Effects of Chlorpyrifos and *Pythium splendens* on Growth of Rex Begonia

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ABSTRACT

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Incorporating granular chlorpyrifos (7.42 g a.i./m^3) or drenching with an equivalent amount of a wettable powder formulation significantly reduced growth of *Begonia* × *rex-cultorum*. Untreated plants inoculated with *Pythium splendens* were smaller than control plants. When plants were treated with chlorpyrifos and inoculated with *P. splendens*, an interaction between these two factors resulted in a significantly synergistic reduction in plant growth. Importance of the interaction was influenced by formulation and rate of chlorpyrifos used. Stunting caused by this interaction was reduced when plants were treated with metalaxyl, which is effective in controlling Pythium root rot.

Chlorpyrifos has been used in Florida to prevent infestation of potting media for ornamental plants with *Diaprepes abbreviatus* (L.) (sugarcane rootstalk borer weevil) and *Solenopsis invicta* Buren (red imported fire ant). Only granular formulations of chlorpyrifos and heptachlor were legally available for use to satisfy quarantine restrictions for these pests during the first half of 1981. At this time, growers began to note that certain plants died when planted in potting media treated with chlorpyrifos. Root tissue from affected plants was collected, and *Pythium* spp. were isolated from some root systems. Phytotoxicity studies with chlorpyrifos conducted in sterile potting medium indicated that the insecticide caused significant growth reductions of a number of greenhouse-grown ornamental plants including *Begonia* × *rex-cultorum* L. H. Bailey (Rex begonia). None of the plants treated with chlorpyrifos died (L. S. Osborne, *unpublished*).

Chlorpyrifos has been shown to have fungicidal activity in vitro against a number of pathogens including species of *Pythium, Fusarium, Rhizoctonia,* and *Curvularia* from turf (3,5). This insecticide also has been shown to suppress white mold disease of peanuts caused by *Sclerotium rolfsii* Sacc. (1,2,6). Other soil pesticides have been found to increase severity of sugar beet root rot caused by *Rhizoctonia solani* Kühn (4) and cucumber root rots caused by *Rhizoctonia* spp., *Fusarium oxysporum*, and *Pythium* spp. (7). Our studies were initiated to evaluate the effects of chlorpyrifos and Pythium root rot, alone and in combination, on growth and survival of Rex begonia.

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MATERIALS AND METHODS

Healthy leaf cuttings of Rex begonia were rooted in steam-treated potting medium under intermittent mist. The potting medium (50% Canadian peat, 25% cypress shavings, and 25% pine bark, v/v) was steam-treated (1.5 hr at 190 C) before amendment with 4.4 kg of Osmocote 19-6-12, a slow-release fertilizer (Sierra Chemical Co., Milpitas, CA), 4.2 kg of dolomite, and 0.9 kg of Micromax (micronutrient source, Sierra) per cubic meter of medium. Plants were grown in 10-cm plastic pots in a glasshouse with air temperatures ranging from 15 to 37 C, depending on time of year, and a maximum light level of 200 μmol s^{-1} m^{-2}. An isolate of *P. splendens* Braun originally obtained from diseased roots of *Epipremnum aureum* (Linden & Andre) Bunt (pothos) was used in all trials. Inoculum was prepared from cultures of *P. splendens* grown on cornmeal agar medium (CMA; Difco Laboratories, Detroit, MI) for 3 days in the dark. Each plate was blended with 200 ml of sterilized, deionized water in a Waring Blendor for 15 sec. The resulting slurry (primarily mycelia) was added at a rate of 10 ml/pot by syringe injection into the potting medium. Control plants were treated similarly with a slurry made from uninoculated CMA plates.

Insecticide treatments in each of four tests consisted of either soil incorporation by mixing a single application of a 5% granular formulation of chlorpyrifos into the potting mix before planting or drenching each pot after planting with 40 ml of a solution containing a 50% wettable powder formulation (Dursban 50WP) of this pesticide. Unless otherwise noted, plants in all tests that were drenched or had chlorpyrifos incorporated into the soil received 7.42 g a.i. of pesticide per cubic meter of potting mix (1× rate).

Because tests were replicated at different times of the year, all tests were blocked by time and thus arranged in a randomized complete block design. Data were evaluated by analysis of variance.

Test 1. Effect of a granular formulation of chlorpyrifos. The first experiment was conducted as a 2 × 2 factorial test with 10 pots per treatment. The first factor was medium amended or not amended with with the 1× rate of granular chlorpyrifos and the second factor was inoculation with *P. splendens* or treatment with CMA as described earlier. Inoculation was done the day after potting in the appropriate soil. This experiment was performed three times beginning 23 January, 1 February, and 21 June 1984. Numbers of leaves per plant were recorded at the beginning of the experiment and again after about 1 mo.

Test 2. Effect of chlorpyrifos formulation. The second experiment was conducted as a 3 × 2 factorial test consisting of six treatments with 10 pots per treatment. The first factor was soil amended with the granular formulation of chlorpyrifos, drenched with the 50% wettable powder formulation, or untreated, and the second factor was inoculation with *P. splendens* or CMA alone. This experiment was performed twice beginning on 28 June and 28 August 1984. Numbers of leaves per plant were recorded at the beginning of the experiment and again after about 1 mo.

Test 3. Effect of chlorpyrifos rate. A third experiment was conducted as a 2 × 4 factorial test with 10 pots per treatment. The first factor was inoculation with *P. splendens* or treatment with CMA alone. The second factor consisted of four drench rates of the wettable powder formulation of chlorpyrifos: 0.0, 0.25, 0.5, or 1× (× rate = 0.58 g a.i./L). This experiment was performed twice with the day of pesticide application beginning on 26 July and 3 October 1984. Plants were inoculated with *P. splendens* or treated with uninoculated CMA the next day. Numbers of leaves per plant were recorded at the beginning of the experiment and again after about 1 mo.

Test 4. Effect of metalaxyl and chlorpyrifos drenches. A fourth experi-

Table 1. Effects of chlorpyrifos treatment and *Pythium* inoculations on growth of Rex begonia[a]

Chemical treatment	*P. splendens* inoculation	Mean no. leaves per plant
Chlorpyrifos		
50WP	+	6.8[b]
drench	−	15.5
Chlorpyrifos 5G	+	11.8
incorporation	−	15.2
Water	+	14.3
Water	−	16.7

[a] This test was repeated twice and was thus blocked accordingly in the analysis of variance. Values represent the mean number of leaves for 20 plants (10 from each block). Chlorpyrifos was applied at the rate of 7.42 g a.i./m^3.
[b] Differences between chemical and *Pythium* treatments were significant at $P = 0.01$, and the interaction between chemical and *Pythium* treatments was significant at $P = 0.05$.

ment was conducted as a 2 × 2 × 2 factorial test with 10 pots per treatment. The first factor was a potting medium drench with 40 ml of water or a solution containing the 1× rate of chlorpyrifos. The second factor was a drench with 40 ml of water or a solution containing 6.3 g a.i. metalaxyl (Subdue 2E) per 100 L of water. The third factor was inoculation with *P. splendens* or treatment with CMA alone. Chlorpyrifos was applied the day rooted plants were potted, metalaxyl was applied the following day, and the *P. splendens* inoculation or CMA treatment occurred 2 days later. This experiment was performed twice beginning 22 June and 8 August 1984. Numbers of leaves per plant were recorded at the beginning of the experiment and again after about 1 mo.

Test 5. Recovery of *P. splendens*. A fifth experiment was conducted as a 2×2 factorial test with 10 pots per treatment to verify involvement of *P. splendens* in the interaction. The first factor was a potting medium drench with 40 ml of water or a solution containing the 1× rate of chlorpyrifos. The second factor was inoculation with *P. splendens* or treatment with CMA alone. Chlorpyrifos was applied the day rooted plants were potted, and the *P. splendens* inoculation or CMA treatment occurred 2 days later. This experiment was performed once beginning 10 July 1986. Numbers of leaves per plant were recorded at the beginning of the experiment and again after about 1 mo. After 1 mo, fresh top weights were determined for each replicate. Root tissue was sampled for five replicates per treatment. Tissue samples were disinfested in 95% ethanol for 5 sec and rinsed in sterilized, deionized water before being placed on PVP agar in plastic petri plates. Plates were incubated for 3 days at 26 C and then evaluated for the presence of *P. splendens*.

Table 2. Effects of chlorpyrifos rate and *Pythium* inoculation on growth of Rex begonia[a]

Chlorpyrifos rate (g a.i./L)	*P. splendens* inoculation	Test 1 Mean no. leaves	Test 1 Fresh top wt (g)	Test 2 Mean no. leaves	Test 2 Fresh top wt (g)
0.00	−	16.0	30.8	35.9	127.0
0.00	+	16.0	27.4	35.8	119.5
0.15	−	14.0	26.1	26.2	90.3
0.15	+	13.1	18.4	27.7	66.2
0.29	−	12.8	21.6	27.2	77.4
0.29	+	9.5	8.3	19.9	39.1
0.58	−	13.8	17.1	19.0	46.4
0.58	+	6.0	8.0	14.8	17.7

	Significance[b] Leaf no.	Significance[b] Top wt
Test (T)	$P = 0.01$	$P = 0.01$
Chlorpyrifos (C)	$P = 0.01$	$P = 0.01$
Pythium (P)	$P = 0.01$	$P = 0.01$
T × C	$P = 0.01$	$P = 0.01$
T × P	NS[c]	$P = 0.01$
C × P	$P = 0.10$	$P = 0.05$
T × C × P	NS	NS

[a] This test was repeated three times and thus blocked accordingly in the analysis of variance. Values represent the mean number or weight of leaves for 30 plants (10 from each block).
[b] Significant effects are given for the combined data from both tests.
[c] NS = not significant.

Table 3. Effects of metalaxyl, chlorpyrifos, and *Pythium* inoculations on growth of Rex begonia[a,b]

Treatment Chlorpyrifos	Treatment Metalaxyl	Treatment *Pythium*	Fresh top wt (g)
+	+	+	33.3
+	+	−	37.0
+	−	+	13.8
+	−	−	35.9
−	+	+	67.5
−	+	−	63.3
−	−	+	56.3
−	−	−	68.5

[a] This test was repeated twice and thus blocked accordingly in the analysis of variance. Values represent the mean top weight for 20 plants (10 from each test). Chlorpyrifos was applied at the rate of 7.42 g a.i./m^3. Forty milliliters of metalaxyl (6.3 g a.i./100 L of water) was applied to each of the appropriate pots.
[b] Differences between test (T), chlorpyrifos (C), metalaxyl (M), *Pythium* (P), and interactions for T × C and M × P were significant at $P = 0.01$. The interactions for P × C, T × M × P, and T × M × C were significant at $P = 0.05$.

RESULTS AND DISCUSSION

Test 1. Effect of a granular formulation of chlorpyrifos. When means for the two uninoculated treatments were compared, a 22% reduction in mean number of leaves was observed resulting from medium amendment with chlorpyrifos. Plants potted in unamended medium and inoculated with *P. splendens* were about 8% larger than uninoculated plants (mean number of leaves 13.1 and 12.1, respectively). This difference was not significant; however, plants potted in amended medium and inoculated with *P. splendens* were about 35% smaller than uninoculated plants (mean number of leaves 6.1 and 9.4, respectively). A significant ($P = 0.01$) interaction between the two factors occurred.

Test 2. Effect of chlorpyrifos formulation. All plants inoculated with *P. splendens* had significantly ($P = 0.01$) fewer leaves than uninoculated plants (Table 1). In addition, plants treated with chlorpyrifos granules and inoculated showed the same trend as noted in test 1, although the effect was less dramatic. When plants were drenched with the equivalent amount of chlorpyrifos in the wettable powder formulation, they were severely stunted. The difference noted between inoculated plants drenched with chlorpyrifos and those planted into potting medium amended with granular chlorpyrifos may be due to the differential rates at which the toxicant was available. The toxicant is released more slowly and over a longer period of time from the granules than the wettable powder formulation. As in test 1, chlorpyrifos was phytotoxic, with the interaction between *P. splendens* and chlorpyrifos significant ($P < 0.05$). The effect of inoculating with *P. splendens* was significant ($P < 0.01$) and resulted in a 14% reduction in mean number of leaves in the water-treated plants and a 31%

reduction over all inoculated treatments.

Test 3. Effect of chlorpyrifos rate. The interaction between chlorpyrifos and *P. splendens* was influenced by pesticide concentration (Table 2). As chlorpyrifos concentration increased, both fresh weight of tops and mean numbers of leaves decreased, with significant differences at the $P = 0.05$ and 0.10, respectively. This experiment also demonstrated that top weight was a more sensitive indicator of this interaction than leaf numbers.

Test 4. Effect of metalaxyl and chlorpyrifos drenches. A significant ($P < 0.05$) interaction between chlorpyrifos and *P. splendens* was observed (Table 3). This interaction was significantly reduced when plants also were treated with metalaxyl. In treatments without metalaxyl drenches, inoculated plants treated with chlorpyrifos showed about a 62% reduction in top growth compared with uninoculated plants. Uninoculated plants treated with metalaxyl and chlorpyrifos had a mean top weight of 37.0 g compared with 33.3 g for those treated with the same material but inoculated (about a 10% reduction). By reducing the effects from *Pythium* inoculations with the fungicide, all inoculated plants had about the same mean top weight (50.4 g = the mean of 33.3 and 67.5 g) as all uninoculated plants (50.2 g = the mean of 37.0 and 63.3 g) regardless of chlorpyrifos treatment.

Test 5. Recovery of *P. splendens. P. splendens* was not isolated from either of the uninoculated treatments but was isolated from root tissue in inoculated treatments. The recovery, however, was less in the chlorpyrifos-*Pythium* treatment than in the *Pythium* treatment. The mean top weights followed the same trends as observed in previous experiments with 49.7, 31.2, 29.0, and 16.5 g/plant for the water control, *Pythium* only, chlorpyrifos only, and *Pythium*-chlorpyrifos treatments, respectively.

The results of these studies demonstrate that an adverse interaction exists between chlorpyrifos and *P. splendens* that reduces or limits growth of Rex begonia. The severity of the interaction can be influenced by the formulation and rate of chlorpyrifos used, and the negative effects from the interaction can be greatly reduced by treating the soil with metalaxyl to limit the effects of Pythium root rot. The interaction observed may have been the result of reducing plant vigor by the application of a phytotoxic chemical, making the plant more susceptible to disease. These results also emphasize the need to be cautious when recommending soil drenches of chlorpyrifos to control either insect pests or fungal pathogens unless all of the elements of the plant production system have been carefully evaluated. Pesticides influence the entire system, and even single applications can produce dramatic effects on other biotic components of that system.

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