Sensitivity of North American Isolates of *Phytophthora erythroseptica* and *Pythium ultimum* to Mefenoxam (Metalaxyl)

R. J. Taylor, B. Salas, G. A. Secor, V. Rivera, and **N. C. Gudmestad,** Department of Plant Pathology, North Dakota State University, Fargo 58105

ABSTRACT

Taylor, R. J., Salas, B., Secor, G. A., Rivera, V., and Gudmestad, N. C. 2002. Sensitivity of North American isolates of *Phytophthora erythroseptica* and *Pythium ultimum* to mefenoxam (metalaxyl). Plant Dis. 86:797-802.

A 4-year study (1997 to 2000) was conducted to determine the sensitivities of the potato tuber rot pathogens, *Phytophthora erythroseptica* and *Pythium ultimum*, to mefenoxam (metalaxyl). A total of 2,277 tubers showing symptoms of "water rot" were collected from 16 states and 2 Canadian provinces. From these, 849 isolates of *P. erythroseptica* and 213 isolates of *P. ultimum* were obtained, and 805 and 190 isolates, respectively, were tested for their ability to grow on V8 medium amended at 0.01 to 100 µg/ml with fungicide. Isolates ranged widely in their responses to mefenoxam. The presence of resistant isolates (EC_{50} >100 µg ml^{-1}) of *P. erythroseptica* in the potato producing areas of Maine was confirmed. The presence of *P. erythroseptica* isolates in Idaho and Minnesota resistant to mefenoxam is reported for the first time. The proportion of *P. erythroseptica* isolates resistant to mefenoxam varied from 2.9 to 36.2% between 1997 and 2000. The proportion of resistant *P. ultimum* isolates represented only a small proportion of the isolates tested (3.7%). A single resistant *P. ultimum* isolate was recovered from Washington, whereas most of the resistant isolates obtained (5 of 7) were collected in Minnesota during the final year of the study. This is the first report of resistance in *P. ultimum* pathogenic to potato tubers. These observations suggest that pink rot and leak could become significant problems in the future, particularly in those areas where resistance has been detected. Our results have implications for the effective management of water rot. Monitoring the sensitivity of the pathogen population to mefenoxam in all production areas should be considered and integrated as a part of the overall disease management strategy.

Additional keywords: fungicide resistance, leak, pink rot, potato, Ridomil, *Solanum tuberosum*

Since their introduction in the late 1970s, phenylamide fungicides have been used effectively to control diseases caused by the Peronosporales. These fungicides have a very specific mode of action; inhibiting fungal ribosomal RNA polymerases (7). Because of this specificity, there is a relatively high risk that resistance to the fungicide may appear quickly in the target fungal population (3). Phenylamides were used initially as a straight product (unaccompanied by a second fungicide) and as a result, within 2 years of their introduction, insensitive isolates of *Pseudoperonospora cubensis* were collected from cucumber in Israel (33), and isolates of *Phytophthora infestans* insensitive to metalaxyl also were found in potato crops in Ireland (9) and the Netherlands (8). A short time later, resistance was documented in a variety of Oomycete species from diverse crops (grapes, lettuce, tobacco, and turf). Morton and Urech (25) reviewed these and other reports of the initial development of resistance to this class of fungicides.

Phenylamide fungicides have been used primarily to control foliar diseases since the initial Environmental Protection Agency (EPA) conditional registration of metalaxyl on tobacco in 1980 (25). Resistance to metalaxyl was first found in North America in 1984 when this fungicide failed to control Pythium turf blight in Pennsylvania (35). Most of the information gathered on resistance in North America has come from studies involving foliar pathosystems. As phenylamides lost their effectiveness in controlling some foliar diseases, their use against soilborne diseases has increased. This poses a potential problem since resistance probably would develop under these conditions as well (41), although this is likely to occur at a slower rate due to the monocyclic nature of most soilborne pathogens. Intrasite and intersite movement of resistance also should be slower in this group.

Two important soilborne diseases of potato, pink rot and leak, often collectively known as "water rot", are caused by the Oomycetes, *Phytophthora erythroseptica* and *Pythium ultimum*, respectively. Either disease can be found during or immediately following harvest, especially in areas and in years with high moisture (14,20). Pink rot and leak can be widely distributed in potato fields and storage bins (31,36). Strategies commonly used to manage water rots include crop rotation, planting the potato crop in well-drained soils, avoiding excessive irrigation at the end of the growing season, modifying handling procedures to reduce wounding, and applications of mefenoxam (31,36). Most commonly grown potato cultivars are susceptible to *P. erythroseptica* and *Pythium ultimum* (authors, *unpublished data*) so resistance is not currently a practical control measure for the water rot diseases.

Mefenoxam (Ridomil Gold EC and Ultrafluorish EC) is the only fungicide used to control pink rot and leak (26,43). The effectiveness of this fungicide may be compromised if insensitive isolates of *P. erythroseptica* and *Pythium ultimum* become widespread. Currently, mefenoxam-resistant isolates of *P. erythroseptica* are known to be present in Maine, New York, and Idaho (13,15,23). This and recent outbreaks of pink rot in the Red River Valley of North Dakota and Minnesota (24) have caused concern among growers and industry representatives. Aside from our preliminary reports (15,16,34,38), a thorough survey of the incidence of resistance to phenylamide fungicides in populations of the water rot fungi has not been conducted. The objective of this study was to determine the level of sensitivity to mefenoxam in isolates of *P. erythroseptica* and *Pythium ultimum* collected throughout North America and to monitor the distribution of these isolates.

MATERIALS AND METHODS

Tuber samples and isolations. Tubers with symptoms of water rot were collected from commercial potato fields and storages, and shipped to our laboratory for analysis. Isolations were made by placing small pieces (4 × 4 mm) of infected tissue in petri dishes containing water agar amended with ampicillin (100 µg ml^{-1}), and incubated in darkness at 17 to 20°C for 3 to 5 days. Fungal colonies with mycelia resembling those of *P. erythroseptica* or *Pythium* spp. were selected and purified by hyphal tipping. All isolates were identified based on morphological characteristics given by Ho and Jong (17) for *P. erythroseptica* and the key for *Pythium* spp. found in Plaats-Niterink (28). A number of

Corresponding Author: Neil C. Gudmestad
E-mail: Neil.Gudmestad@ndsu.nodak.edu

Current address of second author: Department of Plant Pathology, University of Minnesota, St. Paul, 55108.

Accepted for publication 8 March 2002

Publication no. D-2002-0503-01R

isolates from California, Oregon, and Washington were received in 1999 as a group collection made by P. Hamm (Oregon State University, Corvallis) between 1996 and 1998. The selective medium P_5ARPH (19) was used to isolate *P. erythroseptica* from badly rotted tubers or from tuber samples arriving in late fall or winter. Isolates of *P. erythroseptica* were maintained on clarified V8 juice agar medium (10% V8 juice filtered through four layers of cheesecloth, 0.1% $CaCO_3$ and 2% agar) and those of *Pythium* spp. were kept on corn meal agar (CMA).

Fungicide sensitivity testing. The effect of metalaxyl (Ridomil 2E WPS) in 1997 and mefenoxam (Ridomil Gold 4EC) in 1998 to 2000 on mycelial growth in vitro was determined on modified V8 juice agar (5% V8 juice filtered through four layers of cheesecloth and 2% agar). Fungicide was incorporated into the medium in a 10-fold dilution series ranging from 0.1 to 100 μg ml^{-1} (1997 and 1998) and 0.01 to 100 μg ml^{-1} (1999 and 2000) before autoclaving. The test culture plates were stored in darkness at room temperature (20 to 25°C) for 3 days prior to use to minimize photodegradation of the fungicide. Isolates with 50% effective concentration (EC_{50}) values greater than 100 μg ml^{-1} were retested up to 300 μg ml^{-1} in follow-up trials in 1999 and 2000.

Mycelial plugs, 5 mm in diameter, were cut from the margin of actively growing colonies of 4- to 6-day-old cultures of *P. erythroseptica* on clarified 10% V8 juice agar as described above. One plug was positioned in the center of a 9-cm culture dish with the mycelium in contact with the medium. Duplicate culture dishes, each containing 17 ml of test medium, were used to test each isolate at each concentration. In the tests of *Pythium ultimum* isolates, 48-h-old cultures were used. Pathogenicity of the *Pythium* isolates was determined prior to testing and only those isolates capable of infecting potato tubers were tested for their sensitivity to mefenoxam.

Growth of isolates at each concentration was determined by measuring colony diameters in two perpendicular directions on each culture plate after 6 days (*P. erythroseptica*) and 36 h (*Pythium ultimum*) of incubation in darkness at 20 ± 1°C. Measurements were averaged, and the diameter of the mycelial plug was subtracted (30,41). The relative growth reduction for each rate of fungicide was calculated as follows: [100 – (growth with fungicide/growth in control plate)*100]. The concentration causing 50% relative reduction of mycelial growth (EC_{50}) compared to the control was estimated by plotting the percent inhibition against the log-scale of fungicide concentration (1997 and 1998) or by regression curve fitting (1999 and 2000). Individual sensitivity curves were generally sigmoid in shape unless the isolates were highly insensitive to the fungicide. Therefore, data sets were fit to nonlinear functions such as the logistic and Gompertz. Only the Gompertz function yielded solutions on a consistent basis. The Gompertz model is a 3 parameter sigmoidal function with the general form

$$y = \alpha\{\exp[-\exp(\beta - \gamma * x)]\}$$

It has frequently been used in population studies and to model growth in animals and plants (10). Each of the parameters, or some monotonic transformation thereof, relates to aspects of the sigmoidal curve (32). As such, α is associated with the asymptote or maximum value, β is associated with the *y*-intercept, and γ relates to the rate at which the response increases as one proceeds from the initial value (β) to the final value (α). Once obtained, data parameters were input into the Gompertz function and the EC_{50} was estimated by solving for the concentration at which 50% sensitivity would occur. In those cases where the Gompertz function would not yield estimates, a simple linear interpolation between the two concentrations bracketing 50% sensitivity was performed to estimate the EC_{50}.

Comparative isolates. Mefenoxam sensitivity of 87 isolates of *P. erythroseptica* obtained from the culture collection of Robert W. Stack (Department of Plant Pathology, North Dakota State University, Fargo) was assessed during the final year of the survey. These isolates were collected at various locations approximately 5 years prior to the start of the current project (1991 and 1992) and were reported previously (38).

RESULTS

Survey. During the course of this study (1997 to 2000), a total of 2,277 tubers were obtained from 16 states and 2 Canadian provinces. Much of this material came from problem areas in fields treated with metalaxyl/mefenoxam. Isolates of *P. erythroseptica* were collected from 47.3% of the infected tubers, and 36.6% of the samples yielded isolates of *Pythium ultimum*. Tubers exhibiting symptoms of multiple infections were encountered during the survey, and pathogens such as *Fusarium* spp, *Verticillium* spp., *Pythium* spp., and various other "contaminating" microorganisms were occasionally recovered but these were discarded without further documentation. Based on symptomology, *P. infestans* was undoubtedly present in some multiple infections but attempts were not made to recover that pathogen. A total of 849 isolates of *P. erythroseptica* and 213 isolates of *Pythium ultimum* were recovered from pink rot (1,750) and leak (527) infected tubers, respectively. Of these, 805 isolates of *P. erythroseptica* and 190 isolates of *Pythium ultimum* were subsequently tested for their sensitivity to metalaxyl/mefenoxam.

Sensitivity of *P. erythroseptica*. Of the 69 isolates of *P. erythroseptica* tested in 1997, only two with EC_{50} values ≥100 μg ml^{-1} were identified (Table 1). These represented 2.9% of isolates tested and both were from Maine. The remaining 97.1% of the isolates fell into a wide grouping with EC_{50} <1.0 μg ml^{-1}. No isolates with EC_{50} values between 1.0 μg ml^{-1} and 99.9 μg ml^{-1} were recovered in 1997.

A total of 137 *P. erythroseptica* isolates were tested in 1998. Most of the isolates, representing 83.9% of the total population from all sites, fell within the group with EC_{50} values in the <1.0 μg ml^{-1} range. As in the previous year, eight isolates from Maine had EC_{50} values higher than 100 μg ml^{-1}. However, nine isolates from Idaho with EC_{50} values greater than 100 μg ml^{-1} were found for the first time. Isolates with high EC_{50} values (>100 μg ml^{-1}) from these two states represented 12.4% of the total number tested in 1998. A small portion of the Maine and Idaho isolates (3.6%) had EC_{50} values ranging between 10.0 μg ml^{-1} and 99.9 μg ml^{-1} (Table 1).

A total of 232 isolates were tested for mefenoxam sensitivity in 1999. The results confirmed the presence of isolates with high EC_{50} values (in excess of 100 μg ml^{-1}) in Idaho and Maine. In 1998, 19.6% of isolates from Idaho and 61.5% isolates from Maine fit this grouping. These proportions increased to 53.6 and 75.0%, respectively, in 1999. Isolates with high EC_{50} values represented 27.2% of all isolates received from seven states that year. In contrast, more that half of the isolates (71.9%) tested had EC_{50} values less than 1.0 μg ml^{-1}, but this represented a lower proportion of the total population relative to 1998.

In the final year of this study, we tested more isolates than in the first 3 years combined. Of these, the majority was received from Idaho and Minnesota. Again, isolates with EC_{50} values greater than 100 μg ml^{-1} were obtained from Maine (60.0%) and Idaho (43.2%), but 18 isolates with very high EC_{50} values from Minnesota were identified for the first time. This represented a substantial proportion (21.4%) of the isolates tested from that state. The proportion of the total population with EC_{50} values in the lower grouping (<1.0 μg ml^{-1}) was considerably lower in the latter 2 years of this study as sensitivity to mefenoxam shifted toward the ≥100 μg ml^{-1} group (36.2% of all isolates tested). More of the intermediate types (9.3% of all isolates tested) were found than in the previous 3 years. All of these came from the three states having isolates with high EC_{50} values (Idaho, Maine, and Minnesota). Upon reassay, the sensitivities of isolates with EC_{50} values ≥100 μg ml^{-1} were found to range between 127 μg ml^{-1} and 184 μg ml^{-1} mefenoxam in the 1999 population and from 109 μg ml^{-1} to 205 μg ml^{-1} in the 2000 population.

Sensitivity of comparative isolates. None of the isolates collected in 1991 and 1992 was resistant to mefenoxam (Table 2). With the exception of a single isolate from Washington that fit into the lowest ranking category of the intermediate group (EC_{50} = 1.56 μg ml^{-1}), all of the isolates tested were sensitive to mefenoxam. Nearly 30% of the isolates were highly sensitive, falling into the group with EC_{50} values $\leq$0.05 μg ml^{-1}. This group represents a much greater proportion of the sample population than observed in the samples collected during the 4 years of this study. The proportion of highly sensitive isolates present in the population sampled during that time ranged from 7.3% (1998 and 1999) to 10.1% (1997) (Table 1).

Sensitivity of *Pythium ultimum*. Only seven isolates of *Pythium ultimum* pathogenic to potato were recovered in the initial year of this study (1997), and because of the range of the dilutions selected to assess sensitivity we did not determine their exact EC_{50} values that year. These values were below 1.0 μg ml^{-1} because none of the isolates was able to grow at this concentration. During the 3 years of this study, 1998 to 2000, 190 isolates of the *Pythium ultimum* were screened, most of which (94.2%) had EC_{50} values <1.0 μg ml^{-1} (Table 3). A single isolate from Idaho with an EC_{50} >100 μg ml^{-1} was found in 1998, but none was found in the 41 isolates tested in 1999. In 2000, six resistant isolates were found in the population of 93 tested. Of these, five came from Minnesota and one from Washington. The Washington isolate had an EC_{50} of 101 μg ml^{-1} and all five of the isolates from Minnesota tested were highly resistant to mefenoxam (EC_{50} >300 μg ml^{-1}). Resistance was not observed in the isolates recovered from Washington during 1998 and 1999, and only two isolates fitting the intermediate group were obtained from that state prior to 2000.

DISCUSSION

Successful implementation of any fungicide resistance management program must include an assessment of the initial level of fungicide sensitivity, or "baseline" in the pathogen population. Numerous studies involving a variety of fungal pathogens have demonstrated that isolates can range widely in their responses to phenylamide fungicides (2,5,6,11–13,18,27,39,40,42). The 1,082 isolates tested in this study also varied markedly in their sensitivities to mefenoxam. Some isolates were extremely sensitive (EC_{50} values below 0.05 μg ml^{-1}), whereas others were extremely resistant (EC_{50} >100 μg ml^{-1}), and readily grew on

Table 1. Distribution and ranking of EC_{50} values of *Phytophthora erythroseptica* isolates collected during a survey (1997 to 2000) of infected tubers obtained from the United States and Canada

		Distribution of isolate EC_{50} values (μg ml^{-1})							
		Sensitive				Intermediate			Resistant
Year and source	**No. isolates tested**	**≤0.05**	**0.06-0.1**	**0.11-0.15**	**0.16-0.99**	**1.0-1.99**	**2.0-9.9**	**10.0-99.9**	**≥100**
1997									
Idaho	16	1	8	6	1	0	0	0	0
Maine	3	0	1	0	0	0	0	0	2
Minnesota	22	3	9	9	1	0	0	0	0
New Brunswick, Canada	4	0	0	1	3	0	0	0	0
North Dakota	24	3	8	11	2	0	0	0	0
Totals for year	69	7	26	27	7	0	0	0	2
Frequency (%)		(10.1)	(37.7)	(39.1)	(10.1)	(0.0)	(0.0)	(0.0)	(2.9)
1998									
Idaho	46	0	16	17	2	0	0	2	9
Maine	13	1	1	0	0	0	0	3	8
Minnesota	46	4	36	6	0	0	0	0	0
Nebraska	6	3	3	0	0	0	0	0	0
North Dakota	20	1	16	3	0	0	0	0	0
South Dakota	1	1	0	0	0	0	0	0	0
Washington	3	0	2	1	0	0	0	0	0
Wisconsin	2	0	1	1	0	0	0	0	0
Totals for year	137	10	75	28	2	0	0	5	17
Frequency (%)		(7.3)	(54.7)	(20.4)	(1.5)	(0.0)	(0.0)	(3.6)	(12.4)
1999									
Colorado	11	0	11	0	0	0	0	0	0
Idaho	84	6	31	2	0	0	0	0	45
Maine	24	0	3	0	1	1	1	0	18
Minnesota	58	4	54	0	0	0	0	0	0
Nebraska	1	0	1	0	0	0	0	0	0
North Dakota	14	3	11	0	0	0	0	0	0
Oregon	32	3	28	0	1	0	0	0	0
Washington	8	1	6	1	0	0	0	0	0
Totals for year	232	17	145	3	2	1	1	0	63
Frequency (%)		(7.3)	(62.5)	(1.3)	(0.9)	(0.4)	(0.4)	(0.0)	(27.2)
2000									
Colorado	1	1	0	0	0	0	0	0	0
Delaware	2	2	0	0	0	0	0	0	0
Idaho	247	12	92	6	11	1	9	9	107
Maine	15	0	1	0	1	0	3	2	8
Minnesota	84	10	38	4	4	0	4	6	18
North Dakota	7	6	1	0	0	0	0	0	0
Washington	4	2	0	1	1	0	0	0	0
Wisconsin	7	0	4	2	1	0	0	0	0
Totals for year	367	33	136	13	18	1	16	17	133
Frequency (%)		(9.0)	(37.1)	(3.5)	(4.9)	(0.3)	(4.4)	(4.6)	(36.2)
Survey totals	805	67	382	71	29	2	17	22	215
Frequency (%)		(8.3)	(47.5)	(8.8)	(3.6)	(0.2)	(2.1)	(2.7)	(26.7)
Group totals	805			549			41		215
Group frequency (%)				(68.2)			(5.1)		(26.7)

media amended with mefenoxam. The growth responses of many others fell somewhere between these values (EC_{50} 0.05 µg ml^{-1} to 98.9 µg ml^{-1}).

Levels of metalaxyl in tuber tissue can range from 0.01 to 0.04 µg g^{-1} 1 month after harvest (1,4,29), and it would logically follow that isolates able to grow at or above such levels in vitro could be identified and classified as resistant. Unfortunately, the case is not that simple. Host physiology, morphology, compartmentalization, and distribution of the compound within host tissue and the ability to maintain antifungal activity in vivo may all affect disease development, and as a result, the perceived sensitivity of the pathogen. In studies involving *Phytophthora* species (18,21,22,37,41), EC_{50} values above 100 µg ml^{-1} were equated with resistance and those below 0.1 µg ml^{-1} with sensitivity. Similarly, since our work involves related but soilborne species, we consider isolates with in vitro EC_{50} values below 1.0 µg ml^{-1}

Table 2. Distribution and ranking of EC_{50} values of *Phytophthora erythroseptica* isolates collected in 1991 and 1992

		Distribution of isolate EC_{50} values (µg ml^{-1})							
		Sensitive				Intermediate			Resistant
Year and source	No. isolates tested	≤0.05	0.06-0.1	0.11-0.15	0.16-0.99	1.0-1.99	2.0-9.9	10.0-99.9	≥100
1991									
Colorado	3	0	1	1	1	0	0	0	0
Idaho	1	1	0	0	0	0	0	0	0
Minnesota	32	15	12	3	2	0	0	0	0
North Dakota	1	0	0	0	1	0	0	0	0
Oregon	2	1	1	0	0	0	0	0	0
Prince Edward Island, Canada	2	1	1	0	0	0	0	0	0
Washington	2	1	0	0	0	1	0	0	0
Wisconsin	4	1	3	0	0	0	0	0	0
Totals for year	47	20	18	4	4	1	0	0	0
Frequency (%)		(36.4)	(45.4)	(9.1)	(9.1)	(0.0)	(0.0)	(0.0)	(0.0)
1992									
Minnesota	17	5	7	1	4	0	0	0	0
Idaho	21	1	16	0	4	0	0	0	0
Totals for year	38	6	23	1	8	0	0	0	0
Frequency (%)		(16.1)	(54.8)	(3.2)	(25.8)	(0.0)	(0.0)	(0.0)	(0.0)
Combined Total	85	26	41	5	12	1	0	0	0
Frequency (%)		(28.7)	(49.4)	(6.9)	(13.8)	(1.1)	(0.0)	(0.0)	(0.0)
Group totals	85			84			1		0
Group frequency (%)				(98.9)			(1.1)		(0.0)

Table 3. Distribution and ranking of EC_{50} values of *Pythium ultimum* isolates collected during a survey (1998 to 2000) of infected tubers obtained from the United States and Canada

		Distribution of isolate EC_{50} values (µg ml^{-1})							
		Sensitive				Intermediate			Resistant
Year and source	No. isolates tested	≤0.05	0.06-0.1	0.11-0.15	0.16-0.99	1.0-1.99	2.0-9.9	10.0-99.9	≥100
1998									
Idaho	11	5	5	0	0	0	0	0	1
Maine	1	0	0	1	0	0	0	0	0
Minnesota	19	3	8	7	1	0	0	0	0
North Dakota	8	1	4	2	0	0	1	0	0
South Dakota	3	0	3	0	0	0	0	0	0
Washington	7	2	3	1	0	0	0	1	0
Wisconsin	2	1	1	0	0	0	0	0	0
Totals for year	51	12	24	11	1	0	1	1	1
Frequency (%)		(23.5)	(47.1)	(21.6)	(2.0)	(0.0)	(2.0)	(2.0)	(2.0)
1999									
California	1	1	0	0	0	0	0	0	0
Colorado	2	0	2	0	0	0	0	0	0
Minnesota	4	4	0	0	0	0	0	0	0
Oregon	25	15	8	1	1	0	0	0	0
Washington	14	4	6	0	3	0	0	1	0
Totals for year	46	24	16	1	4	0	0	1	0
Frequency (%)		(53.7)	(34.1)	(0.0)	(7.3)	(0.0)	(0.0)	(2.2)	(0.0)
2000									
British Columbia, Canada	4	2	1	0	0	0	1	00	
Idaho	6	1	5	0	0	0	0	0	0
Minnesota	57	14	27	3	8	0	0	0	5
North Dakota	9	1	7	0	1	0	0	0	0
Washington	5	2	2	0	0	0	0	0	1
Wisconsin	12	4	8	0	0	0	0	0	0
Totals for year	93	24	50	3	9	0	1	0	6
Frequency (%)		(25.8)	(53.8)	(3.8)	(7.3)	(0.0)	(1.1)	(0.0)	(6.5)
Survey totals	190	60	90	15	14	0	2	2	7
Frequency (%)		(31.6)	(47.4)	(7.9)	(7.4)	(0.0)	(1.1)	(1.1)	(3.7)
Group totals	190			179			4		7
Group frequency (%)				(94.2)			(2.1)		(3.7)

to be sensitive to the fungicide and those at EC_{50} 100 µg ml^{-1} or greater to be resistant. Isolates that fall between these two groups are considered "intermediate" types. This is consistent with the ranking ranges defined and characterized in a recent survey of metalaxyl resistance in the *P. erythroseptica* population collected from potatoes on Prince Edward Island (30). That study recognized three categories of sensitivity (metalaxyl sensitive, moderately metalaxyl resistant, highly metalaxyl resistant). The moderately resistant isolates appear to be intermediate in their responses to mefenoxam in vitro, but the level of resistance these isolates possess might be variable within a biological system particularly under field or storage conditions. Determination of the "biological significance" of the sensitivities of such isolates was not within the scope of the present study but should be addressed in future work.

Since a 2- to 3-year crop rotation cycle is typically employed in potato production, it is unlikely that individual sites (fields) were recurrently sampled during the course of this study. Therefore, although our data might imply that resistance is increasing temporarily in certain areas, the results are actually more indicative of the spatial distribution of resistance in the North American populations of these soilborne pathogens. Prior to our current work, a preliminary survey was undertaken in 1992 to ascertain the sensitivities of *P. erythroseptica* isolates from Minnesota, Wisconsin, and Idaho. All 46 isolates recovered and tested in that study were found to be sensitive to metalaxyl (38). However, soon after completion of that study, *P. erythroseptica* isolates insensitive to metalaxyl were discovered in Maine (23) and New York (13). We subsequently confirmed metalaxyl resistance in *P. erythroseptica* in Maine and documented the presence of resistant isolates in Idaho (15,16,34). The results obtained with the comparative isolates appear to confirm the absence of mefenoxam resistance in these states prior to 1996. Resistance was found in two states (Maine and Idaho) during the first 3 years of the present study. However, with the 2000 survey, we report the occurrence of mefenoxam-resistant isolates of *P. erythroseptica* in Minnesota for the first time.

Results we obtained from testing a limited number of isolates from Canada suggest that the population may have been sensitive to mefenoxam prior to 1997. Peters et al. (30) in 2001, found that all 62 field isolates of *P. erythroseptica* obtained from the major potato growing area of Prince Edward Island remained highly sensitive to metalaxyl (EC_{50} <0.5 µg ml^{-1}). These results are encouraging because this is a high use area for mefenoxam. It is not likely that resistance would appear first in areas of minimum mefenoxam use.

We documented only a single case of resistance in *Pythium ultimum* in the first 3 years of this study (1997 to 1999). This isolate from Idaho represented only 1% of the total population tested over that time. In 2000, insensitive isolates were recovered from 6.4% of the population. This is a modest increase in the number of sites found to have insensitive isolates but these still represent a very small portion of the overall *Pythium ultimum* population. However, changes observed over the last 2 years of the survey suggest that the resistance to mefenoxam is becoming more widespread. This trend is particularly evident in Minnesota as 5 of 6 resistant isolates recovered in 2000 were from that state. Previous work with *Pythium* isolates collected from sugar beet in the Red River Valley from 1991 to 1993 found no evidence of resistance in the 97 isolates tested (2). The majority of the isolates we recovered from this area were also highly sensitive with EC_{50} values ranging between 0.05 µg ml^{-1} and 0.99 µg ml^{-1}.

The increase in the number of resistant isolates of *P. erythroseptica* and *Pythium ultimum* recovered from Minnesota, and the very high level of resistance they appear to possess is particularly alarming. Mefenoxan is still the only fungicide used to control leak and pink rot (26,42), so significant problems controlling these diseases in that state may arise in the immediate future. Although the incidence of resistance remains low, we did find intermediate types at approximately the same frequency as the resistant types in the *Pythium ultimum* populations. These moderately insensitive isolates of *P. erythroseptica* and *Pythium ultimum* may be indicators of a population in transitional flux from sensitivity to resistance. Additional work is needed to determine the significance of this group.

Many of the infected tubers received in the survey came from fields and storages where fungicide apparently failed to control water rot. Thus, by design, our survey is probably skewed toward finding insensitivity within the pathogen population. As a result, our observations may not necessarily represent the frequency of resistance that exists in the field. These results only demonstrate that resistance exists in field populations of these water rot fungi collected from our survey areas. Further studies should be initiated to obtain soil populations of *P. erythroseptica* and *Pythium ultimum* and subsequently monitor the level of mefenoxam resistance in these populations, if effective control measures for pink rot and leak are to be maintained or developed.

The data presented here confirms that populations of mefenoxam insensitive isolates of *P. erythroseptica* are now present in the potato-growing areas of Maine, Idaho, and Minnesota. Displacement of mefenoxam-sensitive isolates may be due to the intense selection pressure provided by mefenoxam fungicide usage. The incidence of insensitivity to mefenoxam is still at a very low level in the North American population of *Pythium ultimum* relative to that of *P. erythroseptica*. However, the discovery of a significant number of resistant isolates of these pathogens in Minnesota in 2000, demonstrates that insensitivity has reached detectable levels in the populations there. This should trigger concern and prompt adjustments in management practices directed toward the water rot pathogens. It is therefore, important to continue monitoring these areas, but it is also essential to survey other potato growing areas more extensively to assess the composition of the pathogen populations.

ACKNOWLEDGMENTS

We thank Phil Hamm for providing some of the isolates used in this study; the following individuals for supplying "water rot" infected tubers for our survey: P. Nolte, J. Fuller, P. Hamm, D. Johnson, S. Erickson, J. Vaadeland, D. Steevens, D. Lambert, W. Stevenson, R. Zink, L. Morrow, C. Larson, G. Taylor, T. Dayton, M. Bittner, D. Miller, G. McCoy, and J. Elmhirst; and Robert W. Stack for allowing us to access and test his collection of *P. erythroseptica* isolates. We also wish to gratefully acknowledge the financial support of Syngenta Crop Protection, Inc. and the statistical analysis assistance of Curt Doetkott. Without the support of our colleagues, this work would not have been possible.

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