Effects of Metalaxyl on *Phytophthora cinnamomi* Root Rot of *Abies fraseri*

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ABSTRACT

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Greenhouse and field experiments were initiated in 1980 to determine the efficacy of metalaxyl (Subdue 2E and 15G) as a control for Phytophthora root rot. Two-year-old greenhouse-grown fir trees were protected from *P. cinnamomi* when treated with metalaxyl either before, during, or up to 7 days after soil infestation with fungal propagules. Three-year-old greenhouse-grown seedlings treated with the highest metalaxyl rate (250 μg a.i./ml) as late as 15 days after soil infestation remained asymptomatic and attempts to recover *P. cinnamomi* from their roots were unsuccessful. Survival of *P. cinnamomi* propagules living free in the soil was completely retarded only at a high fungicide rate (250 μg a.i./ml) 7 days posttreatment; however, after 48 days, no propagules were detected at rates >1.0 μg a.i./ml. In nursery bed trials, Fraser fir seedlings were protected from root rot and spread of the fungus within nursery beds was arrested. Metalaxyl may be effectively employed as a prophylactic root rot fungicide against Phytophthora root rot of Fraser fir.

Fraser fir (*Abies fraseri* (Pursh) Poir.) is the premium conifer species grown in western North Carolina for the Christmas tree market. This species represents 70% of the total revenue from Christmas tree sales in North Carolina and is cultivated on about 2,000 acres. Cultivation of Fraser fir is initiated by direct seeding of nursery beds where the germinated seedlings are grown for 3 yr. After this period, the seedlings are lifted and graded and large individuals are transplanted directly into plantations. Seedlings less than 20 cm high are transplanted into line-out beds where they are grown for an additional 2–3 yr before outplanting in plantations. Regardless of plant age, Fraser fir is susceptible to root rot disease incited by *Phytophthora cinnamomi* Rands. The fungus may overwinter as thick-walled chlamydospores free in soil, organic debris, or root fragments or as propagules in the roots of asymptomatic seedlings (5). Simultaneously with moist soil conditions or an increase in soil temperature and root flush, these propagules may germinate directly or indirectly and subsequently infect other fir seedlings, transplants, and trees. Fraser fir remains susceptible to infection by *P. cinnamomi* propagules until late fall, when soil temperatures drop below values favorable for infection (6).

Management of Phytophthora root rot has been directed at reducing soil inoculum levels by plant-bed fumigation and by prevention of spread and transport of the pathogen by employing sanitation practices (2). These management strategies have met with only moderate success. Plant-bed fumigation does reduce inoculum levels, but because Fraser fir is extremely susceptible to *P. cinnamomi*, a propagule density as low as 0.01/g dry soil can result in infection (7). Also, infected asymptomatic seedlings have been found to be infected, with their subsequent outplanting spreading the pathogen to previously uninfested line-out beds and plantations (5).

The systemic acylalanine fungicide metalaxyl Subdue 2E and 15G is highly effective against oomycetous fungi (1,4,8). This study was initiated to determine the efficacy of metalaxyl on *P. cinnamomi* root rot of Fraser fir. Preliminary reports have been published (3).

MATERIALS AND METHODS

Greenhouse studies. Two- and 3-yr-old disease-free Fraser fir seedlings were obtained from the Linville River Nursery (N.C. Forest Service) in Crossnore. Seedlings were planted in 8-cm-diameter clay pots in a 1:1:1 (v/v/v) mixture of peat, vermiculite, and sterile (fumigated) nursery bed soil. Nursery bed soil naturally infested with propagules of *P. cinnamomi* was used as the source of inoculum for all greenhouse studies.

The soil was drenched with 70 ml of the desired concentration of metalaxyl (10–250 μg a.i./ml). This volume saturated the potted soil (200 cm^3) with the desired fungicide solution with a minimum of runoff. Fungicide applications were made 1 day before infesting pots, the day of infestation, or 1, 5, 7, 10, or 15 days after infestation. The seedlings were at the budbreak stage when the fungicide was applied. The pots were infested by incorporating 20 g of the naturally infested nursery bed soil (range 17–23 propagules of *P. cinnamomi* per gram dry soil [PPG]) into the top 2 cm of the pots. All pots were watered daily and allowed to drain freely except on the day of fungicide application. Percent fir mortality based on symptom expression was recorded 45 days after infestation. Data from all treatments were collected from three replicates (10 potted seedlings each) and the experiment was repeated twice.

Propagule survival study. Clay pots 8 cm in diameter were filled (volume 200 cm^3) with naturally infested nursery soil and drenched with 70 ml of the desired concentration of metalaxyl (range 0.1–250 μg a.i./ml). The pots were watered daily for the duration of the experiment. The initial propagule density of *P. cinnamomi* was determined by plating 5 g of soil slurry (5 g moist soil suspended in 120 ml distilled water) on PCH medium, which is highly selective for *P. cinnamomi* (7). Seven and 48 days after the fungicide drench, each potted soil sample was stirred and a 5-g sample was removed and again assayed on PCH medium for *P. cinnamomi*. Data from all treatments were collected from three replicates (10 potted soils each) and all experiments were repeated twice.

Nursery bed studies. In 1980 at the Linville River Nursery, Crossnore, NC, 15 plots were established in a 1.2-ha nursery bed of 5-yr-old fir transplants. Mortality caused by *P. cinnamomi* infection was estimated to be 30%. Confirmation of *P. cinnamomi* infection at each plot was made by randomly selecting 10 seedlings per plot and plating their entire root systems on PCH medium. Three contiguous 1-m^2 blocks were established at each plot containing about 30 trees each. One block in each plot was treated with metalaxyl 15G at a rate of 1.1 kg a.i./ha, another block was treated with 2.8 kg a.i./ha, and the third block was left untreated. Four seedlings

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were removed from each block during 10 sampling periods from 1 May to 9 October 1980. Incidence of *P. cinnamomi* among these samples was determined by plating root systems on PCH medium.

On 29 April 1981, a 0.65-ha nursery bed of 2-yr-old seedlings was divided into five randomized complete blocks. Four rates of metalaxyl (Subdue 2E) ranging from 1.10 to 0.1 kg a.i./ha and controls (unsprayed) were applied by a tractor-mounted hydraulic pump sprayer and diluted in a volume of 467 L/ha at 40 psi. Twenty-five seedlings were randomly removed from each row adjacent to known *P. cinnamomi* disease foci on 10 occasions from 29 April to 6 December 1981. Seedlings were assayed for *P. cinnamomi* as described previously.

The third study was conducted in 1981 in a line-out nursery bed from which 5-yr-old Fraser fir seedlings had been recently harvested. Five plots with three age classes of Fraser fir (1-, 2-, and 3-yr-old transplants) were established within the line-out bed. The plots were infested by incorporating soil from other beds within the Linville River Nursery known to contain *P. cinnamomi* propagules. The plots were subdivided into six sections with 1-, 2-, and 3-yr-old fir seedlings transplanted into each of two sections. Three sections (containing each of three age classes) were hand sprayed with metalaxyl (Subdue 2E) at a rate of 1.10 kg a.i./ha. A 60-cm buffer was established between treated and untreated sections. Propagule density of *P. cinnamomi* was determined in each section before fungicide application and at five other dates throughout the growing season. Mortality based on symptom expression was assessed throughout the 5-mo sampling period.

In addition to the studies at the Linville River Nursery, a 0.51-ha line-out bed containing about 60,300 3-yr-old Fraser fir seedlings at Wolf Mountain, NC, was assessed for Phytophthora root rot on 5 May 1981. All dead seedlings from 12 randomly selected rows (1.2×70 m) were counted. One hundred dead seedlings were randomly removed from these 12 rows and assayed for *P. cinnamomi*. Because *P. cinnamomi* was isolated from 100% of these randomly lifted dead seedlings, the entire field except for two end rows was treated on 12 May with metalaxyl (Subdue 2E) at the rate of 1.1 kg a.i./ha on a tractor-mounted sprayer as described before. All dead seedlings were carefully rogued from the transplant beds on the same day. On 13 November, the field was surveyed to determine percent mortality and 20 randomly selected asymptomatic trees were removed from each row to determine percent infection.

RESULTS

Greenhouse studies. When potted 2-yr-old Fraser fir plants were treated with all rates of metalaxyl either before, during, or up to 7 days after infestation with *P. cinnamomi* propagules, they were effectively protected from root rot development (Fig. 1). Mortality of the seedlings was observed when metalaxyl at rates of 10–100 μg a.i./ml was applied 10 or 15 days after infestation (Fig. 1). The fungus, however, was not isolated from any potted seedlings treated with 150 μg a.i./ml or greater, regardless of when the application was made.

Potted 3-yr-old fir trees were protected from root rot when treated at all rates of metalaxyl either 1 day before, during, or 1 day after infestation of the soil with *P. cinnamomi* propagules (Fig. 2). Treatment with metalaxyl at 100 μg a.i./ml was effective up to 7 days after infestation; however, 250 μg a.i./ml was required for protection when treatments were made 10 or 15 days after infestation (Fig. 2).

Propagule survival study. *P. cinnamomi* propagule survival was adversely affected by soil treatment with metalaxyl. On the day of treatment, the infested soil had an average of 34 propagules per gram (dry wt) of soil (PPG). Seven days after treatment, control (untreated) soils contained 29 PPG, whereas soils treated with 0.1, 1, 10, 25, 100, and 250 μg a.i./ml contained 27, 25, 21, 16, 3, and 0 PPG, respectively. Forty-eight days after metalaxyl application, soils treated with 0, 0.1, and 1 μg a.i./ml contained 19, 2, and 0 PPG, respectively (Fig. 3).

Nursery bed studies. The 1980 field trial resulted in a high degree of disease control in the 5-yr-old transplants at both treatment rates (1.1 or 2.8 kg a.i./ha). *P. cinnamomi* was isolated from roots of about 23% of all trees from the 15 experimental plots on 1 May (Table 1).

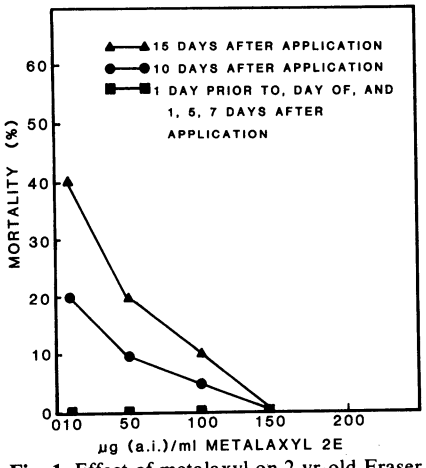

Fig. 1. Effect of metalaxyl on 2-yr-old Fraser fir mortality. Potted seedlings were treated in the greenhouse 1 day before, during, or 1, 5, 7, 10, or 15 days after infestation with *P. cinnamomi* propagules. Mortality is based on visual assessment.

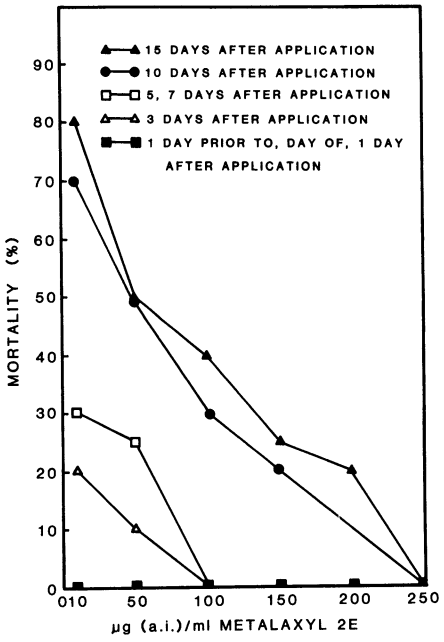

Fig. 2. Effect of metalaxyl on 3-yr-old greenhouse-grown Fraser fir mortality. Seedlings were treated 1 day before, during, or 1, 3, 5, 7, 10, or 15 days after infestation with *P. cinnamomi* propagules. Mortality is based on visual assessment.

Table 1. Percent *P. cinnamomi* isolation from Fraser fir treated with metalaxyl at two rates in 1980 and assessed on 10 dates in nursery bed plots

Date	Metalaxyl 15G rate (kg/ha)		
	0	1.1	2.8
1 May[a]	25	22	25
29 May	33	10	5
9 June	33	0	0
24 June	25	0	0
30 June	40	0	0
15 July	50	0	0
20 July	33	0	0
13 August	10	0	0
2 September	40	0	0
9 October	50	0	0

[a]Day of treatment after sampling for *P. cinnamomi*.

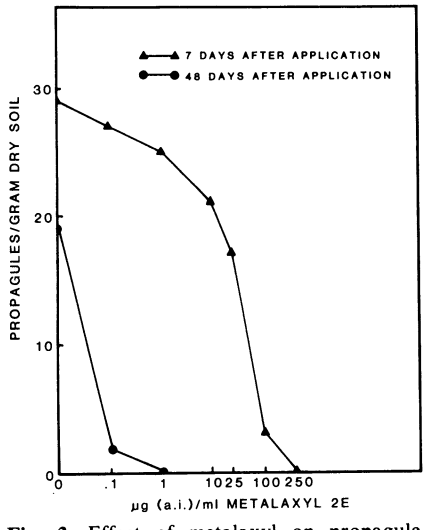

Fig. 3. Effect of metalaxyl on propagule populations. Potted soils infested with *P. cinnamomi* propagules were treated with metalaxyl (0–250 μg a.i./ml) and assayed for number of colony-forming units on PCH medium 7 and 48 days later.

Table 2. Effect of metalaxyl rate on control of *P. cinnamomi* root rot of Fraser fir as determined by percent fungus isolation from 2-yr-old nursery grown fir in 1981

Date	Metalaxyl 2E rate (kg/ha)				
	0	0.14	0.28	0.56	1.1
29 April[a]	61	70	65	48	77
5 May	48	0	0	0	0
19 May	52	0	0	0	0
26 May	40	0	0	0	0
2 June	59	0	0	0	0
16 June	71	0	0	0	0
5 July	90	0	0	0	0
12 October	85	1	0	0	0
7 November	50	0	0	0	0
5 December	40	0	0	0	0

[a] Day of treatment after sampling for *P. cinnamomi*.

Twenty-eight days after application of metalaxyl, the fungus was isolated from 33, 10, and 5% of the sampled trees from plots treated with 0, 1.1, and 2.8 kg a.i./ha, respectively. After 40 days posttreatment, *P. cinnamomi* could not be recovered from any of the fungicide-treated plots (Table 1). *P. cinnamomi* was recovered from 35% of untreated control plots.

Metalaxyl as 2E formulation was highly effective in controlling Phytophthora root rot in the 2-yr-old nursery bed at Linville during 1981 (Table 2). All seedlings sampled adjacent to the *P. cinnamomi* foci were asymptomatic. Before metalaxyl treatment on 29 April, an average of 64% of all trees sampled were infected with *P. cinnamomi*. With the exception of one tree (on 12 October), the fungus was never recovered from any asymptomatic trees adjacent to metalaxyl-treated disease foci. The fungus was consistently isolated from dead trees within the disease foci, however. Disease progress of the mapped foci was essentially halted within 6 days of treatment but continued in the control rows. Five assessments of cumulative mortality in the control rows were made. Mortality of Fraser fir ranged from 9.2 to 2.0% with a mean total mortality of 5.8% for all control rows.

In the line-out nursery bed where seedlings of three different age classes were transplanted into artificially infested soil, initial propagule density within plots was not significantly different ($\bar{x}$ = 0.07 PPG). After the metalaxyl application, propagule density within the treated sections of each plot was significantly reduced ($\bar{x}$ >0.01 PPG) compared with control sections. In 90% of the samples taken from the treated sections, no propagules were detected during the 5-mo sampling period. The mean propagule density (PPG) for the metalaxyl-treated plots on the final sampling date (16 September) were as follows: 1-yr-old, 0; 2-yr-old, 0; 3-yr-old, 0.02 (0–0.08). In contrast, 92% of the soil samples taken from the control plots during the 5-mo sampling period resulted in detection of pathogen propagules. The final propagule densities (PPG) were as follows: 1 yr old, 0.93 (0.05–3.96); 2 yr old, 1.47 (0.07–6.97); and 3 yr old, 9.00 (1.94–24.00).

Final mortality was significantly different between control and metalaxyl-treated sections for all age classes. For metalaxyl-treated sections, mortality per age class was: 1 yr old, 7%; 2 yr old, 2%; and 3 yr old, 0%. When these dead seedlings were assayed for *P. cinnamomi*, however, the fungus could not be recovered from any of them. The mean final mortality among the age classes in the control section was 1 yr old, 24%; 2 yr old, 16%; and 3 yr old, 59%. The percent fungus recovery from a random sample of five dead trees per section was: 1 yr old, 25%; 2 yr old, 85%; and 3 yr old, 100%.

At the Wolf Mountain Nursery line-out bed, a single observation at the end of the growing season was made to determine the efficacy of the application of metalaxyl. A total of 432 dead trees was observed in all metalaxyl-treated rows. This represents mortality of less than 1% of all trees in the nursery beds. From the two end rows that were left untreated, 6.3% of the trees had died. The fungus was recovered from 100% of the subsamples of dead trees taken from treated and control rows.

DISCUSSION

Two-year-old fir seedlings were protected from *P. cinnamomi* root rot when treated with metalaxyl either before, during, or up to 7 days after infestation of their soil with fungal propagules. It has been observed that under greenhouse conditions, fir seedlings may become infected as early as 2 days after exposure to naturally infested soils; infection usually takes place within 4 days (H. D. Shew, *personal communication*). These data suggest that metalaxyl possesses both prophylactic and a degree of therapeutic activity. Three-year-old seedlings treated with the highest metalaxyl rate (250 μg a.i./ml) as long as 15 days after soil infestation remained asymptomatic and the fungus was not recovered from their roots (Fig. 2). The 3-yr-old trees were generally more susceptible to root rot than the 2-yr-old trees. This may have been due to greater root mass and surface area, increasing the probability of a root tip encountering a fungal propagule.

Survival of *P. cinnamomi* propagules was greatly affected by metalaxyl treatments. In the greenhouse study with potted infested soils, the propagule density of the infested soils treated with metalaxyl at the highest rate tested (250 μg a.i./ml) was reduced from 34 (at day of treatment) to 0 within 7 days. When the same potted soils were sampled 48 days after treatment, however, no propagules were detected from infested soils receiving rates ⩾1 μg a.i./ml. The toxicity of metalaxyl in this system toward propagule survival apparently increased over time. A significant reduction of *P. cinnamomi* propagules as assayed on PCH was also seen in the nursery bed study among the three age classes of seedlings.

During both years of the experiments at the Linville River Nursery and the transplant experiment on Wolf Mountain, highly effective Phytophthora root rot control was realized using metalaxyl. We conclude that metalaxyl may be effectively employed as a prophylactic root rot fungicide for the control of Phytophthora root rot of Fraser fir. In addition, by maintaining disease-free nursery beds, the incidence of carrying infected seedlings to the plantation may be reduced; hence further economic loss and infestation of previously uninfested plantations may be avoided.

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