In Vitro Screening of Fungicides to Control Artillery Fungi[1]

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Abstract

We evaluated the influence of 26 fungicides at four different concentrations (0.1, 1.0, 10.0, and 100.0 a.i.) on *in vitro* growth of an artillery fungus (*Sphaerobolus stellatus* isolate SS8) in Petri plate culture. The most effective fungicides, as determined by comparison of mean EC_{50} values, were polyoxin-D and azoxystrobin, followed by pyraclostrobin, triademifon, tebuconazole, propiconazole, thiophante-methyl, triticonazole, thiram, and fludioxonil. These *in vitro* laboratory findings need to be evaluated in the field before specific fungicides can be recommended to control artillery fungi in landscape mulch.

Index words: landscape mulch, *Sphaerobolus.*

Fungicides used in this study: refer to Table 1.

Significance to the Nursery Industry

There has been a dramatic increase in the use of landscape mulch within rapidly urbanizing areas and expanding housing developments (16). In Pennsylvania alone, more than 2,000,000 m^3 (nearly 3,000,000 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, PA). Concomitantly, artillery fungi (*Sphaerobolus* spp.), which are wood decay fungi in the Basidiomycota, are an ever-increasing problem in the United States, paralleling increased use of landscape mulch (3, 6). Moist landscape mulch is an attractive substrate for artillery fungi, especially in cool, wet springtime weather. Artillery fungi are notoriously difficult to control. Mushroom compost, when blended into landscape mulch at ≥ 40% by volume, suppresses sporulation of artillery fungi (4, 5). However, used mushroom compost is not available in many parts of the United States. This *in vitro* laboratory study revealed that several fungicides commonly used in turf and ornamental horticultural industries show promise in controlling artillery fungi. However, these laboratory results must be evaluated in the field before fungicides can be registered for use in landscape beds for control of artillery fungi.

Introduction

Artillery fungi in mulched foundation plantings are a major problem to homeowners across Pennsylvania and much of the United States (5). Such nuisance fungi (2, 7) thrive in damp, cool landscape mulch along foundations and have become a plague in recent years for many homeowners and housing developments. Artillery fungi shoot sticky spore masses towards sunlight or light reflected from light-colored surfaces such as house siding. These sticky spore masses (gleba) adhere tenaciously to siding, as well as onto the sides of cars parked near landscaped/mulched areas (3, 6, 14). Increasingly, property owners have become aware of damage from artillery fungi on their homes and often contact their homeowner's insurance agent, seeking to recover cost of re-siding their house. However, owners often discover that 'artillery fungi' have been specifically listed as exemptions or exclusions (like mold, mildew, and dry rot) in their homeowner's insurance policy. If damage from artillery fungi is excluded, the insurance claim is usually denied. Homeowners may then file a lawsuit against the landscape professional perceived responsible for the presence of artillery fungi in the mulch. The occurrence of undesirable organisms (*i.e.,* artillery fungi, bird's nest fungi, slime molds, and stinkhorns) in landscape mulch may be erroneously blamed on the mulch producer, mulch sales yard, or contractor applying the mulch, when in reality these organisms are naturally occurring.

Control of artillery fungi is needed, especially in areas of the country that do not have access to recycled mushroom compost. In a preliminary laboratory study, we reported that the fungicide active ingredients epoxiconazole, thiophanate-methyl, or triphenyltin acetate inhibited *in vitro* growth of artillery fungi in Petri plate culture plates (8). However, only a limited number of fungicides at two concentrations were evaluated. In addition, many of the fungicides tested are not commercially available for lawn and landscape use, or are not legal for use in the United States. The objective of this paper was to expand this previous preliminary laboratory study by evaluating the efficacy of 26 commonly used fungicides at four concentrations each to reduce *in vitro* colony growth of isolate SS8 of *Sphaerobolus stellatus* (Tode) Pers.

Materials and Methods

Inoculum and fungicides. Gleba (fruiting bodies containing spores) of *S. stellatus* were originally obtained from the outside glass walls of a greenhouse at The Pennsylvania State University, University Park, PA. Gleba were plated onto oatmeal agar (8), one per Petri plate, and one isolate (SS8) selected based on abundant sporulation in culture. Isolate SS8 was further subcultured onto oatmeal agar and fresh gleba removed from Petri plate lids. These new gleba were used as inoculum in this study.

Fungicides selected for this study (Table 1) were commercially available plant protection products widely used in the U.S. and European green industry (*i.e.,* lawn, landscape/

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Table 1. List of fungicides by common name (active ingredient), FRAC Code (fungicide chemical family group), and trade name (formulated product).

Common name	FRAC code[z]	Trade name[y]
Azoxystrobin	11	Heritage 50WG
Boscalid	7	Emerald 70WG
Chloroneb	14	Terraneb SP 30F
Chlorothalonil	M5	Daconil Ultrex 82.5WDG
Copper hydroxide	M1	Junction 46WDG
Fenarimol	3	Rubigan 1AS
Fenhexamid	17	Decree 50WDG
Fludioxonil	12	Medallion 50WP
Flutolanil	7	Prostar 70WP
Iprodione	2	Chipco 26GT 2SC
Kresoxim-methyl	11	Cygnus 50WDG
Mancozeb	M3	Protect 80WP
Mefenoxam	4	Subdue MAXX 1ME
Myclobutanil	3	Eagle 40WP
Polyoxin-D	19	Endorse 2.5WP
Potassium bicarbonate	NC	Kaligreen 82WDG
Prochloraz	3	Prochloraz 40EC
Propamocarb	28	Banol 6L
Propiconazole	3	Banner MAXX 1.24ME
Pyraclostrobin	11	Insignia 20WG
Tebuconazole	3	Lynx 45WP
Thiophanate-methyl	1	Clearys 3336 50WP
Thiram	M3	Spotrete 4L
Triademifon	3	Bayleton 50DF
Triticonazole	3	Chipco Triton 1.67SC
Vinclozalin	2	Curalan 50EG

[z]Information on fungicide chemical family groups is available through the FRAC (Fungicide Resistance Action Committee) website at www.frac.info.

[y]Information on product trade names and manufacturers is found in Turf & Ornamental Reference for Plant Protection Products, available at www.greenbook.net.

turfgrass, and ornamentals) market (9). Agar for fungicide incorporation was prepared by adding 7.5 g (0.26 oz) DIFCO® agar (Becton Dickinson Microbiology Systems, Sparks, MD) and 10.0 g (0.35 oz) baby-food oatmeal to 500 ml (30.5 in^3) distilled water. Media was autoclaved, cooled, and 100 mg (0.004 oz) chloramphenical (Sigma-Aldrich Corp, St. Louis, MO) antibiotic added prior to solidification to retard bacterial growth. Each of the 26 fungicides was incorporated into the agar at 0.1, 1, 10, or 100 ppm (1 ppm = 1 $\mu g \cdot ml^{-1}$) active ingredient of the formulated product. Concentrations selected were based on product labeling and reported research results (10, 11). Fungicides were not added to 15 control plates (0 ppm).

Agar in each Petri plate was inoculated with a 4-mm (0.16 in) diameter plug removed from the growing edge of oatmeal agar colonized by *S. stellatus* isolate SS8. Inoculated plates were sealed with Parafilm® (American National Can, Chicago, IL) to reduce dehydration and incubated on a laboratory benchtop for 42 days at room temperature and natural daylight from adjacent windows.

Data collection and analyses. The experiment was conducted twice, using a randomized complete block design, with experiments as blocks. The first experiment was conducted during June–July and the second during August–September 2007. In the first experiment, treatments were 26 fungicides × 4 concentrations × 4 replications (Petri plates) + 15 controls; total n = 431. Treatments were identical in the second experiment, but replications were increased to 5; total n = 535. After 42 days incubation, colony growth (mm) was measured along two diameters at right angles and averaged.

EC_{50} values (effective concentration to cause 50% fungal growth inhibition) were determined for each fungicide treatment by calculating percent inhibition (1 – mean colony diameter on fungicide-amended media ÷ mean colony diameter on unamended media) and subjecting data to probit analysis (11, 12). Probit transformation was used to linearize the dose-response curve, providing an accurate estimation of EC_{50} values (17). EC_{50} values for fungicide treatments were analyzed using a one-way analysis of variance followed by a Fisher's Protected Least Significant Difference Test to compare treatment means at $P = 0.05$ (18). In terms of general fungicide efficiency, an EC_{50} value ≤ 0.5 ppm indicated best growth inhibition, 0.5–1.0 ppm indicated very good inhibition, 1.0–2.0 ppm indicated marginal inhibition, and an EC_{50} value ≥ 2.0 ppm indicated poor or weak inhibition.

Results and Discussion

There were no significant differences between blocks or among replications, so these data were pooled. The artillery fungus (*S. stellatus* SS8) was most sensitive, as indicated by an EC_{50} value ≤ 0.5 ppm) to polyoxin-D and azoxystrobin (Table 2). Polyoxin-D is a Group 19 (Table 1) fungicide, with the group name of polyoxin. The mode of action of polyoxin fungicides is to inhibit or disrupt glucan synthesis. Azoxystrobin is a Group 11 (Table 1) fungicide that is classified as a QoI, or quinine outside inhibitor. QoI fungicides target specific sites of cellular respiration. Fungicide products containing azoxystrobin or polyoxin-D are commonly used on golf courses to manage turfgrass diseases (1).

The artillery fungus exhibited very good sensitivity, as indicated by an EC_{50} value of 0.5–1.0 ppm, to pyraclostrobin, triademifon, tebuconazole, propiconazole, thiophante-methyl, triticonazole, thiram, and fludioxonil (Table 2). EC_{50} value for pyraclostrobin, also a QoI fungicide similar to azoxystrobin, ranged from 0.5–1.0 ppm, indicating very good sensitivity although not statistically different than azoxystrobin. Kresoxim-methyl, the other QoI tested, did not perform as well as azoxystrobin or pyraclostrobin. Within the QoI grouping, azoxystrobin is also further sub-classified as a methoxy-acrylate, and pyraclostrobin is a methoxy-carbamate, whereas kresoxim-methyl is an oximino-acetate. As was observed with demethylation inhibitor (DMI) fungicides, there was a range of fungicidal activity among the QoI fungicides against *S. stellatus*.

Triademifon, tebuconazole, propriconazole, and triticonazole are all Group 3 fungicides (Table 1), classified as sterol biosynthesis inhibitors, or more commonly as DMIs. In this study, artillery fungus growth exhibited high sensitivity towards these four DMI fungicides. Wide ranges of fungicidal or fungistatic activity exist among the many DMI fungicides (13). In our study, other DMI fungicides (fenarimol, myclobutanil, and prochloraz) tested did not perform as well as the four listed above. Geml et al. (8) reported that epoxiconazole (also a Group 3 fungicide) provided the best control of artillery fungi *in vitro*. However, it is difficult to compare their results with ours, since they used only two concentrations (5 and 20 ppm) of epoxiconazole.

Thiophanate-methyl is a Group 1 (Table 1) fungicide classified as methyl benzimidazole carbamate. This fungicide

Table 2. Sensitivity of *Sphaerobolus stellatus* (isolate SS8) *in vitro* culture growth to 26 fungicides as determined by mean EC_{50} (ppm) value.

Common name	FRAC code[z]	Trade name[y]	EC_{50} (ppm)[x]
polyoxin-D	19	Endorse 2.5WP	0.201g
azoxystrobin	11	Heritage 50WG	0.432fg
pyraclostrobin	11	Insignia 20WG	0.540efg
triademifon	3	Bayleton 50DF	0.597efg
tebuconazole	3	Lynx 45WP	0.828efg
propiconazole	3	Banner MAXX 1.24ME	0.848efg
thiophanate-methyl	1	Clearys 3336 50WP	0.946efg
triticonazole	3	Chipco Triton 1.67SC	0.957efg
thiram	M3	Spotrete 4L	0.987efg
fludioxonil	12	Medallion 50WP	0.989efg
chlorothalonil	M5	Daconil Ultrex 82.5WDG	1.258defg
fenarimol	3	Rubigan 1AS	1.368defg
boscalid	7	Emerald 70WG	1.611defg
kresoxim-methyl	11	Cygnus 50WDG	1.773defg
iprodione	2	Chipco 26GT 2SC	1.846defg
mancozeb	M3	Protect 80WP	1.859def
flutolanil	7	Prostar 70WP	1.951def
myclobutanil	3	Eagle 40WP	2.134de
copper hydroxide	M1	Junction 46WDG	2.136de
chloroneb	14	Terraneb SP 30F	2.769cd
prochloraz	3	Prochloraz 40EC	2.887cd
potassium bicarbonate	NC	Kaligreen 82WDG	3.829cd
mefenoxam	4	Subdue MAXX 1ME	5.652b
fenhexamid	17	Decree 50WDG	6.418b
vinclozalin	2	Curalan 50EG	12.019a
propamocarb	28	Banol 6L	12.729a

[z]Information on fungicide chemical family groups is available through FRAC (Fungicide Resistance Action Committee) website at www.frac.info.

[y]Information on product trade names and manufacturers is found in Turf & Ornamental Reference for Plant Protection Products at www.greenbook.net.

[x]The means of 9 replications combined over two experimental runs of EC_{50} values (i.e., effective concentration of the active ingredient to cause 50% fungal growth inhibition) followed by the same letter are not significantly different according to Fisher's Protected Least Significance Difference Test at P = 0.05. An EC_{50} value ≤ 0.5 ppm = best sensitivity of the artillery fungus to the fungicide treatments *in vitro*, 0.5 to 1.0 ppm = very good sensitivity, 1.0 to 2.0 = good to marginal sensitivity, and ≥ 2 = poor sensitivity or essentially ineffective.

targets cell mitosis and division. Geml et al. (8) reported that thiophanate-methyl suppressed the growth of artillery fungi, but only at the higher of two concentrations used. A similar pattern of fungal sensitivity to thiophanate-methyl was observed in our study, as indicated by a higher EC_{50} value (0.5–1.0 ppm) needed to suppress growth. Thiophanate-methyl is common fungicide in green industry markets used for control of both foliar and root diseases of turfgrass and ornamentals (15).

Thiram is a Group M3 fungicide (Table 1) and is classified as a dithiocarbamate. The artillery fungus exhibited more sensitivity to thiram than mancozeb, the other dithiocarbamate tested. Mancozeb is a common active ingredient found in many lawn and garden-type fungicide products due to its broad-spectrum activity against many common plant pathogenic fungi, whereas products containing thiram are restricted to professional pesticide applications within green industry markets (15).

Fludioxonil is a Group 12 fungicide (Table 1) and was the only phenyl-pyrrole used in our study. This fungicide targets osmotic signal transduction in susceptible fungi. Fludioxonil is used to control diseases of golf course turfgrass and greenhouse crops (1).

All other fungicides tested did not provide effective or noticeable suppression of the artillery fungus *in vitro* (Table 2). Of interest, flutolanil is used extensively for control of Basidiomycota-caused diseases in turfgrass, but provided marginal (EC_{50} value 1.0–2.0 ppm) inhibition of the artillery fungus. As reported by Geml et al. (8), the artillery fungus exhibited little or no sensitivity to chloroneb. Also, mefenoxam or propamocarb were essentially ineffective. Those two fungicides are specifically used for control of diseases in turfgrass caused by *Pythium* spp. (19). Since *Pythium* is not a fungus, it is not surprising that the artillery fungus had very little sensitivity towards mefenoxam or propamocarb.

Based on EC_{50} results of this *in vitro* study, the artillery fungus (isolate SS8 of *S. stellatus*) was most sensitive to polyoxin-D or azoxystrobin, followed-by pyraclostrobin, triademifon, tebuconazole, propiconazole, thiophante-methyl, triticonazole, thiram, and fludioxonil. Many of these fungicides are commercially available for use in turfgrass and ornamental markets, and have proven effective in controlling a variety of plant pathogenic fungi that cause foliar and root diseases in green industry markets. However, none are labeled for control of artillery fungi in mulch beds at this time. Although these fungicides may offer a possible solution to suppress artillery fungi, these fungicides need to be evaluated in field tests before control of artillery fungi in landscape mulch can be added to the product label.

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