Influence of Selected Fungicides on *in vitro* Growth of Artillery Fungi (*Sphaerobolus* spp.)[1]

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Abstract

We tested the inhibitory effect of 14 fungicides, 13 of which were known to be effective against the basidiomycete *Agaricus*, at 5 and 20 ppm on the *in vitro* growth of three species of artillery fungi: *Sphaerobolus iowensis*, *S. stellatus*, and a previously undescribed *Sphaerobolus* sp. Captafol, epoxiconazole, thiophanate-methyl, triflumizole, and triphenyltin acetate were the most effective inhibitors against all three *Sphaerobolus* species, and the reduction in growth was directly related to fungicide concentration. Chloroneb, chlorothalonil/zinc oxide, fuberidazole, glyodin, and tolylfluanid showed varying results, depending on fungal species and fungicide concentration; however, they were much less effective than the previous five fungicides. Dazomet, dinocap, folpet, and ferbam failed to slow the growth of any artillery fungi at either concentration. This preliminary study revealed that certain fungicides suppress growth of artillery fungi and should be further tested in the field.

Index words: artillery fungus, fungicidal control, *Sphaerobolus*.

Fungicides used in this study: captafol, (Difolatan, Folcid), *N*-(1,1,2,2-tetrachloroethylthio)cyclohex-4-ene-1,2-dicarboximide; chloroneb, (Demosan, Tersan SP), 1,4-dichloro-2,5-dimethoxybenzene; chlorothalonil/zinc oxide, (Bravo Zn), 2,4,5,6-tetrachloroisophthalonitrile; dazomet, (Preservit, Mylone), 3,5-dimethyl-1,3,5-thiadiazinane-2-thione; dinocap, (Dinocap, Karathane, Mildex, Isocothane), 2,4-dinitro-6-octylphenyl crotonates; epoxiconazole/naptha, (Opus), (2*RS*,3*SR*)-1-[3-(2-chlorophenyl)-2,3-epoxy-2-(4-fluorophenyl)propyl]-1*H*-1,2,4-triazole; ferbam, (Ferberk, Fermate, Carbamate), iron(3+) dimethyldithiocarbamate; folpet, (Phaltan, Thiophal), *N*-(trichloromethylthio)phthalimide; fuberidazole, (Fuberidazole, Voronit), 2-(2'-furyl)benzimidazole; glyodin, (Glyoxide), 2-heptadecyl-2-imidazoline acetate; thiophanate-methyl, (Topsin M, Cercobin-M, Fungitox), dimethyl 4,4'-(*o*-phenylene)bis(3-thioallophanate); tolylfluanid, (Euparen M), *N*-dichlorofluoromethylthio-*N*',*N*'-dimethyl-*N*-*p*-tolylsulfamide; triflumizole, (Terraguard 50W, Procure, Trifmine), [[4-chloro-2(trifluoromethyl)phenyl]imino]-2-Propoxy-ethyl-1H-imidazole; triphenyltin acetate, (Triacetane, Brestan, Suzu), triphenyltin acetate.

Significance to the Nursery Industry

Artillery fungi (*Sphaerobolus* spp.) grow in landscape mulch within various regions of the United States. They produce and expel spore masses that adhere to various surfaces, including house siding, automobiles, and plant tissues. Fourteen fungicides were evaluated *in vitro* at 5 and 20 ppm to assess their potential to control growth of three species of artillery fungi. Captafol, epoxiconazole, thiophanate-methyl, triflumizole, and triphenyltin acetate were the most effective in controlling growth of *Sphaerobolus* spp. Future studies are needed to test whether mulch applications of these five fungicides will suppress the growth or sporulation of artillery fungi on wood mulch under landscape conditions.

Introduction

Artillery fungi are white-rotting, wood-decay basidiomycetes that are often found on decomposing landscape mulch. The genus *Sphaerobolus* contains three species: *S. iowensis* Walker, *S. stellatus* (Tode) Pers., and a previously undescribed *Sphaerobolus* sp. (8, 9, 10).

Temperatures between 68–78F (20–26C) are ideal for the fungus to grow vegetatively, but cooler temperatures between 50–68F (10–20C) are considered ideal for sporulation (2). For this latter reason, artillery fungi are categorized as cool-season fungi. When the environmental conditions are suitable for sporulation in the spring or fall, spherical, whitish or orange-colored basidiocarps approximately 2 mm (0.1 in) in diameter, that contain a single spore mass (gleba), are formed on wood substrates. Fungal fruiting bodies are phototropic, and at maturity orient themselves towards the brightest direct or reflected light (2, 12). Prior to glebal expulsion, hyphae begin to decompose and coat the gleba with amorphous cellular debris that is responsible for its adhesive nature (7, 15). The sticky spore mass is expelled when the inner layer of the fruiting body suddenly inverts, propelling the gleba up to approximately 2 m (6 ft) vertically and 6 m (20 ft) horizontally (5).

Because artillery fungi are commonly found on landscape mulch, and the adhesive nature of the gleba, property owners often express dismay when finding an abundance of brown-black spherical masses (expelled gleba) on house siding, windows, and automobiles (1, 3, 4, 11). When dry, gleba are extremely difficult to remove and permanently stain the surface to which they adhere (1, 3, 4).

Species of fungi (i.e., *Trichoderma*) and bacteria (i.e., *Bacillus*) have shown to be potentially effective biological control agents against artillery fungi (3). An alternative control method is prevention, i.e., the use of certain types of landscape mulch that discourage growth and sporulation of artillery fungi (4, 6). Although these control strategies have shown promising results, there has recently been an increasing interest in use of fungicides to quickly control artillery fungi. The objective of this study was to determine the effect of selected fungicides on the *in vitro* mycelial growth of the three known species of *Sphaerobolus*.

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Table 1. Original sources of *Sphaerobolus* isolates investigated in this study. All isolates except the ATCC (American Type Culture Collection, Manassas, VA) isolate are maintained at The Pennsylvania State University, Department of Plant Pathology, University Park, PA.

Species	Isolate code	Geographic origin
Sphaerobolus sp.	T-800	Kellogg Biological Station Long Term Ecological Research site, Michigan
	SS19	Atlanta, Georgia
Sphaerobolus iowensis Walker	ATCC 52850	East Lansing, Michigan
	SS5	State College, Pennsylvania
Sphaerobolus stellatus (Tode) Pers.	SS8	State College, Pennsylvania
	SS13	Erie, Pennsylvania

Materials and Methods

Isolates and experimental design. Two isolates of each species, as determined by our molecular phylogenetic and morphological analyses (8, 9), were selected for this study (Table 1). Thirteen fungicides, known to suppress the basidiomycete *Agaricus* were selected, as well as thiophanate-methyl, a broad-spectrum fungicide used for disease control in a variety of crops. All fungicides except for chlorothalonil/zinc oxide (ISK Biotech), dinocap (May & Baker), epoxiconazole/naptha (BASF), fuberidazole (Bayer), and triflumizole (Uniroyal) were technical grade, active ingredients, obtained from ChemService, West Chester, PA. Fungicides were incorporated into DIFCO® potato-dextrose agar (PDA) (Becton Dickinson Microbiology Systems, Sparks, MD) in Petri plates at 0 ppm (control), 5 ppm, or 20 ppm. The 20-ppm concentration had been shown to suppress growth of the basidiomycete *Agaricus* (14), whereas the 5-ppm concentration was selected to test whether control could be achieved using less fungicide.

Each plate was inoculated with a 4-mm diameter plug of agar, that had been colonized by the artillery fungus, and sealed with Parafilm® (American National Can, Chicago, IL) to prevent dehydration. Inoculated plates were incubated in growth chambers for 21 days at 77F (25C) and 95–100% relative humidity in darkness. Three replicates in a completely randomized design were used within each treatment.

Collection and analysis of data. Colony diameters (Fig. 1) were measured 21 days after inoculation along two diameters at right angles to each other. Diameter values for the two isolates per species were averaged. Data were analyzed using a two-way analysis of variance (ANOVA) using 'species' and 'fungicide treatment' as factors. However, since significant *P*-values were obtained for both main effects and the interaction, data were then analyzed in a one-way ANOVA to compare: 1) colony diameters among 15 fungicide treatments (including the fungicide-free control) and 2) colony diameters for each species of *Sphaerobolus*. Fisher's least significant difference (*LSD*) multiple range test (13) was used to test for significant ($P = 0.05$) differences in growth between the fungicide treatments and the control, as well as significant differences in growth among species within each treatment.

Results and Discussion

Fungicide treatments vs. control. The one-way ANOVA for the fungicide treatment data showed highly significant *P*-values ($P = 0.001$) among diameters of the artillery fungi *vs* the control. Both concentrations of captafol, epoxiconazole, thiophanate-methyl, triflumizole, and triphenyltin acetate significantly suppressed growth of all three species of artillery fungi as compared to the control (Table 2). Chlorothalonil/zinc oxide moderately (yet, significantly) inhibited the growth of all three *Sphaerobolus* species, with no significant difference between growth at the two concentrations. While moderate growth inhibition was observed in all species by 5 ppm of triphenyltin acetate and thiophanate-methyl, fungicide efficacy increased to almost full inhibition at 20 ppm for all three species. Epoxiconazole significantly inhibited growth at both 5 and 20 ppm, although the 5-ppm treatment resulted in less growth inhibition in all three species.

Among fungicides exhibiting a wide range of inhibition, 5 ppm of captafol showed virtually no inhibition of growth as

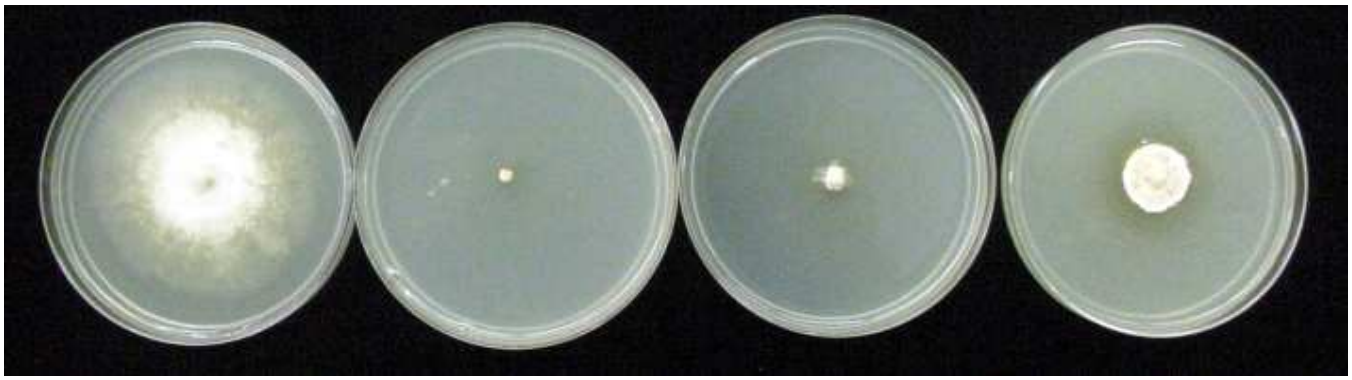

Fig. 1. Three-week-old cultures of *Sphaerobolus* sp. (isolate T-800) showing colony diameters on PDA amended with different fungicides treatments. The plate on the far left is the no-fungicide control.

Table 2. Influence of 14 fungicides on *in vitro* diameter growth of *Sphaerobolus*. Mean colony diameters (mm) are given for each treatment after 21 days of growth at 77F (25C). Petri plates were initially inoculated with colonized agar plugs 4 mm in diameter; therefore, a 4.0-mm colony diameter indicates no growth.

Fungicide	Conc. (ppm)	Mean colony diameter (mm) by species			LSD ($P = 0.05$)
		Sphaerobolus sp.	*Sphaerobolus iowensis*	*Sphaerobolus stellatus*	
Tolylfluanid	5	33.9	56.8	49.9	13.2
	20	40.7	59.7	42.8	NS
Chloroneb	5	39.2	55.7	41.3	NS
	20	33.1	43.8	32.5	NS
Fuberidazole	5	47.4	57.6	39.9	NS
	20	34.6	47.2	37.6	NS
Triphenyltin acetate	5	14.2	14.2	11.5	NS
	20	9.2	6.4	8.0	2.1
Triflumizole	5	38.5	47.5	40.1	NS
	20	29.9	10.8	10.0	4.2
Glyodin	5	47.2	47.8	33.4	NS
	20	36.9	29.4	18.8	8.5
Captafol	5	60.8	56.7	47.2	NS
	20	9.2	8.6	8.2	NS
Dinocap	5	56.3	56.8	44.4	NS
	20	45.7	55.2	45.4	NS
Folpet	5	46.4	61.3	45.2	NS
	20	56.7	60.8	40.5	13.9
Dazomet	5	62.2	51.4	43.6	NS
	20	42.1	58.2	44.2	NS
Ferbam	5	58.4	56.5	37.4	17.1
	20	52.4	53.1	33.6	16.8
Epoxiconazole	5	14.9	4.4	4.8	0.9
	20	7.3	4.0	4.0	0.2
Chlorothalonil/zinc oxide	5	16.8	31.1	28.1	NS
	20	15.3	37.6	30.0	17.4
Thiophanate-methyl	5	24.2	30.1	20.1	NS
	20	4.2	4.0	4.0	0.2
No fungicide		53.1	58.3	51.0	NS
LSD ($P = 0.05$)		11.2	9.9	19.8	

compared to the control, while 20 ppm of this fungicide greatly suppressed growth in all three species of artillery fungi. Similar results were obtained for glyodin and triflumizole, where all of the 20 ppm and some of the 5-ppm treatments resulted in significant growth inhibition, although the difference between the growth values for the two concentrations was not as great as with captafol. Chloroneb, chlorothalonil/zinc oxide, fuberidazole, glyodin, and tolylfluanid gave variable results, showing moderate growth inhibition in every treatment, but with few effects being significant at 5 or 20 ppm. Dazomet, dinocap, folpet, and ferbam did not significantly inhibit growth of any artillery fungus species at either concentration.

Influence of species. The one-way ANOVA of colony diameters among species showed highly variable *P*-values (from $P = 0.001$ to $P = 0.729$) within the fungicide treatments. Since the growth of the three species did not differ from each other in the control, we were able to compare the values among species directly without normalizing the data for each species relative to control values. Among the three species, 5 ppm of tolylfluanid significantly reduced growth of *Sphaerobolus* sp., as compared to *S. iowensis* and *S. stellatus*, which had similar colony diameters. At 20 ppm of triphenyltin acetate, *S. iowensis* showed significantly less growth than *Sphaerobolus* sp., whereas the colony diameters of *S. stellatus* did not differ significantly from either species. For 20 ppm of triflumizole, colony diameters of *S. iowensis* and *S. stellatus* did not differ significantly; however, colonies of both species were significantly smaller than those of *Sphaerobolus* sp. *Sphaerobolus stellatus* showed less growth at 20 ppm of glyodin than both *Sphaerobolus* sp. and *S. iowensis*. Growth of *S. stellatus* was significantly less than that of *S. iowensis* and *Sphaerobolus* sp. at the rate of 20

ppm folpet, but was similar to that of the *S. stellatus* control (0 ppm).

Although ferbam failed to significantly suppress growth, colony diameters differed significantly among species; at 5 and 20 ppm of ferbam, *S. iowensis* and *Sphaerobolus* sp. had greater diameters than *S. stellatus*. Epoxiconazole greatly suppressed the growth of all three species, but was slightly less effective in suppressing growth of *Sphaerobolus* sp. than *S. iowensis* and *S. stellatus* at both rates. In contrast, chlorothalonil/zinc oxide proved to be more effective against *Sphaerobolus* sp. than *S. iowensis*, whereas the colony diameter of *S. stellatus* was no statistically different from the other two species. Thiophanate-methyl was effective against all three species, but had much greater inhibition at 20 than at 5 ppm.

Triflumizole was only moderately effective against *Sphaerobolus* sp., but greatly inhibited growth of *S. iowensis* and *S. stellatus* at 20 ppm. Since our previous research (8, 9) revealed that these latter two species were more abundant than *Sphaerobolus* sp., triflumizole might give effective control of the artillery fungi in some areas of the United States. Sensitivity to glyodin differed among species, but this fungicide was not as effective as triflumizole. In contrast, chlorothalonil/zinc oxide was particularly effective against *Sphaerobolus* sp., showing high growth inhibition, but was only moderately inhibitory against *S. iowensis* and *S. stellatus*.

Results of our study indicate that epoxiconazole, triphenyltin acetate, and thiophanate-methyl were the most effective in suppressing growth of all three *Sphaerobolus* species at the higher rate, and show promise for further field-testing against the artillery fungi. However, the three *Sphaerobolus* species differed significantly in the amount of growth suppression. Epoxiconazole had the most suppressive activity; field-testing of this compound or others in its general fungicide class (class = triazoles) is warranted. Triphenyltin acetate was quite effective and also warrants further consideration. Future field research using thiophanate-methyl (class = carbamates) of should also be conducted since forms of this fungicide are registered for wide use in the United States. However, this fungicide is also known to suppress the growth of *Trichoderma* (D. Royse, The Pennsylvania State University, Department of Plant Pathology, personal communication), a potential biocontrol agent that may suppress artillery fungi (14). Further field studies involving captafol, which suppressed diameter growth of all three artillery fungi species at 20 ppm, might prove useful since captafol has been used in the lumber and timber industries to reduce losses from wood rot fungi in logs and wood products. Other fungicides within the same classes of the more promising fungicides should be field-tested. Although colony growth suppression was often directly related to fungicide concentration, other fungicide rates must be evaluated, as the efficacy of 5 or 20 ppm shown here *in vitro* may not be related to the rate needed to suppress growth and sporulation of artillery fungi *in vivo*.

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