Control of Pythium Blight on Penncross Bentgrass with Pyroxychlor

P. L. Sanders, C. G. Warren, and H. Cole, Jr.

Research Assistant, Research Aide, and Professor, respectively, Department of Plant Pathology, The Pennsylvania State University, University Park 16802.

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ABSTRACT

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The efficacy of the experimental systemic fungicide, pyroxychlor (Nurelle®), was tested in vitro against species of *Pythium*, and in the greenhouse and field against Pythium blight on Penncross creeping bentgrass *(Agrostis palustris)*. In vitro toxicity against 17 species of *Pythium* was variable. Field and greenhouse experiments indicate that the chemical was immediately effective against Pythium foliar blight, regardless of the method of application. Residual activity was significantly affected by application technique. Foliar sprays, not watered-in, provided control for less than 7 days, watered-in foliar sprays were effective for up to 21 days, and drenches exhibited the longest residual activity. When the experiment was terminated at 35 days post-treatment, the drench treatments significantly suppressed disease at all treatment rates.

Additional key words: Agrostis palustris, Nurelle®.

Pythium blight (cottony blight, grease spot, spot blight), which is caused by several *Pythium* spp., is one of the most common and most devastating of all turfgrass diseases. When environmental conditions are favorable, this disease can completely decimate an established stand of turf within 24 hours. Pythium blight of foliage occurs in hot, wet weather. The causal organisms are most aggressive at temperatures of 29-35 C, under high humidity conditions (2).

Because the disease develops rapidly when the environment is favorable, control measures ideally should be of a preventive nature. Cultural practices used to minimize infection chiefly are aimed at avoiding the accumulation of free water, especially when temperatures are high. Chemical control is dependent either on preventive treatment when environmental conditions favor disease development, or on early diagnosis of symptoms and appropriate curative measures. Because of the high cost of preventive chemical treatment, control attempts commonly are curative; i.e., stopping further disease development after symptom appearance. The fungicides 1, 4-dichloro-2, 5-dimethoxybenzene (chloroneb), *p*-(dimethylamino)benzenediazo sodium sulfonate (Dexon), and 5-ethoxy-3-(trichloromethyl)-1, 2, 4-thiadiazole (Koban) currently are among the fungicides used to control this disease. These are primarily protectant materials, requiring repeated and frequent application (as often as every 3-4 days) for effective disease suppression.

The experimental systemic fungicide, pyroxychlor (Nurelle®) [2-chloro-6-methoxy-4-(trichloromethyl) pyridine] offers promise for longer-lasting control of Pythium blight on turf because it is systemic (6). Pyroxychlor has been successfully used to control Phycomycetous root rots and damping-off of tobacco (6), diffenbachia (4), carnation (8), African violet (7), azalea (5), peperomia (1), rhododendron, and soybean (3).

The experiments reported here were undertaken to determine the efficacy and residual activity of this new systemic fungicide under various conditions, against Pythium foliar blight on Penncross bentgrass (*Agrostis palustris* Huds.).

MATERIALS AND METHODS

Laboratory experiments.—The in vitro toxicity of pyroxychlor to 17 *Pythium* spp. was evaluated by inhibition of mycelial growth on potato-dextrose agar (PDA) amended with the chemical. The fungicide was suspended in sterile distilled water and added in appropriate quantities to partially cooled PDA. All concentrations were calculated on a w/v, active ingredient basis. The amended medium was poured into sterile glass petri plates, allowed to cool, and used immediately. Rates tested were 0, 1, 10, 100, and 1,000 μg active ingredient fungicide per ml of PDA. Agar plugs of mycelium were taken from the periphery of actively growing *Pythium* colonies and transferred to the center of the fungicide-amended PDA plates. Each chemical treatment was replicated three times. All cultures were incubated at 24 $\pm$ 1 C. Colony diameter was recorded at 24 and 48 hours.

Greenhouse experiments.—Penncross creeping bentgrass (*Agrostis palustris* Huds.) was seeded into 10-cm diameter plastic pots of sterilized greenhouse mixture of soil, sand, and peat (2:1:1, v/v). Throughout the experiment, the grass regularly was irrigated and maintained at about 2.5 cm above the soil surface. The chemical treatments were applied at 2-4 weeks after seeding. Pyroxychlor was tested both as a foliar spray and as a soil drench.

The sprays were applied at 474, 947, and 1,894 ml pyroxychlor (7.2% EC) per 93 centares in 23 liters of water per 93 centares. Spray application methods evaluated were: (i) 23 liters per 93 centares (dilute spray); (ii) dilute spray, followed immediately by irrigation of the foliage with 0.6 cm of water; (iii) dilute spray, followed by irrigation 15 minutes later; and (iv) dilute spray allowed to dry on foliage, and irrigated about 4 hours later. Chloroneb, at 114 g per 93 centares, was included for comparison. The grass was inoculated at the following intervals after chemical treatment: immediately, 1, and 2 weeks.

Drenches were applied at 474, 947, and 1,894 ml pyroxychlor (7.2% EC) per 93 centares in water equivalent to 1.2 cm of irrigation. Care was taken not to wet the grass foliage during the drenching procedure. Inoculations were done immediately, 1, 2, 3, and 4 weeks after treatment.

Inoculum was prepared by growing three pathogenic isolates of *Pythium aphanidermatum* (P-1, P-3, and P-15) on autoclaved rye grain. The rye inoculum was blended with a small quantity of sterile water in a blender to make a uniform, thick homogenate. Each pot of grass was inoculated by placing about 2 ml of the pooled homogenate in the center of the grass area. After inoculation, the pots were placed on a shaded greenhouse bench under individual transparent plastic covers to maintain high humidity. The greenhouse temperature during the experiment was 30 ± 3 C.

One week after inoculation, the plastic covers were removed and the grass was evaluated for disease severity. A 0-10 visual severity scale was employed, with 0 = no disease, 1 = 1-10% of the grass blighted, 2 = 11-20%, 3 = 21-30%, 4 = 31-40%, 5 = 41-50%, 6 = 51-60%, 7 = 61-70%, 8 = 71-80%, 9 = 81-90%, and 10 = 91-100% (essentially complete death of the foliage) of the grass blighted.

Field experiments.—A mixed stand of bentgrass under standard fairway management and maintained at 1.2-cm cutting height was used in this experiment. Pyroxychlor (7.2% EC) at 474, 947, and 1,894 ml per 93 centares was sprayed on 91-cm strips in each of three replications. Immediately after spraying, and before the foliage had dried, the area was irrigated with 1.2 cm of water. Chloroneb was applied at 114 g per 93 centares for comparison, but was not irrigated.

Inoculations were made at 4, 12, and 26 days after treatment. A 91-cm band across all treatments in each replicate was inoculated with *P. aphanidermatum* growing on autoclaved rye grain. The inoculated area was covered from 1700 hours to 0800 hours with a 91- × 455-cm wooden frame with a transparent plastic top to maintain high humidity and minimize radiational cooling. Pythium blight severity was estimated by employing the following visual rating scale: 0 = no detectable infection, 1 = trace to 5% of area blighted, 2 = 6-10% blighted, 3 = 11-20% blighted, 4 = 21-40% blighted, 5 = 41% or more of the area blighted. The plots inoculated at 4, 12, and 26 days after treatment were rated at 8, 18, and 31 days, respectively.

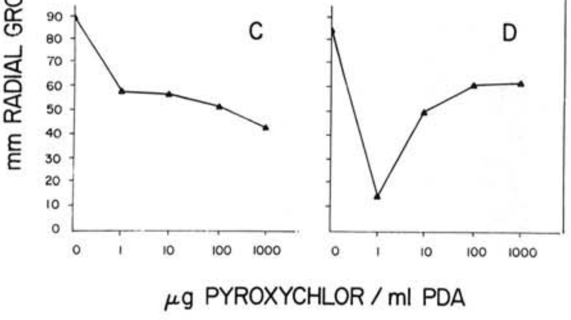

Fig. 1-(A to D). Rate-response curves of four representative *Pythium* spp. on pyroxychlor-amended potato-dextrose agar (PDA): A) Sensitive, – *Pythium aphanidermatum* (P-1); B) Sensitive, bimodal – *Pythium ultimum* (P-17); C) Insensitive – *Pythium mamillata* (P-6); D) Insensitive, bimodal – *Pythium aphanidermatum* (P-15).

RESULTS

Laboratory experiments.—There was great variation in response to pyroxychlor among the 17 species of *Pythium* tested in vitro (Table 1). Figure 1 illustrates the four general rate response curves obtained. Eleven isolates gave sensitive responses; i.e., ED_{50} concentrations of <1 µg/ml. Of these sensitive isolates, eight gave responses in which the colony diameter decreased as the

TABLE 1. Response of *Pythium* spp. to pyroxychlor in potato-dextrose agar

Isolate no.	Species name	Response to pyroxychlor[a]
P-1	*Pythium aphanidermatum*[b]	Sensitive[c]
P-2	*Pythium* sp.	Sensitive, bimodal
P-3	*Pythium aphanidermatum*[b]	Sensitive
P-4	*Pythium* sp.	Insensitive[d]
P-5	*Pythium* sp.	Sensitive, bimodal
P-6	*Pythium mamillata*	Insensitive, bimodal
P-7	*Pythium torulosum*	Sensitive
P-8	*Pythium dissorticum*	Sensitive
P-9	*Pythium artotrogas*	Insensitive, bimodal
P-10	*Pythium* sp.	Insensitive
P-11	*Pythium irregulare*	Sensitive, bimodal
P-12	*Pythium torulosum*	Sensitive
P-13	*Pythium periplocum*	Sensitive
P-14	*Pythium graminicola*	Sensitive
P-15	*Pythium aphanidermatum*[b]	Insensitive, bimodal
P-16	*Pythium vanterpoolii*	Sensitive
P-17	*Pythium ultimum*	Sensitive, bimodal

[a]Types of response curve are illustrated in Fig. 1.
[b]Pathogenic on bentgrass.
[c]ED_{50} <1 µg/m.
[d]ED_{50} >100 µg/m.

rate increased (Fig. 1-A). The other three sensitive isolates produced bimodal response curves (Fig. 1-B). The other six isolates tested were relatively insensitive to pyroxychlor and had ED_{50} concentrations of >100 μg/ml. Of these six insensitive isolates, two gave decreasing growth response to increasing chemical concentration (Fig. 1-C) and four gave a bimodal response (Fig. 1-D).

Greenhouse experiments.—All concentrations of pyroxychlor and all application methods tested immediately protected bentgrass against Pythium foliar blight (Tables 1 and 2). Residual disease control, however, appeared to be markedly affected by the application technique.

In the spray experiment (Table 2), when the plants were inoculated 7 days after treatment, only the spray applications combined with irrigation were effective. The spray application followed immediately by irrigation was active at the 947- and 1,894-ml rates, and delaying irrigation for 15 minutes was satisfactory only at the 1,894-ml rate. When the spray experiment was terminated at 21 days after treatment, the group of plants that had been sprayed and immediately irrigated showed 83% and 90% disease suppression at the 947- and 1,894-ml rates, respectively, when compared to the untreated control. The sprayed plants that were irrigated after 15 minutes provided partial suppression (56%) at the 1,894-ml rate.

In the drench experiment (Table 3), pyroxychlor significantly reduced disease at all rates throughout the entire 5 weeks of the test. When the drench experiment was terminated at 35 days, the 474- and 947-ml rates reduced disease 62% and 65%, respectively, compared to

TABLE 2. Pythium blight control and residual effectiveness of pyroxychlor applied as a foliar spray to bentgrass

Fungicide and dosage per 93 centares	Mean disease severity[a] with post-spray treatment of: No irrigation		Immediate irrigation		Irrigation 15 minutes after treatment		Irrigation 4 hours after treatment	
Inoculated immediately after treatment–evaluated 7 days after treatment:								
Unsprayed control	10[b]	A[c]	10	A	10	A	10	A
Pyroxychlor 474 ml (7.2% EC)	0	G	0	G	0	G	0	G
Pyroxychlor 947 ml (7.2% EC)	0†	G	0	G	0	G	0†	G
Pyroxychlor 1,894 ml (7.2% EC)	0††	G	0†	G	0†	G	0††	G
Chloroneb 114 g (65% WP)	0	G	0	G	0	G	0	G
Inoculated 7 days after treatment—evaluated 14 days after treatment:								
Unsprayed control	10	A	10	A	10	A	10	A
Pyroxychlor 474 ml (7.2% EC)	8.7	AB	10	A	10	A	10	A
Pyroxychlor 947 ml (7.2% EC)	10	A†	2.0	FG	7.0	BC	9.7†	A
Pyroxychlor 1,894 ml (7.2% EC)	9.3	A††	0†	G	0†	G	6.3††	CD
Chloroneb 114 g (65% WP)	9.3	A	7.0	BC	6.3	CD	9.7	A
Inoculated 14 days after treatment–evaluated 21 days after treatment:								
Unsprayed control	5.0	DE	6.0	CD	6.3	CD	5.3	DE
Pyroxychlor 474 ml (7.2% EC)	3.7	E	6.7	CD	4.3	EF	5.0	DE
Pyroxychlor 947 ml (7.2% EC)	4.8†	DE	1.0	G	5.0	DE	5.0†	DE
Pyroxychlor 1,894 ml (7.2% EC)	4.0††	EF	0.6†	G	2.7†	FG	6.7††	CD
Chloroneb 114 g (65% WP)	6.3	CD	9.0	A	8.7	AB	9.7	A

[a]Mean of three replications.
[b]Rating scale: 0 = no disease, 1 = 1-10% of the grass blighted, 2 = 11-20%, 3 = 21-30%, 4 = 31-40%, 5 = 41-50%, 6 = 51-60%, 7 = 61-70%, 8 = 71-80%, 9 = 81-90%, and 10 = 91-100%.
[c]Disease severity values followed by the same letter are not significantly different by Duncan's multiple range test ($P = 0.05$).
†Moderate foliage tip burn induced by chemical treatment.
††Severe chemical tip burn induced by chemical treatment.

TABLE 3. Pythium blight control and residual effectiveness of pyroxychlor (7.2% EC) applied as a soil drench to bentgrass

Post-treatment inoculation time (days)	Mean disease severity[a] with formulated product drenches per 93 centares of: Untreated control		474 ml		947 ml		1,894 ml	
0	10[c]	E[d]	0	A	0	A	0	A
7-14[b]	10	E	1.1	AB	1.9	A	0.3	AB
14-21[b]	9.8	DE	2.5	C	1.4	BC	1.4	BC
21-28[b]	9.5	DE	2.1	C	1.3	C	0.4	AB
28-35[b]	8.1	D	3.1	C	2.8	C	0.3	AB

[a]Mean of eight replications.
[b]Plants were inoculated on the first day of the period and evaluated for disease at the end of the period.
[c]Rating scale: 0 = no disease, 1 = 1-10% of the grass blighted, 2 = 11-20%, 3 = 21-30%, 4 = 31-40%, 5 = 41-50%, 6 = 51-60%, 7 = 61-70%, 8 = 71-80%, 9 = 81-90%, and 10 = 91-100%.
[d]Severity values followed by the same letter are not significantly different by Duncan's multiple range test ($P = 0.05$).

TABLE 4. Field control of Pythium blight of bentgrass and residual effectiveness of pyroxychlor applied as a spray followed by immediate irrigation

Fungicide treatment per 93 centares	Mean disease severity[a] at various times after spray treatment: 8 days (inoculated at 4 days)		18 days (inoculated at 12 days)		31 days (inoculated at 26 days)	
Unsprayed control	2.0	B	2.3	B	1.7	B
Pyroxychlor (7.2% EC)						
474 ml	0.3[b]	A[c]	1.3	AB	0.3	A
947 ml	0.3	A	0.3	A	0.7	A
1,894 ml	0†	A	0.3†	A	0.7†	A
Chloroneb (65% WP)						
114 g	0.7	A	2.0	B	1.7	B

[a]Mean of three replications.
[b]Rating scale: 0 = no detectable infection, 1 = trace to 5% of area blighted, 2 = 5-10% blighted, 3 = 11-20% blighted, 4 = 21-40% blighted, 5 = 41% or more of the area blighted.
[c]Severity values followed by the same letter are not significantly different (LSD $P = 0.05$).
†Moderate foliage tip burn induced by chemical treatment.

the untreated control. At this time, the 1,894-ml rate gave excellent disease suppression, 96% reduction when compared to the untreated control.

Field experiments.—Pyroxychlor suppressed Pythium blight at all rates tested in the field (Table 4). At the 18-day evaluation, chloroneb was no longer effective. There was no significant difference in degree of control among the three rates of pyroxychlor tested. The slight variation in the amount of disease present in the control plots at 8, 18, and 31 days was a reflection of the temperature at the time of inoculation.

DISCUSSION

The results of our laboratory studies indicate that the response of different species of *Pythium* to pyroxychlor is extremely variable. These results agree with Richardson (9) who reported similar variation in response of species of *Pythium* and *Phytophthora* to pyroxychlor. Because only three of the species of *Pythium* tested were pathogenic on bentgrass, it was not possible to determine the degree of correlation between laboratory and field results, but our results suggest that pyroxychlor may not control all species of *Pythium* spp. equally well under greenhouse and field conditions. Field studies with naturally-occurring *Pythium* spp. pathogens are needed to establish the overall effectiveness of pyroxychlor.

Our experiments indicate that application procedure can significantly influence the effectiveness of pyroxychlor for controlling Pythium blight on bentgrass. The drench and the spray application immediately followed by irrigation produced significantly greater residual control than did the nonirrigated foliar spray application. These results agree with those of Raabe and Hurlimann (7), who compared the effectiveness of pyroxychlor as a foliar spray and as a soil drench in the control of Phytophthora crown rot in African violet.

Since the application of pyroxychlor as a soil drench provided immediate protection from foliar blighting incited by inoculum placed directly on the foliage, and since care was taken not to wet the foliage during drenching, the inference that pyroxychlor is absorbed by the roots and rapidly translocated acropetally in bentgrass appears to be justified. In addition, because the grass regularly was irrigated and was maintained throughout the experiments at a height of 2.5 cm by removing clippings at regular intervals, it would appear that the fungicide remained available for uptake and or translocation over the 35 days covered by the drench experiment. Foliar uptake and basipetal translocation in bentgrass would appear to be minimal, since foliar sprays without irrigation produced no residual protection. These results on bentgrass differ from the findings of Small and Edgington (10) who investigated root and foliar uptake of pyroxychlor on tomato and those of Noveroske (6) who studied the activity of pyroxychlor against *Phytophthora parasitica* on tobacco.

Studies currently underway indicate that texture and organic matter of the soil markedly influence efficacy and residual activity of pyroxychlor against Pythium blight. These studies, as well as greenhouse experiments to determine residual activity of pyroxychlor on mature, thatched bentgrass presently are in progress.

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