Efficacy and in vitro Activity of Two Systemic Acylalanines and Ethazole for Control of Phytophthora cinnamomi Root Rot of Azalea

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ABSTRACT

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One drench of the acylalanines CGA-38140 (72 μg/ml) or CGA-48988 (18 μg/ml) applied 3 days before inoculation controlled Phytophthora root rot of azalea for 24 wk. Rates of 72 and 144 μg/ml of CGA-48988 were phytotoxic to Hershey Red azalea if applied more frequently than three times in 24 wk. Lower rates of CGA-48988 (18 μg/ml) were not phytotoxic. Ethazole (222 μg/ml) was as effective as the acylalanines in control of root rot only if applied at least four times in 28 wk. Systemic activity of CGA-48988 was demonstrated 6 days after drenching azaleas at rates 12–36 times the rate that effectively controlled root rot (18 μg/ml). After 5 days, ED_{50} for linear extension of *Phytophthora cinnamomi* in vitro was 0.11, 0.16, and 0.32 μg/ml for CGA-48988, CGA-38140, and ethazole, respectively. Inhibition of sporangial formation was similar for all three compounds with ED_{50} values ranging between 0.11 and 0.17 μg/ml. The ED_{50} values for inhibition of chlamydospore formation were 0.01, 0.04, and 1.4 μg/ml for CGA-38140, CGA-48988, and ethazole, respectively. When the compounds were drenched on 6-cm soil columns containing an agar disk of *P. cinnamomi* at 2 cm, ED_{50} values for subsequent growth on cornmeal agar were 1.9, 2.2, and 320.0 μg/ml for CGA-48988, CGA-38140, and ethazole, respectively. Ethazole mobility in soil columns was not enhanced by additional water. At the concentration of the acylalanines and ethazole effectively controlling root rot, these materials acted like fungistats rather than fungitoxicants.

Additional key words: leaf-disk bioassay, *Rhododendron obtusum*, simple inoculator.

The soilborne fungus, *Phytophthora cinnamomi* Rands causes severe root rot of azaleas (*Rhododendron* spp.) in nurseries and landscape plantings in North Carolina. Although most nurserymen fumigate soil used in potting mixes, the 2 to 3-yr growing cycle increases the possibility that the potting mix may become contaminated by propagules carried in surface water from surrounding areas (10). Soil drenches are of value in nursery-container blocks with a small percent of infected plants to prevent dissemination of propagules and subsequent infection of nearby healthy plants.

Diazoben and ethazole are soil fungicides that have been used to control Phythophthora and Pythium diseases on ornamentals. Recently, two systemic acylalanine fungicides were developed with activity toward fungi in the class Oomycetes (1,12,13,15). The purpose of this study was to compare the efficacy of these acylalanine fungicides with that of a standard fungicide for control of Phytophthora root rot of azalea and to investigate the in vitro activity of these compounds against *P. cinnamomi*.

MATERIALS AND METHODS

Greenhouse and field studies. Ten month old liners of the azalea cultivars Hershey Red and Snow (*Rhododendron obtusum* Planch.) grown in peat/perlite (1:1) were transplanted from 6-cm diameter pots to 15-cm diameter pots containing sand/soil/peat (1:1:1, by volume) at pH 5. Lime and superphosphate each at the rate of 3.8 kg/m^3 were incorporated in the potting mix. Plants then were placed in the greenhouse or outdoors in a container nursery. Plants in the greenhouse were fertilized bimonthly with liquid 21-7-7 (N-P-K) at a rate of 1.8 μg/ml applied with a hose-on proportionator. Plants in the container nursery were fertilized with 5 cm^3 of slow-release 19-6-12.

Chemicals evaluated included Ciba-Geigy 38140 (50 WP), *N*-(2,6-dimethylphenyl)-*N*-(2-furanylcarbonyl)-alanine methyl ester; CGA-48988 (50 WP), *N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)-alanine methyl ester; and ethazole, (Truban, 30 WP), 5-ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole. Various concentrations and schedules of drenches were tested. All concentrations are expressed on an active ingredient basis. Plants growing in 15-cm diameter pots received 250 ml of drench. An additional 250 ml of water was applied to enhance movement of ethazole into the soil.

Four isolates of *Phytophthora cinnamomi* (A^2 mating type) from azalea and rhododendron were used as inoculum. Thirty day old cultures of oat grains colonized by the *P. cinnamomi* isolates were blended by hand to ensure thorough mixing of inoculum. Plants were inoculated by placing the inoculum at the margin of the liner root ball after transplanting.

A simple inoculator was devised to increase the efficiency of the inoculation process. The inoculator consisted of a plunger (a 14.5 mm diameter cork borer with stopper) and a barrel (a 15.5 mm diameter cork borer). Stainless steel components were most durable. The inoculator was calibrated to hold 30 oat grains that were inserted 6 cm into the potting mix by holding the plunger stationary and sliding the barrel up. The inoculum then was covered by releveling the potting mix in the container. Three deposits of inoculum were spaced equidistant around the margin of the liner root ball. Cornmeal-sand inoculum was successfully inserted with the inoculator in other experiments. More than 100 plants per hour could be inoculated with this device.

Plants grown in the greenhouse and field were under different soil water regimes. The pots in the greenhouse were placed in saucers to maintain water in the potting mix near field capacity. The containers in the field had no saucers, and during the summer months, the plants were irrigated with 1.5 cm/day. A series of three experiments in a randomized complete block design with six replications per treatment were conducted, terminating 24–32 wk after inoculation. Plants were rated for root rot severity by taking the fresh top-weight and by assigning a root rot rating. The root rot

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index was based on a scale where 1 = healthy roots, 2 = fine roots necrotic, 3 = coarse roots necrotic, 4 = crown rot, and 5 = dead plant (8). A hacksaw was used to split the root ball for rating. Root samples were placed on a modified pimaricin-penicillin-polymyxin selective medium (4), with 10 mg/L instead of 100 mg/L pimaricin to assay for *P. cinnamomi*.

A bioassay technique was used to determine the systemic activity of CGA-48988 under greenhouse conditions. Ten month old Hershey Red azaleas were transplanted to 10-cm diameter pots containing the sand/soil/peat mix. Plants were allowed to grow 30 days and then drenched with 100 ml of CGA-48988 at concentrations ranging from 9 to 648 μg/ml. There were three plants per concentration. At various intervals after drenching, two terminal leaves (first fully-expanded) per plant were removed, dipped momentarily in 70% EtOH, soaked in 0.525% NaOCl for 1 min, and then transferred through five sterile-water rinses in 1 min.

Three 8-mm diameter disks were cut aseptically from each leaf, plated on cornmeal agar, and positioned with a premarked template at 60 degree angles and 2.5 cm from the center of the dish. At the same time, a 6-mm diameter cornmeal agar disk of *P. cinnamomi* isolate 101 (A^2 mating type) from *Rhododendron* sp. was transferred to the center of the assay dish. The fungus grew to the edge of the control leaf-disks in 2 days and the edge of the petri dish in 5 days. Growth inhibition was calculated by comparing the distances of growth of the drenched and control leaf disk along a line from the edge of the agar disk through the leaf disk.

Laboratory studies. Toxicity of the fungicides toward linear extension of *P. cinnamomi* was measured in vitro by incorporating various concentrations of fungicide in cornmeal agar or by the soil-vial technique (16). Isolate 101 of *P. cinnamomi* was used in all laboratory studies. In the agar incorporation technique, a 6-mm agar disk from a 5 to 7 day old cornmeal agar culture was placed at the center of a petri dish. Linear extension at 25 C was measured after 3 and 5 days. Growth inhibition was expressed as percent inhibition of linear extension relative to the no fungicide control.

In the soil-vial technique, a 6-mm agar disk was placed in a 21-mm diameter vial containing about 4 cm of sterile soil. Soil was added to cover the disk to a depth of 2 cm. The soil and disk were drenched with enough of the test fungicide suspension (4 ml) to wet the soil to the bottom of the vial. A sterile water drench of 4 ml was used in the control. After the soil vials were incubated at 25 C for 24 hr, the disks were transferred to cornmeal agar in petri dishes. Linear extension was measured at 3 and 5 days. Calculations are based on measurements at 5 days when growth extended almost to the edge of the petri dish for disks not exposed to a fungicide drench. Concentrations of fungicide that prevented uniform growth of the fungus from the disks were not used in the calculations.

Toxicity of the fungicides toward sporangium formation was determinated by growing *P. cinnamomi* on lima bean extract broth for 2 days at 25 C. After aspirating off the broth, the mycelial disks (approximately 2.5 cm in diameter) were rinsed twice in sterile distilled water, followed by two 1-hr soaks in a modified Chen-Zentmyer salt solution (11) containing fungicide. The mycelial disks were incubated an additional 17–19 hr in the salt solution and fungicide mixture at 25 C. Sporangia in a 12.5 mm^2 area of each disk were counted with the aid of a dissecting microscope. Inhibition was expressed as percent inhibition of sporangium formation relative to the no fungicide control. Sporangia from three mycelial disks per petri dish, with three petri dishes per treatment, were counted.

Chlamydospore development in the presence of the fungicides was determined with slight modification of the procedures used in sporangium formation tests. After 2 days in lima bean extract broth, mycelial disks were rinsed twice in lima bean extract broth plus fungicide and then incubated in lima bean extract broth plus fungicide for 5 days before counts were made. Experiments were repeated at least three times. Dosage-response data were plotted on semilogarithmic and logarithmic-probability paper (3). Linear regression equations were fitted to data on logarithmic-probability plots so that slope values and ED_{50} values (concentration giving 50% inhibition) could be interpolated.

RESULTS

In a greenhouse experiment, one drench of CGA-38140 at 144 μg/ml 3 days before inoculation controlled Phytophthora root rot of Hershey Red azalea as long as 24 wk (Fig. 1-A). No increase in top weight or decrease in root rot index was observed with more frequent applications. A lower rate of CGA-38140 (72 μg/ml) controlled root rot when applied three times in 24 wk but not when applied once at the beginning of the 24-wk experiment.

P. cinnamomi was not isolated from roots of plants drenched three times in 24 wk or more frequently with CGA-38140, but 60 and 20% of the plants drenched once with 72 and 144 μg/ml, respectively, were infected. The oat-grain inoculum was viable after 24 wk in all containers drenched once, however. All inoculated, undrenched plants became infected.

At 72 μg/ml CGA-48988 was more effective than only one application of CGA-38140 in root rot control (Fig. 1-A). More frequent applications or the higher rate (144 μg/ml) of CGA-48988 were not effective in increasing top weight or in decreasing root rot index compared with the inoculated, undrenched control. The fungus was not isolated from any drenched plants regardless of rate or frequency of application. However, the fungus was isolated from all inoculated, undrenched plants.

Severe phytotoxicity of foliage and root development was observed on Hershey Red azaleas drenched three times in 24 wk or

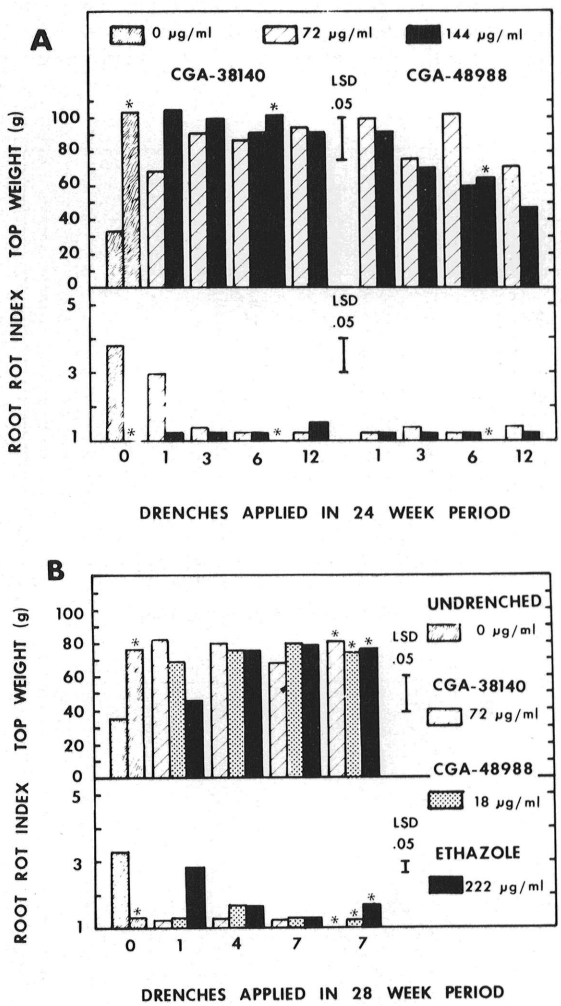

Fig. 1. A) Comparison of CGA-38140 and CGA-48988 for control of *Phytophthora cinnamomi* root rot on Hershey Red azalea after 24 wk in the greenhouse. **B)** Comparison of CGA-38140, CGA-48988, and ethazole for control of *P. cinnamomi* root rot of Snow azalea after 28 wk in the greenhouse. Root rot index: 1 = healthy roots; 2 = fine roots, necrotic; 3 = coarse roots, necrotic; 4 = crown rot; 5 = dead plant. Asterisk indicates that plants were not inoculated.

more frequently with CGA-48988 at both rates. Phytotoxicity symptoms on leaves appeared as a marginal chlorosis extending toward the midrib along the veins. Eventually this tissue became necrotic. Root development was inhibited on the noninoculated plants drenched six times with 144 μg/ml of CGA-48988 compared with the noninoculated, undrenched plants. Plants drenched three times in 24 wk or more frequently with the 144 μg/ml rate had 32–55% less top weight than the noninoculated, undrenched control. The range in top weight reduction was 1–37% for similar plants treated at 72 μg/ml, however. No phytotoxicity symptoms were observed on plants drenched once in 24 wk at either rate of CGA-48988.

Phytotoxicity symptoms also were observed on plants drenched 6 or 12 times with 144 μg/ml of CGA-38140. Top weight was 10–11% less for these plants than for the noninoculated, undrenched control.

In a second greenhouse experiment, ethazole at 222 μg/ml was compared with CGA-31840 at 72 μg/ml and CGA-48988 at 18 μg/ml for root rot control on Snow azalea. Phytophthora root rot was controlled for 28 wk with an initial drench application of CGA-38140 or CGA-48988 (Fig. 1-B). Ethazole did not give control with one application, but it was as effective as the acylalanines when applied four times or more.

P. cinnamomi was isolated from 100, 33, and 0% of the plants drenched one, four, and seven times with ethazole, respectively. The fungus was not isolated from any plants drenched with CGA-38140 or CGA-48988. All inoculated control plants were infected.

Control of *P. cinnamomi* on Hershey Red azalea with CGA-48988 and ethazole was not complete when plants were grown unshaded in an outdoor containter area. No differences were found in top weight of plants. Plants drenched eight times in 32 wk with 36 μg/ml of CGA-48988 had less root rot than the inoculated control. However, plants drenched once with 444 μg/ml of ethazole also had less root rot. *P. cinnamomi* was isolated from 66.6 and 16.6% of the plants drenched one and four times in 32 wk, respectively, with 18 μg/ml of CGA-48988, but at 36 μg/ml of CGA-48988, infection was 16.6% for both one and four drenches in 32 wk. In corresponding drench treatments with ethazole, the fungus was recovered from 66.6 and 83.3% of the plants drenched with 222 μg/ml and from 33.3 and 16.6% of the plants drenched with 444 μg/ml. No phytotoxicity symptoms were observed on Hershey Red azaleas treated with the 18 or 36 μg/ml rate of CGA-48988.

In the leaf-disk bioassay experiment, systemic activity of CGA-48988 was not detected as long as 18 days after drenching at rates of 9–72 μg/ml. At 42 days, there was 15.2% inhibition of *P. cinnamomi* with leaf-disks from plants treated at 72 μg/ml. In a second experiment, CGA-48988 at rates of 108, 216, 324, and 648 μg/ml produced 2.2, 22.4, 39.2, and 40.6% inhibition around leaf disks, respectively, 6 days after drenching. No inhibition was found 2 days after drenching, however.

Toxicity of CGA-38140, CGA-48988, and ethazole toward *P. cinnamomi* was measured in vitro by the inhibition of linear extension and sporangial and chlamydospore formation. A sigmoid shaped dosage-response curve was found when percent inhibition of linear extension on cornmeal agar was plotted against the log of fungicide concentration (Fig. 2-A). This dosage-response curve was straightened by plotting on a log-probit basis (3). The ED_{50} values were 0.11, 0.16, and 0.32 μg/ml for CGA-48988, CGA-38140, and ethazole, respectively, at 5 days (Fig. 2-B). Slope values indicating rate of inhibition ranged from 1.1 to 1.2 for CGA-38140 and CGA-48988 to 2.1 for ethazole. Thus as the concentration of ethazole was increased, *P. cinnamomi* was inhibited at about double the rate of that with the acylalanines. The coefficient of determination was 0.98 for all three fungicides. In all subsequent laboratory tests correlation coefficients were significant at $P \leqslant 0.01$ with r^2 values at 0.95 or above.

When the fungicides were drenched onto 6-cm soil columns containing an agar disk of *P. cinnamomi*, higher concentrations were required to inhibit fungal growth once the disks were transferred to cornmeal agar. Concentrations ranging from 0.5 to 5.0 μg/ml of CGA-38140 or CGA-48988 and 50 to 900 μg/ml of ethazole produced a typical dosage-response curve. Log-probit transformation of the data resulted in ED_{50} values of 1.9, 2.2, and 320.0 μg/ml for CGA-48988, CGA-38140, and ethazole, respectively (Fig. 3). Thus, the acylalanine compounds were about 160 times more active than ethazole against *P. cinnamomi* in the soil environment.

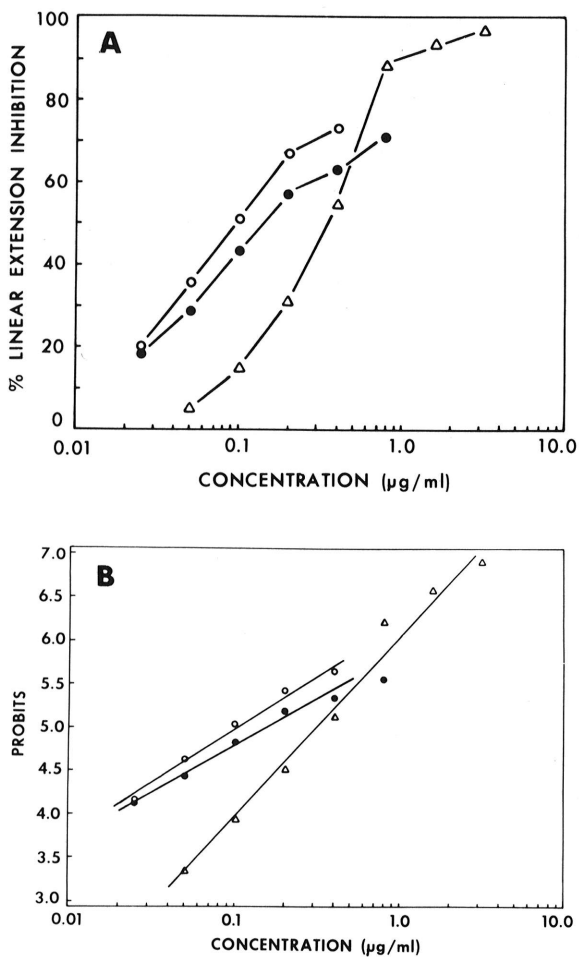

Fig. 2. Dosage-response curve for *Phytophthora cinnamomi*. Inhibition of linear extension on cornmeal agar incorporated with CGA-38140 (●), CGA-48988 (○), or ethazole (Δ). **A)** Semilogarithmic plot, **B)** log-probit plot. Correlation coefficients were significantly positive at $P \leqslant 0.01$.

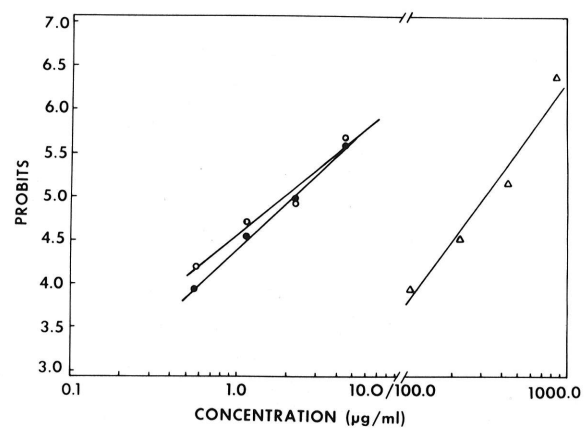

Fig. 3. Dosage-response curve for *Phytophthora cinnamomi* in the soil-vial test. Fungicide was drenched on a soil column containing an agar disk of *P. cinnamomi* buried at 2 cm. After 24 hr, the disk was transferred to cornmeal agar, and inhibition of linear extension was determined after 5 days. ● = CGA-38140, ○ = CGA-48988, and Δ = ethazole. Correlation coefficients were significantly positive at $P \leqslant 0.01$.

The soil-vial technique was modified for ethazole in another experiment by increasing the height of the soil column beneath the agar disk. This allowed addition of 4 ml of distilled water after the fungicide drench to facilitate any additional movement of the material into the soil. At ethazole concentrations of 111–888 μg/ml, no significant dosage-response shift in toxicity to *P. cinnamomi* occurred. An ED_{50} value of 355 μg/ml was interpolated from the log-probit plot. This value is comparable to that found in the standard soil vial test (ED_{50} = 320 μg/ml) in which no additional water was added.

In a soil-vial test to measure fungitoxic properties, soil was drenched at rates of 888, 1,776, and 3,552 μg/ml of the three compounds. Agar disks of the fungus that failed to grow within 5 days (control had reached edge of plate) were transferred to fresh cornmeal agar. This was repeated every 5–7 days. Growth of *P. cinnamomi* started 8 days after the original plating for all rates of CGA-38140. After 11 days, the fungus grew from disks drenched with CGA-48988 at 888 and 1,776 μg/ml. In one experiment, the fungus grew from disks drenched with ethazole at 888 μg/ml after 5 days. The fungus did not grow from disks drenched at the higher ethazole rates after 56 days. In a second experiment, no growth occurred after 42 days at any of the ethazole rates.

The dosage-response curves for sporangium inhibition of *P. cinnamomi* appeared similar on a semilog basis for the three fungicides. Indeed log-probit transformation and interpolation of ED_{50} values gave 0.11, 0.14, and 0.17 μg/ml for CGA-38140, CGA-48988, and ethazole, respectively (Fig. 4). Slope values were 1.6 for the acylalanines and 2.9 for ethazole. In all sporangium inhibition experiments, CGA-38140 was slightly more inhibitory than CGA-48988. This order of toxicity was reversed in the linear extension experiments.

Ethazole did not inhibit chlamydospore formation as much as did CGA-38140 and CGA-48988. Fewer chlamydospores formed as fungicide concentration increased, and their diameter also decreased. Interpolation of ED_{50} from the log-probit plot gave values of 0.01, 0.04, and 1.4 μg/ml for CGA-38140, CGA-48988, and ethazole, respectively (Fig. 5). Slope values ranged from 2.08 to 3.84 for the three fungicides, which indicates no differences between rates of inhibition of chlamydospore formation among the fungicides.

DISCUSSION

In the greenhouse, Snow azalea was protected 24 wk from infection by *P. cinnamomi* with one drench of CGA-48988 at 18 μg/ml, if applied 3 days before inoculation. Protection was similar for plants drenched with CGA-38140 at 72 μg/ml. The acylalanines at rates below those needed with standard fungicides also have controlled diseases on other crops (13,15).

Ethazole (Truban 30% WP) at the recommended rate of 222 μg/ml and applied four times in 28 wk gave control of Phytophthora root rot equal to that of the acylalanines. Hoitink and Schmitthenner (9) controlled root rot of rhododendron caused by *P. cinnamomi* for 6 wk with one soil drench of ethazole at 375 μg/ml if applied 2 days before inoculation. Lower rates were ineffective. The higher rate required for control on rhododendron than on azalea may be due to the greater susceptibility of the former to *P. cinnamomi*.

Phytotoxicity occurred on Hershey Red azalea when CGA-48988 at 72 μg/ml and CGA-38140 at 144 μg/ml were applied more frequently than three or six times in 24 wk, respectively. These rates were four to eight times higher than required for effective control. At effective rates, phytotoxicity was not apparent in Hershey Red and Snow azalea. The reaction of other azalea cultivars to these fungicides is unknown.

The systemic activity of CGA-48988 was demonstrated in Hershey Red azalea using the leaf-disk bioassay but only at rates 12 to 36 times greater than the effective rate (18 μg/ml) for root rot control. Because the percent inhibition of fungal growth was determined in a similar manner to that in the linear extension experiments, Fig. 2-B can be used to interpolate the apparent concentration of fungicide diffusing into the agar from the leaf-disk. At drench rates of 216, 328, and 648 μg/ml of CGA-48988, 0.025, 0.065, and 0.070 μg/ml, respectively, of the fungicide diffused from the 50.2 mm^2 leaf disk into the agar. Because accumulated fungicide from one disk diffused into approximately 6 ml of agar (three leaf disks per 18 ml of culture medium), 0.15–0.42 μg of CGA-48988 had accumulated in each leaf disk at the 216 and 648 μg/ml rate of drench, respectively. Although the amount of accumulated fungicide per leaf disk was relatively small in relation to the amount applied, concentrations developed in the leaf disks that inhibited *P. cinnamomi*. The relationship of leaf concentration of CGA-48988 to root concentration is unknown, but in root rot experiments control was obtained 24–28 wk after treatment. Thus, long-term action of CGA-48988 is probably related to the systemic nature of this fungicide, even though it is highly effective in the soil environment.

The dosage-response relations for inhibition of linear extension in agar can be used to demonstrate that the acylalanine compounds were 2.0–2.9 times more active than ethazole against *P. cinnamomi*. *P. cinnamomi* was more sensitive (ED_{50} = 0.32 μg/ml) than *Pythium ultimum* (ED_{50} = 4.2 μg/ml [5]) to ethazole. Zentmyer (17) found that 500 μg/ml of diazoben retarded growth of *P. cinnamomi* by 80% in agar culture. As little as 0.5 μg/ml of CGA-48988 inhibited growth by 80% in our studies. Schwinn et al (12) reported an ED_{50} for *P. cinnamomi* of 0.6 μg/ml for CGA-38140, compared with 0.2 μg/ml in this study.

The slope value for ethazole inhibition was greater than the values for acylalanine inhibition. In addition to differences in rate of inhibition among fungicides, different slope values between

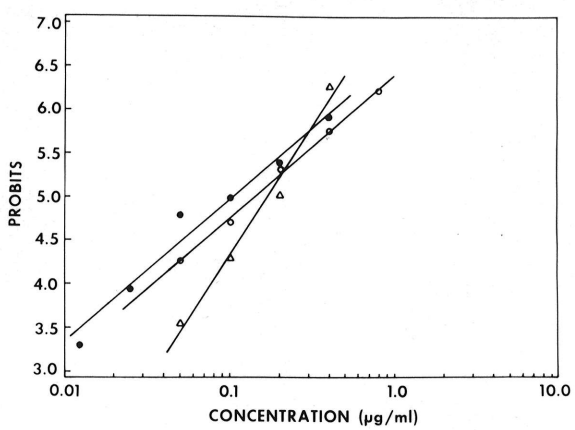

Fig. 4. Dosage-response curve for sporangium inhibition of *Phytophthora cinnamomi* with CGA-38140 (•), CGA-48988 (○), and ethazole (Δ). Correlation coefficients were significantly positive at $P \leq 0.01$.

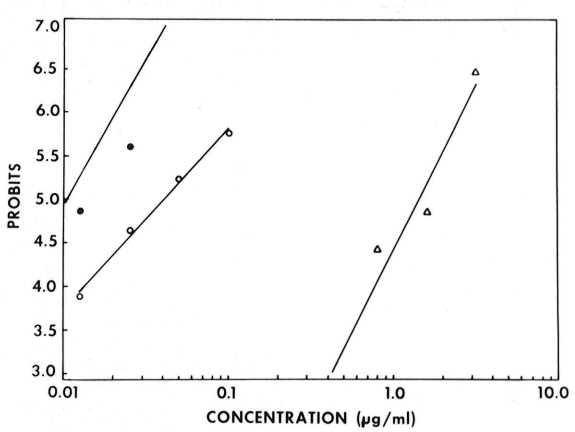

Fig. 5. Dosage-response curve for chlamydospore inhibition of *Phytophthora cinnamomi* with CGA-38140 (•), CGA-48988 (○), and ethazole (Δ). Correlation coefficients were significantly positive at $P \leq 0.01$.

fungicides may indicate a difference in mode of action (2,3). Halos and Huisman (6) demonstrated that ethazole may inhibit respira- in *Pythium* spp. by blocking electron transport.

Inhibition of sporangial production was similar for the acylalanine compounds and ethazole. These fungicides were three to eight times more toxic than diazoben (ED_{95} = 5 μg/ml [17]) toward inhibition of sporangial production in *P. cinnamomi*. As in linear extension experiments, the slope values for ethazole and acylalanine compounds differed, which may indicate that the mechanism of sporangial inhibition is different in these compounds.

The acylalanines were 35–140 times more toxic than ethazole toward chlamydospore formation in *P. cinnamomi*. Chlamydospore formation was 4 to 10 times more sensitive than linear extension or sporangial formation to the acylalanines. In disease development, the increased sensitivity toward the acylalanines could reduce significantly the number of chlamydospores formed in infected tissue. No information is available on the effect of the acylalanines on chlamydospore germination, but Zentmyer (17) found a flat dosage-response relation with diazoben. In his studies, 10 μg/ml of diazoben inhibited chlamydospore germination 56%, but as much as 200 μg/ml did not completely inhibit germination.

Toxicity of the acylalanine compounds and ethazole toward *P. cinnamomi* varied most in the soil-vial test. The acylalanines were about 160 times more active than ethazole against *P. cinnamomi*. Even at the recommended rate of 222 μg/ml, ethazole only inhibited fungal growth 35%. The ED_{50} value was 320 μg/ml. Supplemental water did not enhance ethazole mobility in the soil. Lack of soil mobility probably best explains the wide variation in ED_{50} values for ethazole between agar incorporation and soil-vial tests. Soil mobility studies were used to demonstrate that ethazole, when spotted on a thin soil film on thin-layer chromatography plates, did not move from the origin after a 10 cm water front had passed (7). Soil drenches of ethazole at 500 μg/ml also were not effective in eliminating *Pythium aphanidermatum* or *Phytophthora palmivora* from infested seed at a 5 mm depth (14).

Halos and Huisman (5) reported that at 416 and 1,390 μg/ml, ethazole was fungitoxic to *Pythium ultimum*. Failure of mycelium to grow within 5 days of treatment was the criterion used to assess fungicidal action. In the present study, mycelium of *P. cinnamomi* in agar disks drenched at 888 μg/ml of ethazole in the soil vial test was inhibited initially, but grew in one experiment but not in a second experiment 42 days after transferring to fresh substrate. Neither CGA-48988 nor CGA-38140 were fungitoxic at rates to 1,776 and 3,552 μg/ml, respectively. Hence, ethazole is fungitoxic at four times the recommended rates, but both ethazole and the acylalanines act as fungistats at the rates effective for root rot control. This fungistatic action of ethazole was reported previously (9,14). In future studies, care should be taken to determine fungistatic and fungitoxic properties, especially because the fungus may survive at high concentrations before it is killed.

The acylalanine compounds when labeled will offer the nurseryman several advantages over materials presently labeled for Pythium and Phytophthora root rot control. These include greater soil mobility, greater toxicity toward *P. cinnamomi*, systemic activity, and reduced labor costs associated with less frequent applications.

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