SPENT MUSHROOM SUBSTRATE: A NOVEL MULTIFUNCTIONAL CONSTITUENT OF A POTTING MEDIUM FOR PLANTS

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Fresh spent mushroom substrate (SMS), when properly sized by sieving, leached of salts, and blended with vermiculite, represents an ideal growth medium for plants, offering exceptional aeration porosity, water-holding capacity and nutrition. Moreover, SMS has a plant disease suppressive quality, rendering it a unique multifunctional constituent of a potting mix. Considering its abundance and remarkable physical, chemical and biological attributes, SMS could conceivably replace peat in some soilless mixes used throughout the vast bedding plant industry. This prospect is potentiated by the extensive network of greenhouses lying in close proximity to many of the major mushroom growing centers.

INTRODUCTION

The commercial production of the button mushroom, *Agaricus bisporus*, generates as a co-product a virtually inexhaustible supply of spent mushroom substrate (SMS). The volume of SMS produced annually by the mushroom industry in the U.S. is estimated to be in excess of 1.2 billion cubic feet (American Mushroom Institute). In recent years, the industry has faced increasing challenges from regulatory agencies demanding an environmentally friendly treatment for SMS. Nowhere is the SMS disposal issue of a greater concern than in Pennsylvania, where intensive mushroom production focused in the southeastern corner of the state accounts for approximately 50% of the U.S. crop. The enormous volume of SMS emanating from this production area far and away exceeds the existing demand. This constant overage has forced into practice the widespread and year-round field storage of SMS. In other areas of the U.S., the supply of SMS is offset by the local demand. Even so, this demand tends to be seasonal, and so considerable stockpiling occurs during certain times of the year.

An obvious solution to the disposal problem is to increase the demand for SMS through the exploration and development of new applications for usage. The basic property of SMS restricting its implementation in the agricultural arena is a high concentration of soluble salts. Leachates and suspensions of fresh SMS contain up to 40-fold the concentration of inorganic salts that is known to be injurious to most plant species (Plaster, 1992; Szmidt and Chong 1995; Chorover *et al.* 2000).

Apart from the high salinity, fresh SMS is a rich source of nitrogen, carbon and other elements, which makes it well suited for supporting luxurious plant growth. Depending on the analytical method, the nitrogen content of fresh SMS was estimated to be in the range of 0.4% to 13.7% with a C:N ratio between 9-15:1 (Szmidt and Chong 1995; Chorover *et al.* 2000). SMS also contains an abundance of the inorganic cations (K^+, Na^+, Ca^{2+}, and Mg^{2+}) and anions (Cl^-, So_4^{2-}, and No_3^-), all of which are essential for optimal plant growth and development. Those who have had experience with SMS as an organic fertilizer or soil conditioner will attest to its beneficial effect on plants. And, these anecdotal observations have been validated time and again by scientific experimentation.

SMS has been applied to the propagation of fruits (Robbins *et al.* 1986), vegetables (Male 1981; Lohr *et al.* 1984a, 1984b; Wang *et al.* 1984; Lohr and Coffey 1987; Maher 1991, 1994), flower and foliage crops (White 1975, 1976a, 1976b; Henny 1979), and turfgrass (Maher 1994). For the most part, these applications have not been reduced to practice for reasons including the use of formulations relying on aged SMS and the unsatisfactory performance of formulations having elevated levels of fresh SMS. Bark-and peat-amended mixes enriched with fresh SMS have been well characterized and shown to support excellent growth of woody ornamentals (Chong *et al.* 1991a, 1991b, 1994; Chong and Rinker 1994; Chong and Hamersma 1997). Fresh SMS has also been employed as a shallow top dress for field crops (Wang 1977; Wuest 1991), but only on a limited scale owing to its low bulk density and high moisture content making long distance transport cost prohibitive. In recent years, there has been some interest in recomposting fresh SMS. The recomposted end product is prescribed for direct use on established ornamental plantings or for rationing with other materials to create a propagative medium for seedlings. Despite these real or potential applications, large inventories of field-stored SMS still remain.

Plant propagators have long recognized the seemingly curative power of composts over plant health. In fact, scientific scrutiny has revealed that composts, sewage sludge, and organic fertilizers have disease suppressive characteristics, protecting plants from pathogens (Hoitink and Fahy 1986; Hoitink *et al.* 1997, 1998). On the other hand, most of the sphagnum peat used in plant growth media is of a decomposition level that does not offer disease control (Hoitink *et al.* 1998). Of these various organic substrates, the use of composts for the management of plant disease has been investigated most thoroughly. The application of composts as top dressings and soil amendments promotes a population of antagonistic microorganisms that interfere with the activity of pathogenic fungi. Aged composts, when recolonized by mesophilic bacteria, heterotrophic fungi, or actinomycetes, have been found to mitigate plant diseases as well (Hoitink *et al.* 1993; Grebus *et al.* 1994; Craft and Nelson 1996). There is new evidence that composts also stimulate a natural disease defense system in plants (Hoitink *et al.* 1998; Zhang *et al.* 1998). Though a wealth of scientific evidence documenting the control of diseases with composts is available, nothing is known about the therapeutic value of SMS for plants.

PURPOSE OF THE STUDY

The primary goal of our research was to broaden the scope of use for fresh SMS within the horticultural industry in an effort to stimulate its demand, and thereby reduce field inventories. Specifically, we sought to devise a process by which fresh SMS could be substituted for peat in a propagative medium for the vast vegetable and flower bedding plant industry. With this sole objective, our research proceeded along two interrelated lines. First, we set out to characterize fresh SMS with respect to its possible suppressive effect on plant disease. Second, we strived to formulate a fresh SMS-based medium that allowed the propagation of plants from seed. Herein, we describe a simple growth medium composed of size-fractionated, fresh SMS and vermiculite. Our data suggest that, unlike the components ordinarily found in artificial soil mixes, size-fractionated SMS represents a novel multifunctional constituent possessing the physical, chemical, and biological characteristics promoting exceptional plant growth and health.

MATERIALS & METHODS

General Preparation of SMS

Batches of fresh SMS were obtained from commercial mushroom operations located in Pennsylvania and from the Mushroom Test Demonstration Facility, The Pennsylvania State University (PSU). In all cases, the SMS was sampled within 48 hours of removal from the production facility, and screened through a coarse sieve (2-cm x 3.5-cm mesh). For the disease control studies, the SMS was leached with water to an electrical conductivity (EC) of 1-2 mmhos/cm, and then sieved through a 2.4-cm square plastic mesh. For the plant growth trials, the SMS was sieved through a 2-mm square mesh screen to generate two size classes as follows: coarse (>2 mm) and fine (<2 mm) SMS. Sieved and unsieved SMS was mixed with other components as described below, and leached over a two-day period to an EC<2 mmhos/cm directly in the growing containers.

Isolation of SMS Bacteria

Samples of SMS were mechanically stirred with several volumes of sterile water. The SMS suspension was clarified by centrifugation, serially diluted with sterile water, and plated on nutrient media. The media used were nutrient agar, potato dextrose agar (PDA), and King's B agar. Bacteria observed growing on the plates were arbitrarily selected and transferred to new PDA plates.

Screening of SMS Bacteria for Disease Control Properties

The disease suppressive activity of SMS bacteria was evaluated in a dual plate assay against a panel of fungal pathogens. The fungi were obtained from PSU and included the mushroom pathogens, *Verticillium fungicola, Trichoderma harzianum* and *Mycogone perniciosa*. For one bacterium, an antagonistic activity was screened using a pathogen of ryegrass, *Pyricularia grisea* (W. Uddin, PSU). The bacteria and fungi were arranged in an opposing fashion on PDA plates. The plates were maintained at room temperature and observed for the appearance of a zone of inhibition surrounding the bacterial colony where the mycelium failed to grow.

Identification of SMS Bacteria

Antagonistic bacteria isolated from SMS were identified by fatty acid profile analysis (Microbial ID, Inc., Newark, DE).

Disease Control Studies with SMS-based Media

The incidence of *Pythium* damping-off disease on tomato grown from seed was evaluated in mixes composed of SMS blended on a volume basis with either MetroMix 200 (MM200, Scotts-Sierra Horticultural Products Co, Marysville, OH) or perlite (The Schundler Co., Metuchen, NJ) in proportions of 0, 25, 50, 75, and 100%. The various mixes were packed into ~10-cm pots and each pot was sown with 30 seeds of 'Ace 55' (Olds Seed Co., Madison, WI) tomato.

Pythium ultimum isolate 42 (G. Moorman, PSU) was grown at room temperature on 15-cm PDA plates. When the fungus had completely colonized the agar medium, the agar disc was removed from the plate and homogenized in a Waring blender with 100-ml sterile water. Immediately after the seed was sown, 10 ml of the mycelial-agar homogenate was applied to the surface of the mix in each pot. Non-inoculated treatments received 10 ml of a homogenate prepared from blank agar discs.

Four or five replicate pots for each treatment were arranged in an incomplete randomized design in controlled-environment growth chambers maintained at 18°C with constant low-level fluorescent illumination. The plants were watered by sub-irrigation without fertilization. After 3-4 weeks, each pot was scored for seedling survival. Statistical analysis of data was done by the student's t test at $p = 0.05$ (SAS Institute Inc. 1999).

Plant Growth Studies with SMS-based Media

Coarse, fine and unsieved SMS were mixed on a volume basis with either perlite or vermiculite (The Schundler Co.) in proportions of 0, 50, and 100%. The various mixes were packed into ~10-cm pots,

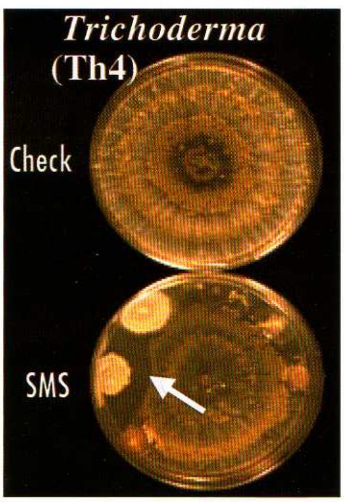

Figure 1: Dual plate assay for an antagonistic effect of several bacteria isolated from SMS on the mycelial growth of the green mold pathogen, *Trichoderma harzianum* biotype Th4 (Th4). Shown is the growth of Th4 in the absence (Check) and presence (SMS) of several bacteria situated at the periphery of the plate. The arrow indicates the zone of inhibition surrounding one bacterial isolate where the Th4 mycelium failed to grow.

Table 1: Effect of the proportion of SMS to perlite in the potting medium on the survival of tomato inoculated with the fungal pathogen, *Pythium ultimum*, causing damping-off disease

	Seedling Survival (%)			
	Exp. 1		Exp. 2	
Potting Medium	-Pythium	+Pythium	-Pythium	+Pythium
100% Perlite	75 a	29 c	76 a	21 c
Perlite + 25% SMS	70 a	59 b	75 a	63 ab
Perlite + 50% SMS	72 a	78 a	74 ab	60 ab
Perlite + 75% SMS	81 a	78 a	75 a	55 b
100% SMS	68 ab	75 a	69 ab	59 ab

Means within an experiment followed by the same letter are not significantly different based on the student's t test at $p = 0.05$

Figure 2: Dual plate assay for an antagonistic effect of a single bacterial isolate from SMS on several fungal pathogens of the mushroom. Plates were inoculated at their peripheries with either four biotypes of *Trichoderma harzianum (Trichoderma)*, four isolates of *Verticillium fungicola (Verticillium)* or three isolates of *Mycogone perniciosa (Mycogone)*, and at their centers with the bacterium (SMS) or a blank agar plug (Check). In each case, notice how the presence of the bacterium impeded the advance of the fungal mycelium towards the center of the plate.

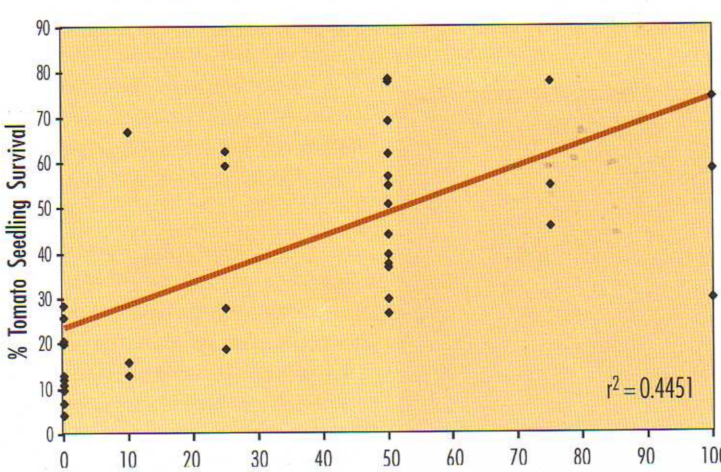

Figure 3: Scatter plot depicting the relationship between the proportion of SMS in the potting medium and the survival of tomato seedlings in the presence of the fungal pathogen, *Pythium ultimum*.

and each pot was sown with five 'Ace 55' tomato seeds. The pots were maintained in a growth chamber maintained at 22°C with a 12-hr photoperiod. After the initial 2-day leaching, plants were placed in an ebb and flood sub-irrigation system with a reservoir containing water soluble fertilizer (20-10-20 or 21-7-7; N-P-K) at 100 ppm N. After 4 to 6 weeks, plants were excised at the soil level, and the fresh and dry weights of the aerial portions were determined.

Determination of Physical Properties

The various media used in the plant growth trials were analyzed for bulk density, aeration porosity and water-holding capacity (Contrisciano and Holcomb 1995).

RESULTS & CONCLUSIONS

Characterization of SMS Bacteria

Five bacterial species isolated from fresh SMS inhibited the mycelial growth of one or more of the fungal pathogens tested in the dual plate assay (Figs. 1 & 2). The bacteria were identified by fatty acid analysis as *Bacillus licheniformis, B. subtilis, Paenibacillus macerans, Pseudomonas aeruginosa*, and *Streptomyces albidoflavus*. Four of the bacteria were tested and found to be antagonistic to the fungal pathogens of *A. bisporus*, namely *V. fungicola, T. harzianum*, and *M. perniciosa*. The fifth bacterial species, *P. aeruginosa*, retarded the growth of *Pyricularia grisea* in the laboratory test and controlled gray leaf spot disease on perennial rye turfgrass in the field (Viji *et al.* 2002). Similarly, Phae *et al.* (1990) found that bacteria such as *Pseudomonas* and *Bacillus* isolated from composts were effective in suppressing root and soil-borne pathogens.

Disease Control Studies with SMS-based Media

The disease suppressiveness of SMS was evaluated for *P. ultimum*, the fungal pathogen causing damping-off disease. In experiment 1 (Table I), the addition of 25% SMS to perlite significantly increased tomato seedling survival from 29% to 59%, although this survival rate was lower than that observed in the healthy control treatments (~75%). However, increasing the level of SMS in the growing medium to 50-100% afforded a seedling survival rate (75-78%) that was equal to that observed in the control treatments. In the absence of P. ultimum, SMS over the range of 25% to 100% in the medium did not have an adverse effect on tomato seedling survival.

Experiment 1 was repeated with similar results. In experiment 2 (Table I), amending perlite with from 25% to 100% SMS provided effective control of damping-off disease. Seedling survival in the SMS-based media was approximately three-fold higher (55-63%) than observed in the 100% perlite medium (21%). This compares favorably to a 69-76% survival rate for the healthy control treatments. Once again, there was no negative impact of SMS on healthy seedling survival.

Figure 3 depicts the relationship between tomato seedling survival involving *Pythium* damping-off disease and the level of SMS in the growing medium. Based on the findings of 13 experiments, there was a general trend for seedling survival to increase as the proportion of SMS increased up to 100%. SMS at a level of 50% or higher provided highly effective disease control (Figs. 3 and 4). The coefficient of correlation (r2) for this relationship was 0.4451, but a high variation was expected considering the undoubtedly complex nature of the biological control mechanism. Also, the majority of the experiments were conducted using SMS blended with the commercial peat-based MM200. Peat offers no biological control of disease (Hoitink *et al.* 1998), and we suspect that it provided a source of experimental variation by interfering with the disease suppressiveness of SMS (Romaine and Holcomb 2001a, 2001b).

In the absence of *Pythium*, SMS at levels up to 100% in the growing medium had no toxic side effect on the germination and survival of tomato seedlings (r2=0.0013) as long as an adequate moisture level was maintained (Fig. 5).

We have shown that the use of SMS as a component in a potting medium provides highly effective control of *Pythium* damping-off disease on tomato. Microorganisms isolated from natural organic substrates such as compost have been employed for the control of a variety of plant diseases (Hoitink and Fahy 1986; Phae *et al.* 1990; Nelson and Craft 1992; Grebus et al. 1994; Hoitink *et al.* 1998). Our data suggest that fresh SMS is comparable to other composts in that it is replete with microorganisms that are generally antagonistic to pathogenic fungi. In all likelihood, it is the totality of this antagonistic microflora that contributes to the selectivity of mushroom compost for *A. bisporus* to the exclusion of other fungi. Our data indicate that this suppressive effect extends to pathogenic fungi on plants, which casts a new light on SMS as a potentially valuable, organic disease control additive for potting mixes.

Plant Growth Studies with SMS-based Media

Initial plant growth studies were carried out with tomatoes in mixes of unsieved or sieved (fine or coarse) SMS with perlite. However, tomato seeds did not germinated well in perlite mixtures with unsieved or coarse SMS, although they germinated at a 100% rate in mixtures with fine SMS. Physical analyses revealed that the coarse SMS had an extremely low water-holding capacity and, consequently, an undesirably high aeration porosity. The fine SMS had a higher water-holding capacity of 62% and, in turn, a reduced aeration porosity of 18%. It should be noted that 10-15% aeration would be considered excellent, and many commercial mixes would have values less than 10%. The values for unsieved SMS were intermediate between the coarse and fine SMS. Because the unsieved and coarse SMS had low water-holding capacities, the media dried rapidly and inhibited seed germination. In summary, the results of these experiments showed that fresh SMS had a surprisingly high aeration porosity and that perlite, which has a low water-holding capacity, mixed with SMS created a medium with an inadequate water-holding capacity.

Vermiculite has a higher water-holding capacity than perlite, so it was selected as the companion amendment for additional plant growth trials. Of the various SMS and perlite formulations tested, we found that the rate of growth of tomatoes was highest in a medium containing fine SMS, intermediate with unsieved SMS, and lowest with coarse SMS (Figs. 6 & 7). Plant growth was comparably poor in 100% unsieved SMS, 100% coarse SMS, and 100% vermiculite, and relatively improved in 100% fine SMS.

The physical property data explained the differences in plant growth observed with the different media (Fig. 8). Compared to a mixture of coarse SMS and vermiculite, 100% coarse SMS has a lower water-holding capacity, which impaired growth. Reducing the particle size of the SMS (i.e., fine) improved plant growth somewhat, but the maximal response was observed with the medium composed of fine SMS and vermiculite. This is because the aeration porosity of this formulation was near ideal and there was an excellent water-holding capacity. Vermiculite alone has good aeration and water-holding

Figure 4: Effect of SMS in the potting medium on the development of damping-off disease on tomato caused by *Pythium ultimum*. Shown is the survival of tomato seedlings grown in (A) 100% perlite and (B) 50% perlite-50% SMS and in the presence (right) or absence (left) of *Pythium ultimum*. It should be noted that many of the seedlings shown in the medium composed of 100% perlite and inoculated with the pathogen subsequently succumbed to a post-emergent form of the disease involving a girdling of the stem.

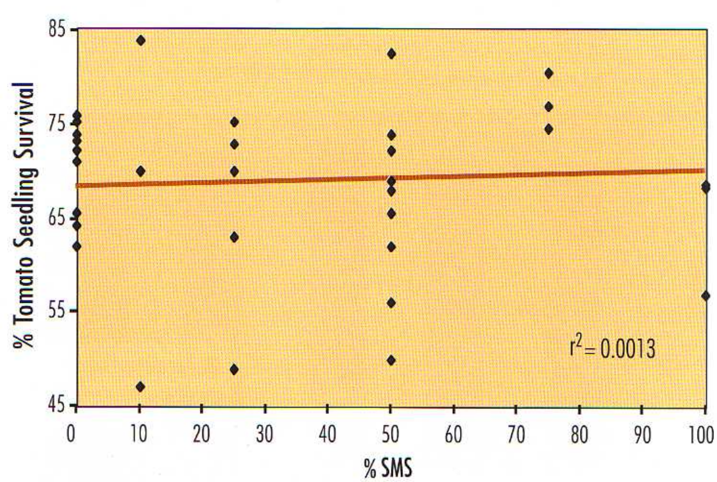

Figure 5: Scatter plot depicting the relationship between the proportion of SMS in the potting medium and the survival of healthy (non-inoculated) tomato seedlings.

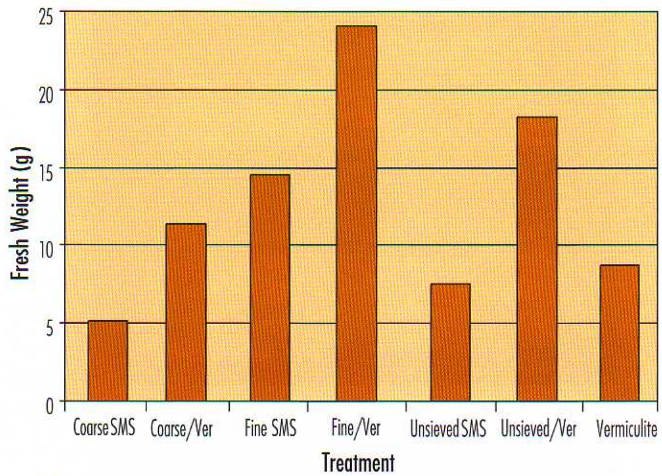

Figure 6: Fresh weight of tomato plants grown in various potting media. The media consisted of coarse 100% SMS (Coarse SMS), 50% coarse SMS-50% vermiculite (Coarse/Verm), 100% fine SMS (Fine SMS), 50% fine SMS-50% vermiculite (Fine Verm), 100% unsieved SMS (Unsieved SMS), 50% unsieved SMS-50% vermiculite (Unsieved/Ver), and 100% vermiculite (Vermiculite).

Figure 7: Effect of the SMS particle size on the growth of tomato plants. The potting media consisted of 50% coarse SMS-50% vermiculite (50% GT2-50% Verm), 100% coarse SMS (SMS GT2), 50% fine SMS-50% vermiculite (50% LT2-50% Verm), 100% fine SMS (SMS LT2), and 100% vermiculite (Vermiculite).

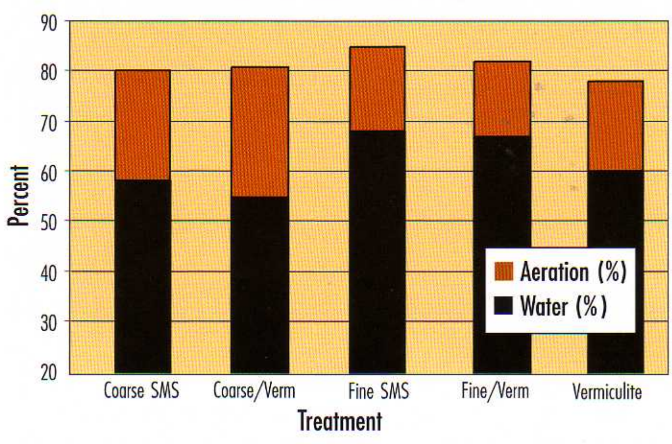

Figure 8: A comparison of the aeration porosity and water-holding capacity for various formulations of SMS and vermiculite. The formulations consisted of 100% coarse SMS (Coarse SMS), 50% coarse SMS-50% vermiculite (Coarse/Verm), 100% fine SMS (Fine SMS), 50% fine SMS-50% vermiculite (Fine/Verm), and 100% vermiculite (Vermiculite).

capacity, yet growth was improved by the addition of SMS, suggesting that the plants were responding to the nutrients that are likely being released from the SMS and possibly to desirable microbial activity.

In summary, a medium composed of a 50-50 mix of fine SMS (<2 mm particle size) and vermiculite on a volume basis demonstrated excellent tomato growth, because it had an optimal combination of aeration porosity and water-holding capacity. It may be that the unfavorable plant growth response observed by other investigators with media containing high levels of fresh SMS was related to its structural features rather than salts per se. This shortcoming can be rectified by physically shearing the SMS to reduce the particle size.

We prefer a mechanical method to size the SMS rather than recomposting, because the latter process would involve the microbial conversion of ammonical nitrogen to nitrate nitrogen (Chorover *et al.* 2000). A higher level of nitrates in the SMS product would pose a greater environmental threat. Instead, physically-sheared SMS as a component of a potting mix would undergo mineralization at a time when the accumulating nitrates could be immediately absorbed by the roots and converted into plant biomass. And, in fact, our results suggested that the nutrients being released from the SMS as it decomposes benefited the plant.

We also conclude from our studies that rapid leaching directly in the pots effectively removes excess salts, such that the germination and growth of tomatoes in a medium containing a high proportion of fresh SMS was exceptional. Chong and coworkers (1994) arrived at a similar conclusion in an evaluation of several mixes of bark, peat, and fresh SMS for woody ornamental species.

For commercial-scale production of the SMS-based potting medium, we envision one scheme whereby individual mushroom growers or consortiums would mechanically size fresh SMS and then blend it in the proper proportion with vermiculite. The mix prepared in this manner would be ready for delivery in bulk to nearby greenhouses and nurseries. This seems to be a plausible proposition, as a large bedding plant and nursery industry is situated in close proximity to many of the major mushroom production centers. An alternative scheme would involve all aspects of processing and handling being carried out by the plant propagator.

The salinity level of the SMS-based mix as we have described would prove injurious to most plant species. Consequently, the plant propagator would be instructed to leach the mix directly in the growing container and prior to establishing the seedlings. The greenhouse or nursery operation would be a reasonable location for the leaching to take place, particularly if the irrigation water containing the leachate was recycled for use in the fertilization program. This reuse of the SMS leachate as a plant fertilizer is the subject of an on-going research project at PSU (Holcomb *et al.* 2001). Thus, the expense and burden of leaching the SMS would be transferred from the mushroom industry to the horticultural industry.

The economic feasibility of producing an SMS-based potting mix cannot be determined at this time, because not all of the variables in the cost-benefit equation are known. For example, which is the most economical method to size fresh SMS? Must a drying step be introduced for sizing, and if so, is this cost justified by the real value of the product to the plant propagator? How does the cost of shipping the SMS-based medium to a local market compare to the long distance transit fee now paid for peat-based mixes? The real value of the product to the propagator would be a function of the actual savings derived from the anticipated lower cost of a SMS-based mix compared to a peat-based mix, the tangible benefits of improved plant growth and health, and the reduced cost for fertilizers and fungicides.

Our future research plans are to 1) evaluate physical methods for sizing SMS, 2) optimize the ratio of SMS to vermiculite for plant growth, 3) explore the versatility of the SMS-based medium using a variety of bedding plant species and 4) examine the robustness of disease control with other plant pathogens.

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