Effectiveness of Three Phenylamide Fungicides Against *Phytophthora cryptogea* Isolated From Kiwi and Their Mobility in Soil

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ABSTRACT

Zaviezo, T., Latorre, B. A., and Torres, R. 1993. Effectiveness of three phenylamide fungicides against *Phytophthora cryptogea* isolated from kiwi and their mobility in soil. Plant Dis. 77:1239-1243.

The mobility of phenylamide fungicides in sand and clay loam soil columns was demonstrated using *Phytophthora cryptogea* from kiwi to assess for the fungicides. Mobility appears to be a function of the chemical nature of the fungicide and soil texture. Regardless of the soil type, metalaxyl was highest, ofurace was intermediate, and oxadixyl was lowest in the degree of mobility observed. There was a tendency toward less mobility in clay loam soil than in sandy soil. Similarly, water that percolated through soil columns treated with metalaxyl inhibited *P. cryptogea* more than did water that percolated through soil columns treated with ofurace or oxadixyl. There was significant variation in the response to ofurace, oxadixyl, and metalaxyl among the species of *Phytophthora*. The ED_{50} values obtained for *P. cryptogea* were 1.77, 1.1, and 0.22 mg/L for ofurace, oxadixyl, and metalaxyl. A single application of each phenylamide fungicide to 2-yr-old kiwi plants inoculated with *P. cryptogea* provided significant ($P < 0.05$) control of root rot. Protection persisted for 120 days after treatment with metalaxyl, the most active fungicide tested.

Phytophthora cryptogea Pethybr. & Lafferty is the major causal agent of Phytophthora root rot of kiwi (*Actinidia chinensis* Planch.) in Chile (13,14). It has also been reported as a pathogen of kiwi in California (7). Several phenylamide fungicides, including metalaxyl, ofurace, and oxadixyl, are being developed for the control of diseases caused by soilborne *Phytophthora* (3,5,8-12,20,21). Metalaxyl is one of the most active phenylamide compounds with curative as well as protective activity. It has a relatively high water solubility and low degree of soil adsorption, which accounts for the good performance obtained with soil applications to control root rot diseases caused by *Phytophthora*. Metalaxyl is taken up by the roots and moves systemically (1,5,6,18,20,22,24). Ofurace and oxadixyl appear to have biological properties similar to those of metalaxyl (5,15,20,21); however, their performance as soil fungicides has not been well documented. Furthermore, their activity against root rot of kiwi caused by *P. cryptogea* has not been investigated. This study was undertaken to verify the in vitro and in vivo sensitivity of *P. cryptogea* to ofurace, oxadixyl, and metalaxyl and to study the mobility of these fungicides in a soil profile.

MATERIALS AND METHODS

Fungicides. The following compounds were used: ofurace (=milfuram, Ofurace 50WP), oxadixyl (Sandofan 25WP), and metalaxyl (Apron 35SD).

In vitro tests. Sensitivity tests were conducted with *P. cryptogea* isolate K-13-4, previously tested for pathogenicity on kiwi plants (14). The following species were included for comparison: *P. cactorum* (Lebert & Cohn) Schröt., *P. cinnamomi* Rands, *P. citricola* Sawada, *P. citrophthora* (R.E. Sm. & E.H. Sm.) Leonian, *P. megasperma* Drechs. var. *sojae* Hildebrand, and *P. capsici* Leonian. All tests were performed on amended corn meal agar (ACMA) containing per liter: 17 g of corn meal agar plus 150 mg of ampicillin (100% a.i.), 10 mg of pimaricin (Delvocid, 50% a.i.), 16 mg of rifampicin (Rifaldin, 100% a.i.), 10 mg of benomyl (Benlate, 50% a.i.), 100 mg of PCNB (Brassicol, 20% a.i.), and 30 mg of hymexazol (Tachigaren, 99.5% a.i.) (14). Each fungicide was suspended in 10 ml of 95% ethanol and added at the desired concentration to ACMA at 50 C. A 5-mm disk of actively growing mycelium was placed in the center of each of three plates per concentration. An equal number of plates with ACMA without the fungicide to be tested were left as controls. All plates were then incubated in the dark at 18-20 C for 7 days or until the mycelium completely covered the control plates. The radial growth of the mycelium was determined. This experiment was repeated twice.

The effective dose to inhibit 50% (ED_{50}) of the mycelial growth was estimated by linear regression analysis where Y = probit of the percent inhibition of the mycelium and X = log of the fungicide concentration. The ED_{50} values were used to compare the relative sensitivity of the *Phytophthora* spp. to each fungicide.

Mobility of fungicides in soil and drainage water. *Experiment 1.* Soil columns were established to test the mobility of ofurace and oxadixyl relative to metalaxyl (17). The experimental design was a split-split plot with fungicide as the main plot, soil type as the subplot, and soil depth as the sub-subplot. Natural field soils from the Clarillo series (Pirque, Región Metropolitana, Chile) were used to create two different soil columns. Each column was made from a 10.5 × 50 cm polyvinyl chloride (PVC) cylinder. Column A was filled and packed with sandy soil to a bulk density of 1.4 g/ml; column B with clay loam soil to a bulk density of 1.28 g/ml (Table 1). Three replicate columns were established per treatment. This experiment was performed twice.

Soil moisture in each column was adjusted to field capacity before suspensions of ofurace (0.7 mg/cm^2), oxadixyl (0.7 mg/cm^2), and metalaxyl (0.25 mg/cm^2) in 25 ml of sterile distilled water (DW) were added to each column. The concentration of each fungicide was equivalent to the concentration suggested by the manufacturer for soil treatment. Soil columns A and B were subsequently leached with 830 ml and 1,700 ml of DW, respectively. The columns were maintained at room temperature (18-23 C) for 24 to 48 hr. Each column was frozen at −18 C for 18 hr, and three 2-cm sections of each frozen soil column were obtained by cutting the cylinder at 2, 20, and 40 cm depths. To avoid any possible error arising from channeling or capillary effects, the outer 10 mm of each section next to the cylinder wall was discarded. The soil of each section was dried separately at 58 C for 18 hr, mixed 1:1 (w/w) with DW, shaken for 2 hr on a rotary shaker, and centrifuged at 400 rpm for 15 min.

Table 1. Physical characteristics of two soils used in this research

Character	Soil A	Soil B
pH	7.1	7.8
Organic matter (%)	0.1	2.8
Soil texture (%)		
Sand	82.0	28.0
Silt	10.5	42.5
Clay	7.5	29.5
Textural class	Sandy	Clay loam

Accepted for publication 27 August 1993.

The supernatant was diluted 1:10 with DW; and 5 ml of this dilution was mixed with 5 ml of double strength ACMA, molten at 50 C, agitated, and poured into 9-cm petri plates. A 5-mm disk of actively growing mycelium of *P. cryptogea* isolate K-13-4 on ACMA was placed in the center of each of three plates per assay and incubated in the dark at 23 C for 6 days. The radial growth of the mycelium was used as an index of the fungicidal activity in the soil.

Analysis of variance was performed for the mobility of the fungicides in the soil columns according to the split-split plot design (19). Data were square root transformed before analysis.

The leachate from each soil column was collected immediately, diluted 1:10 using DW, and bioassayed as described above for the soil samples. Analysis of variance was performed using a split plot design where the main plots were fungicide and the subplots were soil types A and B. Mean differences among treatments were separated according to Duncan's multiple range test.

Experiment 2. Columns with sandy soil were treated with 0.31, 0.47, and 0.62 mg/cm^2 of oxadixyl; 0.33, 0.65, and 0.98 mg/cm^2 of ofurace; and 0.10, 0.25, and 0.50 mg/cm^2 of metalaxyl. Fungicides were carefully added in 25 ml of DW to each soil column, then the columns were rinsed with 830 ml of DW. Movement of the fungicides through the soil column was determined as described above. Leachates were also collected and bioassayed as described above.

Effectiveness of fungicides against the root rot caused by *P. cryptogea*. The effectiveness of ofurace, oxadixyl, and metalaxyl for preventing infections by *P. cryptogea* was evaluated in 2-yr-old kiwi plants cultivated in disinfested soil (2:1:1, organic soil:sand:loam soil) in plastic bags (14 × 30 cm). The inoculum, consisting of 4- to 5-day-old mycelial mats of *P. cryptogea* (K-13-4) from carrot juice broth (14), was washed twice in sterile DW and then macerated in a blender for 15 min at high speed. Plants were injured by cutting the roots around each trunk with a knife. Then 100 ml of inoculum suspension, adjusted to 10^6 mycelial fragments per milliliter with a hemacytometer, was poured into 15–20 cm deep holes in the soil made with a knife. Plants were kept for 24 hr before treatment with ofurace (0.05, 0.5, 0.95, and 1.4 mg/cm^2), oxadixyl (0.08, 0.5, 1.2, and 1.85 mg/cm^2), or metalaxyl (0.03, 0.1, 0.18, and 0.25 mg/cm^2). The fungicide for each plant was suspended in 50 ml of DW and delivered to the soil surface (153.9 cm^2). Nontreated but inoculated plants were left as controls. The experiment was designed as a complete block with four replications and a single plant as an experimental unit. All plants were arranged on trays containing 5 cm of water to assure a very high soil moisture level and left for 120 days under greenhouse conditions with a minimum air temperature of 15.5 C and a maximum of 30.4 C (average 21.5 C). Soil temperatures measured at noon, 10 cm below the soil surface, varied from 13 to 28 C (average 19.6 C). About 14 hr of light were recorded daily during the experiment. The percent root rot, root fresh weight, and percent reisolation of *P. cryptogea* were recorded. Data were first analyzed for variance, and correlations analyses were conducted between dosage and each of the parameters evaluated.

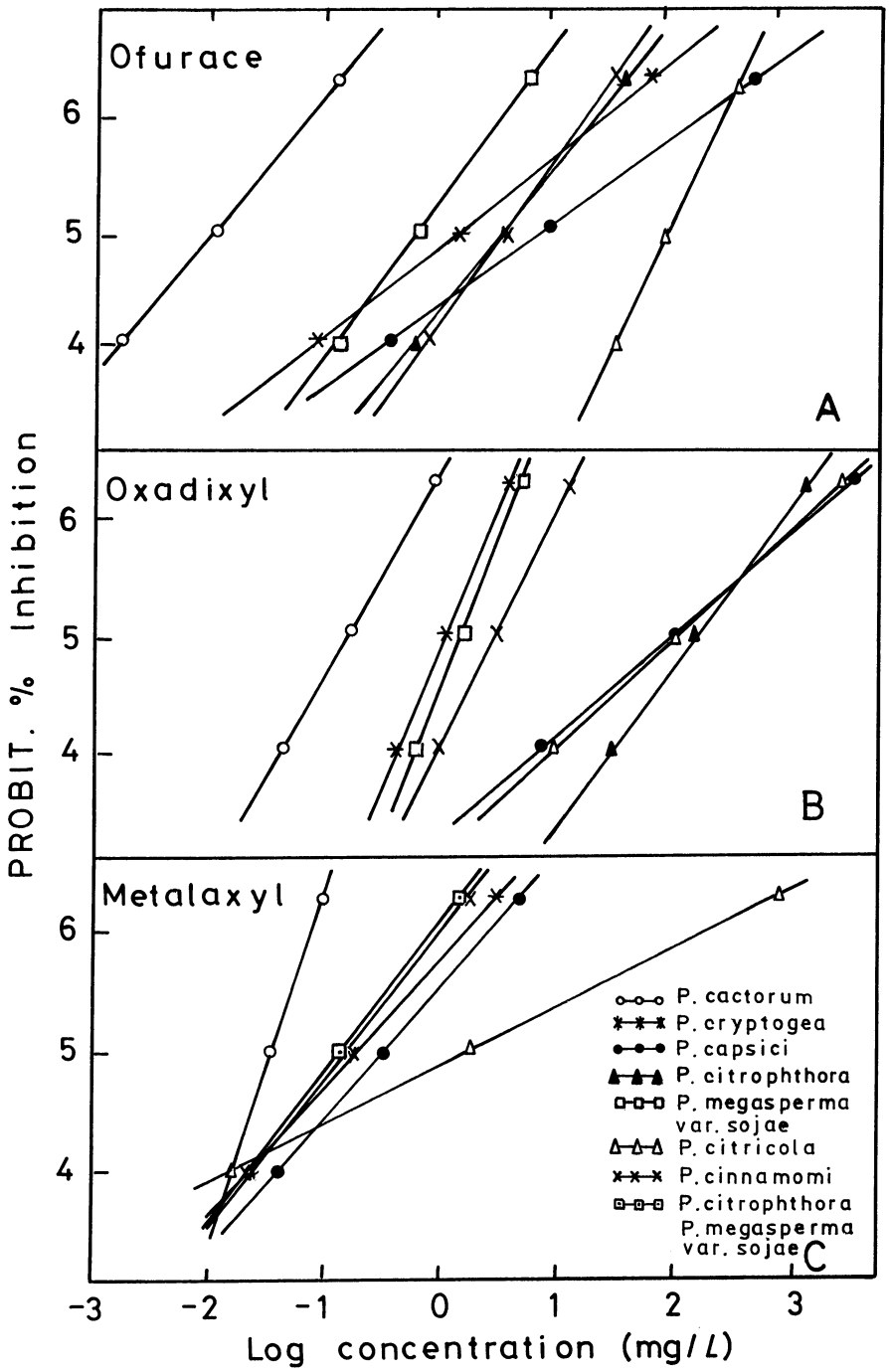

Fig. 1. Dosage response curves obtained for mycelial growth inhibition with seven *Phytophthora* spp. on corn meal agar amended with: **(A)** ofurace, **(B)** oxadixyl, and **(C)** metalaxyl.

RESULTS

In vitro tests. *P. cactorum* exhibited the highest sensitivity to metalaxyl, ofurace, and oxadixyl (ED_{50} values of 0.04, 0.01, and 0.17 mg/L, respectively); *P. citricola* the least sensitivity to metalaxyl and ofurace (ED_{50} values of 1.3 and 91.6 mg/L, respectively); and *P. citrophthora* the least sensitivity to oxadixyl (ED_{50} value of 144.8 mg/L). *P. cryptogea* was intermediate in sensitivity to ofurace, oxadixyl, and metalaxyl with ED_{50} values of 1.77, 1.1, and 0.22 mg/L, respectively (Fig. 1).

Mobility of fungicides in soil and drainage water. Fungicide activity was detected via the bioassay for the three compounds tested in both sandy and clay

loam soil columns up to 40 cm below the soil surface, as well as in leachate collected from the columns (Tables 2, 3, and 4).

In the analysis of variance of experiment 1, the effects of fungicide (F) ($P = 0.0054$), soil type (ST) ($P = 0.0027$), and soil sampling depth (SSD) ($P = 0.0001$) on fungicide mobility were highly significant. In addition, statistically significant interactions were found for F × SSD ($P = 0.0001$), F × ST ($P = 0.0066$), and F × SSD × ST ($P = 0.0002$); however, the ST × SSD was not significant ($P = 0.3766$). Except for ofurace at a depth of 2 cm and metalaxyl at a depth of 40 cm, no significant differences were found between sandy and clay loam soils (Table 2). In sandy soil columns, ofurace was the most active compound at 2 cm of soil depth sampling; but at 20 and 40 cm of depth, the activity of metalaxyl was the highest. In clay loam soil columns, no significant differences were detected between fungicides at 2 and 40 cm depths of soil sampling; but at 20 cm soil depth, metalaxyl was the most active fungicide.

Fungicide activity was found in the water that percolated through soil columns treated with each fungicide. Fungicide and soil type ($P = 0.0001$) significantly affected the amount of fungicidal activity present in the water. However, no F × ST interaction was detected ($P = 0.0205$). Regardless of the soil type, water collected from columns treated with metalaxyl inhibited *P. cryptogea* more than water from columns treated with ofurace or oxadixyl. Regardless of fungicide, leachates collected from sandy soil exhibited significantly ($P < 0.05$) more inhibition of *P. cryptogea* than leachates from clay loam soil (Table 3).

In experiment 2, all three fungicides were detected in sandy soil columns; but the probit percent inhibition of the mycelial growth of *P. cryptogea* obtained was a function of the log of fungicide concentration initially applied, with R^2 values ranging from 0.89 to 0.98, 0.86 to 0.88, and 0.64 to 0.96, respectively, for metalaxyl, ofurace, and oxadixyl. A partial inhibition of the mycelial growth was obtained at the 40-cm depth with metalaxyl varying from 6 to 29%, depending on the fungicide concentration applied. Regardless of the fungicide concentration, no fungicide activity was observed at the 40-cm depth with ofurace and oxadixyl; but at the 2-cm depth, all three fungicides were detected, with growth inhibitions varying from 19 to 29% for ofurace, 3 to 20% for oxadixyl, and 4 to 63% for metalaxyl (Table 4).

For experiment 2, 100% inhibition of the mycelial growth was obtained for metalaxyl in the water that leached out of the soil column for each of the concentrations tested; and a partial inhibition was obtained for ofurace and oxadixyl varying from 61.3 to 73.7% and from 70 to 82.8%, respectively.

Effectiveness of fungicides against the root rot caused by *P. cryptogea*. All three fungicides significantly ($P < 0.05$) reduced the level of root rot and increased root fresh weight of kiwi plants inoculated with *P. cryptogea*. Similarly, each fungicide treatment significantly ($P < 0.05$) reduced the percent pathogen reisolation after 120 days of treatment. A significant ($P < 0.05$) correlation between fungicide concentration and the root fresh weight was obtained for ofurace ($Y = 40.8 + 22.5X$, $R^2 = 0.84$), oxadixyl ($Y = 37.6 + 13.6X$, $R^2 = 0.86$), and metalaxyl ($Y = 36.6 + 136.9X$, $R^2 = 0.97$) (Fig. 2A). A negative linear correlation between fungicide concentration and the percent root rot (square root transformed data) was significant ($P < 0.05$) for ofurace ($Y = 7.6 - 3.9X$, $R^2 = 0.95$), oxadixyl ($Y = 7.4 - 2.9X$, $R^2 = 0.95$), and metalaxyl ($Y = 8.1 - 24.2X$, $R^2 = 0.94$) (Fig. 2B). Similarly, a negative and significant ($P < 0.05$) correlation between fungicide concentration and the percent pathogen reisolation of *P. cryptogea* (angular transformed data) was ob-

Table 2. Inhibition of growth of *Phytophthora cryptogea* on corn meal agar amended with sandy and clay soil taken at three depths from soil columns treated with fungicide suspensions

Soil type[x]	Column depth (cm)	Growth inhibition of *P. cryptogea* (%)[y] Metalaxyl (0.25 mg/cm²)	Ofurace (0.70 mg/cm²)	Oxadixyl (0.70 mg/cm²)
Sandy	2	15.5 aB[z]	51.3 bC	0.0 aA
Clay loam	2	11.4 aA	3.0 aA	3.3 aA
Sandy	20	38.7 aB	8.2 aA	5.8 aA
Clay loam	20	37.5 aB	5.4 aA	4.5 aA
Sandy	40	23.0 bB	1.1 aA	0.0 aA
Clay loam	40	0.0 aA	3.4 aA	2.2 aA

[x] Soil columns were built in 10.5 × 50 cm polyvinyl chloride cylinders filled and packed to bulk densities of 1.4 and 1.28 g/ml for the sandy and clay loam soils, respectively.
[y] Growth inhibition after 6 days of incubation at 23 C on corn meal agar amended with 5% (v/v) soil extract from a given depth after completely draining each soil column.
[z] Average of three replicates. Means in columns followed by the same lowercase letter and means in rows followed by the same capital letter are not significantly different according to Duncan's multiple range test at $P = 0.05$.

Table 3. Inhibition of growth of *Phytophthora cryptogea* on corn meal agar amended with water that drained out of soil columns treated with fungicide suspensions

Soil type	Growth inhibition of *P. cryptogea* (%)[y] Metalaxyl (0.25 mg/cm²)	Ofurace (0.70 mg/cm²)	Oxadixyl (0.70 mg/cm²)
Sandy	86.1 bB[z]	49.2 bA	46.9 bA
Clay loam	20.2 aC	7.4 aB	1.6 aA

[y] Growth inhibition after 6 days of incubation at 23 C in corn meal agar amended with 5% (v/v) leachate after each soil column was drained with 830 and 1,700 ml of water, respectively, for sandy and clay loam soil types.
[z] Average of three replicates. Means in columns followed by the same lowercase letters and means in rows followed by the same capital letters are not significantly different according to Duncan's multiple range test at $P = 0.05$.

Table 4. Fungicide activity in sandy soil columns detected by bioassay using *Phytophthora cryptogea* as the indicator organism

Fungicide	Dosage (mg/cm²)	Inhibition of *P. cryptogea* (%) at the following depths of soil in columns (cm)[y] 2	20	40
Ofurace	0.33	19	15	0
	0.65	21	34	0
	0.98	26	35	0
Correlation coefficient (R)[z]		0.92**	0.94**	ND
Oxadixyl	0.31	3	17	0
	0.47	3	26	0
	0.62	20	29	0
Correlation coefficient (R)		0.80	0.98**	ND
Metalaxyl	0.10	4	0	6
	0.25	18	21	20
	0.50	63	49	29
Correlation coefficient (R)		0.98**	0.94**	0.99**

[y] Average of three observations.
[z] Correlation coefficient estimated by linear regression analysis where X = log dosage and Y = probit % inhibition. ** = highly significant at $P < 0.01$. ND = not determined.

tained for ofurace ($Y = 30 - 49.5X + 21.8X^2$, $R^2 = 0.91$), oxadixyl ($Y = 31.3 - 48.3X + 18.1X^2$, $R^2 = 0.96$), and metalaxyl ($Y = 31.4 - 338.9X + 910.6X^2$, $R^2 = 0.94$) (Fig. 2C).

DISCUSSION

The phenylamides, metalaxyl, ofurace, and oxadixyl, significantly inhibited growth of *P. cryptogea* in vitro and recovery in vivo. However, relative to the ED_{50} values, *P. cryptogea* was 8.2 and 5 times more sensitive to metalaxyl in vitro than to ofurace and oxadixyl, respectively. A similar trend was observed when the effectiveness of these fungicides was tested in vivo (Fig. 2).

Several reports have demonstrated variation in sensitivity to metalaxyl within the genus and among isolates of a given species of *Phytophthora* (4,11). This study revealed a significant variation in response of the mycelial growth to ofurace, oxadixyl, and metalaxyl among seven species of *Phytophthora.* Based on the dosage-response curves (Fig. 1), *P. cactorum* was the most sensitive species to the phenylamides tested. *P. citrophthora* was the least sensitive species to metalaxyl and ofurace, while *P. citricola* was the least sensitive to oxadixyl. In relation to the ED_{50} value of *P. cactorum, P. cryptogea* was 5.5 times less sensitive to metalaxyl and oxadixyl, and 180 times less sensitive to ofurace. The ED_{50} values obtained in this study were within the range already reported in the literature for metalaxyl (4,11,20).

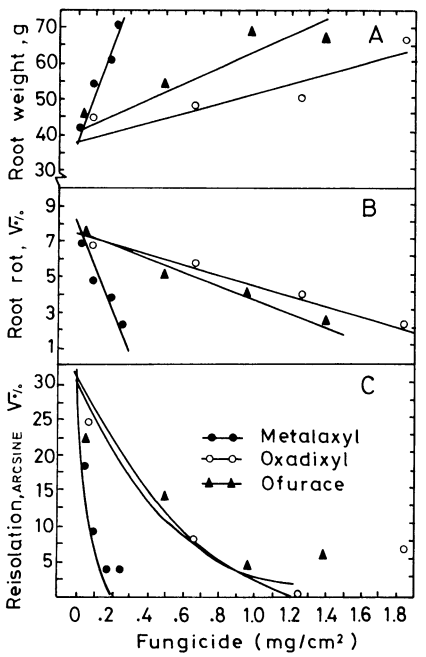

Fig. 2. Effectiveness of ofurace, oxadixyl, and metalaxyl to control the root rot of kiwi caused by *Phytophthora cryptogea* on 1-yr-old plants. **(A)** Dosage response curves obtained by linear regression analysis between X = log dosage and Y = root rot fresh weight; R^2 values were 0.84, 0.86, and 0.97 for ofurace, oxadixyl, and metalaxyl, respectively. **(B)** Dosage response curves obtained by linear regression analysis between X = log dosage and Y = % root rot transformed as square root; R^2 values were 0.95, 0.95, and 0.94 for ofurace, oxadixyl, and metalaxyl, respectively. **(C)** Percent reisolation of *P. cryptogea* after 120 days of treatment; R^2 values were 0.91, 0.96, and 0.94 for ofurace, oxadixyl, and metalaxyl, respectively.

Fungicide mobility in soil appears to be a function of the compound and is dependent upon the physical properties of the soil. Although positive evidence was obtained for the movement of the three phenylamide fungicides in the soil profile, our results corroborated previous information demonstrating a high degree of mobility for metalaxyl in the soil (2,22). Of the three fungicides tested, metalaxyl exhibited the highest mobility in sandy soil and was present in the highest concentration in leachates from sandy soil columns. The lowest mobility was demonstrated by oxadixyl. The relatively high water solubility of metalaxyl, 0.71% (w/v), which is 50.7 and 2.1 times higher than ofurace and oxadixyl, respectively, may partially explain the differences in fungicide mobility observed among these compounds in soil (24,25).

As observed by other authors (2,3,22), there was a tendency toward a lower mobility in clay loam than in sandy soil in this study. Significant differences ($P < 0.05$) were found in the water that drained from clay loam vs. sandy soil columns (Table 4). A possible higher adsorption of these fungicides to clay loam than to sandy soil may explain these differences. However, a low adsorption coefficient has been reported for metalaxyl regardless of the soil texture (2,22).

A single soil drench application of these phenylamide fungicides was effective against root rot of kiwi caused by *P. cryptogea,* and significant protection was obtained even 120 days after application. The relatively long residual activity reported for metalaxyl and other phenylamide compounds may explain these results (2,3,16).

The dosage-response curves showed that ofurace and oxadixyl were less active than metalaxyl in their fungicidal performance as soil treatment for *P. cryptogea* (Figs. 2A and B). The slope values (b) estimated for metalaxyl varied from b = 13.2 to b = 136.9 and were 6 to 10 times higher than those estimated for ofurace and oxadixyl, respectively. The degree of control achieved in this experiment with metalaxyl was similar to the effectiveness previously reported for *P. cryptogea* from other crops (23).

The extended residual activity in the soil and the mobility in the soil profile for the phenylamide fungicides tested can be valuable in developing disease control strategies for Phytophthora root rot, particularly for preventing a possible infection of kiwi plants that may occur at the nursery or during transplant. Similar results were obtained with the same fungicides on the root rot of kiwi caused by *P. citrophthora* (13), and numerous reports (3,9,16,23,25) have demonstrated the effectiveness of metalaxyl on different species of *Phytophthora* affecting other crops.

ACKNOWLEDGMENTS

This research was supported, in part, by grants from FONDECYT, project 517-89. We thank R. Piña and M. Castiglioni for their help during this study, and we also thank J. C. Broome for her valuable advice given during the conduct of the research.

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