Occurrence of *Phytophthora cinnamomi* on Roots of Azalea Treated with Preinoculation and Postinoculation Applications of Metalaxyl

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ABSTRACT

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Phytophthora cinnamomi was isolated from roots of azaleas treated with metalaxyl at 37 or 75 mg a. i./L before, on the day of, or after inoculation with colonized oat grains of the pathogen. Recovery of *P. cinnamomi* from roots of treated plants ranged from 0 to 100% depending on timing of the metalaxyl application and year of the experiment. Recovery of *P. cinnamomi* was lowest from roots of plants treated on the day of inoculation. In general, percent recovery of *P. cinnamomi* was lower when plants were treated with 75 mg/L of metalaxyl rather than 37 mg/L. Fresh top weights and root rot ratings for plants treated with metalaxyl did not differ significantly from those of untreated, uninoculated plants for azaleas treated up to 14 days after inoculation even though recovery of *P. cinnamomi* ranged from 0 to 83% depending on time of treatment after inoculation and year of the experiment. Preinoculation drenches of metalaxyl were ineffective in preventing subsequent root infection even though top weights and root rot ratings did not differ from those of untreated, uninoculated controls. Mobility of the fungicide in soil may account for the frequent isolation of *P. cinnamomi* from plants given preinoculation drenches of metalaxyl.

In the past few years, several new systemic fungicides have been developed to control fungal pathogens in the Oomycetes (9). These fungicides are fungistatic rather than fungicidal and therefore need to be used in a preventative manner in disease control programs (9). Application of these systemics after infection occurs could lead to sale of infected but apparently healthy nursery stock. Baker and Cook (3) have emphasized the importance of avoiding the sale of infected but apparently healthy nursery stock, not only because the plant may die but also because the soil in the landscape planting site may become permanently infested with the pathogen.

Metalaxyl (Subdue) has been used successfully to prevent Phytophthora root rot of ornamentals and other crops (4,7–9,11–14). On the azalea cultivar Snow (*Rhododendron obtusum* Planch), only one drench of metalaxyl at 18 mg/L during a 24-wk period was required to control Phytophthora root rot caused by *Phytophthora cinnamomi* Rands under greenhouse conditions (4). In greenhouse studies, *P. cinnamomi* could not be isolated from roots of plants treated with metalaxyl but was isolated readily from untreated plants growing in infested soil (4). In the nursery, control was not complete; *P. cinnamomi* was isolated from 17% of the plants treated with 37 mg of metalaxyl per liter of water (4).

The purpose of this work was to determine the occurrence of *P. cinnamomi* on roots of azaleas treated with metalaxyl applied at registered rates at various times before, on the day of, or after introduction of *P. cinnamomi* inoculum to container-grown plants. A portion of this work has been reported previously (6).

MATERIALS AND METHODS

Preliminary experiments were conducted between 1981 and 1983 to determine appropriate container medium, quantity of inoculum, fertility regime based on horticultural recommendations, and application rate and timing of metalaxyl drenches. Results from these experiments were used to design the experiments described in this paper.

Experiments between 1981 and 1986 were conducted with container-grown azaleas spaced about 15 cm apart within rows and 25–30 cm apart between rows on a gravel base under 50% shade cloth in a research nursery in Raleigh, NC. Sprinkler irrigation in the morning and in the evening provided a total of 0.9 cm of water per day in addition to natural rainfall that averages 10 cm/mo from June through September. Supplemental fertilization with a liquid 21-7-7 (NPK) at a rate of 1.8 μg/ml was applied with a water siphon proportionator weekly or biweekly depending on year.

Plant culture. Ten-month-old plants of azalea cultivar Hinodegiri were transplanted to 2.6-L containers in mid to late May each year from a 6.4-cm-diameter pot with a peat moss-perlite root ball. In 1985 and 1986, a pine bark:sand medium (3:1, v/v) at pH 6.8 with lime incorporated at 3.3 kg/m^3, superphosphate at 1.2 kg/m^3, micronutrient (C-trel, Coors Farm Supply, Smithfield, NC) at 0.9 kg/m^3, and sulfur-coated fertilizer (21-6-12) at 0.5 kg/m^3 was used. In 1984, no lime was incorporated into the pine bark:sand medium (pH 5.5), although superphosphate, micronutrient, and 21-6-12 were added.

Inoculum. Several isolates of *P. cinnamomi* from azalea and rhododendron including isolates 100 (from H. A. J. Hoitink, Ohio State), 101 (ATCC 46292), 116, 128, and 150 were used. Thirty-day-old oat grain cultures of the isolates were mixed before use each year as described previously (4). Thirty oat grains from the combined cultures were placed in three holes 5 cm deep equidistant from each other at the edge of the azalea root ball. Inoculum was covered with container medium, and the plants were irrigated immediately after inoculation. Plants were inoculated during the first week of June each year.

Concurrent but independent experiments conducted between 1984 and 1986 demonstrated that a linear relationship existed between number of colonized oat grains per container and rate of symptom development on cultivar Hinodegiri (*unpublished*). In all years but 1986, 30 oat grains per container resulted in 100% of the plants (80% in 1986) developing symptoms of Phytophthora root rot within 60 days of inoculation.

Fungicide application. Metalaxyl (Subdue 2EC) at 37 or 75 mg a.i./L of water was applied as a soil drench in 250 ml of drench solution to the surface of the container medium. The 37-mg/L rate was used in all years except 1984, and the 75-mg/L rate was used in 1984–1986. Application was at various intervals before inoculation, on the day of inoculation, or after inoculation. A second drench was applied 8 wk later. Containers given the various fungicide treatments were arranged in a randomized complete block design with eight replicates in 1984, four replicates in 1985, and 10 replicates in 1986.

Disease assessment. Disease severity and occurrence of *P. cinnamomi* on roots of treated azalea were assessed 16–18 wk after inoculation depending on the year. Severity was assessed by measuring fresh top weights of plants and by comparing fungicide-treated plants with untreated, inoculated and uninoculated controls. Severity was assessed by rating roots for

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extent of Phytophthora root rot. A subjective rating scale of 1–5 was used where 1 = roots apparently healthy, 2 = small roots necrotic, 3 = large roots necrotic, 4 = crown rot, and 5 = dead plant (4). Top weight and root rot rating data were analyzed with analysis of variance, and means were separated with an *F*-protected LSD.

Occurrence of *P. cinnamomi* was assessed by collecting a portion of the root system from at least 50% of the plants in a given treatment. The root sample was washed under tap water, blotted free of surface moisture on paper towels, and cut into about 1-cm-long clumps each containing eight to 15 root pieces. Five clumps from each treatment were placed on an agar medium containing penicillium-polymixin-pimaricin (10) but modified by reducing the pimaricin concentration to 10 mg/L. A plant was considered infected if *P. cinnamomi* developed from any clump of roots.

RESULTS

Comparison of results in 1984 and 1985. Data from 1984 and 1985 for root rot rating and top weight are presented as a comparison of the difference in variation of environmental factors between years on overall plant growth and severity of Phytophthora root rot. In 1984, conditions were favorable for growth of azalea; top weights in untreated and uninoculated plants averaged 129 g (Fig. 1A). In contrast, 1985 conditions were unfavorable for plant growth; untreated, uninoculated plants averaged only 60 g fresh top weight. Root rot development, on the other hand, was as severe in 1984 as in 1985 in the untreated, inoculated control (Fig. 1B).

Occurrence of *P. cinnamomi* on roots of azalea treated with metalaxyl. Recovery of *P. cinnamomi* was frequent in most years when 37 mg/L of metalaxyl was used. Year-to-year variation in percent recovery of *P. cinnamomi* from azaleas treated with metalaxyl was observed, however. Recovery of *P. cinnamomi* over all application intervals was lowest in 1986 (range 0–20%) and highest in 1984 (range 0–100%). There were no differences in recovery of *P. cinnamomi* from roots of azalea treated with metalaxyl at 37 or 75 mg/L in the 2 yr that both rates were used.

P. cinnamomi was recovered from azalea roots treated with a preinoculation application of metalaxyl. Recovery of *P. cinnamomi* was lowest from plants treated on the day of inoculation and increased as the length of time between inoculation and application increased (Fig. 1C). Recovery of *P. cinnamomi* from plants treated 7 days after inoculation ranged from 0 to 75% depending on year. Plants treated with metalaxyl 21 days after inoculation had a 0–91% rate of recovery depending on rate of application and year (Fig. 1C).

Relation of fresh top weight and root rot rating to occurrence of *P. cinnamomi*. In 1984, fresh top weights or root rot rating of azaleas treated before, on the day of, or up to 14 days after inoculation with 75 mg/L did not differ significantly

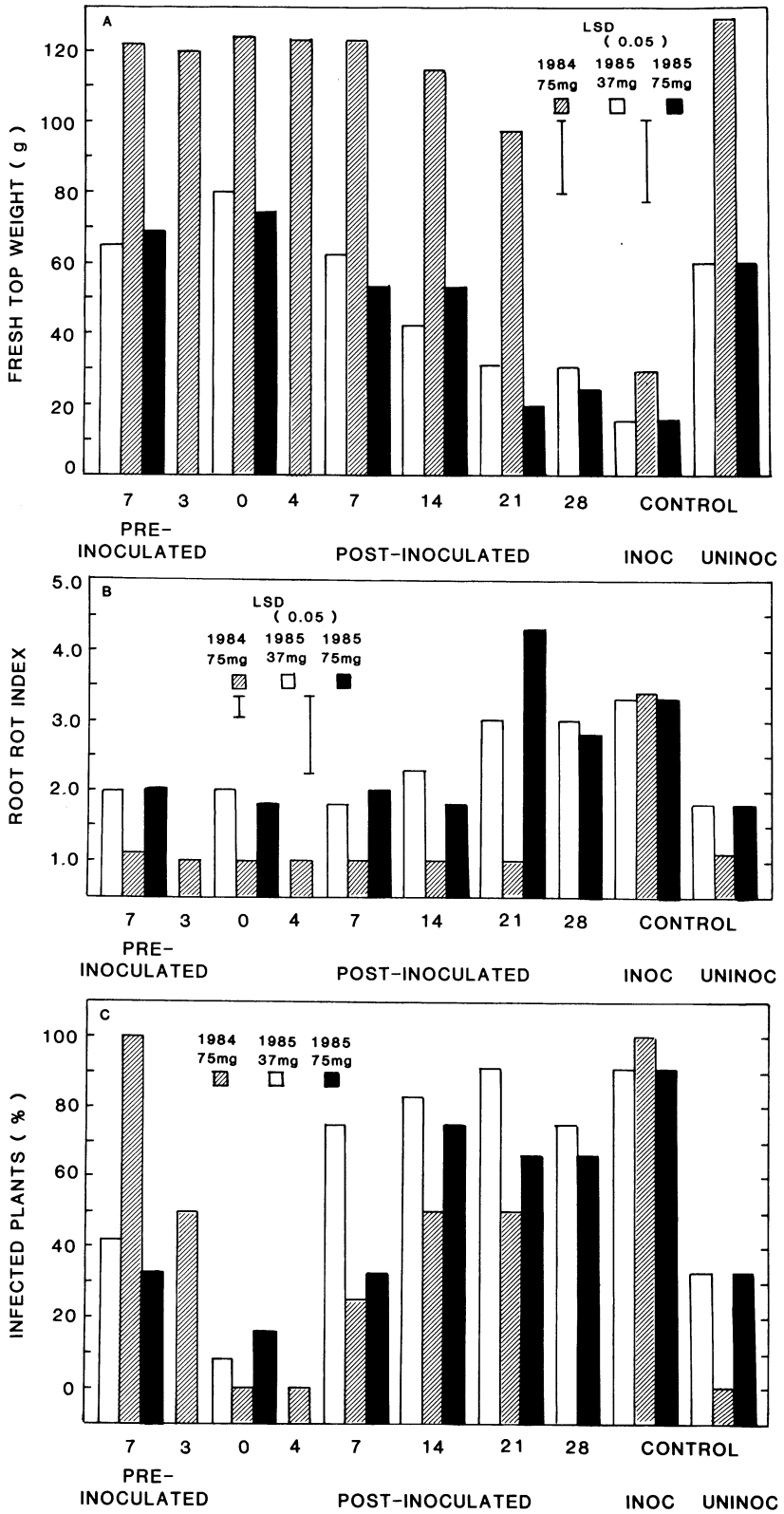

Fig 1. (A) Fresh top weight, **(B)** root rot rating, and **(C)** infection of azalea cultivar Hinodegiri treated with either 37 or 75 mg/L of metalaxyl at various times before, on the day of, or after inoculation with oat grains colonized by *Phytophthora cinnamomi*. Results are data from experiments in 1984 and 1985. Inoculated or uninoculated plants were untreated as a control. Root rot rating is based on 1 = healthy-appearing roots, 2 = small roots necrotic, 3 = large roots necrotic, 4 = crown rot, and 5 = dead plant. Percent infected plants was determined on a plant-by-plant basis by culturing a portion of the roots from at least 50% of the plants in a treatment in each experiment on a selective medium (10).

($P = 0.05$) from those of the untreated, uninoculated control (Fig. 1A,B). During this same period, occurrence of *P. cinnamomi* on roots of these plants decreased from 100% on plants treated 7 days before inoculation to 0% recovery on plants treated on the day of inoculation (Fig. 1C). In postinoculation applications of metalaxyl, occurrence of *P. cinnamomi* on roots was directly proportional to the length of time application of metalaxyl was delayed after inoculation. In 1985, a similar pattern was seen among fresh top weights, root rot ratings, and occurrence of *P. cinnamomi* on roots, except a higher percentage of roots had *P. cinnamomi* regardless of treatment time (Fig. 1C).

DISCUSSION

P. cinnamomi was recovered frequently from roots of treated plants at the end of the growing season, even when metalaxyl was applied on the day of inoculation. In 1984–1986, recovery was lowest from plants treated with metalaxyl on the day of inoculation. Although no rate effect was found, *P. cinnamomi* was recovered least frequently when the 75-mg/L rate was used on the day of inoculation. Lambe and Wills (12) reported 50% recovery of *P. cinnamomi* from azaleas treated with 37 mg/L 4 wk before inoculation but no recovery from plants treated at 72 or 144 mg/L. In experiments with *Pieris japonica*, Lambe et al (11,14) found 7.4–25% recovery of *P. cinnamomi* from roots of plants treated with metalaxyl at 37 mg/L 14 or 19 days before inoculation. At rates of 72 or 144 mg/L, *P. cinnamomi* was not recovered from *Pieris* plants in the greenhouse experiments (11,14). Similar results were also found for recovery of *P. cinnamomi* from *Taxus cuspidate* 'Hicksii' treated with metalaxyl (13).

Occurrence of *P. cinnamomi* from roots of apples dipped in solutions of metalaxyl also has been reported, although no frequency data were provided (15). Naturally infected rootstocks dipped in 1,000 ppm metalaxyl for 10 or 60 min produced healthy-appearing plants (15). In 1984, azaleas treated up to 14 days after inoculation with metalaxyl at 75 mg/L were apparently healthy because no evidence of root rot was seen on the roots and top weights were not different from those of uninoculated controls. Although *P. cinnamomi* was not recovered from the roots of plants treated on the day of inoculation, the fungus was recovered from plants treated 7 and 14 days after inoculation.

In 1986, occurrence of *P. cinnamomi* on roots of azalea was very low regardless of application timing of metalaxyl. Inoculum of *P. cinnamomi* may have been inactivated in June after inoculation by a period of extremely high temperatures (near 38 C for a 2-wk period) and dry conditions that plagued the southeastern United States that year, even though plants were irrigated daily. Nevertheless, *P. cinnamomi* was recovered from plants treated with metalaxyl 14–28 days after inoculation.

In 1984, when root rot ratings were low and top weight values for plants treated up to 14 days after inoculation were not different from those of uninoculated, untreated controls, recovery of *P. cinnamomi* was 100% for plants treated 7 days before inoculation. In general, *P. cinnamomi* was recovered from plants treated before inoculation regardless of year. It is possible that occurence of *P. cinnamomi* on preinoculated, treated plants resulted because the drench front of the fungicide solution did not contact the oat grain inoculum in the initial treatment. The high mobility of metalaxyl in soil (1,2,16) might account for a low residual of the fungicide in the zone where inoculum was placed, thus resulting in the high rate of recovery of *P. cinnamomi*. In an experiment to test commercial application methods for applying metalaxyl, a 7-day delay in inoculation after application resulted in 56–100% recovery of *P. cinnamomi* from roots of azalea cultivar Hinodegiri regardless of application method (7).

Timing of fungicide applications could have a tremendous impact on efficacy, because preinoculation applications of metalaxyl resulted in a high rate of recovery of *P. cinnamomi*. Introduction of naturally occurring inoculum from various sources in a commercial nursery at times several days to weeks after fungicide application could result in development of Phytophthora root rot. Thus, preventative applications of metalaxyl for control of Phytophthora root rot may be only partially successful if the application precedes introduction of inoculum by more than a few days and residual activity in the medium or systemic activity in the host is low.

In other experiments, *P. cinnamomi* was recovered from azalea roots treated with foliar applications of fosetyl-Al, a systemic fungicide also used for control of Phytophthora root rot (5,7). As with results with metalaxyl, even plants that appeared healthy after treatment with fosetyl-Al were infected. Recovery of *P. cinnamomi* ranged from 11 to 89% from azaleas treated with fosetyl-Al depending on application method. Plants given granular applications to the container surface at monthly intervals had the lowest occurrence of *P. cinnamomi*, however (7). In a second study with fosetyl-Al, foliar spray or drench applications resulted in 70–100% recovery of *P. cinnamomi* even though treated plants appeared healthy in one of two experiments (5).

These results suggest that preinoculation and postinoculation applications of metalaxyl may result in the occurrence of *P. cinnamomi* in roots of a portion of an azalea crop that appears healthy. However, because introduction of natural inoculum in a commercial nursery is variable, preventative applications on a regular basis continue to provide the most effective control strategy for use of metalaxyl.

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