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NON-COMPOSTED AND SPENT MUSHROOM SUBSTRATES FOR

PRODUCTION OF *AGARICUS BISPORUS*

A Thesis in

Plant Pathology

by

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ABSTRACT

Commercial production of *Agaricus bisporus* is dependent upon a substrate prepared by composting. Traditional composting is associated with a number of environmental problems such as the emission of offensive odors and waste water run-off during handling. In addition, after the production of a crop of mushrooms, there is the problem of disposal of spent mushroom substrate (SMS). Thus, preparation of a suitable substrate for mushroom production without the generation of offensive odors and the possible re-use of SMS for the production of a second crop of mushrooms are desirable. The goals of this research were to evaluate two substrates for the production of *A. bisporus*: a) non-composted substrate (NCS) consisting of red oak sawdust (28%), millet (29%), rye (8%), peat (8%), ground alfalfa (4%), ground soybean meal (4%), wheat bran (9%) and $CaCO_3$ (10%) and b) SMS. Treatments included 1) NCS and SMS alone and in combination, 2) spawn carriers, 3) strains, 4) supplements, 5) time of supplementation, and 6) substrate moisture content. The substrates were sterilized in very high porosity filter plastic bags and then spawned and incubated. Mushrooms were harvested for two flushes and yield, biological efficiency (BE) and mushroom size were determined. The highest yield and BE (27.2 kg/m^2, 144.3%) were from 1:1 NCS/SMS spawned with casing inoculum (CI) and supplemented with 10% Target® at casing. The largest mushrooms were obtained from NCS spawned with CI and supplemented at spawning with 3,000 mg/kg Micromax® (57.3 g/mushroom). Mushrooms containing the highest solids content (9.9%) were harvested from NCS spawned with CI (60% moisture content). Supplementation of NCS/SMS with Micromax®, a commercial micronutrient,

had no significant effect on yield and BE. However, the addition of 0.9% Micromax® (d.w.) to NCS significantly increased yield by 72% (from 8.5 kg/m^2 to 14.6 kg/m^2). This work shows the potential to produce relatively high yields on NCS or on mixtures of NCS/SMS and that a combination of supplements that contain protein, carbohydrate and micronutrients added at spawning or at casing resulted in improved mushroom yields.

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Chapter 1: General Introduction

1.1 Economic value of mushrooms

United States total mushroom production during 2005-06 was 379,350 t [843 million pounds] (USDA, 2006). The value of the mushroom crop was $881 million, down 3 percent from the 2004-05 season and 4 percent below the 2003-04 season. *Agaricus* mushroom value of sales for 2005-06 season totaled $841 million, down 2 percent from previous season and 4 percent below the 2003-04 season. As a leading state in mushroom production, Pennsylvania accounted for 59% of the total volume of sales followed by California which produced 14%. The sales total of the *Agaricus* crop was estimated at 373,500 t (830 million pounds), down 1 percent from both the 2004-05 and 2003-04 seasons. Brown *Agaricus*, including Portobello and Crimini varieties, accounted for 45,450 t (117 million pounds), up 17% from last season and 21% higher than the 2003-04 crop year. The value of sales for the brown mushrooms for 2005-06 season was $152 million, which was 18% of total *Agaricus* value. Brown mushrooms accounted for 14% of the total *Agaricus* volume sold in 2005-06 season.

1.2 Environmental impact

Agaricus bisporus is produced on a composted substrate of various raw materials including straw-bedded horse manure, hay, corncobs, cottonseed hulls, poultry manure,

brewer's grain, cottonseed meal, hardwood bark and gypsum. All commercial production of *A. bisporus* is on composted materials. During the preparation of compost, offensive odors (Duns et al., 2004) may be emitted and the environment may be fouled with waste water runoff. After mushrooms are harvested, the remaining spent mushroom substrate (SMS) may be considered environmentally unfriendly and may represent a solid waste disposal problem for growers (Duns et al., 2004).

1.3 History of *Agaricus bisporus* cultivation

Mushrooms have been appreciated as a table delicacy since ancient times (Houghton, 1885; Buller, 1914). The cultivation of mushrooms for food reportedly began as early as 600 A.D. in China (Chang, 1993). The cultivation of *A. bisporus* in Europe (France) began in the 1650's but the first scientific information on the cultivation of *A. bisporus* was by Tournefort (1707). The discovery that mycelium from old horse manure may be inoculated into newly prepared horse manure marked the beginning of rational mushroom growing in limestone caves surrounding Paris. Cultivation was so successful that, by 1900, there were more than 900 km of bed-space devoted to mushroom culture (Treschow, 1944; Pinkerton, 1954). Mushroom growing was soon introduced into England by Muller in 1731(Treschow, 1944). In Britain, mushrooms were first grown in beds in caves. In 1883, mycelium was exported from England to America (Treschow, 1944) where the mushroom industry began in greenhouses (mushroom substrate was placed under benches). In 1905, Duggar, in the U. S., published a tissue culture method of making pure spawn. The method consisted of removing a piece of tissue from the

mushroom cap and culturing it on a suitable medium under sterile conditions. In the U. S., mushrooms proved so profitable that houses fitted with shelves were built solely for mushroom production (Pinkerton, 1954).

1.4 Cultivation methods of *Agaricus bisporus*

Button mushroom growing may arbitrarily be divided into six steps as follows: Phase I composting, Phase II composting, spawning, casing, pinning and cropping (Wuest et al., 1981). The process begins with preparation of wheat straw bedded-horse manure or synthetic compost. Both types of compost require the addition of nitrogen supplements and a conditioning agent, gypsum. The substrate is supplemented according to crop requirements and individual farmer's preferences. The supplements commonly used in button mushroom cultivation may include gypsum, chicken manure, hay, corncobs, cottonseed meal and brewer's grain. These are typically used to adjust nitrogen and carbohydrate contents (Schisler and Wuest, 1982), or as flocculating agent, as in the case of gypsum. Shredded hardwood bark, cotton seed hulls, neutralized grape pomace, and cocoa bean hulls may be used as a substitute for corn cobs.

Phase I composting starts by mixing and wetting the compost ingredients, followed by placing it in piles approximately 2 m wide by 2 m high. The length of the pile is determined by the individual grower. The pile should have a loose center and tight sides for aeration to avoid anaerobic conditions. The pile is turned and watered at 2-3 day intervals. Phase I composting lasts from 7-14 days and is characterized by a strong ammonia and sulfide odor and high temperatures ($63^{o}C$ - $77^{o}C$). At the end of Phase I, the

compost is chocolate brown in color, straws are soft and pliable and moisture content may range from 68-74%.

The second step (Phase II) starts by pasteurization of Phase I compost to obtain Phase II compost that is free of ammonia and rich in complex carbohydrates. The phase II process may last 6-10 days depending on how the air and compost temperatures are managed to control microbial activity.

In the third step *Agaricus bisporus* spawn is distributed onto the compost which is then thoroughly mixed. The mycelium is allowed to grow until it spreads throughout the compost. The time needed for spawn to colonize the compost depends on the spawning rate and its distribution, the compost moisture and temperature and the quality of the compost. Complete, spawn-run compost requires 12-21 days of incubation.

The fourth step in the production cycle is the application of a casing layer (such as neutralized peat) to the compost. Light watering of the casing then continues until mushrooms begin to appear.

The fifth step is pinning. Mushroom primordia develop after rhizomophs have formed in the casing. Mushroom primordia develop into pins, which then grow into the button, and ultimately, a button enlarges to a mushroom. From the time of casing to first flush takes about 15-21 days and 50-60 days after composting first began.

The last step is cropping and harvesting the mushrooms by twisting or cutting. A flush, the name given to 3- to 5- day harvest periods during the cropping cycle, is followed by few days (3-4) when no mushrooms are available to harvest. This cycle repeats itself in a rhythmic fashion (6-8 days) and harvest may continue as long as

mushrooms grow and develop. The harvest period may last as long as 150 days (Wuest et al., 1981).

1.5 Varieties of *Agaricus bisporus*

There are generally four strain types found within *A. bisporus*. These are white, off-white, hybrid and brown strains (Fritsche and Sonnenberg, 1988). White strains are characterized by white fruit-bodies without scales, and their relatively small unit weight. They are grouped into slow and rapid fruiting where rapid fruiting white strains have a longer and thinner stalk, the picking begins sooner and less regular distribution of breaks (Fritsche and Sonnenberg, 1988). The smooth white variety or snow-white is the whitest of all mushrooms under cultivation and was discovered by L. F. Lambert in 1925 and was registered in the USA patent office (Lambert, 1946).

The brown mushrooms were extensively cultivated in 1929 in the USA (Kligman, 1942) and then gained popularity again in the late 1990's. Crimini are brown mushrooms harvested before the veils break and the lamellae are exposed. Portobellos are mature, "open" mushrooms with their dark brown gills clearly visible. The stipes of brown mushroom strains remains white.

Off-whites are intermediates between the snow-white and the brown cultivar (Kligman, 1942; Fritsche and Sonnenberg, 1988). They have white pilei, but are as large as the brown strains. They turn off-white especially during the later breaks. The scales are often very light brown in color. Off-white strains are suitable for mechanical harvesting and are often grown for the processors.

Hybrid mushrooms are crosses between white and off-white strains and white and brown strains. In 1976, at the Mushroom Experimental Station in Horst, breeders crossed the white strains with the off-white strains and obtained the hybrid Horst U3 and Horst U1 strains (Fritsche and Sonnenberg, 1988). U3 is smooth white, with pink-staying gills and a short, thick stalk. U3 looks more like a white strain than an off-white one. U1 is more like an off-white strain. Under the right production conditions, U1 may form very large fruit-bodies with thick caps that remain closed for a considerable time. Hybrids are grown preferentially because of high quality and yield of the fruit-bodies (Fritsche and Sonnenberg, 1988). Today, 90% of U1 is produced as spawn under various names (Sylvan 140, Sylvan 130, Amycel 2100, Amycel 2500 [Europe only], Amycel Delta etc).

1.6 Spawn types used in *Agaricus bisporus* cultivation

The first spawn for the commercial market was French flake spawn. This was spawn that had been dug up from fields where mushrooms were growing in the wild. The spawn was first dried, and then dispatched to growers along with any 'pests' that happened to be infesting the particular field from which the spawn was taken (Pinkerton, 1954). Another problem with the French flake spawn was the impossibility of foretelling what variety of mushroom would be produced from such spawn. An improvement to French flake spawn was the British 'brick spawn' that was made by collecting wild spawn from the fields and inoculated into 'bricks' made of compressed manure and leaf-mould. After the spawn had grown through, the brick was dried and sold.

In 1893, a method of germinating the spores of mushrooms was discovered by two French scientists, Constantin and Matruchot and pure culture spawn was made (Pinkerton, 1954). Constantin and Matruchot were the first to patent mushroom spawn made using "pure culture" techniques in 1894 (Ferry, 1894). Later, Duggar (1905), in U.S., published a method for mushroom spawn making in pure culture.

Flake spawn is the viable mycelial culture found in the 'spent' substrate. Flake spawn was obtained from compost beds ("spent" compost) or other substrate after mushroom production had become scanty (Pinkerton, 1954). Flake spawn was an important source of spawn for the cultivation of mushrooms especially in cases where pure-culture spawn was unobtainable or too expensive. The 'spent' substrates that have passed their peak production period were chopped into pieces and used for the next cultivation cycle.

Cultivation of mycelium in pure synthetic liquid media (liquid spawn) may be started either from a 1-spore culture, from cultures of spore masses or from tissue cultures. Quantity of mycelial fungal growth in fluid media was determined by dry matter production. Treschow (1944) found the amount of mycelial dry matter production to be 19% by weight.

Manure spawn is the mushroom mycelium grown on sterilized composted manure. Manure spawn is less sensitive than grain spawn to the high concentrations of ammonia present when the compost is spawned before it is fully ready (Fritsche and Sonnenberg, 1988). However, the invention of grain spawn rendered manure spawn obsolete.

Grain spawn was invented by Sinden (1932). Rye, wheat, millet and sorghum are commonly used to make spawn. The grain, colonized with mycelia on the surface, may readily be mixed with various substrate formulations, thus providing many points of inoculum. Since spawn is normally used on a weight basis, small grains such as millet give a greater number of inoculation points per kg than large grains such as rye. However, large grains have a greater food reserve that can sustain the mycelium for longer periods of time (Fritsche and Sonnenberg, 1988).

1.7 Supplementation in *Agaricus bisporus* cultivation

Nutrients added to the compost sometime after the completion of Phase I composting is referred to as supplementation (Gerrits, 1988). The idea of supplementation was developed by Sinden and Schisler (1962) and Schisler and Sinden (1962). They obtained slight yield increases when 100 ml of 10% solutions of peptone and xylose per tray (25-28 kg of wet compost) were added to the compost at spawning. This was the first indication that nutrient supplements could be utilized by the mushrooms. Supplementing can be carried out at various cropping stages (at filling, at spawning and at casing) but for practical reasons it is not done at harvest because the casing soil would be disturbed (Gerrits, 1988).

Compost which consists of lignin-humus complex, cellulose, protein and oils should provide the mushroom mycelia with all nutrients required for growth and development. If compost is nutrient-deficient it requires supplementation to enhance the protein and lipid availability for the mushrooms (Schisler and Patton, 1971). Yields may

increase by 10% when compost is supplemented (Schisler and Sinden, 1962). Commercial supplements have been treated to delay the availability of the nutrient for the mushrooms. Supplements consisting of high-protein contents have included soybean, corn and feather. Commercial supplements have been treated to delay the availability of the nutrient for the mushrooms. Mushroom supplements consisting of carbohydrates such as cellulose have been found to have a positive linear relationship with an increase in mushroom yield (Dahlberg, 2004).

Macroelements such as phosphorus, sulfur, potassium, calcium and magnesium are required for physiological processes. Microelements such as copper, zinc, manganese and molybdenum are required in small quantities. Six to ten percent of mushroom weight consists of dry matter. Only one tenth of this is inorganic matter. The minerals extracted from compost by mushrooms are limited to 2-3% of the quantity present (Gerrits, 1988), therefore compost contains sufficient minerals and are unlikely to be a limiting factor on the mushroom yield. However, after addition of 0.25, 0.5 and 0.75% Micromax® at casing average yield increases of 1.0, 4.9 and 4.5%, respectively were achieved when compared to non-supplemented compost (control) (Desrumaux et al., 2000; Weil et al., 2004).

Minerals such as manganese, iron, copper, sodium, aluminum, and zinc that are naturally present or added to compost raw materials are incorporated into the microflora of the compost. The minerals indirectly influence the performance of the crop through catalyzing enzymatic reactions without being taken up by the mushrooms (Desrumaux et al., 2000). Although it has been shown that copper is a vital cofactor for enzymes such as tyrosinases (Munger et al., 1982), excessive copper accumulation in *A. bisporus* may

have negative effects on yield and quality (whiteness) of the mushrooms (Beelman et al., 2004).

1.8 Problem statement

Since the beginning of commercial production, composting has been a reliable source of *Agaricus bisporus*-specific substrate. Offensive odors (Duns et al., 2004) and water run-off produced by the preparation of mushroom compost, are increasingly becoming a problem for mushroom farms, due to peoples' sensitivity to environmental issues. Although the time and nature of the composting has been modified, no one has been successful in eliminating this step from the preparation of a suitable medium for *A. bisporus* production (Till, 1962; Mee, 1978; Sánchez and Royse, 2001; San Antonio, 1971; Bechara et al., 2005; 2006). The composting process requires considerable machinery (loaders, compost turners, conveyor belt systems), time (up to 4 weeks) and is labor intensive. In addition, considerable land requirements are needed for composting operations. Odor containment, which has an environmentally positive impact, is achieved through a totally enclosed biofiltered bulk Phase I composting facility but is capital intensive. Water run-off that contains soluble nutrients from the composting process may contaminate sources of domestic water. A novel method of mushroom production without negative environmental impact and cost-effective operations is desirable (Bechara et al., 2006).

Spent mushroom substrate (SMS) is the material remaining after the mushroom growing process (AMI, 2005). The SMS is steam pasteurized for 6 h at 68°C before it is

discarded. Aged (2 years) SMS is an ideal growth medium for container-produced plants (Chong et al., 1991; Maher, 1991; Chong and Hamersma, 1997; Romaine and Holcomb, 2001). It also is added to mulch to suppress the artillery fungus (*Sphaerobolus stellatus*) (Davis and Kuhns, 2005; Davis et al., 2005) and is used as a soil amendment to improve turf (Landschoot and McNitt, 2005), fruit (Robbins et al., 1986), and vegetable production (Male, 1981; Wang et al., 1984a, b; Kaddous and Morgans, 1986; Webber et al., 1997). Currently, however, these applications only use a small portion of the SMS generated each year. Spent mushroom substrate is considered environmentally unfriendly and represents a solid waste disposal problem for mushroom growers. In Pennsylvania alone, more than 21 million m^3 of SMS is produced and in the U.S. it is estimated to exceed 36 million m^3 annually (AMI, 2005; Davis and Kuhns, 2005). Spent mushroom substrate disposal is an issue of great concern to the industry, especially in PA.

1.9 Objectives

The overarching goal of this project was to develop alternative methods [non-composted substrate (NCS) and SMS] to alleviate problems associated with traditional mushroom production systems.

Using NCS and SMS, five mushroom cropping experiments were conducted to test distinct hypotheses. This dissertation is thus divided into four chapters whereby chapter 1 address introductory aspects of *A. bisporus* and chapters 2, 3 and 4 cover the hypotheses. Chapter 2 addresses the H_o: Strain and spawn types used with NCS and SMS do not have an influence on mushroom yield, size and mushroom solids content.

Mushroom cropping experiments were carried out at the Mushroom Research Center, The Pennsylvania State University, to determine the effect of mixtures of NCS and SMS, strain (white and brown) and spawn types (casing inoculum [CI], millet, 1:1 CI/Millet, NCS) on mushroom yield and size. Chapter 3 addresses the H_o: Different levels of nutrient supplements, time of application of commercial delayed release nutrients and micronutrients do not have an influence on mushroom yield, size and solids. Wheat bran, soybean meal, Target® and Macromax® in NCS and SMS were used to investigate this hypothesis. Chapter 4 examines the H_o: Moisture contents of NCS and mixtures of NCS and SMS do not influence mycelial linear growth, yield and size. Experiments on various levels of substrate moisture content were conducted using different types of substrate mixtures.

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Chapter 2: The influence of spawn carrier, strain and substrate on yield, size and mushroom solids content of *Agaricus bisporus*

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2.1 Abstract

Five substrate mixtures of non-composted substrate (NCS), spent mushroom substrate (SMS) and Phase II compost (control) as well as spawn carriers and strains of *Agaricus bisporus* (J. Lange) Imbach were evaluated for effects on yield, biological efficiency (BE), size and mushroom solids content. Substrates tested were: 1:3 NCS/SMS, 1:1 NCS/SMS, 3:1 NCS/SMS, NCS and Phase II compost. The substrates were spawned with various spawn carriers (millet, casing inoculum (CI), 1:1 CI/millet or NCS) and brown and white strains. The substrate mixtures of NCS and SMS produced mushroom yields and BEs comparable to non-supplemented Phase II compost. A substrate mixture of 1:1 NCS/SMS and spawn carrier NCS produced the highest yield (12.8 kg/m^2) and BE (70.9%) of all substrates, followed by a mixture of 3:1 NCS/SMS. The highest mushroom dry weight (7.1% solids) was obtained from the brown strain produced on 1:1 NCS/SMS.

2.2 Introduction

The button mushroom, *Agaricus bisporus* (J. Lange) Imbach, is a mainstay of Pennsylvania agriculture, contributing almost one-half billion dollars annually to the economy of the state. It is produced on a composted substrate from various raw materials including straw-bedded horse manure, hay, corncobs, cottonseed hulls, poultry manure, brewer's grain, cottonseed meal, hardwood bark and gypsum.

Offensive odors produced by the preparation of mushroom compost are a problem for mushroom farms, due to a combination of residential encroachment into rural areas, and the heightened sensitivity of the general population to environmental issues (Duns et al., 2004). Several measures have been introduced to reduce the offensive odors produced during the preparation of mushroom compost. The most common measure is the use of forced aeration of Phase I compost contained in bunkers or tunnels. Some studies have reported volatile sulfur compounds, the most offensive odors, were reduced under forced aeration (Op den Camp et al., 1991; Noble et al., 2001) but others have shown no significant difference in detectable offensive odors with either conventional or forced air composting (Duns et al., 2004). Since sulfur compounds such as mercaptans and sulfides have very low odor thresholds, near-complete elimination of these compounds would be required to reduce offensive odors from compost. Therefore, a novel method of mushroom substrate preparation without the generation of offensive odors is desirable.

It is possible to produce *A. bisporus* on non-composted substrates. Till (1962) and Lemke (1965) obtained yields comparable to Phase II compost after grinding, mixing,

filling, sterilizing and spawning substrate in 200 L steel barrels. San Antonio (1971) produced mushrooms on cased grain spawn, and Murphy (1972), was able to obtain "good" mushroom yields by supplementing spent mushroom substrate (SMS) with corncobs, cottonseed meal and hardwood sawdust. He concluded that up to 25% SMS could be mixed directly with fresh Phase I compost at fill with no adverse effects on yield. Mee (1978) obtained "good quality" mushrooms on a non-composted substrate (NCS) mixture of cold manure, *Sphagnum* peat moss and gypsum. Biological efficiency (BE) ranging from 30-77% was obtained when NCS was used to produce brown (Portobello) *A. bisporus* (Sánchez and Royse, 2001; Sánchez et al., 2002). Bechara et al. (2005, 2006b) produced white mushrooms with a BE of over 150% when grain spawn was supplemented with delayed-release nutrient and placed on top of an auxiliary water reservoir of Perlite®. Since liquid cultures can be utilized to grow mycelia of *A. bisporus*, there are some reports on production of mushrooms using hydroponics systems (Aksu and Gunay, 1999; Bechara et al., 2006a). They were able to produce fruiting bodies of *A. bisporus* but yields were significantly lower than yields from traditional Phase II compost.

Spent mushroom substrate, spent mushroom compost, mushroom soil and recycled mushroom compost are all terms used to describe the production material that remains after mushrooms are harvested (AMI, 2005). Spent mushroom substrate can be used as growth medium for plants (Romaine and Holcomb, 2001), an organic biocontrol agent that suppresses the artillery fungus (*Sphaerobolus* spp) (Davis et al., 2005; Davis and Kuhns, 2005), and as a soil amendment to improve turf (Landshoot and McNitt, 2005), fruit (Robbins et al., 1986), vegetable (Kaddous and Morgans, 1986; Male, 1981;

Wang et al., 1984a, b), corn (Webber et al., 1997) and container-grown plants (Chong and Hamersma, 1997; Maher, 1991; Chong et al., 1991). Spent mushroom substrate from one genus of mushroom can be used as growth medium for another genus of mushroom (Rinker, 2002). For example, SMS obtained after a crop of *Pleurotus ostreatus* can be used as compost for cultivation of *A. bisporus* (Harsh and Bisht, 1984).

Spent mushroom substrate often is considered environmentally unfriendly, undesirable and represents a solid waste disposal problem for mushroom growers. Approximately 60% of the U.S. mushroom crop is produced in Pennsylvania; nearly 21.6 million m^3 of SMS is produced each year as a by product of the mushroom industry (Davis and Kuhns, 2005). In the U.S., this by product exceeds 36 million m^3 annually (AMI, 2005). A method of utilizing SMS to produce a second crop of mushrooms would help alleviate the problem of solid waste disposal in the mushroom industry.

Schisler (1990) was able to obtain BEs of up to 78% after supplementing SMS with 1.22 kg/m^2 of SpawnMate II, is a commercial delayed released nutrient, and 1 kg/m^2 Bonaparte peat. Till (1962) obtained higher mushroom yields from autoclaved SMS composed of chopped straw and additional nutritional supplements but had little success when the SMS was pasteurized. Huhnke and Sengbusch (1968) amended Till's procedure by further treating the substrate using a fermentation process after substrate sterilization. This process inhibited and prevented the subsequent development of mushroom competitors. Flegg and Randle (1968) used a pasteurized mixture of SMS, straw and Phase II compost to produce *A. bisporus,* but, the number and weight of sporophores were not comparable to those from Phase II compost. Spent mushroom substrate pasteurized at 66°C for 24 h, and then stored under cover after some time was found to

contain several molds such as *Monilia* sp. and *Trichoderma viride* (Flegg and Randle, 1968). A method of utilizing a mixture of NCS and SMS to produce *A. bisporus* has not been reported.

Most spawn of *A. bisporus* is prepared by commercial manufacturers (Fritsche, 1988). Grain spawn, first developed by Sinden (1932), is made by sterilizing a cereal substrate, inoculating the substrate with a pure culture of mother spawn and incubating the substrate until fully colonized. Grain spawn typically is composed of rye, millet or sorghum, supplemented with chalk, and contains a moisture content of 45-48%. The grain, colonized with mycelia on the surface, may readily be mixed with various substrate formulations, thus providing many points of inoculation. Since spawn is normally sold on a weight basis, grain that has small seeds such as millet give a greater number of inoculation points per kg than large grain such as rye. However, large grain has a greater food reserve and can sustain the mycelium for longer periods of time during stress (Fritsche, 1988). Thus, different types of spawn carrier may influence productivity.

Casing inoculum (CI) is a low-nutrient, fully-colonized substrate added to the casing material to speed up colonization of the casing layer. Commercial production of CI evolved in the late 1980's as an alternative to farm-prepared, fully-colonized compost added at casing, termed CACing (Bodine, 2005). The beneficial aspects of using CI to inoculate casing material include shortening of the mushroom production cycle, elimination of the pathological risk, and cost effectiveness. The additional points of inoculum available in CI increase the growth and development of mushroom mycelium in the casing layer. When used as a spawn carrier to inoculate NCS or SMS, CI may

increase the colonization rate of the substrate and thereby reduce the time required to complete a crop cycle.

The objectives of this research were to examine the effect of NCS and SMS on mushroom yield, size, and percentage solids, and to examine the influence of strain and type of spawn carrier on growth and development of *A. bisporus* in NCS and SMS.

2.3 Methods

2.3.1. Non-composted substrate (NCS)

Ingredients used for non-composted substrate were adopted from Sánchez and Royse (2001). Ingredients used (percentage based on oven dry wt) were red oak sawdust 28%, millet 29%, rye 8%, peat 8%, ground alfalfa 4%, ground soybean 4%, wheat bran 9% and $CaCO_3$ 10%.

2.3.2. Spent mushroom substrate (SMS)

Spent mushroom substrate was obtained from the Mushroom Test Demonstration Facility at The Pennsylvania State University. Spent mushroom substrate was post-crop pasteurized at $60^{\circ}C$ for 24-48 h with steam to kill pests or pathogens that might interfere with subsequent cropping trials. Pasteurized SMS, including the casing layer, was mixed before removal from the production facility, bagged in plastic trash bags (94 cm x 75 cm), and stored at $2^{\circ}C$ until use.

2.3.3. Spawn and spawn carriers

The brown strain (Sylvan SB-65) of *A. bisporus* (Portobello) was selected for this study in the first cropping experiment because it is increasing in popularity in the United States (Fig. 2.1a). In the second cropping experiment, we included a white strain (Sylvan 140) (Fig. 2.1b) because this is the major variety grown in the U.S. The commercial spawn carriers used were millet spawn and CI obtained from Sylvan Spawn Laboratories, Kittanning, Pennsylvania. Non-composted substrate spawn was prepared by placing 350 g (w.w.) of NCS mixture in Erlenmeyer flasks (1000 ml), and autoclaving at $121^{o}C$ for 90 min. The NCS spawn substrate was allowed to cool and inoculated with 4% (w.w.) millet spawn (Sylvan SB-65 or Sylvan 140). Flasks were placed on the laboratory bench at room temperature ($23 \pm 2^{o}C$), and shaken every 2-4 days, depending on rate of mycelial growth, to enhance uniform growth. The mature NCS spawn was stored at $4^{o}C$ until use.

2.3.4. Experimental design and data analysis

Two experiments were conducted. The first cropping experiment was designed as a two-factor (5 x 3) factorial in a randomized complete block design (RCBD) where treatment combinations were randomly assigned to the units within each block with four replicates per treatment (Kuehl, 2000). The experiment contained five substrate types and three spawn carriers. The experiment was repeated three times (blocks) and the mushrooms were harvested for two flushes (35-50 days from day of casing). This experiment, with repetitions, was designated Crop I. The second experiment was a (2 x 3

x 3) factorial in RCBD where treatment combinations were randomly assigned to the units within each block with three replicates per treatment. The experiment had three substrate types across two mushroom strains and three spawn carriers. The experiment was repeated twice (blocks) and the mushrooms were harvested for two flushes. This experiment, with repetitions, was designated Crop II. The general linear model SAS procedure (SAS Institute, Cary, N.C) was used for an analysis of variance and treatment means were separated using Fisher's least significant difference test at $p<0.05$.

2.3.5. Mushroom cropping trials

Cropping trials were carried out at the Mushroom Research Center (MRC) of The Pennsylvania State University. Ingredients were hand mixed, moistened to 65%, filled in high porosity filter plastic bags (Unicorn Bags, Garland, TX, Fig. 2.1c), autoclaved ($121^{o}C$ for 3 h), aseptically cooled and spawned with 30 g spawn per 2.5 kg substrate mixture (1.2%, w.w.). Fresh Phase II compost was used as a control. After spawning, the bags were heat-sealed and transferred to the MRC for spawn run (18-19°C, 18-21 days). The bags were opened and the fully colonized substrate was broken by hand and placed in 6.1 L plastic tubs (29.5 cm x 15.75 cm x 8.75 cm). Neutralized peat (2.5 cm) was overlaid on the substrate surface as casing. Case hold lasted for 18-21 days at 18-19°C; during this period water was applied daily or as needed with a hand sprinkler until the casing layer was saturated. Relative humidity in the production room was maintained at 90-95%.

2.3.6. Harvesting, determination of yield, BE and size

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

2.3.7. Mushroom solids content

Fresh mushrooms for solids content determination were randomly sampled from each treatment from Crop II. The mushrooms were sliced and 100 g of each sample was placed in a paper bag and oven dried at 60^oC for 48 h. Dried mushrooms were placed on laboratory bench for 2 h to cool before weighing. Ten replicates per treatment were used and solids content was recorded as percentage of dry mushroom weight.

2.4 Results

2.4.1. Mushroom yield and BE

Both crops had significant sources of variation in the analysis of variance for yield and BE for the treatments of substrate mixtures and spawn carriers including blocks (Table 2.1). There were no significant interaction effects ($p<0.05$) for any of the factors for yield or BE for either Crop (Table 2.1).

For Crop I, the highest mushroom yield (11.9 kg/m^2) and BE (67.3%) were obtained from the 3:1 NCS/SMS mix; however, yield and BE were not significantly different from the 1:1 NCS/SMS mixture or from Phase II compost (Table 2.2). Both NCS alone and 1:3 NCS/SMS were significantly lower than Phase II compost (Table 2.2). For Crop II, the highest mushroom yield (12.1 kg/m^2) and BE (66.8%) were obtained from a substrate mixture of 1:1 NCS/SMS (Table 2.2). Both 1:1 NCS/SMS and NCS alone were significantly higher in both yield and BEs than Phase II compost (Table 2.2).

Highest yield (10.8 kg/m^2) and BE (61.1%) in Crop I were obtained from substrate spawned with a mixture of 1:1 CI/millet spawn carrier (Table 2.3). The highest yield (12.8 kg/m^2) and BE (70.9%) in Crop II were obtained from substrate spawned with NCS spawn (Table 2.3).

2.4.2. Mushroom size

There was a significant source of variation for mushroom size in Crop II for strain types (Table 2.1). Crop II showed a significant difference in mushroom size between strains. The brown strain had larger ($p<0.05$) mushrooms (21.3 g) than the white strain (13.5 g) yet there had no significant effect on size between substrate mixtures and spawn carriers. There was no significant interaction effect ($p<0.05$) for size between substrate mixtures and spawn carriers in Crops I and II. Furthermore, substrate mixtures, spawn carriers, and blocks in Crop II had no significant effect on mushroom size. However, there was a significant difference ($p<0.05$) in mushroom size between blocks in Crop I.

2.4.3. Mushroom solids content

The brown strain, produced on substrates spawned with CI, tended to have the highest solids contents when compared to the white strain (Table 2.4). The highest solids content (7.1%) was obtained from the 1:1 NCS/SMS substrate, whereas the lowest solids content (5.9%) occurred in mushrooms produced on Phase II compost spawned with the white strain.

2.5 Discussion

Agaricus bisporus is produced commercially on a composted substrate. Although the time and nature of composting has been modified, no one has been successful in

eliminating this step from the preparation of a suitable medium for *A. bisporus* production (Schisler, 1982). We have shown that it is possible to obtain substantial yields of both brown and white *A. bisporus* by using mixtures of NCS and SMS. While yields obtained on NCS/SMS mixtures were not as high as those reported by Bechara et al. (2005) on grain spawn and Murphy (1972) with 1:1 SMS/Phase II compost they were comparable to those of Schisler (1990) on supplemented SMS. The reason for the lower yields may be because Bechara et al. (2005) supplemented the grain spawn with a delayed-released nutrient, Full House S41. Bechara et al. (2005) obtained a yield of 13.5 kg/m^2 and a BE of 166% when using grain spawn supplemented with S-41 placed on a water reservoir, and cased. The Phase II compost used by Murphy (1972) consisted of SMS, corn cobs, cottonseed meal and hardwood sawdust. He obtained yields of 18.8-26.1 kg/m^2 and BEs of 61-82%. The higher yields obtained by Murphy (1972) may be due to the addition of cottonseed meal in the substrate formulation. Cottonseed meal contains vegetable oil which is one of the sources of lipids required by *A. bisporus* sporophores. Lipids are stimulatory to mushroom growth (Schisler and Parton, 1971), and developing sporophores require a supply of lipids and proteins (ratio of 1:1), that are necessary for structural lipids in expanding cells in membranes (Schisler, 1982). Our yields are comparable to those reported by Schisler (1990) who obtained yields of 10.9-15.9 kg/m^2 when pasteurized SMS was supplemented with SpawnMate II and Bonaparte peat. The SMS and casing were mixed and pasteurized for 4 h at 63°C and cooled to 27°C before spawning.

We obtained a yield of 12.8 kg/m^2 and BE of 70.9% from the 1:1 NCS/SMS substrate and NCS spawn. Although the yield (on a per m^2 basis) obtained by us and

Bechara et al. (2005) may seem relatively low compared to commercial standards, it is associated with substrate depth and number of breaks harvested. Murphy (1972) showed that the substrate depth influences mushroom yield. He obtained higher yields from trays 30 cm deep. However, Murphys' (1972) BEs were the same for both substrate depths (22.5 cm and 30.0 cm) tested. Our substrate depth was about 6 cm while that of Bechara et al. (2005) was approximately 2 cm. The common Phase II compost depth used in the mushroom industry is about 16-20 cm. On the other hand, the BEs obtained by Bechara et al. (2005) were very high relative to commercial standards. This probably is due to the very aseptic conditions maintained in grain spawn making and to the water reservoir placed beneath the colonized NCS before casing. Our BEs from 1:1 NCS/SMS and 3:1 NCS/SMS were higher than those obtained from Phase II compost and were consistently between 40-70%, in line with previous research (Sánchez and Royse, 2001; Sánchez et al., 2002; Schisler, 1982).

Sánchez and Royse (2001) and Sánchez et al. (2002) obtained BEs of 30-70% and 42-77%, respectively, when NCS was used to produce the brown strain (Portobello). Schisler (1982) mentioned BEs of 50-70% as average and BEs of 70-90% as good for the white strain of *A. bisporus*. However, Schisler's (1982) ratings were made before the widespread adoption of white hybrid mushrooms. The use of various spawn carriers resulted in BEs ranging from 51-71%. Our BEs were average according to ratings by Schisler (1982) but higher than Sánchez and Royse (2001) and Sánchez et al. (2002). The lack of significant interaction ($p<0.05$) between substrate mixtures and spawn carriers on yields and BEs in both crops indicates that each treatment had a major contribution to mushroom yield and BE. Furthermore, the lack of significant interaction between

substrate mixtures and spawn carriers and strains in Crop II, would also indicate that each treatment had a major contribution to mushroom yield and BE.

Modern mushroom growers use rye or millet grain spawn and non-grain spawn such as SpeedSpawn® to inoculate their compost. In an apparent relationship between the substrate types and spawn carrier, it was observed that NCS, a non-grain spawn carrier, produced the highest yields (12.8 kg/m^2, BE of 70.9%) in Crop II as shown in Table 2.3. Spawn carrier NCS was more adaptable to the 1:1 NCS/SMS and NCS substrates because the ingredients are similar. It was not surprising that the spawn carrier mixture of 1:1 CI/millet gave the highest yield (10.8 kg/m^2 and BE (61.1%) in Crop I, since the large grains of millet contribute a greater food reservoir, whereas CI provided a greater number of inoculation points. Fritsche (1988) previously observed this phenomenon when comparing the spawn of two carriers made from grain.

In both crops, spawn carriers had a significant effect ($p<0.05$) on yields and BEs, but not on mushroom size. Mushroom yield and BE were not significantly different when CI was used as a spawn carrier compared to millet ($p<0.05$). Rinker and Alm (1998) obtained higher yields when Phase II compost was spawned with non-grain spawn (Speedspawn®). Non-grain spawn may also be effective against Trichoderma green mold (Rinker and Alm, 1998; Romaine et al., 1998). Casing inoculum, for example, contains a lesser nutrient level than grain but provides a sufficient amount to support vigorous vegetative growth of mycelium (Dahlberg and LaPolt, 1996). Vigorous substrate colonization by the mycelium during spawn run is desirable because it reduces mushroom cropping time and may allow mycelium to outgrow competitors in the substrate. This

work is the first report of use of a mixture of CI and grain spawn to obtain improved mushroom yields.

As expected, mushroom size of the brown strain (Portobello) was significantly larger than the white strain. This was due primarily to the stage of growth at harvest. Portobellos are harvested when the mushrooms are open and the gills are exposed, as compared to the white strains which are harvested with closed veils.

There was a significant ($p<0.05$) difference in mushroom solids contents produced from different substrates and strain types. Portobellos from 1:1 NCS/SMS substrate had the highest solids content (7.1%) while the white mushrooms from Phase II compost had the lowest solids content (5.9%). There was a significant difference ($p<0.05$) in mushroom solids within a strain for each substrates. The substrate 1:1 NCS/SMS produced a significantly higher dry weight in both strains because it may have contained more nutrients than Phase II compost. Shroeder and Schisler (1981) obtained dry weights of mushrooms grown on Phase II compost supplemented with Spawn Mate ranging between 7.5-8.6%, whereas Weil et al. (2004; 2006) obtained solids contents of 7.2-8.7% from Phase II compost supplemented with Micromax®. Micromax® is a commercially available micro-nutrient that is composed of six micro-elements (Bo, Cu, Fe, Mo, Mn, Zn). From this study the solids content obtained from 1:1 NCS/SMS without supplements were lower than those obtained by Shroeder and Schisler (1981), but comparable to those obtained from Micromax® supplemented Phase II compost.

Our results show that it is possible to obtain substantial yields from autoclaved mixtures of NCS and SMS for the production of both brown and white mushrooms and that a mixture of the two is better than either one alone. Although results from this study

are promising, more work is required to improve yields and BEs from an NCS/SMS mixture. Such improved technology may benefit small scale mushroom growers who are growing specialty mushrooms and want to expand into the production of Portobellos. These growers would not need compost turning and filling machines and would not need to devote specific land for a composting yard. The use of mixtures of NCS and SMS in the production of mushrooms may also help to reduce the odor problems associated with composting. We did not notice undesirable odors associated with the preparation and autoclaving of substrates used in this study.

Recycling SMS may help to reduce the amount of SMS disposed of each year. However, there would still be the problem of disposal of mixtures of spent NCS/SMS. Additional work is needed to determine the possibility of reusing spent NCS/SMS mixtures for a third crop of mushrooms or possibly for animal feed.

Acknowledgements

2.6 References

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Table 2.1

Probabilities greater than F from analysis of variance for three factors tested for yield,

biological efficiency and size of *Agaricus bisporus* for two crops

Source		Probability > F^c		
	df	Yield	BE	Size
Crop I[a]				
Block	2	<0.0001	<0.0001	0.0022
Substrate (SB)	4	<0.0001	<0.0001	0.5200
Spawn carrier (SC)	2	0.0436	0.0445	0.2570
SB x SC	8	0.6541	0.6914	0.4668
Crop II[b]				
Block	1	0.0001	0.0029	0.5483
Substrate (SB)	2	0.0024	0.0148	0.6443
Spawn carrier (SC)	2	0.0008	0.0007	0.4254
Strain (ST)	1	0.5085	0.4623	<0.0001
SB x SC	4	0.8362	0.8546	0.1071
SB x ST	2	0.8530	0.8503	0.5145
SC x ST	2	0.7424	0.7699	0.9955
SB x SC x ST	4	0.0777	0.0700	0.3777

[a] Error, df, 163; coefficient of variation, 37.86, 38.28, and 47.17 for yield, BE and size, respectively.

[b] Error, df, 89; coefficient of variation, 28.64, 28.02, and 28.42 for yield, BE and size, respectively.

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

Table 2.2

Mean yield (kg/m^2), percentage biological efficiency (% BE) and mushroom size (g/mushroom) of *Agaricus bisporus* grown on non-composted substrate (NCS), mixtures of NCS and spent mushroom substrate (SMS), and Phase II compost for two crops

Substrate type	Yield (kg/m^2)[abc]	BE (%)[cd]	Size (g)[bc]
Crop I			
NCS	7.4b	42.0b	21.9a
1:1 NCS/SMS	10.9a	61.5a	24.8a
3:1 NCS/SMS	11.9a	67.3a	25.9a
1:3 NCS/SMS	8.7b	49.3b	26.4a
Phase II compost	10.5a	60.6a	25.1a
Crop II			
NCS	11.5a	63.4a	16.8a
1:1 NCS/SMS	12.1a	66.8a	17.8a
Phase II compost	9.6b	55.0b	17.7a

[a] Yield = fresh mushrooms harvested when pilei were open (lamellae visible) per 2.5 kg substrate (w.w.).

[b] Values are the means of four replicates.

[c] Means followed by the same letter in the same column in the same crop are not significantly different according to Fisher's LSD.

[d] % BE = ratio of fresh mushrooms harvested/dry substrate wt expressed as a percentage.

Table 2.3

Mean yield (kg/m^2), percentage biological efficiency (%BE) and mushroom size (g/mushroom) of *Agaricus bisporus*, grown on substrate and spawned with three types of spawn carrier, casing inoculum (CI), millet and non-composted substrate (NCS) for two crops

Spawn carrier	Yield (kg/m^2)^{abc}	BE (%)^{cd}	Size (g)^{bc}
Crop I			
CI	9.0b	51.2b	26.4a
Millet	9.8ab	56.0ab	22.9a
1:1 CI/millet	10.8a	61.1a	25.1a
Crop II			
CI	10.5b	58.9b	17.5a
Millet	10.0b	55.4b	16.6a
NCS	12.8a	70.9a	18.1a

[a] Yield = fresh mushrooms harvested when pilei are open (lamellae visible) per 2.5 kg substrate (w.w.).

[b] Values are the means of four replicates.

[c] Means followed by the same letter in the same column in the same crop are not significantly different according to Fisher's LSD.

[d] % BE = ratio of fresh mushrooms harvested/dry substrate wt expressed as a percentage.

Table 2.4

Mean mushroom solids content of *Agaricus bisporus* mushrooms grown on non-composted substrate, spent mushroom substrate and Phase II compost spawned with casing inoculum

Treatment	Strain	Solids (%)[a]
NCS	Brown	6.8ab
1:1 NCS/SMS	Brown	7.1a
Phase II compost	Brown	6.6abc
NCS	White	6.0bc
1:1 NCS/SMS	White	6.0bc
Phase II compost	White	5.9c

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

Fig. 2.1. *Agaricus bisporus*: (A) brown (Portobello), (B) smooth white hybrid and (C) substrate contained in plastic bags with very high porosity filter patches used for sterilization and spawn run of non-composted and spent mushroom substrates.

Chapter 3: Yield, size, and solids content of *Agaricus bisporus* mushrooms grown on supplemented non-composted and spent mushroom substrates

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3.1 Abstract

Four crops of *Agaricus bisporus* were grown on a mixture of sterilized non-composted (NCS) and spent mushroom substrate (SMS), sterilized SMS, or pasteurized Phase II compost. Substrates were spawned with casing inoculum at a rate of 30 g per 2.5 kg substrate and were non-supplemented or supplemented with: 10% Target® or 10% soybean meal at spawning or at casing for Crop I, wheat bran (0%, 5%, 10%) and soybean meal (0%, 5%, 10%, 15%) at spawning for Crop II, Micromax® (0 mg/kg, 6,000 mg/kg, 7,350 mg/kg) at spawning for Crop III and Micromax® (0 mg/kg, 3,000 mg/kg, 6,000 mg/kg, 7,350 mg/kg, 9,000 mg/kg, 12,000 mg/kg) at spawning for Crop IV. Highest yield (27.2 kg/m^2) and biological efficiency (BE) (144.3%) for Crop I were obtained from a 1:1 NCS/SMS mixture supplemented with Target® at casing. For Crop II, the highest yield and BE (18.7 kg/m^2, 99.5%, respectively) were obtained from Phase II compost supplemented with 10% soybean meal and 5% wheat bran. For Crop III, highest yield and BE (15.6 kg/m^2, 82.5%, respectively) were obtained from Phase II

compost supplemented with 7,350 mg/kg Micromax®. For Crop IV, highest yield and BE (14.6 kg/m^2, 77.7%, respectively) were obtained from NCS supplemented with 9,000 mg/kg Micromax®. Supplementation of substrates at casing with either 10% Target® or 10% soybean meal resulted in yields and BEs that were significantly higher than those obtained from substrates supplemented at spawning. For Phase II compost, supplementation with 10% soybean meal at spawning resulted in a complete loss of mushroom production due to contaminants. In Crop III, where various concentrations of Micromax® were used as supplements, non-supplemented 1:1 NCS/SMS produced a yield (14.0 kg/m^2) and BE (74.4%) not significantly different ($p<0.05$) from Phase II compost supplemented with 6,000 mg/kg (14.8 kg/m^2, 78.7%) or with 7,350 mg/kg (15.6 kg/m^2, 82.5%). Similarly, non-supplemented 1:1 NCS/SMS produced a yield (14.0 kg/m^2) and BE (74.4%) not significantly different ($p<0.05$) from 1:1 NCS/SMS supplemented with 7,350 mg/kg (14.8 kg/m^2, 78.4%) or 6,000 mg/kg (13.6 kg/m^2, 72.3%). In Crop IV, where various concentrations of Micromax® were used as supplements in NCS, the addition of 9,000 mg/kg Micromax® gave the highest yield (14.6 kg/m^2) of any of the Micromax® supplemented treatments. The highest mushroom solids content (9.8%) was obtained from NCS supplemented with 7,350 mg/kg Micromax® (Crop III). Our results show the potential to produce relatively high yields on mixtures of NCS/SMS, and that a combination of supplements that contain protein, carbohydrate and micronutrients added at spawning or at casing may lead to improved mushroom yields.

3.2 Introduction

Organic and inorganic supplements added to Phase II compost are known to stimulate yield of *Agaricus bisporus* (Lange) Imbach (Schisler and Sinden, 1962; Sinden and Schisler, 1962; Gerrits, 1974; Carrol and Schisler, 1976; Schisler, 1982; Beyer and Muthersbaugh, 1996; Desrumaux et al., 2000; Weil et al., 2006). Yield increases of 10-60% were realized when small amounts of protein supplements were added to Phase II compost at spawning (Schisler and Sinden, 1962). A large fraction of the supplements known to stimulate mushroom yield consists of protein (Schisler and Sinden, 1962; Sinden and Schisler, 1962). However, materials other than those containing high protein levels are reported to function as supplements. Dahlberg (2004) obtained a positive linear relationship between the amount of carbohydrate (cellulose) added to the compost at spawning and mushroom yield.

Micronutrients such as manganese are required by several fungi, including *A. bisporus,* at concentrations of 0.005 to 0.01 ppm for the catalysis of many enzymes including those involved in the TCA cycle, and as a cofactor for Mn peroxidase that is responsible for lignin degradation (Lankinen et al., 2004). Manganese (Mn^{2+}) is known to stimulate yield and enhance quality of fresh mushrooms (Racz and Tasnadi, 1998; Weil et al., 2004; Weil et al., 2006).

Compost may be supplemented with organic or inorganic micronutrients at spawning, casing, or later during cropping. Supplementation of mixtures of SMS and Phase I compost prior to Phase II composting stimulated yield and increased BE (Murphy, 1972). Albuminous substances such as cottonseed meal, soybean meal and

grated coconut, when added to SMS, also stimulate mushroom yield (Till, 1962). While considerable research has been done on supplementation of Phase II compost with organic (Schisler, 1967; Schisler and Sinden, 1962; Schisler and Sinden, 1966; Schisler and Patton, 1970; Schisler and Patton, 1971; Schisler and Patton, 1974; Carrol and Schisler, 1976; Shroeder and Schisler, 1981; Lehrian et al., 1976; Bech and Rasmussen, 1969; Gerrits, 1974) and inorganic (Desrumaux et al. 2000; Weil et al. 2004; Weil et al. 2006) nutrients, the effects of supplementation of substrate mixtures of NCS and SMS have not been reported.

Offensive odors produced by the preparation of mushroom compost are a problem for mushroom farms, due to a combination of residential encroachment into rural areas, and the heightened sensitivity of the general population to environmental issues (Duns et al., 2004). Several measures have been introduced to reduce the offensive odors produced during the preparation of mushroom compost. The most common measure is the use of forced aeration of Phase I compost contained in bunkers or tunnels (Op den Camp et al., 1991; Noble et al., 2001). Other researchers (Till, 1962; San Antonio, 1971; Murphy, 1972; Mee, 1978; Sánchez and Royse, 2001; Sánchez et al., 2002; Bechara et al., 2005a, b; 2006a, b) have shown that it is possible to produce *A. bisporus* on NCS that can be prepared without generating offensive ordors.

Spent mushroom substrate, spent mushroom compost, mushroom soil, and recycled mushroom compost are all terms used to describe the production material that remains after mushrooms are harvested (AMI, 2005). Spent mushroom substrate often is considered environmentally unfriendly, undesirable and represents a solid waste disposal problem for mushroom growers.

Approximately 60% of the U.S. mushroom crop is produced in Pennsylvania; nearly 21.6 million m^3 of SMS is produced each year as a by-product of the mushroom industry (Davis and Kuhns, 2005). In the U.S., this by-product exceeds 36 million m^3 annually (AMI, 2005). Utilizing SMS to produce a second crop of mushrooms would help alleviate the problem of solid waste disposal in the mushroom industry (Schisler, 1990). Therefore, we conducted mushroom cropping experiments with SMS, NCS and Phase II compost to examine the effect of organic and inorganic supplements and their possible interactions on yield, size, and solids of *A. bisporus*. We also sought to determine the most optimum time to add organic supplements to stimulate mushroom yield.

3.3 Methods

3.3.1 Substrate

Ingredients used for NCS, adopted from Sánchez and Royse (2001), 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%).

Spent mushroom substrate, obtained from the Mushroom Test Demonstration Facility at The Pennsylvania State University, was post-crop pasteurized with steam at $60^{o}C$ for 24-48 h to kill pests or pathogens that might interfere with subsequent cropping trials. Pasteurized SMS, including the casing layer, was mixed before it was removed

from the production facility, bagged in plastic bags (94 cm x 75 cm), and stored at 2°C until used.

3.3.2 Strain and spawn

A brown strain of *A. bisporus* (Portobello) was selected since it is commercially produced and is becoming increasingly popular in the United States. The spawn used was commercial casing inoculum (CI) composed of a mixture of neutralized peat, wheat bran, and vermiculite. The CI (SB-65) was obtained from Sylvan Spawn Laboratories, Kittanning, PA.

3.3.3 Experimental design and data analyses

Four cropping experiments (Crops I, II, III and IV) were conducted at the Mushroom Research Center. Crop I was designed as a 3 x 2 x 2 factorial in a completely randomized design, 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. Crop II was a 2 x 12 factorial in a completely randomized block design (CRBD) where treatment combinations were randomly assigned to each unit with three replicates per treatment. The experiment had two substrate types across 12 supplement combination treatments in three blocks. Each supplement combination treatment had a mixture of various levels of soybean meal and wheat bran. Crop III was a 4 x 3 factorial in a CRBD

where treatment combinations were randomly assigned to each unit with six replicates per treatment. The experiment had four substrates across three levels of micronutrient supplement in three blocks. Crop IV was a 1 x 6 factorial in a CRBD where treatment combinations were randomly assigned to each unit with six replicates per treatment. The experiment had one substrate across six levels of Micromax® in three blocks. 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).

3.3.4 Mushroom cropping trials

For Crop I, the substrates 1:1 NCS/SMS, SMS and Phase II compost were supplemented either at spawning or at casing with 10% (oven dry wt basis) soybean meal (Archer Daniels Midland, P. O. Box 1470, Decatur, IL) or 10% Target® (Spawn Mate, Inc., Watsonville, California). For Crop II, SMS and Phase II compost were supplemented at spawning with 0%, 5%, 10% and 15% soybean meal and/or 0%, 5% and 10% soft red winter wheat bran (Bi-pro Marketing Limited, Princeton, ON). For Crop III, the substrates 1:1 NCS/SMS, NCS, SMS and Phase II compost were supplemented at spawning with either 0, 6,000 or 7,350 mg/kg Micromax® (Scotts-Sierra Horticultural Products Co., Marysville, OH). For Crop IV, NCS was supplemented at spawning with 0, 3,000, 6,000, 7,350, 9,000 or 12,000 mg/kg Micromax® (Scotts-Sierra Horticultural

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Products Co., Marysville, OH). Ingredients were hand mixed, moistened to 65%, placed

in very high porosity filter bags (Unicorn Bags, Garland, TX), autoclaved (121^oC for 3

h), aseptically cooled and spawned with 30,000 mg spawn per 2.5 kg substrate mixture

(1.2%, w.w.). Phase II compost was included in the experiments (except for Crop IV) as a

control treatment. After spawning, the bags were heat-sealed and transferred to the MRC

for spawn run at 18-19^oC for 18-21 days. The bags were opened and the fully colonized

substrate was fragmented and placed in 6.1 L plastic tubs (29.5 cm x 15.75 cm x 8.75

cm). Neutralized peat (2.5 cm) was overlaid on the substrate surface as casing. Case hold

lasted for 18-21 days at 18±1^oC; during this period, water was applied daily or as needed

until the casing layer was saturated. Relative humidity in the production room was

maintained at 90-95%.

3.3.5 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 BE were

determined and average mushroom size was 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 III and IV. 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.

3.4 Results

3.4.1 Yield and BE

3.4.1.1 Time of supplementation

A significant difference in treatments and treatment interactions was observed for time of supplementation, substrate mixture, and supplements for yield and BE in Crop I (Table 3.1). There was a significant difference in yield and BE when the substrates were supplemented at spawning or at casing in Crop I (Table 3.2). The two supplements, Target® and soybean meal, and the four substrates tested for different supplementation times gave a significant difference for yield and BE. Mushroom yield was stimulated, ranging from +24.3% to +145.0%, when substrates (SMS, NCS/SMS, Phase II compost) were supplemented at casing with either 10% Target® or 10% soybean meal (except SMS supplemented with 10% soybean meal) when compared to the control (non-supplemented Phase II compost). No mushrooms were harvested from Phase II compost supplemented with 10% soybean meal at spawning.

3.4.1.2 Substrate mixtures

There was a significant source of variation for substrates for Crops I and II for yield and BE (Table 3.1). Mushroom yield (27.2 kg/m^2) and BE (144.3%) in Crop I were highest from a substrate mixture of NCS/SMS supplemented with Target® at casing and were significantly higher than both controls, SMS (yield of 4.9 kg/m^2, BE of 25.7%) and Phase II compost (yield of 11.1 kg/m^2, BE of 58.8%) (Table 3.2). Percentage yield increase over the control (Phase II compost) was 145% (Table 3.2).

There was a significant yield increase when SMS was supplemented with various levels and combinations of soybean meal and wheat bran in Crop II (Table 3.3). The highest yield (18.7 kg/m^2) and BE (99.5%) were obtained from Phase II compost supplemented with 10% soybean meal and 5% wheat bran. However, these values were not significantly different from Phase II compost supplemented with 5% soybean meal and 5% wheat bran, but were different from both non-supplemented Phase II compost (yield of 12.5 7 kg/m^2, BE of 66.3%) and non-supplemented SMS (yield of 8.3 kg/m^2, BE of 44.4%). Regardless of the amount of supplement, SMS treatments that included soybean meal yielded between 11.9-16.3 kg/m^2 with BEs ranging between 63.2 to 86.7% (Table 3.3). Mushrooms were not produced from Phase II compost supplemented with 10% soybean meal, or on a mixture of 10% soybean meal and 10% wheat bran. Regardless of the amount of wheat bran used, Phase II compost only produced mushroom yields ranging from 15.5-15.6 kg/m^2 and BEs ranging from 82.6-82.8% which were neither significantly different from each other, nor significantly different from non-supplemented Phase II compost (control, yield of 12.5 kg/m^2 and BE of 66.3%).

3.4.1.3 Supplements

In Crops I and II, there was a significant source of variation for supplements for yield and BE (Table 3.1). Supplementation of NCS/SMS with 10% Target® at casing, produced a maximum mushroom yield of 27.2 kg/m^2 and a BE of 144.3% in Crop I (Table 3.2).

The effect of various amounts of soybean meal and/or wheat bran added to SMS and Phase II compost is shown in Table 3.3. The highest yield (18.7 kg/m^2) and BE (99.5%) was obtained when Phase II compost was supplemented with 10% soybean meal and 5% wheat bran. Some treatments, containing various levels of soybean meal alone or in combination with wheat bran, reduced mushroom yield.

3.4.1.4 Interactions

There was a significant interaction effect for factors as follows: 1) supplementation time and substrates 2) substrates and supplements, and 3) supplementation time and supplements for yield and BE (Table 3.1). There was no significant interaction in Crop I between supplementation time, substrates and supplements. For Crop II, a significant interaction effect was observed between substrates and supplements for yield and BE. There was no significant interaction between substrates and supplements for mushroom size (Table 3.1).

3.4.1.5 Micromax®

Mushroom yield and BE were significantly affected by Micromax® concentration, and the interaction between substrate mixture x Micromax® concentration (Table 3.1). However, Micromax® concentration alone did not significantly affect mushroom size.

The highest yield (15.6 kg/m^2) and BE (82.5%) were obtained from Phase II compost supplemented with 7,350 mg/kg Micromax®, while the lowest yield (8.3 kg/m^2) and BE (44.1%) were from SMS with no Micromax® (Table 3.4). Relatively low yield (8.7 kg/m^2) and BE (46.1%) also were obtained from NCS with no Micromax®. The highest levels of Micromax® stimulated mushroom yield from both Phase II compost and NCS when compared to controls without Micromax®. When NCS was supplemented with higher levels of Micromax® (9,000 mg/kg), both yield (14.6 kg/m^2) and BE (77.7%) increased (Table 3.5). However, increasing Micromax® levels further to 12,000 mg/kg, resulted in a significant yield decrease (to 12.4 kg/m^2). Adding Micromax® to SMS neither increased nor suppressed mushroom yield. At 7,350 mg/kg Micromax®, yields were similar for NCS, NCS/SMS and Phase II compost (Table 3.4).

3.4.1.6 Experimental blocking

The necessity for blocking in three of our experiments (Crops II, III and IV) was management of tasks, viz a viz insufficient autoclave capacity. Blocking was required to account for the variation in different autoclave runs. We observed a significant difference

in blocks for Crops III and IV for yield and BE while a significant difference for mushroom size was observed for Crops II, III and IV. The highest mushroom yield and BE for Crops III (12.7 kg/m^2, 67.3%) and IV (14.5 kg/m^2, 77.2%) were from the first blocks, respectively (Table 3.6).

3.4.2 Size

3.4.2.1 Substrate mixture

Substrate mixtures significantly influenced mushroom size in Crops II and III (Table 3.1). For Crop II (Table 3.3), the largest mushrooms were obtained from Phase II compost supplemented with 5% soybean meal and 5% wheat bran (54.1 g/mushroom). Mushrooms obtained from non-supplemented or supplemented SMS were significantly larger than mushrooms obtained from non-supplemented Phase II compost (one of the controls). The one exception was mushroom size for SMS supplemented with a combination of 5% wheat bran and 5% or 15% soybean meal.

In Crop III, the largest mushrooms (38.3 g/mushroom) were obtained from NCS supplemented with 6,000 mg/kg Micromax®. The smallest mushrooms were obtained from NCS/SMS supplemented with either 7,350 mg/kg (27.0 g/mushroom) or 6,000 mg/kg (27.1 g/mushroom) or Micromax®.

3.4.2.2 Interactions

There was no significant interaction for mushroom size in Crop I. For Crop II, a significant interaction effect was observed between substrates and supplements for mushroom size (Table 3.1), where the largest mushroom size (54.1 g/mushroom) was from Phase II compost supplemented at spawning with 5% soybean meal and 5% wheat bran (Table 3.3). Phase II compost supplemented at spawning with either 10% soybean meal only or 10% soybean meal and 10% wheat bran produced no mushrooms. The smallest mushrooms (20.0 g/mushroom) were obtained from non-supplemented Phase II compost (control).

A significant interaction effect was observed between substrates and Micromax® concentration for mushroom size (Crop III, Table 3.1). The largest mushrooms (38.8 g/mushroom) were obtained from NCS supplemented with 6,000 mg/kg while the smallest mushrooms were obtained from NCS/SMS supplemented with either 6,000 mg/kg or 7,350 mg/kg Micromax® (Table 3.4).

3.4.2.3 Experimental blocking

A significant difference for mushroom size for experiment blocking was observed for Crops II, III and IV (Table 3.1). The largest mushroom sizes were obtained from block 1 Crop II (42.9 g/mushroom), block 3 in Crop III (35 g/mushroom)(Table 3.6), and block 2 Crop IV (57.9 g/mushroom). Mushroom sizes from blocks 2 (28.6 g/mushroom) and 3 (25.3 g/mushroom) in Crop II, blocks 2 (33.2 g/mushroom) and 3 (35.0

g/mushroom) in Crop III, and from blocks (57.9 g/mushroom) and 3 (55.1 g/mushroom) in Crop IV were not significantly different (Table 3.6).

3.4.3 Mushroom solids content

There was a significant difference in mushroom solids content when various levels of Micromax® were added to production substrates (Tables 3.4 and 3.5). Mushroom solids, 9.8% (Table 3.4, Crop III) and 9.6% (Table 3.5, Crop IV), were significantly higher from non-composted substrate supplemented with 7,350 mg/kg Micromax® (Table 3.4). The lowest (5.9%) solids content was obtained from Phase II compost with no Micromax®. Solids content of mushrooms harvested from Phase II compost supplemented with 7,350 mg/kg Micromax®, was 6.8% while mushrooms harvested from Phase II compost supplemented with 6,000 mg/kg Micromax® and Phase II compost with no Micromax® contained 6.1 and 5.9% solids, respectively. There was no discernible pattern observed for mushroom size for any of the Micromax®/substrate combinations other than the Micromax®/Phase II compost substrate combination.

3.5 Discussion

A 1:1 mixture of NCS/SMS supplemented with either 10% Target® or 10% soybean meal at casing resulted in BEs greater than 120%. The results, showing that supplemented substrates produce significantly higher yields than non-supplemented substrates, agree with those of Sinden and Schisler (1962). Sinden and Schisler (1962)

supplemented Phase II compost at casing with up to 150 g cottonseed meal per kg (d.w.) of compost. They obtained yield increases nearly proportionally to the amount of supplement added to the compost. Schisler (1990) showed that mushroom yields were stimulated when delayed release nutrients were added to pasteurized SMS. When he supplemented SMS with 2.44 kg/m^2 SpawnMate II and 24.4 kg/m^2 Bonaparte peat, he obtained a mushroom yield (14.9 kg/m^2) that was significantly greater than the non-supplemented control (SMS, 5.7 kg/m^2).

Target® is a commercially-available delayed-release supplement that is high in protein concentrate and specifically formulated to stimulate mushroom mycelial growth and crop yields. It is formulated to minimize risk associated with heat generated during spawn run or case hold and can be used in Phase II or Phase III compost. In our work, BE was as high as 144% when Target® was added to 1:1 NCS/SMS at casing (similar to Phase III compost). Except for SMS, there was a significant increase in yield and BE when substrate was supplemented at casing. Yields and BEs were significantly greater when substrates were supplemented at casing compared to supplementation at spawning. Our results agree with the findings of Sinden and Schisler (1962) who obtained higher mushroom yields from Phase II compost supplemented with cottonseed meal at casing, as compared to supplementation at spawning. A 1:1 mixture of NCS/SMS supplemented at casing with 10% Target® resulted in a mushroom yield of 27.2 kg/m^2. The average yield obtained from commercial mushroom houses in the United States when mushrooms are produced on supplemented Phase II compost is approximately 28.9 kg/m^2 (USDA, 2006). Although the yield on a per m^2 basis obtained in our work may seem relatively low compared to commercial standards, it could be explained by depth of the substrate and

number of breaks harvested. The depth of our substrate was about 6 cm compared to the common Phase II compost depth used in the mushroom industry of about 16-20 cm. Murphy (1972) and Sinden and Schisler (1962) showed that the substrate depth influences mushroom yield. Murphy (1972) obtained greater yields from trays 30 cm deep as compared to the yields from trays 22.5 cm deep. Sinden and Schisler (1962) obtained greater yields from trays 25 cm deep as compared to the yields from 15 cm deep trays. Supplementation of NCS/SMS with 10% Target®, even at spawning, resulted in yields higher than 20 kg/m^2.

Carroll and Schisler (1976) pointed out that it was disadvantageous to supplement at casing because of the necessity of through-mixing the supplements into the mycelial-knitted compost. Other problems mentioned were excessive heat production after supplementation, and the possibility of contamination with weed molds. In our work, it was not a problem to through-mix the supplements because the spawn run was carried out axenically in plastic bags. It was possible to break up the substrate before opening the bags or to open the bags before fragmenting the substrate and placing it into tubs.

No mushrooms were obtained from Phase II compost supplemented at spawning with 10% soybean meal because the substrate was contaminated with bacteria and weed molds. This was due to the non-delayed release nature of the proteinaceous supplements. Similar observations were made by Schisler and Sinden (1962) during their pioneering work on supplements added at spawning. They observed mold infestations that caused severe yield reduction when Phase II compost was supplemented with raw proteinaceous plant materials. Bechara et al., (2006b) obtained no yield when they used millet grain-spawn substrate mixed with 25% soybean. This was apparently due to the production of

ammonia which is toxic to the mushroom mycelia. However, when 1:1 NCS/SMS was supplemented with 10% soybean meal at spawning or at casing, there was a yield increase of 65% and 107%, respectively. Bechara et al., (2006a) also observed a significant yield increase when millet grain-spawn substrate was supplemented with 15% soybean and 5% S41. They obtained a yield of 16.9 kg/m^2 and BE of 196%. Our results and those of Bechara et al., (2006a) show that soybean stimulates yield. However, the magnitude of the yield response is dependent upon substrate type and crop stage of supplementation. Bechara et al., (2006a) observed clustered and malformed mushrooms when 30% soybean and 5% S41 were used to supplement millet grain-spawn substrate at casing.

The significant difference in blocks observed in Crops II, III and IV indicates that some of variation in the responses is due to variation associated with autoclave processing. By blocking, this variation was accounted for in the experimental design and therefore, improved the precision of the experiment.

Both yield increases and decreases were observed when SMS and Phase II compost were supplemented at spawning with various ratios of soybean meal and or wheat bran. Materials that are low in protein content and high in carbohydrate content, when added at spawning, stimulate yield and decrease competition of mushroom mycelia with molds (Dahlberg, 2004). Wheat bran supplement stimulates production of manganese peroxidase (MnP) in *A. bisporus* (Lankinen et al., 2004). Manganese peroxidase, together with laccase, is produced by *A. bisporus* to degrade lignin. Lignin degradation primarily occurs during the growth stage of the mycelium before primordium formation (Durrant et al., 1991). Lignin is one of the carbon sources required by

mushrooms (Treschow, 1944; Schisler, 1982). Lignin, lignin-N complexes and phenolic compounds are degraded by laccase to produce lower molecular weight aromatic compounds such as soluble quinines and aromatics. Schisler (1982) stated that extracellular laccase increases during spawn run and after casing which indicates more utilization of lignin. Our experiment showed higher yields and BEs when wheat bran was mixed with soybean meal than when each component was used alone. These results indicate that further improvement in commercial supplements may be possible using a combination of both high protein and high carbohydrate.

Incorporation of Micromax® into the various treatments caused a darkening of the substrates. This may be associated with the manganese content in Micromax®. Rantcheva (1972) also reported the darkening of synthetic compost during the composting process after the addition of trace elements. Likewise, Royse (personal communication) observed a darkening of shiitake substrate after autoclaving when manganese was added at 250 mg/kg to the substrate.

The yield and BE of non-supplemented NCS/SMS were not significantly different from NCS/SMS supplemented with Micromax®. These results remain unexplained, but may be related to the NCS/SMS containing a sufficient supply of these micronutrients. Non-supplemented NCS and SMS individually produced the lowest mushroom yields and BEs but, when these substrates were combined, they resulted in a significantly higher yield (14.0 kg/m^2) and BE (74.4%) in Crop III (Table 3.4). Mixtures of NCS and SMS may provide a balance of organic and micronutrients responsible for the observed yield increases.

We used a combination of containers to manipulate the substrates used in these experiments. Spawn runs for all experiments were carried out in plastic bags fitted with very high porosity filter patches and fructification occurred in plastic tubs. Before placing the fully colonized Phase II compost in the tubs for casing, the mycelia-knitted substrates were fragmented, which may have caused stress on the mushroom mycelia. However, stress (fragmentation) may actually improve mushroom quality (Gerrits, 1988; R. B. Beelman, personal communication) and increase levels of antioxidants (Dubost, 2006). Post-harvest shelf-life of mushrooms may improve when compost is fragmented prior to casing (R.B. Beelman, personal communication). Dubost (2006) found that levels of ergothioneine, an antioxidant found in *A. bisporus*, increased significantly after colonized the compost was fragmented. Antioxidants are chemicals, enzymes or organic molecules that may reduce the oxidative damage to human or animal cells and biomolecules in tissues (DiSilvestro, 2001; Halliwell, 2001).

In the commercial mushroom industry, fully colonized Phase II compost is not fragmented before casing on bed farms; however, growers do fragment the compost when they use Phase III tunnels for bulk spawn run. Also it has been observed that anastomosis and recovery of the fragmented mycelium just before casing of the fully colonized Phase II compost is more rapid and mushroom production starts 1 or 2 days earlier compared to the undisturbed Phase II compost (Sinden and Schisler, 1962). Thus, fragmentation may increase earliness of production and mushroom quality.

We have demonstrated that it is possible to obtain reasonably high yields of brown *A. bisporus* on mixtures of NCS/SMS and SMS after the addition of either organic or inorganic supplements at spawning or at casing. Supplemented NCS/SMS and SMS

produced yields and BEs higher than those obtained from non-supplemented standard Phase II compost and equivalent yields and BEs to supplemented Phase II compost. While the processes we developed here may not have immediate commercial application, an increased understanding of nutrition of the mushroom at various stages and production on various substrates may lead to new avenues of research. Additional work is needed to determine the effect of adding inorganic supplements at casing or later, and the effect of adding both organic and inorganic supplements in the same treatments.

Acknowledgements

3.6 References

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Table 3.1

Probabilities greater than F from analysis of variance for three factors tested for yield,

biological efficiency and size of *Agaricus bisporus* for four crops

Source		Probability > F^e		
	df	Yield	BE	Size
Crop I[a]				
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[b]				
Block	2	0.2910	0.3344	<0.0001
Substrate (SB)	1	0.0533	0.0553	0.0064
Supplement (SP)	11	<0.0001	<0.0001	0.4682
SB x SP	11	<0.0001	<0.0001	0.2824
Crop III[c]				
Block	2	0.0149	0.0159	0.0070
Substrate (SB)	3	<0.0001	<0.0001	0.0002
Micromax concentration (MC)	2	<0.0001	<0.0001	0.5561
SB x MC	6	<0.0001	<0.0001	0.2375
Crop IV[d]				
Block	2	<0.0001	<0.0001	0.0018
Treatment	5	<0.0001	<0.0001	0.3849

[a] Error, df, 53 coefficient of variation, 14.26, 14.20, and 28.02 for yield, BE and size,

respectively.

[b] Error, df, 144 coefficient of variation, 27.11, 26.48, and 34.14 for yield, BE and size, respectively.

[c] Error, df, 196 coefficient of variation, 18.85, 18.82, and 29.63 for yield, BE and size, respectively.

[d] Error, df, 91 coefficient of variation, 21.5, 21.5, and 39.6 for yield, BE and size, respectively.

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

Table 3.2

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 substrate, non-composted substrate and Phase II compost supplemented with either 10% Target® or 10% soybean meal at spawning or at casing (Crop I)

Substrate[a]	Suppl. time	Supplement (10%)	Size (g/mush)[b]	BE (%)[bc]	Yield (kg/m^2)[b]	Difference (%)[d]
PIIC	Control	None	40.9a	58.8f	11.1f	0.0
SMS	Control	None	45.9a	25.7h	4.9h	-55.9
SMS	Spawning	Soybean meal	47.2a	53.5fg	10.1fg	-9.0
SMS	Spawning	Target®	43.0a	41.2g	7.8g	-29.7
1:1NCS/SMS	Spawning	Soybean meal	34.3a	96.9d	18.3d	+64.9
1:1NCS/SMS	Spawning	Target®	35.8a	106.5cd	20.1cd	+81.1
PIIC	Spawning	Soybean meal	---	---	---	---
PIIC	Spawning	Target®	41.0a	109.4bcd	20.6bcd	+85.6
SMS	Casing	Soybean meal	44.0a	57.9f	10.9f	-1.8
SMS	Casing	Target®	41.1a	73.4e	13.8e	+24.3
1:1NCS/SMS	Casing	Soybean meal	34.7a	122.2b	23.0b	+107.2
1:1NCS/SMS	Casing	Target®	38.6a	144.3a	27.2a	+145.0
PIIC	Casing	Soybean meal	38.6a	75.3e	14.2e	+27.9
PIIC	Casing	Target®	40.1a	114.6bc	21.6bc	+94.6

[a] SMS = spent mushroom substrate, NCS = non-composted substrate, PIIC =Phase II compost, BE = biological efficiency.

[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) x 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 from control.

Table 3.3

Mean yield (kg/m^2), percentage yield difference compared to the control (Phase II compost), percentage biological efficiency (% BE) and mushroom size (g/mushroom) of sterilized spent mushroom substrate (SMS) and pasteurized Phase II compost (PIIC) supplemented with various levels of wheat bran and soybean meal at spawning (Crop II)

Substrate	Supplement (%)		Yield (kg/m^2)^{acd}	Difference (%)^e	BE (%)^{bcd}	Size (g)^{cd}
	Soybean meal	Wheat bran				
SMS	0	0	8.3gh	-33.6	44.4ghi	38.2bcd
PIIC	0	0	12.5cde	0.0	66.3def	20.0e
SMS	0	5	7.9gh	-36.8	41.9hi	38.5bcd
SMS	0	10	8.4fgh	-32.8	44.7ghi	35.5bcd
SMS	5	0	11.9def	-4.8	63.2efg	33.2cd
SMS	10	0	13.4cde	+7.2	71.1bcdef	35.9bcd
SMS	15	0	14.0bcde	+12.0	74.2bcdef	35.5bcd
SMS	5	5	11.2efg	-10.4	59.3fgh	29.3de
SMS	10	5	14.7bcd	+17.6	78.2bcde	36.5bcd
SMS	15	5	16.0abc	+28.0	85.1abc	28.0de
SMS	5	10	15.9abc	+27.2	84.4abcd	31.9cd
SMS	10	10	15.3abcd	+22.4	81.1abcde	32.7cd
SMS	15	10	16.3ab	+30.4	86.7ab	33.0cd
PIIC	0	5	15.5abc	+24.0	82.6abcd	28.8de
PIIC	0	10	15.6abc	+24.8	82.8abcd	28.0de
PIIC	5	0	8.5fgh	-32.0	45.0ghi	41.8bc
PIIC	10	0	---	---	---	---
PIIC	15	0	14.5bcde	+16.0	77.0bcdef	36.4bcd
PIIC	5	5	18.4a	+47.2	97.4a	54.1a

PIIC	10	5	18.7a	+49.6	99.5a	36.2bcd
PIIC	15	5	13.1bcde	+4.8	69.2bcdef	30.4de
PIIC	5	10	5.8h	-53.6	30.9i	46.6ab
PIIC	10	10	---	---	---	---
PIIC	15	10	12.6cde	+0.8	67.0cdef	27.7de

[a] Yield = g fresh mushrooms harvested when pilei are open and lamellae were visible (per 2.5 kg moist substrate wt).

[b] %BE = (g fresh mushrooms/g dry substrate) x 100.

[c] Values are the means of three replicates.

[d] Means followed by the same letter in the same column are not significantly different according to Fisher's LSD.

[e] Control used to calculate % difference was non-supplemented Phase II compost. Difference (%) = $[(a-x)/x]100$ where a = yield from non-control treatment, x = yield from control.

Table 3.4

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 substrate (SMS), non-composted substrate
(NCS) and Phase II compost (Crop III)

Substrate	Micromax® (mg/kg)	Yield (kg/m^2)[ab]	BE (%)[a]	Size (g)[ab]	Solids (%)[ac]
NCS	0	8.7c	46.1c	29.6de	8.5b
SMS	0	8.3c	44.1c	31.1bcde	7.5de
1:1NCS/SMS	0	14.0b	74.4b	30.5cde	7.6cde
Phase II compost	0	13.9b	73.7b	35.3abcd	5.9f
NCS	6,000	9.3c	49.4c	38.3a	8.5b
SMS	6,000	8.4c	44.7c	32.9abcde	7.8bcd
1:1NCS/SMS	6,000	13.6b	72.3b	27.1e	8.3bc
Phase II compost	6,000	14.8ab	78.7ab	36.7abc	6.1f
NCS	7,350	14.0b	74.3b	37.2ab	9.8a
SMS	7,350	9.0c	47.6c	31.6bcde	7.0e
1:1NCS/SMS	7,350	14.8ab	78.4ab	27.0e	7.6cde
Phase II compost	7,350	15.6a	82.5a	35.0abcd	6.8e

[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] Values are means of ten replicates.

Table 3.5

Influence of Micromax® micronutrients on yield (kg/m^2), percentage biological

efficiency (% BE), size and basidioma (*Agaricus bisporus)* solids content of mushrooms

grown on non-composted substrate (NCS) (Crop IV)

Micromax® (mg/kg)	Yield (kg/m^2)[ab]	Difference (%)[c]	BE (%)[a]	Size (g)[ab]	Solids (%)[ad]
0	8.5e	0	45.0e	54.1a	8.3bc
3,000	9.6de	+12.9	51.1de	57.3a	8.8b
6,000	10.9cd	+28.2	58.0cd	53.9a	8.4b
7,350	12.9b	+51.8	68.4b	50.3a	9.6a
9,000	14.6a	+71.8	77.7a	44.2a	7.8c
12,000	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 (%) =

[$(a$-$x)/x$]100 where a = yield from non-control treatment, x = yield from control.

[d] Values are means of ten replicates.

Table 3.6

Mean yield (kg/m^2), percentage biological efficiency (% BE) and mushroom size

(g/mushroom) of blocks of mushrooms grown on sterilized spent mushroom substrate

(SMS) and pasteurized Phase II compost supplemented with various levels of wheat bran

and soybean meal at spawning (Crop II), mushrooms grown on SMS, non-composted

substrate (NCS) and pasteurized Phase II compost supplemented with various

Micromax® concentrations at spawning (Crop III), and mushrooms grown on NCS

supplemented with various Micromax® concentrations at spawning (Crop IV)

Crop	Blocks	Yield (kg/m^2)^{acd}	BE (%)^{bcd}	Size (g)^{cd}
II	1	12.8a	68.1a	42.9a
	2	13.6a	72.0a	28.6b
	3	13.2a	70.4a	25.3b
III	1	12.7a	67.3a	29.9b
	2	11.5b	61.3b	33.2a
	3	12.1ab	64.4ab	35.0a
IV	1	14.5a	77.2a	41.0b
	2	8.1c	42.6c	57.9a
	3	11.6b	61.5b	55.1a

[a] Yield = g fresh mushrooms harvested when pilei are open and lamellae were visible (per
2.5 kg moist substrate wt).

[b] %BE = (g fresh mushrooms/g dry substrate) x 100.

[c] Values are the means of three replicates for Crop II and six replicates for Crop III.

[d] Means followed by the same letter in the same column within the same crop are not
significantly different according to Fisher's LSD.

Chapter 4: Yield and mushroom solids of *Agaricus bisporus* as influenced by moisture content of non-composted substrate (NCS) and a mixture of spent mushroom compost and NCS

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4.1 Abstract

Yield, biological efficiency (BE), basidioma size, and mushroom solids were determined from mushrooms harvested from non-composted substrate (NCS) and a 1:1 mixture of NCS and spent mushroom substrate (SMS) at three moisture contents (55%, 60%, and 65%). Substrate type and moisture content significantly influenced yield, BE, and mushroom solids. Moisture content also significantly influenced basidioma size. Mushroom yield (14.5 kg/m^2) was highest on a 1:1 mixture of NCS and SMS at 55% moisture, whereas BE (60.5%) was highest from Phase II compost (control). The largest mushrooms (23.8 g/mushroom) and highest mushroom solids (9.9%) were obtained from NCS at a moisture content of 60%. Optimum substrate moisture contents for yield and BE varied depending on substrate type.

4.2 Introduction

The ongoing concern for odor reduction has focused the need to develop a substrate for mushroom production that does not emit offensive odors. In addition, SMS, a medium that remains from the mushroom production process, is sometimes considered environmentally unfriendly, undesirable and represents a solid waste disposal problem for mushroom growers (Duns et al., 2004). Production of *A. bisporus* using NCS and/or SMS has been demonstrated previously (Till, 1962; Fleg and Randle, 1968; San Antonio, 1971; Murphy, 1972; Mee, 1978; Schisler, 1990; Sánchez and Royse, 2001; Sánchez et al., 2002; Bechara et al., 2005; 2006a, b). However, the influence of substrate moisture contents on mushroom yield, BE, size and mushroom solids content has not been examined.

Agaricus bisporus (Lange) Imbach basidiomata contain more than 90% water (Carey and O'Connor, 1991). The basidiomata receive 54-83% of their water from the substrate and 17-46% from the casing depending on the depth and water potential of the casing (Kalberer, 1990). Too low or too high moisture availability in the substrate at spawning may negatively affect the yield of the crop (Gerrits, 1971; Kalberer, 1991; Carey and O'Connor, 1991). Low compost moisture (<61.6%) content is associated with with few visible mycelial strands whereas compost with higher moisture content is associated with dense and thick mycelial growth (Shroeder and Schisler, 1981). Gerrits (1971) suggested that optimum moisture content of compost was 63-68% at spawning. Phase II compost moisture content below 61.6% may limit mycelial growth and limit mushroom yield (Shroeder and Schisler, 1981). Shroeder and Schisler (1981)

observed that a compost moisture content of 65% gave optimum yield while compost moisture did not influence mushroom size.

For the production of brown *A. bisporus* on non-composted substrate (NCS), a moisture content of 48-55% was used by Sánchez and Royse (2001). These levels may seem low when compared to the moisture contents of 63% to 68% typically found in Phase II compost. However, they are in the range of mushroom spawn that may be cased and directly used to produce mushrooms (Bechara et al., 2005, 2006a, b; San Antonio, 1971). Optimal moisture content for mushroom production and quality may depend on the type of substrate being used. For example, Phase II compost is substantially different from NCS, so the optimum moisture content for maximum yields may not be comparable. It is not known if substrate moisture contents higher than 55% for NCS may lead to higher yields and mushroom size. Therefore, the main goal of our experiments was to determine the effect of substrate moisture content for two formulations on *A. bisporus* yield, BE, mushroom size, solids content, and mycelial growth.

4.3 Methods

4.3.1 Substrate

Ingredients used for NCS, adopted from Sánchez and Royse (2001), 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%).

Spent mushroom substrate, obtained from the Mushroom Test Demonstration Facility (MTDF) at the Pennsylvania State University, was post-crop pasteurized with steam at $60^{o}C$ for 24-48 h to kill pests or pathogens that might interfere with subsequent cropping trials. Pasteurized SMS, including the casing layer, was mixed, removed from the production facility, bagged in plastic bags (94 cm x 75 cm), and stored at $2^{o}C$ until used.

Phase II compost was obtained from the MTDF with a moisture content of approximately 68%. The compost was used as received and no attempt was made to adjust its moisture content.

4.3.2 Spawn

A brown strain (Sylvan SB-65, Sylvan Spawn Laboratories, Kittanning, PA) of *A. bisporus* (Portobello) was selected since it is commercially produced and is becoming increasingly popular with consumers in the United States. The spawn used was commercial casing inoculum (CI) that consisted of a mixture of peat, wheat bran and vermiculite. Casing inoculum was used as spawn because it contains many fine particles that may serve as additional points of inoculum.

4.3.3 Mushroom cropping trials

A moisture content of each substrate component was obtained by using a drying oven. Four samples, (100 g each) from substrate components were collected after

thorough mixing. Samples were placed in the oven for 24 h at $60^{\circ}C$. Moisture contents were as follows: red oak sawdust (31%), millet (10%), rye (10%), peat moss (41%), alfalfa meal (10%), soybean meal (10%), wheat bran (10%), $CaCO_3$ (0%), SMS (67%), and Phase II compost (68%).

The substrate moisture contents were adjusted as shown in Table 4.1. Ingredients were hand mixed, moistened to 55%, 60%, and 65%. The substrate mixtures were placed into plastic bags with very high porosity filters (Unicorn Bags, Garland, TX), autoclaved at ($121^{\circ}C$ for 3 h), cooled, and aseptically spawned with 30 g spawn per 2.5 kg moist substrate (1.2%, w.w.). After mixing the spawn with the substrate, the bagged substrates were transferred to the Mushroom Research Center for spawn run at $18\pm1^{\circ}C$ for 18-21 days. The bags were opened and the fully colonized substrate was fragmented and placed in 6.1 L plastic tubs (29.5 cm x 15.75 cm x 8.75 cm, with a surface area of 0.0465 m^2). Neutralized peat (2.5 cm) was overlaid on the substrate surface as casing. Case hold lasted for 18-21 days at $18\pm1^{\circ}C$; during this period, water was applied daily or as needed until the casing layer was saturated. Relative humidity in the production room was maintained at 90-95%.

The experimental design was a factorial with a variance (Kuehl 2000). The cropping experiment was the 2 x 3 factorial with the addition of fresh Phase II compost as a control and was replicated 12 times except the control which was replicated eight times. The experiment contained two substrates (NCS and 1:1 NCS/SMS) and three substrate moisture contents (55%, 60%, and 65%) in a completely randomized design.

4.3.4 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 BE were determined and average mushroom size calculated as (fresh mushroom weight)/(number of mushrooms harvested). Biological efficiency was determined as the ratio of (g fresh mushrooms harvested)/(g dry substrate weight) and expressed as a percentage. Yield was expressed as kg/m^2.

Mushrooms for solids content determination were randomly sampled from each treatment from the mushroom cropping trials. Mushrooms were sliced into fourths or eighths depending on the initial size of each mushroom. Each sample (100 g) was 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 percentage weight of oven-dry mushrooms.

4.3.5 Mycelial growth

The experiment was designed to examine the effect of substrate moisture content on vegetative mycelial growth. Phase II compost was included in the experiment as a control. The NCS and NCS/SMS were tested at moisture contents of 55%, 60%, and 65%, whereas Phase II compost had a moisture content of 68% (oven dry wt). The NCS and NCS/SMS were placed in plastic bags containing very high porosity filter patches and autoclaved at 121°C for 90 min. The substrates were cooled overnight and 5 g CI (brown strain) (Sylvan SB-65) was placed in the bottom of sterile 130 mm long x 35 mm

diameter glass tubes. Sterile substrates (100-120 g) were added until the tubes were full. Inoculated substrates (12 tubes per treatment) were incubated at 25°C for 18 days. Mycelial growth was measured at 3-day intervals.

4.3.6 Statistical analysis

Data from the mushroom cropping experiment were subjected to two analyses of variance (ANOVA). The first analysis considered the 2 x 3 factorial design alone, and was performed using the general linear model (GLM) (SAS, 2001). The second analysis utilized one-way ANOVA with each treatment combination and the control as one of the levels, for a total of seven treatments. Treatment means were separated using Fisher's least significant difference (LSD) test at $p<0.05$. The treatment combinations were further subjected to pairwise comparisons with Phase II compost (control) according to Dunnett's test (Zar, 1999; Kuehl, 2000) since Dunnett specifically addresses making pairwise comparisons between a control group (Phase II compost in our case) and each of the other conditions, such as substrate-moisture content treatments (Kuehl, 2000).

A linear regression of mycelial growth (mm) versus time (days) for each treatment (MINITAB, 2004) was carried out to obtain mycelial growth rate. Slope means were separated according to Fisher's LSD test at $p<0.05$.

4.4 Results

4.4.1 Mushroom yield and BE

Significant sources of variation in the ANOVA included yield and BE for substrates and substrate moisture contents (Table 4.2). For the NCS/SMS mixture, mushroom yields tended to decrease as moisture content increased (Table 4.3). For NCS, the opposite trend was observed, i.e. as substrate moisture increased, yield increased. For BE, however, no differences were observed for the NCS/SMS mixture. However, BE was higher for the NCS containing 65% moisture. Biological efficiency was higher on Phase II compost (60.5%), but was not significantly different from the other treatments except for NCS at 60% moisture (42.0%). No mushrooms were obtained from NCS at 55% moisture.

4.4.2 Mushroom size

A significant source of variation was found for mushroom size due to substrate moisture content (Table 4.2). Mushroom sizes ranged from 14.6 g/mushroom (NCS/SMS @ 55%) to 23.8 g/mushroom (NCS @ 60%) (Table 4.3). Mushrooms harvested from NCS/SMS tended to increase in size as moisture content increased. However, differences within the NCS/SMS were not significant. Conversely, mushrooms produced on NCS tended to decrease in size as moisture content increased, reflecting an increase in total

number of mushrooms harvested (data not shown). The largest mushrooms (23.8 g/mushroom) were obtained from NCS.

4.4.3 Mushroom solids content

Significant sources of variation for mushroom solids content were observed for the three substrates and for different levels of substrate moisture contents (Table 4.2). Mushrooms with the highest solids content (9.9%) were obtained from NCS with a substrate moisture content of 60% (Table 4.3). The lowest solids content (6.6%) was obtained from the NCS/SMS mixture with a substrate moisture content of 65%.

4.4.4 Mycelial growth rates

Mycelial growth rate on Phase II compost was more than twice that of any of the other substrates (Table 4.4). In general, mycelial growth on the NCS/SMS substrate was faster than on NCS alone. The NCS/SMS mixture containing a moisture content of 60% resulted in the most rapid mycelial growth rate (2.35 mm/day) (Table 4.4) (Fig 4.1), while sparse mycelial growth was observed on NCS at 55% moisture. The slowest growth rate (1.61 mm/day) was observed on NCS with a substrate moisture content of 65%.

4.5 Discussion

The reasons for the differential yield and size response to different substrates and moisture contents remain unexplained. However, these responses may be related to the physical nature of the substrate, to gas exchange or to water availability within the substrate. Ohga (1990) demonstrated that the vegetative growth rate of *Lentinula edodes* (shiitake) mycelium was different at the surface compared to the interior of substrate prepared with various woodchip particle sizes. As particle size decreased, the radial mycelial extension rate increased whereas mycelial biomass decreased. Ohga (1990) suggested that oxygen (O_2) depletion was the cause of reduced mycelial biomass development in substrates containing smaller particle size. Donoghue and Dennison (1995, 1996) demonstrated that O_2 and CO_2 levels in the airspace above the inoculated substrate were correlated with subsequent mushroom yields. Royse and Sánchez-Vazquez (2001) found that shiitake yields from substrates prepared with wood chips smaller than 0.85 mm were lower by about 15% compared to large chips (>0.85 mm). We did not measure the size of the woodchips used in our experiments, so it is not possible to compare our profile with others. However, it is known that particle size distribution for commercial sawdust may vary as much as 300% within a sieve size (Royse and Sànche-Vazquez, 2001). In their work with composted sawdust as a medium for *A. bisporus*, Block and Rao (1960) used oak sawdust with 91% of the particle sizes >0.85 mm. They suggested that smaller particle sizes may lead to insufficient aeration during composting and production. Additional work would be required to elucidate the effect of particle size distribution on yield and size of *A. bisporus* produced on NCS.

No mushrooms were obtained from NCS at 55% moisture due to poor mycelial growth. Bechara et al., (2005) observed a similar outcome, i.e., large variation in mushroom yield due to inadequate moisture (58% in cased) grain spawn. The desired moisture level of Phase II compost is 65-68% (Shroeder and Schisler, 1981). Bechara et al., (2005) were able to increase yield dramatically by placing a layer of water-saturated Perlite® below the cased grain spawn.

Our work is the first report on how moisture content of NCS and a mixture of NCS/SMS influenced mushroom size. As moisture content increased from 55% to 65% in NCS/SMS substrate, mushroom size increased from 14.6 g/mushrooms to 19.0 g/mushroom, but this increase was not significant. Also, mushroom size increased 5.5 g/mushroom (although not significant) when the moisture content of NCS decreased from 65% to 60%. The reason for an increase in mushroom size as substrate moisture content decreased may be due to the number of mushrooms forming on the casing. A higher number of mushrooms were produced when the NCS moisture content decreased from 65% to 60% (data not shown). It is known that the number of mushrooms maturing on the casing is indirectly related to the overall size of the mushrooms (Sinden and Schisler, 1962).

We observed a negative correlation between substrate moisture and mushroom solids content, i.e., greater solids content were obtained from drier substrates of both types. This supported the report by Gormely (1969) who observed an increase in dry mushroom weight as compost moisture content decreased from 80% to 66%. However, our findings were different from those of Shroeder and Schisler (1981) who found that

mushrooms from drier compost had lower percent dry weights than mushrooms from higher compost moisture treatments.

Mycelial growth of *A. bisporus* on both NCS and mixtures of NCS/SMS contained in test tubes appeared to have a common growth pattern. Mycelial growth increased as moisture content increased from 55% to 60%. However, there was a decrease in mycelial growth as substrate moisture content was increased from 60% to 65% in both substrates. Gerrits (1971) observed less mycelial growth in compost with moisture contents greater than 72%. He observed thin mycelial growth from very dry (<60%) Phase II compost at spawning. Both NCS and NCS/SMS at a moisture content of 65% had the slowest mycelial growth rate. On the driest (55%) NCS, mycelial growth was sparse. This was similar to the observations of Shroeder and Schisler (1981) who observed that on dry (61.6%) substrate, mycelial growth was visibly less whereas growth on the higher moisture compost was dense, numerous and thick.

We have shown that the optimum moisture contents for yield and BE for NCS/SMS and NCS were 55% and 65%, respectively. However, the optimum moisture contents for mushroom size for NCS/SMS and NCS were 65% and 60%, respectively. Gerrits (1971) suggested that the optimum moisture content of compost at spawning is in the range of 63 to 68%. In the future, higher levels of moisture content such as 70% and 74% should be evaluated for NCS and NCS/SMS. In addition, the effect of casing moisture on yield and size of mushrooms grown on NCS and NCS/SMS should be examined. The work of Schroeder and Schisler (1981) demonstrated the importance of the interaction of substrate moisture and casing moisture on yield and mushroom size. It

is not known if a similar pattern would be observed for the NCS and NCS/SMS treatments.

Acknowledgements

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Table 4.1

Calculations of the amount of water added to substrate mixtures of 1:1 NCS/SMS to attain 55% moisture. Calculations were based on 12 bags of substrate mixture @ 2,500 g/bag = 30,000 g + 10% (extra material to account for loss) = 33,000 g. Therefore, 33,000 x 0.45 dry wt = 14,850 g (d.w.). 33,000 g (w.w.) – 14,850 g (d.w.) = 18,150 g (total H_2O needed); 18,150 g – 16,869 g (already present) = 1,281 g of water added to the substrate to attain 55% moisture. The same procedure was used to calculate the moisture for substrates 1:1 NCS/SMS and NCS at 55%, 60%, and 65% moisture

Substrate component (%)	Moisture content (%)	Dry weight (g)	Wet weight (g)	Amount of water (g)
Red oak sawdust	31	2,079	3,013	934
Millet	10	2,153	2,393	240
Rye	10	594	660	66
Peat moss	41	594	1,007	413
Alfalfa meal	10	297	330	33
Soybean meal	10	297	330	33
Wheat bran	10	668	743	75
$CaCO_3$	0	743	743	0
SMS	67	7,425	22,500	15,075
Total	-	14,850	31,719	16,869

Table 4.2

Probabilities greater than F from analysis of variance for two factors and their

interactions tested for yield, biological efficiency (BE) and size of *Agaricus bisporus* for

two crops grown at the Mushroom Research Center

		Probability > F^b			
Source[a]	*df*	Yield	BE[c]	Size	Solids
Substrate (SB)	1	<0.0001	<0.0001	0.1013	<0.0001
Moisture content (MC)	2	0.0012	<0.0001	0.0306	<0.0001
SB x MC	2	<0.0001	<0.0001	0.1127	0.4884

[a] Error df 66, coefficient of variation, 33.95, 34.58, 29.71 and 7.52 for yield, BE, size and

solid contents respectively.

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

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

Table 4.3

Influence of substrate moisture content on yield (kg/m^2), percentage biological efficiency (% BE), size (g/mushroom) and mushroom solids content (%) of *Agaricus bisporus* grown on spent mushroom substrate (SMS), non-composted substrate (NCS) and mixtures of NCS/SMS

Substrate	Dry wt (kg)	Moisture content (%)	Yield (kg/m^2)[abc]	BE (%)[cd]	Size (g)[bc]	Solids (%)[ce]
NCS/SMS	1.125	55	14.5a	59.1a	14.6b	7.6c
	1.000	60	12.7ab	58.3a	18.8ab	7.6c
	0.875	65	10.7bc	56.4a	19.0ab	6.6d
NCS	1.125	55	---[f]	---	---	---
	1.000	60	9.1c	42.0b	23.8a	9.9a
	0.875	65	10.3c	54.4a	18.3ab	8.7b
Phase II compost	0.800	68	10.5bc	60.5a	16.0b	8.2b

[a] Yield = fresh mushrooms harvested when pilei were open (lamellae visible) per 2.5 kg substrate (w.w.).

[b] Values are means of 12 replicates except for Phase II compost (control) which had eight replicates.

[c] 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 10 replicates.

[d] % BE = ratio of fresh mushrooms harvested/dry substrate wt expressed as a percentage.

[e] Values are means of 10 replicates.

[f] Treatment did not produce mushrooms.

Table 4.4

Influence of substrate moisture content on linear mycelial growth rate of a brown (Sylvan

SB-65) strain of *Agaricus bisporus* grown on spent mushroom substrate (SMS) and non-

composted substrate (NCS)

Substrate	Moisture content (%)	Mycelial growth (mm/day)[ab]
1:1 NCS/SMS	55	2.06cd
	60	2.35b
	65	2.09c
NCS	55	1.85d
	60	1.93cd
	65	1.61e
Phase II compost	68	5.89a

[a] Means within a column followed by the same letter are not significantly different

 ($p<0.05$) according to Fisher's LSD.
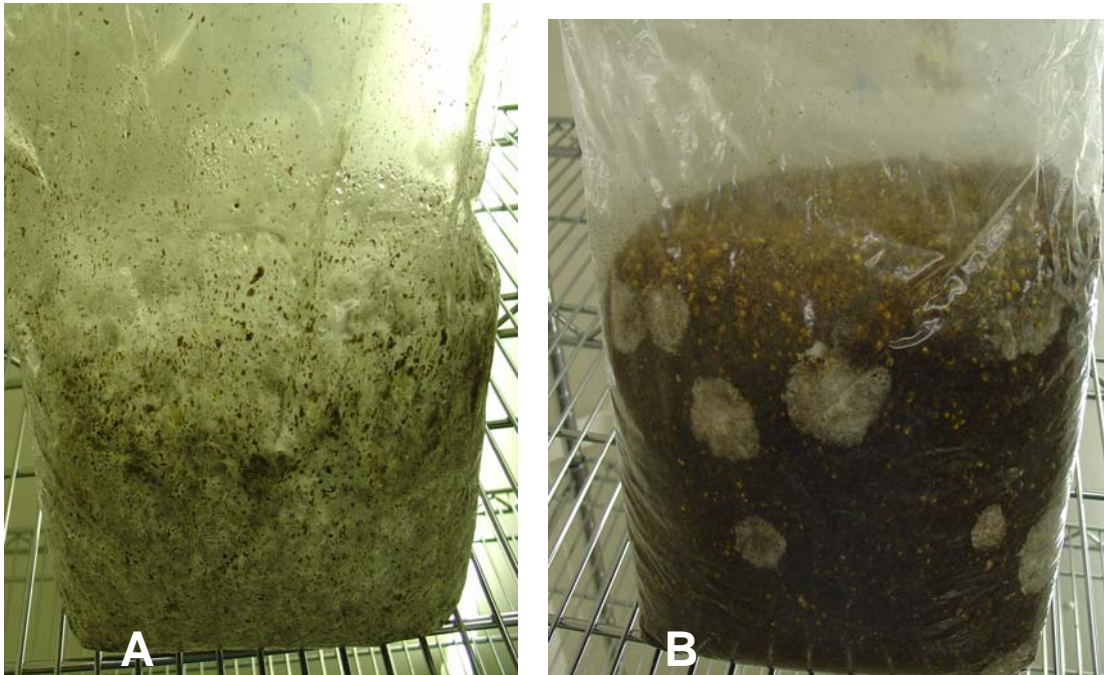
[b] Values are means of 12 replicates

Fig 4.1. Effect of substrate moisture content on substrate colonization of non-composted substrate after 18 da incubation (A) 60% moisture with fully colonized substrate (B) 55% moisture showing restricted mycelial growth.

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