Induction of Fungal Resistance to Metalaxyl by Ultraviolet Irradiation

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This investigation was supported by the Natural Sciences and Engineering Research Council of Canada.
Accepted for publication 9 June 1981.

ABSTRACT

Bruin, G. C. A., and Edgington, L. V. 1982. Induction of fungal resistance to metalaxyl by ultraviolet irradiation. Phytopathology 72:476-480.

Three minutes of ultraviolet (UV) irradiation of a suspension containing 3×10^6 zoospores of *Phytophthora capsici* resulted in the development of 12 metalaxyl-resistant strains and 35 min of UV irradiation of mycelium of *P. capsici* and *Pythium ultimum* produced many resistant strains. The resistance was initially low, but a few transfers on metalaxyl-amended V-8 agar followed by 12 transfers on unamended V-8 agar resulted in a number of stable, highly resistant mutants that retained pathogenicity to their respective pea and pepper hosts. There was a high correlation between resistance expressed in vitro and in vivo. Resistance to metalaxyl was coupled with resistance to furalaxyl, Galben, milfuram, RE26745, and RE26940. UV irradiation of 1.05×10^8 spores of *Peronospora parasitica* yielded no resistant strains. Large differences in levels of metalaxyl sensitivity among single zoospore isolates of *P. capsici* were demonstrated and may indicate the coexistence of metalaxyl-'sensitive' and 'resistant' nuclei in the coenocytic mycelium.

Additional key words: Peronosporales, acylalanine-type fungicides, heterokaryosis.

Resistance to systemic fungicides is a frequently reported and well-documented phenomenon (4,7) but, until very recently, not known for the new systemic fungicide metalaxyl (*N*-[2,6-dimethylphenyl]-*N*-[methoxyacetyl] alanine methyl ester, Ridomil 50WP). Metalaxyl is an effective fungicide specifically acting against pathogens belonging to the Peronosporales (11). Application methods for disease control include foliar sprays, seed dressings, in-furrow granules, and soil drenches. Metalaxyl does not affect spore germination, but inhibits subsequent fungal growth both in culture (11) and in plant tissue (9). In 1980, metalaxyl was released for commercial use in North America for the control of potato late blight and tobacco blue mold. It had previously been used for 3 yr in Australia against tobacco blue mold and in Israel against downy mildew of cucurbits.

The potential for members of the Peronosporales to become resistant to acylalanine fungicides was studied by Staub et al (10) with *Phytophthora infestans* (Mont.) de By. on tomato under laboratory conditions. Some isolates of *P. infestans* quickly lost sensitivity to the fungicides on agar, but most of those resistant strains did not infect metalaxyl-treated plants. They concluded that resistance to acylalanine fungicides in vitro was not necessarily related to resistance in vivo. Further, if resistance to a fungicide is linked with loss of pathogenicity or decreased vitality, as is reported for fenarimol (12) and triforine (6), then resistant mutants will not be competitive enough in nature to become epidemiologically significant. However, in 1979, a metalaxyl-resistant strain of *Pseudoperonospora cubensis* (Berk. & Curt.) Rostow appeared under field conditions in Israel (8).

Our work with strains of *Phytophthora capsici* Leonian, selected against high concentrations of metalaxyl on agar showed that resistant strains remained pathogenic on pepper and could not be controlled in vivo even with doses of metalaxyl that were phytotoxic (1,2).

In the present research, the propensity of *Pythium ultimum* Trow, *P. capsici*, and *Peronospora parasitica* (Pers. ex Fr.) Fr. to develop metalaxyl resistance was studied as well as the stability of the pathogenicity and fungicide resistance of the resistant strains.

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0031-949X/82/05047605/$03.00/0

MATERIALS AND METHODS

Fungicides. The following acylalanine-type fungicides were compared: metalaxyl, and furalaxyl (*N*-[2,6-dimethylphenyl]-*N*-[2-furoyl] alanine methyl ester, Fongarid 50WP) both supplied by CIBA-Geigy Canada Ltd., Agrochemicals Division, Cambridge, Ontario; milfuram (2-chloro-*N*-[2,6-dimethylphenyl]-*N*-[tetrahydro-2-oxo-3-furanyl] acetamide), RE26745 (2-methoxy-*N*-[2,6-dimethylphenyl]-*N*-[tetrahydro-2-oxo-3-furanyl] acetamide), and RE26940 (*N*-[2,6-dimethylphenyl]-2-methoxy-*N*-[tetrahydro-2-oxothiophen-3-yl] acetamide), all 50WP, supplied by Chevron Chemical Canada Ltd., Burlington, Ontario; and Galben (*N*-[2,6-dimethylphenyl]-*N*-[phenylacetyl] alanine methyl ester, M9834), synthesized in Montedison Research Laboratories, Italy, and supplied by Pfizer Chemicals and Genetics Ltd., London, Ontario.

Fungi. *P. capsici* (ATCC 15399) was supplied by G. Lazarovits, Agriculture Canada, London, Ontario; *P. ultimum* was obtained from R. Hall and *P. parasitica* from H. Hartmann, both from the Department of Environmental Biology, University of Guelph, Ontario.

Culture methods and induction of resistance. The fungi were grown on V-8 agar containing 180 ml V-8 juice (clarified by centrifuging for 10 min at 4,100 *g*), 3 g $CaCO_3$, and 16 g agar per liter. *P. capsici* readily produced zoospores on this medium when young mycelium was placed 25 cm below two 40 W cool-white fluorescent light tubes for 2 days and subsequently flooded with water. Zoospore suspensions containing 200,000–350,000 zoospores in 5 ml sterile water per petri dish were placed 20 cm below an 11 W 254 nm UV lamp and irradiated for 3 min. Previous experiments indicated that 30–40% of the spores survived this treatment. The irradiated spores were incubated in liquid V-8 medium (200 ml clarified V-8 juice and 3 g $CaCO_3$ per liter) containing 250 μg metalaxyl per milliliter, a concentration that inhibits growth of sensitive mycelium of *P. capsici* by 90%. After 5 days, well-developed colonies were transferred to V-8 agar.

P. ultimum does not produce zoospores and therefore young growing mycelium was used for irradiation studies. Both *P. capsici* and *P. ultimum* were transferred by placing agar disks, taken from colony margins, 3 cm apart on V-8 agar plates. The new fungal colonies were allowed to grow to 2 cm in diameter. The plates were then irradiated for 35 min with UV under the same conditions as described before, after which the colonies were excised and placed inverted on agar containing the test fungicide at 2.5 $\mu g \cdot ml^{-1}$ for *P. ultimum* and 300 $\mu g \cdot ml^{-1}$ for *P. capsici*. Subsequently, isolations were made from mycelium growing on the amended medium and

sensitivity to the fungicides was determined by a modified agar strip technique developed by Edgington et al (5).

To study induction of resistance in the biotrophic parasite *P. parasitica*, the causal agent of downy mildew of crucifers, spores were produced on infected cabbage seedlings (*Brassica oleracea* var. *capitata* 'Copenhagen Market Early'). A spore suspension containing 2×10^7 spores in 20 ml water was irradiated in thin layers in petri dishes for 20 min with UV light and sprayed on 5,000 5-day-old cabbage seedlings, previously treated with an average of 1 ng metalaxyl per seedling. As control, 0.7×10^7 nonirradiated spores in 7 ml of water were inoculated on 1,000 treated seedlings. The seedlings were grown in 27 × 19 × 10-cm clear plastic boxes, each containing about 1,000 seedlings. In a second experiment, a suspension containing 8.5×10^7 spores was irradiated for 10 min and sprayed on seedlings treated with 10 ng metalaxyl per plant. The plants were kept at 15 C during a 16-hr light period and at 11 C for an 8-hr dark period, with high relative humidity.

Determination of stability of resistant mutants. All isolates obtained in vitro after UV irradiation of zoospores or mycelium were subcultured for a few transfers on metalaxyl-amended V-8 agar and thereafter for at least 12 transfers on either V-8 agar with up to 300 μg metalaxyl per milliliter or V-8 agar without any fungicide amendment. After 12 such transfers, during 8 mo, sensitivity to metalaxyl and related fungicides was again evaluated. The isolates were also screened in vivo for pathogenicity and sensitivity to metalaxyl.

Pathogenicity tests and in vivo sensitivity to metalaxyl. Pathogenicity of *P. ultimum* was tested on pea (*Pisum sativum* 'Improved Laxton's Progress') by planting pea seeds in infested soil. A peat-soil-perlite mixture (1:1:1, v/v) was sterilized and a 3-cm layer was placed in 52 × 26 × 5-cm plastic flats. After 2 days, the soils were infested with the strains of *Pythium* by mixing finely chopped mycelium from two petri dish cultures per flat through the soil. The fungal inoculum was allowed to colonize the soil for 3 days at room temperature before the seeds were planted.

Pea seeds were surface sterilized for 10 min in 5.5% NaOCl and 95% ethanol (1:1, v/v), rinsed three times with sterile water and blotted dry with filter paper. They were put in Erlenmeyer flasks and treated by shaking with Ridomil 50WP, 1WP, or 0.1WP until they were evenly covered. The rates of fungicide were 0, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, and 30 g a.i. per 100 kg seed (100 kg is the amount of seed necessary to plant 1 ha). The 1WP and 0.1WP were prepared by diluting Ridomil 50WP with inert powder obtained from Ciba-Geigy. Ten rows were planted, 15 seeds per row, in each flat, including two rows of untreated seed. After 1 wk, the number of emerged plants per row was counted.

Pathogenicity of *P. capsici* was tested on pepper (*Capsium frutescens* 'Vinedale'). Pepper seedlings were grown for 4 wk in flats of peat-soil-perlite mixture and then transplanted, three plants per 10-cm-diameter plastic pot containing 0.33 kg of the soil mix. Three days after they were transplanted, the seedlings were treated with 10 ml of either a metalaxyl solution or water per pot as a soil drench. Three days after metalaxyl treatment, zoospore suspensions of the strains of *P. capsici* were poured along the stems of the young pepper plants, when they were 3–5 cm high. Pathogenic strains caused darkening and constriction of the crown or stem within 4 days of inoculation, followed by a rapid collapse of the entire plant.

Cross-resistance. Cross-resistance to other acylalanine-type compounds (furalaxyl, Galben, milfuram, RE26745, and RE26940) was tested after 12 transfers of the mutants on unamended or metalaxyl-containing V-8 agar.

Determination of variation in sensitivity to metalaxyl between single-zoospore cultures of *Phytophthora capsici*. *P. capsici* mutant Z9⁻ which was obtained after 3 min of UV irradiation of zoospores, and subsequently transferred 18 times on V-8 agar without fungicide, was shown to have an intermediate level of resistance to metalaxyl. This isolate was transferred to a glucose-water agar, containing 15 g agar and 5 g glucose per liter distilled water, and after 2 days, eight hyphal tips ranging ~0.5–1.0 mm long were isolated from the sparsely growing mycelium. Colonies that developed from these tips were cultured on V-8 agar and assessed for sensitivity to metalaxyl. In turn, single zoospores from these colonies were isolated and the colonies that developed from them were assessed for sensitivity to metalaxyl.

RESULTS

Induction of resistance to metalaxyl. Following 3 min of UV irradiation of 3×10^6 zoospores of *P. capsici*, 12 isolates (strains Z1 to Z12) were obtained that grew relatively well on V-8 agar containing 100 μg metalaxyl per milliliter. None of 10^6 nonirradiated zoospores produced a resistant isolate. Irradiation of 80 small colonies of *P. capsici* resulted in 20 resistant strains and irradiation of 50 colonies of *P. ultimum* produced 15 resistant strains. Two of 10 nonirradiated colonies of *P. capsici* also produced some growth. In certain cases, growth of resistant mycelium clearly originated from a single hypha. Upon initial isolation most of the strains tested for metalaxyl sensitivity exhibited poor and irregular growth with EC_{50} values only five to 20 times higher than those of the parent strain. An example is *P. ultimum* mutant II[b] (Fig. 1). Just after isolation this mutant had an EC_{50} of 0.1, only 10 times higher than the parent strain, but it produced a growth response slope 30% less steep than that of the parent strain. Thus, the mutant could grow on 30 μg ml^{-1} while 1 μg ml^{-1} was lethal to the parent. The viability of several mutants was so low that they were lost during the subsequent transfer period.

TABLE 1. Stability of UV-induced metalaxyl-resistant mutants of *Pythium ultimum*

P. ultimum strain[y]	EC_{50} of metalaxyl after 12 transfers on V-8 agar[x]: Without fungicide (μg · ml^{-1})	With 100 μg·ml^{-1} metalaxyl (μg · ml^{-1})
Parent strain	0.01[z]	
Mutant II[a]	400	500
II[b]	400	500
II[c]	10	500
VII	0.02	300
IX	2	400
XV	0.25	800

[x] EC_{50} is the concentration that inhibits fungal growth by 50%.
[y] Mutation was induced by 35 min of UV irradiation of mycelium.
[z] Each value is the mean of three replicate cultures.

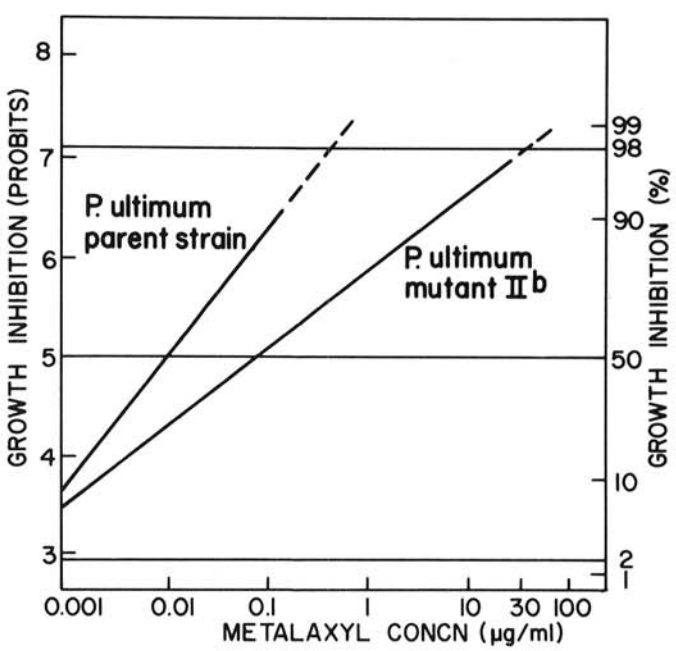

Fig. 1. Sensitivity of *Pythium ultimum* mutant II[b] to metalaxyl in comparison to that of the parent strain, determined shortly after emergence of the mutant.

Five days after inoculation with both untreated and irradiated spores of *P. parasitica* the cabbage seedlings treated with 1 ng metalaxyl per plant developed many dark, pinpoint lesions characteristic of penetration by *P. parasitica* and some of these showed sporulation. However, the spores produced did not infect cabbage seedlings that were treated with a dose of metalaxyl 10 times higher than the maximum dose the parent strain could tolerate. In the second experiment, lesions on none of the 6,000 inoculated cabbage seedlings treated with 10 ng metalaxyl per seedling had sporulated.

Stability of resistant mutants. After 12 transfers on metalaxyl-amended agar, all surviving mutants of *P. ultimum* had high degrees of resistance to metalaxyl with EC_{50} values about 50,000 times higher than that of the parent strain (Table 1). Similarly, strains of *P. capsici* were obtained with EC_{50} values 500 times higher than that of the parent strain (Table 2). The mutants kept on V-8 agar without fungicide, differentiated into either resistant strains that retained a high level of resistance during 12 successive transfers on agar without fungicide (*P. ultimum* mutants II^a and II^b, *P. capsici* mutants Z5, Z8, and IV) or into strains that lost their resistance partially or entirely.

Pathogenicity of resistant mutants. Most of the metalaxyl-resistant mutants of *P. ultimum* tested in vivo were still pathogenic on pea and infected pea seeds dressed with relatively high amounts of metalaxyl (Table 3). In soil infested with the parent strain only 8% of the untreated seedlings emerged. As the rate of metalaxyl applied as seed dressing was increased logarithmically to 1 g per 100 kg seed, the amount of disease decreased to zero. Seeds planted in soil infested with the mutants II^b and II^{c+} (II^c grown for 12 transfers on agar containing 100 $\mu g\ ml^{-1}$ metalaxyl) exhibited very poor emergence, even when the seeds had been treated with up to 30 g of metalaxyl per 100 kg. The mutant II^{c-} gave an intermediate response (Table 3). The metalaxyl sensitivity of strains of *P. ultimum* in vivo was closely related to their sensitivity in vitro (Tables 1 and 3).

Some mutants of *P. capsici* lost, while other retained, pathogenicity to pepper. The reaction of pepper plants, untreated or treated with different doses of metalaxyl, to inoculation of those strains, is summarized in Table 2.

Cross-resistance. Resistance to metalaxyl in *P. ultimum* was coupled with resistance to the other acylalanine fungicides: furalaxyl, Galben, milfuram, RE26745, and RE26940 (Table 4). Resistance to metalaxyl in *P. capsici* was only coupled with resistance to furalaxyl and RE26745, but not to Galben, milfuram, and RE26940, since the latter compounds initially were not very toxic to the *P. capsici* parent strain.

Variation in sensitivity to metalaxyl among single-zoospore cultures of *P. capsici*. Colonies grown from hyphal tips of *P. capsici* $Z9^-$, an isolate with an intermediate level of resistance to metalaxyl (Table 2), varied slightly in sensitivity to metalaxyl, while colonies grown from single zoospores, produced from these tips, varied widely in metalaxyl sensitivity (Table 5).

TABLE 2. Stability, pathogenicity, and sensitivity in vivo of UV-induced mutants of *Phytophthora capsici* to metalaxyl

	Sensitivity to metalaxyl after 12 transfers on V-8 media containing:			
	No fungicide		100 $\mu g \cdot ml^{-1}$ metalaxyl	
P. capsici strain	In vitro EC_{50}[v] ($\mu g \cdot ml^{-1}$)	In vivo EC_{50}[w] ($mg \cdot kg^{-1}$)	In vitro EC_{50} ($\mu g \cdot ml^{-1}$)	In vivo EC_{50} ($mg \cdot kg^{-1}$)
Parent strain	0.8	< 5		
Mutant[y] A	0.2	< 5	620	>30[x]
K	0.35	< 5	480	10
L	1	NP[z]	330	NP
M	0.7	< 5	500	20
III	1	10	500	>30
IV	260	>30	300	>30
Z5	400	< 5	600	>30
Z7	1	< 5	600	>30
Z8	440	< 5	500	>30
Z9	20	< 5	500	>30

[v] Fungicide concentration in agar that inhibits fungal growth for 50%.
[w] Fungicide concentration in soil that provides 50% disease control.
[x] 15 mg of metalaxyl per kilogram of soil caused slight, and 30 mg severe, phytotoxic symptoms on pepper plants, curling leaves and necrotic leaf margins.
[y] Mutants A to IV obtained from 35 min UV irradiation of mycelium, mutants Z5 to Z9 from 3 min UV irradiation of zoospores.
[z] NP = not pathogenic.

TABLE 3. Control of parent and mutant strains of *Pythium ultimum* with metalaxyl seed treatment of peas planted in infested soil

Strain of *P. ultimum* as soil inoculum	Metalaxyl in transfer-media[y]	Diseased plants after metalaxyl treatment (%)[z] Seed treatment g a.i. 100 kg^{-1} seed								
		0	0.01	0.03	0.1	0.3	1	3	10	30
Parent strain	−	92	85	62	15	15	0	0	0	0
Mutant II^b	0	85	77	62	77	46	0	15	15	8
	−	77	92	77	85	77	92	85	100	85
	+	100	100	100	100	100	100	100	100	100
Mutant II^c	−	92	100	100	92	62	31	8	0	0
	+	92	92	100	100	85	100	92	100	100

[y] Strains initially isolated (0) or after 12 transfers on media with (+) or without (−) metalaxyl in agar media.
[z] Based on average numbers of plants emerged out of 15 seeds (having a germination rate of 85%).

DISCUSSION

Exposure of *P. ultimum* and *P. capsici* to UV resulted in emergence of a number of strains with decreased sensitivity to metalaxyl. This agrees with research by Davidse (3) who obtained metalaxyl-resistant mutants of *Phytophthora megasperma* f. sp. *medicaginis* after treatment of zoospores with the mutagenic agent *N'*-nitro-*N*-nitroso-guanidine (NG); about half of the NG-induced mutants were as virulent on alfalfa seedlings as the parent strain and some were able to kill seedlings in the presence of 20 mg metalaxyl per kilogram soil applied as a soil drench. In our experiments, irradiation of zoospores and mycelium resulted in the development of mutants that were resistant, stable, and still pathogenic. Irradiation of 1.05×10^8 conidia of *P. parasitica*, however, yielded no resistant mutants in the in vivo tests. The failure of certain biotrophic fungi to develop resistance to acylalanine fungicides was reported by Staub et al (10) for *P. infestans* on tomato. They found that isolates of *P. infestans* in vitro easily adapted to high doses of metalaxyl, but concluded that this kind of resistance was not related to in vivo resistance. The in vivo mode of action of acylalanine-type fungicides, therefore, is more complicated than that discerned in vitro, and may involve induction of hypersensitive reactions in plants, accompanied by accumulation of phytoalexins. Such a mechanism was indicated by Ward et al (13). They found that a compatible interaction between a race of *Phytophthora megasperma* var. *sojae* and soybean changed to an incompatible interaction in the presence of metalaxyl, resulting in reduced lesion size and accumulation of the phytoalexin, glyceollin. Metalaxyl alone, without inoculation, did not stimulate glyceollin accumulation, nor did the compatible race by itself.

The observation that isolates of *P. infestans* that were resistant to metalaxyl in vitro (10), and even were stimulated in growth by metalaxyl concentrations up to 1 $\mu g\ ml^{-1}$ (2) but unable to infect plant tissue containing as low a concentration as 0.02 μg metalaxyl per gram tissue, indicates that direct inhibition of fungal growth is unlikely to be the mechanism of action of metalaxyl in vivo for these isolates.

In the present work, the resistant mutants were initially only slightly less sensitive to metalaxyl than their parent strains (Fig. 1) and exhibited irregular growth in vitro and intermediate resistance in vivo (*P. ultimum* mutant II^{b0} in Table 3). The mutants apparently needed time to become stabilized, which possibly gives some indication of the genetic evolution of the strains. In this respect we

TABLE 4. Cross-resistance of UV-induced metalaxyl-resistant mutants of *Pythium ultimum* and *Phytophthora capsici* to related fungicides

Fungal strain	Transfer history	EC_{50} in $\mu g \cdot ml^{-1}$[w]					
		Metalaxyl	Furalaxyl	RE26745	RE26940	Milfuram	Galben
P. ultimum							
Parent strain		0.01	0.2	0.03	0.3	0.4	4.5
Mutant[x] II^{b-y}	(30)[z]	400	175	300	160	300	200
II^{b+}	(28)	500	190	330	160	320	200
II^{c-}	(38)	0.15	0.03	0.2	0.2	0.02	2
II^{c+}	(28)	500	230	370	230	400	260
P. capsici							
Parent strain		0.8	10	30	200	200	150
Mutant IV^-	(26)	260	80	290	180	230	500
IV^+	(21)	225	105	300	220	800	200
$Z5^-$	(27)	400	30	100	110	110	60
$Z5^+$	(24)	600	85	200	180	130	250
$Z7^-$	(20)	1	1.2	1	130	115	200
$Z7^+$	(26)	600	200	330	170	400	250
$Z8^-$	(26)	440	50	160	180	170	75
$Z8^+$	(25)	500	60	390	225	360	160
$Z9^-$	(26)	20	30	125	250	180	70
$Z9^+$	(27)	500	90	250	190	250	125

[w] Concentration of fungicide in agar that inhibits fungal growth for 50%.
[x] Mutants II^b, II^c, and IV were obtained by 35 min UV irradiation of mycelium, mutants Z5 to Z9 by 3 min UV irradiation of zoospores.
[y] Mutant maintained on V-8 agar without (−), or amended with (+) 100 $\mu g \cdot ml^{-1}$ metalaxyl.
[z] Numbers in parentheses indicate the number of transfers the isolate went through before EC_{50}s were determined.

TABLE 5. Sensitivity of single-zoospore colonies of *Phytophthora capsici* mutant $Z9^-$ to metalaxyl

Isolate of *P. capsici* $Z9^-$	$EC_{50}(\mu g \cdot ml^{-1})$[y]	
P. capsici $Z9^-$ (18)[z]	90	
hyphal tip a	450	
zoospore colony a1		32
a2		25
a3		0.1
a4		0.1
a5		0.1
hyphal tip b	130	
zoospore colony b1		3
b2		70
b3		1
b4		3

[y] Each value is the mean of three replicate cultures.
[z] Grown after emergence for 18 transfers on fungicide-free V-8 media.

should consider a coenocytic colony of *Pythium* or *Phytophthora* not as one entity, but as a population of many nuclei. If one of the nuclei becomes 'resistant' to metalaxyl, that nucleus has an advantage over the others in the presence of the fungicide and may permit the associated hypha to grow. After 12 transfers of the different mutants on medium with or without the fungicide, the different paths along which mutants can evolve were shown: a number of mutants did not survive; others reverted completely to the parental strain level of sensitivity; some only partially reverted (*P. ultimum* II^c, IX, and *P. capsici* Z9); and some retained a highly stable resistance in absence of the fungicide (*P. ultimum* II^a, II^b, *P. capsici* Z5, Z8, and IV). The large differences in metalaxyl sensitivity between isolates of *P. capsici* grown from single zoospores (Table 5) indicate that the intermediate level of sensitivity of the original isolate *P. capsici* $Z9^-$ was due to differences in metalaxyl sensitivity of its component nuclei. Thus, nuclei of different genotypes may exist together in the coenocytic mycelium.

From Table 3 it is evident that the mutant *P. ultimum* II^b is at least as pathogenic as the parent strain. This together with the observation of Reuveni et al (8) that the metalaxyl-resistant strain of *P. cubensis* was not less pathogenic on cucumber than the parent strain, suggests that isolates resistant to metalaxyl may readily become established in the field. Comparison of Tables 1 and 3 shows a close correlation between sensitivity in vitro and in vivo. The same correlation holds for *P. capsici* on pepper (Table 2), where high resistance in vivo always was expressed by mutants with high resistance in vitro.

The observed cross-resistance to the related fungicides furalaxyl, RE26745, RE26940, milfuram, and Galben supports the hypothesis that all these acylalanine-type fungicides have the same mode of action.

In the present study induction of resistance in the biotrophic parasite *P. parasitica* could not be established with the limited number of spores that were used. In contrast, certain species of *Phytophthora* and *Pythium* readily developed resistance to metalaxyl when exposed to UV irradiation, without losing virulence or pathogenicity. Thus, development of metalaxyl-resistant strains in the field is not only possible, but likely to occur in these fungal species.

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