Activity of Propiconazole and Other Sterol-Inhibiting Fungicides Against *Phymatotrichum omnivorum*

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ABSTRACT

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Eight sterol-inhibiting fungicides were evaluated for in vitro activity against *Phymatotrichum omnivorum*, the causal fungus of Phymatotrichum root rot of cotton. Mycelial growth of *P. omnivorum* was inhibited at concentrations as low as 0.0001 μg/ml. Significant stunting of cotton seedlings occurred after preplant applications of propiconazole at rates equivalent to 0.56–2.24 kg/ha. When this stunting of cotton seedlings was used as a bioassay, propiconazole was observed to persist in unsterile soil for as long as 5 mo. Results of field evaluations of propiconazole in 1982 and 1983 determined that foliar applications at 0.56–1.12 kg/ha or granular side-dress applications at 1.12–2.24 kg/ha applied 6–9 wk after planting provided statistically significant control of root rot in cotton. In an evaluation of basipetal translocation, about 0.25% of the radioactivity from ^{14}C-labeled propiconazole applied to the leaves of cotton plants accumulated in the roots.

Phymatotrichum root rot, caused by *Phymatotrichum omnivorum* (Shear) Duggar, is the most serious soilborne disease of cotton in the alkaline, low-organic-matter soils of the southwestern United States and northern Mexico. It is unusual among soilborne diseases because it appears annually in the same localized areas and is not spread by tillage or irrigation. At present, no consistently effective measures exist to control Phymatotrichum root rot of cotton.

In 1982, we determined that mycelial growth of several isolates of *P. omnivorum* was significantly reduced by propiconazole in vitro at concentrations as low as 0.0001 μg/ml (5). This unusual activity stimulated us to evaluate propiconazole for disease control. The purpose of this report is to discuss the results of laboratory, greenhouse, and field research performed with propiconazole and related fungicides for control of this important root disease. Preliminary studies have been published (5,6).

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MATERIALS AND METHODS

In vitro activity. Fungicides evaluated in these tests were propiconazole, etaconazole, bitertanol, triadimefon, triadimenol, imazalil, fenarimol, XE-779, and benomyl. The sensitivity of two isolates of *P. omnivorum* to the fungicides tested was determined by transferring an 8-mm-diameter plug of mycelium from a 2-wk-old culture to petri dishes of fungicide-amended or nonamended potato-dextrose agar (PDA) and incubating at 28 ± 1 C. Five replicates of each isolate were prepared for each fungicide and concentration, and the entire evaluation was replicated a minimum of three times during successive weeks. Measurements of the greatest radial growth of the mycelium were made after 96 hr.

Phytotoxicity and soil persistence evaluations of propiconazole. To simulate the application of propiconazole as an in-furrow treatment at planting, 15-cm-diameter pots were filled with unsterile soil (Gila silt loam, pH 7.8, organic matter < 1%) and placed in the greenhouse at 24–35 C. Propiconazole granules equivalent to field applications of 0.56, 1.12, and 2.24 kg a.i./ha were applied as an in-furrow band. Upland cotton (DPL-55, treated with captan, carboxin, malathion, and methoxychlor) was planted (six seeds per pot) in the furrow

after the application and covered with soil. Periodic observations were made on plant height.

In other studies, the granular formulation of propiconazole (2.5% a.i.) was mixed thoroughly with air-dried soil to produce concentrations of 6.25, 12.5, 25, and 50 mg a.i./kg (ppm). Ten 10-cm-diameter pots were prepared for each concentration, along with 10 nonamended controls, planted with Upland cotton (DPL-55, five seeds per pot) and watered. At monthly intervals, plants were removed from the soil and stem length (soil line to apical meristem) was measured. Once the plants were removed, the soil from the 10 pots of each concentration was mixed, redistributed in the pots, and replanted. These propiconazole-amended soils were planted and harvested in this manner once a month for 5 mo after the original soil treatment.

Field evaluations. Replicated field trials with propiconazole were conducted at Marana, AZ, during the growing seasons of 1982 and 1983. Cotton fields infested with Phymatotrichum were located with aerial infrared photographs. Plots of Upland cotton (DPL-55) were single rows 15.2 m long with a border row between plots. Each treatment was replicated four times. Treatments consisted of foliar sprays with the emulsifiable concentrate (EC) at rates ranging from 0.14 to 1.12 kg a.i./ha, in 450 L of water per hectare and granular side-dress applications (10 cm from the plant row and 10 cm deep) at rates ranging from 0.56 to 2.24 kg a.i./ha. The fields were irrigated within 48 hr of treatment to activate the granules. Control of Phymatotrichum root rot was evaluated by determining the percentage of plants killed or dying from Phymatotrichum root rot in each plot before harvest and by determining yield (estimated by hand-picking the lint from a 2-m section selected at random from each plot and calculated on the basis of weight [kg/ha]).

Systemic translocation of propiconazole in cotton plants. ^{14}C-labeled propiconazole was obtained from the Ciba-Geigy Corporation, Greensboro, NC. Cotton plants (DPL-55) were grown in a field soil:peat moss (2:1) mixture contained in 1-m sections of 10-cm-diameter PVC sewer pipe to allow development of a taproot. The cotton (one plant per container) was planted in mid-April, and the containers were placed outdoors.

The first group of plants was treated when 5 wk old (six or seven true leaves). Each plant was treated with 1 ml of an aqueous solution of ^{14}C-propiconazole (0.25 μCi/ml) by carefully placing drops of the solution along leaf veins (maximum of 0.25 ml per leaf) of the upper four or five leaves and allowing the drops to evaporate. Aluminum foil was placed at the base of the plant to prevent soil contamination. A second group of plants was treated when 8 wk old (eight to 10 true leaves) in the same manner, except 1.5 ml of the ^{14}C-propiconazole solution was distributed on the upper six or seven leaves. Control plants were treated in a similar manner with a solution of the emulsifier used in preparing the ^{14}C-propiconazole solution. Plants from both groups (three treated and one control) were removed from the containers 1, 2, 3, 4, and 8 wk after treatment and prepared for analysis.

The plants were weighed (fresh and air-dry weights), measured, divided into small samples of various plant tissues (apical meristem, leaves, petioles, roots, etc.), and prepared for analysis. The tissues were oxidized to CO_2 with a Packard Tri-Carb model B306 tissue oxidizer. The ^{14}C contained in the samples was analyzed on a Beckman LS 7000 liquid scintillation counter.

Movement of propiconazole into the taproot from foliar sprays was also evaluated by a bioassay method. Four-week-old cotton plants were sprayed with the emulsifiable concentrate formulation of propiconazole at rates of 0.5 and 1 kg a.i./ha in 450 L of water per hectare. Ten roots from the treated and control plots were collected nine times during September and October. The cortex was removed and cross sections 0.3 cm in diameter were cut from the midsection of each root and surface-sterilized for 5 min in 0.5% sodium hypochlorite.

The root disks were placed in the centers of petri dishes containing water agar amended with streptomycin sulfate (200 mg/L). Three 1-cm plugs of mycelium from a vigorously growing culture of *P. omnivorum* (on potato-dextrose agar) were placed 1.5 cm from the root disks. Plates were incubated at 28 C, and mycelial growth was measured periodically.

RESULTS

In vitro activity. EC_{50} values for the fungicides were calculated from the in vitro data (Table 1). Mycelial growth of *P. omnivorum* was affected at very low concentrations by all the sterol-inhibiting fungicides tested. Propiconazole and XE-779 showed the greatest activity, with significant growth reductions of 17 and 19%, respectively, at 0.0001 μg/ml ($P = 0.05$). Overall, the EC_{50} values for the sterol-inhibiting fungicides were two to 60 times lower than that of benomyl, the standard.

Phytotoxicity and soil persistence of propiconazole. Application of propiconazole to field soil at planting (in the greenhouse) resulted in severe plant stunting and reduced seedling emergence. Seedling emergence was reduced 77, 57, and 13% in the simulated in-furrow studies with propiconazole granules at rates of 2.24, 1.12, and 0.56 kg a.i./ha, respectively. Heights of surviving plants were also severely retarded. After 1 mo, plants treated at rates of 2.24, 1.12, 0.56, and 0 (control) averaged 8, 11.6, 13.2, and 23.9 cm high, respectively.

Table 2 presents the percent reduction in stem length of 1-mo-old cotton plants after one to five plantings in soil amended with propiconazole at initial concentrations of 6.25, 12.5, 25, and 50 ppm (a.i.) based on air-dry soil weight. Reduction in stem length was less severe over time, with the rate of recovery related to the initial concentration. These data indicate that propiconazole was not rapidly degraded and remained active for at least 5 mo in field soil.

Field trials. In 1982, foliar sprays and granular side-dress applications of propiconazole significantly reduced Phymatotrichum root rot. Significant

Table 1. EC_{50} values of eight sterol-inhibiting fungicides and benomyl for isolates of *Phymatotrichum omnivorum* collected from cotton roots at Rillito and Marana, AZ

Fungicide	Isolate of *P. omnivorum*	
	Rillito	Marana
XE-779	3[a]	1[a]
Propiconazole	6	3
Etaconazole	5	3
Imazalil	19	9
Bitertanol	29	18
Triadimenol	30	15
Triadimefon	31	28
Fenarimol	38	17
Benomyl	80	61

[a] EC_{50} values are expressed as parts per billion (ng/ml) of active ingredient.

Table 2. Percent reduction in stem length of 1-mo-old cotton plants grown in propiconazole-amended soil over a period of 5 mo as a bioassay of the persistence of propiconazole

Concentration[a] of propiconazole (ppm)	Number of plantings into the soil[b]				
	First	Second	Third	Fourth	Fifth
6.25	56	47	13	0*[c]	0*
12.50	67	58	33	15*	16
25.00	70	65	47	33	14
50.00	75	70	63	65	39

[a] Initial concentration (mg a.i./kg) in air-dry Gila silt loam.

[b] Cotton (DPL-55) was planted into 10-cm-diameter pots containing propiconazole-amended and nonamended field soil. The plants were removed after 1 mo, measured, and the pots replanted monthly for 5 mo.

[c] Values followed by an asterisk do not differ significantly from control plants grown in nonamended soil (LSD, $P = 0.05$).

yield increases occurred with foliar applications at 0.56 kg a.i./ha and granular side-dress applications at 2.24 kg a.i./ha (Table 3). No yield data were collected for the 1983 field trials because heavy rains (>22 cm) and flooding destroyed any potential differences that may have existed between the plots. However, data on incidence of disease were collected before the rains (Table 4) and are indicative of the performance of the chemicals through early October. Only a late foliar application of propiconazole at 1.12 kg a.i./ha, early granular side-dress applications at 2.24 kg a.i./ha, and a split granular application at 1.12 + 1.12 kg a.i./ha produced significant reductions in the percentage of root rot.

Systemic translocation of propiconazole in cotton plants. Table 5 presents the recovery of ^{14}C (following application of ^{14}C-propiconazole) from various tissues of treated cotton plants. Most of the radioactivity was concentrated in the leaves and petioles (both dosed and newly developed leaves), with less than 0.25% of the applied dose accumulating in the roots. These data show that propiconazole moves systemically within the cotton plant and that a small amount of basipetal translocation does occur.

Table 6 presents a theoretical estimate of the concentration of propiconazole within the root after a foliar application. These estimates were calculated from the amount of propiconazole that would be applied to a cotton plant in the field (100-cm rows, about 97,000 plants per hectare) at a rate of 0.56 kg a.i./ha (about 5 mg/plant), the fresh weight of the roots sampled in these studies, and the percentage of the applied dose of radioactivity detected in these roots. If it is assumed that the radioactivity detected in the roots represents propiconazole and/or its metabolites and that these metabolites are toxic to *P. omnivorum*, the estimated values in Table 6 indicate that the concentration of propiconazole in the roots after a foliar application would be 30–600 times the EC_{50} value of propiconazole for *P. omnivorum* (Table 1). These estimates indicate that the concentration of propiconazole would be sufficient to inhibit the mycelial growth of the fungus for at least 8 wk after a foliar application of 0.56 kg a.i./ha. These data also suggest that an application to smaller, more actively growing plants could result in more of the fungicide reaching the roots.

Bioassay studies also indicated that propiconazole and/or a breakdown product toxic to mycelial growth of *P. omnivorum* moved from the foliage into the taproot. Zones of inhibition ranging from 6 to 8 mm occurred when mycelial plugs of *P. omnivorum* were placed 1.5 cm from root disks taken from plants that had been sprayed with propiconazole at rates of 0.5 and 1 kg a.i./ha. No zones of

Table 3. Control of Phymatotrichum root rot of cotton in two field plots at Marana, AZ, with foliar sprays or granular side-dress applications of propiconazole in 1982

Application method and rate (kg a.i./ha)	Disease incidence[v]	Yield[w]
Foliar spray[x]		
Control	92 a[y]	1,336 a
0.14	78 ab	2,016 ab
0.28	65 b	2,064 ab
0.56	35 c	2,525 b
Side-dress[z]		
Control	99 a	754 a
1.12	76 ab	1,774 ab
2.24	55 b	2,680 b

[v] Percentage of dead plants.
[w] Estimated by hand-picking 2 m of row (1/5,000 ha) on 1 November 1982. Values expressed as kilograms of unginned seed cotton per hectare.
[x] The emulsifiable concentrate was applied in 450 L of water per hectare when plants were 6 wk old.
[y] Values followed by the same letter do not differ significantly (LSD, $P = 0.05$). Disease incidence was analyzed by arc sine transformation.
[z] Granules were applied in a trench 10 cm from the plant row and 10 cm deep when plants were 6 wk old. Irrigation followed within 48 hr.

Table 4. Evaluation of application timing for control of Phymatotrichum root rot of cotton (DPL-55) with different formulations of propiconazole at Marana, AZ, during 1983

Application timing and rate (kg a.i./ha)[y]	Disease incidence[z]	
	Foliar spray	Granular side-dressing
Control	47 a	35 ab
Early (0.28)	29 ab	···
Late (0.28)	37 ab	···
Split (0.28 + 0.28)	32 ab	···
Early (0.56)	33 ab	18 bc
Late (0.56)	31 ab	20 abc
Split (0.56 + 0.56)	28 ab	10 bc
Early (1.12)	···	14 bc
Late (1.12)	20 b	21 abc
Split (1.12 + 1.12)	···	3 c
Early (2.24)	···	4 c

[y] Early, late, and split applications were made when plants were 6 and 9 wk old. ··· = Rate not tested or application not made.
[z] Percentage of plants dead on 1 October 1983. Values followed by the same letter do not differ significantly (arc sine, LSD, $P = 0.05$).

Table 5. Percentage of the applied dose of radioactivity remaining in various tissues of container-grown cotton plants after an application of ^{14}C-labeled propiconazole to leaves of 5- and 8-wk-old plants

Sampling interval (wk) after treatment	Tissue[a]				
	Apical meristem	Stem	Roots	Leaves and petioles	Entire plant[b]
Fifth-week treatment[c]					
1	0.34	1.76	0.07	73.21	75.45
2	0.38	0.47	0.22	64.47	65.56
3	0.44	0.42	0.23	19.61	20.57
4	0.10	0.29	0.13	7.74	8.34
8	0.10	0.28	0.11	1.63	2.25
Eighth-week treatment[c]					
1	0.10	0.31	0.11	61.11	62.00
2	0.20	0.24	0.12	38.90	39.71
3	0.10	0.23	0.06	32.22	32.86
4	0.06	0.16	0.04	18.45	18.84
8	0.05	0.12	0.05	5.01	5.27

[a] Values are the percentage of the radioactivity applied to the foliage recovered in each tissue at each sampling interval.
[b] Sum of the radioactivity recovered from all tissues. (Not all tissues sampled are presented.)
[c] An aqueous solution of ^{14}C-labeled propiconazole (0.25 μCi/ml) was applied along the veins (0.25 ml/leaf) of the upper four to six leaves of 5-wk-old (1 ml/plant) and 8-wk-old (1.5 ml/plant) cotton plants.

Table 6. Theoretical concentration of propiconazole in cotton roots after an application of ^{14}C-labeled propiconazole to leaves of 5- and 8-wk-old plants

Sampling interval (wk) after treatment	Entire root[a] (ppm)	Root tip[b] (ppm)
Fifth-week treatment[c]		
1	2.59	20.66
2	4.31	10.29
3	3.94	12.47
4	1.80	4.16
8	1.52	1.65
Eighth-week treatment[c]		
1	1.72	1.80
2	1.60	1.47
3	0.65	0.63
4	0.64	0.33
8	0.73	0.50

[a] Concentration estimates calculated from the following information: percentage of radioactivity recovered in root tissues after a foliar application of ^{14}C-labeled propiconazole to container-grown plants, fresh weight of the roots from these studies, and amount of propiconazole applied per plant (about 5 mg) during a field application of 0.56 kg a.i./ha.
[b] Root from 20 cm below the soil surface and deeper.
[c] An aqueous solution of ^{14}C-labeled propiconazole (0.25 μCi/ml) was applied along the veins (0.25 ml/leaf) of the upper four to six leaves of 5-wk-old (1 ml/plant) and 8-wk-old (1.5 ml/plant) cotton plants.

inhibition occurred with root disks taken from plants in the control plots. The zones of inhibition occurred with roots sampled 8–14 wk after application.

DISCUSSION

In vitro, sterol-inhibiting fungicides are highly active against *P. omnivorum*. They are at least twice as active as benomyl, previously the most effective fungicide evaluated (4,7). The most active fungicides (XE-779 and propiconazole) were 13–60 times more active than benomyl and produced significant growth reduction at extremely low concentrations (0.0001 μg/ml).

The sclerotia of *P. omnivorum* are less sensitive to propiconazole than the mycelium (9). It was speculated, on the basis of the greater sensitivity of pregerminated sclerotia, that the greater levels of sterols in sclerotia (2,3) may initially mask the inhibition of sterol synthesis by propiconazole (9).

Propiconazole was fungistatic rather than fungicidal to *P. omnivorum*. If the mycelium from propiconazole-amended media was transferred to nonamended media, greater than normal growth resumed (9). Presumably, this was the result of an accumulation of sterol precursors (9) that is common with these fungicides (8).

Greenhouse evaluations of propiconazole indicated that this fungicide should not be used at planting for control of Phymatotrichum root rot of cotton because it causes severe stunting of seedlings. This stunting was probably the result of an inhibition of the synthesis of gibberellins (1). Because the stunting of the cotton seedlings and the growth inhibition of the fungus are the result of the same or very similar mode of action (inhibition of sterol and gibberellin synthesis) (8), cotton seedlings were used as a bioassay for measuring the persistence of propiconazole in unsterile soil. Propiconazole remained active in the soil from 3 to 5 mo (depending on the rate applied), which should be a factor in providing adequate protection against root rot in the field.

Field evaluations determined that propiconazole does show potential for control of Phymatotrichum root rot of cotton. Results to date indicate that granular side-dress applications at 1.12 to 2.24 kg/ha or foliar spray applications at 0.56 to 1.12 kg/ha are the most promising application methods and rates. The best control would be achieved if the application were made 4–8 wk after planting and before irrigation when using granules. Control has been inconsistent and requires further evaluation. Therefore, field evaluations of propiconazole and other sterol-inhibiting fungicides will be continued.

Despite the low percentage of radioactivity translocated to the roots of cotton plants after application of ^{14}C-propiconazole to leaves, the theoretical concentration of propiconazole accumulating in roots after a foliar application was calculated to be sufficient to inhibit the growth of *P. omnivorum*. Bioassay data from roots of foliar-treated plants support this thesis.

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LITERATURE CITED

1. Buchenauer, H., and Rohner, E. 1981. Effect of triadimefon and triadimenol on growth of various plant species as well as on gibberellin content and sterol metabolism in shoots of barley seedlings. Pest. Biochem. Phys. 15:58-70.
2. Gunasekaran, M., Hess, W. M., and Weber, D. J. 1974. Lipids and ultrastructure of *Phymatotrichum omnivorum*. Trans. Br. Mycol. Soc. 63:519-525.
3. Gunasekaran, M., and Weber, D. J. 1974. Physiological studies on *Phymatotrichum omnivorum* VI. Lipid composition of mycelium and sclerotia. Mycopathol. Mycol. Appl. 52:261-266.
4. Hine, R. B., Johnson, D. L., and Wenger, C. J. 1969. The persistence of two benzimidazole fungicides in soil and their fungistatic activity against *Phymatotrichum omnivorum*. Phytopathology 59:798-801.
5. Hine, R. B., and Whitson, R. S. 1982. Activity of CGA-64250 (Propiconazol) against *Phymatotrichum omnivorum*. (Abstr.) Phytopathology 72:980.
6. Hine, R. B., Whitson, R. S., and Lyda, S. D. 1983. Control of Phymatotrichum root rot in Arizona with propiconazole. (Abstr.) Phytopathology 73:959.
7. Lyda, S. D., and Burnett, E. 1970. Influence of benzimidazole fungicides on *Phymatotrichum omnivorum* and Phymatotrichum root rot of cotton. Phytopathology 60:726-728.
8. Siegel, M. R. 1981. Sterol-inhibiting fungicides: Effect on sterol biosynthesis and sites of action. Plant Dis. 65:986-989.
9. Whitson, R. S. 1984. Evaluations of sterol-inhibiting fungicides for control of Phymatotrichum root rot of cotton. Ph.D. dissertation. University of Arizona, Tucson. 57 pp.