2002; Rodriguez Estrada and Royse, 2007; Royse et al., 2004; Schisler, 1990; Schisler and Sinden, 1962; Sinden and Schisler, 1962). For *A. bisporus*, yields for each successive break decrease mainly as a consequence of nutrient depletion in the production medium (Royse et al., 2008; Schisler, 1964). Therefore, supplementation at spawning or casing with delayed-release nutrients is a practice commonly used in the commercial cultivation of *A. bisporus*. Although available commercial supplements have been developed specifically for use with this species, researchers have found that many supplements are effective in stimulating yields of some specialty mushrooms such as *Pleurotus* spp. (Royse, 1999).

Development of cost-efficient production methods to improve yield and BE without sacrificing mushroom quality is a major focus of many researchers and growers. In this work, we sought to increase BE and yield of *P. eryngii* var. *eryngii* using a casing overlay and by the addition of a delayed-release supplement to the substrate. We also evaluated the effects of these two factors on the number of mushrooms produced, pileus color, and mushroom solids content. Finally, we compared the influence of three production methods on mushroom yield: (1) "standard" method using no casing, (2) "casing", i.e., application of a casing overlay after substrate colonization, and (3) "casing after first flush" i.e., adding a casing layer after harvest of first break.

2. Methods

2.1. Experimental design

Part 1 of the experiment was a 2×3 factorial in a completely randomized design where influence of a casing layer (cased vs.

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non-cased) and delayed-release nutrient supplementation (non-supplemented, supplemented at spawning, and supplemented at casing) were evaluated for their effects on yield, BE and number of mushrooms. Each treatment had 6 replicates resulting in 36 experimental units (Table 1). Part 2 of the experiment consisted of applying additional treatments to non-cased substrate that had produced only one break of mushrooms. Part 2 of the experiment is described in Section 2.6 below.

2.2. Spawn

Spawn of *P. eryngii* var. *eryngii* (WC888) was prepared in 500 ml flasks by autoclaving a mixture of Hesco (Watertown, SD) mushroom rye grain (91 g), 3 month aged Northern red oak (*Quercus rubra* L.) sawdust (13 g w.w., 18% moisture), $CaSO_4$ (3 g) and 120 ml of warm tap water. After ingredients were cooled, five mycelial-agar plugs (5 mm diameter) were placed into the grain mixture and then incubated for one week at room temperature. Spawn was then shaken in order to evenly distribute mycelia on the grain. Spawn was incubated for an additional week, shaken again and then stored at 4 °C until used.

2.3. Substrate preparation, inoculation and incubation

Mushrooms were produced on a substrate that contained: cottonseed hulls (56% in formula, 12% moisture), corn distiller's waste (4% in formula, 11% moisture), calcium sulfate (1%), ground soybean (12% in formula, 7% moisture) and aged Northern red oak (*Q. rubra* L.) sawdust (27% in formula, 18% moisture). Dry ingredients were mixed for 2 min in a paddle mixer at the Mushroom Research Center (MRC) then warm tap water was added to reach 60% moisture content. Final moisture content of substrate for each treatment was determined in triplicate with an Ohaus moisture analyzer (Model MB35). Moistened substrate was packed into polypropylene (PP) bags (2.5 kg) with medium porosity filters (Unicorn Import and Manufacturing, Commerce, TX) and then autoclaved at 121 °C for 90 min. Cooled substrate was inoculated with 30 g (±0.1 g) of grain spawn. Bags were heat-sealed and then vigorously shaken to uniformly distribute spawn. Substrate was incubated for 25 days in a 9 m^2 room at the MRC. Room temperature was maintained at 21 °C with a cycle of 8 h light/16 h dark using cool-white fluorescent bulbs.

2.4. Supplementation at spawning

Treatments 2 and 5 (NC/S/AS and C/S/AS, respectively, Table 1) were supplemented at time of substrate preparation. Because the substrate was inoculated the day after it was prepared, it was referred as "supplementation at spawning" (AS). A commercial delayed-release nutrient supplement (Remo's, corn and soybean based) was added to the substrate (4% d.w.) and mixed prior to addition of water. The substrate was packed, sterilized, spawned and incubated as outlined above.

Table 1
Experimental design used to evaluate the influence of a casing layer and delayed-release nutrient supplement on yield, biological efficiency and number of mushrooms (*Pleurotus eryngii* var. *eryngii*).

Treatment number	Treatment designation[a]	Casing layer	Supplement[b]
1	NC	No	No
2	NC/S[b]/AS	No	Spawning
3	NC/S/AF	No	Fragmentation
4	C	Yes	No
5	C/S/AS	Yes	Spawning
6	C/S/AF	Yes	Fragmentation

[a] NC: non-cased; NC/S/AS: non-cased, supplemented (Remo's) at spawning; NC/S/AF: non-cased, supplemented at fragmentation; C: cased; C/S/AS: cased, supplemented at spawning; C/S/AF: cased, supplemented at fragmentation.
[b] Remo's supplement added at 4% dry substrate weight.

2.5. Substrate fragmentation, supplementation at fragmentation and casing

After incubation, colonized substrate from all treatments was removed from bags and manually fragmented. Treatments 3 and 6 (NC/S/AF and C/S/AF, respectively) were supplemented at time of fragmentation by adding 4% (d.w.) delayed-release nutrient (Remo's) and uniformly mixed by hand. Non-supplemented and supplemented substrates were compacted into 6.1 L plastic bins (33 cm long × 18.5 cm wide × 12 cm deep, Rubbermaid®) and then either non-cased (NC) or cased (C). Non-cased bins (Treatments 1, 2, and 3) were covered with perforated plastic sheeting (holes 7 mm in diameter, separated by 44 mm × 94 mm spaces, Fig. 1a). Bins for treatments 4, 5 and 6 were cased with a mixture of 2:1 d.w. peat moss and calcium carbonate (78.7% moisture). Casing (1 kg, w.w.) was overlaid on the surface of exposed substrate (0.44 g/cm^2 d.w., 2.5 cm deep) (Fig. 1d). Bins were transferred to a production room set at 90% relative humidity (RH), 16 °C and 8 h light/16 h dark cycle. Plastic sheeting was removed once primordia made contact with the film (Fig. 1b). Cased substrate in bins was watered (field capacity) at intervals of three to four days. Primordia formed below the casing layer and mushrooms emerged through the layer as shown in Fig. 1e. Mushrooms were manually harvested when mature (Fig. 1c and f); second and third flushes were obtained from cased substrates (4, 5 and 6) without further treatment. Additional flushes for the non-cased treatments (1, 2 and 3) were obtained from the substrate by adding a casing layer after first flush was harvested (Part 2 of the experiment described below).

2.6. Induction of subsequent flushes in non-cased substrates

In order to increase productivity of non-cased substrates that otherwise would be discarded, a casing overlay was used to induce subsequent flushes. The additional set of treatments applied to "spent" non-cased substrates remaining after first flush is shown in Table 2. These are referred to as "later-cased" treatments. A second procedure of substrate fragmentation and supplementation was performed for half of the replicates. Therefore, for each original non-cased treatment (NC, NC/S/AS and NC/S/AF) three replicates randomly selected were directly cased and three replicates were fragmented, supplemented and cased. Non-fragmented/non-supplemented (NFNS) substrates (Fig. 2a) were cased with a mixture of 1 kg of casing soil and 1.4% (w.w.) casing inoculum (1st break-colonized substrate) per bin. The fragmented/supplemented treatment (FS) consisted of removal of the substrate from bins, manually fragmenting the substrate and then adding Remo's supplement at 4% (d.w.) initial substrate weight (Fig. 2b). Substrates were thoroughly mixed by hand and then returned to original bins. These treatments were cased as explained above (Fig. 2c).

2.7. Solids

Solids contents of mushrooms harvested from first flush were determined by assessing two mushrooms from each of three bins per treatment (6 replicates per treatment). Whole mushrooms where chopped (10–15 mm^3) and weighed in polystyrene weigh boats (14 × 14 × 2.5 cm, VWR®). Mushroom cubes were dried in an oven (Shel Lab, model 1330GM) at 65 °C for 36 h and then trans-

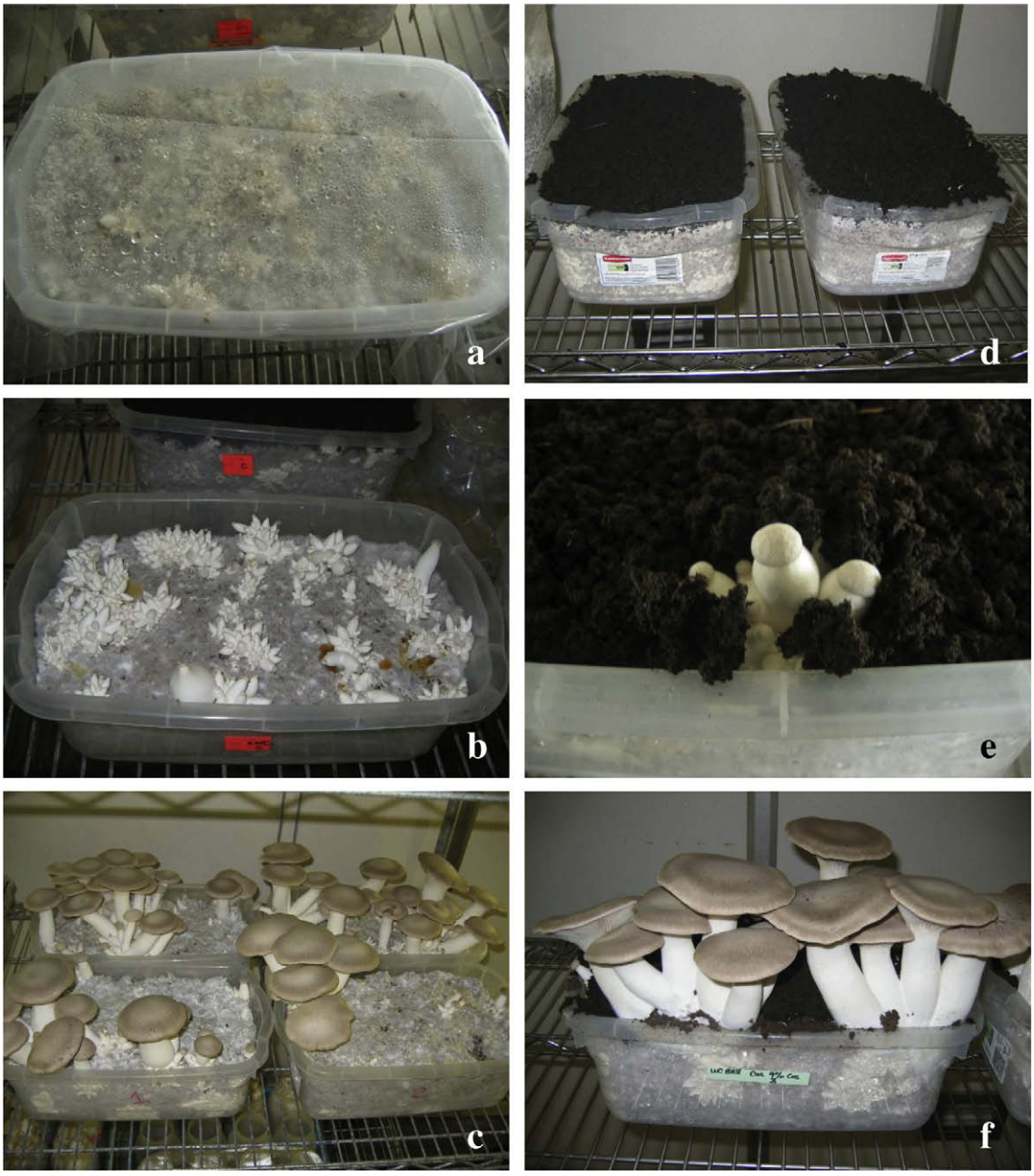

Fig. 1. Primordia and basidiomata development of *Pleurotus eryngii* var. *eryngii* on cased and non-cased substrates (“casing” and “standard” production methods). (a) Colonized substrate was fragmented, placed in a bin and covered with plastic sheeting to maintain moisture and induce primordia formation. (b) Plastic sheeting was removed from the bin when primordia reached the film. (c) Mature basidiomata growing from non-cased substrate. (d) Fragmented substrate covered with a casing layer (3 cm in depth). (e) Primordia emerging from beneath the casing layer. (f) Mature basidiomata growing from cased substrate.

ferred to a new dish (8.9 × 8.9 × 2.5 cm) and re-weighed. Dividing dry weight of cubes by their fresh weight and multiplying the result by 100 calculated percentage solids.

2.8. Pileus color

Pileus color was evaluated for mushrooms harvested during first flush. Three mushrooms per bin were arbitrarily selected and these represented one replicate; three replicates were evaluated per treatment. For each mushroom, three measurements were taken at approximately equal distances around the circumference of the pileus and at a middle distance from center to the margin. Hence, a total of 27 measurements were made per treatment. Brightness of color (L-values) was measured using a chromameter Minolta (model CR-200) (Weil et al. 2004).

2.9. Yield, BE and number of mushrooms

Mushrooms were harvested when fully mature (pileus and margins flat) (Rodriguez Estrada and Royse, 2006). Each harvesting cycle was completed within 2–4 days since not all mushrooms in a bin and across bins matured uniformly. Yield, BE and number of mushrooms were determined for all treatments and flushes. Yield was expressed as fresh mushroom weight (g) per bin; BE is the ratio of basidiomata fresh weight to dry substrate weight, expressed as a percentage. Number of mushrooms obtained per bin was also recorded.

2.10. Statistical analyses

Analysis of variance (ANOVA) and mean separations were performed using SAS statistical software package JMP® (version 7,

Table 2
Description of second treatments (Part 2) applied to substrates for the production of *Pleurotus eryngii* var. *eryngii*. Second treatments were applied to "spent" non-cased substrates to induce additional breaks of mushrooms.

First treatments[a]	Second treatments[b]	Treatment abbreviation[c]
1. NC	(a) Casing (3)	NC-NFNS
	(b) Fragmentation, supplementation and casing (3)	NC-FS
2. NC/S/AS	(a) Casing (3)	NC/S/AS-NFNS
	(b) Fragmentation, supplementation and casing (3)	NC/S/AS-FS
3. NC/S/AF	(a) Casing (3)	NC/S/AF-NFNS
	(b) Fragmentation, supplementation and casing (3)	NC/S/AF-FS

[a] NC: non-cased; NC/S/AS: non-cased, supplemented at spawning; NC/S/AF: non-cased, supplemented at time of fragmentation. Six replicates were used per treatment.
[b] (a) Spent substrate in bins was cased without fragmentation/supplementation; (b) spent substrate was removed from the bins, manually fragmented, supplemented with Remo's (4% d.w.), returned to the bin and cased; (3) three replicates per treatment.
[c] NFNS: non-fragmented/non-supplemented substrate; FS: fragmented/supplemented substrate.

2007). Data for first break (yield, BE, number of mushrooms, solids, and color) were analyzed using a standard least squares procedure in a two-way ANOVA. The model included factors casing layer, delayed-release nutrient, and their interactions. Tukey–Kramer Honestly Significant Difference (HSD) test was used to separate treatment means. In order to compare different production methods ("standard", "casing", and "casing after first flush"), data for yield, BE and number of mushrooms were analyzed using a one-way ANOVA. Because "standard" production method (non-casing) resulted in only one flush and "casing" method produced up to three flushes, data from a single flush obtained from non-cased substrates (treatments 1, 2, and 3) and data from all flushes obtained from cased substrates (treatments 4, 5, and 6) were included in the ANOVA "standard vs. casing". To compare a production method where substrates were cased after first flush vs. casing method, data from all flushes were combined and analyzed using a one-way ANOVA "casing after first flush vs. casing".

3. Results

3.1. First break

First mushroom harvest began 38 days after spawning and lasted 7 days. Yield, BE, and solids were significantly affected by casing layer and supplementation (Table 3). Highest yield (678.2 g) and BE (67%) were observed for treatment C/S/AF (cased substrate supplemented at fragmentation), while lowest yield (367.6 g) and BE (35.9%) were observed for NC/S/AS (non-cased substrate supplemented at spawning) (Table 4). Solids contents were higher in non-cased treatments (10.6–11.8%) compared to cased treatments (7.3–9.1%). Number of mushrooms was affected only by use of a casing layer. Number of mushrooms for cased treatments ranged from 11 to 12, considerably lower than the non-cased treatments NC and NC/S/AF (16 and 21, respectively). Number of mushrooms in treatment NC/S/AS (12) was comparable to cased treatments. Color was significantly affected by casing, supplement and their interactions. Overall L-values (brightness of the pileus) were significantly higher (lighter) for non-cased treatments (68.4–74.1) compared to cased treatments (55.3–57.3).

Fig. 2. "Second treatment" of substrate used to produce 2nd and 3rd breaks of *Pleurotus eryngii* var. *eryngii* (later-cased treatment). (a) Substrate as it remained after first flush. (b) Fragmented, supplemented (Remo's 4% d.w.) substrate. (c) Substrate re-packed into the bin (right) and cased (left).

Table 3
P-values from analysis of variance (ANOVA) for casing layer, supplementation and their interactions influencing yield, BE, number of mushrooms, solids, and pileus color for first break production of *Pleurotus eryngii* var. *eryngii*.

Source	DF	Yield (g)	BE (%)	Number of mushrooms	Solids (%)	Color (L-value)
Model	5	0.0004*	0.0003*	0.0133*	<0.0001*	<0.0001*
Effects tested						
Casing (C)	1	0.0002*	0.0002*	0.0081*	<0.0001*	<0.0001*
Supplement (S)	1	0.0029*	0.0020*	0.1117	0.0119*	0.0015*
C × S	1	0.8828	0.8782	0.1415	0.6340	<0.0001*

* *P* values <0.05 were considered significant.

Table 4
Means and standard deviation for yield, biological efficiency, number of mushrooms, solids and color (brightness) for first break production of *Pleurotus eryngii* var. *eryngii* as influenced by substrate supplementation and application of a casing overlay.

Treatment[a]	Yield[b] (g)	BE[b] (%)	Number of mushrooms[b]	Solids[b] (%)	Color[b] (L-value)
1. NC	481.5 ± 34.2 bc	47.6 ± 3.4 bc	16.3 ± 4.0 ab	10.6 ± 1.2 ab	68.4 ± 2.9 b
2. NC/S/AS	367.6 ± 167.3 c	35.9 ± 16.3 c	11.6 ± 9.6 ab	11.8 ± 0.7 a	74.1 ± 3.8 a
3. NC/S/AF	537.5 ± 45.36 abc	53.1 ± 4.5 abc	21.0 ± 6.6 a	11.6 ± 1.1 a	71.1 ± 4.5 ab
4. C	664.7 ± 114.6 ab	65.7 ± 11.3 ab	12.0 ± 3.1 ab	7.3 ± 1.0 c	55.3 ± 3.6 c
5. C/S/AS	517.5 ± 139.6 abc	50.5 ± 13.6 abc	10.5 ± 4.1 b	9.1 ± 0.2 bc	54.7 ± 4.2 c
6. C/S/AF	678.2 ± 104.1 a	67.0 ± 10.3 a	10.8 ± 3.1 b	8.1 ± 1.3 c	57.3 ± 4.1 c

[a] NC: non-cased; NC/S/AS: non-cased, supplemented at spawning; NC/S/AF: non-cased, supplemented at time of fragmentation; C: cased; C/S/AS: cased, supplemented at spawning; C/S/AF: cased, supplemented at time of fragmentation.
[b] Means ± standard deviation followed by different letters indicates significant differences according to Tukey–Kramer HSD ($p < 0.05$).

3.2. Second and third breaks

Beginning and end of second and third breaks were difficult to determine due to non-uniform primordia formation and development of basidiomata after first break. Therefore, in order to delimit breaks, yield distribution obtained for the treatments was pooled into a histogram (data not shown). Harvest peaks were considered middle of each break. Based on this distribution, second and third flushes started at days 45 and 53 after spawning, respectively. Both breaks lasted for 8 days. In order to facilitate analysis of data, second and third breaks for each treatment were combined for analysis. Hence, we refer to second and third breaks as "subsequent flushes". Yield, BE and number of mushrooms for subsequent flushes obtained in later-cased treatments are presented in Table 5. Highest yield and BE were obtained from fragmented/supplemented treatments (FS). Yields from these treatments ranged from 492 to 687 g/bin while BE ranged from 48.6% to 67.1%. As a comparison, yields from non-fragmented/non-supplemented treatments (NFNS) ranged from 332 to 471 g/bin and from 32.4% to 46.5% BE. When data for FS and NFNS treatments were combined, significant differences were found for yield and BE (Table 5). Average yield per bin from FS was 607 g, while mushroom yield for NFNS was 396.4 g. BE varied from 59.7% to 39%, respectively. Number of mushrooms from FS was 14.1 vs. 9.8 for NFNS.

3.3. Comparison of production methods

Yield for a single flush ("standard", non-casing method) obtained in the non-cased treatments (1, 2, and 3) ranged from 367.6 to 537.5 g/bin. However, yield for 3 flushes obtained with the "casing" production method (treatments 4, 5, and 6), was significantly higher, reaching 1.3 kg/bin. Differences in BE were also significant, ranging from 35.9% to 53.1% in non-cased treatments to 114.8–132.8% in cased treatments (Table 6, "standard vs. casing"). Yields, BEs and number of mushrooms/bin from "casing" and "casing after first flush" treatments are presented in Table 6. "Casing after first flush" includes first flush obtained without casing plus subsequent flushes induced from "spent" substrate. Yield, BE and number of mushrooms obtained with the "casing" method were higher than "casing after first flush" although some values where similar. For example, yield from treatment NC/S/AF (1 kg/bin) was not significantly different than yield from C/S/AF (1.3 kg/bin). BEs were higher than 100% for most treatments, except for NC and NC/S/AS (98.4% and 88.1%, respectively). Highest

Table 5
Means and standard deviations for yield, biological efficiency and number of mushrooms produced per bin for 2nd and 3rd breaks of *Pleurotus eryngii* var. *eryngii* induced by casing or casing/supplementation of "spent" substrate (later-cased treatments).

Treatment[a]	Yield[e] (g)	BE[e] (%)	Number of mushrooms[e]	Total yield[d] (g)	Total BE[d] (%)	Total number of mushrooms[d]
1. NC-FS[b]	642.0 ± 143.4 a	63.4 ± 14.2 a	14.3 ± 0.6 ab	607.0 a ± 131.1	59.7 a ± 12.8	14.1 ± 4.5
2. NC/S/AS-FS[b]	687.0 ± 113.4 a	67.1 ± 11.1 a	18.3 ± 1.5 a			
3. NC/S/AF-FS[b]	492.0 ± 63.7 ab	48.6 ± 6.3 ab	9.7 ± 4.7 ab			
4. NC-NFNS[c]	386.3 ± 63.5 b	38.2 ± 6.3 b	14.7 ± 9.6 ab	396.4 b ± 83.0	39.0 b ± 8.3	9.8 ± 6.4
5. NC/S/AS-NFNS[c]	332.0 ± 47.3 b	32.4 ± 4.6 b	5.3 ± 1.2 b			
6. NC/S/AF-NFNS[c]	471.0 ± 81.2 ab	46.5 ± 8.0 ab	9.3 ± 1.5 ab			

[a] NC: non-cased; NC/S/AS: non-cased, supplemented at spawning; NC/S/AF: non-cased, supplemented at time of fragmentation.
[b] FS: fragmented/supplemented "spent" substrate.
[c] NFNS: non-fragmented/non-supplemented substrate.
[d] Data combined.
[e] Means ± standard deviation followed by different letters indicates significant differences according to Tukey–Kramer HSD ($p < 0.05$).

Table 6
Means and standard deviations for yield, biological efficiency and number of mushrooms for three production methods: "standard", "casing", and "casing after first flush" of *Pleurotus eryngii* var. *eryngii*. Standard vs. casing includes data for all flushes in the cased substrates and the single flush obtained in the non-cased substrates. Casing after first flush vs. casing includes data obtained in the subsequent flushes induced in non-cased substrates and all flushes for the cased substrates.

Treatment[a]	Method of cultivation					
	Standard vs. casing			Casing after first flush vs. casing		
	Yield[b] (g)	BE[b] (%)	Number of mushrooms[b]	Yield[b] (g)	BE[b] (%)	Number of mushrooms[b]
	Standard			*Casing after first flush*		
1. NC	481.5 ± 34.2 b	47.6 ± 3.4 b	16.3 ± 4.0 b	995.7 ± 188.5 bc	98.4 ± 18.6 bc	30.8 ± 7.5 ab
2. NC/S/AS	367.6 ± 167.3 b	35.9 ± 16.3 b	11.6 ± 9.6 b	902.0 ± 371.1 c	88.1 ± 36.2 c	24.6 ± 15.0 b
3. NC/S/AF	537.5 ± 45.4 b	53.1 ± 4.5 b	21.0 ± 6.3 b	1019.0 ± 89.5 abc	100.7 ± 8.8 bc	30.5 ± 8.9 ab
	Casing			*Casing*		
4. C	1162.0 ± 105.9 a	114.8 ± 10.5 a	36.0 ± 6.6 a	1162.0 ± 105.9 abc	114.8 ± 10.5 abc	36.0 ± 6.6 ab
5. C/S/AS	1248.2 ± 74.0 a	121.9 ± 7.2 a	39.8 ± 5.2 a	1248.2 ± 74.0 ab	121.9 ± 7.2 ab	39.8 ± 5.2 ab
6. C/S/AF	1344.7 ± 169.1 a	132.8 ± 16.7 a	40.2 ± 4.2 a	1344.7 ± 169.1 a	132.8 ± 16.7 a	40.2 ± 4.2 a

[a] NC: non-cased; NC/S/AS: non-cased, supplemented at spawning; NC/S/AF: non-cased, supplemented at time of fragmentation; C: cased; C/S/AS: cased and supplemented at spawning; C/S/AF: cased and supplemented at time of fragmentation.
[b] Means ± standard deviation followed by different letters indicates significant differences according to Tukey–Kramer HSD ($p < 0.05$).

number of mushrooms was observed for treatments representing "casing" production method (36–40) and lowest for "standard" production method (12–21).

4. Discussion

A preliminary experiment (data not shown) revealed that cased treatments were likely to result in higher yields than non-cased treatments. In that preliminary trial, supplementation with delayed-release nutrient (Remo's 4% d.w.) was added only after substrates were completely colonized by mycelium. However, in present experiments we observed that two treatments involving supplementation at spawning performed poorly during first break. Yield, BE and number of mushrooms were lowest on NC/S/AS. On average, supplement added at spawning (cased and non-cased treatments) resulted in lower yield (442.6 g) and BE (43.2%) compared to treatments supplemented at time of substrate fragmentation (607.8 g and 60%) (data calculated from Table 4). The non-supplemented treatments produced intermediate yields (573.1 g and 56.6%). Therefore, influence of supplement on yield and BE from first break appears dependent upon time of supplementation (as indicated also from the ANOVA analysis, Table 3). Supplementation of substrate at spawning appears to negatively affect productivity during first flush. The reason for this remains unknown, but may be related to an excessive supply of nutrients during spawn run.

Fragmentation and supplementation of "spent" substrate and use of a casing overlay (later-cased treatments) resulted in higher yield, BE and number of mushrooms compared to non-fragmented/non-supplemented "spent" substrates. Overall yield, BE, and number of mushrooms for fragmented/supplemented substrates (FS) increased by 53%, 51%, and 44% over non-fragmented/non-supplemented substrates (NFNS). Since substrate fragmentation and supplementation were always applied together in this experiment, it is not possible to elucidate influence of one factor over the other. However, Rasmussen (1959) showed a stimulatory effect of substrate fragmentation during the production cycle of *A. bisporus*. He demonstrated that yields increased when colonized compost was fragmented 7–14 days after spawning. Schisler (1964) demonstrated that fragmentation of compost after second break increased yield substantially over non-fragmented second break compost. Fragmentation of the substrate is important in order to promote vigorous re-growth of the mycelium and primordia formation. Observations by the authors indicate that after first break, *P. eryngii* mycelium rarely forms additional primordia if the substrate remains unaltered. However, primordia formation can be induced by placing a casing layer over an undisturbed colonized substrate even though number of developing mushrooms might be relatively low.

Biological efficiencies of *P. eryngii* obtained from cased/supplemented substrates in these experiments were significantly higher than values reported by other authors who produced this fungus on bagged/non-cased substrates and obtained mushrooms from only one break. Gaitan-Hernandez (2005) reported a BE of 58% for *P. eryngii* grown on barley straw substrate supplemented with oak powder. Peng (1996a) obtained a BE of 31% for mushrooms harvested from sawdust-based substrates while Royse (1999) reported a BE as low as 7% for a sawdust-based substrate supplemented with a commercial nutrient (SpawnMate IISE®). Peng (1996b) applied a casing overlay to colonized substrate (rice straw, 70% moisture) and reported relatively low BEs (47%). In contrast, we demonstrated that a casing overlay may increase yield and BE by 141% compared to a one-break-crop ("standard" method) without casing. When a casing overlay and nutrient supplementation at fragmentation were used together, yield increased by 179% over non-cased/non-supplemented substrates.

Use of a casing layer for *A. bisporus* production is essential. Factors such as casing layer depth, chemical and microbial composition, physical properties and moisture content of the casing layer play important roles in yield and quality of mushrooms (Cochet et al., 1992; Gulser and Peksen, 2003; Hayes, 1981; Kalberer, 1985; Pardo et al., 2002; Schroeder and Schisler, 1981). Limited information is available regarding casing soils used for production of *P. eryngii*. Rana et al. (2000) reported that certain isolates of *P. eryngii* cased with different materials may be prone to development of warts and yellowing and may be more susceptible to *Trichoderma viridae*, *Cladobotryum dendroides* and bacterial infections. In this work, green molds or pathogens that negatively affect yield or quality of mushrooms were not observed. Although pilei brightness was significantly reduced as measured with a chromameter, changes were nearly imperceptible to the naked eye. On the other hand, the effect of lower solids content of basidiomata obtained as result of use of a casing overlay should be further evaluated to determine its influence on shelf life. Other studies regarding nutritional qualities and texture of the basidiomata should be undertaken in order to obtain a more comprehensive evaluation of influence of a casing overlay on quality of basidiomata. Availability of peat (casing material) is a concern in some regions around the world where button mushrooms are produced and some research efforts have been devoted to a search for alternative materials that may be used as a substitute or in combination with peat (Gulser and Peksen, 2003; Noble and Dobrovin-Pennington, 2004). This

task has not been easily accomplished, since microbial populations in casing material are important in promoting fructification in *A. bisporus*. However, microorganisms do not appear to play a role in promoting primordia formation of *P. eryngii* and different soils may be used as casing materials for this specialty mushroom (Rana et al., 2000; Zervakis and Venturella, 2002). Casing layer residues on mushrooms may not represent a major problem since manual or mechanical cleaning or washing could be performed similar to other edible species (Kopytowski Filho et al., 2006).

Casing and supplementation of substrate used to produce *P. eryngii* var. *eryngii* are relatively easy and low-cost cultural practices that may be successfully used to enhance yields, BE and maximize utilization of substrate. Commercial mushroom farms that produce other varieties of mushrooms (i.e., *Pleurotus* spp., *L. edodes*, etc.) on substrate contained in bags may readily adapt their technologies for cultivation of *P. eryngii*. Supplementing substrates at casing may allow three breaks with an overall BE of more than 130%.

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