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ORIGINAL PAPER

Yield, size, and mushroom solids content of *Agaricus bisporus* produced on non-composted substrate and spent mushroom compost

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Abstract Three crops of *Agaricus bisporus* were grown on non-composted substrate (NCS), spent mushroom compost (SMC), a 50/50 mixture of NSC/SMC, or pasteurized Phase II compost. NCS consisted of oak sawdust (28% oven dry wt), millet (29%), rye (8%), peat (8%), ground alfalfa (4%), ground soybean (4%), wheat bran (9%) and $CaCO_3$ (10%). Substrates were non-supplemented or supplemented with Target® (a commercial delayed release nutrient for mushroom culture) or soybean meal at spawning or casing, or with Micromax® (a mixture of nine micronutrients) at spawning. Mushroom yield (27.2 kg/m^2) was greatest on a 50/50 mixture of NCS/SMC supplemented with 10% (dry wt) Target® at casing. The same substrate supplemented with Target® at spawning yielded 20.1 kg/m^2. By comparison, mushroom yield on Phase II compost supplemented at casing or at spawning with Target® was 21.6 kg/m^2 and 20.6 kg/m^2, respectively. On NCS amended with 0.74% or 0.9% Micromax® at spawning, yields increased by 51.8% (12.9 kg/m^2) and 71.8% (14.6 kg/m^2), respectively, over non-amended NCS (8.5 kg/m^2). Conversely, mushroom yields were not affected when Micromax® was added to a 50/50 mixture of NCS/SMC. Mushroom solids content was higher in mushrooms harvested from NCS amended with 0.74% Micromax® (9.6%) compared to non-amended NCS (8.3%).

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Introduction

Nuisance complaints, a result of mushroom compost preparation and disposal in close proximity to residential areas, are an increasing problem for many mushroom farms. Offensive odors and surface and ground water contamination associated with the preparation of mushroom compost and disposal of spent mushroom compost or substrate (SMC) are the primary reasons for these complaints. A combination of sub-urbanization and the heightened sensitivity of the general population to environmental issues have focused public attention on these issues (Kelsey and Singletary 1997; Duns et al. 2004). Growers have adopted several measures to reduce the environmental impact of mushroom farming, including the practice of forced aeration of Phase I compost contained in bunkers or tunnels (Op den Camp et al. 1991; Noble et al. 2001) and the development of new uses for SMC (AMI 2005; Davis and Kuhns 2005). However, the issues of offensive odor generation and SMC disposal continue to place severe pressure on mushroom growers.

The preparation of compost for commercial mushroom farms is a major undertaking requiring large turning machines, front-end loaders, and conveyor systems. Traditional compost preparation has followed the shortened two-phase system first described by Sinden and Hauser (1950, 1954). Phase I lasts from 5–10 days in compost windrows or in forced aeration bunkers while Phase II lasts from 3–7 days indoors in beds, trays or tunnels. While the use of forced aeration

bunkers for Phase I composting has empirically reduced the major malodorous compounds over windrow composting, reduced sulfur compounds are produced by both systems that exceed the human detection threshold (Duns et al. 2004). Thus, odor emissions remain a source of complaints and calls for government regulation have continued to increase.

The disposal of SMC is a major problem in Pennsylvania where approximately 60% of the U.S. mushroom crop is produced (USDA 2006). Nearly 21.6 million m^3 of SMC is produced in Pennsylvania each year as a by-product of the mushroom industry (AMI 2005). The total U.S. production of SMC exceeds 36 million m^3 annually (AMI 2005) prompting some researchers to suggest using SMC to produce a second crop of mushrooms. Schisler (1990) demonstrated that it was possible to obtain a second crop of mushrooms on SMC (including the casing layer) that was pasteurized and supplemented with a delayed release nutrient and *Hypnum* peat. While this was possible experimentally, it has not been adopted commercially because of the development of competitor molds during crop production (Phil Coles, personal communication).

Mushroom production is possible on sterilized, non-composted substrate (NCS). Several researchers (Till 1962; San Antonio 1971; Murphy 1972; Mee 1978; Sanchez and Royse 2001; Sanchez et al. 2002; Bechara et al. 2005a, b; 2006a, b) have shown that it is possible to produce *A. bisporus* on NCS and that yields may be equal to or exceed those on traditional Phase II compost. However, to date, *A. bisporus* is not produced commercially on NCS. The economic feasibility of producing *A. bisporus* on sterilized NCS remains to be determined and the most economical and productive processes for producing mushrooms on this substrate are still evolving. Factors such as substrate ingredients, types and time of supplementation, and mushroom quality require optimization to improve commercial feasibility for production of *A. bisporus* on both NCS and SMC. The objective of this study was to determine the effect of substrate composition and the addition of organic and inorganic supplements at spawning or at casing to NCS and SMC and their possible interactions on yield, size, and solids content of *A. bisporus*.

Materials and methods

Substrate

Ingredients for NCS included oak sawdust (28% oven dry wt), millet (29%), rye (8%), peat (8%), ground alfalfa (4%), ground soybean (4%), wheat bran (9%) and $CaCO_3$ (10%) (Sanchez and Royse 2001). ''Spent'' mushroom compost (three breaks of mushrooms were harvested prior to termination of the crop), obtained from the Mushroom Test Demonstration Facility (MTDF) at The Pennsylvania State University, was post-crop pasteurized with steam at 60 °C for 24–48 h to kill pests or pathogens that might interfere with subsequent cropping trials. Pasteurized SMC, including the casing layer (consisting of a mixture of milled, compressed sphagnum peat and pulverized limestone [107 kg limestone/m^3 sphagnum]), was mixed before removal from the production facility, bagged in plastic bags (94×75 cm^2), and stored at 2 °C until used. Phase II compost, used as a control treatment was obtained from the MTDF.

Strain and spawn

A brown strain of *A. bisporus* (Crimini and Portabello) was selected because it is commercially produced and it is becoming increasingly popular in the USA (USDA 2006). The spawn used was commercial casing inoculum (CI) composed of a mixture of neutralized peat, wheat bran, and vermiculite. CI (SB-65, Sylvan Spawn Laboratories Kittanning, PA) was used because of the higher number of inoculum points per g of spawn compared to conventional spawn on rye or millet.

Experimental design and data analyses

Three cropping experiments (Crops I, II, and III) were conducted at the Mushroom Research Center (MRC). Crop I was designed as a $3 \times 2 \times 2$ factorial in a completely randomized design (CRD), where treatment combinations were randomly assigned to each unit with six replicates per treatment (Kuehl 2000). Crop I contained three substrate types, two supplement types, two time periods for supplementation, and two additional controls (72 experimental units total). However, 8 experimental units were discarded because of contamination with competitor fungi resulting in 64 units for Crop I. Crop II was a 4×3 factorial in a randomized complete block design (RCBD) where treatment combinations were randomly assigned to each unit with six replicates per treatment. The experiment had four substrates across three levels of micronutrient amendment in three blocks (216 experimental units). However, 6 experimental units were discarded because of contamination with competitor fungi resulting in 210

units for Crop II. Crop III was a 1×6 factorial in a RCBD where treatment combinations were randomly assigned to each unit with six replicates per treatment. The experiment had one substrate (NCS) across six levels of Micromax® (granular, a mixture of nine micronutrients; Scotts–Sierra Horticultural Products Co., Marysville, OH) in three blocks (108 experimental units). In all experiments, mushrooms were harvested for two flushes (35–50 days from day of casing). The general linear model (SAS 2001) procedure was used for an analysis of variance. Treatment means were separated according to Fisher's least significant difference test ($p < 0.05$) and whenever necessary, treatment means comparisons with the controls were made according to Dunnett's procedure (Kuehl 2000).

Mushroom cropping trials

For Crop I, a 50/50 mixture of NCS/SMC, SMC and Phase II compost were supplemented either at spawning or at casing with 10% (oven dry wt basis) soybean meal (Archer Daniels Midland, Decatur, IL) or 10% Target®, a commercial delayed release nutrient for mushroom culture (Spawn Mate, Inc., Watsonville, CA). For Crop II, a 50/50 mixture of NCS/SMC, NCS, SMC and Phase II compost were amended at spawning with either 0.0%, 0.6% or 0.74% (oven dry wt basis) Micromax®. For Crop III, NCS was amended at spawning with 0.0%, 0.3%, 0.6%, 0.74%, 0.9% or 1.2% Micromax®. A Micromax® concentration of 0.74% was included in order to provide a comparison with the results of Weil et al. (2004, 2006). Ingredients were hand mixed, moistened to 65%, placed in very high porosity filter bags (Unicorn Bags, Garland, TX), autoclaved at 121 °C for 3 h, aseptically cooled and spawned with 30 g spawn per 2.5 kg substrate mixture (1.2%, wet wt). Phase II compost was included in the experiments (except for Crop III) as a control treatment. After spawning, the bags were heat-sealed and transferred to the MRC for spawn run at 18–19 °C for 18–21 days. The bags were opened and the fully colonized substrate was fragmented and placed in 6.1–l plastic bins ($29.5 \times 15.8 \times 8.8$ cm^3). Neutralized peat (pH 7.5; composition as described under section substrate) was overlaid (2.5 cm) on the substrate surface as casing. Case hold (period between casing application and first mushroom harvest) lasted for 18–21 days at 18 ± 1 °C; during this period, tap water was applied daily or as needed until the casing layer was saturated. Relative humidity in the production room was maintained at 90–95%.

Harvesting and determination of yield

Mushrooms were harvested, counted and weighed daily when the pilei were open and the lamellae were exposed. At the end of the second flush, yield and biological efficiency (BE) were determined and average mushroom size calculated as fresh mushroom weight divided by the number of mushrooms harvested. Biological efficiency was determined as the ratio of (g) of fresh mushrooms harvested per dry substrate weight (g) and expressed as a percentage. Yield was expressed as kg/m^2.

Mushrooms for solids content determination were randomly sampled from each treatment from Crops II and III. Mushrooms were sliced into quarters or eighths depending on the original mushroom size. Samples (100 g) were placed in a paper bag and oven-dried at 99 °C for 48 h. Ten replicates per treatment were used and solids contents were recorded as percent dry mushroom weight.

Results

Yield and BE

Time of supplementation, substrate mixture, and supplements significantly influenced yield and BE in Crop I (Table 1). Yields ranged from a low of 4.9 kg/m^2

Table 1 Probabilities greater than F from analysis of variance for three factors tested for yield, biological efficiency and size of *Agaricus bisporus* for three crops

Source	Probability > F[a]				
	df	Yield	BE	Size	Solids
Crop I					
Supplementation time (SPT)	1	<0.0001	<0.0001	0.8202	–
Substrate (SB)	2	<0.0001	<0.0001	0.0548	–
Supplement (SP)	1	<0.0001	<0.0001	0.9719	–
SPT x SB	2	0.0003	0.0003	0.7892	–
SB x SP	2	0.0009	0.0008	0.6266	–
SPT x SP	1	0.0105	0.0106	0.7912	–
SPT x SB x SP	2	0.3187	0.3172	0.9274	–
Crop II					
Block	2	0.0149	0.0159	0.0070	0.4759
Substrate (SB)	3	<0.0001	<0.0001	0.0002	<0.0001
Micromax® (M)	2	<0.0001	<0.0001	0.5561	<0.0001
SB x M	6	<0.0001	<0.0001	0.2375	<0.0001
Crop III					
Block	2	<0.0001	<0.0001	0.0018	0.7931
Micromax®	5	<0.0001	<0.0001	0.3849	<0.0001

[a] Values of less than 0.05 were considered significant according to Fisher's LSD

on non-supplemented SMC to a high of 27.2 kg/m^2 on a 50/50 mixture of NCS/SMC supplemented with Target® at casing (Table 2). In general, yields were highest from NCS/SMC and lowest from SMC (Table 3). Yields were also higher when supplements were added at casing rather than at spawning (Table 3). Supplementation of substrates with Target® resulted in higher overall yields than supplementation with soybean meal (Table 3). There were two exceptions to this, however; Target® was equally effective in stimulating yield when added to Phase II compost either at spawning or at casing and soybean meal was equally effective when added to SMC at spawning or at casing (Table 2). No mushrooms were produced when Phase II compost was supplemented with 10% soybean meal at spawning (Table 2).

Mushroom yield and BE were significantly affected by Micromax® amendment, and the interaction between substrate mixture x Micromax® (Table 1, Crop II). In general, as the level of Micromax® increased, yield increased (Tables 4, 5). However, on SMC and 50/50 mixtures of NCS/SMC yields were not significantly influenced by Micromax® amendment. The highest yield (15.6 kg/m^2) and BE (82.5%) were obtained from Phase II compost amended with 0.74% Micromax®, while the lowest yield (8.3 kg/m^2) and BE (44.1%) were from SMC with no Micromax® (Table 4). Across all treatments, mushroom yield generally increased as the level of Micromax® increased from 0% to 0.74% (Table 5). On NCS, yields increased with increasing levels of Micromax® up to 0.9% then declined as Micromax® levels reached 1.2% (Table 6).

The necessity for blocking in two of our experiments (Crops II, III) was due to insufficient autoclave capacity. We observed a significant difference in blocks (autoclave runs) for Crops II and III for both yield and BE (data not shown). Through blocking, this variation (probably due to growing room environment and not autoclaving conditions) was accounted for in the experimental design, improving precision.

Size

Substrate mixtures significantly influenced mushroom size only in Crop II (Table 1). However, the addition of Micromax® had no significant effect on mushroom size (Tables 1, 5, 6). The largest mushrooms were obtained from NCS and Phase II compost (Table 4).

Mushroom solids content

Mushroom solids contents were significantly influenced by substrates (SB), Micromax® (M) and SB x M interactions (Table 1, Crop II). Solids contents were higher in mushrooms grown on NCS and Phase II compost amended with Micromax® but were not influenced by Micromax®-amended SMC and NCS/SMC (Table 4). Solids contents in harvested mushrooms, in descending

Table 2 Mushroom size (g/mushroom), biological efficiency (% BE), yield (kg/m^2) and percentage yield difference compared to the control (Phase II compost) of *Agaricus bisporus* produced from substrates of spent mushroom compost, non-composted substrate and Phase II compost supplemented with either 10% Target® or 10% soybean meal (SM) at spawning or at casing (Crop I)

Substrate[a]	Time of supplementation	Supplement (10% dry wt)	Yield (kg/m^2)[b]	Difference (%)[d]	BE (%)[bc]	Size (g/)[b]
SMC	Spawning	SM	10.1fg	–9.0	53.5fg	47.2a
SMC	Spawning	Target®	7.8g	–29.7	41.2g	43.0a
SMC	Casing	SM	10.9f	–1.8	57.9f	44.0a
SMC	Casing	Target®	13.8e	+24.3	73.4e	41.1a
NCS/SMC	Spawning	SM	18.3d	+64.9	96.9d	34.3a
NCS/SMC	Spawning	Target®	20.1cd	+81.1	106.5cd	35.8a
NCS/SMC	Casing	SM	23.0b	+107.2	122.2b	34.7a
NCS/SMC	Casing	Target®	27.2a	+145.0	144.3a	38.6a
PIIC	Spawning	SM	–	–	–	–
PIIC	Spawning	Target®	20.6bcd	+85.6	109.4bcd	41.0a
PIIC	Casing	SM	14.2e	+27.9	75.3e	38.6a
PIIC	Casing	Target®	21.6bc	+94.6	114.6bc	40.1a
PIIC	–	None	11.1f	0.0	58.8f	40.9a
SMC	–	None	4.9h	–55.9	25.7h	45.9a

[a] SMC = Spent mushroom compost, NCS = Non-composted substrate, PIIC = Phase II compost

[b] Means within a column followed by the same letter are not significantly different $p < 0.05$ according to Fisher's LSD; values are means of six replicates

[c] %BE = (g fresh mushrooms/g dry substrate) × 100

[d] Control used to calculate % difference was non-supplemented Phase II compost Difference (%) = $[(a\text{-}x)/x]100$ where a = yield from non-control treatment, x = yield of control

Table 3 Means and groupings from analysis of variance for three factors (substrate, time of supplementation, supplements) for (*Agaricus bisporus*) yield, biological efficiency (BE) and mushroom size (Crop I)

Treatment	No. of reps[b]	Yield (kg/m^2)[c]	BE (%)[cd]	Size (g)[b]
Substrate[a]				
SMC	23	10.3c	54.8c	42.1a
PIIC	17	18.7b	99.2b	39.8a
NCS/SMC	24	22.1a	117.5a	35.9a
Time of supplementation				
Spawning	28	11.7b	63.8b	31.2a
Casing	36	18.4a	97.9a	39.5a
Supplement				
Soybean meal	30	12.4b	80.7b	39.3a
Target®	34	17.3a	99.2a	39.5a

[a] SMC = Spent mushroom compost, NCS = Non-composted substrate, PIIC = Phase II compost

[b] Number of replications not equal due to missing values (see M and M)

[c] Means within a column followed by the same letter are not significantly different $p < 0.05$ according to Fisher's LSD

[d] BE (%) = Percentage biological efficiency including weight of supplement

order, of the various substrates were as follows: NCS (8.9%), NCS/SMC (7.8%), SMC (7.4%) and Phase II compost (6.3%) (Table 5). Mushroom solids were significantly higher from NCS amended with 0.74% Micromax® compared to all other levels and to non-amended NCS (Tables 4,6). Solids were significantly higher from Phase II compost amended with 0.74% Micromax® (6.8%) compared to non-amended Phase II compost (5.9%) (Table 4).

Discussion

Across all treatments, BE of mushroom production in terms of overall production could be ranked as follows: SMC (54.8%), PIIC (99.2%) and NCS/SMC (117.5%). When compared to findings reported by Schisler (1982), BEs for the substrates used in our experiments were average (50 to 70%) for SMC, good (70 to 90%) for PIIC, and excellent (>100%) for NCS/SMC.

Overall, yields from substrates supplemented at the time of casing were higher than those from substrates supplemented at time of spawning (equal dry substrate wt). Yields were an average of 6.6 kg/m^2 higher (57%) when supplements were added at casing rather than at spawning. These findings are in general agreement with Sinden and Schisler (1962) who showed that yields were often higher on PIIC when supplements were added at casing rather than at spawning. With sterilized NCS and mixtures of NCS/SMC, supplementation at casing may be commercially feasible because the substrate is fragmented before filling into containers at casing. A delayed release supplement, such as Target® could be added at this time to stimulate yields. Target® is a commercially available supplement high in protein concentrate and specifically formulated to stimulate mushroom mycelial growth and crop yields. It is formulated to minimize risk associated with heat

Table 4 Influence of Micromax® micronutrients on yield (kg/m^2), percentage biological efficiency (% BE), size and basidioma (*Agaricus bisporus)* solids content of mushrooms grown on substrates of spent mushroom compost (SMC), non-composted substrate (NCS) and Phase II compost (PIIC) (Crop II)

Substrate[a]	Micromax® (% dry wt)	Yield (kg/m^2)[bc]	BE (%)[a]	Size (g)[bc]	Solids (%)[bd]
NCS	0	8.7c	46.1c	29.6de	8.5b
NCS	0.6	9.3c	49.4c	38.3a	8.5b
NCS	0.74	14.0b	74.3b	37.2ab	9.8a
SMC	0	8.3c	44.1c	31.1bcde	7.5de
SMC	0.6	8.4c	44.7c	32.9abcde	7.8bcd
SMC	0.74	9.0c	47.6c	31.6bcde	7.0e
NCS/SMC[e]	0	14.0b	74.4b	30.5cde	7.6cde
NCS/SMC	0.6	13.6b	72.3b	27.1e	8.3bc
NCS/SMC	0.74	14.8ab	78.4ab	27.0e	7.6cde
PIIC	0	13.9b	73.7b	35.3abcd	5.9f
PIIC	0.6	14.8ab	78.7ab	36.7abc	6.1f
PIIC	0.74	15.6a	82.5a	35.0abcd	6.8e

[a] NCS = Non-composted substrate, SMC = Spent mushroom compost, PIIC = Phase II compost. BE (%) = Percentage biological efficiency including weight of supplement

[b] Means within a column followed by the same letter are not significantly different $p < 0.05$ according to Fisher's LSD

[c] Values are means of three blocks, each block contained six replicates

[d] Values are means of ten replicates

[e] 50/50 mixture

Table 5 Means and groupings from analysis of variance for two factors (substrate, Micromax® concentrations) for *Agaricus bisporus* yield, biological efficiency (BE) and mushroom size (Crop II)

Treatment	No. of reps[b]	Yield (kg/m^2)[b]	BE (%)[cd]	Size (g)[c]	Solids (%)
Substrate [a]					
SMC	54	8.6c	45.4c	31.9bc	7.4c
NCS	48	10.9b	57.8b	35.3ab	8.9a
NCS/SMC	54	14.1a	75.0a	28.2c	7.8b
PIIC	54	14.8a	78.3a	35.7a	6.3d
Micromax®					
0	68	11.4b	60.4b	31.7a	7.3b
0.6%	70	11.6b	61.6b	33.6a	7.7a
0.74%	72	13.3a	70.7a	32.7a	7.8a

[a] SMC = Spent mushroom compost, NCS = Non-composted substrate, PIIC = Phase II compost

[b] Number of replications not equal due to missing values (see M and M)

[c] Means within a column followed by the same letter are not significantly different $p < 0.05$ according to Fisher's LSD

[d] %BE = (g fresh mushrooms/g dry substrate) × 100

generated during case-hold and can be used in Phase II or Phase III (bulk-colonized) compost. In our work, adding Target® to colonized substrate would be similar to supplementing Phase III compost.

The average mushroom yield obtained from commercial mushroom houses on supplemented Phase II compost is approximately 28.9 kg/m^2 (USDA, 2006). This is similar to the highest yields obtained in our work (27.2 kg/m^2 on NCS/SMC supplemented at casing with Target®). However, compost depth used for commercial production ranges from 16–20 cm, while the depth of our substrate was approximately 6 cm. Thus, average compost depth for commercial production is 3 times that of our substrate. On the other hand, Bechara et al. (2005a, b) reported yields as high as 16.9 kg/m^2 on a 2-cm deep layer of grain spawn supplemented with S-41 and overlain on a water-saturated substratum of perlite, a granular siliceous material of volcanic origin. Use of the hydrated, perlite substratum (5 cm depth) increased yields by 69% compared to the same grain substrate without perlite (Bechara et al. 2005a, b). We did not investigate perlite as a hydrating medium for our substrate, but it is possible that the yields we reported could be further enhanced with its use.

Table 6 Influence of Micromax® on yield (kg/m^2), biological efficiency (BE), size and basidioma (*Agaricus bisporus)* solids content of mushrooms grown on non-composted substrate (NCS) (Crop III)

Micromax® (% dry wt)	Yield (kg/m^2)[ab]	Difference (%)[c]	BE (%)[a]	Size (g)[ab]	Solids (%)[ad]
0	8.5e	0	45.0e	54.1a	8.3bc
0.3	9.6de	+12.9	51.1de	57.3a	8.8b
0.6	10.9cd	+28.2	58.0cd	53.9a	8.4b
0.74	12.9b	+51.8	68.4b	50.3a	9.6a
0.9	14.6a	+71.8	77.7a	44.2a	7.8c
1.2	12.4bc	+45.9	65.9bc	47.1a	8.6b

[a] Means within a column followed by the same letter are not significantly different $p < 0.05$ according to Fisher's LSD

[b] Values are means of three blocks, each block contained six replicates

[c] Control used to calculate % difference was non-supplemented NCS. Difference (%) = $[(a\text{-}x)/x]100$ where a = yield from non-control treatment, x = yield from control

[d] Values are means of ten replicates

Amendment of mushroom substrate with Micromax® is a potential opportunity for growers to improve the yield capacity of their Phase II compost (Weil et al. 2006). Micromax® contains a mixture of nine micronutrients including (percentage dry wt basis): Ca (12%), Mg (3%), S (12%), B (0.1%), Cu (1%), Fe (17%), Mn (2.5%), Mo (0.05%), Zn (1%), and inert ingredients (57.35%). Weil et al. (2004, 2006) examined the effect of 0.74% Micromax® as well as six of the individual minerals present in that level of Micromax®. They determined that approximately 70% of the yield increase was due to Mn. None of the individual six minerals (Mg, Mo, B, Cu, Zn, Fe) examined increased yield; in fact, four of the minerals (Mo, B, Cu, Zn) resulted in significant yield decreases (Weil et al. 2006). Three minerals (Ca, S, Mg) were not individually examined so it may be that these minerals or combinations of these and the other minerals present contributed to the yield increases observed with Micromax®.

The yield and BE of NCS/SMC non-amended and amended with Micromax® were not significantly different. These results remain unexplained, but may be related to the NCS/SMC containing a sufficient supply of these micronutrients. Mixtures of NCS and SMC may provide a more balanced combination of both organic and inorganic nutrients. Furthermore, the S concentration in Micromax® may contribute to mushroom yield. SMC may contain higher levels of S due to the presence of gypsum ($CaSO_4$) added during the composting process. Coupled with the unknown individual effect of S on mushroom yield, further experimentation will be necessary to determine the potential role, if any, of S concentration in NCS. Considering the importance of reduced S compounds in odor generation during composting, this aspect warrants further exploration.

Incorporation of Micromax® into the various treatments caused a darkening of substrates that may be

associated with the Mn content in Micromax®. Rantcheva (1972) reported the darkening of synthetic compost during the composting process after the addition of trace elements containing Mn. Likewise, we observed a darkening of shiitake substrate, after autoclaving, when 250 mg/kg Mn was added to substrate (D.J. Royse, personal communication).

The solids content of harvested mushrooms were significantly higher on NCS followed, in descending order, by NCS/SMC, SMC and Phase II compost. In addition, solids were significantly higher in mushrooms harvested from NCS amended with 0.74% Micromax® compared to non-amended or to other levels of Micromax®. A negative impact on solids as a result of adding Micromax® to Phase II compost has been observed by Weil (2003). It was speculated that cation imbalance in the compost or casing may be responsible for this response (Reid 2001). Such an imbalance also may exist in NCS; however, the addition of Micromax® to NCS may have had the opposite effect, i.e., providing a more balanced mineral composition.

We used a combination of containers to manipulate the substrates in these experiments. Spawn runs were carried out in plastic bags while production occurred in plastic bins. The fully colonized substrate was fragmented before the substrate was placed in plastic bins. Fragmentation actually improves mushroom quality (Gerrits 1988), shelf life (R. B. Beelman, personal communication) and may increase levels of antioxidants (Dubost 2006). Antioxidants are known to reduce the oxidative damage to human or animal cells (DiSilvestro 2001; Halliwell 2001). Dubost (2006) found that levels of ergothioneine, an antioxidant found but not produced in human tissues, increased significantly in mushrooms when colonized compost was fragmented prior to casing. In the commercial mushroom industry, fully colonized Phase II compost is not fragmented before casing on bed farms; however, when Phase III tunnels are used for bulk spawn run, fragmentation is an inherent part of the process. Anastomosis and recovery of fragmented mycelium is more rapid and mushroom production begins 1 or 2 days earlier compared to colonized but non-disturbed Phase II compost (Sinden and Schisler 1962). Thus, fragmentation may not only increase earliness of production but also may improve quality, shelf life and the healthful qualities of mushrooms.

We have demonstrated that it is possible to obtain reasonably high yields of brown *A. bisporus* on NCS and mixtures of NCS/SMC following the additions of either organic or inorganic supplements at spawning or at casing. While the economics of producing *A. bisporus* on NCS remain to be determined, the potential positive environmental impact of such a system clearly is apparent. Additional research is needed to determine if the addition of inorganic supplements at casing or later, and the addition of both organic and inorganic supplements in the same treatments would further enhance productivity. Improved efficiency, coupled with the potential to improve product quality with a lessened environmental impact may drive the use of NCS into a new realm for mushroom production.

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