Metalaxyl and Efosite Aluminum for Control of Phytophthora Gummosis and Root Rot on Citrus

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ABSTRACT

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A soil drench or stem paint of metalaxyl and efosite aluminum controlled Phytophthora gummosis of sweet orange (*Citrus sinensis*) seedlings. Applied as soil drench before inoculation with *Phytophthora*, metalaxyl at 50 mg/L reduced the size of stem lesions caused by *P. parasitica* and *P. citrophthora* by 64 and 79%, respectively. Soil drench treatment with efosite Al at 3,000 mg/L resulted in 58 and 96% reduction of the lesion size with *P. parasitica* and *P. citrophthora*, respectively. Applied as a stem paint after stem inoculation with *Phytophthora*, metalaxyl at 60 g/L reduced lesion size by 51 and 52%, and efosite Al at 300 g/L reduced lesion size by 32 and 91%, with *P. parasitica* and *P. citrophthora*, respectively. Under field conditions, when applied as stem paint at 60 g/L, metalaxyl reduced by 88% the size of lesions caused by *P. citrophthora* on lemon trees (*C. limon*). Root rot incidence was also reduced in sweet orange seedlings treated by a soil drench of metalaxyl at 50 mg/L but not of efosite Al at 3,000 mg/L. The percentage of healthy roots, number of healthy root tips, and root weight were greater in seedlings treated with metalaxyl than in untreated seedlings grown in infested soil. *Phytophthora* was not detected in soil or roots from plants treated with metalaxyl but was recovered from those treated with efosite Al.

Two recently developed systemic fungicides, metalaxyl (Ridomil, Subdue) and efosite aluminum (Aliette), control diseases caused by fungi in the Oomycetes (1–4,7,18,19,21). On citrus, they control Phytophthora root rot, gummosis, and brown rot disease (7,10,13).

The purpose of this study was to investigate the protectant or curative activity of these two fungicides against Phytophthora gummosis on citrus seedlings and mature trees when applied before or after infection and to determine the efficacy of these fungicides against Phytophthora root rot of citrus seedlings.

MATERIALS AND METHODS

Fungi. The two species of *Phytophthora* pathogenic on citrus used were *P. parasitica* Dast, isolate T131, and *P. citrophthora* (R. E. Sm. and E. H. Sm.) Leonian, isolate P1156. *P. citrophthora* was used only for stem inoculation experiments.

Seedlings. Sweet orange (*Citrus sinensis* (L.) Osbeck 'Madam Vinus' or 'Pineapple') seedlings were used in greenhouse experiments. They were grown in steamed UC mix (peat moss/sand = 1:1, v/v) in flats for 5–6 mo, then transplanted and used in soil infestation experiments. Seedlings were also transplanted into 12-cm clay pots (one seedling per pot) and grown for 1–50 mo before being used in stem inoculation experiments.

Soil. A clay soil, with a pH of 7.1 and a maximum moisture holding capacity (MHC) of 50%, was obtained from a citrus orchard in Santa Paula, CA, and used for soil infestation experiments.

Stem inoculation. Before inoculation, the lower leaves on the seedling were cut off and a 4-mm-diameter plug of the bark was removed about 7–15 cm above the soil line. Four-millimeter mycelium-agar plugs, taken from the margin of a 5-day-old culture of *Phytophthora* on V8-$CaCO_3$ agar medium (20% Campbell V8 juice, 0.2% $CaCO_3$, 1.5% agar), were immersed in sterile distilled water for at least 1 hr. Each agar plug was inserted into the hole in the bark, covered by a 15-mm disk of wax paper and a surgical adhesive tape (26 mm × 7 cm) pressed slightly to crush the agar plug into the hole. Each noninoculated seedling received a *Phytophthora*-free agar plug.

Seedlings were left in a headhouse overnight to allow infection and then arranged in a randomized block design on a greenhouse bench, with the inoculated side of the stem facing north to avoid direct sunlight. Temperatures in the greenhouse ranged from 22 to 32 C but occasionally rose to 36 C.

Soil infestation. A soil-inoculum mix was prepared by adding *P. parasitica* chlamydospores to soil at high concentrations (1,000–1,500 spores per gram of soil, dry weight); concentrations were later diluted to 30 or 100/g. Chlamydospores were produced and collected in water by Tsao's method (14). Viability of these spores was 92–95% as determined by rose bengal staining (9). The germination of chlamydospores after 16–24 hr of incubation at 25 C in the dark was 80% or more in a solution of 0.01 M glucose and 0.01 M asparagine (9).

The soil-inoculum mix was prepared as follows: A large volume of soil was pasteurized with aerated steam at 60 C for 30 min and allowed to cool. A 2–3 kg volume was taken and the amount of water necessary to adjust the soil to 25% MHC was calculated. Chlamydospores were suspended in this water and the spore suspension was divided into 10-ml increments to determine the number of soil layers that would each receive 10 ml of the suspension. A small volume of soil, obtained by dividing the total soil volume by number of layers of soil, was layered into a 2,000-ml beaker, and 10 ml of chlamydospore suspension was sprinkled dropwise evenly over the surface with a pipette. Another layer was added plus 10 ml of chlamydospore suspension, and so on, until the soil and spore suspension were used up.

The content of the beaker was then spread onto a plastic sheet on a bench and thoroughly mixed by hand, first with each small portion (about 100 cc) then together with all other portions by making mounds (pyramids) back and forth three times. The thoroughly mixed soil-inoculum was stored in jars in the dark at 15 C for 4 days or more to allow

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microbial equilibrium and lysis of mycelium.

The inoculum density of *P. parasitica* in this soil-inoculum mix was determined by using the selective medium containing pimaricin, vancomycin, pentachloronitrobenzene, and hymexazol (PVPH) and using the soil dilution plate method (16). This allowed us to determine the quantity of soil-inoculum mix to be added to the large volume of soil in the greenhouse experiments to obtain the final concentration of 30 or 100 propagules per gram. Soil to be infested was spread into a thin layer over which the soil-inoculum mix was then sprinkled. A calculated amount of water was also sprinkled to bring the final moisture content of the soil to 25% MHC. Soil was mixed thoroughly using the pyramid method described. Noninfested control soil was treated similarly but with water only instead of soil-inoculum mix.

Seedlings were transplanted into 10- or 12-cm clay pots (one seedling per pot) containing infested or noninfested soil. About 10 g of inoculum of the mycorrhizal fungus, *Glomus fasciculatus* (Thaxter) Gerd. and Trappe, was added and placed near the root system of each seedling. The inoculum consisted of soil, roots, and spores from a pot containing Sudangrass (*Sorghum vulgare* Pers.) infested with *G. fasciculatus*.

Watering. In root rot experiments, soil was waterlogged for 3 days followed by 4 days of normal watering (15). Soil saturation during the 3-day waterlogging was accomplished by filling saucers with water daily, in addition to watering the soil surface. Plants in all the experiments were watered with deionized water.

Fungicidal treatments. In root rot experiments, fungicides were applied as soil drenches three times, at monthly intervals, beginning 3–5 days after the seedlings were transplanted to the infested soil. Each treatment was replicated 10 times. Plants were harvested 1 mo after the last application. Metalaxyl (formulation 2EC, 240 g a.i./L [2 lb a.i./gal]) was used at 25, 50, or 100 mg/L; efosite Al (wettable powder, 80%) was used at 3,000 mg/L. Each plant received 100 or 200 ml of fungicide solution at each application, depending on the experiment. Untreated seedlings received 100 or 200 ml of distilled water.

In stem inoculation experiments, soil drenches with metalaxyl were applied at 50 or 100 mg/L whereas efosite Al was applied at 3,000 mg/L. With each fungicide, two applications were made at weekly intervals, the last one 2 or 3 days before stem inoculation. Each treatment was replicated seven times.

As stem paint, metalaxyl was applied at 60 g/L and efosite Al at 300 g/L. To apply the fungicide to the stem, we removed the adhesive tape 3 or 4 days after stem inoculation and painted the fungicide over the inoculated site using a small brush, until runoff. Untreated control seedlings were painted with sterile distilled water. Each treatment was replicated seven times unless otherwise indicated. All concentrations are reported on the basis of active ingredient.

Root infection and dry weight of seedlings. After more than 3 mo of growth, seedlings were removed and washed under running water. The number of healthy root tips (15) and the percentage of healthy roots were recorded. Roots that were discolored or necrotic were considered infected. Shoot and root weights were determined after drying for 48 hr at 60 C.

Recovery of *Phytophthora* from soil. The number of propagules of *Phytophthora* in the rhizosphere soil of each root system was determined at harvest unless otherwise indicated. Soil that remained on the roots after the bulk of the soil was shaken off was considered as rhizosphere soil and was gently scraped off the roots. Two 1-g samples from each treatment were each divided into five subsamples. By the direct inoculation method (17), each subsample (about 200 mg) was sprinkled onto a petri plate containing the selective PVPH medium (16). Root samples, about 1 cm long, from each treatment were also plated on the PVPH medium. Plates were incubated at 25 C for 3 or 4 days, at which time colonies of *Phytophthora* were easily recognized if present.

Field trial. Seventeen-year-old lemon trees (*C. limon* (L.) Burm.) on *C. macrophylla* Wester rootstock were treated with metalaxyl as a trunk paint. All trees were showing gummosis caused by *P. citrophthora*. Seventeen trees were selected for similar age and lesion size. Before treatment, the lesions were delimited by lightly scratching the bark and then outlined in white acrylic paint. Seven control trees were painted with water and the other 10 were painted with metalaxyl at 60 g/L. Painting of the bark included lesion area and the 8–10 cm area around the lesion.

Determination of lesion size. For greenhouse experiments, stem bark of each seedling was stripped from the lesion area 2–3 wk after inoculation. The margins of the lesions were traced on transparent tape and then tranferred to a white sheet of paper. The surface area of the lesion was then determined by following the traced outline with a polar-compensating planimeter (Salmoiraghi 236A, Lietz No. 3651-00, Filotecnica Salmoiraghi, S.P.A., Milano, Italy), and data were reported in square millimeters.

For field experiments, another method was used in which a mathematical relationship between weight and area of clear plastic sheets was first determined. The increased area of the lesion on the tree trunk 6 mo after fungicide treatment was traced onto the plastic sheet. By cutting out the traced lesion and weighing the plastic, the size of the new lesion on the tree was calculated and reported in square centimeters.

RESULTS

Gummosis control. Treatment of 6- to 7-mo-old sweet orange seedlings, with soil drenches of metalaxyl or efosite Al before stem inoculation with *Phytophthora* spp., reduced lesion size in two greenhouse experiments. The size of lesions caused by *P. parasitica* was reduced by 64 and 79% on seedlings treated with metalaxyl at 50 and 100 mg/L, respectively. With *P. citrophthora*, the reduction was 79 and 88%, respectively (Table 1). Efosite Al at 3,000 mg/L reduced lesion size by 58 and 96% on seedlings inoculated with *P. parasitica* and *P. citrophthora*, respectively (Table 1).

Metalaxyl applied as a stem paint at 60 g/L 4 days after inoculation resulted in 51 and 52% reduction in lesion size on seedlings inoculated with *P. parasitica* and *P. citrophthora*, respectively (Table 2). Stem painting with efosite Al at 300 g/L reduced the lesion size caused by *P. parasitica* and *P. citrophthora* by 32 and 91%, respectively (Table 2).

In the field experiment on 17-yr-old lemon trees infected with *P. citrophthora*, metalaxyl at 60 g/L as a stem paint reduced the size of new lesion growth by 88% (Table 3). No increase in lesion size was observed in 50% of the treated trees.

Root rot control. In the greenhouse, sweet orange seedlings grown in soil infested with *P. parasitica* developed characteristic symptoms of root rot. The incidence of root rot was reduced among seedlings treated with metalaxyl as a soil drench (Tables 4 and 5). The percentage of healthy roots and number of healthy root tips were greater in plants treated with metalaxyl, at as low as 25 mg/L, than in untreated seedlings. Metalaxyl-

Table 1. Effect of metalaxyl or efosite aluminum soil drenches applied before stem inoculation, on Phytophthora lesions on sweet orange seedlings[y]

Treatment	Lesion size (mm^2)	
	P. parasitica	*P. citrophthora*
Control	66 a[z]	394 a
Metalaxyl, 50 mg/L	24 b	84 b
Efosite Al, 3,000 mg/L	28 b	14 c
Control	101 a	828 a
Metalaxyl, 100 mg/L	21 b	100 b

[y] Seedlings, 6 to 7 mo old, were inoculated 3 days after the last soil drench treatment. Each seedling received 200 ml of fungicide solution twice with a 1-wk interval between treatments. Data were collected 1 mo after inoculation.

[z] Each number is an average of seven replicates. Figures with same letter in each column are not significantly different ($P = 0.01$ by Duncan's multiple range test, but $P = 0.05$ for *P. citrophthora* in the first treatment group; log transformation was used for analysis of the latter data).

treated plants averaged 76% healthy roots compared with 38% for the untreated plants (Table 5). Root weight was greater in seedlings treated with 50 mg/L or more of metalaxyl (Tables 4 and 5) than in untreated plants. At the end of the experiment, 12.8 propagules of *P. parasitica* were recovered per gram of untreated soil, but none were recovered from soil treated with metalaxyl at 100 mg/L (Table 4). In all other experiments (Table 5), *Phytophthora* was not recovered from soil or roots at any metalaxyl concentration.

Efosite Al as soil drench at 3,000 mg/L was not sufficient to protect the seedlings from root rot caused by *P. parasitica*. No differences were found in the percentage of healthy roots, the number of healthy root tips, or root weight among seedlings treated with efosite Al and untreated seedlings (Table 5). *Phytophthora* was recovered from soil and roots from all plants treated wth efosite Al.

Table 2. Effect of metalaxyl or efosite aluminum, applied as stem paint after inoculation with *Phytophthora*, on lesions on sweet orange seedlings[w]

Treatment	Lesion size (mm^2)[x]	
	P. parasitica	*P. citrophthora*
4 days after inoculation[y]		
Control	74 a	479 a
Metalaxyl, 60 g/L	36 b	232 b
3 days after inoculation[z]		
Control	84 a	1,030 a
Efosite Al, 300 g/L	57 b	92 b

[w] Lesion size before treatment, on 5-yr-old seedlings, was 20 mm^2 for *P. parasitica* and 143 mm^2 for *P. citrophthora*.
[x] Figures with same letter in each column are not significantly different ($P = 0.01$, except for *P. parasitica* 3 days after inoculation P = 0.05, Duncan's multiple range test).
[y] Each number is an average of seven replicates.
[z] Each number is an average of five replicates.

Table 3. Effect of metalaxyl stem paint on *Phytophthora citrophthora* gummosis lesions on 17-yr-old lemon trees under field conditions

Treatment	New lesion growth (cm^2)[z]	Trees with new lesion growth after treatment (%)
Control	784 a	100
Metalaxyl, 60 g/L	94 b	50

[z] Each number is an average of seven replicates (control) and 10 replicates (metalaxyl). Figures with same letter in each column are not significantly different ($P = 0.01$, Duncan's multiple range test). Metalaxyl was applied in October 1979 and data were collected in April 1980.

DISCUSSION

Applied as a soil drench before stem inoculation with *P. parasitica* or *P. citrophthora*, metalaxyl was taken up by roots and translocated to the stem of citrus seedlings where it reduced lesion size. Only 50 mg/L was sufficient to reduce the lesion size by more than 60% for either *Phytophthora* species. Rapid root uptake and translocation of metalaxyl to the aboveground parts have been reported in many different plants (4,12, 13,21). Although less fungicide remained in the stem than in the foliage or roots of *Persea indica* (20) when metalaxyl was used as a soil drench, apparently the low concentration in citrus stems was sufficient to reduce the size of *Phytophthora* lesions significantly in our study.

Metalaxyl is highly inhibitory to in vitro mycelial growth of both *P. parasitica* and *P. citrophthora* (5). The initial infection and the subsequent lesion development in citrus stems involve growth of *Phytophthora* mycelium, which also may explain why the percentage of the lesion reduction by metalaxyl was similar for both *Phytophthora* species. Efosite Al was more effective in reducing size of stem lesions caused by *P. citrophthora* than of those caused by *P. parasitica*. In vitro studies (6) showed that efosite Al at low concentrations was more inhibitory to *P. citrophthora* than to *P. parasitica* mycelial growth. Biweekly sprays of efosite Al at 2 g/L controlled citrus gummosis caused by *P. parasitica* on orange seedlings (7).

Metalaxyl applied as stem paint at 60 g/L before inoculation protects grapefruit trees against *P. parasitica* for more than 186 days (13). Our study showed that metalaxyl at the same concentration used as a stem paint 4 days after inoculation also reduced the incidence of gummosis caused by *P. parasitica* or *P. citrophthora* by 51–52%. Use of efosite Al as stem paint to control Phytophthora diseases has not been previously reported. Our findings show that a stem paint with this fungicide can effectively reduce stem lesion size, mainly where *P. citrophthora* is the causal agent.

Metalaxyl used as soil drench also showed good control of Phytophthora root rot of citrus caused by *P. parasitica* and *P. citrophthora*. Even at very low concentrations (25 mg/L) with three applications at monthly intervals, metalaxyl reduced Phytophthora root rot. Monthly soil drenches of metalaxyl at 5 and 10 ppm control avocado root rot caused by *P. cinnamomi* in the greenhouse (21), and Phytophthora root rot of azalea has also been controlled with one drench of metalaxyl (18 ppm) applied 3 days before inoculation (1). In our study,

Table 4. Effect of metalaxyl soil drenches[y] on Phytophthora root rot of sweet orange seedlings and on *Phytophthora parasitica* population in rhizosphere soil

Soil	Metalaxyl (mg/L)	Healthy roots (%)	Healthy root tips (no./plant)	Dry weight (g)		*Phytophthora* propagules (no./g of soil)	
				Shoots	Roots	At 5 wk	At 12 wk
Not infested	0	90.5 a	173 a	1.9 a	1.7 a	...	0 b
	100	92.5 a	170.4 a	1.45 b	1.2 b	...	0 b
Infested[z]	0	66 b	92.8 b	1.35 b	0.78 c	6.8 a	12.8 a
	100	83.8 a	129.3 ab	1.65 ab	1.14 b	0.6 b	0 b

[y] Applied in 200 ml solution per pot, three times at monthly intervals. Each number is an average of 10 replicates except for *Phytophthora* population data at 5 wk (average of five replicates). Figures with same letter in each column are not significantly different ($P = 0.01$, Duncan's multiple range test). Duration of the experiment was 12 weeks.
[z] Infested soil received 30 chlamydospores of *P. parasitica* per gram of soil.

Table 5. Effect of metalaxyl or efosite aluminum soil drenches[y] on Phytophthora root rot of sweet orange seedlings

Soil	Treatment	Concn (mg/L)	Healthy roots (%)	Healthy root tips (no./plant)	Dry weight (g)	
					Shoots	Roots
Not infested	Metalaxyl	0	89 a	135 a	2.68 a	1.75 a
		25	90 a	129 a	2.63 a	1.37 ab
		50	88 a	148 a	2.74 a	1.58 a
Infested[z]	Metalaxyl	0	38 b	57 b	2.40 a	0.90 c
		25	76 a	116 a	2.76 a	1.04 bc
		50	84 a	127 a	2.80 a	1.45 ab
Not infested	Control	0	90 a	135 ab	4.73 a	1.87 ab
	Metalaxyl	50	95 a	130 ab	4.37 a	1.81 ab
	Efosite Al	3,000	94 a	113 ab	4.16 a	1.38 ab
Infested	Control	0	58 b	75 b	4.11 a	1.64 ab
	Metalaxyl	50	84 a	142 a	4.60 a	2.10 a
	Efosite Al	3,000	57 b	89 ab	4.37 a	1.14 b

[y] Applied in 100 ml solution per pot, three times at monthly intervals. Each number is an average of 10 replicates. Figures with same letter in each column are not significantly different ($P = 0.01$, except for root weight $P = 0.05$, Duncan's multiple range test). Duration of the experiment was about 12 wk.
[z] Infested soil received 100 propagules of *P. parasitica* per gram of soil.

citrus root weight was greater in soil treated with 50 mg/L or more of metalaxyl than in untreated soil. Greenhalgh (8) found that root weight of peaches is also greater in soil drenched with metalaxyl than in untreated soil infested with *P. cinnamomi*. In all our experiments with metalaxyl, no *Phytophthora* propagules were recovered from soil or roots at any of the concentrations used. Greenhalgh (8) also found that *P. cinnamomi* was not detected in soil drenched with metalaxyl. In our experiments efosite Al at 3,000 mg/L was not sufficient to control Phytophthora root rot of citrus seedlings. Zentmyer (21), however, found that avocado root rot caused by *P. cinnamomi* was controlled by weekly, but not monthly, applications of efosite Al at 50 or 100 ppm.

Our data indicate that efosite Al also moved systemically from the roots to the aboveground part of citrus seedlings and reduced stem lesion size. Since *Phytophthora* mycelium was used as the inoculum and since Farih et al (6) showed that efosite Al is not highly inhibitory to linear mycelial growth of *P. parasitica* and *P. citrophthora*, the fungicide thus must be reducing disease after being metabolized or otherwise changed to another chemical form that is highly toxic to *Phytophthora*. Other workers (3,11,22) have made similar observations.

Metalaxyl used as soil drench or stem paint appears to be a promising fungicide for control of Phytophthora gummosis and root rot of citrus. It is effective when used with only few applications and at low concentrations. Efosite Al can also be a promising fungicide for control of Phytophthora gummosis mainly where *P. citrophthora* is the causal agent. However, higher concentrations of efosite Al than of metalaxyl are needed to assure adequate disease control.

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