In Vivo Expression of Resistance to Metalaxyl by a Nursery Isolate of *Phytophthora parasitica* from *Catharanthus roseus*

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ABSTRACT

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Populations of a metalaxyl-insensitive isolate of *Phytophthora parasitica* from a nursery in southern California were determined every 2 wk for 10 wk from the root zones of container-grown *Catharanthus roseus* either treated or not treated with metalaxyl or fosetyl-Al. When compared with no treatment, metalaxyl applied every 4 wk as soil drenches at 18.8 or 37.4 mg a.i./L and fosetyl-Al applied every 4 wk as foliar sprays at 4.8 g a.i./L had no effect on populations of the isolate. However, increase of the pathogen population was delayed and the population was significantly less ($P < 0.05$) than that of the nontreated control at 6 and 10 wk when fosetyl-Al soil drenches at 1.92 g a.i./L were applied every 4 wk. Based on determinations of fresh shoot and root weights, metalaxyl failed to control disease and fosetyl-Al provided acceptable control.

Additional keywords: Aliette, Subdue

Catharanthus roseus (L.) G. Don is one of the more commonly used bedding plants in the desert areas of southern California because it tolerates heat and strong, direct sunlight. Root and crown rot, caused by *Phytophthora parasitica* Dastur, is a common problem on this host. Symptoms typically are associated with the final stages of disease development, but infected plant roots can support a readily detectable population of the pathogen without manifest symptoms.

Since its registration, metalaxyl (Subdue) has been the primary fungicide used by nursery personnel to control root and crown rots caused by *Phytophthora* spp. Insensitivity to metalaxyl in vitro was reported for two isolates of *P. parasitica* from *C. roseus* in southern California (6). However, the in vivo expression of resistance to metalaxyl has not always correlated with that expressed in vitro (3). The objective of this study was to assess the in vivo sensitivity of one of these metalaxyl-insensitive isolates of *P. parasitica* to metalaxyl and fosetyl-Al.

MATERIALS AND METHODS

A monozoosporic culture of an isolate of *P. parasitica* (P-015F) recovered from *C. roseus* from southern California that had previously been shown to be insensitive to metalaxyl in vitro (6) was used in this study. Experimental procedures followed those described previously (7).

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Chlamydospores for infesting the potting medium were produced by the method of Tsao (14), harvested by the method of Ramirez and Mitchell (10), and quantified with a hemacytometer. Steam-pasteurized U.C. mix (fine sand and peat, 1:1) was infested at five chlamydospores per gram dry weight of the medium. The potting medium was infested by adding aliquots of the chlamydospore suspension to 18 2-kg batches of the medium, each of which was mixed in a Hobart mixer for 5 min. All infested U.C. mix then was combined in a cement mixer and mixed for an additional 10 min. Six samples were removed and assayed to determine the actual inoculum level achieved and the uniformity of infestation.

A magnetic stirrer was used to mix a 50-g subsample from each sample in a 250-ml beaker with 100 ml of 0.25% water agar amended with 10 mg of rifampicin and 250 mg of ampicillin per liter. Then, 1-ml subsamples were spread over the surface of each of 20 petri plates containing PARPH, a medium selective for *Phytophthora* spp. (9); additional 1-ml subsamples were dried overnight at 50 C. Plates were incubated in the dark at 25 C for 72 hr, the U.C. mix was rinsed from the agar under a gentle stream of water, and the colonies of *P. parasitica* were counted. All plates were examined immediately after washing and again 24 hr later. The pathogen was identified either macroscopically on the basis of distinct colony morphology or microscopically on the basis of hyphal characteristics. Populations were expressed as propagules per gram dry weight of the potting medium (ppg). The actual level of inoculum achieved was 7.1 chlamydospores per gram of potting medium.

Infested U.C. mix (200 g) was added to each of 75 10-cm-diameter plastic pots and covered with noninfested U.C. mix (400 g). Noninfested U.C. mix (600 g) was used in 15 pots. A single, 3-wk-old seedling of *C. roseus* was transplanted into each pot. The pots were separated into six treatments, each with five replications of three pots each, and arranged on a greenhouse bench in a randomized complete block design. Plants were fertilized three times a week with dilute Hoagland's solution applied by means of drip tubes so as not to splash inoculum between pots. Enough water was added each time so as to thoroughly wet the root zone.

Fungicide applications were initiated immediately after seedling transplantation. Metalaxyl (Subdue 2E) was applied as a soil drench of 50 ml per pot every 4 wk at a concentration of 18.7 or 37.4 mg a.i./L. Fosetyl-Al (Aliette 80WP) was applied every 4 wk as a soil drench of 50 ml per pot or as a foliar spray to runoff but with care not to introduce the fungicide directly into the potting medium. Because the seedlings were small when transplanted, the first application of fosetyl-Al was as a soil drench for both soil drench and foliar spray treatments. Fosetyl-Al concentrations were 1.92 g a.i./L for soil drenches and 4.8 g a.i./L for foliar sprays.

Samples of the potting medium were taken from all pots beginning 2 wk after seedling transplantation and every 2 wk thereafter through 10 wk. The 4- and 8-wk samples were taken before fungicides were applied. On each assessment date, a single core was removed from each pot with a 1-cm-diameter cork borer, and the cores from the three pots within a replicate were combined into a single sample. Each sample (approximately 5.4 g) was placed in a 50-ml beaker with a magnetic stir bar and 15 ml of 0.25% water agar amended with antibiotics, then plated as described above. Then, 15 ml of 0.25% water agar was added to the suspension to provide a second dilution, and eight 1-ml subsamples were plated. All plates were incubated, washed, and examined for colonies of *P. parasitica* as described above. Dilutions were adjusted as needed during the course of the experiments. Fresh shoot and root weights were determined for all plants after the final population assessment. The experiment was repeated once.

Analysis of variance was calculated for comparisons of population data at each sample date after transformation of the data by $\log_{10}$ (ppg+1). Analysis of variance and LSD values were calculated for comparisons of plant growth data. All statistical analyses were performed with the SAS System (SAS Institute Inc., Cary, NC).

RESULTS

Populations of *P. parasitica* were relatively stable across all treatments during the first 4 wk after seedlings were transplanted, then increased rapidly between the fourth and eighth weeks (Fig. 1). When compared with no treatment, soil drenches of metalaxyl at 18.8 or 37.4 mg a.i./L applied every 4 wk had no effect on populations of the metalaxyl-insensitive isolate. Fosetyl-Al had no effect on populations when applied every 4 wk as a foliar spray at 4.8 g a.i./L but delayed the increase of populations when applied every 4 wk as a soil drench at 1.92 g a.i./L. However, populations differed significantly ($P < 0.05$) from those of the nontreated control only at the 6- and 10-wk sample dates, 2 wk after the fungicide drenches. By the fourth week after drenches of fosetyl-Al, populations had risen to levels comparable to those of the nontreated controls. Similar trends were observed when the experiment was repeated.

Shoot weights of plants treated with either concentration of metalaxyl were significantly less ($P < 0.05$) than those of plants in noninfested U.C. mix or plants treated with fosetyl-Al and did not differ significantly from those of control plants in infested U.C. mix (Fig. 2). Shoot weights of plants treated with either soil drenches or foliar sprays of fosetyl-Al did not differ significantly from those of control plants in noninfested U.C. mix. Root weights of plants treated with metalaxyl were significantly less than those of control plants in infested U.C. mix (Fig. 2). Root weights of plants treated with fosetyl-Al were significantly less than those of plants in noninfested U.C. mix and, for the soil drench treatment, greater than those of control plants in infested U.C. mix.

DISCUSSION

The isolate of *P. parasitica* used in this study was one of two obtained from *C. roseus* from a nursery in southern California that previously had been shown to be insensitive to metalaxyl in vitro (6). The mean EC_{50} for inhibition of linear growth of this isolate was 717.4 μg a.i./ml. In contrast, mean EC_{50} values ranged from 0.255 to 3.080 μg a.i./ml for 24 metalaxyl-sensitive isolates of *P. parasitica*. This level of resistance is approximately 19 times greater than the highest labeled rate for use of metalaxyl on *C. roseus*.

Previously we found that the increase in the population of a metalaxyl-sensitive isolate of *P. parasitica* was delayed dramatically by soil drenches of metalaxyl at 37.4 mg a.i./L applied every 4 wk (7). Based on determinations of fresh shoot and root weights, metalaxyl provided excellent control of disease caused by that particular isolate. In contrast, metalaxyl at this rate and frequency of application had no effect on populations of the metalaxyl-insensitive isolate of *P. parasitica* used in the current study. Based on determinations of fresh shoot and root weights, metalaxyl failed to control disease caused by this isolate. As with the metalaxyl-sensitive isolate used in the previous study (7), however, soil drenches of fosetyl-Al at 1.92 g a.i./L did affect populations of the metalaxyl-insensitive isolate and did provide acceptable disease control even though it did not prevent infection. These results are similar to those with metalaxyl-resistant isolates of *Pythium aphanidermatum* (Edson) Fitzp. from turfgrass in Pennsylvania (11) and Kentucky (12).

Although widespread failure of metalaxyl to control disease was not observed in the nursery from which the two metalaxyl-insensitive isolates of *P. parasitica* were recovered, resistance to metalaxyl was expressed in vivo by the isolate chosen for study. Furthermore, this metalaxyl-resistant isolate appears to be as virulent as sensitive wild-type isolates. For nurseries where plants are grown in containers, widespread failures in disease control would not necessarily be expected immediately after the appearance of resistance because of the low frequency with which such resistance ap-

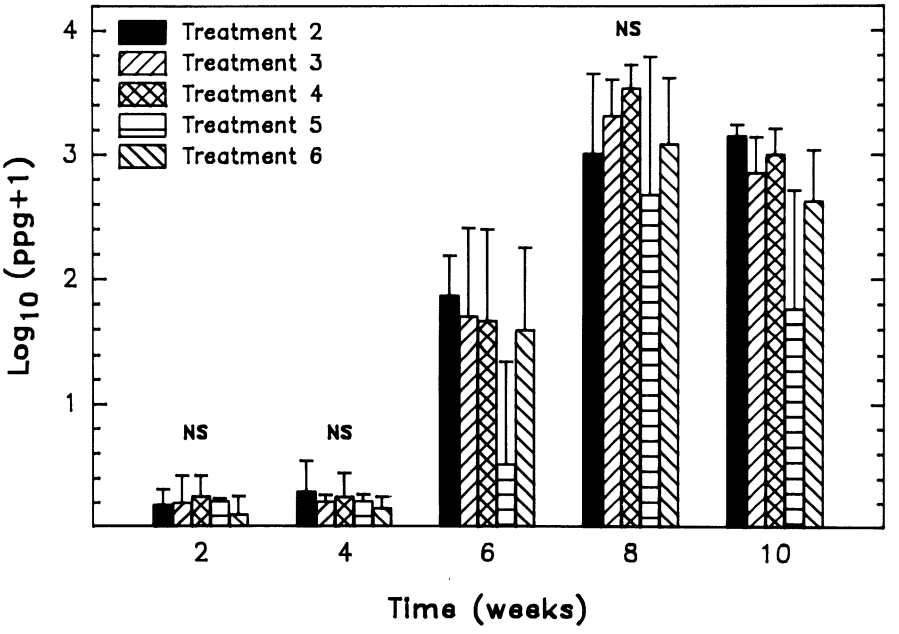

Fig. 1. Populations of a metalaxyl-insensitive isolate of *Phytophthora parasitica* within the root zones of container-grown *Catharanthus roseus* in relation to application rate of metalaxyl and application rate and method of fosetyl-Al. Fungicides were applied every 4 wk beginning at week 0. Treatment 1 (not shown) = noninfested control, treatment 2 = no fungicide, treatment 3 = metalaxyl soil drenches at 18.8 mg a.i./L, treatment 4 = metalaxyl soil drenches at 37.4 mg a.i./L, treatment 5 = fosetyl-Al soil drenches at 1.92 g a.i./L, and treatment 6 = fosetyl-Al foliar spray at 4.8 g a.i./L. Vertical lines represent standard deviations; NS denotes no significant differences ($P < 0.05$) observed among treatments according to analysis of variance.

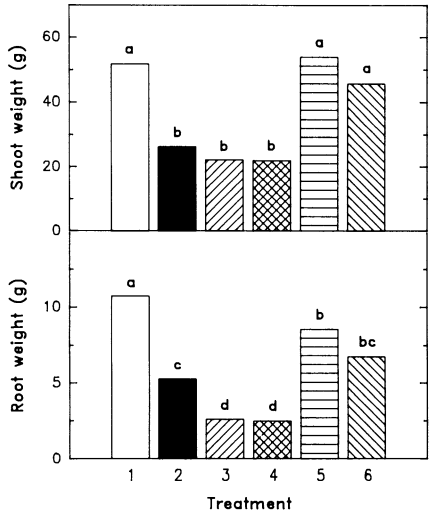

Fig. 2. Fresh shoot and root weights of *Catharanthus roseus* in relation to application rate of metalaxyl and application rate and method of fosetyl-Al. Weights were determined 10 wk after seedlings were transplanted into U.C. mix either infested or not infested with chlamydospores of *Phytophthora parasitica*. Fungicides were applied every 4 wk beginning at week 0. Treatment 1 = no fungicide and no inoculum, treatment 2 = no fungicide, treatment 3 = metalaxyl soil drenches at 18.8 mg a.i./L, treatment 4 = metalaxyl soil drenches at 37.4 mg a.i./L, treatment 5 = fosetyl-Al soil drenches at 1.92 g a.i./L, and treatment 6 = fosetyl-Al foliar spray at 4.8 g a.i./L. Treatments capped with the same letter are not significantly different ($P < 0.05$) according to Fisher's LSD.

pears (2,8,13) and the time needed for the pathogen population to increase and be dispersed. Widespread disease control failures occur only after a large part of the pathogen population has become resistant. The rate at which this resistant population becomes established depends largely on the stability of the resistance, the selection pressure exerted on the pathogen population, and the ability of the pathogen to disperse. Because species of *Phytophthora* and *Pythium* have been detected in recycled irrigation water in nurseries in California (1), the requirement that commercial nurseries in certain areas of California trap and recycle all runoff water greatly increases the risk of recycling fungicide-resistant populations of these pathogens in those nurseries. Thus, the appearance of resistance could eventually result in control failures if the treatment of recirculated water is not sufficient to eliminate propagules of *Phytophthora* spp. and the selection pressure due to the continued use of the fungicide is maintained. That the isolate used in this study still responded to fosetyl-Al supports the recommendations to alternate or to use mixtures of fungicides (4,5,15) for the control of soilborne root-infecting species of *Phytophthora*.

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LITERATURE CITED

1. Ali-Shtayeh, M. S., MacDonald, J. D., and Kabashima, J. 1991. A method for using commercial ELISA tests to detect zoospores of *Phytophthora* and *Pythium* species in irrigation water. Plant Dis. 75:305-311.
2. Davidse, L. C. 1981. Resistance to acylalanine fungicides in *Phytophthora megasperma* f. sp. *medicaginis*. Neth. J. Plant Pathol. 87:11-24.
3. Davidse, L. C. 1982. Acylalanines: Resistance in downy mildews, *Pythium* and *Phytophthora* spp. Pages 118-127 in: Fungicide Resistance in Crop Protection. J. Dekker and S. G. Georgopoulos, eds. Pudoc, Wageningen.
4. Dekker, J. 1982. Countermeasures for avoiding fungicide resistance. Pages 177-186 in: Fungicide Resistance in Crop Protection. J. Dekker and S. G. Georgopoulos, eds. Pudoc, Wageningen.
5. Delp, C. J. 1980. Coping with resistance to plant disease control agents. Plant Dis. 64:652-657.
6. Ferrin, D. M., and Kabashima, J. N. 1991. In vitro insensitivity to metalaxyl of isolates of *Phytophthora citricola* and *P. parasitica* from ornamental hosts in southern California. Plant Dis. 75:1041-1044.
7. Ferrin, D. M., and Rohde, R. G. 1992. Population dynamics of *Phytophthora parasitica*, the cause of root and crown rot of *Catharanthus roseus*, in relation to fungicide use. Plant Dis. 76:60-63.
8. Joseph, M. C., and Coffey, M. D. 1984. Development of laboratory resistance to metalaxyl in *Phytophthora citricola*. Phytopathology 74:1411-1414.
9. Mitchell, D. J., Kannwischer-Mitchell, M. E., and Zentmyer, G. A. 1986. Isolating, identifying, and producing inoculum of *Phytophthora* spp. Pages 63-66 in: Methods for Evaluating Pesticides for Control of Plant Pathogens. K. D. Hickey, ed. American Phytopathological Society, St. Paul, MN.
10. Ramirez, B. N., and Mitchell, D. J. 1975. Relationship of density of chlamydospores and zoospores of *Phytophthora palmivora* in soil to infection of papaya. Phytopathology 65:780-785.
11. Sanders, P. L. 1984. Failure of metalaxyl to control Pythium blight on turfgrass in Pennsylvania. Plant Dis. 68:776-777.
12. Sanders, P. L. 1987. Failure of metalaxyl to control Pythium blight on Kentucky golf courses. (Abstr.) Phytopathology 77:121.
13. Stack, J. P., and Millar, R. L. 1985. Isolation and characterization of a metalaxyl-insensitive isolate of *Phytophthora megasperma* f. sp. *medicaginis*. Phytopathology 75:1387-1392.
14. Tsao, P. H. 1971. Chlamydospore formation in sporangium-free liquid cultures of *Phytophthora parasitica*. Phytopathology 61:1412-1413.
15. Wolfe, M. S. 1982. Dynamics of the pathogen population in relation to fungicide resistance. Pages 139-148 in: Fungicide Resistance in Crop Protection. J. Dekker and S. G. Georgopoulos, eds. Pudoc, Wageningen.