Residual Activity of Metalaxyl and Population Dynamics of *Phytophthora cinnamomi* in Landscape Beds of Azalea

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ABSTRACT

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Metalaxyl applied at 0.0014 kg a.i./5.5 m^2 of soil surface on a 30-day schedule during the growing season for two seasons resulted in continued suppression of inoculum of *Phytophthora cinnamomi* up to 18 mo after the last fungicide application. Inoculum density of *P. cinnamomi* in plots treated with metalaxyl on a 60-day schedule was similar to that in untreated plots after 18 mo, but plant mortality was nil and plant size was significantly greater than in the untreated plots. No suppression of inoculum of *P. cinnamomi* was found after 18 mo in plots treated with fosetyl-Al, even though plant mortality was low compared with that of plants in untreated plots. The accuracy of the semiautomatic elutriator assay method for *P. cinnamomi* was improved by including a 9% correction factor to account for propagules that passed through the smallest sieve used. The precision of the elutriator method did not increase with more than three subsamples assayed per soil sample as determined by estimating the variance of the general mean procedure with increasing numbers of subsamples. Inoculum density of *P. cinnamomi* was greatest in the root zones of plants with no foliar symptoms or with only initial symptoms of root rot and lowest in soil from plants with severe symptoms or in plots without plants.

Application of fungicides to control Phytophthora root rot of azalea caused by *Phytophthora cinnamomi* in landscape beds was a successful management practice during a recent 2-yr study in North Carolina (4). Symptoms of Phytophthora root rot were not observed on plants in any of the treatments when the fungicides were applied on 30- or 60-day schedules during the first growing season (1983). Symptoms developed on plants between May and September of the second year (1984), but plants in plots treated with metalaxyl showed few symptoms compared with those treated with other fungicides. Metalaxyl at 0.0014 kg a.i./5.5 m^2 of soil surface gave better control of Phytophthora root rot and greater suppression of inoculum of *P. cinnamomi* than either ethazol or fosetyl-Al at 0.025 and 0.044 kg a.i./5.5 m^2, respectively. During the third growing season (1985), the test area was maintained without further fungicide applications. In the spring of 1986, inoculum density of *P. cinnamomi* in soil samples collected 18 mo after the last fungicide application was suppressed in plots treated with metalaxyl on a 30-day schedule compared with that in plots treated on a 60-day schedule, plots treated with fosetyl-Al or ethazol on either schedule, and plots left untreated.

Extended residual activity of fungicides applied to soil can have a significant effect in the development of disease control strategies for root pathogens. Previous research has demonstrated that the half-life of metalaxyl in soils varies from 70 days in sandy loam soils not previously treated with metalaxyl to as few as 14–28 days in similar soil with a history of metalaxyl treatment (2). In addition, the organic matter content of a soil is directly proportional to the half-life of metalaxyl in that soil (14). Long-term residual activity of metalaxyl in soil has not been observed previously, because previous research has involved relatively short-term experiments (2,14).

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The purpose of this report is to describe the residual effect of metalaxyl on inoculum density of *P. cinnamomi* in soil, the development of symptoms of Phytophthora root rot on azalea plants in landscape beds after treatment with fungicides is discontinued, the population dynamics of *P. cinnamomi* in landscape beds, and the reliability of the assay method for propagules of *P. cinnamomi*.

MATERIALS AND METHODS

The landscape beds used, the establishment of azaleas in the fall of 1982, the fungicides used, and the rate and timing of applications in 1983 and 1984 have been described (4). Briefly, two landscape beds each 3.04 × 32.8 m that were naturally infested with *P. cinnamomi* were each divided into 18 plots (1.8 × 3.04 m) and treated with either ethazol (Truban 5G), fosetyl-Al (Aliette 10G), or metalaxyl (Subdue 2E) at rates of 0.025, 0.044, or 0.0014 kg a.i./5.5 m^2 of soil surface, respectively, in October of 1982 (4). Control plots were left untreated or fumigated with a methyl bromide-chloropicrin mixture. Each plot was planted with four 2-yr-old azaleas (*Rhododendron obtusum* Planch. 'Hinodegiri'). Additional fungicide applications were made on either 30- or 60-day schedules during the 1983 and 1984 growing seasons such that plots received seven and four applications, respectively (4).

During the 1985 growing season, no fungicides were applied but cultural practices for weed control and sprinkler irrigation (2.8 cm/wk) were continued. Symptom development was assessed for plants on 7 September 1984 and on 21 June and on 6 August 1985 with an index where 1 = healthy, 2 = initial symptoms (chlorosis, dwarfed leaves, poor shoot growth), 3 = severe symptoms (plant stunted, necrotic leaves present), and 4 = plant dead and reported (4). A final assessment for symptom expression was made 21 March 1986. Plant size was assessed as (average plant height + average plant width)/2 on 7 September 1984 and on 27 May 1986. Plant mortality was recorded as zero and was included in means for plant size per plot.

In February and March 1986, two replicates in one of the two landscape beds were selected for sampling inoculum of *P. cinnamomi*. The 18 plots in the bed were divided into 144 quadrats of 1 m^2; 72 of the quadrats were centered on individual plants in the bed, and the other 72 quadrats were centered in the fallow space between rows of plants. Ten soil cores (2.5 cm diameter × 15 cm) were collected around the circumference of the drip line of each plant or from the circumference of an imaginary plant in the center of the fallow plots. The 10 soil cores from each quadrat were combined to provide one bulked soil sample per quadrat and stored at ambient temperature in plastic bags until assayed for *P. cinnamomi*. To avoid storing samples for prolonged periods before assay, 12 quadrats were sampled and assayed starting 25 February 1986 and ending 21 March 1986. Samples were normally collected the day before the assay was performed.

Because soil was assayed from quadrats containing individual plants as well as from quadrats without plants, comparison of plant vs. no-plant effects on inoculum density was possible. In addition, the data were used to compare inoculum density with individual plant size and symptom rating. Analysis of variance was used with the *F*-protected LSD calculated to separate treatment means.

Assay procedure. The method of Shew and Benson (15) was used to assay soil for *P. cinnamomi*. Briefly, the method consisted of a 100-g subsample taken from the bulked soil sample, stirred in 200 ml of tap water for 6 min, and then processed on a semiautomatic elutriator (6). Elutriation time was 6 min with air and water flow rates of 30–40 and 80 ml/sec, respectively (15). One-fifth of the sample was collected from the sample splitter on a 15-cm-diameter sieve with 38-μm openings. The residue on the 38-μm-mesh sieve was rinsed into a 100-ml beaker with a volume of 30–40 ml of tap water. The residue and water mixture was then dispensed as uniformly as possible onto 10 plates of a pimaricin-chloramphenicol-hymexazol agar medium (PCH) (15). The plates were incubated at 20 C in the dark for 2–3 days prior to counting microscopically the developing colonies of *P. cinnamomi*. Additional subsamples of about 10 g each were dried in an oven at 105 C overnight to determine percent soil moisture so that counts could be expressed as propagules per gram (p/g) of air-dried soil.

Reliability of assay procedure. The accuracy of the assay method was tested by comparing results of assays done by hand with nested sieves and assays done on the semiautomatic elutriator with nested sieves. Extra soil samples were collected from around plants in control plots and bulked for subsequent subsampling. Forty-gram subsamples of soil were wet-sieved by hand on a series of four nested sieves from 850- to 20-μm mesh. The fractions from the 125-, 38-, and 20-μm-mesh sieves were plated on PCH medium as described. The hand assay method was run four times with eight 40-g subsamples of soil each run.

The elutriation assay method used 200-g subsamples of the extra soil processed on the semiautomatic elutriator. The method described previously was modified by placing a 125-μm-mesh sieve nested on a 38-μm-mesh sieve, which was nested on a 20-μm-mesh sieve below the sample splitter of the semiautomatic elutriator. The larger soil sample was used on the semiautomatic elutriator because the one-fifth portion collected corresponded to the 40-g sample size used with the hand assay method. Air and water flow rates and elutriation time were as before. Residual from the 125- to 20-μm-mesh sieves was plated on PCH medium. There were four runs with eight subsamples each for this method.

The precision of the hand and elutriator methods was evaluated by processing five subsamples per method from each of four bulked soil samples from the landscape bed. Residue in fractions smaller than 850 μm and larger than 20 μm was plated on PCH medium. The method of Campbell and Nelson (7) was used to determine the variance of the general mean (VGM) for one to five subsamples for each technique based on an analysis of variance of inoculum density data from the four samples.

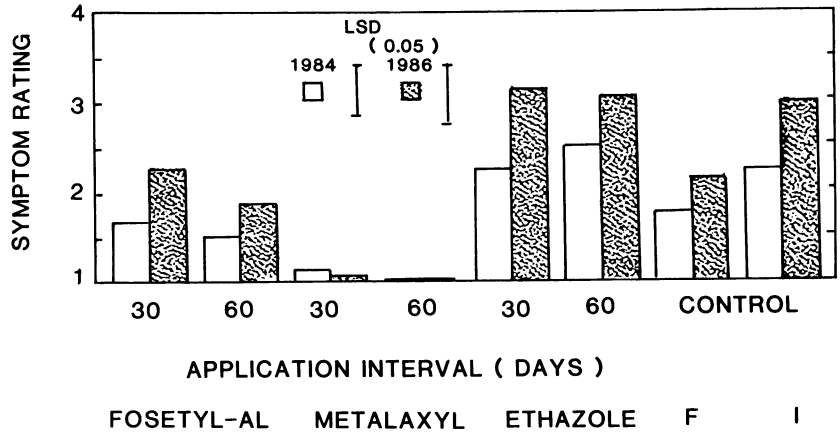

Fig 1. A comparison of azalea symptom ratings for Phytophthora root rot in 1984 (open histogram) in landscape beds treated on 30- or 60-day schedules during the growing season with ethazol, fosetyl-Al, or metalaxyl with symptom ratings in 1986 (solid histogram), 18 mo after the last fungicide application. Controls were either plots fumigated (F) or infested plots left untreated (I). Symptom rating was 1 = healthy, 2 = initial symptoms (chlorosis, dwarfed leaves, poor shoot growth), 3 = severe symptoms (plant stunted, necrotic leaves present, defoliation), and 4 = dead plant.

RESULTS

Symptom development and mortality. Depending on the original fungicide used, symptom development on plants increased only 10% or less during 1985, when no fungicides were applied. Symptom development did not reach 100% on plants in any of the treated or untreated plots. Symptom rating in 1986 on plants originally treated with metalaxyl on a 30- or 60-day schedule was near 1.0 (healthy appearing), with no increase in symptom rating from that observed in 1984 (Fig. 1). The greatest increase in symptom rating between 1984 and 1986 occurred on plants in plots originally treated with fosetyl-Al and ethazol on a 30-day schedule and on plants in infested, untreated plots. Final symptom ratings for plants originally treated with metalaxyl and fosetyl-Al and for plants in fumigated plots were lower ($P = 0.05$) than those for plants in infested, untreated plots (Fig. 1).

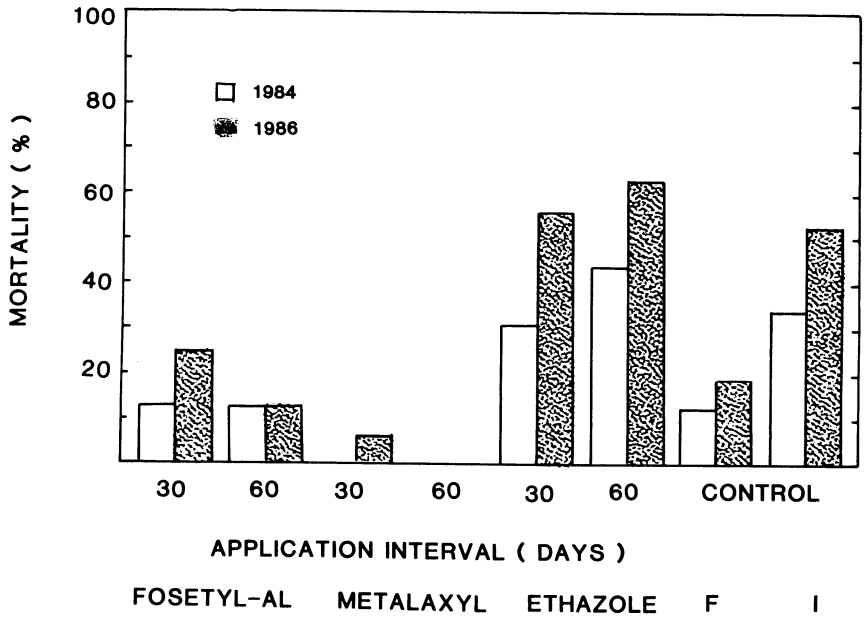

Fig. 2. Plant mortality in landscape beds in 1984 (open histogram) for plants treated on 30- or 60-day schedules during the growing season with ethazol, fosetyl-Al, or metalaxyl compared with mortality in 1986 (solid histogram), 18 mo after the last fungicide application. Controls were either plots fumigated (F) or infested plots left untreated (I).

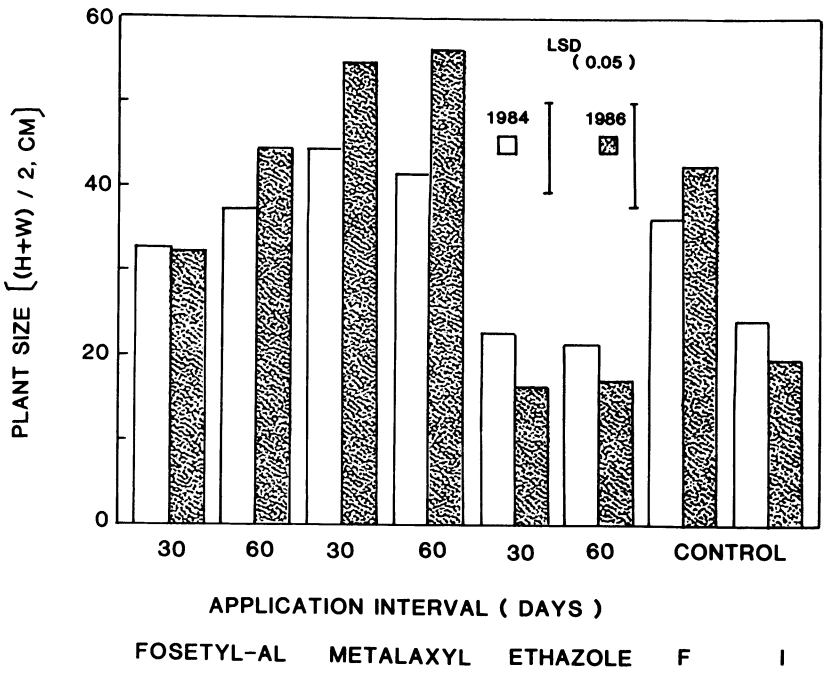

Fig. 3. Plant size [(height plus width)/2] for azaleas in landscape beds in 1984 (open histogram) treated on 30- or 60-day schedules during the growing season with ethazol, fosetyl-Al, or metalaxyl compared with plant size in 1986 (solid histogram), 18 mo after the last fungicide application. Controls were either plots fumigated (F) or plots left untreated (I).

Plant mortality increased the least on plants in metalaxyl-treated, fosetyl-Al-treated, and fumigated plots between 1984 and 1986 (Fig. 2). Between 1984 and 1986, no mortality occurred in plots originally treated with metalaxyl on a 60-day schedule and only one of 16 plants (6%) died in plots originally treated with metalaxyl on a 30-day schedule. Mortality of plants in fosetyl-Al-treated plots in 1986 ranged from 13 to 25% for plants that had been on 60- and 30-day application schedules, respectively. Plants in infested, untreated plots had 53% mortality by May 1986, whereas plants in ethazol-treated plots had 56–63% mortality, depending on fungicide application interval (Fig. 2).

Plant size. The greatest increase in plant size between 1984 and 1986 occurred in plants in plots treated with metalaxyl on either schedule and with fosetyl-Al on a 60-day schedule and in fumigated plots (Fig. 3). Plants in plots originally treated with metalaxyl or fosetyl-Al on a 60-day schedule and in fumigated plots were larger ($P = 0.05$) than plants in infested untreated plots. Mean plant size decreased between 1984 and 1986 for plants in ethazol-treated and infested, untreated plots, reflecting the mortality that had occurred.

Reliability of assay method. Total inoculum densities expressed as p/g were 1.6 and 2.2 times greater in two of four runs of the hand assay method than in the elutriator method. Inoculum density in the other two runs did not differ between methods. Because two different batches of extra soil were collected, comparisons of actual inoculum densities among runs was not possible.

Inoculum density of *P. cinnamomi* in the residue on the 20-μm-mesh sieve averaged 6.4 and 9.1% of the total inoculum density from all sieve fractions for the hand and elutriation methods, respectively. Therefore, p/g counts were increased by 9% for the inoculum densities reported below, because the 20-μm-mesh sieve was not used routinely on the elutriator.

The precision of the two methods as compared by the method of Campbell and Nelson (7) for five subsamples from four soil samples was similar. The variance among samples (σ_s^2) for the two methods was 1.80 and 0.75 with subsample variance (σ^2) of 0.28 and 0.05 for the hand and elutriation methods, respectively. The VGM was then calculated and plotted. There was a decrease in VGM as number of subsamples increased from one to five per sample (Fig. 4). A plateau was reached between two and three subsamples per sample for both methods. This suggests that the precision of the inoculum density estimates given below and based on three subsamples would not have been improved materially if additional subsamples had been assayed per sample.

Population dynamics. Inoculum density around plants in plots originally treated with metalaxyl or fosetyl-Al on a 30-day schedule and around plants in fumigated plots was relatively unchanged between September 1984 and March 1986 (Fig. 5). Inoculum density around plants in plots originally treated with metalaxyl on a 30-day schedule remained near 0.03 p/g, 18 mo after the last fungicide application. Increases in inoculum density between 1984 and 1986 occurred around plants in plots originally treated with metalaxyl or fosetyl-Al on a 60-day schedule and in plots originally treated with ethazol on a 30-day schedule. The largest increase in inoculum density (from 0.07 p/g in 1984 to 1.1 p/g in 1986) occurred in soil around plants in plots originally treated with metalaxyl on a 60-day schedule. Inoculum density in soil around plants in infested, untreated plots decreased from 4.6 p/g in 1984 to 1.8 p/g in 1986. In 1986, plots originally treated with metalaxyl on a 30-day schedule had significantly lower ($P = 0.05$) inoculum density than the infested, untreated control.

Striking differences among quadrats were found when inoculum densities of individual quadrats were compared, based on the presence or absence of a plant and whether the plant was alive or dead. Regardless of original fungicide or control treatment, inoculum density averaged 2.1, 0.4, and 0.03 p/g for soil around plants that were alive, around plants that were dead, and from quadrats without plants, respectively.

A significant correlation ($r = -0.32$, $P = 0.0054$, $n = 72$) was found between symptom rating and inoculum density. For instance, healthy-appearing plants had an inoculum density of 1.8 p/g, plants with initial symptoms averaged 3.1 p/g, plants with severe symptoms averaged 1.2 p/g, and dead plants averaged 0.4 p/g. Plants with initial symptoms of Phytophthora root rot had greater ($P = 0.05$) inoculum densities than plants in the other categories.

Inoculum density in quadrats with plants in March 1986 was also highly correlated ($r = 0.40$, $P = 0.0005$, $n = 72$) with plant size when previous treatments were ignored. Regression of plant size in May 1986 with inoculum density in March 1986 was highly significant ($P = 0.001$). The regression equation was $Y = 31.01 + 0.189x$, where Y = plant size and x = inoculum density. The R^2 value was 0.16. The slope was significantly different from zero by the t test. The regression model is confounded because plants with inoculum densities near zero could either be healthy and hence large or dead and hence small. A scatter plot for plant size by symptom rating vs. inoculum density illustrates the phenomenon (Fig. 6A). While plots with dead plants averaged 0.4 p/g, 10 of 14 plants had inoculum densities of *P. cinnamomi* near zero. Inoculum densities in plots with healthy plants varied depending on original fungicide treatment. Healthy-appearing plants from plots originally treated with metalaxyl had inoculum densities near zero, but healthy-appearing plants originally treated with fosetyl-Al had inoculum densities between 1 and 3.5 p/g (Fig. 6B).

DISCUSSION

The ability of metalaxyl to suppress inoculum of *P. cinnamomi* in plots originally treated on a 30-day schedule 18 mo after the last application was unexpected. Metalaxyl has high mobility and low adsorption in soil (1,14). In sandy loam soils with a history of metalaxyl treatment, microorganisms were capable of degrading half of the applied sample within 14–28 days, whereas similar soils not previously treated with metalaxyl had a half-life of more than 70 days (2). In the clay soil used in my study, the tendency of metalaxyl to be adsorbed may be greater than in a sandy loam soil (14).

Because *P. cinnamomi* survives in soil as chlamydospores (12,15) and the dosage-response relation for 50% inhibition (ED_{50}) of chlamydospore formation in culture is 0.14 μg/ml of metalaxyl (3), the residual concentration in plots treated on a 30-day schedule must have remained above the ED_{50} value. Incomplete degradation of metalaxyl applied at other times during 1984 and subsequent persistence of a portion of the metalaxyl in these applications might account for the residual effect of metalaxyl after 18 mo. In contrast, inoculum density of *P. cinnamomi* had increased sharply 18 mo after the last application around plants in plots treated with metalaxyl on a 60-day schedule. Apparently, the residual concentration of metalaxyl in these plots was not sufficient to prevent chlamydospore formation.

Although plants appeared healthy in plots treated with fosetyl-Al on a 60-day schedule and plant size was not significantly different from that of plants treated with metalaxyl, inoculum density was very high 18 mo after the last application of fungicide. The high incidence of inoculum in March 1986 coupled with 30% infection in September 1984 (4) indicates a poor residual effect for this fungicide. The extremely short

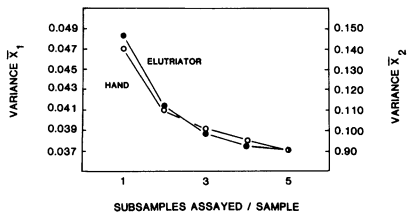

Fig. 4. Comparison of the precision of the hand (x_1) and elutriator (x_2) assay methods based on the variance of the general mean of different numbers of subsamples assayed per bulk soil sample.

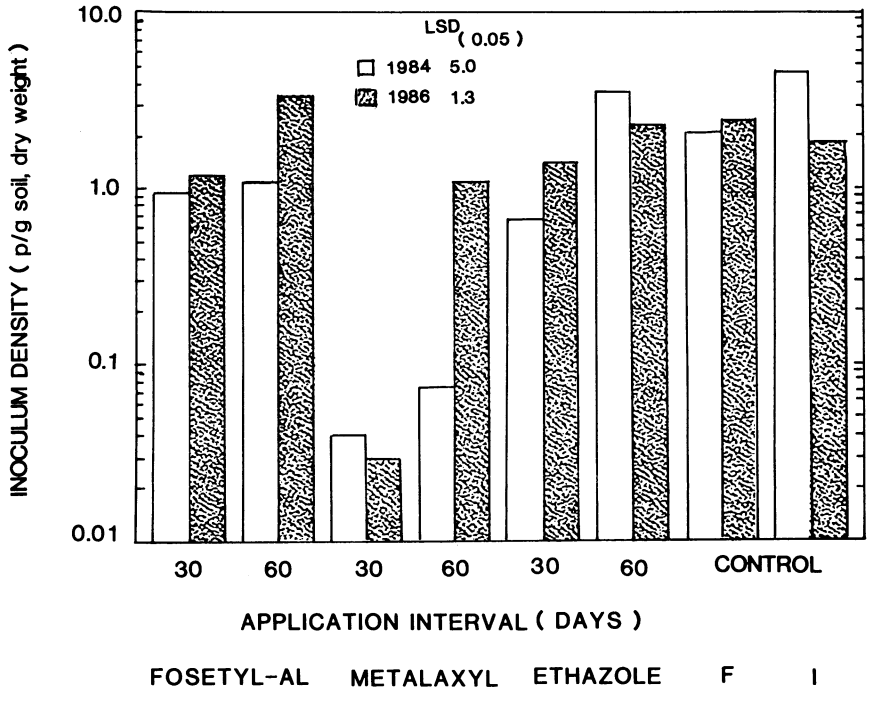

Fig. 5. Inoculum density of *Phytophthora cinnamomi* in soil from the root zones of plants in plots treated on 30- or 60-day schedules during the growing season with ethazol, fosetyl-Al, or metalaxyl in 1984 (open histogram) compared with inoculum density in 1986 (solid histogram), 18 mo after the last fungicide application. Controls were either plots fumigated (F) or plots left untreated (I). Soil samples were assayed by the elutriator method.

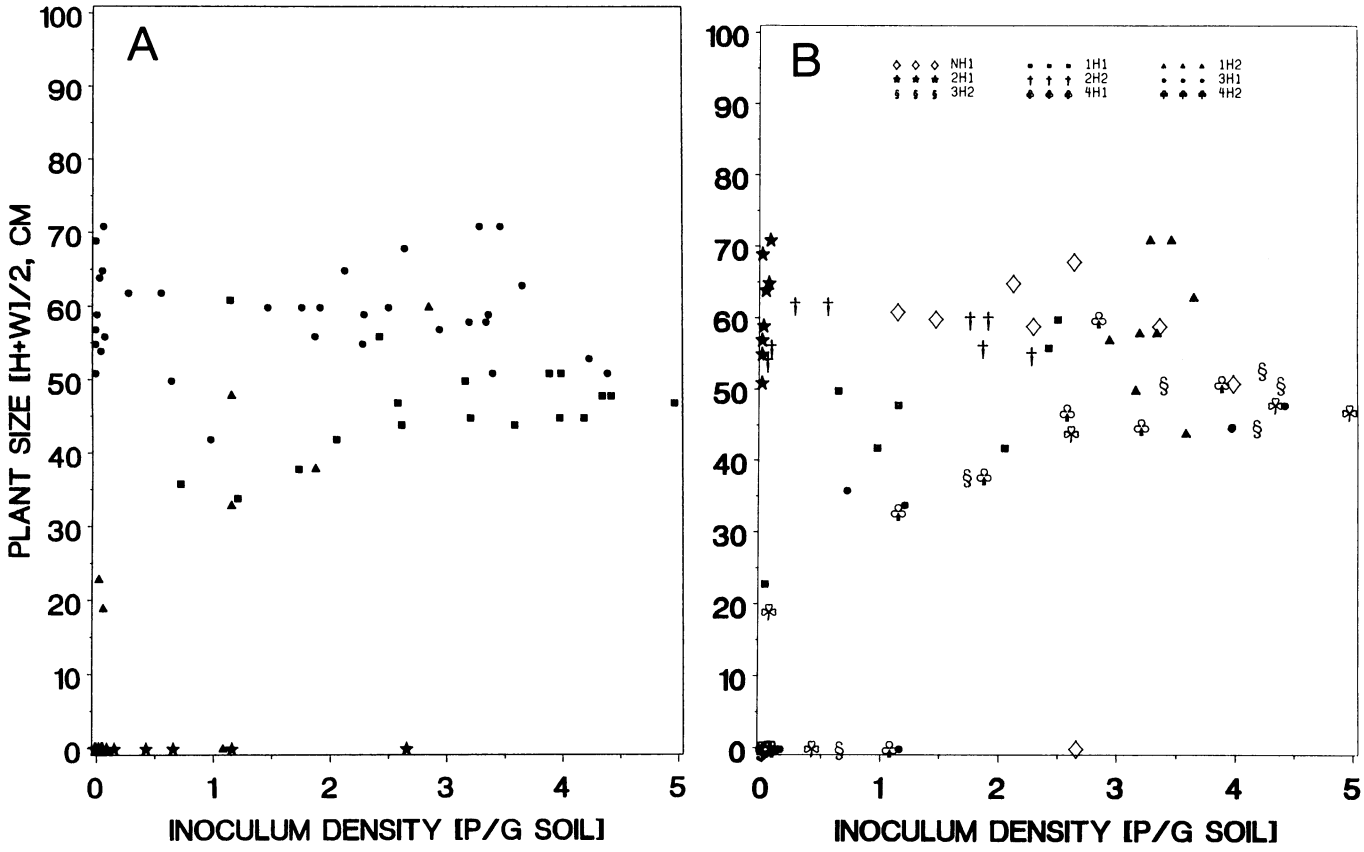

Fig. 6. (A) Scatter plot for estimated plant size [(height plus width)/2] by symptom rating (● = 1, healthy; ■ = 2, initial symptoms [chlorosis, dwarfed leaves, poor shoot growth]; ▲ = 3, severe symptoms [plant stunted, necrotic leaves present, defoliation]; and ★ = 4, dead plant) against inoculum density for each plan in 1986. **(B)** Same scatter plot for estimated plant size vs. inoculum density, only plotted by fungicide treatment instead. NH1 = fumigated control; 1H1 = fosetyl-Al, 30-day schedule; 1H2 = fosetyl-Al, 60-day schedule; 2H1 = metalaxyl, 30-day schedule; 2H2 = metalaxyl, 60-day schedule; 3H1 = ethazol, 30-day schedule; 3H2 = ethazol, 60-day schedule; and 4H1 and 4H2 = untreated, infested control.

half-life of fosetyl-Al in soil (0.3–1.9 days, depending on amount of organic matter [8]) and the inability of the plant to maintain toxic levels of phosphorous acid, the active agent in fosetyl-Al (9,10), for prolonged periods may account for the lack of residual activity of this fungicide.

The hand assay method for determining inoculum density of *P. cinnamomi* in soil resulted in a higher estimate of inoculum density than the elutriation method in two of four runs. Because the hand assay method was more time consuming, however, the elutriator method was used for routine assays. The accuracy of the elutriator method was increased by adjusting propagule counts by 9% to account for the portion of the population passing through the standard 38-μm-mesh sieve. The precision of the elutriation method with three subsamples per sample for inoculum density determinations was as good as running more than three subsamples. In a similar study with *Macrophomina phaseolina*, the precision of the assay procedure was best with as few as two subsamples per sample (7). When cost considerations for the assay procedure were included, however, only one subsample per sample could be justified. No cost considerations were included in the present study.

The effect of host plant health on inoculum density of *P. cinnamomi* was similar to other reports (5,11,13,16). Survival of *P. cinnamomi* in soil was poorest in the absence of a host or in soil where the host had died. Plants growing most vigorously often had the highest inoculum density in the root zone. As severity of foliar symptoms increased, inoculum density decreased.

Multiple applications of fungicides to soil may result in an apparent long-term persistence of the fungicide that is not accounted for by straightforward considerations of estimated half-life values. Although the results presented here suggest that lower rates of metalaxyl or longer intervals between application may be equally effective in suppression of inoculum of *P. cinnamomi* and development of Phytophthora root rot, further experimentation is warranted.

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