Resistance to Mefenoxam and Metalaxyl Among Field Isolates of *Phytophthora capsici* Causing Phytophthora Blight of Bell Pepper

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ABSTRACT

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Incidence of Phytophthora blight in bell pepper fields that were sprayed for the first time with Ridomil Gold (mefenoxam) according to labeled recommendations was higher in North Carolina in 1997 than in previous years. Mefenoxam is the more active enantiomer contained in the racemic fungicide metalaxyl. A total of 150 isolates were obtained from 17 fields at eight grower locations. Among isolates from all locations, 30% were classified as sensitive, 10% as intermediate, and 59% were resistant to mefenoxam. Mefenoxam-resistant isolates were found in 82% of the fields sampled (14 of 17 fields). The proportion of resistant isolates in individual fields ranged from 28 to 100%. The mean effective concentration (EC_{50}) values for mefenoxam-sensitive isolates was 0.568 μg ml^{-1} (ranging from 0.12 to 1.1 μg ml^{-1}), whereas the mean EC_{50} value for mefenoxam-resistant isolates was 366.5 μg ml^{-1} (ranging from 3 to 863 μg ml^{-1}). The mean EC_{50} value for metalaxyl-sensitive isolates was 0.27 μg ml^{-1} (ranging from 0.00002 to 1.3 μg ml^{-1}) and for metalaxyl-resistant isolates was 470.34 μg ml^{-1} (ranging from 10 to 966 μg ml^{-1}). The greatest proportion of resistant isolates came from fields where mefenoxam was used alone rather than in combination with other fungicides. Both mating types were found among resistant isolates, suggesting that these isolates may persist in soil in subsequent years. Field isolates of *Phytophthora capsici* resistant to mefenoxam on pepper have not been reported previously and now pose new challenges for management of this important disease.

Additional keywords: epidemiology, fungicide resistance, fungicide sensitivity, Phytophthora root and crown rot

Phytophthora blight, caused by the oomycete plant pathogen *Phytophthora capsici*, is an important disease of bell pepper and cucurbit crops and causes significant economic loss. The incidence of the disease has increased in recent years in the United States and worldwide (19,35). *P. capsici* can infect all parts of the plant and the pathogen can be dispersed within the soil, with surface water, and via water splashing from the soil to foliage (36). The pathogen has both a sexual and asexual life cycle, thus making disease management difficult. Disease is often spatially aggregated in fields and initial disease foci can quickly initiate new foci via pathogen spread in surface water (36). Management of *P. capsici* currently relies on modifications in cultural practices, crop rotation, and judicious use of selective fungicides (35).

The phenylalamide fungicide metalaxyl (trade name: Ridomil; Syngenta, Greensboro, NC) provides systemic protection against oomycete pathogens (7,8,40). Metalaxyl was introduced in 1977 and provided excellent control of Phytophthora diseases (40). Its benefits included systemic activity, which enabled growers to extend spray intervals when used alone in disease management programs (38). Metalaxyl has been used extensively for control of many different oomycete pathogens, including *P. infestans*, *Peronospora tabacina*, and *Bremia lactucae* (31). The intensive use of metalaxyl led to the rapid selection for metalaxyl-resistant strains of *Phytophthora infestans* in Europe within 1 year of its introduction (10,13,38). This was primarily due to the use of metalaxyl as a curative on large populations of *P. infestans* (30). In the United States, isolates of *P. infestans* resistant to metalaxyl were first reported in Washington State in 1991 (11,12). Isolates of *P. infestans* resistant to metalaxyl also have been recovered from nontreated fields, demonstrating the importance of migration of resistant isolates of *P. infestans* in disease spread (6,16,18,23). Metalaxyl resistance has been reported among many oomycete pathogens, including *Plasmopara viticola, Pseudoperonospora cubensis*, *Peronospora tabacina*, *B. lactucae*, and *Pythium* spp. (31). As a result, metalaxyl was removed from some markets until new management strategies were deployed, including its use in combination with other fungicides (38).

Metalaxyl historically has been used to control the soil and crown (stem) rot phase of Phytophthora blight on bell pepper (20,21,28,32,36,39). In the early 1990s, metalaxyl received a section 3 Federal registration for the control of Phytophthora blight of peppers in the United States. Recently, metalaxyl was replaced with mefenoxam (trade name: Ridomil Gold; Syngenta), the active enantiomer contained in the racemic fungicide metalaxyl. The fungicide is applied at frequencies similar to those used with metalaxyl, but at lower rates. In 1997, mefenoxam was widely used for the first time in field production of bell peppers in North Carolina.

Resistance of isolates of *Phytophthora capsici* to metalaxyl has been induced in laboratory studies by chemical mutagenesis, ultraviolet irradiation, or exposure to sublethal concentrations of metalaxyl (1,3–5). Metalaxyl-resistant isolates of *P. capsici* that were cross-resistant in in vitro studies to other phenylamide fungicides also have been observed. However, cross-resistance has not been observed in this pathogen to different classes of fungicides (1,3). Adaptive resistance of isolates of *P. capsici* to metalaxyl was demonstrated in vitro after successive transfer on metalaxyl-amended media, but some isolates lost their insensitivity after successive transfer on media amended with sublethal concentrations of metalaxyl (4). It was suggested that sublethal exposure of propagules to the compound in soil might occur. Based on these studies and the documented cases of field resistance to metalaxyl in other oomycetes, it was predicted that field resistance to metalaxyl by *P. capsici* was likely (5). Despite results from laboratory studies, development of fungicide resistance in *P. capsici,* which has an important soilborne phase, has been slower to develop than in species of *Phytophthora* that are primarily dispersed aerially and infect foliage.

Production of peppers in the Southeast can require frequent fungicide applications to ensure a quality harvest and, therefore, greatly increase the risk of resistance development. In the spring and summer of

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1997, an alarming increase in Phytophthora blight occurred in North Carolina bell pepper fields that were sprayed with mefenoxam. Many of the growers whose fields were affected had applied mefenoxam for the first time in 1997 for the management of Phytophthora blight. High disease severity in fungicide-treated fields indicated the possibility of mefenoxam resistance in field populations of *P. capsici* in North Carolina and elsewhere (25,26,29). The objectives of this research were to collect isolates of *P. capsici* from fields where disease control failures had occurred in North Carolina and New Jersey and to determine the in vitro sensitivity of these isolates to mefenoxam. In addition, the mating type and pathogenicity of the isolates were examined. Evaluation of the metalaxyl sensitivity of isolates of *P. capsici* collected in the same pepper-growing region of North Carolina prior to 1997 was also conducted. A preliminary report of this research has been published (33).

MATERIALS AND METHODS

Culture collection and maintenance. Plants infected with *P. capsici* were collected from bell pepper fields at 13 locations among seven farms in North Carolina (Table 1). In addition, Stephen Johnston (Rutgers University, Bridgeton, NJ) provided diseased plants from four additional fields from one grower's farm in New Jersey. Initially, isolations from fields in North Carolina were from a small number of random samples submitted by county extension agents and university extension faculty. Data from our preliminary screen indicated that several isolates of *P. capsici* were highly resistant to metalaxyl (Ridomil 2E, 240 mg a.i./ml). Subsequently, six of the North Carolina fields were sampled more extensively, and 50 or more plants were collected from each site (Table 1). Data from the combined collections are shown in Table 1.

Sections of plant stem tissue with visible black lesions were surface disinfested in a 0.05% NaOCl solution for 1 min, rinsed three times in sterile distilled water, and plated onto Kannwisher Mitchell agar amended with 50 μg ml^{-1} hymexazol (24). Petri dishes were incubated for 5 to 7 days at 24°C in the dark and colonies with growth characteristic of *P. capsici* were transferred to clarified V8 juice agar (200 ml of V8 clarified juice, 800 ml of deionized water, and 17 g of agar). V8 juice was clarified by filtration through a Whatman number 4 filter paper after the addition of 2 g of $CaCO_3$, followed by centrifugation at 4,340 × *g* for 10 min. The pathogen was identified based on colony morphology and sporangial characteristics.

Polymerase chain reaction identification of isolates. A subset of the isolates was tested by polymerase chain reaction (PCR) to confirm identification as *P. capsici*. DNA was extracted from mycelium of the isolates by a NaOH method (42). DNA was also extracted from 16 previously identified isolates of *P. capsici* in our collection for comparison (37). DNA was amplified via PCR utilizing the *P. capsici* specific primer, PCAP. The PCAP primer, in combination with the universal primer ITS1, amplifies an approximately 172-bp fragment of ribosomal DNA from isolates of *P. capsici* (37). PCR products were separated by gel electrophoresis on 2% agarose gels. Product size was determined by comparison with a molecular size standard included in each gel.

Fungicide sensitivity and EC_{50} assays. Thirty-five isolates of *P. capsici* collected prior to 1997 were assayed for sensitivity to metalaxyl. These isolates were collected from pepper, tomato, pumpkin, eggplant, cucumber, and squash (Table 2). Stock cultures from the isolates were grown on V8 juice agar for 10 days in ambient light. Agar disks (6 mm in diameter) were removed from actively growing margins of the cultures and transferred to clarified V8 juice agar media previously amended with metalaxyl (Ridomil 2E, 240 mg a.i. ml^{-1}) at 0, 0.1, 1.0, 5.0, 10.0, or 100 μg ml^{-1}. The fungicide (emulsifiable concentrate) was diluted in sterile water before adding it to the autoclaved media. Three replicate petri dishes per fungicide level were tested for each isolate. Dishes were incubated at 24°C for 7 to 10 days in constant light. Colony diameters of *P. capsici* were measured in two directions for each individual dish, averaged, and compared to average colony diameters from non-amended media. The percent growth of each isolate relative to the nonamended control was plotted against the $\log_{10}$ of the metalaxyl concentration. The effective concentration (EC_{50}) for each isolate was calculated. Analysis involved fitting regression lines for isolate radial growth values expressed as a percentage of the nonamended controls plotted against $\log_{10}$ of the metalaxyl concentration for each isolate. The point on the regression line at which 50% of the isolate growth was inhibited is the ED_{50} value. Statistical analysis was conducted with the Statistical Analysis System (SAS Institute, Inc., Cary NC) as described below.

Table 1. Sensitivity of isolates of *Phytophthora capsici* collected in 1997 from bell pepper fields in North Carolina and New Jersey to the fungicide mefenoxam

		Number collected		Number of isolates[a]				
Farm, county or state	Field	Plants	Isolates	Sensitive	Intermediate	Resistant	Resistant (%)	Fungicide[b]
1, Wilson, NC	1	50	28	12	6	10	36	RG
2, Sampson, NC	1	62	13	4	2	7	53	R2E, M/Cu
	2	54	8	3	2	3	38	RG
	3	62	20	6	2	12	60	MBR, RG
	4	7	4	2	0	2	50	RG
3, Sampson, NC	1	60	26	5	0	21	81	RG
	2	57	15	1	0	14	93	RG
4, Sampson, NC	1	7	2	2	0	0	0	Chl, R2E
	2	8	5	3	0	2	40	RG/Cu
	3	1	1	1	0	0	0	RG
5, Sampson , NC	1	1	1	0	0	1	100	RG
6, Greene, NC	1	8	7	4	1	2	29	RG 3×
7, Sampson, NC	1	1	1	1	0	0	0	No RG
8, New Jersey	1	3	3	0	0	3	100	RG
	2	1	1	0	0	1	100	RG
	3	5	5	0	1	4	80	RG
	4	10	10	2	0	8	80	RG
Total	17	397	150	46	14	90	…	…

[a] Isolates were characterized as sensitive to mefenoxam if colony growth at 5 μg ml^{-1} was less than 40% of the isolate's growth on the nonamended media. Intermediate isolates exhibited growth greater than 40% of the nonamended media control at 5 μg ml^{-1}, but less than 40% of the nonamended media control with mefenoxam at 100 μg ml^{-1}. Resistant isolates exhibited growth greater than 40% of the nonamended media control with mefenoxam at 100 μg ml^{-1}.

[b] Use history during season: RG = Ridomil Gold (mefenoxam), R2E = Ridomil 2E (metalaxyl), M/Cu = Maneb/copper, MBR = methyl bromide/Chloropicrin), Chl = Chloropicrin.

In all, 150 isolates collected during the summer of 1997 were evaluated for resistance to mefenoxam (Table 1). Isolates were grown on clarified V8 juice agar for 10 days at 24°C in ambient light prior to use. Clarified V8 juice agar was amended with mefenoxam (Ridomil Gold EC, 480 mg a.i. ml^{-1}) at 0, 5, and 100 μg ml^{-1} (11). Solutions of the fungicide were prepared in sterile water prior to amendment of the agar media. Agar disks from each isolate were transferred onto two replicate petri dishes of media amended with mefenoxam at each of the three fungicide concentrations. The dishes were incubated at 24°C in ambient light for 7 to 10 days. Colony growth was determined by measuring two colony diameters for each replicate dish and calculating the mean diameter for each isolate. The experiment was repeated two times for each isolate.

Isolates were characterized as sensitive if colony growth on media amended with 5 μg ml^{-1} of metalaxyl or mefenoxam was less than 40% of the isolates' growth on nonamended media. Intermediate isolates exhibited growth on media amended with 5 μg ml^{-1} greater than 40% of that on nonamended media, but growth on media amended with 100 μg ml^{-1} less than 40% of that on nonamended media. Resistant isolates had growth with mefenoxam at 100 μg ml^{-1} greater than 40% of that on nonamended media.

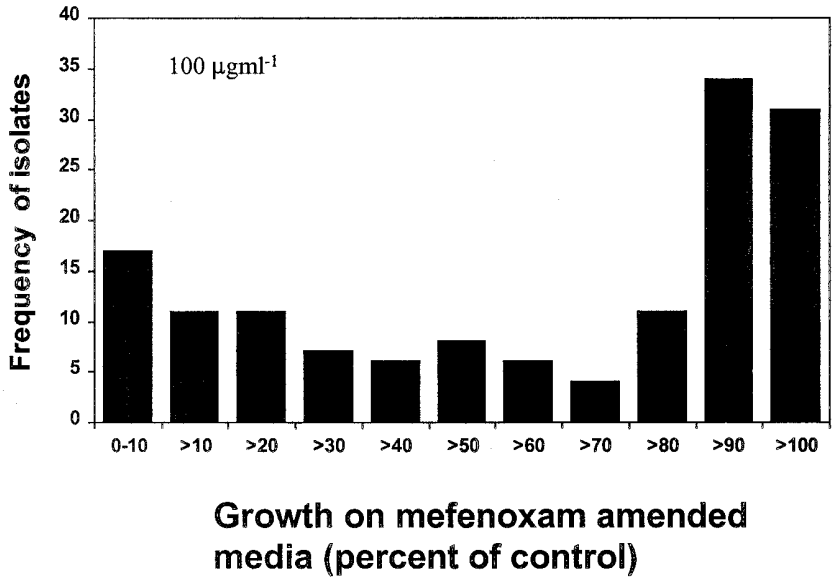

Fig 1. Frequency of mefenoxam resistance in isolates of *Phytophthora capsici* collected in 1997 in 13 locations in North Carolina and four locations in New Jersey. Isolates were grouped according to percentage of growth on mefenoxam-amended media at 100 μg ml^{-1} relative to nonamended control. Isolates with intermediate resistance had growth with mefenoxam at 100 μg/ml less than 40% of that on nonamended media. Resistant isolates had growth with mefenoxam at 100 μg/ml greater than 40% of that on nonamended media.

Table 2. Isolate designation, county, state, host, year of isolation, mating type and effective concentration (EC_{50}) value on metalaxyl-amended media of isolates of *Phytophthora capsici* collected from pepper, cucumber, squash, pumpkin, eggplant, and tomato between 1987 and 1996

					EC_{50} (μg ml^{-1})	
Isolate number, name	**County, state**	**Host**	**Year isolated**	**Mating type**	**Mean**	**Range[a]**
18 Fruit	Sampson, NC	Pepper	1989	A1	0.78	0.54–1.39
19 War 1	Sampson, NC	Pepper	1989	A1	1.34	1.11–1.7
20 War 2	Sampson, NC	Pepper	1989	A1	0.17	0.17–0.17
21 RT 403#1	Sampson, NC	Pepper	1989	A1	0.60	0.53–0.7
22 RT 403 #2	Sampson, NC	Pepper	1989	A1	1.20	0.86–1.96
23 B&W #1	Sampson, NC	Pepper	1989	A1	0.97	0.89–1.06
25 King-1	Sampson, NC	Pepper	1990	A1	0.67	0.53–0.94
26 King-2	Sampson, NC	Pepper	1990	A1	9.15	7.62–11.45
27 King-3	Sampson, NC	Pepper	1990	A1	0.88	NV
29 D. Wilson-1	Sampson, NC	Pepper	1990	A1	1.32	1.15–1.53
30 Ruff 6-6	Polk, NC	Pepper	1991	A1	2.09	1.36–4.48
31 Ruff 2-15	Polk, NC	Pepper	1991	A1	0.74	0.58–1.04
32 Ruff 7-1	Polk, NC	Pepper	1991	A1	1.19	0.066–5.62
33 Ruff 5-4	Polk, NC	Pepper	1991	A1	0.73	0.65–0.83
34 1CA	California	Tomato	1986	A2	0.77	0.74–0.81
35 Tom2	Henderson, NC	Tomato	1990	A2	0.39	0.32–0.51
36 Tom3	Henderson, NC	Tomato	1990	A2	2.94	2.18–4.51
37 Tom4	Henderson, NC	Tomato	1990	A1	0.19	NV
38 Tom5	Henderson, NC	Tomato	1990	A2	1.95	1.71–2.27
39 Pump1	California	Pumpkin	1986	A2	0.17	NV
40 Pump2	Wake, NC	Pumpkin	1990	A2	0.76	0.55–1.22
41 Pump3	Wake, NC	Pumpkin	1990	A2	0.67	0.55–0.87
42 Flaim	New Jersey	Eggplant	1988	A1	2.58	2.04–3.51
52 Acorn2	Sampson, NC	Squash	1988	A2	0.48	0.41–0.58
55 But2	Sampson, NC	Squash	1988	A1	2.66	2.17–3.45
57 Patti4	Sampson, NC	Squash	1988	A1	0.89	0.78–1.04
59 Spag2	Sampson, NC	Squash	1988	A1	0.53	0.51–0.55
62 Ginseng	Henderson, NC	Ginseng	1991	A2	0.33	0.32–0.34
82 B92-1	Sampson, NC	Pepper	1982	A1	2.51	2.06–3.23
83 B92-2	Sampson, NC	Pepper	1992	A2	0.68	0.54–0.89
84 K92-1	Sampson, NC	Pepper	1992	A1	7.81	6.96–8.88
85 K92-2	Sampson, NC	Pepper	1992	A1	0.73	0.6–0.96
87 B1HB14	Sampson, NC	Pepper	1993	A1	0.70	0.47–1.43
88 B2HH4	Sampson, NC	Pepper	1993	A2	0.53	0.49–0.59
98 CL5-Phy2	Sampson, NC	Cucumber	1994	A2	2.60	2.06–3.54

[a] NV = no variation, values were the same between reps.

lates exhibited growth on media amended with 100 μg ml^{-1} greater than 40% of that on nonamended media (16).

The EC_{50} values for sensitivity to mefenoxam were estimated for six mefenoxam-resistant (isolates numbers 317, 363, 414, 428, 399, and 391) and six mefenoxam-sensitive (isolate numbers 358, 299, 376, 297, 378, and 427) isolates of *P. capsici*. Clarified V8 juice agar was amended with mefenoxam at levels of 0, 0.1, 1.0, 10, 100, and 1,000 μg ml^{-1}. Agar disks from each of the 12 isolates of *P. capsici* were placed onto two replicate plates of the mefenoxam-amended media. The plates were incubated in ambient light at 24°C for 7 to 10 days. Colony growth was determined by measurement of colony diameters in two directions for each replicate petri dish. The experiment was done three times for each isolate. Percent growth was determined relative to the nonamended control for each isolate. The percent growth of each isolate compared to the nonamended control was plotted against log_{10} concentration of mefenoxam. EC_{50} values were calculated for each isolate. The experiment was repeated substituting metalaxyl (Ridomil 2E) for mefenoxam and the EC_{50} values for the same 12 isolates were determined.

EC_{50} values were calculated for each isolate by nonlinear regression using the Statistical Analysis System Software (SAS Institute, Inc.). The log-logistic model (Pr $(y > c) = 1/\{1 + (\lambda * c)^{**} p\}$, where EC_{50} = 1/lambda, provided the best fit of the data for mefenoxam- and metalaxyl-sensitive isolates. The Weibull distribution function (Pr $(y > c) = \exp\{-1 (\lambda * c)^{**} p\}$, where $EC_{50} = [(0.6931)^{**}(1/p)]/\lambda$), provided the best fit of the data for mefenoxam- and metalaxyl-resistant isolates.

Mating type. Mating type was determined for a subset of isolates from each field by pairing each isolate of *P. capsici* with a known A1 (isolate B1BH14) or A2 (isolate B2HH4) tester isolate. All crosses were conducted by

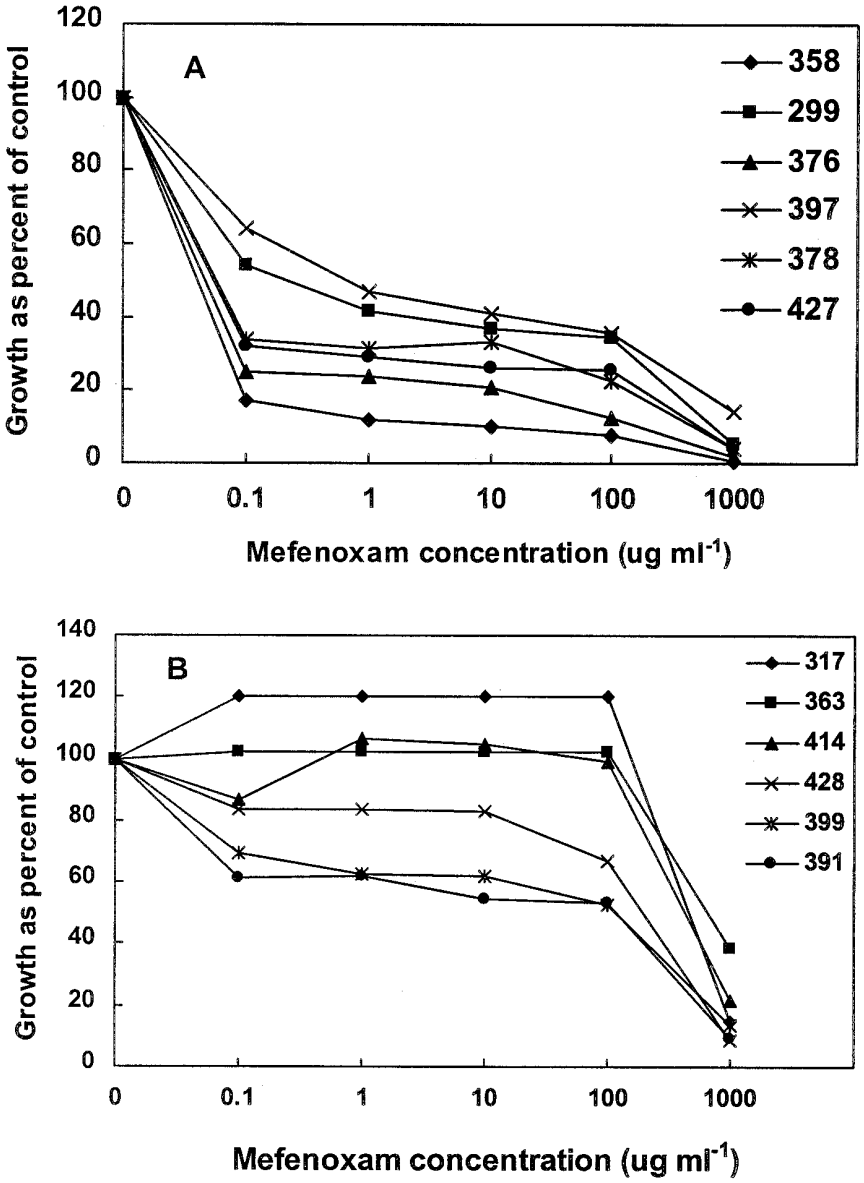

Fig 2. Growth with mefenoxam at 100 μg/ml expressed as a percentage of that on nonamended media versus log_{10} mefenoxam concentration of **A,** six sensitive and **B,** six resistant isolates of *Phytophthora capsici* collected in 1997. Isolates were grown on clarified V8 juice agar amended with mefenoxam at 0, 0.1, 1.0, 10, 100, or 1,000 μg/ml. Each line represents a different isolate

Table 3. Mean effective concentration (EC_{50}) values of six isolates of *Phytophthora capsici* that were sensitive or resistant to mefenoxam and metalaxyl

			Mefenoxam (μg/ml)		Metalaxyl (μg/ml)	
Isolate number[a]	**Isolate name**	**Mating type**	**Mean EC_{50}**	**Range EC_{50}**	**Mean EC_{50}**	**Range EC_{50}[b]**
Resistant						
414	SH A	A1	512	170–840	723.07	551–1050
391	2950	A2	3	0.4–19	10.16	4.18–23.54
317	NJ SE	A2	863	NV[b]	877.94	NV
399	SH(W)A	A1	7	0.2–100	87.34	10.23–170.30
363	TriW2A	A2	787	400–920	966	NV
428	JH(C)E	ND[c]	27	0.5–>1,000	157.53	100.9–352.3
Sensitive						
427	JH(C)G	ND	0.12	0.02–0.29	0.0008	0
299	HAM	A2	1.00	0.4–2.5	0.30	0.13–1.08
378	JH(C)D	A2	0.12	0.07–0.19	0.003	0
376	SH(HS)J	A2	0.94	0.6–1.5	0.0003	0
297	HAM	A2	1.10	0.15–4.1	1.33	0.82–3.53
358	SH(HS)F	A1	0.13	0.08–0.2	0.00002	0

[a] Isolates were previously characterized as sensitive or resistant to mefenoxam on mefenoxam at 0, 5, and 100 μg/ml.
[b] NV = no variation observed.
[c] ND = not described.

placing agar disks removed from 1-week-old cultures of each isolate on clarified V8 juice agar and incubating them for 21 to 28 days in the dark at 24°C. In each test, the absence of oospores at the interface between colonies indicated the same mating type. The positive control was a cross between two tester isolates of opposite mating type, whereas the negative control was a cross between isolates of the same mating type.

RESULTS

PCR identification of isolates. Isolates originally identified as *P. capsici* through the use of selective media, colony, and sporangial morphology were confirmed as *P. capsici* through the use of PCR and the PCAP primer. All of the isolates tested with the PCR technique yielded the expected 172-bp product.

Fungicide sensitivity and EC_{50} values for isolates collected between 1987 and 1994. No isolates of *P. capsici* collected between 1987 and 1994 were resistant to metalaxyl (Table 2). In all, 23 isolates had EC_{50} values between 0.1 and 1.0 μg ml^{-1}, 10 isolates had EC_{50} values between 1.0 and 5.0 μg ml^{-1}, and 2 isolates had EC_{50} values between 5 and 10 μg ml^{-1} (Table 2). These two isolates were classified as intermediate in sensitivity to metalaxyl and came from the same grower field in different years with a history of metalaxyl use.

Fungicide sensitivity and EC_{50} values for isolates collected in 1997. Among 150 isolates of *P. capsici* collected from bell pepper fields in 1997, 30% were classified as sensitive, 10% as intermediate, and 59% as resistant to mefenoxam (Table 1). Mefenoxam-resistant isolates were detected in 82% of the fields sampled (14 of 17 fields) and the percentage of resistant isolates among individual fields ranged from 29 to 100%. Isolates that were sensitive to mefenoxam were also found in most fields sampled, and the percentage of sensitive isolates among individual fields was 7 to 100% (Table 1).

The largest frequency of isolates exhibited growth greater than 90% of the nonamended control on mefenoxam at 100 μg ml^{-1}. (Fig. 1). Sixty-five isolates were highly resistant to mefenoxam and exhibited enhanced growth of 90% or more on mefenoxam-amended media when compared to growth on the nonamended control. Twenty percent of the isolates exhibited enhanced growth of 100% or greater on mefenoxam at 100 μg ml^{-1} (Fig 1).

More plant samples were removed from three of the grower's farms where disease incidence was highest (growers 1, 2, and 3; Table 1). Growers 1, 3, and 8 applied mefenoxam to their fields during the season for management of *Phytophthora* spp., whereas growers 2 and 4 applied a preplant soil fumigant of methyl bromide/chloropicrin or chloropicrin alone, followed by a combination of different fungicides, including metalaxyl (Ridomil 2E), maneb/copper, metalaxyl/copper (Ridomil 2E/copper), or mefenoxam (Ridomil Gold) during the season (Table 1). Highest percentages of mefenoxam-resistant isolates (>80%) came from fields of growers 3 and 8, where only mefenoxam was applied (Table 1). However, in other fields where mefenoxam was used alone (growers 1 and 6), lower percentages of mefenoxam-resistant isolates were found. A lower percentage of mefenoxam-resistant isolates (38 to 60%) was found in fields of grower 2, where mixtures of fungicides were used during the season (Table 1).

The mean EC_{50} value for a subset of six sensitive isolates collected in 1997 was 0.568 μg ml^{-1} and EC_{50} values ranged from 0.12 to 1.1 μg ml^{-1} (Table 3). The mean EC_{50} value for mefenoxam-resistant isolates was 366.5 μg ml^{-1} and EC_{50} values ranged from 3 to 863 μg ml^{-1} (Table 3). The relative sensitivities to mefenoxam of six sensitive and six resistant isolates are shown in Figure 2. Some of the mefenoxam-resistant isolates exhibited enhanced growth on media amended with mefenoxam at 100 μg ml^{-1} (Fig. 2B), while sensitive isolates had reduced growth on media with mefenoxam at 100 μg ml^{-1}; Fig. 2A).

The relative sensitivities of six sensitive and six resistant isolates of *P. capsici* to metalaxyl are shown in Figure 3. The mean EC_{50} value for the sensitive isolates was 0.27 μg ml^{-1} and EC_{50} values ranged from .00002 to 1.33 μg ml^{-1} (Table 3). The mean

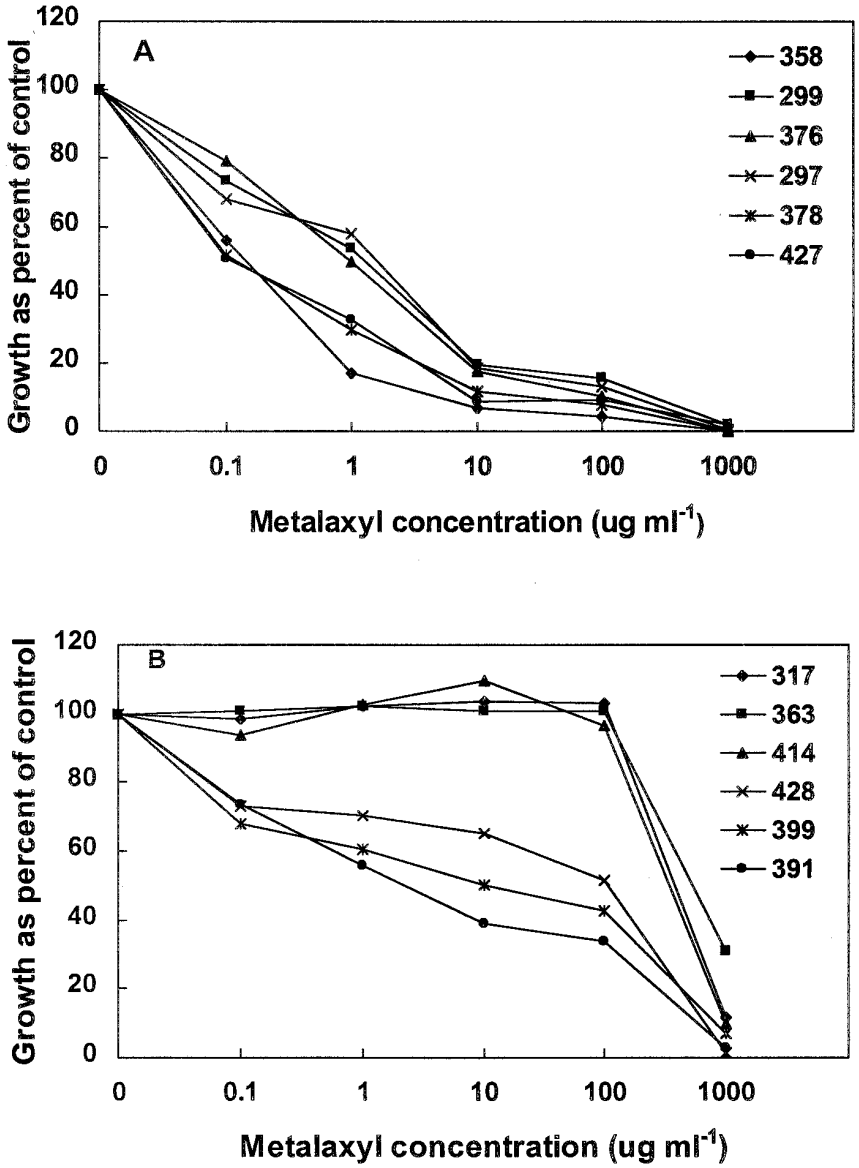

Fig 3. Growth with metalaxyl at 100 μg/ml expressed as a percentage of that on nonamended media versus metalaxyl concentration of **A,** six sensitive and **B,** six resistant isolates of *Phytophthora capsici* collected in 1997. Isolates were grown on clarified V8 juice agar amended with metalaxyl at 0, 0.1, 1.0, 10, 100, or 1,000 μg ml^{-1}. Each line represents a different isolate.

EC_{50} value for resistant isolates was 470.34 μg ml^{-1} and EC_{50} values ranged from 10 to 966 μg ml^{-1} (Table 3).

Mating type determination. A1 and A2 mating types were recovered from most of the fields that were sampled in North Carolina, as well as the fields in New Jersey (Table 4). In nine fields located on four grower farms, both A1 and A2 mating types were found among mefenoxam-resistant isolates.

DISCUSSION

Isolates of *P. capsici* collected between 1987 and 1994 from a range of vegetable crops in North Carolina fields were highly sensitive to metalaxyl. Our data show that isolates of *P. capsici* collected in fields in 1997 in North Carolina and New Jersey have developed resistance to the fungicide mefenoxam. Mefenoxam-resistant isolates were widespread in North Carolina fields and occurred in 10 of 13 fields sampled. Metalaxyl-resistant isolates were initially discovered in 1997 via routine screening for metalaxyl sensitivity of isolates obtained from the Plant Disease and Insect Clinic at North Carolina State University. Isolates that were resistant to metalaxyl were also highly resistant to mefenoxam. However, mean EC_{50} values were higher for metalaxyl than mefenoxam for all isolates tested. Metalaxyl has been used more frequently than mefenoxam in the past in both regions, which may explain the higher EC_{50} values for metalaxyl. Mefenoxam is applied at lower rates in the field than metalaxyl, but contains the more active enantiomer of metalaxyl. The rate of resistance development in populations of the pathogen may vary for the two fungicides.

The high percentage of mefenoxam-resistant isolates collected was surprising considering that neither metalaxyl- nor mefenoxam-resistant isolates of *P. capsici* had been reported prior to 1997 in the field to our knowledge. After our initial report (33), isolates of *P. capsici* collected between 1992 to 1997 from greenhouse peppers grown in Italy were screened by others and intermediate levels of resistance to metalaxyl were reported (34). These workers suggested that caution should be exercised in the use of metalaxyl in greenhouse-grown pepper crops in Italy. More recently, mefenoxam resistance has been reported in Michigan on field-grown cucurbits and on peppers and cucurbits in Georgia (25,26,29).

A shift in sensitivity to mefenoxam within populations of *P. capsici* has occurred in bell pepper fields in North Carolina and New Jersey in a relatively short period of time. Due to the risk-averse nature of many growers, it is possible that more fields have received applications of mefenoxam as disease incidence has increased in recent years with high rainfall conditions in many areas of the northeast and southeast. Frequently, bell pepper production in North Carolina is followed by cucurbit production in the same field during the same season. Metalaxyl and mefenoxam are also used for disease management in adjacent tobacco fields. In addition, tobacco fields where phenylamide fungicides are used are rotated into vegetable production. Intensive use of phenylamide fungicides has been shown to rapidly select for resistance in other oomycetes (10). Other work on nonoomycete pathogens has demonstrated that pathogens can become more resistant to a fungicide when it is used frequently over an extended period of time (43).

Some mefenoxam-resistant isolates were not effectively controlled and were pathogenic on plants in greenhouse assays when treated with labeled rates of the fungicide (J. B. Ristaino, *unpublished data*). Further research is needed to determine whether mefenoxam-resistant isolates are more fit than sensitive isolates and to measure their aggressiveness on plants. Metalaxyl can promote growth of some resistant isolates of *P. infestans* and can cause normally heterothallic single mating type isolates to form oospores in culture (41,44). Some studies have demonstrated that metalaxyl-resistant isolates of *P. infestans* are more fit than sensitive isolates (6,8). However, other studies have suggested that no correlation exists between fitness and metalaxyl resistance in *P. infestans* (17,27). Some of the mefenoxam-resistant isolates of *P. capsici* in our study grew to a greater extent than the nonamended controls in the presence of mefenoxam (Fig. 1). Variation in fitness could occur among isolates of *P. capsici* and fungicide resistance may not present a fitness cost in *P. capsici* (17,25). The frequency of mefenoxam-resistant isolates of *P. capsici* from cucurbits did not decrease over a period of 2 years in the absence of mefenoxam use in Michigan (26).

Oospores are known to play a role in the survival of *P. capsici* in the field (35). Both mating types of *P. capsici* were found among resistant isolates within 9 of 17 grower fields sampled. Oospores survive in soil; therefore, mefenoxam-resistant isolates may persist for prolonged periods and cause disease in subsequent years in the absence of selection pressure from fungicide use. Further studies are needed to test individual fitness components of sensitive and resistant isolates in quantitative assays in vivo and in soil. Mefenoxam-resistant, intermediate, and mefenoxam-sensitive isolates of *P. capsici* were found in a single cucurbit fruit in Michigan and oospore progeny derived from isolates from those fruit exhibited all six mefenoxam sensitivity and mating type combinations (25). Sexual reproduction of the pathogen enhances genetic variation and may enhance survival of resistant isolates (25).

It is believed that the mode of action of metalaxyl is by the selective inhibition of ribosomal RNA synthesis (8,9,15). RNA polymerase is the target site for metalaxyl and an alteration of this target site can lead to resistance in some oomycete pathogens (9). Genetic studies of *P. infestans* and *P. sojae* have indicated that resistance is

Table 4. Isolates of *Phytophthora capsici* classified by mefenoxam sensitivity and mating type within populations from different locations on eight grower farms in North Carolina and New Jersey in 1997

		Number of isolates[a]					
		Sensitive		Intermediate		Resistant	
Grower	Field	A1[b]	A2[b]	A1	A2	A1	A2
1	1	3	9	4	1	6	3
2	1	2	2	1	1	3	1
	2	1	0	2	0	2	0
	3	2	4	1	1	6	5
	4	0	2	0	0	1	1
3	1	4	1	0	0	11	4
	2	0	1	0	0	9	5
4	1	2	0	0	0	0	0
	2	0	2	0	0	1	0
	3	1	0	0	0	0	0
5	1	0	0	0	0	1	0
6	1	2	1	0	0	0	2
7	1	0	1	0	0	0	0
8	1	0	0	0	0	1	2
	2	0	0	0	0	1	0
	3	0	0	0	1	2	2
	4	2	0	0	0	2	2
Total	19	23	8	4	46	27	…

[a] Isolates were characterized as sensitive to mefenoxam if colony growth at 5 μg ml^{-1} was less than 40% of the isolate's growth on the nonamended control media. Intermediate isolates exhibited growth greater than 40% of the nonamended media control at 5 μg ml^{-1}, but less than 40% of the nonamended media control with mefenoxam at 100 μg ml^{-1}. Resistant isolates exhibited growth greater than 40% of the nonamended media control with mefenoxam at 100 μg ml^{-1}.

[b] A1 indicates A1 mating type and A2 indicates A2 mating type determined by crosses with known tester isolates.

linked to a single dominant gene, with variations in resistance accounted for by the influence of minor genes (2,27). Recently, DNA markers that are linked to loci associated with metalaxyl resistance in isolates of *P. infestans* were reported (14,22). Loci found to confer metalaxyl resistance in isolates of *P. infestans* from Mexico and the Netherlands were different than in British isolates of *P. infestans*, indicating that metalaxyl resistance may have developed independently in *P. infestans* isolates in different areas of the world (14). A single target site for metalaxyl resistance in *P. infestans* is highly unlikely (22). Mefenoxam sensitivity in isolates of *P. capsici* from cucurbits is controlled by a single, incompletely dominant gene that is not linked to mating type (25). Further work is needed to examine the genetic mechanisms of mefenoxam resistance in pepper isolates of *P. capsici* and to determine whether the same or different loci confer resistance in populations of *P. capsici* found in different areas or on different hosts (25).

Management of Phytophthora blight in bell pepper will continue to rely on integrated approaches. Phenylamide fungicides should not be used for disease management in fields where mefenoxam-resistant isolates have been found. Alternative, low-risk fungicides with different modes of action will be needed in problem fields. Phytophthora blight can be most effectively managed through the use of resistant cultivars and cultural practices, including water management and the use of cover crops (35).

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