Responses to Metalaxyl of Sensitive and Resistant Isolates of *Phytophthora infestans*

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ABSTRACT

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Zoospore release by three isolates of *P. infestans* was completely inhibited by 200–300 μg of metalaxyl per milliliter. Inhibition of cyst germination of the isolates ranged from 10 to 44% with 5 μg of the fungicide per milliliter. An ultrastructural analysis of the effects of metalaxyl revealed no observable change in fine structure of either cysts or zoospores. In vitro mycelial growth of a metalaxyl-sensitive isolate was inhibited 69%, while two metalaxyl-resistant isolates were inhibited only 23–25% at 1 μg/ml. In vitro sporulation of all three isolates was inhibited 69–85% by metalaxyl at 1 μg/ml; there was no correlation between the in vitro and in vivo metalaxyl resistance of the isolates. In vivo sporulation was reduced to zero by metalaxyl at 0.1 μg/ml in the case of the sensitive isolate, but was still profuse at 100 μg/ml for two resistant isolates on potato cultivar Kerr's Pink. The sporulation was dependent on both the fungal isolate and host cultivar involved. On cultivar Arran Victory, metalaxyl-resistant isolate P1296 was completely inhibited by metalaxyl at 10 μg/ml, whereas isolate P1297 still sporulated at 600 μg/ml. In contrast, on cultivar Kerr's Pink, which has foliar disease resistance identical to Arran Victory, P1296 sporulated sparsely on leaves treated with 500 μg of the fungicide per milliliter.

In Ireland, the systemic fungicide metalaxyl, prepared as the formulated product Ridomil 25WP®, was first used commercially in 1977 to control late blight on potatoes. Its initial high efficacy against *Phytophthora infestans* on potato led to its widespread use by growers. During the extremely blight-favorable meteorological conditions that prevailed in Ireland during the summer of 1980, however, late blight developed rapidly on metalaxyl-sprayed potato crops. It was determined that the presence of metalaxyl-resistant strains of *P. infestans* accounted for the failure of disease control with this fungicide (9). Failure of late blight control on potatoes due to the development of metalaxyl-resistant strains of the pathogen has also been reported from Holland (8), Northern Ireland (5), and more recently from Israel (3).

The studies reported in this paper compare the in vitro and in vivo responses of three strains of *P. infestans*, one sensitive and two resistant, to metalaxyl. In particular, it examines the differences between the two resistant isolates, in terms of their in vitro, and more particularly, their in vivo responses to metalaxyl. Electron microscopy is used to determine whether metalaxyl is fungistatic or fungitoxic at concentrations up to 600 μg/ml.

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MATERIALS AND METHODS

Organisms. Isolate P1292, a metalaxyl-sensitive isolate of *P. infestans*, race 4,11, was originally collected in N. Wales, U.K., in 1972 by R. C. Shattock (14) prior to the release of the fungicide. Two cultures with field resistance to metalaxyl were isolated from infected tubers by L. J. Dowley in 1980 in Ireland (9). Isolate P1297 was obtained in County Meath from potatoes that had been treated with metalaxyl; isolate P1296 was obtained in County Kilkenny from potatoes that had been sprayed with mancozeb, although adjacent fields had been sprayed with metalaxyl. The cultures were maintained in the Phytophthora Collection at the University of California, Riverside, and were grown on rye seed agar medium (RSM) at 15 C (19).

Fungicide. Metalaxyl was used either as a formulated 25% a.i. wettable powder (25WP) or as the technical grade (93%). The wettable powder formulation contained 3% silicic acid, 3% Ultraton W300 (wetting agent), and kaolin. All metalaxyl concentrations were expressed in micrograms per milliliter (a.i.).

Zoospore release and survival. Sporangial suspensions in distilled water were produced from 12-day-old cultures grown on RSM. Serial dilutions of both the technical and 25WP grades of metalaxyl were made to obtain solutions with concentrations of 1, 5, 10, 15, 25, 50, 75, 100, 150, 300, and 600 μg/ml. To 1 ml of each solution was added 10 μl of a sporangial suspension containing 10^5 spores per milliliter. The sporangia were incubated for 2 hr at 10 C to allow zoospore release and the number of zoospores was counted by using a hemacytometer. The mean of 10 replicate counts per treatment was calculated.

Cyst germination. Zoospore release was achieved as described in the previous section. After 2 hr at 10 C, the spore suspension was passed through Whatman No. 4 filter paper to obtain a zoospore suspension free of sporangia. The zoospores were centrifuged to induce cyst formation. The cysts were suspended, and 10 μl of the suspension containing 10^5 cysts per milliliter was added to 1 ml of each of a series of metalaxyl solutions identical to those used for zoospore release. The cysts were incubated for 3 hr at 25 C, the numbers of germinated cysts were counted by using a hemacytometer, and a mean of 10 replicate counts per treatment was calculated.

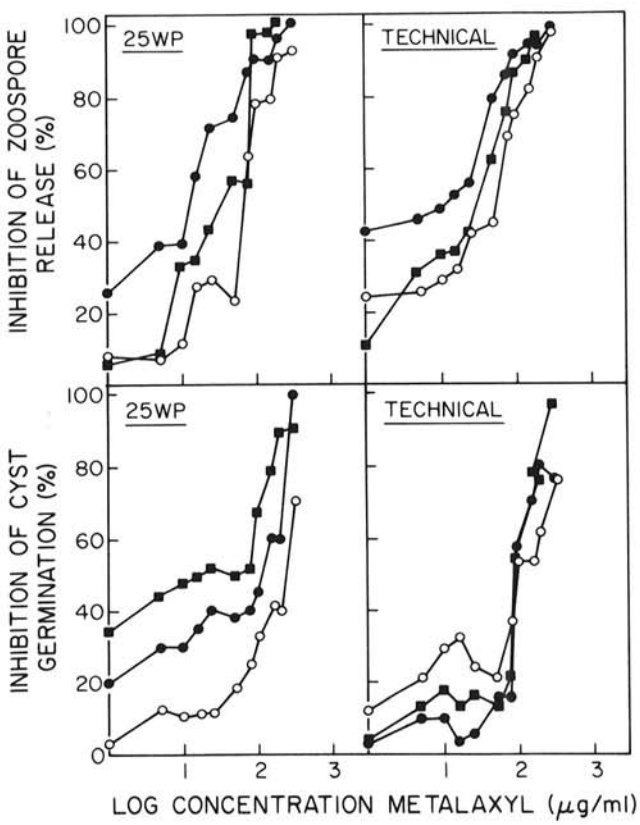


Fig. 1. The effect of concentration (active ingredient) of metalaxyl, both 25% wettable powder (25WP) and technical grade (TECHNICAL), on the inhibition of zoospore release and survival as well as inhibition of cyst germination of three isolates of *Phytophthora infestans*. Isolate P1292 (■) is metalaxyl-sensitive, isolate P1296 (●) is field-resistant, and isolate P1297 (○) is also field-resistant to metalaxyl.

Mycelial growth and sporulation in vitro. Mycelial disks, 11 mm in diameter, of the three isolates of *P. infestans* were plated on agar plates containing either 0, 75, 100, 300, or 600 μg of metalaxyl (technical grade) per milliliter. The medium was cleared RSM incubated at 20 C, and there were five replicate plates per treatment. Growth measurements were made from 3 to 21 days, and the amount of sporulation was determined with a hemacytometer at 21 days by using 5 ml of distilled water for each agar plate.

In vivo response to metalaxyl. Disks, 14 mm in diameter, were cut from leaves of potato cultivars King Edward, Kerr's Pink, and Arran Victory. They were floated on test solutions with their abaxial surfaces uppermost, five disks per 5-cm-diameter plastic petri dish, each containing 5 ml of metalaxyl 25WP at 0, 0.01, 0.1, 1.0, 10, 100, 300, 400, 500, or 600 μg/ml. Each disk was immediately inoculated with 10 μl of a suspension containing ~ 200 sporangia. The petri dishes were incubated at 15 C by using a 16-hr photoperiod with illumination at ~250 μW/cm^2 provided by 40W warm-white fluorescent lamps. At 9 days after inoculation, the disease severity was assessed according to an index rating scale of 0, 1, 2, 3, or 4 representing 0, 0–25, 25–50, 50–75, and 75–100%, respectively, of the leaf surface covered by sporangia (8).

Fine structural observations on the in vitro effects of metalaxyl. Sporangia, zoospores, and cysts of isolate P1292 were sampled at successive stages in their development and fixed in 1.5% glutaraldehyde in 0.025 M potassium phosphate buffer, pH 6.8, for 1.5 hr at 20 C. Following centrifugation, the spore pellets were mixed with molten 3% Difco agar held at 40 C. The solidified agar was cut into 1 mm^3 blocks and washed in 0.05 M phosphate buffer for 30 min. Postfixation and staining was in 2% osmium tetroxide in the same buffer for 2 hr. Following dehydration in an acetone series and epoxypropane, the agar blocks containing the spores were embedded in a resin mixture of Epon 812, Araldite 6005, and dodecynyl succinic anhydride (3:3:8, v/v) and using DMP 30 (tris-[dimethyl aminomethyl] phenol) as an accelerator. The resin mixture was polymerized at 70 C for 24 hr. Thin sections were cut with a diamond knife, mounted on 149 × 96-μm (75 × 300-mesh) grids, and poststained with 2% aqueous uranyl acetate followed by lead citrate, prior to examination in a Hitachi HU12A transmission electron microscope.

Pathological and cytological observations on the in vivo responses to metalaxyl. Individual detached leaflets of the breeding line 2863/11 (Irene × Maris Peer, from the National Potato Breeding Program, Ireland) were inoculated with 0.1 ml of a suspension of 10^5 zoospores of P1292 (race 4,11) per milliliter as described previously (19). Either 24 hr before or 48 hr after inoculation the leaflets were sprayed on both surfaces to runoff with metalaxyl 25WP at either 100, 300, or 600 μg/ml. Leaflet samples inoculated 48 hr prior to the treatment with metalaxyl at 600 μg/ml were dissected into 1-mm^2 pieces in 3% glutaraldehyde in 0.05 M potassium phosphate buffer, pH 6.8, at 24, 48, and 72 hr following fungicide application. The tissue pieces were fixed, dehydrated, and embedded in Epon-Araldite resin for light microscopy as described previously (2). Five leaflets, each representing a different plant, were employed at each experimental stage. Ten randomized samples of resin-embedded material were sectioned, and the extent of fungal colonization was determined as a percentage of the thickness of the leaf lamina.

RESULTS

Zoospore release and survival. Zoospore release and survival was affected by as little as 1 μg of metalaxyl per milliliter (Fig. 1). Metalaxyl-resistant isolate P1296 was inhibited more than either the other resistant isolate P1297, or the sensitive isolate P1292.

With progressive increase in concentration of metalaxyl there was further inhibition of zoospore production, and complete inhibition was achieved between 200 and 300 μg/ml (Fig. 1).

Cyst germination. The pattern of inhibition of cyst germination in response to increasing metalaxyl concentration was similar to that of zoospore release (Fig. 1). At 5 μg/ml, inhibition ranged from 10 to 44% depending on the isolate and metalaxyl formulation. With isolates P1292 and P1297, but not with isolate P1296, the 25WP formulation was always more inhibitory than the technical grade over the range of concentrations tested (Fig. 1).

Mycelial growth and sporulation in vitro. The sensitive isolate P1292 had its growth rate reduced from 10.2 to 3.2 mm/day in the presence of metalaxyl at 1 μg/ml (Table 1). From 10 to 100 μg/ml there was an identical 72% reduction in growth rate compared to the control. The metalaxyl-resistant isolates were much less inhibited by the fungicide. At 1 μg/ml there was 23–25% growth inhibition, while at 150 μg/ml this inhibition had only increased to 31–36% (Table 1).

All three isolates produced abundant sporangia on RSM incubated in the dark at 20 C for 21 days (Table 2). With a metalaxyl concentration of 1 μg/ml, the three isolates had a much reduced sporulation capacity. P1297 was slightly more inhibited than the sensitive isolate P1292 (Table 2). P1296, which was less inhibited than the other isolates, still exhibited a flat dosage-response pattern with 1 μg/ml being as effective as 150 μg/ml in causing significant inhibition of sporulation.

Sporulation in vivo. The sporulation of the sensitive isolate P1292 was completely inhibited by 0.1 μg/ml or greater of metalaxyl when potato leaf disks, inoculated with zoospores, were floated on fungicide solutions (Table 3). Heavy sporulation occurred on both the controls and on those treated with metalaxyl at 0.01 μg/ml.

With the field-resistant isolate P1296, 0.1 μg/ml had little effect on sporulation. At 10 μg/ml, sporulation was completely inhibited in one cultivar, Arran Victory, but was only reduced in one other cultivar, King Edward. At 200 μg/ml, sporulation was still profuse on cultivar Kerr's Pink (Table 3).

With resistant isolate P1297, sporulation was not affected by concentrations of metalaxyl up to 200 μg/ml. Indeed there was some sporulation on the cultivar Arran Victory, even at 600 μg/ml (Table 3). This contrasted with the behavior of the resistant isolate P1296, in which sporulation was completely inhibited by 10 μg/ml on this same cultivar.

The in vitro effects of metalaxyl on the fine structure of *P. infestans*. The fine structure of the sporangia was not affected by metalaxyl. In both control (Fig. 2) and metalaxyl-treated sporangia (Fig. 3) the ultrastructures of the nuclei, mitochondria, dictyosomes, endoplasmic reticulum, and fingerprint vacuoles were similar. Generally, sporangia treated with very high concentrations of metalaxyl (300 to 600 μg/ml) did not produce zoospores (Fig. 1). At lower concentrations of metalaxyl (100 μg/ml or less), some zoospores were produced (Fig. 1) and their fine structure was similar to that of the controls (cf. Figs. 4 and 5).

Pathological and cytological observations on the in vivo response to metalaxyl. Preventive treatment with metalaxyl 24 hr prior to inoculation with isolate P1292 resulted in complete suppression of disease symptoms, both necrosis and sporulation (Table 4). When metalaxyl was used as a curative treatment at 48 hr following inoculation with P1292, sporulation was greatly reduced at 100 and 300 μg/ml, and was eliminated at 600 μg/ml (Table 4).

The effect of metalaxyl on the spread of *P. infestans* through potato leaves was evaluated histologically in tissue that had received the 600 μg/ml curative treatment. The depth of colonization of leaves was significantly less ($P = 0.01$) at 24 and 48 hr following metalaxyl treatment (Table 5). However, at 72 hr, the depths of colonization of the control and metalaxyl treatments were not significantly different (Table 5).

TABLE 1. Effect of metalaxyl on the rate of growth in vitro of mycelium of three isolates of *Phytophthora infestans* on cleared rye seed medium at 20 C

P. infestans isolates[x]	Mean growth rate (mm/day) of mycelium exposed to metalaxyl at (μg [a.i.]/ml): 0	1	10	50	100	150
Sensitive						
P1292	10.2[y]	3.2 b (69)[z]	2.9 b (72)	2.9 b (72)	2.8 b (72)	3.8 b (63)
Resistant						
P1297	8.9 a	6.6 b (25)	6.3 b (29)	6.0 b (33)	6.0 b (33)	5.7 b (36)
P1296	6.2 a	4.8 b (23)	4.9 b (21)	4.8 b (23)	4.8 b (23)	4.3 b (31)

[x] Isolate P1292 is sensitive and isolates P1296 and P1297 are resistant to metalaxyl.
[y] Means within each row followed by different letters are significantly different, $P = 0.01$, according to Duncan's multiple range test.
[z] Figures in parentheses represent the percent inhibition of growth compared to the control.

TABLE 2. Effect of metalaxyl on the in vitro sporulation capacity of isolates of *Phytophthora infestans* growing on rye seed agar medium at 20 C

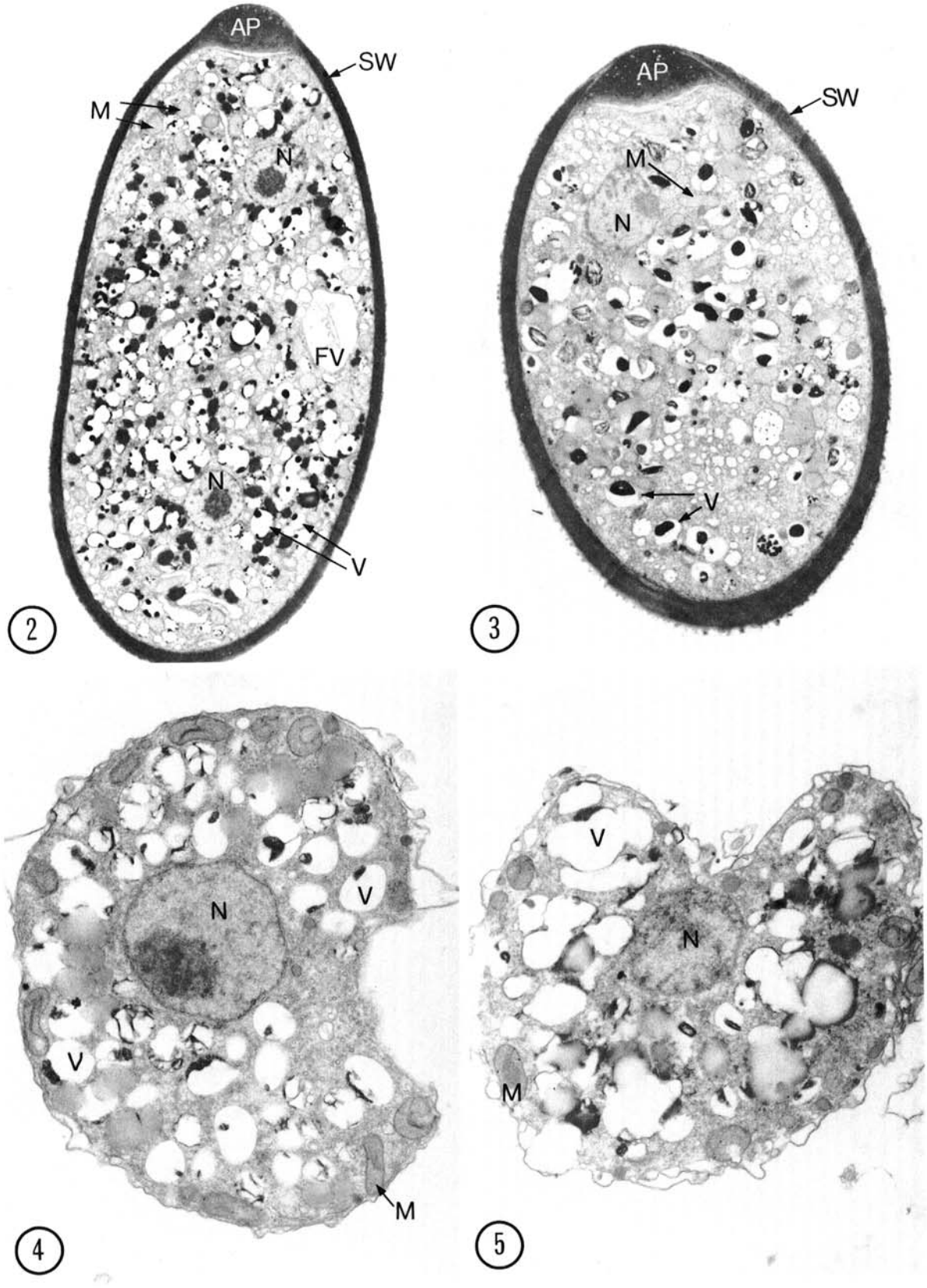
Metalaxyl (μg [a.i.]/ml)	Sporulation in vitro (sporangia/ml)[x] of isolate: P1292	P1297	P1296
0	578,000 a[y]	606,000 a	796,000 a
1	136,000 b (76)[z]	92,000 b (85)	248,000 b (69)
10	104,000 b (82)	76,000 b (87)	250,000 b (69)
50	98,000 b (83)	64,000 b (89)	256,000 b (68)
100	96,000 b (83)	66,000 b (89)	272,000 b (66)
150	56,000 b (90)	70,000 b (88)	252,000 b (68)

[x] At 21 days, the sporangia produced in each culture plate were suspended in 5 ml of water.
[y] Means within each column followed by different letters are significantly different, using $P = 0.05$, according to Duncan's multiple range test.
[z] Figures in parentheses are percent inhibition of sporulation compared to the control.

TABLE 3. Disease severity indices[y] of leaf disks of potato cultivars Arran Victory (AV), Kerr's Pink (KP), and King Edward (KE) floating on different concentrations of metalaxyl 9 days after inoculation with three different isolates of *Phytophthora infestans*

Metalaxyl concentration (μg [a.i.]/ml)	Isolate P1292 AV	KP	KE	P1296 AV	KP	KE	P1297 AV	KP	KE
0.0	4.0 a[z]	4.0 a	4.0 a	4.0 a	4.0 a	4.0 a	4.0 a	4.0 a	4.0 a
0.01	4.0 a	4.0 a	4.0 a	3.6 a	4.0 a	3.8 a	4.0 a	4.0 a	4.0 a
0.1	0.0 c	0.0 c	0.0 c	3.4 b	4.0 a	3.8 b	4.0 a	4.0 a	4.0 a
1.0	0.0 d	0.0 d	0.0 d	3.0 c	3.0 c	3.6 b	4.0 a	4.0 a	4.0 a
10	0.0 d	0.0 d	0.0 d	0.0 d	3.0 b	1.2 c	4.0 a	4.0 a	4.0 a
100	0.0 b	0.0 b	0.0 b	0.0 b	3.0 a	0.0 b	3.6 a	3.2 a	3.6 a
200	0.0 c	0.0 c	0.0 c	0.0 c	2.8 b	0.0 c	3.8 a	3.4 a	3.4 a
300	0.0 d	0.0 d	0.0 d	0.0 d	0.6 d	0.0 d	4.0 a	2.2 c	3.2 b
400	0.0 d	0.0 d	0.0 d	0.0 d	1.4 c	0.0 d	3.8 a	1.8 bc	2.2 b
500	0.0 d	0.0 d	0.0 d	0.0 d	0.8 c	0.0 d	3.8 a	1.6 b	0.6 c
600	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c	0.4 bc	2.6 a	0.6 b

[y] The disease severity index is based on the system of Davidse et al (8) and rated by using the scale: 0 = no sporulation; 1, 2, 3, and 4 = 0–25, 25–50, 50–75, and 75–100%, respectively, of the surface of the leaf disk covered with sporangia.
[z] Within each row, indices followed by different letters are significantly different, $P = 0.05$, according to Duncan's multiple range test.



Figs. 2–5. 2. Longitudinal ultrathin section of a sporangium of *Phytophthora infestans* isolate P1292 illustrating the typical fine structure of the cytoplasm containing nuclei (N), mitochondria (M), fingerprint vacuoles (V), and larger vacuoles containing flagella (FV). Note the characteristic apical papilla (AP), which is distinguishable from the sporangial wall (SW) (×4,000). **3.** A near-longitudinal ultrathin section of a sporangium of *P. infestans* P1292 treated for 3 hr with 50 μg of metalaxyl per milliliter. The fine structure of the cytoplasm is unaltered by the fungicide. Nuclei (N), mitochondria (M), and fingerprint vacuoles (V) are similar in structure to those in Fig. 3 (×6,000). **4.** Ultrathin section through a zoospore of *P. infestans* P1292 showing its characteristic pyriform shape. There is a central nucleus (N), mitochondria (M), and fingerprint vacuoles (V) (×11,000). **5.** Ultrathin section of a zoospore of *P. infestans* P1292 released in the presence of 100 μg/ml of metalaxyl. The cytoplasm is unaffected by the fungicide with the nucleus (N), mitochondria (M), and vacuoles (V) having a typical fine structure (×12,000).

DISCUSSION

Isolates P1296 and P1297 were highly resistant to metalaxyl, both being capable of sporulating profusely on cultivar Kerr's Pink in the presence of as much as 200 μg/ml of the fungicide. In contrast, the sensitive isolate P1292 was completely inhibited by only 0.1 μg/ml. These findings were similar to those of Davidse et al (8), and indicated that the most tolerant isolates were in the range of 2,000 times more resistant in vivo to metalaxyl than were the wild types.

The two tolerant isolates were readily differentiated on the basis of their growth rates on RSM; P1297 grew faster. They were also clearly differentiated by their in vivo reaction to metalaxyl on the three host cultivars. The effect of metalaxyl 25WP on zoospore release differed, the ED_{50} for P1296 being only 9 μg/ml, while that for P1297 was 24 μg/ml. In addition, sporulation in vitro in the presence of metalaxyl differed; at a concentration of 100 μg/ml it was reduced by 89% with P1297, but only by 66% with P1296. These differing responses to metalaxyl indicated that the two isolates have distinctly different biological properties both in vitro and in vivo. The likelihood is that they arose as separate mutants with high tolerance to metalaxyl.

In this study, the in vivo response to metalaxyl was dependent not only on the fungal isolate, but also on the host cultivar. For instance, P1296 on the cultivar Arran Victory was completely inhibited by metalaxyl at 10 μg/ml, but on Kerr's Pink was not prevented from sporulating even at 500 μg/ml. Moreover, P1297 sporulated profusely at 600 μg/ml on Arran Victory, while there was only limited sporulation on Kerr's Pink at the same metalaxyl concentration.

Definitive evidence for an involvement of host metabolism in responses to metalaxyl in vivo was provided in a study of the *Phytophthora megasperma* (Pm)-soybean interaction (18). In the presence of metalaxyl the level of the phytoalexin glyceollin in soybean increased during the first 24 hr following inoculation with Pm. Some of the observed changes in the fine structure of the Pm cytoplasm, especially the marked convolutions of the plasmalemma, were attributed to the effects of glyceollin, rather than metalaxyl (17). However, an assessment of the actual levels of metalaxyl and glyceollin present in the disease lesions indicated that the amount of fungicide present could have inhibited Pm (13).

TABLE 4. Sporulation of *Phytophthora infestans* on leaves sprayed with metalaxyl 24 hr before inoculation (preventive) and 48 hr after inoculation (curative)

Metalaxyl (μg [a.i.]/ml)	Sporulation capacity (sporangia/mm²)	
	Preventive	Curative
Control	257.5 a[z]	227.9 a
100	0 b	5.1 b
300	0 b	10.4 b
600	0 b	0 c

[z] Different letters denote significant differences within each column using Duncan's multiple range test, $P = 0.01$.

TABLE 5. Colonization by *Phytophthora infestans* within potato leaves treated with a curative application of metalaxyl 48 hr after inoculation with zoospores

Control		Metalaxyl (600 μg/ml [a.i.])	
Time after inoculation (hr)	Depth of colonization (%)	Time after treatment (hr)	Depth of colonization (%)
24	6.0	...	...
48	49.0	...	...
72	78.4 a[z]	24	59.2 b
96	92.1 a	48	76.7 b
120	100.0 a	72	92.3 a

[z] Values followed by different letters within each horizontal row are significantly different based on analysis of variance, $P = 0.01$.

In the present study, it was evident from the differential reactions of the three potato cultivars that apparently more was involved than a direct action of metalaxyl against the fungus. Kerr's Pink and Arran Victory possess very similar levels of general resistance. Involvement of a host reaction would appear necessary to explain why P1296 was completely inhibited by 10 μg/ml on Arran Victory, and yet as much as 300 μg/ml was required to reduce sporulation on cultivar Kerr's Pink. One possible explanation could be that a much stronger host resistance reaction was triggered in Arran Victory treated with metalaxyl. Alternatively, different cultivars may accumulate metalaxyl at the infection sites in different concentrations. However, the responses on detached leaflets may not reflect reactions that would occur under field conditions.

In vitro, sensitive isolate P1292 was not completely inhibited by metalaxyl even at 600 μg/ml, and yet 0.1 μg/ml was sufficient for complete inhibition in vivo. In another study with *P. infestans*, one isolate was unaffected in vitro by up to 350 μg/ml, and yet in vivo it was controlled by 1.0 μg/ml of metalaxyl sprayed on leaflets at the time of inoculation (1).

Some lines of investigation indicate that metalaxyl has a fungistatic, rather than a directly fungitoxic, effect on the physiology of the pathogen. In this study, there were no visible changes in the ultrastructure of metalaxyl-treated zoospores or sporangia. There were also no obvious changes in the fungal cytoplasm following metalaxyl application in the interaction between *Peronospora pisi* and *Pisum sativum* (12). Again, in the Pm-soybean interaction some of the ultrastructural changes that were observed in the fungus were attributed to the effects of glyceollin rather than metalaxyl (18). The fungistatic mode of action of metalaxyl is believed to involve the inhibition of RNA synthesis (6,11). However, the level of inhibition of RNA synthesis, even at high concentrations of metalaxyl, never exceeded 80% (7). The ability of the fungus to continue developing slowly when metalaxyl is used as a curative treatment 48 hr following infection, may reflect this partial fungistatic inhibition. The essentially fungistatic nature of metalaxyl, even at high concentrations, may in part explain the rapidity with which resistance can develop (3,5,8), since there will be a large population of fungal nuclei from which selection may occur.

Cohen et al (4) observed that zoospore release in *P. infestans* was reduced 80% by metalaxyl at 50 μg/ml. In the present study, the ED_{50} values at 6–25 μg/ml for inhibition of zoospore release for the three isolates agree with their findings. As Cohen et al (4) demonstrated, metalaxyl can exert a direct effect on zoospore release and survival, though high levels of 1,000 μg/ml were required to give a completely preventive effect when the fungicide was mixed with the fungal inoculum in an in vivo test.

Cyst germination was influenced by metalaxyl, with ED_{50} values for the wettable powder formulation ranging from 13 to 55 μg/ml. Whether this effect would have any consequence in vivo is doubtful since even 600 μg/ml of metalaxyl was reported to have no effect on the infection process (15). The cyst germination of other *Phytophthora* species appears to be much less sensitive to metalaxyl. With *P. parasitica* var. *nicotianae* even 100 μg/ml had no effect upon germination (16). Similarly, with the citrus pathogens, *P. parasitica* and *P. citrophthora*, high levels of metalaxyl did not inhibit the germination process (10).

In summary, this study has focused on the apparent discrepancies between the in vitro and in vivo responses to metalaxyl of three different isolates of *P. infestans*. More information is required on the extent to which a range of isolates of *Phytophthora* may differ in their inherent in vitro and in vivo sensitivity to metalaxyl. In addition, the apparent in vivo role of host metabolism in possibly enhancing the direct effects of metalaxyl on the pathogen deserves further investigation.

LITERATURE CITED

1. Bruck, R. I., Fry, W. E., and Apple, A. E. 1980. Effect of metalaxyl, an acylalanine fungicide, on developmental stages of *Phytophthora infestans*. Phytopathology 70:597-601.
2. Coffey, M. D., and Wilson, U. E. 1983. An ultrastructural study of the

late-blight fungus *Phytophthora infestans* and its interaction with the foliage of two potato cultivars possessing different levels of general (field) resistance. Can. J. Bot. 61:2669-2685.
3. Cohen, Y., and Reuveni, M. 1983. Occurrence of metalaxyl-resistant isolates of *Phytophthora infestans* in potato fields in Israel. Phytopathology 73:925-927.
4. Cohen, Y., Reuveni, M., and Eyal, H. 1979. The systemic antifungal activity of Ridomil against *Phytophthora infestans* on tomato plants. Phytopathology 69:645-649.
5. Cooke, L. R. 1981. Resistance to metalaxyl in *Phytophthora infestans* in Northern Ireland. Proc. 1981 Br. Crop Prot. Conf.—Pests and Diseases 2:641-650.
6. Davidse, L. C. 1981. Mechanism of action of metalaxyl in *Phytophthora megasperma* f. sp. *medicaginis*. Neth. J. Plant Pathol. 87:254-255.
7. Davidse, L. C., Hofman, A. E., and Velthuis, G. C. M. 1983. Specific interference of metalaxyl with endogenous RNA polymerase activity in isolated nuclei from *Phytophthora megasperma* f. sp. *medicaginis*. Exp. Mycol. 7:344-361.
8. Davidse, L. C., Looijen, D., Turkensteen, L. J., and Van Der Wal, D. 1981. Occurrence of metalaxyl-resistant strains of *Phytophthora infestans* in Dutch potato fields. Neth. J. Plant Pathol. 87:65-68.
9. Dowley, L. J., and O'Sullivan, E. 1981. Metalaxyl-resistant strains of *Phytophthora infestans* (Mont.) de Bary in Ireland. Potato Res. 24:417-421.
10. Farih, A., Tsao, P. H., and Menge, J. A. 1981. In vitro effects of metalaxyl on growth, sporulation, and germination of *Phytophthora parasitica* and *P. citrophthora*. Plant Dis. 65:651-654.
11. Fisher, D. J., and Hayes, A. L. 1982. Mode of action of the systemic fungicides furalaxyl, metalaxyl and ofurace. Pestic. Sci. 13:330-339.
12. Hickey, E. L., and Coffey, M. D. 1980. The effects of Ridomil on *Peronospora pisi* parasitizing *Pisum sativum*: an ultrastructural investigation. Physiol. Plant Pathol. 17:199-204.
13. Lazarovits, G., and Ward, E. W. B. 1982. Relationship between localized glyceollin accumulation and metalaxyl treatment in the control of Phytophthora root rot in soybean hypocotyls. Phytopathology 72:1217-1221.
14. Shattock, R. C., and Shaw, D. S. 1976. Novel phenotypes of *Phytophthora infestans* from mixed culture of antibiotic resistant mutants. Trans. Br. Mycol. Soc. 67:201-206.
15. Staub, T., and Dahmen, H. 1980. Effects of Ridomil on the development of *Plasmopara viticola* and *Phytophthora infestans* on their host plants. Z. Pflanzenkrankh. Pflanzenschutz 87:83-91.
16. Staub, T. H., and Young, T. R. 1980. Fungitoxicity of metalaxyl against *Phytophthora parasitica* var. *nicotianae*. Phytopathology 70:797-801.
17. Stössel, P., Lazarovits, G., and Ward, E. W. B. 1982. Light and electron microscopy of Phytophthora root rot in soybeans treated with metalaxyl. Phytopathology 72:106-111.
18. Ward, E. W. B., Lazarovits, G., Stössel, P., Barrie, S. D., and Unwin, C. H. 1980. Glyceollin production associated with control of Phytophthora rot of soybeans by the systemic fungicide, metalaxyl. Phytopathology 70:738-740.
19. Wilson, U. E., and Coffey, M. D. 1980. Cytological evaluation of general resistance to *Phytophthora infestans* in potato foliage. Ann. Bot. 45:81-90.