Cross-Resistance to Four Systemic Fungicides in Metalaxyl-Resistant Strains of *Phytophthora infestans* and *Pseudoperonospora cubensis*

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ABSTRACT

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Although cyprofuram + folpet, SAN 371F, propamocarb, and phosethyl Al controlled metalaxyl-sensitive strains of *Phytophthora infestans* and *Pseudoperonospora cubensis* in potato and cucumbers, respectively, the fungicides failed to do so in plants inoculated with metalaxyl-resistant strains.

Metalaxyl, SAN 371F, propamocarb, and phosethyl Al are systemic fungicides effective against plant-pathogenic Peronosporales. Systemic fungicides must be selective within plant cells, affecting only the target pathogen. To achieve this selective toxicity, only a single site or process must be affected (15). Because of this narrow spectrum of activity, selection of mutants resistant to systemic fungicides occurs at relatively high frequencies of between 1×10^{-4} and 1×10^{-12} (8,11,12). In agrosystems, the buildup of Peronosporales subpopulations resistant to acylalanine fungicides is a well-known problem in disease control (2–7,10,13,19,24). To delay buildup of such subpopulations, alternating unrelated fungicides using mixtures of a systemic and a protectant fungicide is recommended (22). Because a pathogen is unlikely to develop cross-tolerance to unrelated chemicals, these strategies are expected to be efficient in disease control.

This study was undertaken to test whether strains of *Phytophthora infestans* de Bary and *Pseudoperonospora cubensis* (Berk. & Curt.) Rostow resistant to metalaxyl express cross-tolerance to cyprofuram + folpet and SAN 371F, fungicides related to metalaxyl, or to propamocarb and phosethyl Al, fungicides unrelated to metalaxyl.

MATERIALS AND METHODS

Plants, pathogens, and inoculation. Late blight-susceptible potato (*Solanum tuberosum* L. 'Croft') and downy mildew-susceptible cucumber (*Cucumis sativus* L. 'Bet-Alfa') were used. Potato plants were grown in the greenhouse (20–32 C) in 1-L plastic pots (about 1.3 kg air-dried soil mixture) and were used when about 10 compound leaves had developed. Cucumbers were grown in the greenhouse in 0.35-L plastic pots (0.4 kg air-dried soil mixture) and inoculated on day 10 after developing fully expanded cotyledons. Ten to 12 plants were planted per pot. Soil mixture composition was sandy loam, vermiculite, and peat at a 2:1:1 (v/v) ratio.

Potato plants were inoculated by spraying to runoff with sporangial suspensions (5×10^4 sporangia per milliliter) of *Phytophthora infestans* and incubated for 20 hr in a dew chamber at 17 C in the dark. Cucumber plants were inoculated with *Pseudoperonospora cubensis* by placing 10-μl inoculum droplets (10 ± 1 sporangia per droplet) on the adaxial surface of each cotyledon and incubated in a similar manner. All plants were then transferred to growth cabinets for 7 days at 20 C (60–70% RH) illuminated 12 hr/day (about 100 μE m^{-2} s^{-1}) with VHO fluorescent lamps. Disease records were taken 7 days after inoculation. For potatoes, numbers of stems infected and area of lesioned stem (on a visual scale of 0–5, where 0 represents no apparent infection and 1, 2, 3, 4, and 5 represent about 20, 40, 60, 80, and 100% of stem surface area blighted, respectively) were recorded. For cucumbers, numbers of infected cotyledons of the population inoculated were recorded.

Inoculation tests were done with several metalaxyl-sensitive and metalaxyl-resistant isolates of the pathogens. Metalaxyl-sensitive isolates of *Pseudoperonospora cubensis* were collected in nature in 1979 and 1982. These isolates were controlled in cucumber cotyledons growing in pots drenched with 0.25 mg a.i. metalaxyl per pot (20 ml of 12.5 μg/ml a.i. metalaxyl/0.4 kg soil). The metalaxyl-resistant strains of this fungus were collected in 1979 and 1982 from cucumber plants growing in commercial plastic houses. These strains produced disease and sporulated profusely in plants drenched with 15 mg a.i. metalaxyl per pot (20 ml of 750 μg/ml a.i. metalaxyl/0.4 kg soil) (24).

Metalaxyl-sensitive and metalaxyl-resistant isolates of *Phytophthora infestans* were isolated in nature in 1982 (6). Sensitive isolates were controlled in potato plants drenched with 0.25 mg a.i. metalaxyl per pot (20 ml of 12.5 μg/ml a.i. metalaxyl/1.3 kg soil). The metalaxyl-resistant-1 isolate of this fungus was controlled with 5 mg a.i. metalaxyl per pot (20 ml of 250 μg/ml a.i. metalaxyl/1.3 kg soil) and the metalaxyl-resistant-2 isolate developed and sporulated abundantly on potato plants grown in pots drenched with 20 mg a.i. metalaxyl per pot (20 ml of 1,000 μg/ml a.i. metalaxyl/1.3 kg soil).

Fungicides and fungicide application. Five systemic fungicides were used in this study: 1) metalaxyl, 25% wettable powder; 2) propamocarb (Previcur-N), 70% aqueous solution; 3) phosethyl Al (Aliette), 80% wettable powder; 4) cyprofuram + folpet (Vinicur); and 5) SAN 371F (Sandofan).

Fungicides were applied at the following ranges of concentrations (mg a.i./pot): metalaxyl, 0.25–20; propamocarb, 2.1–8.4; cyprofuram + folpet, 0.67–5.36; SAN 371F, 0.8–6.4; and phosethyl Al, 8–64. Fungicides were dissolved in water and applied as soil drench treatments 48 hr before inoculation, except phosethyl Al and propamocarb, which were drenched 72 hr before inoculation. Twenty-milliliter aliquots of a fungicide suspension were applied to the soil surface of potted plants. Treated plants were kept in the greenhouse until taken for inoculation.

RESULTS

In three experiments conducted with three metalaxyl-sensitive strains of *Pseudoperonospora cubensis*, cyprofuram + folpet, SAN 371F, propamocarb, and phosethyl Al gave complete control of downy mildew in cucumbers at dosages of 0.67, 0.8, 4.2, and 8 mg a.i./pot, respectively. The relative control efficacy of metalaxyl was highest because a soil drench of 0.25 mg a.i./pot gave complete control. All four fungicides at the highest rates used provided very poor or no control of the disease incited by three

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Table 1. Control of potato late blight incited by metalaxyl-sensitive and metalaxyl-resistant strains of *Phytophthora infestans* by four systemic fungicides[a]

Fungicide	Rate (mg a.i./pot)[b]	Metalaxyl-sensitive strains: Percentage of diseased stems	Metalaxyl-sensitive strains: Disease severity[c]	Metalaxyl-resistant strain-1: Percentage of diseased stems	Metalaxyl-resistant strain-1: Disease severity	Metalaxyl-resistant strain-2: Percentage of diseased stems	Metalaxyl-resistant strain-2: Disease severity
None	...	100	4.6 ± 0.7	100	4.5 ± 0.8	100	4.5 ± 0.5
Metalaxyl	0.25	33	0.5 ± 0.1	100	4.5 ± 0.5	Not tested	...
	1.25	25	0.5 ± 0.2	100	4.5 ± 0.5	Not tested	...
	2.50	0	...	100	3.0 ± 0.2	100	5.0 ± 0.0
	5.00	0	...	33	2.0 ± 0.0	100	4.8 ± 0.5
	10.00	0	...	0	...	100	5.0 ± 0.0
	20.00	0	...	0	...	100[d]	5.0 ± 0.0
Propamocarb	2.10	0	...	78	3.0 ± 0.8	100	5.0 ± 0.0
	4.20	0	...	29	2.5 ± 0.7	100	4.5 ± 0.5
	8.40	0	...	22	1.5 ± 0.7	100	4.5 ± 0.7
Cyprofuram + folpet	0.67	0	...	40	3.5 ± 0.7	100	4.6 ± 0.6
	1.34	0	...	25	2.0 ± 0.0	100	4.7 ± 0.6
	2.68	0	...	0	...	100	4.8 ± 0.4
	5.36	0	...	0	...	100	4.7 ± 0.3
SAN 371F	0.80	0	...	80	3.2 ± 0.8	100	4.8 ± 0.5
	1.60	0	...	80	3.2 ± 0.8	100	3.0 ± 1.4
	3.20	0	...	33	1.5 ± 0.7	100	2.6 ± 0.5
	6.40	0	...	29	1.5 ± 0.7	100	3.8 ± 1.2
Phosethyl Al	8.00	0	...	30	1.0 ± 0	100	4.4 ± 0.8
	16.00	0	...	0	...	100	4.4 ± 0.8
	32.00	0	...	0	...	100	4.0 ± 0.7
	64.00	0	...	0	...	100	3.0 ± 0.0

[a]Twenty-one to 30 plants per fungicide per dose with each strain.
[b]Twenty milliliters of aqueous fungicide solution was applied to a pot containing 1.3 kg air-dried soil.
[c]Disease severity based on a visual scale of 0–5, where 0 = no apparent infection, 1 = 20%, 2 = 40%, 3 = 60%, 4 = 80%, and 5 = 100% of stem surface area blighted.
[d]Phytotoxic symptoms.

metalaxyl-resistant strains of *P. cubensis*. Therefore, propamocarb and phosethyl Al gave no control of the mildew at dosages of 8.4 and 64 mg a.i./pot, respectively, whereas cyprofuram + folpet at 5.36 mg a.i./pot and SAN 371 F at 6.4 mg a.i./pot protected only 37 and 36% of the cotyledons inoculated, respectively.

Similar results were obtained when sensitive and resistant strains of *Phytophthora infestans* were used. All five fungicides, at the lowest rates used, controlled late blight in potato incited by three metalaxyl-sensitive strains of the fungus (Table 1). However, none of them was efficient in controlling the disease incited by the metalaxyl-resistant-2 strain of the pathogen. The metalaxyl-resistant-1 strain, which showed moderate resistance to metalaxyl, was partially controlled by the other four fungicides, especially cyprofuram + folpet and phosethyl Al.

DISCUSSION

Our data show that metalaxyl-resistant strains of *Pseudoperonospora cubensis* and *Phytophthora infestans* were also resistant in greenhouse tests to cyprofuram + folpet, SAN 371F, propamocarb, and phosethyl Al, all systemic compounds active against Oomycetes, indicating that these fungicides cannot substitute for metalaxyl in disease control in growing areas where metalaxyl-resistant strains of *P. cubensis* and *P. infestans* are present.

Resistance of fungal strains to structurally related fungicides that act at the same site in a fungal cell is a common phenomenon (11,12,23). It may occur also with structurally unrelated compounds (11,12). Resistance to metalaxyl was reported in *P. infestans* (4,6,7,10,13,26), *P. cubensis* (17,24), *Peronospora hyoscyami* (1), and *Plasmopara viticola* (5,18). Cross-resistance to compounds related to metalaxyl was reported. Bruin and Edgington (2) showed that *Phytophthora capsici* and *Pythium ultimum* resistant to metalaxyl in vitro conferred cross-resistance to the related fungicides furalaxyl, RE 20615, RE 26756, RE 26940, and M 9834 (Galben). Davidse (8) obtained strains of *Phytophthora megasperma* f. sp. *medicaginis* resistant to both metalaxyl and furalaxyl. Katan (20) reported on cross-resistance in *Pseudoperonospora cubensis* to metalaxyl, M 9834, and cyprofuram. Cyprofuram, however, is not an acylalanine compound, as Katan thought, but an anilide. Gisi et al (18) observed a positive cross-resistance between SAN 371F and metalaxyl in *Phytophthora infestans* and *Plasmopara viticola*. However, the level of resistance of SAN 371F was 20–120 times lower compared with that of metalaxyl. Cross-resistance to metalaxyl and chemically unrelated fungicides was not reported. Cooke (7) showed that there were no differences in sensitivity to cymoxanil or mancozeb between metalaxyl-resistant and metalaxyl-sensitive isolates of *Phytophthora infestans*.

Metalaxyl, SAN 371F, and cyprofuram + folpet are anilides containing an aromatic amine (aryl-N-CO-) structure in their molecules. Cyprofuram possesses an amide moiety but appears as a carbamate and not as an anilide. Phosethyl Al is a monoethyl ester of phophonic acid complexed with aluminum. Little information is available on the mode of action of these compounds. Metalaxyl was reported to inhibit synthesis of RNA, DNA, and lipids in *Pythium splendens* (21) and of poly(A)+RNA and poly(A)−RNA in *P. megasperma* f. sp. *medicaginis* (9). Fisher and Hayes (16) showed that the primary effect of metalaxyl on *P. palmivora* probably involves impaired biosynthesis of RNA so that mitosis is inhibited. Phosethyl Al has no fungicidal effect in vitro (14,15,25). Sanders (25) assumed that suppression of Pythium blight in turfgrass by phosethyl Al may be indirect, involving biotransformation or elicitation of antifungal response in the host. It would be interesting to investigate whether phosethyl Al is biotransformed to (or induces in the plant tissue) antifungal anilide or amide compounds similar to metalaxyl or cyprofuram.

More data regarding the mode of action of the compounds, especially of phosethyl Al, are required before any conclusive explanation for this cross-resistance can be drawn. We also need to know whether naturally occurring strains resistant to either cyprofuram + folpet, propamocarb, SAN 371F, or phosethyl Al will possess resistance to metalaxyl and/or to the other three compounds, in other words, whether cross-resistance works in all directions among all five xenobiotics.

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