Variability in Sensitivity to Metalaxyl of Isolates of *Phytophthora cinnamomi* and *Phytophthora citricola*

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ABSTRACT

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Amongst isolates of *Phytophthora cinnamomi*, both A1 and A2 mating types, there was a significant variation in the response of mycelial growth to metalaxyl with ED_{50} values ranging from 0.07 to 0.29 μg/ml. The ED_{50} values amongst A2 isolates from a single host, avocado (*Persea americana*), ranged from 0.07 to 0.14 μg/ml, and ED_{90} values from 0.45 to 1.14 μg/ml. Isolates of another species, *P. citricola* pathogenic on avocado, had ED_{50} values ranging from 1.18 to 4.61 μg/ml, and ED_{90} values from 31.5 to 192.4 μg/ml of metalaxyl. Chlamydospore production in three isolates of *P. cinnamomi* was inhibited more than 60% with 0.1 μg/ml, and was completely inhibited at 0.25 μg/ml. Germination of chlamydospores was much less sensitive than their production, with about 70% inhibition at 10 μg/ml. Sporangium production in *P. cinnamomi* was extremely sensitive to metalaxyl; 0.25 μg/ml generally was completely inhibitory. Isolates of *P. citricola* were less sensitive, with 1.0 μg/ml giving about 70% inhibition. Cyst germination in both *P. cinnamomi* and *P. citricola* was insensitive to metalaxyl, with even 100 μg/ml having essentially no effect. Oospore formation in *P. cinnamomi* was sensitive compared to that of *P. citricola*. With *P. cinnamomi* 1.0 μg/ml prevented oospore formation, while with *P. citricola* some formation still occurred at 10 μg/ml of metalaxyl.

Metalaxyl is a member of a new class of acylalanine fungicides with systemic activity specifically against the Peronosporales, including the important plant pathogenic genus *Phytophthora* (14). The fungicide metalaxyl is very active both in vitro (6,10) and in vivo (14) against *Phytophthora*. A recent study with *P. megasperma* f. sp. *glycinea* on soybean indicated that the concentration of metalaxyl that accumulated in infected tissue in vivo, usually exceeded the ED_{90} values for inhibition of mycelial growth in vitro (11). Consequently, the limited data that are available support the concept that there is a similar mode of action both in vivo and in vitro for direct inhibition of fungal development by metalaxyl. The chemical mode of action of metalaxyl is believed to involve the inhibition of fungal RNA synthesis in both *Pythium* (6,9) and *Phytophthora* (3,6).

The biological mode of action of metalaxyl against various phases of the life cycle of *Phytophthora* has been investigated in vitro, though only with single isolates of *P. parasitica* (5), *P. parasitica* var. *nicotianae* (17), and *P. citrophthora* (5). With concentrations ≤ 1 μg of metalaxyl per milliliter, mycelial growth and sporangium formation were inhibited, but both the indirect germination of sporangia (5) and direct germination of encysted zoospores (5,17) were relatively insensitive even to concentrations of 100 μg of metalaxyl per milliliter. Chlamydospore formation and germination, and oospore formation, were also sensitive to low concentrations of metalaxyl (5,17). In a study of the effects of metalaxyl on a single isolate of *P. cinnamomi*, the ED_{50} value for both inhibition of mycelial growth and sporangium formation was 0.11 μg/ml, while that for chlamydospore formation was determined to be only 0.04 μg/ml (1).

There is little information available on the range of sensitivity to metalaxyl, either among separate isolates within a single species, or between different species, of *Phytophthora*. A study of 35 isolates of *P. megasperma* has shown that marked differences in sensitivity of mycelial growth to metalaxyl can exist within a single species (7).

P. cinnamomi, a heterothallic species which occurs worldwide on nearly 1,000 host plants (20), and *P. citricola*, a homothallic species with a narrower host range (18,22), are pathogenic on avocado (*Persea americana*) (19).

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The experiments reported here examine the in vitro sensitivity to metalaxyl of a number of isolates of *P. cinnamomi* and *P. citricola* selected on the basis of host origin and geographical location.

MATERIALS AND METHODS

The fungicide metalaxyl (*N*-[2,6-dimethylphenyl]-*N*-[methoxyacetyl]-alanine methyl ester, 25% a.i. wettable powder formulation) was dissolved in sterile distilled and deionized water (SDW) before addition to media following autoclaving. With the concentrations of metalaxyl used in this study, no differences were observed between the response to the technical grade and the wettable powder formulation. Concentrations of metalaxyl are given in micrograms (a.i.) per milliliter. Isolates of *P. cinnamomi* (A1 and A2 mating types) and *P. citricola* were taken from the collection of *Phytophthora* at the University of California, Riverside.

Linear growth response. Metalaxyl was incorporated into Difco cornmeal agar (CMA) plates at various concentrations. Plates were inoculated with single 5-mm-diameter disks from 4-day-old cultures of *P. cinnamomi* and *P. citricola* using three replicates per treatment, and then incubated in the dark at 25 C for 4 days.

Chlamydospore production. Mycelial mats of *P. cinnamomi* were produced by placing 5-mm-diameter disks in 6-cm-diameter petri dishes in 7 ml of carrot broth prepared at one half the concentration used by Kaosiri et al (8). Following incubation at 25 C for 24 hr, the broth was replaced with SDW containing metalaxyl. Finally, the mats were minced in a Waring blender and chlamydospore production was determined by using a Hawksley eelworm counting chamber.

Chlamydospore germination. Chlamydospores were produced on mycelial mats grown on carrot broth for 10 days at 25 C. These mats were rinsed with SDW, blended, and filtered through 100-μm nylon mesh. The filtrate was centrifuged for 90 sec, the supernatant discarded, and the pellet resuspended in SDW. Chlamydospore suspensions were pipetted onto CMA containing various concentrations of metalaxyl, and germination determined after 1 day at 25 C in the dark.

Sporangium production. Nonsterile (for *P. cinnamomi*) or sterile (for *P. citricola*) soil extracts (SE) were prepared by mixing 10 g (fresh weight) of soil with 1 L of demineralized water which was allowed to stand overnight and then was filtered through Whatman No. 1 paper. Disks taken from the margins of cultures on cleared

V8-$CaCO_3$ (V8) agar were placed singly in 6-cm-diameter petri dishes containing 7 ml of SE containing different concentrations of metalaxyl. The cultures were incubated under continuous illumination for 2 days and sporangium production was determined by inspection under a dissecting microscope.

Encysted zoospore germination. Sporangia obtained in SE were rinsed in SDW and zoospore release induced by placing them in fresh SDW at 4 C for 20 min. Encysted zoospores were produced by agitation for 2 min in a Vortex mixer. One milliliter of a spore suspension containing ~2×10^4 cysts was pipetted onto CMA containing metalaxyl and percent germination was determined after incubation for 3 hr at 25 C. To be counted as germinated, a cyst must have produced a germ tube more than twice as long as the cyst diameter.

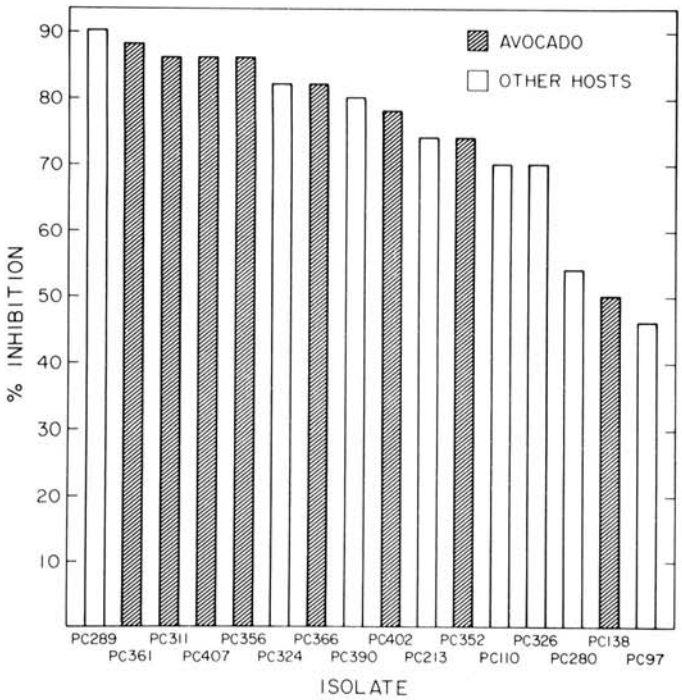

Fig. 1. Comparative sensitivity to metalaxyl of isolates of *Phytophthora cinnamomi* from avocado and other hosts. Pc97 and Pc138 are A1 mating types, while the remainder are A2 mating types.

Oospore formation. Oospores were produced by inoculating V8 agar containing metalaxyl with suspensions of minced mycelium (21). Suspensions of *P. cinnamomi* contained both A1 and A2 mating types, while those of *P. citricola*, which is homothallic, contained oospores of only a single type of isolate. Following 28 days of incubation at 25 C in the dark, the cultures were blended for 1 min and numbers of oospores produced were determined with a Hawksley eelworm counting chamber.

RESULTS

Linear growth. The variation in sensitivity to a concentration of 0.25 μg of metalaxyl per milliliter among 100 isolates of *P. cinnamomi*, selected from 24 different host plants, typically ranged from 47 to 96% inhibition of linear growth on CMA in repeated experiments. The responses of 27 isolates are given in Table 1. Both mating types, A1 and A2, had a similar range of sensitivity, possessing both relatively tolerant and sensitive isolates. Geographical location did not seem related to the level of sensitivity, since selected pairs of isolates, Pc326 and Pc289, and Pc200 and Pc365, from California and the USSR, respectively, differed widely in their response to metalaxyl (Table 1). In Fig. 1 the California A1 and A2 avocado isolates were selected from a total of 100 isolates used in an initial screening test, and they included isolates representing the extremes of sensitivity to metalaxyl. The range of sensitivities of these isolates from avocado in Southern California were similar to those present in the species as a whole, which included isolates from a wide host range and diverse geographical locations (Fig. 1, Table 1). The percent inhibition of

TABLE 1. Relative sensitivity of different isolates of *Phytophthora cinnamomi* to 0.25 μg of metalaxyl per milliliter

Isolate[x]	Host species or other source of isolation	Geographical origin	Mating type	Year of isolation	Inhibition[y] (%)
Pc324	*Syzygium aromaticum*	Malaysia	A2	1977	96 a[z]
Pc390	Soil	Papua New Guinea	A1	1981	88 b
Pc200	*Castanea sativa*	USSR	A2	1973	86 bc
Pc375	*Vitis* sp.	South Africa	A2	1980	86 bc
Pc387	*Piper nigrum*	Costa Rica	A2	1980	85 bcd
Pc270	*Tristania conferta*	Australia	A1	1971	84 bcde
Pc213	Soil	New Zealand	A2	1973	82 bcdef
Pc289	*Cedrus deodara*	California	A2	1975	81 bcdef
Pc271	*Banksia aemula*	Australia	A1	1973	77 cdefg
Pc328	*Ananas comosus*	Guadeloupe	A2	1977	76 defgh
Pc189	*Taxus baccata*	Australia	A2	1967	76 defgh
Pc110	*Cinnamomum burmannii*	Sumatra	A2	1922	76 defghi
Pc373	*Leucadendron rubrum*	South Africa	A1	1980	75 efghi
Pc325	*Cinchona* sp.	Rwanda	A2	1977	75 efghi
Pc392	*Persea americana*	Venezuela	A2	1981	75 efghi
Pc140	*Prunus* sp.	Maryland	A2	1970	74 fghi
Pc244	*Chamaecyparis* sp.	England	A2	1974	74 fghi
Pc365	*Laurus nobilis*	USSR	A2	1978	73 fghi
Pc339	*Calluna* sp.	England	A2	1972	70 ghi[z]
Pc144	*Rhododendron* sp.	Ohio	A2	1971	69 ghi
Pc284	*Erica* sp.	Switzerland	A2	1970	68 ghij
Pc321	*Ananas comosus*	Taiwan	A1	1977	67 hij
Pc319	*Macadamia* sp.	Guatemala	A2	1976	66 ij
Pc170	*Camellia japonica*	California	A1	1972	60 jk
Pc335	*Juglans regia*	France	A2	1977	60 jk
Pc280	*Gaultheria shallon*	Oregon	A2	1975	56 k
Pc326	*Pinus radiata*	California	A2	1977	47 l

[x] Isolate designations are those of the UCR collection of *Phytophthora cinnamomi*; Pc110 is the type culture (IMI22438).
[y] Percentage inhibition of mycelial growth at 25 C on cornmeal agar. Each value is based on the average of six replications from two experiments, measuring inhibition of radial growth after 4 days.
[z] Different letters indicate a significant difference ($P = 0.01$) according to Duncan's new multiple range test.

the avocado isolates by 0.25 μg of metalaxyl per milliliter ranged from 50% for the A1 isolate Pc138 to 88% for the A2 isolate Pc361. In comparison, the non-avocado isolates ranged from 46% for the A1 isolate Pc97 to 90% for the A2 Pc289 (Fig. 1).

A number of isolates of *P. citricola*, from 10 different host plants, also varied in linear growth response to 0.25 μg of metalaxyl per milliliter. A California isolate from *Cytisus racemosus*, P1233, was inhibited 77%, while at the other extreme, P705, an isolate of *Humulus lupulus* from England, was only inhibited 12% (Table 2). If only avocado isolates of *P. citricola* were considered, then a much narrower range of sensitivities emerged with a range from 18 to 32% inhibition at 0.25 μg of metalaxyl per milliliter (Table 2). This contrasts with the wider range of 50–80% inhibition for *P. cinnamomi* A2 avocado isolates at a similar concentration of metalaxyl (Fig. 1). In addition, the isolates of *P. citricola* from avocado were clearly separated from the A2 isolates of *P. cinnamomi* from avocado due to the much higher tolerance of the former group to metalaxyl (compare Fig. 1 and Table 2). The least sensitive A1 and A2 isolates of *P. cinnamomi* were inhibited an average of about 20% by metalaxyl at 0.1 μg/ml and 70–80% by 0.5 μg/ml (Figs. 2 and 3). The most sensitive A1 and A2 isolates of *P. cinnamomi* were inhibited about 25% by metalaxyl at a concentration of only 0.025 μg/ml and about 90% at 0.5 μg/ml (Figs. 2 and 3). In contrast, the isolates of *P. citricola* required about 100 μg of the fungicide per milliliter for 90% inhibition of linear growth (Fig. 4). Linear regression analyses of the relationship between the log metalaxyl concentration and probit percent linear growth inhibition revealed a straight line relationship for isolates of both *P. cinnamomi* (Figs. 2 and 3) and *P. citricola* (Fig. 4).

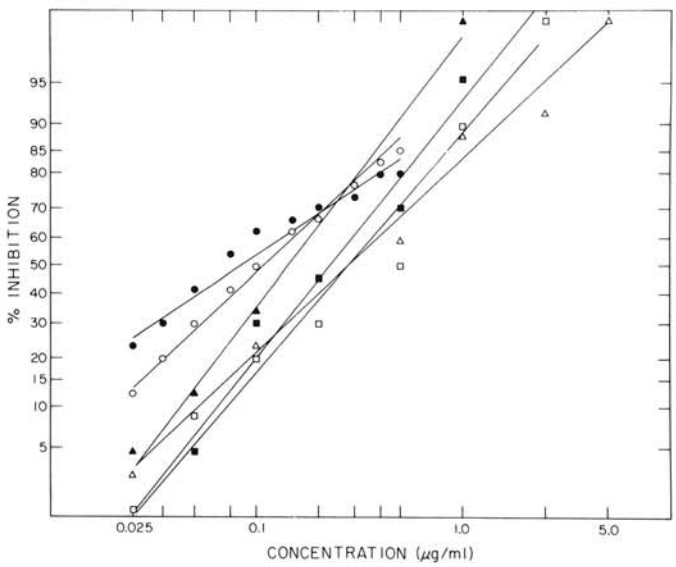

Fig. 2. Linear regression analyses of the relationship between probit percent inhibition of mycelial growth and log concentration of metalaxyl for six A1 isolates of *Phytophthora cinnamomi*. The isolates, selected on the basis of their relative sensitivity or tolerance to metalaxyl, were Pc21 (o——o), Pc97 (□——□), Pc138 (■——■), Pc271 (▲——▲), Pc300 (Δ——Δ), and Pc320 (•——•).

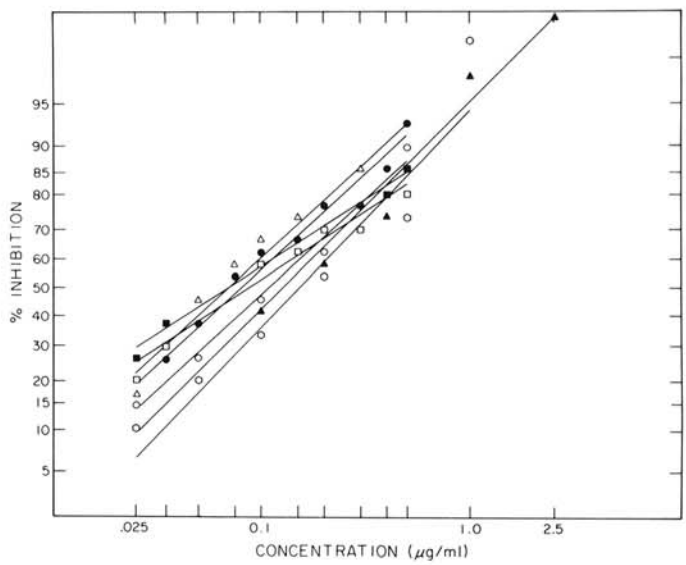

Fig. 3. Linear regression analyses of the relationship between probit percent inhibition of mycelial growth and log concentration of metalaxyl for seven A2 isolates of *Phytophthora cinnamomi*. The isolates, selected on the basis of their relative sensitivity or tolerance to metalaxyl, were Pc289 (•——•), Pc290 (▲——▲), Pc311 (■——■), Pc352 (⬡——⬡), Pc356 (□——□), Pc361 (Δ——Δ), and Pc366 (o——o).

TABLE 2. Relative sensitivity of different isolates of *Phytophthora citricola* to 0.25 μg of metalaxyl per milliliter

Isolate[x]	Host species	Geographical origin	Year of isolation	Inhibition[y] (%)
P1233	*Cytisus racemosus*	California	1980	77 a[z]
P767	*Syringa* sp.	Canada	...	54 b
P1189	*Pieris japonica*	N. Carolina	1979	52 bc
P781	*Juglans nigra*	Indiana	1971	48 bc
P1175	*Rhododendron* sp.	N. Carolina	1978	45 bc
P820	*Rhododendron* sp.	Ohio	...	40 cd
P1273	*Persea americana*	California	1981	32 de
P846	*Citrus sinensis*	Australia	1967	32 de
P912	*Persea americana*	California	1973	31 de
P845	*Citrus limon*	Australia	1965	31 de
P1287	*Persea americana*	California	1982	27 def
P704	*Humulus lupulus*	New Zealand	1960	26 efg
P1275	*Persea americana*	California	1982	25 efgh
P1150	*Persea americana*	California	1978	22 efgh
P980	*Persea americana*	California	1974	20 efgh
P768	*Rubus* sp.	Ohio	...	20 efgh
P513	*Persea americana*	Mexico	1967	18 efgh
P716	*Citrus sinensis*	Formosa	1927	16 fgh[z]
P819	*Syringa* sp.	Massachusetts	1932	13 gh
P705	*Humulus lupulus*	England	1964	12 h

[x] Isolate designations are those of the UCR collection of *Phytophthora* species. P716 is the type culture (1M121173).

[y] Percentage inhibition of mycelial growth at 25 C on cornmeal agar. Each value is based on the average of six replications from two experiments, measuring inhibition of radial growth after 4 days.

[z] Different letters indicate a significant difference ($P = 0.01$) according to Duncan's new multiple range test.

Correlation coefficients for all except one of the isolates of *P. cinnamomi* exceeded 0.980 ($P = 0.01$). The correlation coefficients for the isolates of *P. citricola* were >0.930, giving a significance level of $P \leq 0.05$. Slope values, reflecting the rate of inhibition for the individual A1 isolates of *P. cinnamomi*, ranged from 1.20 for Pc320 to 2.22 for Pc271 (Fig. 2). A similar, though more limited range of slope values, from 1.16 for Pc356 to 1.84 for Pc352, typified the rates of inhibition for A2 isolates of *P. cinnamomi* (Fig. 3). Slope values for rate of inhibition of isolates of *P. citricola* varied from 0.70 for P1281 to 0.97 for P602 (Fig. 4). The ED_{50} values for the A1 isolates of *P. cinnamomi*, calculated from their regression equations, were 0.08 μg/ml for the most sensitive (Pc320) to 0.29 μg/ml for the most tolerant (Pc300). Corresponding ED_{50} values for the A2 isolates were 0.07 for the most sensitive isolate (Pc361) to 0.14 μg/ml for the most tolerant isolate of *P. cinnamomi* (Pc352). Isolates of *P. citricola* had ED_{50} values ranging from 1.18 (P475 and P602) to 4.61 μg (P1277) of metalaxyl per milliliter (Fig. 4). ED_{90} values, also calculated from the regression equations, ranged from 0.53 to 1.84 μg/ml for A1 isolates of *P. cinnamomi* (Fig. 2), and from 0.45 to 1.14 μg/ml for A2 isolates (Fig. 3). Isolates of *P. citricola* had ED_{90} values ranging from 31.5 (P475) to 192.4 (P1277) μg of metalaxyl per milliliter (Fig. 4).

Chlamydospore formation. The production of chlamydospores from vegetative mycelium, grown in the presence of either 0.1 or 0.25 μg/ml of metalaxyl for 24 days, was strongly inhibited (Table 3). With three of the *P. cinnamomi* isolates, production was inhibited by more than 60% at 0.1 μg/ml, and inhibition was complete at 0.25 μg/ml. The fourth, Pc320, a "sensitive" A1 isolate in terms of inhibition of its linear growth (Fig. 2), was significantly less inhibited at 0.1 μg/ml than the other three isolates (Table 3). Pc138, a "tolerant" A1 isolate in terms of growth inhibition (Fig. 2), was much more sensitive than Pc320 with regard to inhibition of chlamydospore production by metalaxyl (Table 3).

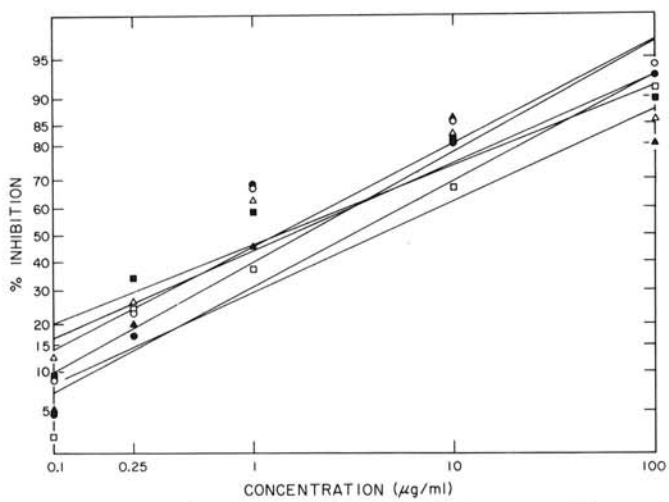

Fig. 4. Linear regression analyses of the relationship between probit percent inhibition of mycelial growth and log concentration of metalaxyl for six isolates of *Phytophthora citricola*. P716 (□——□) is the type culture isolated from *Citrus sinensis*. P475 (o——o), P602 (•——•), P1273 (■——■), P1277 (▲——▲), and P1281 (Δ——Δ) are isolates from avocado.

Mycelium of the A2 isolate Pc356, grown for 1 day in carrot broth, was incubated for 7 days in the presence of concentrations of metalaxyl ranging from 0.25 μg/ml to 100.0 μg/ml. Chlamydospore production was inhibited by 83% at 0.25 μg/ml, and a similar degree of inhibition persisted up to 100 μg of metalaxyl per milliliter.

Chlamydospore germination. Chlamydospores of *P. cinnamomi* (Pc356) were germinated on CMA containing different concentrations of metalaxyl. At 0.25 μg/ml, a level which had a markedly inhibitory effect on chlamydospore production (Table 3), there was only 16% inhibition ($P = 0.01$) of germination. Inhibition of chlamydospore germination was high at 10 and 100 μg of metalaxyl per milliliter, ranging from 73 to 84% ($P = 0.01$).

Sporangium production. Sporangium production in *P. cinnamomi* was extremely sensitive to low concentrations of metalaxyl (Table 4). A comparison of isolates, selected for their relative sensitivity or tolerance to metalaxyl in terms of inhibition of their mycelial growth (Figs. 1–3), revealed similar sensitivities with respect to sporangium production (Table 4).

Sporangium production in *P. citricola* was less sensitive to metalaxyl (Table 5). The most tolerant *P. citricola* isolate P1277 with respect to mycelial growth was also the most tolerant with respect to sporangium production (Table 5). This relationship did not hold for the other isolates of *P. citricola* (Table 5).

Sporangium production in an A1 and A2 isolate of *P. cinnamomi* was almost completely inhibited at metalaxyl concentrations as low as 0.1 μg/ml, contrasting with the situation with the isolates of *P. citricola*, for which even at 100 μg/ml inhibition was not complete (Tables 4 and 5).

Cyst germination. Cysts of *P. cinnamomi* (Pc356) and *P. citricola* (P1273) were germinated on CMA containing different concentrations of metalaxyl. At concentrations up to 100 μg/ml of metalaxyl, cyst germination was very similar to the control for both *P. cinnamomi* and *P. citricola* (Table 6).

Oospore production. Formation of oospores in *P. cinnamomi* (Pc138 × Pc366) was significantly reduced by only 0.1 μg/ml of metalaxyl (Table 7). At a concentration of 1.0 μg/ml, oospore production was essentially eliminated. With *P. citricola* (P1273 and P1287), oospore formation was not appreciably reduced until a concentration of 0.25 μg/ml was reached, and in the case of P1273 there was oospore production even at 1.0 μg of metalaxyl per milliliter (Table 7). Some production occurred, particularly with P1273, at 10 μg/ml of metalaxyl.

DISCUSSION

Using low concentrations of metalaxyl (0.1 to 0.25 μg/ml), which were only partially inhibitory to the linear growth of isolates of *P. cinnamomi*, it was established that significant variation existed in

TABLE 3. Influence of low concentrations of metalaxyl on the number of chlamydospores produced per mycelial mat by four isolates of *Phytophthora cinnamomi*[x]

Isolate[y]	Mating type	Numbers (×10³) of chlamydospores at different concentrations of metalaxyl (μg/ml)			Percent inhibition at different concentrations of metalaxyl (μg/ml)	
		0	0.1	0.25	0.1	0.25
Pc110	A2	22.6	0.4	0	98 a[z]	100 a
Pc356	A2	22.8	3.0	0.2	87 a	99 a
Pc152	A1	32.0	0.4	0.8	98 a	98 a
Pc138	A1	25.6	9.4	0	63 b	100 a
Pc320	A1	31.6	21.8	3.8	32 c	88 a

[x] Mycelial mats were grown in carrot broth for 1 day and then placed in solutions of metalaxyl for 24 days, before counting chlamydospore production.
[y] Pc110 is from *Cinnamomum burmannii*, Pc356 is from *Persea americana*, Pc152 is from *Tristania conferta*, Pc138 is from *Persea americana*, and Pc320 is from *Camellia japonica*.
[z] Different letters indicate a significant difference ($P = 0.01$) according to Duncan's new multiple range test.

in vitro sensitivity to the fungicide. The range of responses to metalaxyl exhibited by a selection of California A2 isolates of *P. cinnamomi* from avocado (*P. americana*) was almost as extensive as that represented by a selection of 100 isolates of *P. cinnamomi* from 24 different host species originating from different geographical locations spread over five continents. Despite the variability, the sensitivity of the majority of isolates fell within a relatively narrow range with ED_{50} values between 0.07 and 0.29 μg of metalaxyl per milliliter. The ED_{50} values for the A2 isolates alone ranged from 0.07 to 0.14 μg/ml. In so far as the linear growth response might reflect corresponding in vivo sensitivity of these isolates, the data indicated that there was little tolerance to the fungicide in either the worldwide or the California isolates of *P. cinnamomi*. The range of growth response to metalaxyl paralleled similar growth responses exhibited by both A1 and A2 isolates of *P. cinnamomi* to the antibiotics streptomycin, chloramphenicol, ethidium bromide, and chlorotetracycline (12). Interestingly, the A1 isolate Pc97, from *Camellia japonica*, and Pc138, from avocado, which were among the most sensitive to the antibiotics, were some of the more tolerant isolates with respect to metalaxyl (Figs. 1 and 2). In contrast, the A2 isolate Pc110, the type culture from *Cinnamomum burmannii*, showed relative tolerance to the antibiotics, but was quite sensitive in its growth response to metalaxyl (Fig. 1). As was found with growth response to temperature (23) and antibiotics (12), the variation in response to the systemic fungicide metalaxyl was unrelated to the mating type, host origin, or geographical location.

This study revealed that the variation in response to metalaxyl was almost as great within the California A2 population as it was in 100 isolates of the A1 and A2 populations of *P. cinnamomi* worldwide. It would be interesting to sample the variation in response to metalaxyl at a population level such as that which occurs within the confines of a single diseased avocado grove, or indeed that associated with the feeder roots of a single tree.

The ED_{50} values for inhibition of mycelial growth with *P. cinnamomi* were similar to those obtained with two isolates of *P. parasitica* (5,15) and an isolate of *P. citrophthora* (5). However, isolates of *P. citricola* were much more tolerant to metalaxyl than isolates of *P. cinnamomi* with ED_{90} values for inhibition of colonial growth ranging from 31.5 to 192.4 μg/ml compared to 1.84 μg/ml for the most tolerant A1 isolate of *P. cinnamomi*. The higher level of tolerance found in isolates of *P. citricola* compared to those of *P. cinnamomi* should significantly influence the efficacy of the residual preventative or curative effects of metalaxyl, assuming that there is a correlation between in vitro and in vivo responses to metalaxyl.

The influence of metalaxyl on different stages of the life cycle of *Phytophthora* has received limited attention (5,15,17). With an isolate of *P. infestans*, it was found that germination of encysted zoospores was insensitive to as much as 600 μg of metalaxyl per milliliter (17). Cyst germination was only slightly inhibited by 10 μg/ml, and it required 1,000 μg/ml to inhibit it completely, in *P. parasitica* (5). In contrast, only 0.1 to 0.2 μg inhibited both chlamydospores and sporangium formation in *P. parasitica* (5,15) and *P. citrophthora* (5). Only 0.04 μg/ml was inhibitory to oospore formation in *P. parasitica* (5).

In our study, chlamydospore formation in most of the isolates of *P. cinnamomi* was inhibited by more than 60% with as little as 0.1 μg of metalaxyl per milliliter. A single isolate was more tolerant, but at 0.25 μg/ml, even this isolate was inhibited by 90%. Interestingly, this isolate (Pc320) was a relatively sensitive isolate with respect to inhibition of its mycelial growth. Chlamydospore formation by *P. parasitica* was less sensitive to metalaxyl than by *P. cinnamomi*, since even at 10 μg/ml it was still about 30% of the control (5), compared to 10% in *P. cinnamomi*.

Chlamydospore germination was inhibited about 50% by 1 μg/ml of metalaxyl in *P. parasitica*, though some (9–27%) still occurred at 100 μg/ml (5,15). Similarly, chlamydospore germination in *P. cinnamomi* was inhibited 34% at 1 μg/ml, though even at 100 μg there was still ~15% germination.

Sporangium production was extremely sensitive to metalaxyl in

TABLE 4. Influence of metalaxyl on the percentage inhibition of production of sporangia by four isolates of *Phytophthora cinnamomi*[x]

Isolate[y]	Mating type	Metalaxyl (μg/ml) 0.1	0.25
Pc289	A2	99.5 a[z]	99.5 a
Pc372	A1	98.5 a	99.0 a
Pc366	A2	90.0 ab	95.0 b
Pc271	A1	64.5 b	87.5 c

[x] Mycelial mats grown for 2 days in nonsterile soil extract containing either 0, 0.1, or 0.25 μg of metalaxyl per milliliter. Data are the averages of two experiments with a total of six replicates.
[y] Isolates were selected on the basis of the relative sensitivity of their mycelial growth to metalaxyl. Pc289 and Pc372 were "sensitive"; Pc366 and Pc271 were "tolerant."
[z] Percentage inhibition of sporangium production. Different letters indicate a significant difference ($P = 0.01$) according to Duncan's new multiple range test.

TABLE 5. Influence of metalaxyl on the percentage inhibition of production of sporangia by six isolates of *Phytophthora citricola*[x]

Isolate[y]	ED_{90} mycelial growth (μg/ml)	Metalaxyl (μg/ml) 0.1	1.0	10.0	100.0
P475	3.15	64.0 cd[z]	78.5 d	88.0 b	96.9 bc
P602	3.95	72.7 bc	83.5 c	94.3 a	96.6 bc
P1273	6.85	56.5 d	78.2 d	85.5 b	91.3 d
P716	8.16	15.6 e	67.2 e	70.3 c	81.3 e
P1281	10.45	62.8 cd	85.8 bc	96.8 a	95.1 c
P1277	19.24	84.7 a	97.7 abc	98.4 a	94.9 c

[x] Mycelial mats grown for 2 days in sterile soil extract containing different concentrations of metalaxyl. Data based on six replicates.
[y] Isolates were from avocado, except P716, which was the type culture from *Citrus sinensis*.
[z] Percentage inhibition of sporangium production. Different letters indicate a significant difference ($P = 0.1$) according to Duncan's new multiple range test.

TABLE 6. Influence of metalaxyl on the germination of cysts of *Phytophthora cinnamomi* (Pc356) and *Phytophthora citricola* (P1273)[y]

Concentration of metalaxyl (μg/ml)	*P. cinnamomi* (Pc356)	*P. citricola* (P1273)
0.0	87 a[z]	99 a
0.1	87 a	...
0.25	86 ab	...
1.0	89 a	95 ab
10.0	84 b	93 bc
100.0	88 a	90 c

[y] Sporangia were produced on soil extract media, zoospores released by chilling, and cysts generated by agitation for 2 min in a vortex mixer.
[z] Percentage germination of cysts. Different letters indicate a significant difference ($P = 0.01$) according to Duncan's new multiple range test.

TABLE 7. Influence of metalaxyl on the production of oospores by *Phytophthora cinnamomi* and *Phytophthora citricola*[y]

Concentration of metalaxyl (μg/ml)	*P. cinnamomi* (Pc138 × Pc366)	*P. citricola* P1273	P1287
0.0	585 a[z]	519 a	458 a
0.1	170 b	724 bc	402 a
0.25	31 c	381 cd	115 b
1.0	0.1 c	291 d	81 b
10.0	0 c	50 e	7 b

[y] Cultures were grown on cleared V8 agar (15 ml per plate) for 28 days at 25 C. Inoculum consisted of minced mycelium.
[z] Number of oospores ($\times 10^3$) per plate. Different letters indicate a significant difference ($P = 0.01$) according to Duncan's new multiple range test.

P. cinnamomi, comparable to results obtained with both *P. parasitica* and *P. citrophthora* (5). The inhibition of sporangia production in *P. cinnamomi* was more pronounced than that of chlamydospore formation at equivalent concentrations of metalaxyl.

Sporangium production by *P. citricola* was generally somewhat less sensitive to metalaxyl, but the differences did not parallel those found for mycelial growth. Isolates of *P. citricola*, more tolerant to metalaxyl in terms of sporangium formation, were not necessarily more tolerant with respect to effects of the fungicide on mycelial growth. The initial stage of germination of encysted zoospores of *P. cinnamomi* and *P. citricola* was remarkably insensitive to metalaxyl, even 100 μg/ml being totally ineffectual, a result which parallels findings with other *Phytophthora* species (5,15,16).

Ultimately, the major effect of a systemic fungicide such as metalaxyl will be in preventing mycelial growth of the fungus in its host. The high in vitro sensitivity of isolates of *P. cinnamomi* to metalaxyl suggested that this aspect of the life cycle was important in terms of the primary biological target of the fungicide. Additionally, sporangium formation, and to a lesser extent, chlamydospore and oospore formation, were important secondary targets of the fungicide.

Wide differences in tolerance to metalaxyl existed among various isolates of *P. megasperma* (7), whereas *P. cinnamomi*, despite its extensive host range, had a comparatively narrow spectrum of sensitivities. However, within the complex species, *P. megasperma*, several types (including several forma speciales) have been recognized (2,4,13). Thus the concept that a particular *Phytophthora* species may have a characteristic spectrum of responses to metalaxyl still remains viable.

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