Fresh Recycled Mushroom Compost Suppresses Artillery Fungi Sporulation: A 4-Year Field Study[1]

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Abstract

Artillery fungi (*Sphaerobolus* spp.) have recently emerged from an interesting mycological curiosity that grow in landscape mulch, to a problem of emotional stress and financial concern to homeowners, as well as insurance companies, mulch producers, and landscape contractors. Artillery fungi shoot their spore masses (gleba) towards sunlight and/or reflective objects such as light-colored house siding or automobiles, peppering the surface with small sticky tar-like black specks. Once dry, gleba are very difficult to remove from surfaces such as vinyl siding. If they can be removed, gleba usually leave a brown stain that is extremely difficult to clean. We previously reported that *aged* (weathered or composted outside) mushroom compost (MC), when blended with landscape mulch at ≥ 40% by volume, suppressed artillery fungi sporulation in the mulch. In this paper, we report that *fresh* MC, taken directly from the mushroom house and blended with landscape mulch without aging, also reduces artillery fungi sporulation as follows: 0% MC (control = 0% reduction), 10% MC (62%), 20% MC (80%), 40% MC (95%), and 100% MC (97% reduction in sporulation). Landscape companies using *fresh* MC, rather than *aged* MC, need not invest time and money in additional MC aging, composting, and processing.

Index words: *Sphaerobolus*, landscape mulch.

Significance to the Nursery Industry

Moist bark and wood of landscape mulch is an excellent growth substrate for wood-decay fungi such as artillery fungi. Artillery fungi growing in landscape mulch shoot sticky spore masses (gleba) towards reflective surfaces such as light-colored house siding or sides of automobiles. Gleba stick tenaciously, and upon removal, leave a permanent stain on surfaces such as vinyl siding. Disfigurement of siding may result in a homeowner filing a claim to his or her insurance company to replace the siding on the affected house. However, if the homeowner's policy specifically excludes artillery fungi, the insurance company may deny the claim and the perceived liability may shift to the mulch producer, mulch sales yard, or contractor applying the mulch.

We previously reported that *aged* (weathered) MC, when blended into landscape mulch at ≥ 40% by volume, suppressed sporulation of artillery fungi and offered an environmentally friendly, 'green' solution to control nuisance fungi in landscape mulch without use of chemical pesticides (14). In this paper we report that *fresh* MC, taken directly from the mushroom house without aging or composting, blended with landscape mulch at ≥ 40% by volume also inhibits artillery fungi. Use of fresh MC eliminates the need to store, compost, or weather the MC, thus saving time, money, and resources for landscapers. Homeowners searching for an environmentally friendly, sustainable solution to control nuisance fungi in their mulch beds may find that blending MC into landscape mulch offers such a solution.

Introduction

Landscape mulches are used to reduce weeds, maintain soil moisture, and enhance aesthetics in horticultural plantings (1). Expanding residential and commercial developments with extensive landscaping require increased volumes of landscape mulch (30, 35). In Pennsylvania alone, more than *ca*. 2M m^3 (3M yd^3) of landscape mulch are sold annually to homeowners in the southeastern part of the state (personal communication, Pennsylvania Landscape and Nursery Association, Harrisburg).

In the past, landscape mulches were comprised mainly of bark, usually obtained as a waste product from sawmills (12). Bark, as opposed to wood, contains fungitoxic and hydrophobic compounds that inhibit fungi (33, 37). However, modern mulches, such as those used by landscapers and available at lawn and garden centers, are often formulated from wood, rather than bark, and may be derived from pallet recycling facilities and scrap wood from sawmills (7, 17), as well as from land-clearing operations where stumps, soil, and rocks are ground and mixed in large tub grinders (20). Most landscape mulch is double- or triple-ground to a fine texture, to enhance appearance and moisture-holding capacity. Use of wood-based mulch, mulch from land-clearing operations, and finely shredded mulch all favor nuisance fungi such as artillery fungi (D.D. Davis, personal observations).

Simultaneously with increased use of landscape mulch, more than *ca*. 2.7M m^3 (3.5M yd^3) of used MC are generated annually as a byproduct of the mushroom industry in southeastern Pennsylvania (personal communication, Giorgi Mushrooms, Temple, PA). Mushroom compost is the steam-pasteurized substrate on which mushrooms are grown. In order to produce high quality, uniform crops of mushrooms, the chemical and physical composition of mushroom compost is governed by a precise formula that has minimal temporal and spatial variability (38). After the final mushroom crop is harvested, the spent MC is steamed a second time, removed from the production house, and is available for recycling. The two steamings eliminate weed seeds and many harmful fungi, leaving a valuable organic byproduct that is a registered fertilizer (*ca*. 1:1:1, N:P:K) with the Pennsylvania Department of Agriculture (13). The physical and chemical composition of used MC has been thoroughly documented based on MC samples from 30 mushroom production facilities in southeastern Pennsylvania (13,16).

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Concurrent with increased use of landscape mulch and generation of used MC, nuisance fungi and organisms such as artillery fungi, bird's nest fungi, slime molds, stinkhorns, and others pose problems within landscaped areas across Pennsylvania (3, 15). The most serious of these nuisance fungi are artillery fungi that live on the wood and bark within moist foundation mulches (11). Artillery fungi are most active during cool moist weather in spring and fall, becoming inactive during hot dry weather of mid-summer. However, during moist summers, artillery fungi may remain active in Pennsylvania from April to November, especially on the cooler north sides of houses (D.D. Davis, personal observations).

Many homeowners wage a continuous, stressful battle against artillery fungi, and ultimately may forsake attractive wood or bark mulches for stone or artificial mulches that do not support artillery fungi (10, 11). Or, upon learning there are no chemical fungicides labeled to control artillery fungi (14), homeowners may seek environmentally friendly green solutions, such as using recycled MC to suppress nuisance fungi in their landscape mulch. Although the mechanisms of suppression are unknown, it is likely that composts contain populations of beneficial microbes that are antagonistic to nuisance fungi and fungal plant pathogens (4, 9, 21, 23–25, 28, 29, 31, 36).

The abundance of used MC and its antagonistic nature towards unwanted mulch fungi make it an ideal, environmentally friendly, sustainable recycling product that can be blended with landscape mulch to suppress nuisance fungi. The objective of this field study was to determine if *fresh* MC, when blended with a common landscape mulch, suppressed artillery fungi sporulation, and if so, what percentages of fresh MC are needed to induce significant levels of suppression.

Materials and Methods

Plot construction for study 1. In Study 1, 20 field plots in two back-to-back linear rows of 10 plots (each row having a common back wall) were constructed in 2006 within eastern Pennsylvania on the grounds of the Center for Horticulture and a Sustainable Environment at The Pennsylvania State University, Berks Campus, Reading, PA (40° 21' 51" N, 75° 58' 36" W). Average monthly rainfall during April 1 to October 31 for each study year was: 2007 (3.9 in, 9.9 cm), 2008 (3.2 in, 8.0 cm), 2009 (4.7 in, 11.9 cm), and 2010 (2.7 in, 6.9 cm). Average monthly temperature during the same period for each year was: 2007 (20.2C, 68.3F), 2008 (20.1C, 68.1F), 2009 (18.3C, 65.0F), and 2010 (20.6C, 69.0F). Local weather information was obtained from the Reading Regional Airport (Reading, PA), located 0.82 miles (1.32 km) from the study site. Wind direction within the region during April 1 to October 31 was generally from the SW (2).

Each plot was 2 ft wide × 3 ft long (0.7 × 1.0 m) with a 2 ft high back wall. Plywood sidewalls, each 2 × 2 ft, were attached at right angles to the back wall to separate each plot. Back walls were painted white to serve as 6 ft^2 (0.56 m^2) 'targets,' whereas sidewalls were stained dark brown. On June 16, 2006, fresh MC was donated by Giorgi Mushroom Company, Temple, PA, and transported to the site directly from a mushroom production facility. Simultaneously, a common landscape mulch composed of ground tree roots, stumps, branches, and wood pallets was delivered to the site by a local mulch supplier. Five mulch treatments were prepared by blending 0 (control), 10, 20, 40, and 100% (by vol) fresh MC with the landscape mulch. Treatments applied to a depth of 5 in (*ca.* 12–13 cm) onto landscape fabric cloth placed over bare soil in a randomized complete block design with four replications per treatment. Total target area for the 4 replications/treatment was 2.24 m^2. Because of the physical plot placement at this location, two replications were aligned with the target area facing a northwest (WNW, 298°) direction and two replications were aligned facing southeast (ESE, 118°). The two replications had a common back wall, and therefore were facing 180° apart.

Plot construction for study 2. As construction of the 20 plots was nearing completion, additional space became available. Therefore, the two back-to-back linear rows were extended by adding 18 more plots, placed 9 per row, also in a back-to-back configuration, during 2006. Construction of the additional 18 plots was similar to the original 20 plots, except that that the area of the white back wall target area of each of the 18 new plots was slightly smaller (0.35 m^2, 3.75 ft^2). Total target area of the 18 new plots was therefore 6.30 m^2. The additional 18 plots received only landscape mulch with no mushroom compost (0% MC) and were used to study the impact of compass direction (aspect) that the target was facing on artillery fungi sporulation, without the interacting influence of MC, as well as to increase the number of replications. The new plots contained 9 replications of 0% MC with the target area facing WNW and 9 replications of 0% MC with targets facing ESE.

All 38 plots received natural rainfall, but were hand-watered to thoroughly soak each plot surface to aid establishment of artillery fungi during dry periods of 2006–2007, following plot inoculation. To control weeds, the non-selective herbicide glyphosate was applied to all plots in spring and early fall of each year.

Inoculum. Gleba of artillery fungi, originally removed from the outside glass walls of a campus greenhouse at University Park, were plated onto oatmeal agar and an isolate selected based on abundant sporulation (5). Inoculum used herein consisted partially of new gleba removed from the Petri plate lids of pure subcultures. However, to increase inoculum diversity, the gleba were randomly mixed with gleba collected during a statewide field survey (D.D. Davis, personal communication). This final inoculum mixture likely contained *S. stellatus* (Tode) Pers. and *S. iowensis* Walker, both common artillery fungi species in Pennsylvania (18). On June 16, 2006, 15 gleba were randomly selected and placed on the mulch surface in each plot for both Study 1 and 2. Gleba were covered with a fine layer of plot substrate to reduce desiccation. To facilitate infestation, all 38 plots were inoculated a second time on October 10, 2006, with 10 randomly selected gleba per plot.

Data collection and analyses. In both Study 1 and Study 2, sporulation was estimated by counting numbers of gleba deposited onto the white back wall target of each plot. Gleba were not observed on targets during year of inoculation (2006). Thereafter, numbers of new gleba deposited on each target were evaluated biannually on eight evaluation dates: July 12 and November 13, 2007; May 7 and November 23, 2008; May13 and November 18, 2009; and May 20 and October 21, 2010. In addition, an extra evaluation was conducted

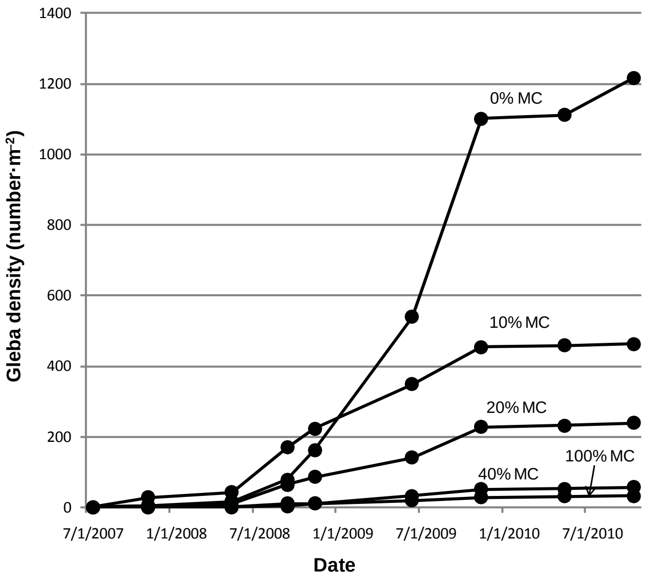

Fig. 1. Mean number of gleba·m^{-2} on 'targets' (white back walls of plots) for each treatment (n = 4 replications per treatment) over time (Study 1). The five treatments were 0 (control), 10, 20, 40, and 100% (by vol) fresh mushroom compost (MC) blended with landscape mulch. The study was initiated on June 16, 2006.

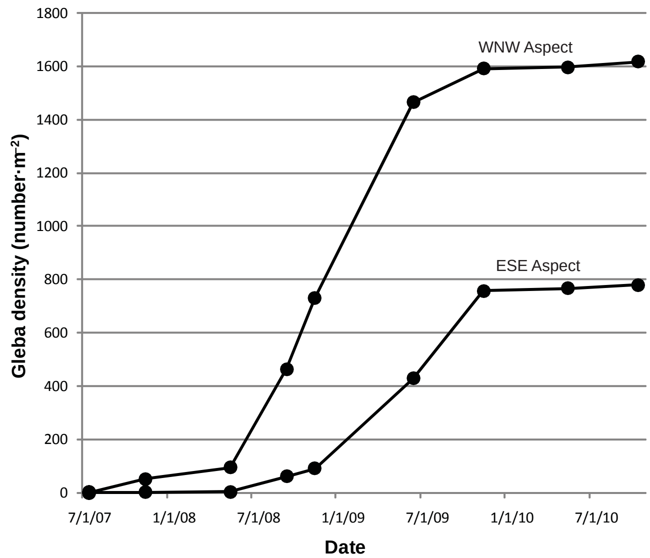

Fig. 2. Mean number of gleba·m^{-2} on 'targets' (white back walls of plots) for facing WNW (298°) or ESE (118°) aspects (Study 2) in the 0% fresh mushroom compost (MC) (control, 100% landscape mulch) treatment. The study was initiated on June 16, 2006.

on September 23, 2008, when it appeared that large numbers of gleba were forming in the mulch during late summer. The September 2008 data were not used in statistical analyses, but were included in graphs to illustrate temporal production of gleba·m^{-2} (Figs. 1 and 2). The accumulated numbers of gleba deposited over the 4 years for each treatment were summarized using area under disease progress curve (AUDPC) using the formula:

$$[(y_i + y_{i+1}) / 2)][t_{i+1} - t_i]$$

where y_i is the number of spores/m^2, t_i is the time of the *i*th rating, and $i = 1, 2, 3 \ldots n - 1$ (8, 26, 32). Analysis of AUDPC data can be envisioned by comparing the area under the 0% MC treatment curve with the area under the other four curves shown in Fig. 1. Data from both Study 1 and Study 2 were subjected to analysis of variance. Treatment means from Study 1 were compared using predetermined single degree-of-freedom orthogonal contrasts at $P \leq 0.05$ using Statistix (version 8.0, Tallahassee, FL) analytical software (27). Treatment means from Study 2 were compared using Fisher's protected least significance difference test at $P \leq 0.05$ (34).

Results and Discussion

Study 1. Sporulation levels within plots facing the two aspects were statistically similar (data not shown) and data were combined for analyses. Mean accumulative number of gleba·m^{-2} for each treatment on the last evaluation date (October 21, 2010) were: 0% MC (1217), 10% MC (463), 20% MC (239), 40% MC (56), and 100% MC (32 gleba) (Table 1). Contrast analysis revealed that the sporulation level in the control (0% MC) was not significantly different from the 10% MC treatment at any evaluation date, likely influenced by the high level of variability in the 10% MC data. The sporulation level in the control was significantly greater than sporulation in the 20, 40, or 100% MC during later evaluation dates (Table 1). The AUDPC index, reflecting sporulation over the entire study period, also revealed that numbers of gleba in the 10% MC treatment were overall not significantly different compared to sporulation levels of the control (0% MC), whereas the 20, 40, and 100% MC treatments all supported significantly fewer gleba (Table 1).

When expressed as % reduction in sporulation due to MC treatment, the final values on October 21, 2010, were as follows: 0% MC (control, 0% reduction in sporulation), 10% MC (62% reduction), 20% MC (80% reduction), 40% MC (95% reduction), and 100% MC (97% reduction). This study with *fresh* MC supports our previous studies with *aged* (weathered) MC, where we recommended blending ≥ 40% aged MC with landscape mulch to suppress nuisance fungi such as artillery fungi (10) and bird's nest fungi (15). It is likely that the fresh MC quickly ages, or weathers, when blended into landscape mulch, and by the end of 1–2 years is similar to an aged product. We now recommend blending ≥ 40% of either *fresh* or *aged* MC with landscape mulch. However, use of *fresh* MC in the landscape blend eliminates the need for landscape companies, or homeowners, to further compost, process, and store the MC or the MC/landscape mulch blend to age or re-compost it.

With regard to time needed for significant reduction in artillery fungus, sporulation in the 20% MC treatment was not significantly suppressed (compared to the 0% MC control) until November 18, 2009. In contrast, sporulation levels in the 40 and 100% MC treatments were significantly suppressed a year earlier (November 5, 2008) than the 20% MC treatment. Thus, although 20% MC blended with landscape mulch ultimately suppressed artillery fungus sporulation, the suppressive nature of 20% MC was delayed 1 year as compared to 40 and 100% MC. Gleba were first observed on the white back walls during the November 13, 2007, evaluation, approximately 17 months after initial inoculation and 13 months after the second inoculation (Fig. 1, Table 1). At this time, the mean number of gleba·m^{-2} for each treatment were: 0% MC (4), 10% MC (28), 20% MC (5), 40% MC (0),

Table 1. **Mean number of accumulated gleba·m^{-2} on targets within landscape mulch plots containing 0% fresh mushroom compost (0% MC, control), as contrasted against mean number of gleba·m^{-2} in 10, 20, 40, or 100% MC treatments. AUDPC index refers to area under disease progress curve as calculated from July 12, 2007, to October 21. 2010.**

	Evaluation date								
	2007		2008		2009		2010		AUDPC index
	Jul 12	Nov 13	May 8	Nov 5	Jun 25	Nov 18	May 20	Oct 21	
Treatment									
0% MC	0	4	17	162	540	1,102	1,112	1,217	602,667
10% MC	0	28	43	223	348	454	458	463	311,716
20% MC	0	5	10	86	140	227	232	239	141,888
40% MC	0	0	2	10	32	52	53	56	30,294
100% MC	0	0	1	10	20	28	31	32	18,241
Contrasts[z]									
0% vs. 10% MC	ns	ns	ns	ns	ns	ns	ns	ns	ns
0% vs. 20% MC	ns	ns	ns	ns	ns	*	*	*	*
0% vs. 40% MC	ns	ns	ns	*	*	*	*	*	*
0% vs. 100% MC	ns	ns	ns	*	*	*	*	*	*

[z]Treatment means (n = 4 replications) were compared from pre-planned orthogonal contrasts where '*' equals statistically significant and 'ns' equals not statistically significant comparisons, respectively, at $P \leq 0.05$.

and 100% MC (0). These initial gleba were deposited at some time between the July 12, 2007, and November 13, 2007, evaluations, but the exact date of deposition is unknown. Nevertheless, this finding indicates that the first sporulation occurred *ca.* 1 to 1.5 years following introduction of artillery fungi into landscape mulch, under the conditions of this study. The length of time between inoculation and artillery fungi sporulation is greatly influenced by weather, especially rain (D.D. Davis, personal observations). During cool wet weather, the length of time may be shortened and sporulation occurs more rapidly. The supplemental hand watering, applied in addition to natural rain events, likely increased the mulch moisture and allowed rapid colonization and sporulation by artillery fungi. Without supplemental watering, the length of time between inoculation and sporulation is likely extended during hot dry weather.

With regard to temporal patterns in sporulation, initial sporulation was observed during the time period from late fall 2007 to spring 2008. By May 8, 2008, sporulation was observed in all treatments. The numbers of spores deposited on the targets increased rapidly from November 5, 2008, through 2009. As described above, this rapidity was likely related to the moisture content of the mulch. By the last year of the study in 2010, sporulation had leveled off and relatively few new spores were deposited on the targets (Fig. 1). Decline in sporulation at the end of the study may have been due to exhaustion of food bases crucial for artillery fungus sporulation, or due to an increase in total microflora antagonistic to artillery fungi (10, 31). In the latter case, populations of competitive antagonistic microflora such as *Bacillus* and *Trichoderma* (4), especially in treatments containing MC, may have increased to levels that interfered with artillery fungi colonization and sporulation.

Study 2. Beginning on May 8, 2008, and throughout the remainder of the study, plots facing the WNW aspect had significantly more sporulation than did plots facing the ESE aspect (Fig. 2, Table 2). The lack of statistical significance for aspect in Study 1, as compared to Study 2, was likely influenced by the large degree of variability in the Study 1 data, as well as the lower number of replications in Study 1 (n = 4 in Study 1, n = 9 in Study 2). By the end of the 4-year study on October 21, 2010, the mean number of accumulated gleba on the 9 plots facing the WNW aspect was 1,617 gleba·m^{-2}, significantly greater than the number of gleba on plots facing the ESE aspect (780 gleba·m^{-2}). Likewise, AUDPC index was significantly greater for the WNW aspect (1,109,188) than for the ESE aspect (414,923). In the field, we observed that plots that had the back wall target facing WNW were generally wetter than plots facing ESE. Rains in the study area generally come from the southwest. It is likely that rain directly impacted plots facing WNW, whereas plots facing the ESE were in a slight 'rain shadow' created by the common back wall of the plots, and therefore received less precipitation.

Table 2. **Mean number of accumulated gleba·m^{-2} with regard to target aspect (compass direction that white back wall was facing) in Study 2. The compass bearing for the ESE aspect was 118° and 298° for the WNW aspect.**

	Evaluation date								
	2007		2008		2009		2010		AUDPC index
	Jul 12	Nov 13	May 8	Nov 5	Jun 25	Nov 18	May 20	Oct 21	
Aspect									
ESE	0a[z]	1a	4b	90b	429b	757b	767b	780b	414,923b
WNW	0a	51a	93a	731a	1,465a	1,590a	1,597a	1,617a	1,109,188a

[z]Treatment means (n = 9 replications) were analyzed with Fisher's protected least significance difference test; means followed-by the same letter were not statistically significant at $P \leq 0.05$.

Artillery fungus sporulation, as estimated by deposition, was very low in 2007 (the year following inoculation) and early 2008, began to increase in late 2008, rose rapidly by the end of 2009, and then leveled off in 2010. This temporal pattern of sporulation, as observed in eastern Pennsylvania (Figs. 1 and 2), was similar to patterns observed in analogous studies from previous years at plots located *ca.* 140 mi (225 km) distant in central Pennsylvania (3, 10, 11). These common patterns indicate the length of time required between inoculation and significant sporulation, as well as the sigmoidal pattern of sporulation over time. These temporal patterns may illustrate a general response pattern of artillery fungus sporulation.

Rapid urbanization with ever-expanding housing and commercial developments require ever-increasing amounts of landscape mulch (19, 30, 35). Utilization of recycled MC can reduce the over-supply of used MC in the mushroom industry that occurs in some areas, and create an environmentally friendly, green solution to the artillery fungus problem. The manner by which MC confers suppression to sporulation of nuisance fungi is unknown, but is likely related to populations of beneficial microbes in MC that are antagonistic to, and/or feed upon nuisance and plant-pathogenic fungi (4, 6, 22, 29, 31). In addition, use of recycled MC also offers numerous other horticultural benefits related to plant and soil health (13, 16).

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