Competitive Parasitic Fitness of Mefenoxam-Sensitive and -Resistant Isolates of *Phytophthora erythroseptica* under Fungicide Selection Pressure

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Abstract

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A 2-year field and laboratory experiment was initiated to study the competitive parasitic fitness of mefenoxam-resistant (50% effective concentration [EC_{50}] > 100 μg ml^{-1}) and mefenoxam-sensitive (EC_{50} = 0.07 μg ml^{-1}) isolates of *Phytophthora erythroseptica* with equal aggressiveness. The competitive ability of the mefenoxam-resistant and -sensitive isolates was tested under no selection pressure (nonfungicide treated) as well as under the influence of mefenoxam and non-mefenoxam (phosphorous acid) fungicides. *P. erythroseptica* isolates were combined in four ratios of mefenoxam-resistant (R) to mefenoxam-susceptible (S) (0R:0S, 1R:1S, 3R:1S, and 1R:3S) and subsequently infested into the soil at the time of planting. In-furrow mefenoxam applications were applied to the soil immediately following infestation with *P. erythroseptica*. Phosphorous acid was applied at tuber initiation and 14 days after tuber initiation. Noninfested, nonfungicide-treated plots served as controls. *P. erythroseptica* isolates recovered from field-infected pink rot tubers at harvest and 3 to 4 weeks after harvest were tested for mefenoxam sensitivity in vitro. In vivo studies were performed by challenge inoculating a zoospore suspension in the four ratios described above onto potato tubers harvested from nontreated, phosphorous acid-treated, or mefenoxam-treated field plots. These field plots were not infested with *P. erythroseptica* at planting. Results from both field and in vivo studies demonstrate that mefenoxam-resistant isolates of *P. erythroseptica* are as fit as sensitive isolates in the absence of selection pressure or in the presence of a phosphorous acid fungicide treatment. Under mefenoxam selection pressure, mefenoxam-resistant *P. erythroseptica* isolates were more parasitically fit than -sensitive isolates. These studies suggest the lack of an apparent fitness penalty in mefenoxam-resistant *P. erythroseptica* populations under field conditions and that these isolates could be stable in most agroecological systems. Based on these results, mefenoxam-based fungicides are no longer recommended for the management of pink rot once mefenoxam-resistant *P. erythroseptica* populations are detected in a specific field.

Pink rot of potato (*Solanum tuberosum* L.), caused by *Phytophthora erythroseptica* Pethybr., is an economically important disease in the United States and Canada (32,35). Management of this disease is based on the integration of several cultural methods and the application of agrochemical fungicides such as mefenoxam (18,45) and phosphorous acid (21,27). Mefenoxam (Ridomil Gold 48% EC and Ultra Flourish 25% EC), an enantiomer of metalaxyl, is site-specific, systemic, and inhibits r-RNA synthesis in oomycete organisms (11). Historically, the most effective pink rot control measure was the application of the protective fungicide mefenoxam (35). Unfortunately, resistance to metalaxyl has been reported in many *Phytophthora* spp. Most notably, resistance was first reported in *P. infestans* on potato in the Netherlands (10). Additionally, metalaxyl-resistant isolates have been reported in *P. cactorum* (43), *P. citricola, P. capsici* (4), *P. parasitica* (14), and *P. nicotianae* (40), as well as in other oomycetes such as *Peronospora tabacina, Bremia lactucae* (29), and *Pythium ultimum* (39). Frequent detections of metalaxyl-resistant isolates of *Phytophthora erythroseptica* in Maine (25), New York (17), Idaho, Minnesota (39), and North Dakota (6) indicate the difficulties in successful management of the disease pink rot in the past two decades. The occurrence of mefenoxam-resistant isolates of *P. erythroseptica* (6,39) and the lack of disease-resistant potato cultivars (34) have contributed to pink rot becoming an economically important disease of potato.

The development and evolution of fungicide resistance in fungal populations is largely dependent on the fitness of resistant isolates, and has important implications for disease management (31). Fitness has been defined as the survival and reproductive success of an allele, individual, or group (30). Parameters including the ability of the pathogen to grow, reproduce, and compete on a susceptible host, as well as pathogenicity and survival through repeated cycles, are used to measure the fitness of an organism (44). Fitness costs also have been measured in terms of predicted fitness (mycelial growth, spore germination, germ tube length, incubation period, virulence, and spore production) and realized fitness (competitive experiments) (1).

The fitness of fungicide-resistant isolates relative to fungicide-sensitive isolates is important in determining whether resistant isolates will persist in the absence of selection pressure (9). However, the fitness of metalaxyl-resistant isolates of oomycetes may not be affected when the fungicide usage has ceased or in the absence of fungicide (8). In reality, results may depend on the characteristic measured as well as the host–pathosystem under investigation. Studies examining the stability of phenylamide resistance in the absence of selection pressure did not demonstrate a change in response to the fungicide in polycyclic oomycete pathogens *Pseudoperonospora cubensis* and *Phytophthora infestans* after several asexual cycles (16,19). In contrast, metalaxyl-resistant isolates of *P. capsici* were determined to be less fit in the absence of selection pressure of metalaxyl after several asexual generations (4). Lesion size on potato leaves increased significantly in the absence of selection pressure when the leaves were inoculated with a metalaxyl-resistant isolate compared with a metalaxyl-sensitive isolate of *P. infestans*; however, there was no significant difference in sporulation capacity (22,23). Metalaxyl-resistant isolates were better competitors than metalaxyl-sensitive isolates in studies conducted with *P. infestans* from potato and *P. nicotianae* from citrus as well as lupin (20,22,40). Additionally, no significant difference was observed between the aggressiveness (as measured by disease incidence and severity) of mefenoxam-resistant and -sensitive *P. erythroseptica* isolates in the absence or presence of mefenoxam

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selection pressure (37). However, when predicted fitness parameters were compared, mefenoxam-resistant *P. erythroseptica* isolates produced more oospores, grew more quickly, and produced significantly fewer zoospores than mefenoxam-sensitive isolates (33). The impact of mefenoxam resistance on realized parasitic fitness (competitive ability) components has not been investigated for *P. erythroseptica*.

Development and application of alternative fungicides, including phosphorous acid, may impact the extent and distribution of mefenoxam resistance within the *P. erythroseptica* population. Phosphorous acid has been registered to control many diseases caused by *Phytophthora* spp. (45). This systemic compound is composed of mono- and dibasic sodium, potassium, and ammonium salts which inhibit oxidative phosphorylation in the metabolism of oomycetes (26). Phosphorous acid is thought to act indirectly, stimulating the plant's natural defense responses against pathogen attack (13), and is effective against several *Phytophthora* spp. such as *P. cinnamomi, P. nicotianae, P. palmivora*, and *P. capsici* in lupin, tobacco, papaya, tomato, and pepper, respectively (15,36). Phosphorous acid is translocated to the roots and, therefore, foliar applications provide protection against tuber rots caused by *P. infestans* and *P. erythroseptica* (21).

The objective of this study was to determine the effects of selection pressure from mefenoxam and non-mefenoxam (phosphorous acid) fungicides on parasitic fitness of mefenoxam-sensitive and -resistant isolates of *P. erythroseptica* in both field and laboratory settings. Competitive fitness was assessed under these conditions with mixed populations of mefenoxam-resistant and mefenoxam-sensitive isolates.

Materials and Methods

Screening *P. erythroseptica* isolates for aggressiveness. A preliminary challenge inoculation study was conducted to determine aggressiveness of four mefenoxam-resistant (R) (50% effective concentration [EC_{50}] value > 100 μg ml^{-1}) and four mefenoxam-sensitive (S) (EC_{50} < 1 μg ml^{-1}) isolates of *P. erythroseptica*. Isolates were collected from infected tubers received during a mefenoxam-resistance survey in Minnesota conducted in 2004.

Zoospores of *P. erythroseptica* were obtained using the method previously described (34,37,38). Agar plugs (5-mm) taken from the margin of an actively growing 3-day-old culture were placed in 12 ml of autoclaved 10% V8 juice broth (100 ml of V8 extract and 900 ml of deionized H_2O) in petri dishes and incubated in the dark at 20°C for 4 days. Mycelia were washed three times with 10 ml of sterile deionized H_2O prior to culture immersion in 10 ml of sterile soil extract (100 g of field soil autoclaved in 900 ml of deionized H_2O) at 20°C for 48 h under continuous fluorescent light. After incubation, the extract was decanted; cultures were washed twice and resuspended in 8 ml of sterile deionized H_2O. Following a chilling treatment at 5°C for 1 h in the dark, cultures were moved to room temperature (22 ± 2°C) for approximately 1 h in the dark to encourage zoospore release. Zoospore concentrations were determined with a hemacytometer and adjusted to 2×10^4 zoospores ml^{-1}.

Isolate aggressiveness was evaluated via tuber challenge inoculations performed using methods similar to those previously described (37). The experiment was conducted twice as a completely randomized design (CRD) with four replications. Ten nonfungicide-treated Russet Burbank potato tubers, selected at random from tubers harvested from noninfested soil, were placed in moist chamber boxes lined at the bottom with number 3 plastic mesh. Challenge inoculations were performed on three apical eyes per tuber by applying 10 μl of the zoospore suspension (approximately 200 zoospores) to each eye. In total, 80 tubers per treatment (four replications × 10 tubers × two inoculation trials) were inoculated within 10 to 60 min of zoospore release. Controls were inoculated with sterile distilled H_2O. The inoculated tubers were covered with four layers of paper towels moistened to saturation with deionized H_2O and chamber boxes were closed to establish a high-humidity environment to promote infection. Following incubation in the dark at 20 to 22°C for 9 days, inoculated tubers were cut longitudinally and exposed to air for 30 min to promote discoloration of infected tissue. Disease incidence (I) and the depth of penetration (P) were used to calculate the aggressiveness index (AI) (AI = I × P) (37). Mefenoxam-resistant isolate 04MN22-3R and mefenoxam-sensitive isolate 04MN22-4S were chosen from the four isolates of each group because of similar AI and used for both field and tuber challenge-inoculation experiments.

Field soil infestation experiments. Field experiments carried out in 2007 and 2009 near Park Rapids, MN were performed under selection pressure from three sources (mefenoxam, phosphorous acid, and no fungicide) and four inoculum ratios (0R:0S, 1R:1S, 3R:1S, and 1R:3S) as a three-by-two factorial in a randomized complete block design with four replications. Application of mefenoxam (Ridomil Gold, 45.3% a.i.; Syngenta Crop Protection Inc., Greensboro, NC), phosphorous acid (Phostrol, 53.6% a.i.; Nufarm Americas Inc., Houston, TX), and the no-fungicide control served as whole-plot while infestation ratios constituted subplots within the split-plot arrangement. Each treatment (application/infestation ratio) was applied to four 6-m rows of 'Russet Burbank' potato planted at 0.3-m in-row seed spacing and 0.9 m between rows.

An infestation slurry was prepared by blending the contents of the culture plates (agar, mycelium, and oospores) of mefenoxam-resistant isolate 04MN22-3R and mefenoxam-sensitive isolate 04MN22-4S in deionized H_2O. The infestation slurry was applied in-furrow at ratios described above followed by the assigned fungicide (mefenoxam) applied to the respective treatment plots. With this configuration, the infestation slurry was applied using a 45° forward-directed nozzle which applied *P. erythroseptica* directly onto seed pieces immediately following the furrow shovel. Using a different nozzle, in-furrow mefenoxam fungicide applications were introduced to the soil at the rate of 200 g a.i. ha^{-1} immediately following the infestation of *P. erythroseptica* using a rear nozzle pointed at the hilling disks as they closed the furrow with soil. In-furrow applications of mefenoxam are the industry standard practice in potato production in the Midwest and are based on the results of previous studies (37). Phosphorous acid was sprayed at tuber initiation and 14 days after tuber initiation at the rate of 100 g a.i. ha^{-1} per application (21).

Controlled challenge inoculations. Healthy tubers produced from nontreated, mefenoxam-treated, and phosphorous acid-treated potato plants in noninfested subplots of experimental field trials conducted in both years were challenge inoculated with *P. erythroseptica* (10 tubers × four replications × three treatments × two inoculation trials per year). Zoospores were extracted and post-harvest challenge inoculations were performed using isolates, isolate ratios, and general methods previously described for determination of isolate aggressiveness (37). Each year, challenge inoculations were conducted within 2 months after harvest as a CRD.

Isolation of *P. erythroseptica.* Isolates of *P. erythroseptica* were obtained from tubers infected in field soil infestation studies and in challenge inoculation studies using the same general methods. *P. erythroseptica* was isolated whenever possible from 20 tubers displaying pink rot symptoms selected from each infested treatment replication at harvest and 30 days after harvest. Challenge-inoculated tubers were incubated for 9 days, tubers with pink rot symptoms were identified, and, again, *P. erythroseptica* was isolated whenever possible. Sections of infected tuber tissue (4 by 4 mm) were excised from the interior of aseptically bisected tubers, placed on petri dishes containing water agar, and incubated in the dark at 17 to 20°C for 3 days. Fungal colonies with mycelia resembling *P. erythroseptica* were selected and purified by hyphal tip isolation and maintained on 10% V8 juice agar medium (100 ml of V8 juice, 15 gm of agar, and 900 ml of deionized H_2O) prior to testing for mefenoxam sensitivity (6,39).

Mefenoxam sensitivity assay. Mefenoxam sensitivity of *P. erythroseptica* isolates recovered from all field trials and challenge inoculation experiments were characterized by growing isolates on

5% V8 juice agar media (50 ml of V8 juice filtered through four layers of cheesecloth, 950 ml of distilled H_2O, and 20 g of agar) containing concentrations of mefenoxam at 0, 0.01, 0.1, 1, 10, and 100 µg a.i. ml^{-1} (Ridomil Gold EC; 48% a.i.) (6). Isolates were arranged in a CRD with two replicates. Mefenoxam-sensitive isolate PR-347 and mefenoxam-resistant isolate PE-89 were included in each experiment as internal controls, and EC_{50} values were calculated by fitting the data sets into a nonlinear Gompertz function (6,39):

$$Y = \alpha \times \exp\{-\exp[\beta - (\gamma \times x)]\}$$

The percentage of mefenoxam-resistant (EC_{50} > 100 µg/ml) and mefenoxam-sensitive (EC_{50} < 1 µg ml^{-1}) isolates was determined, and the frequency was expressed as previously described (6).

Statistical analysis. Homogeneity of variance for aggressiveness index was evaluated across the two trials using Levene's test (28) and a one-way analysis of variance (ANOVA) was conducted with General Linear Model of SAS (version 9.2; SAS Institute, Inc, Cary, NC). Data from each year of field soil infestation and challenge inoculation experiments were analyzed separately. Two-factor ANOVAs were performed to determine whether the main effects of fungicide chemistry applied and inoculum/infestation proportion had a significant effect on the proportion of mefenoxam-resistant isolates recovered from inoculated field trials or tubers challenge inoculated postharvest. In all cases, means were separated using Fisher's protected least significant difference (α = 0.05). To compare the observed ratios with that of the theoretical expected ratios (0R:0S, 1R:1S, 3R:1S, and 1R:3S), exact binomial (goodness-of-fit) tests were performed (α = 0.05). Isolate ratios from noninoculated/noninfested treatments (0R:0S) were used as the nominal variables to which other expected ratios were compared in data analysis.

Results

Isolate selection. A significant difference in aggressiveness indices among the mefenoxam-sensitive and -resistant *P. erythroseptica* isolates was observed in preliminary studies (Fig. 1). Mefenoxam-resistant isolate 04MN22-3R and mefenoxam-sensitive isolate 04MN22-S were selected for field infestation and challenge inoculation trials because these isolates were not significantly different from each other and were nearly numerically identical in aggressiveness index. Additionally, these two isolates were recovered from the same field in 2004.

Competitive parasitic fitness studies with soil infestations of mixed populations of mefenoxam-sensitive and -resistant *P. erythroseptica* isolates. At-harvest analysis in 2007 indicated that there were significant differences in the frequency of mefenoxam-resistant *P. erythroseptica* isolates recovered among whole-plot fungicide treatments (P = 0.0006) and subplot infestation ratios (P = 0.0445). However, there was no significant interaction found between the whole-plot and subplot (P = 0.1104). Among isolates recovered 3 to 4 weeks after harvest, there was a significant whole-plot–infestation ratio interaction (P = 0.0013), as well as significant differences among the whole-plot fungicide treatments (P = 0.0018) and subplot infestation ratios (P = 0.0251). In 2009, significant differences were observed among whole-plot fungicide treatments (P = 0.0189) but not among subplot infestation ratios (P = 0.63). However, a significant whole-plot–subplot interaction was observed (P = 0.0247). Similar to what was observed in 2007, in 2009 at 3 to 4 weeks after harvest, significant differences were observed among the whole-plot treatments (P = 0.0021), the infestation ratios (P < 0.001), and also the interaction among the whole-plot treatments and infestation ratios (P < 0.001).

In 2007, exact binomial analyses indicated that, in the absence of selection pressure, no fungicide applied, the ratio of recovery of mefenoxam-resistant to -sensitive *P. erythroseptica* isolates at harvest was significantly different from the applied 1R:1S ratio. In contrast, no significant differences were found between the observed and expected ratios where 1R:3S and 3R:1S ratios were applied (Table 1). When the application of mefenoxam fungicide provided selection pressure, there were significant differences observed in all the infestation treatments, with at least 90% mefenoxam-resistant isolates recovered regardless of the ratio applied. However, under the influence of phosphorous acid as a selection pressure, no significant differences were observed in the ratio of R:S isolates recovered when compared with the ratios used to infest the soil. Analysis of data taken at 3 to 4 weeks after harvest indicated that, in the absence of selection pressure, the ratio of recovery of mefenoxam-resistant and -sensitive isolates was as expected in the 1R:1S ratio but not with 1R:3S and 3R:1S ratios. Similar results were found when phosphorous acid acted as a selection pressure (Table 1). However, as observed in isolates collected at harvest, under selection pressure of mefenoxam, significantly higher frequencies of mefenoxam-resistant isolates were recovered from tubers grown in plots with all inoculum ratios.

Results obtained in 2009 generally were as expected for each of the fungicide treatment–infestation ratio combinations (Table 1). In the absence of a selection pressure, the ratio of recovery of mefenoxam-resistant and -sensitive isolates was not significantly different than the applied ratio in all four infestation ratios at both harvest and 3 to 4 weeks postharvest. Similar results were obtained in the presence of phosphorous acid as a selection pressure and the ratio of recovery of R:S isolates was not significantly different than applied ratios, with an exception in the 3R:1S infestation ratio at harvest (Table 1). As observed in 2007 at both collection dates, significantly more mefenoxam-resistant isolates were recovered from all infestation ratios when mefenoxam fungicides were applied to the soil.

Controlled challenge inoculations with mixed populations of mefenoxam-sensitive and -resistant *P. erythroseptica.* In both years, no tubers treated with phosphorus acid were infected during challenge inoculations (Table 2). The recovery of mefenoxam-resistant *P. erythroseptica* isolates was largely consistent with that observed with isolates recovered from tubers harvested from soil infestation treatments. Exact binomial analyses indicated that the ratio of recovery of R:S isolates from challenge-inoculated, non-fungicide-treated tubers was not significantly different than the inoculated ratios of 1R:1S and 3R:1S. However, significant differences were found in the recovery of isolates in 2007 when the

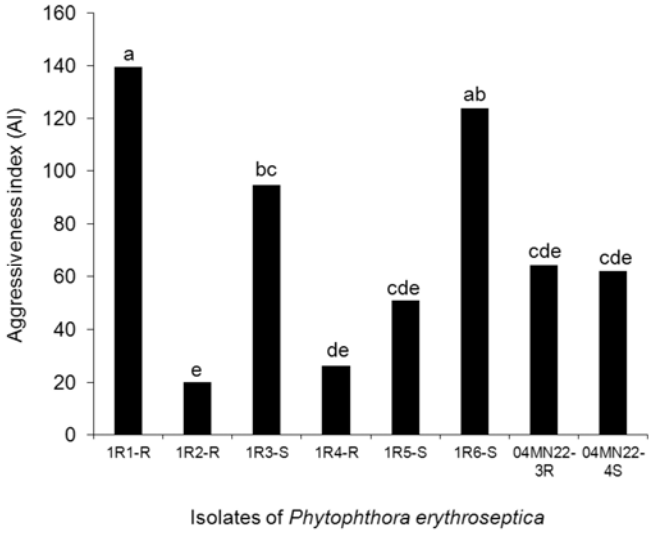

Fig. 1. Aggressiveness index (disease incidence × depth of penetration) of eight *Phytophthora erythroseptica* isolates as determined by challenge inoculation onto nonfungicide-treated tubers of 'Russet Burbank'. Mefenoxam-resistant (50% effective concentration [EC_{50}] > 100 µg ml^{-1}) isolate 04MN22-3R and mefenoxam-sensitive (EC_{50} = 0.07 µg ml^{-1}) isolate 04MN22-4S were selected as inoculum for field soil infestation studies and in vivo challenge-inoculation experiments. Means separated by the same letter are not significantly different according to Fisher's protected least significant difference (α = 0.05).

1R:3S ratio was used for inoculation. Significant differences were observed in the ratio of recovery of mefenoxam-resistant to -sensitive isolates with mefenoxam-treated tubers regardless of the original ratio of isolates used to inoculate potato tubers, because greater than 50% and nearly 100% mefenoxam-resistant isolates were recovered in 2007 and 2009, respectively (Table 2). Finally, challenge inoculations with all ratios of inoculum were unsuccessful in infecting phosphorous acid-treated tubers and, therefore, no analysis could be conducted to evaluate expected versus observed ratios.

Table 1. Exact binomial tests of the ratio of mefenoxam-sensitive and -resistant *Phytophthora erythroseptica* isolates recovered from potato tubers collected at harvest and 3 to 4 weeks postharvest[a]

		At harvest			3 to 4 weeks postharvest		
Year, ratios[b]	**Expected (R:S)**	**Isolates**	**Observed (R:S)**	***P* value**	**Isolates**	**Observed (R:S)**	***P* value**
2007							
Nontreated							
1R:1S	50:50	36	28:72	0.0113*	35	42:58	0.1856 ns
1R:3S	25:75	21	33:67	0.2564 ns	38	06:94	<0.0001*
3R:1S	75:25	18	61:39	0.1390 ns	42	49:51	<0.0001*
Mefenoxam							
1R:1S	50:50	21	90:10	<0.0001*	40	78:22	<0.0001*
1R:3S	25:75	20	95:05	<0.0001*	56	96:04	<0.0001*
3R:1S	75:25	22	91:09	<0.0001*	38	92:08	0.0021*
Phosphorous acid							
1R:1S	50:50	11	45:55	0.5000 ns	22	52:48	0.5000 ns
1R:3S	25:75	15	33:67	0.3135 ns	15	09:91	0.0089*
3R:1S	75:25	8	75:25	0.5675 ns	16	37:63	<0.0001*
2009							
Nontreated							
1R:1Sa	50:50	45	49:51	0.5000 ns	43	53:47	0.3237 ns
1R:3S	25:75	52	29:71	0.3442 ns	30	27:73	0.4165 ns
3R:1S	75:25	39	71:29	0.3513 ns	31	68:32	0.1753 ns
Mefenoxam							
1R:1S	50:50	58	100:0	<0.0001*	36	100:0	<0.0001*
1R:3S	25:75	53	98:02	<0.0001*	36	100:0	<0.0001*
3R:1S	75:25	50	100:0	<0.0001*	38	100:0	0.0002*
Phosphorous acid							
1R:1S	50:50	31	45:55	0.4159 ns	15	47:53	0.3981 ns
1R:3S	25:75	43	40:60	0.1484 ns	29	28:72	0.3739 ns
3R:1S	75:25	27	50:50	0.0271*	37	62:38	0.0713 ns

[a] Tubers were produced in non-fungicide-treated, mefenoxam-treated, and phosphorus acid-treated infested field plots in 2007 and 2009; ns = observed ratios were not significantly different from expected ratios ($\alpha = 0.05$); * = observed ratios were significantly different from expected ratios ($\alpha = 0.05$).
[b] Time of analysis, treatments, and infestation ratios; mefenoxam-resistant:mefenoxam-sensitive (R:S).

Table 2. Exact binomial tests of the ratio of mefenoxam-sensitive and -resistant *Phytophthora erythroseptica* isolates recovered from tubers which were challenge inoculated postharvest[a]

Year, treatment[b]	**Inoculation ratios**	**Expected ratios (R:S)**	**Number of isolates**	**Observed ratios (R:S)**	***P* value**
2007					
Nontreated					
	1R:1S	50:50	27	46:54	0.5000 ns
	1R:3S	25:75	34	41:59	<0.0001*
	3R:1S	75:25	32	70:30	0.3415 ns
Mefenoxam					
	1R:1S	50:50	33	78:22	0.0004*
	1R:3S	25:75	37	51:49	<0.0001*
	3R:1S	75:25	36	89:11	0.0424*
Phosphorous acid					
	1R:1S	50:50	0	0	…
	1R:3S	25:75	0	0	…
	3R:1S	75:25	0	0	…
2009					
Nontreated					
	1R:1S	50:50	35	46:54	0.4450 ns
	1R:3S	25:75	33	27:73	0.4467 ns
	3R:1S	75:25	33	81:19	0.3481 ns
Mefenoxam					
	1R:1S	50:50	28	96:04	<0.0001*
	1R:3S	25:75	29	100:0	<0.0001*
	3R:1S	75:25	29	100:0	0.0013*
Phosphorous acid					
	1R:1S	50:50	0	0	…
	1R:3S	25:75	0	0	…
	3R:1S	75:25	0	0	…

[a] Nonfungicide-treated, mefenoxam-treated, and phosphorus acid-treated potato tubers were collected from noninfested plots of field trials conducted in 2007 and 2009. Infestation ratios: mefenoxam-resistant:mefenoxam-sensitive (R:S); ns = observed ratios were not significantly different from expected ratios ($\alpha = 0.05$); * = observed ratios were significantly different from expected ratios ($\alpha = 0.05$).
[b] Time of analysis and treatment.

Discussion

Rather than test the competitive ability of a single ratio of mefenoxam-resistant and -sensitive isolates, we chose to use four separate combinations of the inoculum derived from isolates of *P. erythroseptica* sensitive and resistant to mefenoxam. This approach was chosen because it more closely reflects mixed populations in nature, where a diversity of strains with varied genetic background competes for dominance, as demonstrated in recent surveys conducted by this research group (6,39).

The parasitic fitness of an individual isolate of a pathogen is likely influenced by characteristics such as aggressiveness, virulence, and the rate of spore production (both sexual and asexual spores; 31). Because these characteristics are likely to vary among isolates, fitness also will vary (31). Experimental variations among parasitic fitness parameters may be due to differences in the genetic background of the isolates tested rather than to fitness costs associated with fungicide resistance genes (31). For these reasons, we chose to use inoculum/infestation ratios of mefenoxam-sensitive and -resistant isolates that were equal in aggressiveness so that true competitive fitness under the influence of various fungicide regimes could be tested.

Results from the current field study and in vivo experiments clearly demonstrate that, when isolates with equal aggressiveness were allowed to compete against each other under the selection pressure provided by mefenoxam, in most cases mefenoxam-resistant isolates emerged as more competitively fit than mefenoxam-sensitive isolates. This was expected; however, in the absence of selection pressure, mefenoxam-resistant isolates were able to compete with mefenoxam-sensitive isolates and emerged at a ratio equal to that at which they were applied. Additionally, in a majority of the observations, when under the selection pressure of phosphorous acid, mefenoxam-resistant and -sensitive isolates were recovered at a ratio similar to that at which they were applied. These results are in contrast to in vitro studies which suggested that mefenoxam-resistant *P. erythroseptica* isolates had a higher reproductive capacity than mefenoxam-sensitive isolates and, therefore, may be more competitive (33). In contrast to the results from the field study, results of the in vivo experiment with phosphorous acid showed that none of the combinations of zoospore inocula were able to infect phosphorous acid treated potato tubers. Although there are no reports available of the length of residual control of phosphorous acid, the inability to successfully infect challenge-inoculated phosphorous acid-treated tubers indicates that phosphorous acid provides residual control of pink rot well beyond harvest. These results are supported by reports of residual phosphite activity for extensive periods in lupin, tobacco, and pawpaw (36).

The parasitic fitness of mefenoxam-resistant isolates under the influence of phosphorus acid appears to differ from fitness levels when mefenoxam is used as the selection pressure. In most cases, the number of isolates recovered from phosphorous acid treatments was very low and the ratio of recovery of mefenoxam-resistant to -sensitive isolates was similar to that obtained under absence of selection pressure, indicating that none of the isolates were totally fit. Our results suggest that phosphorous acid should control *P. erythroseptica* populations composed of mefenoxam-sensitive or -resistant isolates or a mixture of strains. There have been no reports of phosphorous acid-resistant isolates of *P. erythroseptica*. However, laboratory-generated mutant isolates of *P. capsici* demonstrated resistance to phosphorous acid in both in vivo and in vitro studies (2). Phosphorous acid mutant isolates of *P. capsici* were tested for cross-resistance and were found resistant to both fosetyl-Al and fosetyl-Na but not to metalaxyl. Likewise, metalaxyl-resistant isolates were resistant to other acylanilide fungicides but not to phosphorous acid or fosetyl-Al (2). Although it is possible that resistance to phosphorous acid or other phosphonates may develop in the *P. erythroseptica* population, it is important to note that in vivo sensitivity to this group of fungicides does not necessarily correlate with in vitro sensitivity (5,46). On the other hand, rare instances of insensitivity to phosphonates have been documented in field populations of pathogens in areas having a history of extended use of these fungicides (3,12). These observations demonstrate the need to monitor the *P. erythroseptica* population in areas where phosphorous acid applications are utilized to manage pink rot.

Development of resistance within the pathogen population depends upon several factors, such as relative ecological fitness of the resistant isolates under the influence or in the absence of the fungicide, dosage, frequency, and persistence of the fungicide; environmental factors associated with the pathogen survival in the absence of a suitable host; and the method and time of application of the fungicide (32). Several approaches have been suggested as ways to avoid the problem of fungicide resistance. The use of fungicide mixtures rather than reliance upon a single fungicide has been advocated (41). Heterogeneous fungicide mixtures (different plants receiving different fungicides) rather than the homogeneous mixtures (plant receiving the same fungicide mixture) also have been recommended because heterogeneous mixtures do not allow for selection of resistant genotypes and provide a diverse environment (47). Heterogeneous mixtures with varied persistence might allow sensitive genotypes to compete more successfully with resistant genotypes. Prepacked mixtures of the fungicides are the only strategy to delay or prevent the development of resistance (42). It is also possible that fitness of the pathogen populations can be reduced by integrating fungicide use with host resistance (7). Studies indicate that a combination of intermediate resistance of a cultivar with low levels of fungicide application would provide adequate protection while extending the durability of both control measures (38,47). Withdrawal of mefenoxam from use in fields where mefenoxam-resistant isolates are found, even though their proportion of the surveyed population is very low, should be a major component of any pink rot management program (37). If mefenoxam-resistant and -sensitive populations of *P. erythroseptica* are equally able to withstand the environmental conditions in the field, it seems likely that mefenoxam-resistant isolates will persist for a longer period of time in the soil after mefenoxam has been withdrawn from use and, therefore, reintroduction of the fungicide would not be advisable in the near future. Similar predictions were made concerning mefenoxam-resistant isolates in *P. capsici* (5). Studies of the fitness of mefenoxam-resistant and -sensitive isolates on *P. capsici* led to a speculation that the pathogen might have developed different mechanisms of resistance to mefenoxam when applied in the field and in in vivo conditions (5). That was not the case in the current study, where both in vivo and field studies produced similar results.

The current research substantiates the previous findings that mefenoxam-resistant isolates of *P. erythroseptica* are highly fit under the influence of mefenoxam and as fit as mefenoxam-sensitive isolates in the absence of mefenoxam selection pressure. Considering the results of the studies performed here, we no longer recommend applications of mefenoxam for the control of pink rot in fields with known mixed populations of mefenoxam-resistant and -sensitive populations, regardless of the ratio. We believe that the mere presence of mefenoxam-resistant isolates in the agroecosystem will result in a competitive advantage if mefenoxam fungicides are used. The data reported here demonstrate inconsistent responses of mefenoxam-sensitive and -resistant populations to another fungicide (phosphorous acid). The knowledge provided in the current research is important from a disease management point of view.

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