Chemical Disinfestation of Greenhouse Growing Surface Materials Contaminated with *Thielaviopsis basicola*

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ABSTRACT

Copes, W. E., and Hendrix, F. F. 1996. Chemical disinfestation of greenhouse growing surface materials contaminated with *Thielaviopsis basicola*. Plant Dis. 80:885-886.

Sodium hypochlorite and captan significantly reduced propagule viability of *Thielaviopsis basicola* on polypropylene fabric, pressure-treated wood, and galvanized metal hardwarecloth surfaces that had been thoroughly wetted with a solution of endoconidia and aleuriospores of *T. basicola*. Viability of other unidentified fungal propagules was also reduced with captan sprays. Bromine and quaternary ammonium compounds did not reduce propagule viability of *Thielaviopsis basicola* and their effects were not significantly different from that of water.

Black root rot caused by *Thielaviopsis basicola* (Berk. & Broome) Ferraris (= *Chalara elegans* Nag Raj & Kendrick) has caused devastating losses of bedding plants, such as pansy (*Viola* × *wittrockiana* Gams), grown in the southeastern United States. The fungus causes characteristic black necrotic lesions on the main and lateral roots of pansy and on over 137 other plant species including many ornamentals (3,5). Commercial sphagnum peat media have been reported as a source of *T. basicola* (4). In the greenhouse, *T. basicola* was recovered from bench surfaces and peat debris from under benches and endoconidia recovered from air samples 1 and 2 months, respectively, after infected plant material had been removed (4). Aleuriospores (chlamydospores) and endoconidia can survive in field soil for at least 7 months (7). Survival of infective propagules of *T. basicola* on and under greenhouse benches poses a potentially serious problem to subsequent crops.

Disinfection of greenhouse benches is recommended as a sanitation practice but disinfectants are not equally effective on all surfaces and against all pathogens (6). This study was initiated to determine the efficacy of several chemical treatments in reducing propagule viability of *T. basicola* on three greenhouse surfaces.

MATERIALS AND METHODS

Inoculum production. *T. basicola* isolated from diseased pansy roots was grown on V8 agar and nutrient agar with thiamine for the production of endoconidia and aleuriospores, respectively (9). The fungus was grown under continuous light 13 cm below one 15W cool white fluorescent lamp for 14 to 21 days at 20°C. Endoconidia and aleuriospores were harvested from the respective median by flooding cultures with sterilized deionized water, rubbing the agar surface with a rubber policeman or steel spatula, then filtering through four layers of cheesecloth. Spore concentrations were determined with a hemacytometer. Inoculum contained 2×10^6 endoconidia and 1×10^4 aleuriospores per liter in the first experiment and 1×10^5 endoconidia and 1×10^5 aleuriospores per liter in the second experiment.

Experimental design and treatments. Two types of greenhouse bench surfaces and one greenhouse ground surface were simulated with the use of pressure-treated wood slats, galvanized metal wire hardwarecloth, and polypropylene fabric sold for use as a greenhouse ground cover. Wood bench sections were made of five 23 × 3.2 × 0.6 cm pressure-treated wood slats equally spaced (25 mm opening) and nailed on two wood runners (30 × 3.2 × 1.3 cm). Metal and polypropylene sections were 23 × 30 cm.

Experiments were performed in the greenhouse and bench material sections placed on the greenhouse bench. Treatments were replicated four times. In the first experiment, treatments on all three materials were completely randomized. In the second experiment, treatments were completely randomized within blocks of each material.

Inoculum was applied with a hand-squeezed mist bottle that was constantly swirled to maintain a uniform spore suspension. Wood and plastic surfaces were sprayed until the entire surface appeared wet. The metal surface was sprayed until a meniscus shaped droplet formed on the lower cross connections of the wire.

Approximately 24 h after inoculum was applied, chemical treatments were applied with a Solo backpack sprayer fitted with an adjustable hollow cone spray tip. Seven chemical treatments were evaluated: (i) Agribrom, Great Lakes Chemical Corporation (1-Bromo-3-chloro-5,5-dimethyl-2, 4-imidazolidinedione, 93.5% a.i.) at 0.265 g a.i. per liter; (ii) Prevent, The Buffalo Co. (quaternary ammonium, 10% a.i.) at 2.07 ml a.i. per liter; (iii) bleach (sodium hypochlorite, 5.25% a.i.) at 0.525% a.i.; (iv) bleach (sodium hypochlorite 5.25% a.i.) at 0.525% a.i. + Sparkleen, Fischer Scientific Co. (detergent) at 5 g per liter, + scrubbing of upper bench surfaces with a stiff bristled brush (in the first experiment only); (v) bleach (sodium hypochlorite, 5.25% a.i.) at 1.05% a.i. (in the second experiment only); (vi) Captan 50 WP, ICI Corp. (captan, 43.7% a.i.) at 3.0 g a.i. per liter; and (vii) water (control). Chemicals were mixed in 3.875 liters of water. Chemical contamination of neighboring bench surfaces was prevented by containment of the bench section with a four sided wooden frame (26 × 35 cm opening) lined with four layers of 5-mil polypropylene film. Both the sprayer and containment frame were triple rinsed with water between treatments.

Treated sections were air dried for approximately 24 h before being individually placed in polyethylene bags, stacked in a random order in waxed cardboard boxes, and stored at 7°C until sampled. Sampling began the same day and took 2 days. Ten 1-cm^2 pieces were chiseled from each wood bench surface with a 7-mm wood chisel and cut from each plastic ground cover section with dissecting scissors. Ten 4-cm^2 pieces were removed from each metal bench section with tin snips. The 4-cm^2 metal wire pieces always consisted of a square perimeter with cross members of wire in the middle. All tools were flamed between removal of each piece of material. Material pieces were inverted and placed surface down on *Thielaviopsis basicola* media (TBM)–V8 agar (8). Plates were maintained at 22°C in low light (0.23 $\mu E\ s^{-1}\ m^{-2}$). Presence or absence of *T. basicola* and other fungal colonies was determined in each of the 10 pieces from each section (replication) after 14 days.

Statistical analysis. Recovery of *T. basicola* was calculated as a percentage of the 10 pieces sampled from each section. The hypothesis of equal treatments was tested

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Accepted for publication 14 April 1996.

Publication no. D-1996-0510-05R

Table 1. Percent recovery of *Thielaviopsis basicola* from three greenhouse growing surfaces[x]

Treatments	Rate a.i.	Plastic		Wood		Metal	
		Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Water (Control)		92.5 a[y]	87.5 a	75.0 a	77.5 a	100.0 a	90.0 a
Agribrom	(0.27 g)	70.0 b	87.5 a	82.5 a	83.3 a	97.5 a	100.0 a
Prevent	(2.1 ml)	92.5 a	77.5 a	72.5 a	75.0 a	90.0 a	95.0 a
10% sodium hypochlorite	(0.52%)	5.0 c	7.5 b	7.5 b	12.5 b	0.0 c	0.0 b
10% sodium hypochlorite + detergent+scrubbing	(0.52% + 5 g)	0.0 c	. . .[z]	0.0 b	. . .[z]	0.0 c	. . .[z]
20% sodium hypochlorite	(1.04 %)	. . .[z]	0.0 b	. . .[z]	5.0 b	. . .[z]	0.0 b
Captan	(3.0 g)	0.0 c	0.0 b	0.0 b	0.0 b	5.0 b	3.2 b

[x] Recovery was calculated as a precentage from 10 pieces per replicate, four replications.
[y] Values within a column followed by the same letter are not significantly different ($P = 0.05$) according to Tukey's test (df = 21).
[z] Treatment not tested in this experiment.

by analysis of variance (ANOVA) with comparison of multiple means by Tukey's studentized range test (SAS, 6th ed., SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Sodium hypochlorite and captan treatments reduced or eliminated viability of endoconidia and aleuriospores of *T. basicola* on all three greenhouse surfaces (Table 1). Results were not statistically different between experiments. Sodium hypochlorite would be cheaper and require fewer worker safety precautions than captan, which is not normally used as a disinfectant. Use of a 2:8 bleach/water solution and scrubbing bench surfaces treated with a 1:9 bleach/water plus detergent solution consistently reduced recovery of *T. basicola,* compared with use of the 1:9 bleach/water solution, from polypropylene fabric and pressure-treated wood surfaces. Although these treatments were not statistically different, the purpose was complete disinfection of *T. basicola.* Scrubbing bench surfaces treated with a 1:9 bleach/water plus detergent solution would be labor intensive. In commercial operations, for sodium hypochlorite to be efficacious would require prior removal of organic debris to prevent inactivation of chlorine by dirt and organic matter (1,2).

Bromine and quaternary ammonium disinfectants did not substantially reduce propagule viability of *T. basicola* on any of the three greenhouse surfaces (Table 1). The bromine and quaternary ammonium disinfectants are commonly used for elimination of *T. basicola* from greenhouse surfaces.

Counts of fungal colonies other than *T. basicola* were recorded, but fungi were not identified. TBM-V8 medium was used because it was semiselective for *T. basicola* colonies and inhibited growth of many faster-growing microorganisms (8). Different media would be required to provide a representative survey of microbiota. Captan was the only treatment that provided effective elimination of total fungi from all three bench materials (data not shown).

Based on the data presented here, a 1:9 bleach/water solution would be an effective disinfectant for eliminating infective propagules of *T. basicola* from galvanized metal bench surfaces but a 2:8 bleach/ water solution would be more effective on pressure-treated wood and polypropylene fabric surfaces. Bromine and quaternary ammonium compounds were ineffective disinfectants against *T. basicola.* These results show a need to test the efficacy of disinfectants that are commonly used in production systems.

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