Population Dynamics of *Phytophthora parasitica*, the Cause of Root and Crown Rot of *Catharanthus roseus*, in Relation to Fungicide Use

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ABSTRACT

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.

Populations of *Phytophthora parasitica* from the root zones of container-grown *Catharanthus roseus* treated with fosetyl-Al applied as foliar sprays or soil drenches and metalaxyl applied as soil drenches were determined 3, 5, 7, 9, and 11 wk after transplanting. Fosetyl-Al had no effect on pathogen populations when applied every 4 wk as foliar sprays at either 2.4 or 4.8 g a.i./L. Pathogen populations were affected more when fosetyl-Al was applied every 4 wk as soil drenches at 1.92 than at 0.96 g a.i./L. Fresh root and shoot weights of plants treated with fosetyl-Al were not significantly different than those of nontreated plants in infested soil and were significantly less than those of nontreated plants in noninfested soil. Metalaxyl delayed the increase of pathogen populations at all concentrations tested but was most effective when applied every 4 wk at 37.4 mg a.i./L. Fresh root and shoot weights of plants treated with metalaxyl were significantly greater than those of nontreated plants in infested soil and were not significantly different than those of nontreated plants in noninfested soil.

Additional keywords: Aliette, Subdue

Root and crown rot, caused by *Phytophthora parasitica* Dastur, is a common problem on periwinkle (*Catharanthus roseus* (L.) G. Don) in southern California. The development and registration of the two classes of systemic fungicides, the acylalanines and the alkyl phosphonates, has greatly improved our ability to control diseases caused by *P. parasitica* and other plant pathogens within the order Peronosporales (9). The degree of control achieved can be affected by the use patterns of these fungicides and by their mobility and persistence in the soil environment, which is influenced greatly by soil type, frequency and intensity of irrigation or rainfall, and microbial activity (1,20).

Use patterns of these fungicides are determined largely by economics (including costs of materials and labor and the value of the crop), disease intensity, and label restrictions. Label restrictions define both the concentrations and frequencies of application allowed. These restrictions, however, are generally derived from studies in which disease severity is judged as a visual assessment of disease rather than as an actual assessment of root infections or pathogen populations. The visual assessment of disease may be misleading in terms of the actual effect of a fungicide on the pathogen population and the extent of root infection. Although fungicide-treated plants are often symptomless, *Phytophthora* spp. can often be recovered readily from the roots (3,4,7). This is of particular concern for nursery crops such as *C. roseus*, for which even low levels of disease at the time of sale may eventually result in losses for the buyer. Benson (6) found that repeated applications of either metalaxyl or fosetyl-Al after inoculation of azaleas with *P. cinnamomi* Rands were sufficient to prevent root rot after transplanting into pathogen-free landscape beds. However, in this study, the first fungicide application was made immediately after inoculation. Previously, he had demonstrated that the timing of fungicide application in relation to the time of inoculation was critical to the amount of root infection that resulted; the least amount of infection occurred when metalaxyl was applied at the time of inoculation (4).

The objectives of these studies were to assess the influence of the concentration and the method and frequency of application of the fungicides, fosetyl-Al and metalaxyl, on populations of *P. parasitica* in the root zones of container-grown *C. roseus* and to contrast the evaluation of fungicide efficacy based on pathogen population dynamics with that based on plant growth parameters. A portion of this research was reported previously (12).

This research was supported by a grant from the California Association of Nurserymen.

Accepted for publication 23 June 1991 (submitted for electronic processing).

MATERIALS AND METHODS

A single-zoospore isolate of *P. parasitica* (P-012F) obtained from *C. roseus* from southern California was used in these studies. Chlamydospores for infesting potting medium were produced by the method of Tsao (21), harvested by the method of Ramirez and Mitchell (18), and quantified with a hemacytometer. Steam-pasteurized U.C. mix was infested at five chlamydospores per gram dry weight of potting medium. U.C. mix was infested by the addition of aliquots of the chlamydospore suspension to each of 18 2-kg batches of potting 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 assayed to determine the actual inoculum level achieved and the degree of uniformity of infestation.

A 50-g subsample from each sample was mixed 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 with a magnetic stirrer. One-milliliter subsamples were spread over the surface of each of 20 petri plates containing a medium selective for *Phytophthora* spp. (PARPH) (15). 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. Identification of the pathogen was done either macroscopically based on its distinct colony morphology or microscopically based on hyphal characteristics. Populations were expressed as propagules per gram dry weight of potting medium (ppg).

Infested potting medium (450 g) was added to each of 75 10-cm-diameter plastic pots and covered with noninfested potting medium (150 g). An additional 15 pots received noninfested U.C. mix only (600 g). 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 were 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 to avoid splashing inoculum between pots. Enough water was applied each time to thoroughly wet the root zone.

Fungicide applications were initiated immediately after transplanting. Fosetyl-Al (Aliette 80WP) was applied every 4 wk as a soil drench of 50 ml per pot or as a foliar spray applied to runoff so that fungicide was not introduced directly into the potting medium. Because of the small size of the seedlings at the time of transplanting, the first application of fosetyl-Al was made as a soil drench of 0.96 g a.i./L for all foliar spray treatments. Fungicide concentrations were 0.96 and 1.92 g a.i./L for the soil drench treatments and 2.4 and 4.8 g a.i./L for the foliar spray treatments. Metalaxyl (Subdue 2E) was applied as a soil drench of 50 ml per pot every 4 wk at concentrations of 9.35, 18.7, and 37.4 mg a.i./L and every 8 wk at a concentration of 37.4 mg a.i./L.

Samples of potting medium were taken from all pots beginning 3 wk after transplanting and every 2 wk thereafter through 11 wk. On each assessment date, a single core was removed from each pot with a 1-cm-diameter cork borer; cores from the three pots within a replicate were combined into a single sample. The U.C. mix from each sample (approximately 7 g) was placed in a 50-ml beaker with a magnetic stir bar and 15 ml of dilute water agar amended with antibiotics and was plated as described earlier. An additional 15 ml of dilute water agar then was added to the soil suspension to provide a second dilution, and an additional eight 1-ml subsamples were plated. All plates were incubated, washed, and examined for colonies of *P. parasitica* as described earlier. 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. Each experiment was repeated twice.

Analysis of variance and LSD values were calculated for comparisons of populations among treatments for each sample date following transformation of the data by $\log_{10}$ (ppg + 1). Paired *t* tests were used for the comparison of populations within individual treatments between successive sample dates. 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

When applied as a foliar spray, fosetyl-Al at either 2.4 or 4.8 g a.i./L had no effect on populations of *P. parasitica* when compared with the nontreated control (Fig. 1). Fosetyl-Al applied as a soil drench at 1.92 g a.i./L but not at 0.96 g a.i./L resulted in a significantly lower ($P < 0.10$) pathogen population compared with those of the nontreated control at the 9-wk assessment date only. No significant changes in populations within treatments were associated with the application of fosetyl-Al 4 wk after transplanting. Significant decreases ($P < 0.10$) in populations from one assessment date to the next were associated with the application of fosetyl-Al 8 wk after transplanting for all fungicide treatments. Significant increases in populations were observed for all treatments between the fifth and seventh weeks and between the ninth and 11th weeks.

Fresh shoot and root weights of plants in all treatments in infested potting medium were significantly less ($P < 0.05$) than those of plants in noninfested potting medium (Fig. 2). There were no significant differences in shoot or root weights among plants in infested potting medium. Similar trends were observed in repeated experiments (*data not presented*). In those experiments, although fresh shoot and root weights of plants in infested potting medium treated with drenches of fosetyl-Al were significantly greater than those in infested potting medium and not treated, significant differences in pathogen populations among treatments were not observed.

Soil drenches with metalaxyl delayed the buildup of populations of *P. parasitica* to detectable levels (Fig. 3). Once pathogen populations were detectable, they differed significantly ($P < 0.05$) among treatments only at the 7- and 9-wk sample dates. Populations were affected most when metalaxyl was applied at 37.4 mg a.i./L every 4 wk.

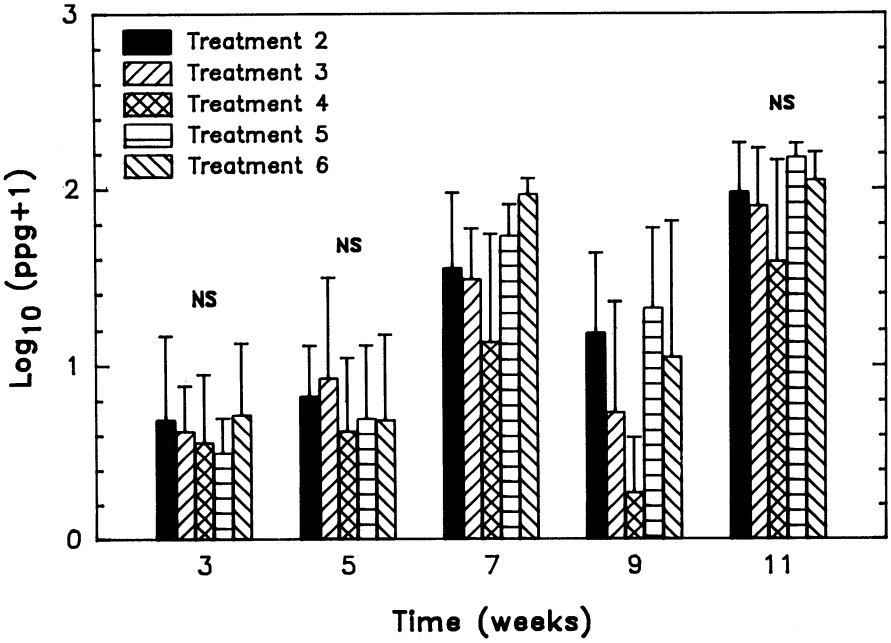

Fig. 1. Populations of *Phytophthora parasitica* from the root zones of container-grown *Catharanthus roseus* in relation to rate and method of application of fosetyl-Al. Fungicide treatments were applied at 0, 4, and 8 wk. Treatment 2 was no fungicide; treatments 3 and 4 were soil drenches at 0.96 and 1.92 g a.i./L, respectively; and treatments 5 and 6 were foliar sprays at 2.4 and 4.8 g a.i./L, respectively. The pathogen was not detected in treatment 1, the noninfested control (*not shown*). Vertical lines represent standard deviations. NS denotes that there were no significant differences ($P < 0.10$) among treatments on that sample date according to analysis of variance.

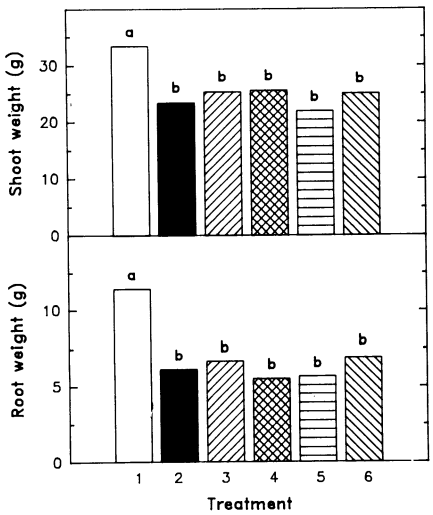

Fig. 2. Fresh shoot and root weights of *Catharanthus roseus* in relation to the rate and method of application of fosetyl-Al. Weights were determined 11 wk after transplanting into U.C. mix either not infested or infested with chlamydospores of *Phytophthora parasitica*. Treatment 1 was no fungicide and no inoculum; treatment 2 was no fungicide; treatments 3 and 4 were applied every 4 wk as soil drenches at 0.96 and 1.92 g a.i./L, respectively; and treatments 5 and 6 were applied every 4 wk as foliar sprays at 2.4 and 4.8 g a.i./L. Treatments capped with the same letter are not significantly different ($P < 0.05$) according to Fisher's LSD.

Pathogen populations were affected similarly when metalaxyl was applied at 18.7 mg a.i./L every 4 wk or 37.4 mg a.i./L every 8 wk. When applied at 9.35 mg a.i./L every 4 wk, populations were not significantly different than for the infested controls. No significant changes in populations within treatments were associated with any of the fungicide applications. Significant increases ($P < 0.05$) in populations were observed between the fifth and seventh weeks after transplanting for application rates of 0, 9.35, and 18.7 mg a.i./L but not for 37.4 mg a.i./L whether applied every 4 or every 8 wk.

Fresh shoot weights of plants in infested potting medium were significantly less than those in noninfested potting medium, and all metalaxyl treatments improved growth to the level of the noninfested control (Fig. 4). Fresh root weights were significantly less ($P < 0.05$) for plants treated with 37.4 mg a.i./L every 8 wk than for plants treated with metalaxyl every 4 wk regardless of the concentration. Root weights of plants treated with metalaxyl were not significantly different than those of the noninfested control plants, but root weights for all treatments were significantly greater than those of the infested control plants. Similar trends were observed in repeated experiments.

DISCUSSION

Strategies for the control of plant diseases are to eliminate or reduce the initial inoculum or delay its appearance, slow the rate of disease development, and shorten the time during which hosts are exposed to the pathogen (8). The use of fungicides for disease control employs the first two of these strategies. When used as protectants, fungicides delay the development of pathogen populations, and when applied repeatedly during the life of the crop, they slow the rate of increase of the pathogen population and disease (13).

Kannwischer and Mitchell (14) found that a single application of metalaxyl as a soil drench at time of transplant delayed the onset of mortality and increases in populations of *P. parasitica* var. *nicotianae* (Breda de Haan) Tucker within the rhizospheres of susceptible and resistant tobacco (*Nicotiana tabacum* L.) cultivars. Farih et al (11) found that monthly applications of metalaxyl to container-grown sweet orange (*Citrus sinensis* (L.) Osbeck) seedlings in the greenhouse resulted in significantly lower rhizosphere populations of *P. parasitica*. Populations were 91 and 100% less than for nontreated plants after 5 and 12 wk, respectively. Similarly, Pond et al (17) reported that both metalaxyl and efosite-Al (fosetyl-Al) resulted in significantly lower populations of *P. parasitica* within the rhizosphere of field-grown sweet orange rootstocks in California when applied through the drip irrigation system. Pathogen populations were 48–71 and 34–38% less than the nontreated trees for metalaxyl and efosite-Al treatments, respectively, after applications at monthly intervals (six applications per year) over 2 or 3 yr. Similarly, in Florida, Sandler et al (19) found that populations of *P. parasitica* from field-grown citrus treated with soil drenches of metalaxyl were generally significantly lower than from nontreated trees. Populations from trees treated with foliar sprays of fosetyl-Al, either at high or low application frequencies (usually four and two applications per season, respectively), were sometimes lower than from nontreated trees but were not consistently lower.

Benson (2) monitored populations of *P. cinnamomi* in the root zones of azaleas in landscape beds over a 2-yr period. Pathogen populations ranged from 0 to 0.07 ppg in plots treated with metalaxyl, 0.1 to 2.2 ppg in plots treated with fosetyl-Al, and 1.0 to 3.0 ppg in the nontreated plots. He concluded that only metalaxyl gave adequate control of Phytophthora root rot under the conditions of the study. The residual activity of these fungicides was greatly influenced by the frequency with which they had been applied (5).

As in most previous studies, we found that foliar applications of fosetyl-Al had no effect on populations of *P. parasitica*, whereas direct application to the potting medium occasionally reduced populations. This is most likely attributable to the limited translocation of fosetyl-Al as phosphonate from foliage to roots compared with the uptake of phosphonate by roots when fosetyl-Al is

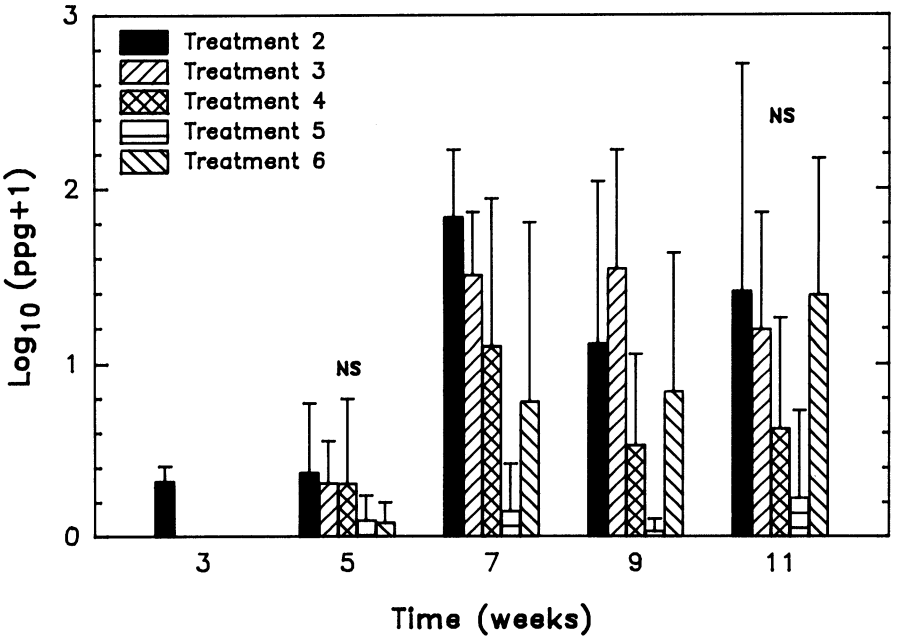

Fig. 3. Populations of *Phytophthora parasitica* from the root zones of container-grown *Catharanthus roseus* in relation to rate and frequency of application of metalaxyl. Fungicide treatments were applied as soil drenches beginning at week 0. Treatment 2 was no fungicide; treatments 3, 4 and 5 were applied every 4 wk at 9.35, 18.8, and 37.4 mg a.i./L, respectively; and treatment 6 was applied every 8 wk at 37.4 mg a.i./L. The pathogen was not detected in treatment 1, the noninfested control (*not shown*). Vertical lines represent standard deviations. NS denotes that no significant differences ($P < 0.05$) were observed among treatments on that sample date according to analysis of variance.

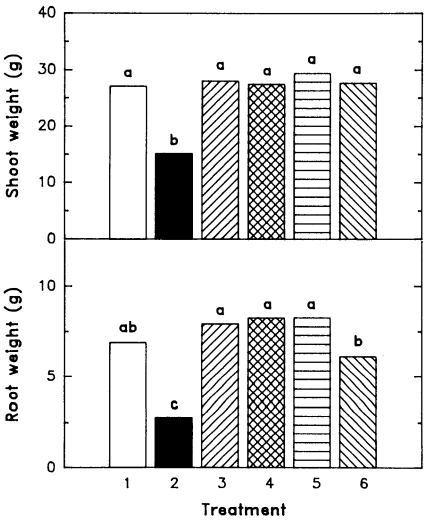

Fig. 4. Fresh shoot and root weights of *Catharanthus roseus* in relation to the rate and frequency of application of metalaxyl. Weights were determined 11 wk after transplanting into U.C. mix either not infested or infested with chlamydospores of *Phytophthora parasitica*. Treatment 1 was no metalaxyl and no inoculum; treatment 2 was no metalaxyl; treatments 3, 4, and 5 were applied every 4 wk as soil drenches at 9.35, 18.7, and 37.4 mg a.i./L, respectively; and treatment 6 was applied every 8 wk as a soil drench at 37.4 mg a.i./L. Treatments capped with the same letter are not significantly different ($P < 0.05$) according to Fisher's LSD.

applied to the soil and the short life of the material within the plant (16). Davis (10) had found that repeated applications of fosetyl-Al to tomato foliage at weekly intervals were required for effective control of root rot caused by *P. parasitica*.

Applications of metalaxyl to potting medium resulted in pathogen populations proportional to the concentration of the fungicide used when applied every 4 wk. For both fungicides, individual fungicide applications did not always result in significant decreases in pathogen populations and pathogen populations tended to increase during the intervals between applications. Significantly lower pathogen populations were not always associated with differences in plant growth. This is important to the nursery industry, particularly for growers whose plants are destined to be sold for use in the landscape. Although use of fungicides enables the production of visually healthy and vigorously growing plants, the assessment of fungicide efficacy based on plant growth may not reflect the true extent of pathogen population development within the root systems. Thus, once fungicide use is halted, pathogen populations may increase, resulting in subsequent plant mortality. An additional concern is that repeated fungicide applications may select for fungicide resistance in the pathogen population.

These results point out the potential danger of assessing the effects of soil fungicides strictly on the basis of plant growth parameters. The evaluation of fungicide efficacy and determination of use rates based on plant growth parameters may be misleading in terms of actual effects of the fungicide on the pathogen in soil. This type of data may be sufficient for field crops, vegetables, and floriculture crops for which yield is the primary concern but may not be sufficient for nursery crops for which the plant itself is the final product. Knowledge of the effects of fungicide treatments on pathogen population dynamics is crucial to their effective use in nurseries and should lead to improved strategies for their use. Our results confirm the importance of the prevention of the introduction and establishment of the pathogen in nursery crops. Once introduced, early detection of the pathogen is essential for effective disease control with fungicides.

ACKNOWLEDGMENTS

We thank Ciba-Geigy Corp., Greensboro, NC, and Rhone-Poulenc, Inc., Research Triangle Park, NC, for providing fungicides used in these studies. No endorsements are implied herein. We also thank S. Duffy, C. Han, E. Johnson, M. Hoang, and D. Johnson for their technical assistance.

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