Effect of Metalaxyl, an Acylalanine Fungicide, on Developmental Stages of *Phytophthora infestans*

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ABSTRACT

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A systemic acylalanine fungicide (metalaxyl) effectively inhibited growth and development of *Phytophthora infestans* in potato foliage. The fungicide, at low concentrations, inhibited lesion appearance, expansion and sporulation from lesions, and reduced the germinance of sporangia produced from lesions on treated leaves. When leaves were treated before inoculation with low concentrations of metalaxyl (10 μg [a.i.]/ml), lesion appearance was suppressed. The number of sporangia produced from lesions and their subsequent germinance was dramatically suppressed by low concentrations of metalaxyl (10–30 μg [a.i]/ml) when applied after inoculation, and by slightly higher concentrations when applied before inoculation. Soil applications also effectively suppressed the development of *P. infestans*. The fungicide at low concentrations (25 μg[a.i.]/ml) suppressed in vitro sporangium germination of one isolate (race 1, 2, 4) but not of another isolate (race 1, 2, 3, 4). These data help explain how this fungicide effectively suppresses established epidemics of potato late blight.

Cultivation of the Irish potato (*Solanum tuberosum* L.) in much of the USA has been hampered by the late blight fungus, *Phytophthora infestans* (Mont.) d By. Although they have been sought, cultivars with high levels of stable resistance have not been widely grown, and contact protectant fungicides are still widely used to suppress late blight (7).

The development of the systemic acylalanine fungicide Ridomil® (CGA 48988, metalaxyl, *N*-[2,6-dimethylphenyl]-*N*-[methoxyacetyl]-alanine methyl ester, CIBA-Geigy Corp., Greensboro, NC 27409 USA) provided the pest manager with a new and potentially more effective tool to control late blight (2). Metalaxyl appears to be specifically effective against Phycomycetes (12). This compound is not phytotoxic, is rapidly transported acropetally, and is effective at low rates (9).

Metalaxyl has been used successfully to suppress epidemics of many different diseases induced by phycomycetes. On potatoes, metalaxyl effectively suppressed late blight on foliage and tubers at dosages less than 10% of those required of contact protectants for effective control (12).

Several authors (9,10,12) note that (compared to normal protectant spray schedules) metalaxyl may be applied at greatly extended intervals, often one-third as frequently.

Metalaxyl was effective whether applied to foliage or applied to roots via the soil; however, soil applications appeared to have longer-lasting effects against *P. infestans* than did foliar applications (4). Metalaxyl was toxic to sporangia and zoospores in vitro and it delayed expansion of young lesions and suppressed sporangium production on treated plants (4,11). When metalaxyl was applied to plants supporting a rapidly progressing epidemic, a suppression of epidemic development occurred within 1–2 days. Tuber blight was greatly reduced and yields increased as compared either to protectant-treated or unsprayed check plots (8).

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This study reports the in vitro and in vivo effects of metalaxyl on *P. infestans*.

MATERIALS AND METHODS

Culture preparation. *P. infestans* [race 1, 2, 3, 4] obtained from H. D. Thurston, Cornell University, was reisolated periodically from potato foliage, and maintained on amended lima bean agar (ALBA) (Table 1) (3). Cultures were grown in the dark at 18 C. Inoculum was prepared from cultures 7–21 days old. Zoospores were prepared by washing sporangia from ALBA in 9-cm-diameter plastic petri dishes with a 1% glucose solution. This sporangial suspension was mixed with an equal volume of ice and incubated at 1 C for 2 hr. The remaining ice was removed and the suspension equilibrated to 21–24 C. Zoospores were liberated within 2 hr.

Plants. *Solanum tuberosum* L. 'Norchip' plants were grown in the greenhouse in 12.7-cm-diameter clay pots (approximate volume 1,040 cm^3) containing a peat-vermiculite mixture (1:1, v/v) amended with 2.3 kg 14-14-14 (N-P-K) per cubic yard of mixture. In all experiments the plants were 28–38 days old at the four- to eight-leaf stage.

In vitro experiments. A suspension of either sporangia or zoospores at a final concentration of 7×10^4/ml was agitated for 20 sec on a Vortex "Genie" mixer (Scientific Industries Inc., Bohemia, NY 11716) with an appropriate concentration of fungicide [Ridomil 50 W] (range 1 to 227 μg[a.i.]/ml of 0.3% glucose solution). This mixture was poured on a 9-cm-diameter petri dish containing 20 ml of solidified ALBA. The plates were swirled to distribute the mixture evenly, and excess liquid was decanted. Plates were incubated at 23 C until at least 90% of the sporangia or zoospores in the absence of the fungicide had germinated (usually within 24 hr). At least 200 sporangia or zoospores were counted by observation of the plate under a compound microscope to determine the percentage of germination. All treatments were repeated two times, and each experiment was replicated at least

twice.

The effect of metalaxyl on radial growth of *P. infestans* was determined. Metalaxyl was incorporated in ALBA in concentrations ranging from 0 to 227 μg (a.i.)/ml of medium. An agar disk (5 mm in diameter) from an actively growing *P. infestans* colony was placed at the center of each test plate. Rates of growth and fungal morphological characteristics were noted at 3, 5, 7, and 9 days post inoculation. Data from all treatments were collected from three replications at each observation period, and all experiments were repeated at least twice.

Effects of metalaxyl on spore germination in vivo. Potted plants were sprayed to runoff with the desired concentration of metalaxyl and then allowed to dry for 1–2 hr. A suspension of zoospores or sporangia, 5,000 or 10,000/ml, respectively, was sprayed on the plant until runoff. The plants were incubated at 100% relative humidity (RH) at 19 C for 72–96 hr. Terminal leaflets were excised from the plant at 72–96 hr after inoculation, and observed under a compound microscope for microscopic lesion development. Four areas (1 cm^2 each) per leaflet were observed. All experiments were repeated at least twice.

Effects of metalaxyl on sporulation and lesion expansion in infected tissue. The effect of dosage and timing of metalaxyl applications on lesion expansion and sporulation was observed. Terminal leaflets of potted potato plants were inoculated by suspending a 10-μl drop of *P. infestans* propagules (2,500 zoospores or 5,000 sporangia per milliliter) on the abaxial surface of the leaflet at the junction of the midvein and a side vein. Inoculated plants were maintained at 100% RH and 19 C for 24 hr and then at 50–85% RH at 22 C for 96 hr. At least six leaflets on two plants were inoculated for each fungicide treatment. Metalaxyl in different concentrations was atomized to runoff onto both surfaces of leaves either before or after inoculation. Four terminal leaflets with well-developed lesions were collected from each plant 5 days after inoculation, and incubated at 18 C on plastic mesh in 9-cm-diameter petri dishes containing moistened filter paper. After incubation for 48 hr, six leaf disks (7 mm in diameter) were cut with a cork borer from the margin of the area where sporulation appeared to be the greatest, and were agitated in 1.5 ml of a 9.5% ethanol solution on a Vortex "Genie" mixer for 20 sec to dislodge the sporangia from sporangiophores. At least two leaves were sampled from each plant, with two plants per treatment. When lesions were small or sporulating poorly, disks from several lesions were combined. Each experiment was replicated at least twice. Sporangia were counted in a haemocytometer and sporulation per unit area was calculated. Sporulation for each treatment was expressed as a percentage of control (no fungicide). Analyses of variance (ANOVA) were performed on arc sine transformed percentage data.

The effect of metalaxyl on lesion expansion was investigated. The greatest width and length of individual selected lesions was measured over time on plants maintained at 22 C and 60–80% RH. Lesion area was calculated by the equation, area = $\pi/4$ width × length.

Viability of sporangia from infected tissue. Sporulating lesions were agitated for 20 sec in 3 ml of an oxytetracycline-dihydrate solution (35 μg/ml). The oxytetracycline-dihydrate was used to suppress bacterial contamination and had a slightly inhibitory effect on sporangium germination (6). The sporangial suspension was distributed onto 10 ml of solidified ALBA in a 9-cm-diameter petri dish and the excess liquid was poured off. Germination was determined after 24 hr at 18 C. When possible, at least 200 sporangia per plate were counted to determine percent germination (viability).

Soil drench experiments. The effectiveness of metalaxyl as a soil drench was determined by pouring 100 ml of the desired concentration of fungicide (range 1–35 μg [a.i.]/ml) on the soil of a 12.7-cm-diameter pot (volume 1,040 cm^3) containing a 28-day-old Norchip plant. Fungicide applications were made either 3 days before or 3 days after inoculation. The plants were not watered for at least 24 hr before the soil drench was applied to assure retention of the fungicide in the pot. Lesion expansion and sporulation of *P. infestans* from lesions were determined as described above.

RESULTS

Within this experimental system, metalaxyl had no in vitro effect on the developmental stages of *P. infestans*, race 1, 2, 3, 4. There was no significant effect ($P = 0.05$) of metalaxyl (up to 227 μg [a.i.]/ml) on the germination of sporangia or zoospores. The speed of germination and shape and size of germ tubes from spores exposed to metalaxyl did not differ from those of propagules not exposed to the fungicide. There was no significant difference ($P = 0.05$) between the rates of growth of *P. infestans* on ALBA containing metalaxyl (from 35–350 μg [a.i.]/ml of medium) and rates of growth on fungicide-free ALBA. *P. infestans* grew from 0.49 to 0.53 cm/day and that rate was not affected by metalaxyl. The colony morphology of *P. infestans* was not affected by metalaxyl incorporated into the medium. The fungus produced abundant aerial hyphae and large numbers of sporangia, regardless of treatment.

Penetration of *P. infestans* race 1, 2, 3, 4 into potato leaves was not affected by metalaxyl. Plants treated with 500, 250, 100, 25, 10, or 0 μg (a.i.)/ml metalaxyl 1–2 hr before inoculation with either zoospores or sporangia had similar numbers of infection sites per square centimeter ($P = 0.05$). Leaves sprayed with metalaxyl at 500 μg (a.i.)/ml had 22 ± 6 lesions per square centimeter while untreated leaves had 19 ± 5 lesions per square centimeter. However, all lesions on metalaxyl-treated leaves failed to expand beyond 0.1 mm in diameter, and were delimited by a distinctive black band of necrotic tissue.

TABLE 1. Components of amended lima bean agar for culture of *Phytophthora infestans*

Major ingredients[a]		Vitamin stock[b]		Trace elements[b]	
Sorbitol	5.0 g	Biotin	0.20 mg	$FeC_6H_5O_7 \cdot 3\ H_2O$	215 mg
Mannitol	5.0 g	Folic acid	0.20 mg	$ZnSO_4 \cdot 7\ H_2O$	150 mg
Dextrose	5.0 g	I-inositol	12.00 mg	$CuSO_4 \cdot 5\ H_2$	30 mg
KNO_3	3.0 g	Nicotinic acid	60.00 mg	$MnSO_4 \cdot H_2O$	15 mg
K_2HPO_4	1.0 g	Pyridoxine-HCl	18.00 mg	H_3BO_3	10 mg
KH_2PO_4	1.0 g	Riboflavin	15.00 mg	MoO_3	7 mg
$MgSO_4$	0.5 g	Thiamine-HCl	38.00 mg	Distilled H_2O to make	400 ml
$CaCl_2$	0.1 g	Coconut milk	50.00 ml		
Vitamin stock	2 ml	Distilled H_2O to make	300 ml		
Trace elements	2 ml				
Yeast extract	2 g				
Lima bean extract[c]	250 ml				
Bacto-agar	15 g				
Distilled H_2O to make	1 L				

[a] Autoclave 30 min before dispensing into sterile containers.
[b] Keep refrigerated.
[c] To prepare lima bean extract, autoclave 100 g of finely ground dried lima beans in 1 L of distilled H_2O for 30 min. Strain through two layers of cheesecloth. Keep frozen until needed.

The sporulation from lesions treated with metalaxyl was greatly affected by the timing of fungicide application in relationship to plant inoculation. Sporulation from lesions in treated tissue increased as the interval between fungicide application and inoculation increased, whether fungicide was applied before or after inoculation. Metalaxyl applied 7 days before inoculation at rates up to 227 μg (a.i.)/ml, reduced sporulation to 24% that of the controls. When fungicide was applied 3 or 5 days before inoculation, sporulation was reduced to less than 1% of control by 150 μg (a.i.)/ml and 175 μg (a.i.)/ml, respectively. Metalaxyl applied at 1 μg (a.i.)/ml on the day of inoculation prevented lesion expansion and no sporulation resulted. When expanding lesions were sprayed 5 days after inoculation, sporulation was reduced to only 10% of control at 227 μg (a.i.)/ml, and when the fungicide was applied to lesions 1 or 3 days after inoculation, sporulation was completely eliminated at 2.5 or 35 μg (a.i.)/ml, respectively (Fig. 1). When areas of a lesion supported different levels of sporulation, the areas including major veins sporulated most densely. Presumably the fungitoxicant effect in this tissue was diluted by the large volume of vascular tissue associated with large veins.

Metalaxyl retarded lesion expansion more effectively when applied 3 days before inoculation than when applied 3 days after inoculation (Fig. 2). When applied 3 days before inoculation, increasing doses of metalaxyl had increasingly suppressive effects on lesion expansion. However, when applied 3 days after inoculation, only the highest concentration of metalaxyl (227 μg [a.i.]/ml) had a large effect in retarding lesion expansion ($P = 0.05$) (Fig. 2). The coefficient of correlation between the curves for the 3

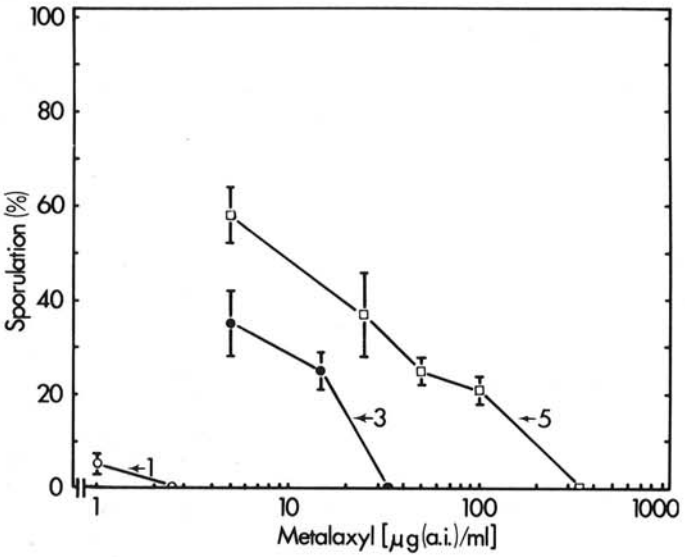

Fig. 1. Effect of metalaxyl on sporulation of *Phytophthora infestans* from lesions on potato (*Solanum tuberosum* 'Norchip') foliage. Metalaxyl was applied 1, 3, and 5 days after inoculation by atomizing the foliage to runoff with the indicated concentration of fungicide. Sporulation from control lesions (no fungicide) ranged from 9.2×10^3 to 2.1×10^4 sporangia/cm^2 and the effect of metalaxyl is quantified in relation to sporulation from control lesions. Sporulation was induced as described in the text. Range bars equal one standard deviation.

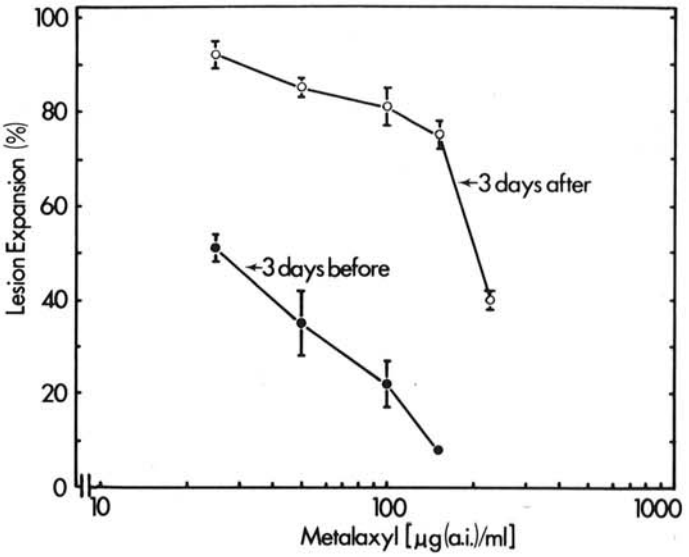

Fig. 2. Effect of metalaxyl on rate of late blight lesion expansion induced by *Phytophthora infestans* in potato (*Solanum tuberosum* 'Norchip') leaves. Rates of expansion are indicated as percent of control, and control lesions (no fungicide) expanded at rates of 1.5–2.0 cm^2/day. Metalaxyl was applied via atomization to runoff at the concentration indicated either 3 days before or 3 days after inoculation. Range bars equal one standard deviation.

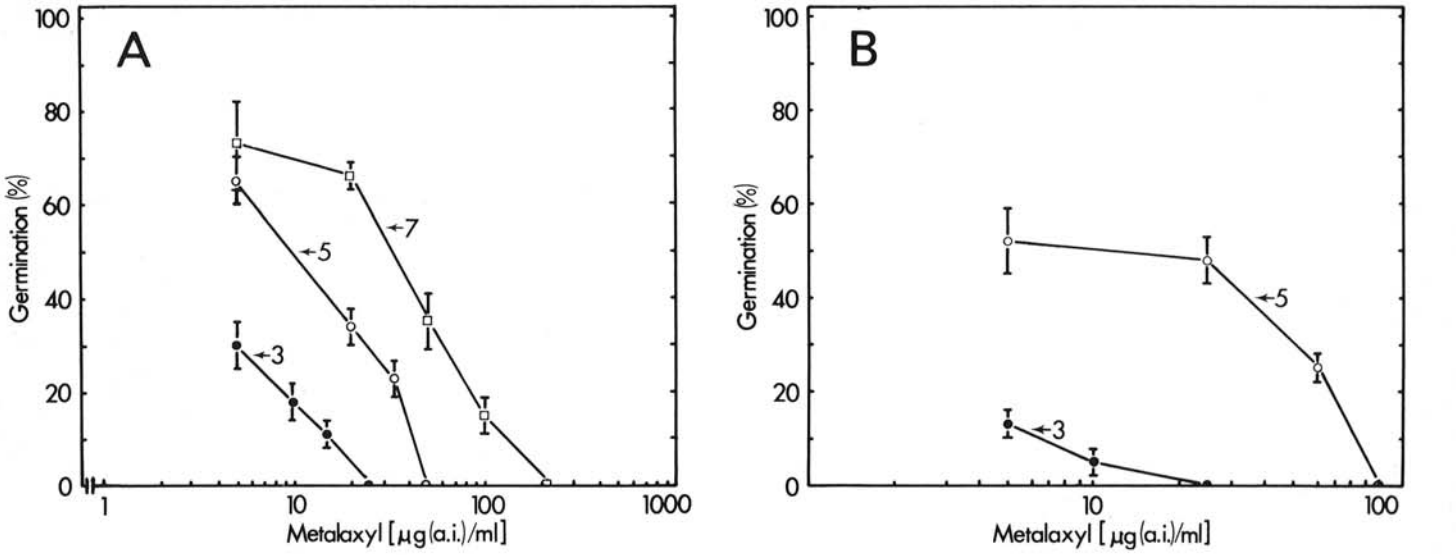

Fig. 3. Effect of metalaxyl on viability of *Phytophthora infestans* sporangia produced from metalaxyl-treated late blight lesions on potato (*Solanum tuberosum* 'Norchip') leaves. Leaves were treated either **A,** 7, 5, or 3 days before inoculation or **B,** 3 or 5 days after inoculation. Metalaxyl was applied via atomization to runoff at the concentration indicated. Sporulation was induced as described in the text, and viability of sporangia (germination in vitro) was determined as described in the text. Data are expressed as percent of control (no fungicide) which was 95–98% germination. Range bars equal one standard deviation.

TABLE 2. Effect of soil or foliar application of metalaxyl[a] on sporulation of *Phytophthora infestans* from potato laminar or vascular leaf tissue disks

Rate (μg [a.i.]/ml)	Sporulation[b] (% of control)	
	Laminar	Vascular
In soil		
0	100 A[c]	100 A
1	...	8 B
5	...	1 C
10	5 B	...
25	0 C	...
Foliar		
0	100 W	100 W
4	38 X	...
5	...	45 X
10	27 Y	...
35	0 Z	18 Y
75	...	1 Z

[a] Metalaxyl was applied 3 days after plants had been inoculated as described in the text. When applied to soil (~ 1,040 ml), metalaxyl was applied in 100 ml of water at the concentration indicated. When applied to the foliage, metalaxyl (at the indicated concentration) was sprayed to runoff.

[b] Sporulation was measured from leaf disks cut from lesions 48 hr after they had been placed into moist chambers as described in the text. Control rates for sporulation ranged from 3.9 to 5.1 × 10^4 sporangia per square centimeter.

[c] Numbers within a column followed by the same letter are not significantly different ($P = 0.05$) as determined by Duncan's new multiple range test.

days before and 3 days after inoculation treatments is not significant (r = 0.115). No lesions were initiated when 227 μg (a.i.)/ml metalaxyl was applied 3 days before inoculation.

The viability of sporangia produced on leaves treated with metalaxyl was greatly affected by the fungicide dosage and time of application (Fig. 3). Fungicide applied after inoculation was more effective in reducing sporangium viability than that applied before. Sporangium viability was reduced to about 1% of the control by 25 or 50 μg (a.i.)/ml when applied 3 or 5 days after inoculation, respectively. When fungicide was applied at 25 μg (a.i.)/ml 3 days before inoculation, germination was suppressed to less than 1% of that of the control. Applications at 5 or 7 days before inoculation had much less of an effect on subsequent sporangium germination (Fig. 3A and 3B). When ALBA-derived sporangia were exposed to healthy leaf disks sprayed with doses of metalaxyl up to 227 μg (a.i.)/ml, there was no inhibition of germination, indicating that the reduced germination of sporangia from metalaxyl-treated lesions was not due to fungitoxicant being washed from treated leaves.

Potted plants that were treated with metalaxyl in a soil drench 3 days before or after inoculation were effectively protected from infection. When metalaxyl (10 μg [a.i.]/ml in 100 ml water [total = 1,000 μg]/pot) was applied to soil 3 days before inoculation, lesion expansion and subsequent sporulation were completely inhibited. When applied 3 days after inoculation, 5 μg (a.i./ml) in 100 ml (total = 500 μg/pot) reduced sporulation to about 1% of the control (Table 2). Sporulation from lesions on plants treated with metalaxyl via the soil was most dense on portions of the lesion furthest from major veins. Areas of lesions which included the main central and side veins had relatively less sporulation than did areas of lesions away from these veins. Presumably fungitoxicant was in greater concentration near these veins when metalaxyl was taken up through the roots.

The lack of in vitro efficacy of metalaxyl against our isolate (race 1, 2, 3, 4) of *P. infestans* prompted an investigation of another isolate (race 1, 2, 4) and three other *Phytophthora* spp. The same in vitro experiments conducted with our test fungus were performed on *P. infestans* race 1, 2, 4, maintained on lima bean agar or amended lima bean agar, and isolates of *P. cinnamomi, P. megasperma,* and *P. cactorum.* Germination of sporangia of all four fungi on ALBA plates was completely inhibited at metalaxyl dosages greater than 25 μg (a.i.)/ml. We concluded, therefore, that our isolate of *P. infestans* race 1, 2, 3, 4 differs significantly from other *Phytophthora* isolates with respect to its in vitro sensitivity to metalaxyl. These observations warrant further investigation.

DISCUSSION

After metalaxyl was applied to potato foliage, the growth rate of *P. infestans* (lesion expansion and sporulation) and viability of sporangia produced were dramatically suppressed. However, with our isolate of *P. infestans* race 1, 2, 3, 4 none of these effects was observed in vitro. These results differ from those of other workers who found an inhibition of spore germination and colony growth when metalaxyl was incorporated into a test medium (1, 11).

Pathogenesis by race 1, 2, 3, 4 was not interrupted by metalaxyl until invasion of the fungus into potato tissue. Sporulation on metalaxyl-treated leaves was greatly reduced in both foliar and soil drench treatments (Fig. 3). These observations are consistent with those from our field experiments in which sporulation on most lesions was dramatically suppressed (Bruck, *unpublished*). We observed that sporulation on foliar-treated plants was greatest in vascular tissue and greatly reduced on laminar tissue, and this also is consistent with our field observations.

Lesion expansion was inhibited by metalaxyl, but application before inoculation had a larger effect than did application after infection. Thus, our data are consistent with those of Cohen et al (5).

Because metalaxyl affects lesion expansion, sporulation, and viability of *P. infestans* sporangia, it has a very large inhibitory effect on potato late blight epidemics. For example, metalaxyl (25 μg [a.i]/ml) applied at 3 days after inoculation suppressed the sporangium viability to less than 1.0% of that for sporangia from nontreated lesions; lesion expansion was suppressed to 92%; and sporulation was suppressed to 10%. The total effect of metalaxyl applied (at 25 μg [a.i.]/ml) to lesions at 3 days after inoculation was to suppress the number of viable sporangia per square centimeter to ~ 0.01% of the number from untreated lesions. These data aid in explaining our field observations (8) in which epidemic development in metalaxyl-treated potato plots was dramatically suppressed within 2 days following metalaxyl application.

Tubers from metalaxyl-treated plots frequently had lower incidences of infection than did tubers from plots sprayed with protectant fungicides, even when the amount of disease in the foliage was comparable (8). The smaller numbers of sporangia produced on metalaxyl-treated lesions and their low viability may explain the low incidence of tuber infection. The compound may be employed effectively in an after-infection therapeutic treatment. Metalaxyl provides growers with a tool to suppress established epidemics, and it should be useful in integrated pest management programs.

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