Inheritance of Mefenoxam Resistance in Selfed Populations of the Homothallic Oomycete *Phytophthora erythroseptica* (Pethybr.), Cause of Pink Rot of Potato

Firas M. Abu-El Samen[1], Kirin Oberoi[2], Raymond J. Taylor[2], Gary A. Secor[2], and Neil C. Gudmestad[2]*

[1]Present address: Department of Plant Pathology, Michigan State University, East Lansing, MI 48824
[2]Department of Plant Pathology, North Dakota State University, Fargo, ND 58105
*Corresponding author: Tel: 701-2317547; Fax: 701-2317851; E-mail: neil.gudmestad@ndsu.nodak.edu

ABSTRACT

The inheritance of mefenoxam resistance in the oomycete *Phytophthora erythroseptica*, causal organism of pink rot of potato, was investigated in two successive selfed generations of single oospore isolates. Fourteen parental isolates from three mefenoxam-sensitivity phenotypes (sensitive, intermediately resistant, and resistant) were used to produce S1 and S2 progeny isolates by selfing each isolate and germinating single oospore cultures from each isolate. The two generations of isolates were tested for mefenoxam sensitivity using the radial growth inhibition assay and calculating EC_{50} values. Twenty-four-hundred isolates from S1 and S2 generations were tested for mefenoxam sensitivity. Results demonstrated the lack of segregation for mefenoxam sensitivity among S1 and S2 progeny isolates from the resistant and sensitive parents. The majority of the S1 progeny isolates from the parents with intermediate resistance also possessed intermediate resistance, but demonstrated substantial quantitative shifts in response to mefenoxam more often toward increased insensitivity. A few isolates from the S1 progeny were sensitive to mefenoxam, and one isolate was mefenoxam-resistant. Similarly, the majority of the S2 progeny isolates from intermediately resistant parents also possessed intermediate resistance with some quantitative shifts in sensitivity to mefenoxam similar to those observed in S1 progeny, as well as a few isolates that were sensitive to mefenoxam. These results do not support the hypothesis that resistance to mefenoxam in *P. erythroseptica* is controlled by a single gene exhibiting incomplete dominance. Alternative hypotheses are proposed such as the genetics of mefenoxam resistance in *P. erythroseptica* is probably under the control of more than one major gene and perhaps some minor genes of additive effect.

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ADDITIONAL KEY WORDS: metalaxyl, phenylamide fungicides, *Solanum tuberosum*

RESUMEN

La herencia de la resistencia del oomycete *Phytophthora erythroseptica* causante de la pudrición rosada de la papa al mefenoxam fue investigada en dos generaciones autofecundadas sucesivas de aislamientos provenientes de una sola oospora. Catorce aislamientos de tres fenotipos sensibles al mefenoxam (sensible, intermedio-resistente y resistente) fueron utilizados para producir los aislamientos progenie S1 y S2, autofecundando cada aislamiento y produciendo aislamientos provenientes de una sola oospora. Se probaron los aislamientos de las dos generaciones para sensibilidad al mefenoxam, utilizando la prueba de inhibición de crecimiento radial y calculando los valores EC_{50}. Se probaron 2,400 aislamientos de las generaciones S1 y S2 para determinar su sensibilidad al mefenoxam. Los resultados demostraron la falta de segregación para sensibilidad a la acción del mefenoxam entre los aislamientos de las progenies S1 y S2 provenientes de progenitores resistentes y sensibles. La mayoría de los aislamientos de la progenie S1 provenientes de progenitores con resistencia intermedia también tuvieron resistencia intermedia, pero demostraron un cambio cuantitativo sustancial en respuesta al mefenoxam, con más frecuencia hacia una mayor insensibilidad. Unos

pocos aislamientos de la progenie S1 mostraron sensibilidad al mefenoxam y un aislamiento mostró resistencia. De la misma manera, la mayoría de los aislamientos de la progenie S2 con progenitores de resistencia intermedia, tuvieron también resistencia intermedia con algunos cambios cuantitativos en sensibilidad, similares a aquellos observados en la progenie S1 e igualmente unos pocos aislamientos fueron sensibles. Estos resultados no apoyan la hipótesis de que la resistencia del *P. erythroseptica* al mefenoxam es controlada por un solo gen de dominancia incompleta. Se propone la hipótesis alternativa de que la resistencia genética del *P. erythroseptica* al mefenoxam está probablemente bajo el control de más de un gen mayor y tal vez de algunos genes menores de efecto aditivo.

INTRODUCTION

Phytophthora erythroseptica Pethybr., causal organism of potato pink rot, is an important soil-borne pathogen worldwide. The disease causes serious post-harvest tuber rotting either in the field or during storage. *Phytophthora erythroseptica*, a homothallic oomycete, is mainly a tuber pathogen, but can also cause stem decay and wilt. Pink rot disease is usually an endemic disease; however, it is more common in poorly drained soils. The disease is usually controlled by a 3- to 4-year rotation with non-host crops such as cereal crops and pulses, by planting potato in well-drained soils, by careful handling of tubers during harvest and storage to avoid tuber wounding, and through the application of fungicides (Lambert and Salas 2001). The phenylamide fungicides metalaxyl and mefenoxam (the R-enantiomer of metalaxyl) have been used effectively to control pink rot during the 1980s. Potato storage surveys conducted in the late 1980s and early 1990s found no evidence of metalaxyl resistance in *P. erythroseptica* (Stack et al. 1993). Metalaxyl-resistant isolates of *P. erythrosptica* have been detected in Maine in 1994 (Lambert and Salas 1994), in New York in 1995 (Goodwin and McGrath 1995), in Idaho in 1998 and in Minnesota in 2000 (Taylor et al. 2002). A recent survey of *P. erythrospetica* populations in the United Sates (Taylor et al. 2002) indicated that mefenoxam resistance is widespread among *P. erythroseptica* populations in North America. This situation has caused concerns among potato growers and industry representatives who currently have no other chemical alternatives to control the disease.

Mefenoxam is a site-specific fungicide that inhibits r-RNA synthesis by interfering with the activity of the RNA polymerase-I template complex in the oomycete organisms (Davidse et al. 1983, 1991). The inheritance of resistance to metalaxyl/mefenoxam has been investigated in the homothallic oomycete *Phytophthora sojae* Kaufm & Gerd. (Bhat et al. 1993) and in some heterothallic oomycete species including *Phytophthora infestans* (Mont.) de Bary (Judelson and Roberts 1999; Lee et al. 1999; Shattock 1988), *Bremia lactucae* Regel (Crute and Harrison 1988) and *Phytophthora parasitica* Dastur (Chang and Ko 1990). The genetics of metalaxyl/mefenoxam resistance in the oomycetes has been reported to be under the control of a single incompletely dominant gene (Bhat et al. 1993; Crute 1987; Crute and Harrison 1988; Goodwin and McGrath 1995; Lucus et al. 1990; Shattock 1988). Resistance to metalaxyl is considered to be conditioned by a single dominant gene in *P. parasitica* (Chabane et al. 1996; Chang and Ko 1990) and in *P. infestans* (Lee et al. 1999). However, Judelson and Roberts (1999) suggested that multiple loci were involved in conveying insensitivity in *P. infestans* to this class of fungicides. Since studies investigating the genetic control of metalaxyl/mefenoxam resistance have produced dissimilar conclusions, it seems likely that the genetic control of metalaxyl resistance differs between various species within a genus or between isolates of the same species. Hence, the objective of this study was to investigate the inheritance of mefenoxam resistance in two generations of *P. erythroseptica* and to test the hypothesis proposed by Goodwin and McGrath (1995) that mefenoxam (metalaxyl) resistance in *P. erythroseptica* is under the genetic control of a single incompletely dominant gene. Accordingly, isolates possessing intermediate resistance to mefenoxam should be heterozygous for the resistance allele and would be expected to segregate to sensitive and resistant phenotypes upon selfing.

MATERIALS AND METHODS

Parental Isolates

Fourteen *P. erythroseptica* isolates belonging to three mefenoxam sensitivity phenotypes (resistant, sensitive, and intermediate) were selected to study the inheritance of mefenoxam resistance in two selfed generations (designated S1 and S2) (Table 1). All parental isolates were of hyphal tip origin, isolated from potato tubers with pink rot symptoms using the protocols described by Salas et al. (2000), and were

purified by repeated hyphal tipping. Sensitivity to mefenoxam (Ridomil Gold 4EC) in parental isolates was determined using *in vitro* radial growth inhibition on mefenoxam amended V8 juice agar plates as described below and is expressed as EC_{50} value (concentration at which 50% growth reduction occurred) (Table 1). All isolates were maintained on V8 juice agar slants at 22 C during the course of the study.

Establishment of S1 and S2 Progenies of P. erythroseptica

Phytophthora erythroseptica parental isolates were selfed by growing each isolate individually on V8 juice media plates for 1 month in the dark at 22 C. The 14 parental isolates consisted of four sensitive isolates (EC_{50} < 1.0 μg mL^{-1}), four resistant isolates (EC_{50} > 100 μg mL^{-1}), and six intermediately resistant isolates (1.0 μg mL^{-1} – EC_{50} ≤ 100 μg mL^{-1}) (Table 1). Plates were examined under the microscope for the presence of mature oospores, and oospores were extracted and germinated using the protocol of Medina and Platt (1999) with slight modifications. Briefly, oospores were harvested by cutting and grinding agar stripes containing oospores in sterile distilled water. The oospore suspension was filtered using a series of mini sieves (60, 100, 150 mesh; Cell dissociation kit, Sigma, cat.# CD-1) to remove agar particles from the suspension. The oospores were purified by digesting mycelia with a lysing enzyme (L1412, Sigma; from *Trichoderma harzianum*). The lysing enzyme was removed by washing the oospore suspension with sterile distilled water and by centrifugation (5 min at 3000 rpm). The oospores were resuspended in sterile distilled water and then filtered through a 20-(m nylon filter (Osmonics Inc. cat# R22SP04700), the filtrate was discarded, and the filter was washed three times with sterile distilled water to collect the oospores (Medina and Platt 1999). The oospore solution was adjusted to $1x10^3$ spores mL^{-1} with the aid of a hemacytometer and 0.1 mL of the diluted oospore suspension was used to inoculate water agar plates. Plates were incubated under fluorescent light at room temperature (20-25 C) for 2 days. Plates were examined for germinating oospores using a compound microscope; oospores germinated by producing germ tubes that developed into hyphae and produced sporangiophores and sporangia. Single oospore cultures were established by transferring germinated oospores to fresh V8 juice agar plates. A total of 100 S1 single oospore cultures were obtained from each parental isolate. The S2 progeny isolates

TABLE 1—Phytophthora erythroseptica *parental and S1 progeny isolates used in studying inheritance of mefenoxam resistance and their EC_{50} values.*

Parental isolate	Year collected	Source	Mean EC_{50} (μg mL^{-1})	Phenotype[a]	S1 progeny isolates[b]	Range EC_{50} (μg mL^{-1})[c]	Mean EC_{50} (μg mL^{-1})[d]	SD[e]
9927-1	1999	Hamer, ID	147	R	9927-1- (1-100)	116.7-195.0	159.9	15.30
484-4d	1999	Hamer, ID	189	R	484-4d- (1-100)	135.3-223.4	176.0	18.85
9926-7	1999	Hamer, ID	188	R	9926-7- (1-100)	107.4-234.3	192.6	24.7
9941-1	1999	Hamer, ID	154	R	9941-1- (1-100)	144.1-238.8	175.0	16.57
PE-89[f]	1995	ME	146	R	———	120.5-183	146.3	16.99
9931-3	1999	Grafton, ND	0.05	S	9931-3- (1-100)	0.004-0.113	0.049	0.019
9942-6	1999	Eastern Idaho	0.04	S	9942-6- (1-100)	0.005-0.083	0.037	0.022
9942-7	1999	Eastern Idaho	0.06	S	9942-7- (1-100)	0.005-0.231	0.047	0.034
97431-1	1997	Morrow Co., OR	0.04	S	97431-1(1-100)	0.005-0.075	0.030	0.018
RB-347[g]	1997	MN	0.05	S	———	0.005-0.52	0.047	0.055
172-3	2000	Burley, ID	60.0	I	172-3- (1-100)	20.0-96.6	68.10	15.58
172-4	2000	Burley, ID	62.0	I	172-4- (1-100)	22.6-170.5	68.90	16.2
252-2	2000	Park Rapids, MN	22.0	I	252-2- (1-100)	0.70-76.0	13.30	14.6
225-2	2000	Hamer, ID	4.0	I	225-2- (1-100)	3.20-58.3	13.00	9.2
230-2	2000	St. Anthony, ID	27.0	I	230-2- (1-100)	17.5-83.5	47.70	11.9
228-3	2000	St. Anthony, ID	4.0	I	228-3- (1-100)	0.50-93.0	15.00	19.22

[a]R, resistant; S, sensitive; I, intermediate.
[b]100 S1 progeny isolates produced by selfing each parental isolate.
[c]Range of the Mean EC_{50} values for the 100 S1 progeny isolates.
[d]Mean EC_{50} values for the 100 S1 progeny isolates.
[e]Standard deviation of the mean EC_{50} values for the 100 S1 progeny isolates.
[f]Internal control isolate included in each test (mefenoxam resistant); mean EC_{50} is based on EC_{50} values obtained in 86 different tests.
[g]Internal control isolate included in each test (mefenoxam sensitive); mean EC_{50} is based on EC_{50} values obtained in 108 different tests.

were obtained by selfing 10 S1 isolates and germinating the resulting oospores. The 10 S1 isolates consisted of two highly sensitive isolates, six intermediately sensitive, and two highly resistant isolates (Table 2). From each of the 10 S1 isolates, 100 S2 single oospore isolates were produced (Table 2).

Mefenoxam Sensitivity Assay

Sensitivity to mefenoxam was determined by growing *P. erythroseptica* isolates on V8 agar plates containing 0, 0.001, 0.01, 0.1, 1.0, 10, and 100 μg mL^{-1} active ingredient of mefenoxam (Ridomil Gold EC; 48% a.i.) suspended in sterile distilled water and added to V8 media before autoclaving. Isolates that demonstrated an EC_{50} value of more than 100 μg mL^{-1} were reevaluated using 1.0, 10, 100, 200, 300μg mL^{-1} of mefenoxam. The assay was performed by placing 4.8 mm agar plugs taken from margins of actively expanding 4- to 5-day-old cultures growing on V8 medium in petri plates. Plates were incubated in the dark at 22 C. Growth of isolates at each concentration was determined by measuring colony diameter in two perpendicular directions on each culture plate after 5 to 7 days of incubation when the control colonies (no mefenoxam) were at least 3.0 cm in diameter. Measurements were averaged, and the diameter of the mycelial plug was subtracted. The relative growth reduction for each concentration of mefenoxam was calculated as follows: [100-(growth with mefenoxam/growth in control plate)*100] (Taylor et al. 2002)

Radial growth, as a percentage of the control, was determined for each isolate at each concentration. There were two replications at each concentration, and two perpendicular measurements of colony diameter were made for each replicate. The trial was performed twice for each isolate. Isolates PE-89 (Maine, 1995, EC_{50} > 100 μg mL^{-1}) and isolate PR-347 (Minnesota 1997, EC_{50} < 0.05 μg mL^{-1}) were included in each test as internal controls.

Data Analysis

The relative growth reduction for each rate of mefenoxam was calculated as follows: [100 - (growth with mefenoxam/growth in control plate)*100]. Each isolate along with internal controls PR-347 (sensitive) and PE-89 (resistant) were evaluated in two independent experiments with two replications for each concentration of mefenoxam. The EC_{50} (concentration causing 50% relative reduction of growth compared to control) was determined for radial growth assays. The EC_{50} value was estimated by plotting the percent inhibition against logarithmic scale of fungicide concentration, producing a typical dose-response curve. The EC_{50} values were determined using a simple linear interpolation between concentrations bracketing 50% sensitivity using Statistical Analysis System software version 8.0 (SAS institute, Inc., Cary, NC). Isolates were scored as sensitive (S) if they exhibited an EC_{50} value of less than 1.0 μg mL^{-1}, as intermediate (I) if they exhib-

TABLE 2—*Summary of EC_{50} values for mefenoxam sensitivity in S2 progeny isolates of* Phytophthora erythroseptica.

S1 Parent	Mean EC_{50} (μg mL^{-1})[a]	Phenotype[b]	S2 progeny isolates[c]	Range of EC_{50} (μg mL^{-1})[d]	Mean EC_{50} (μg mL^{-1})[e]	SD[f]
9942-6-3	0.025	S	9942-6-3 (1-100)	0.045-0.387	0.172	0.116
9942-7-3	0.035	S	9942-7-3 (1-100)	0.025-0.389	0.129	0.117
RB-347[g]	0.043	S	———	0.0009-0.091	0.043	0.022
9927-1-1	173.6	R	9927-1-1 (1-100)	125.0-217.1	156.49	16.47
484-4d-1	178.5	R	484-4d-1 (1-100)	114.6-219.9	169.33	17.58
PE-89[h]	145.7	R	———	115.23-180.26	145.66	16.90
172-3-16	59.9	I	172-3-16 (1-100)	54.4-96.1	81.45	7.95
172-4-56	56.2	I	172-4-56 (1-100)	17.86-86.7	67.15	12.89
230-2-41	32.8	I	230-2-41 (1-100)	4.85-80.85	38.6	13.15
228-3-16	6.3	I	228-3-16 (1-100)	0.70-54.8	10.14	12.00
225-2-59	6.9	I	225-2-59 (1-100)	1.77-88.3	22.24	21.70
252-2-2	11.2	I	252-2-2 (1-100)	0.51-52.9	11.12	10.05

[a]Mean EC_{50} values based on two trials, two replicates each.
[b]R, resistant; S, sensitive; I, intermediate.
[c]100 S2 progeny isolates produced by selfing each S1 parent.
[d]Range of the Mean EC_{50} values (minimum-maximum) for the 100 S2 progeny isolates.
[e]Mean EC_{50} values for the 100 S2 progeny isolates.
[f]Standard deviation of the mean EC_{50} values for the 100 S2 progeny isolates.
[g]Internal control isolate included in each test (mefenoxam resistant); mean EC_{50} is based on 84 EC_{50} values obtained in different tests.
[h]Internal control isolate included in each test (mefenoxam sensitive); mean EC_{50} is based on 84 EC_{50} values obtained in different tests.

ited an EC_{50} value of 1.0-99 μg mL^{-1}, and as resistant (R) if they exhibited an EC_{50} value of 100 μg mL^{-1} or greater (Taylor et al. 2002). Frequencies of phenotypic classes in S1 and S2 progenies were tested for goodness of fit (χ^2- test) to those ratios expected for a Mendelian inheritance of a single gene with incomplete dominance (segregation ratios of 1:2:1, resistant:intermediate:sensitive, respectively). The genetic component of the total variance for each progeny was estimated. The total variance for each progeny isolates tested was calculated using the following equation (Kuehl 1994; Lee et al. 1999):

$$\sigma^2_P = \sigma^2_{error} + \sigma^2_{experiment} + \sigma^2_{progeny} + \sigma^2_{experiment*progeny}$$

The genetic variance of the progeny (σ^2_p) was computed by solving the equation, σ^2_p = MSP - MSEP)/r * a, where MSP is the mean square for progeny, MSEP is the mean square interaction between experiment and progeny, r is the number of replicates within an experiment, and a is the number of experiments. The term σ^2_{error} was the mean square of error (MSR). The term $\sigma^2_{experiment}$ was calculated by solving the equation, $\sigma^2_{experiment}$ = (MSE - MSEP)/r * b, where MSE is the mean square of experiment and b is the number of progeny. The term $\sigma^2_{experiment*progeny}$ was determined by solving the equation, $\sigma^2_{experiment*progeny}$ = (MSEP - MSR)/r. The contribution of the genetic component to total variance was computed. The analysis of variance was performed using the Minitab release 12.1 (Minitab Inc., 1998).

RESULTS

Mefenoxam Sensitivity in S1 Progeny

The S1 progeny isolates were established from the 14 parental isolates (Table 1). A total of 1400 S1 progeny isolates were tested for mefenoxam sensitivity (Table 1). The S1 progeny isolates from the four mefenoxam sensitive isolates (9931-3, 9942-6, 9942-7, and 97431-1) were all sensitive to mefenoxam and none had an EC_{50} value greater than 0.23 μg mL^{-1} (Table 1). No segregation for mefenoxam sensitivity was observed in this set of isolates in the S1 progeny. However, the S1 progeny isolates were generally more sensitive to mefenoxam than the respective parental isolates (data not shown). This case is reflected by the overall population means of EC_{50} values for the S1 progeny isolates being lower than the mean EC_{50} values of the parental isolates (Table 1).

The S1 progeny isolates from the four mefenoxam-resistant isolates (9927-1, 484-4d, 9926-7, and 9941-1) were all mefenoxam resistant with EC_{50} values greater than 100 μg mL^{-1} (Table 1). The S1 progeny from resistant isolates did not segregate for mefenoxam sensitivity. However, in most cases, the S1 progeny isolates demonstrated higher levels of resistance to mefenoxam than did the respective parental isolates (data not shown). This is also reflected by the overall population means of EC_{50} values for the S1 progeny isolates being higher than the mean EC_{50} values of the parental isolates (Table 1).

TABLE 3—*Inheritance of mefenoxam resistance in mefenoxam intermediately resistant isolates of* Phytophthora erythroseptica.

Parental isolate(s)	Generation	No. of single oospore isolates[a] Sensitive	Intermediate	Resistant	Expected ratio[b]	χ^2 Value	df	*P*
172-3	S1	0	100	0	1:2:1	100.00**C	2	<0.00000001
	S2	0	100	0	1:2:1	100.00**	2	<0.00000001
172-4	S1	0	99	1	1:2:1	96.06**	2	<0.00000001
	S2	0	100	0	1:2:1	100.00**	2	<0.00000001
252-2	S1	4	96	0	1:2:1	84.96**	2	<0.00000001
	S2	3	97	0	1:2:1	88.40**	2	<0.00000001
225-2	S1	0	100	0	1:2:1	100.00**	2	<0.00000001
	S2	0	100	0	1:2:1	100.00**	2	<0.00000001
230-2	S1	0	100	0	1:2:1	100.00**	2	<0.00000001
	S2	0	100	0	1:2:1	100.00**	2	<0.00000001
228-3	S1	5	94	1	1:2:1	77.76**	2	<0.00000001
	S2	4	96	0	1:2:1	84.9**	2	<0.00000001

[a]*P. erythroseptica* isolates phenotypic response to mefenoxam based on the estimated EC_{50} values; sensitive: EC_{50} value <1.0 μg mL^{-1}; intermediate EC_{50} value of 1.0-99 μg mL^{-1}; resistant EC_{50} value of 100 μg mL^{-1} or more.

[b]Expected ratios (1 resistant:2 intermediate:1 sensitive) are based on Goodwin and McGrath (1995) proposed model of single incompletely dominant gene control of mefenoxam resistance.

[c]Significantly different from the expected ratio at 95% confidence level ($P = 0.05$).

The S1 progeny isolates derived from mefenoxam intermediately resistant parents (Tables 1 and 3) demonstrated a broad spectrum of sensitivity to mefenoxam (Figure 1). The EC_{50} values of the intermediate parents ranged between 4.0 and 62.0 μg mL^{-1} (Table 1). The S1 progeny from the parental isolates 225-2, 230-2, and 172-3 (Figure 1 A, D, and E) were all intermediately resistant; the EC_{50} values of the progeny isolates were normally distributed around the means at 95% confidence level. The S1 progeny from the parental isolate 172-4 were also intermediately resistant except for one isolate (172-4-12), which was resistant to mefenoxam with mean EC_{50} of 170.5 μg mL^{-1} (Figure 1 F; Table 3). The mean EC_{50} values of the progeny isolates from this parent were normally distributed around the means at 95% confidence level (Figure 1 F). The majority of S1 progeny isolates (94%) from parent 228-3 were intermediately resistant (Table 3); five isolates were mefenoxam sensitive (228-3-11, 228-3-40, 228-3-44, 228-3-91, 228-3-97) with mean EC_{50} values of 0.8, 0.9, 0.5, 0.8, 0.7 μg mL^{-1}, respectively (data not shown). The mean EC_{50} values of the progeny isolates from this parent were normally distributed around the mean at 95% confidence level. Similarly, the majority of S1 progeny isolates from parent 252-2 (96%) were intermediately resistant. Four isolates from this progeny (252-2-37, 252-2-52, 252-2-73, and 252-2-75) demonstrated mean EC_{50} values of 0.7, 0.9, 0.8, and 0.9 μg mL^{-1}, respectively indicative of mefenoxam sensitivity (data not shown). The genetic components of variance in S1 intermediately resistant progeny isolates were large (Table 4), suggesting that the majority of variance in the mefenoxam sensitivity assays was due to the progeny isolates rather than to the experiments or replication within each experiment (Table 4).

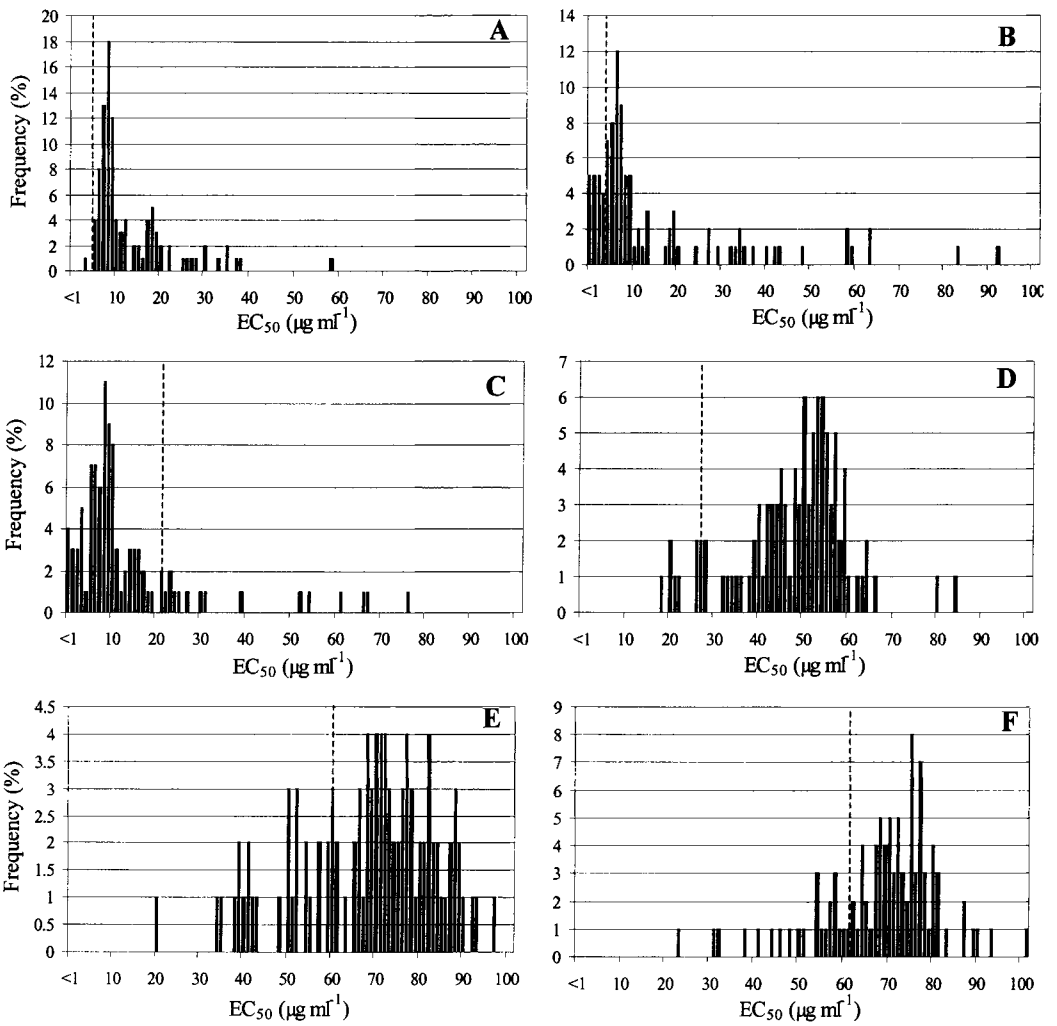

FIGURE 1.
Frequency distribution of mefenoxam sensitivity in S1 progeny isolates of *Phytophthora erythroseptica*, A (225-2), B (228-3), C (252-2), D (230-2), E (172-3) F (172-4), derived from field isolates with intermediate responses to mefenoxam. (EC_{50} values of the parental isolates are indicated by vertical broken lines.)

TABLE 4—*Analysis of variance for mefenoxam resistance in* Phytophthora erythroseptica *intermediate progeny isolates of S1 and S2 populations and isolation of variance components.*

Progeny isolates	Source of variation	Degrees of freedom	Mean squares	% σ^2_p [a]
172-3 (S1)	Exp	1	522.1	
	Isolate	99	971.5	88.01
	Exp* Isolate	99	37.0	
	Error	200	21.8	
172-4 (S1)	Exp	1	589.5	
	Isolate	99	1052.5	92.41
	Exp* Isolate	99	23.1	
	Error	200	13.5	
252-2 (S1)	Exp	1	19.72	
	Isolate	99	848.02	96.94
	Exp* Isolate	99	8.88	
	Error	200	4.42	
225-2 (S1)	Exp	1	34.60	
	Isolate	99	341.56	91.88
	Exp* Isolate	99	9.51	
	Error	200	4.92	
230-2 (S1)	Exp	1	133.06	
	Isolate	99	570.61	79.49
	Exp* Isolate	99	41.39	
	Error	200	25.96	
228-3 (S1)	Exp	1	3.3	
	Isolate	99	1371.6	96.58
	Exp* Isolate	99	16.1	
	Error	200	8.0	
172-3-16(S2)	Exp	1	127.29	
	Isolate	99	253.18	63.16
	Exp* Isolate	99	39.22	
	Error	200	22.29	
172-4-56 (S2)	Exp	1	0.45	
	Isolate	99	664.97	76.47
	Exp* Isolate	99	53.09	
	Error	200	41.57	
252-2-2 (S2)	Exp	1	8.56	
	Isolate	99	403.90	87.63
	Exp* Isolate	99	18.24	
	Error	200	9.08	
225-2-59 (S2)	Exp	1	406.20	
	Isolate	99	1887.00	96.39
	Exp* Isolate	99	19.80	
	Error	200	11.30	
230-2-41 (S2)	Exp	1	277.06	
	Isolate	99	692.01	84.53
	Exp* Isolate	99	38.05	
	Error	200	19.39	
228-3-16 (S2)	Exp	1	10.44	
	Isolate	99	582.12	94.99
	Exp* Isolate	99	10.06	
	Error	200	5.01	

[a]Proportion of total variance due to genetic effects.

Mefenoxam Sensitivity in S2 Progeny

The S2 progeny isolates from the two mefenoxam-sensitive S1 parental isolates (9942-6-3 and 9942-7-3) were all sensitive to mefenoxam (Table 2). No segregation was observed among the 200 S2 progeny isolates for mefenoxam sensitivity. Likewise, the S2 progeny isolates from the mefenoxam-resistant S1 parents (9927-1-1 and 484-4d-1) did not segregate for mefenoxam sensitivity and all were mefenoxam resistant (Table 2).

The S2 progeny isolates from the six intermediately resistant S1 parents demonstrated a wide range of mefenoxam sensitivity based on the calculated EC_{50} values (Table 2; Figure 2). The S2 progeny isolates from the S1 parents 225-2-59, 230-2-41, 172-4-56 and 172-3-16 were all intermediately resistant to mefenoxam (Figure 2 B, D, E, F). No segregation to mefenoxam sensitivity was observed among the 400 S2 oospore isolates from these S1 parents (Table 3). However, as observed in S1 progeny isolates, S2 progeny isolates demonstrated higher EC_{50} values indicative of an increased level of mefenoxam resistance compared to the S1 parents. While the majority of the S2 progeny isolates from the S1 parents 228-3-16 and 252-2-2 were intermediately resistant to mefenoxam (Figure 2 A and C), four S2 isolates from the S1 parent 228-3-16 and three S2 isolates from the S1 parent 252-2-2 demonstrated EC_{50} values of less than 1.0 μg mL^{-1}, indicative of mefenoxam sensitivity (Table 3). As in S1 progeny isolates, partitioning the variance in the mefenoxam sensitivity assays to its components (Table 4) demonstrated that the majority of the variance was due to the progeny isolates (Table 4).

DISCUSSION

Results from this study demonstrated that inheritance of metalaxyl/mefenoxam resistance in *P. erythroseptica* does not fit any of the previously described models proposed for the genus *Phytophthora* (Bhat et al. 1993; Chabane et al. 1996; Chang and Ko 1990; Goodwin and McGrath 1995; Lee et al. 1999; Shattock 1988). Previous studies examined this aspect in *Phytophthora infestans* (Shattock 1988; Lee et al. 1999) also produced contrasting conclusions. Shattock (1988) suggested that resistance to metalaxyl in *P. infestans* was governed by a single nuclear locus exhibiting incomplete domi-

nance. In contrast, Lee et al. (1999) suggested that resistance to metalaxyl is conferred by a single dominant gene and that variation in progeny sensitivity is due to the effect of minor genes. Goodwin and McGrath (1995) hypothesized that insensitivity to metalaxyl in *P. erythroseptica* is conditioned by a single incompletely dominant gene, with the sensitive isolates being homozygous for the sensitivity allele, moderately resistant isolates being heterozygous and resistant isolates being homozygous for the resistance allele. However, the Goodwin and McGrath (1995) hypothesis was not tested by experimentation and was based on the observation that intermediately resistant isolates produced "faster growing" sectors that were metalaxyl resistant.

In our studies, the lack of segregation for mefenoxam resistance in S1 and S2 generations from the resistant and sensitive parents was anticipated, since these phenotypes have been described to be true breeds for this trait in other *Phytophthora* species (Shattock 1988; Fabritius et al. 1997). However, the lack of segregation for mefenoxam resistance in the intermediately resistant isolates in S1 and S2 progeny was not expected. Except for a few isolates, the majority of S1 and S2 progeny isolates from parents with an intermediate level of resistance to mefenoxam were also intermediately resistant. However, in most cases, there were major quantitative shifts in response to mefenoxam among the S1 and S2 progeny isolates. These shifts occurred on both sides of the mefenoxam frequency distribution and in many cases the response of the S1 and S2 progeny isolates from the intermediate class assumed a normal distribution around the parental mean. These results are indicative of quantitative trait genetics.

It has been suggested that resistance to metalaxyl in *P. infestans* may be governed by two major loci (*MEX* 1 and

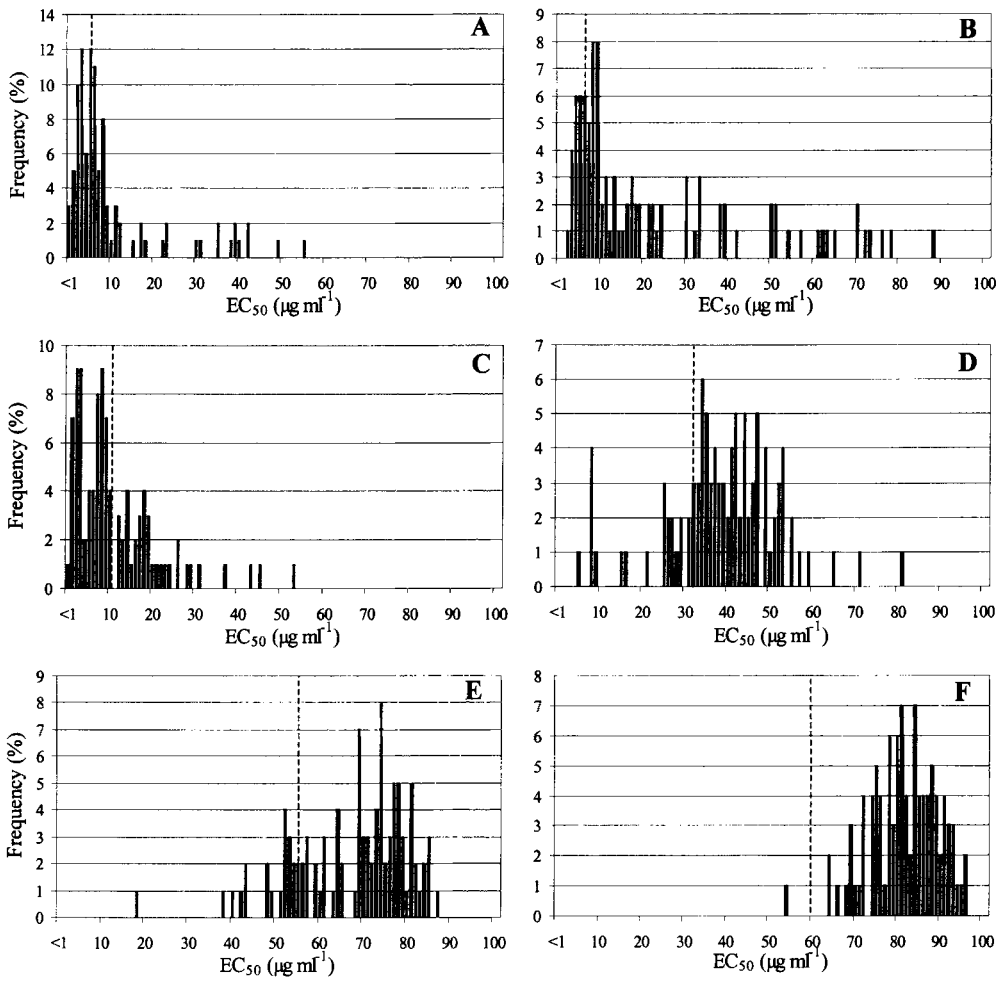

FIGURE 2.
Frequency distribution of mefenoxam sensitivity in S2 progeny isolates of *Phytophthora erythroseptica*, A (228-3-16), B (225-2-59), C (252-2-2), D (230-2-41), E (172-4-56) F (172-3-16), derived from S1 parents with intermediate responses to mefenoxam. (EC_{50} values of the S1 parents are indicated by vertical broken lines.)

MEX 2) and other minor loci contributing additive effects (Fabritius et al. 1997). However, segregation analysis indicated that these two major loci have different chromosomal positions in different isolates of *P. infestans* (Fabritius et al. 1997). In another study, Judelson and Roberts (1999) suggested that resistance to metalaxyl in *P. infestans* is inherited as a quantitative trait in which major *MEX* loci interact with genes of minor effect. They also suggested that allelic differences exist at the *MEX* locus that perhaps contributes to the quantitative variation in metalaxyl sensitivity between different *P. infestans* isolates (Judelson and Roberts 1999).

In the case of *P. infestans*, where two independent loci control resistance to metalaxyl (Judelson and Roberts 1999), it is most likely that these two loci arose from two independent mutations. Thus, if resistance to metalaxyl occurs because of mutation at one or more chromosomal loci, then it is possible that accumulation of more than one mutation would lead to polygenetic control of this trait. If this is occurring in *P. erythroseptica*, it would make the analysis more complicated, considering the genetic relationship between the major loci and minor loci and the possible combinations of homozygosity and heterozygosity at each locus.

The viability of heterozygosity in wild populations of *P. erythroseptica* would be a matter of speculation. Unfortunately, genetic work with this organism is scarce and very little research or information on heterozygosity exists for any *Phytophthora* species, with the exception of *P. infestans*, which is a heterothallic oomycete with a different mating system. However, Goodwin (1997) questioned the idea that homothallic *Phytophthora* species would have low incidence of heterozygosity as compared to heterothallic species due to self-fertilization. Goodwin (1997) reported that some homothallic *Phytophthora* spp. (without specifying which species) had an intermediate fixation index value (near 0.5) and another heterothallic group had fixation index values similar to those of homothallic species. This analysis was done on previously published information, and Goodwin (1997) concluded that many *Phytophthora* species probably have a mixed mating system in nature and cannot be predicted on the basis of heterothallism or homothallism. Goodwin (1997) also indicated that this conclusion was not conclusive and a larger analysis was needed to confirm these findings. The preliminary conclusion from this study is that homothallisim or heterothallism is not the only determinant of the level of homozygoisity or heterozygosity in natural populations of *Phytophthora* spp.

In an effort to investigate the occurrence of major *MEX* loci in *P. erythroseptica*, bulk segregant analysis (Michelmore et al.1991) was used to identify molecular markers that are linked to these putative loci (F.M. Abu-El Samen and N.C. Gudmestad unpublished). One hundred-three RAPD markers, including three markers identified previously by Fabritius et al. (1997) to be linked to *MEX* loci in *P. infestans*, and 16 AFLP primer pairs were used to screen two DNA bulks from resistant and sensitive isolates. In addition, individual sensitive and resistant isolates were included in the analysis. Unfortunately, no major genotypic differences could be identified between the sensitive and resistant isolates with these markers (F.M. Abu-El Samen and N.C. Gudmestad unpublished). However, if such major loci do exist in *P. erythroseptica*, we believe that additional molecular markers would be required to reveal them.

Alternatively, inheritance and expression of mefenoxam resistance in *P. erythroseptica* could be governed by mechanisms similar to that described for the *Qo*I fungicides (Heaney et al. 2000). Two point mutations in the cytochrome *b* gene confer resistance to *Qo*I fungicides, the G143A and the F129L mutations. The G143A mutation has been detected in a number of plant pathogenic fungi including the oomycetes *Plasmopara viticola* Savul. (Heaney et al. 2000) and *Pseudoperonospora cubensis* (Berk. & Curtis) Rostovxev (Ishii et al. 2001) and the F129L mutation has been detected in *Pythium aphanidermatum* (Edson) Fitzp. (G. Olaya pers comm). The G143A mutation is much more commonly detected than the F129L mutation. The expression of these two mutations, relative to the degree of resistance to *Qo*I fungicides, is not equal. For instance in *Pyricularia grisea* (Cooke) Sacc., the resistance factor in isolates possessing the G143A mutation is >100 and in those isolates possessing the F129L mutation it is <20 (Vincelli and Dixon 2002). Therefore, we should not assume that any major gene or genes conferring resistance to mefenoxam in *P. erythroseptica* are expressed with equal intensity, even without the presence of minor or modifying genes that may affect the overall expression of resistance. Rather, it is possible that intermediately resistant isolates of *P. erythroseptica* possess a gene that only conveys an intermediate level of resistance compared to fully resistant isolates that may possess another gene. This would explain the lack of segregation we observed among S1 and S2 progeny isolates of *P. erythroseptica* that exhibited intermediate levels of resistance to mefenoxam.

Results from this study demonstrated that inheritance of mefenoxam resistance does not fit to a single gene mode of inheritance as suggested by Goodwin and McGrath (1995), regardless of whether this gene displayed complete or incomplete dominance. This argument is supported by data obtained from S1 and S2 progeny isolates. In three out of six parental isolates from the intermediately resistant class, no segregation was observed for metalaxyl resistance in either S1 or S2 progeny isolates. In the other three intermediate parents, the segregation ratios were distorted and do not fit to any known Mendelian ratios.

One would expect some segregation to be observed at least in the S2 progeny isolates if a major gene is controlling resistance to metalaxyl. However, the segregant isolates observed in this study were mostly sensitive isolates and were derived from parental isolates that exhibited low levels of mefenoxam resistance (isolates 252-2, EC_{50} = 22.0 μg mL^{-1} and 228-3, EC_{50} = 4.0 μg mL^{-1}). When the phenotypic responses of these segregants were examined carefully using EC_{50} values, they were found to be a segment in the continuum of EC_{50} values we obtained. The EC_{50} values ranged from 0.5 to 92.0 μg mL^{-1} in S1 and from 0.7 to 54.8 μg mL^{-1} in S2 for isolate 228-3. Similarly, the EC_{50} values of progeny isolates from isolate 252-2 have a range of 0.7-76.0 μg mL^{-1} in S1 isolates and 0.51-52.9 μg mL^{-1} in S2 progeny isolates. These results suggest that the sensitive segregants were part of quantitative shifts in response to mefenoxam and did not occur because of major gene-segregation events. The only exception to the aforementioned circumstance is an S1 isolate that demonstrated resistance to mefenoxam with an EC_{50} value of 170.0 μg mL^{-1} (isolate 172-4-12). Unfortunately, this isolate was lost during storage and could not be used for further verification or production of S2 progeny.

In this study, the EC_{50} values have been used to categorize isolates into three phenotypic classes: sensitive, intermediately resistant, and resistant. This method has been used by others to sort the responses of *P. erythroseptica* field isolates to mefenoxam (Gudmestad et al. 2000; Taylor et al. 2002; Peters et al. 2001, 2003). A major difficulty in analyzing inheritance data based on an arbitrary range is the lack of a clear-cut distinction between the three phenotypic classes in response to mefenoxam. The intermediate category, which includes isolates that have an EC_{50} value of 1.0-99 μg mL^{-1}, would contain isolates that have approximately 100-fold difference in response to mefenoxam based on the calculated EC_{50} values. Such a broad range of responses to mefenoxam has been observed among progeny isolates from the intermediate parents in both S1 and S2 generations.

In our attempts to find a better method to categorize isolates into different mefenoxam sensitivity phenotypes, we evaluated another scheme, such as the one described by Shattock (1988) for *P. infestans*. This scheme defines sensitive, intermediate and resistant phenotypes on an arbitrary range as exhibiting <10%, 10% to 60%, and >60% growth, respectively, at metalaxyl-amended media (10 μg mL^{-1}) relative to growth on metalaxyl-free medium. Applying this scheme to our data did not produce a significant difference in the observed segregation ratios, nor did the application of a *P. sojae* categorization scheme (Bhat et al. 1993) or the system used by Goodwin et al. (1996) for *P. infestans*. However, it is important to point out that these categorization schemes are also arbitrary in nature and differ considerably in their ability to distinguish resistant and intermediately resistant categories.

In conclusion, results from the current study do not support the initial hypothesis proposed by Goodwin and McGrath (1995) regarding the genetic control of metalaxyl/mefenoxam resistance in *P. erythroseptica*. The lack of segregation for mefenoxam resistance in S2 progeny isolates from the intermediately resistant phenotype supports our ambiguity of the Goodwin and McGrath (1995) proposition. Results presented here also provide some evidence that the genetics of mefenoxam resistance in *P. erythroseptica* is probably under the control of more than one major gene and perhaps some minor genes of additive effect. In most cases, mefenoxam response of the S1 and S2 progeny isolates from the intermediately resistant parents assumed a continuum of EC_{50} values similar to phenotypic responses of traits under polygenetic control. Based on the results of this study, we predict that mefenoxam resistance in *P. erythroseptica* will continue to increase in magnitude over time, as we observed quantitative shifts in the intermediately resistant progeny toward higher levels of insensitivity. We expect that sensitive isolates will continue to exist and will not disappear entirely even if mefenoxam is not used on all fields cropped to potato.

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