Am. J. Pot Res
DOI 10.1007/s12230-010-9162-3

Prevalence of Mefenoxam Resistance Among *Phytophthora erythroseptica* Pethybridge Isolates in Minnesota and North Dakota

Chapara Venkataramana · Raymond J. Taylor · Julie S. Pasche · Neil. C. Gudmestad

Abstract In an 8 year survey of Minnesota potato fields, a total of 1,772 isolates of *Phytophthora erythroseptica* were collected from potato tubers showing symptoms of pink rot. All isolates were evaluated for sensitivity to the systemic fungicide mefenoxam and categorized as mefenoxam-sensitive, -intermediate or -resistant. A significant yearly increase was observed in the frequency of mefenoxam-resistant *P. erythroseptica* isolates recovered and in the frequency of fields from which mefenoxam-resistant isolates originated. Additionally, isolates with intermediate levels of mefenoxam resistance were recovered in each year of the survey. In a similar survey, greater than 80% of *P. erythroseptica* isolates recovered were mefenoxam-sensitive among 293 total isolates collected over 3 years in North Dakota, and no *P. erythroseptica* isolates with intermediate resistance to mefenoxam were recovered. Results reported here confirm the presence of mefenoxam resistance among isolates of *P. erythroseptica* in Minnesota and North Dakota but at varying levels among years of the survey in both states. The presence of *P. erythroseptica* isolates with intermediate levels of resistance to mefenoxam collected in Minnesota support earlier studies which indicate that isolates displaying intermediate responses to mefenoxam are indicative of a transitional flux from a mefenoxam-sensitive to a mefenoxam-resistant population.

Resumen En inspecciones por ocho años en campos de papa de Minnesota, se colectaron un total de 1,772 aislamientos de *Phytophthora erythroseptica* de tubérculos con síntomas de pudrición rosada. Todos los aislamientos se evaluaron para sensibilidad al fungicida sistémico mefenoxam y se categorizaron como sensitivos al mefenoxam, intermedios o resistentes. Se observó un aumento significativo anual en la frecuencia de los aislamientos de *P. erythroseptica* resistentes recuperados y en la frecuencia de los campos de los cuales se originaron los resistentes al mefenoxam. Adicionalmente, se recuperaron, en cada año del estudio, aislamientos con niveles intermedios de resistencia a mefenoxam. En una inspección similar, se recuperaron más del 80% de aislamientos de *P. erythroseptica* sensibles, entre un total de 293 colectados durante tres años en Dakota del Norte, y no se recuperaron aislamientos de *P. erythroseptica* con resistencia intermedia a mefenoxam. Los resultados que aquí se reportan confirman la presencia de resistencia a mefenoxam entre los aislamientos de *P. erythroseptica* en Minnesota y Dakota del Norte, pero a niveles variables entre los años del estudio en ambos Estados. La presencia de aislamientos de *P. erythroseptica* con niveles intermedios de resistencia a mefenoxam colectados en Minnesota respalda estudios previos que indican que los aislamientos que muestran respuestas intermedias a mefenoxam indican un flujo transicional de una población sensible a una resistente a mefenoxam.

Keywords Fungicide efficacy · Metalaxyl · Potato · *Solanum tuberosum* · Tuber disease · Water rot

R. J. Taylor · J. S. Pasche · N. C. Gudmestad (✉)
Department of Plant Pathology, North Dakota State University, Fargo, ND 58108, USA
e-mail: neil.gudmestad@ndsu.edu

Present Address:
C. Venkataramana
Department of Crop Sciences, University of Illinois, Urbana, IL 61801, USA

Introduction

Pink rot, a soil-borne disease caused by *Phytophthora erythroseptica* Pethybridge, results in significant losses in

Published online: 31 July 2010

storage wherever potatoes (*Solanum tuberosum* subsp. *tuberosum* L.) are grown worldwide (Vargas and Nielsen 1972) and has been reported to be economically important in the United States and Canada (Peters et al. 2001; Secor and Gudmestad 1999). The pathogen has the ability to infect tubers directly through the eyes, roots, stolons, and at the base of the stem via zoospores (Lonsdale et al. 1980; Salas et al. 1997), or through wounds and lenticels (Blodgett 1945; Salas et al. 2000). Oospores of *P. erythroseptica* which survive in soil for many years act as primary inoculum (Vujicic and Park 1964). Although pink rot commonly is noticed in storage, initial infections most likely occur in the field prior to harvest (Blodgett 1945; Cairns and Muskett 1939). The disease usually starts from the stem end of the potato tuber, progressing throughout in a uniform manner, often with a nearly straight line between the healthy and the diseased portions. Diseased tissue typically turns a distinct pink color when exposed to air for a short period of time (Goss 1949; Cairns and Muskett 1933). Affected tissue retains some degree of firmness and has a texture similar to that of boiled potatoes (Miller et al. 2006). Pink rot generally is problematic in production regions where tubers are exposed to high soil moisture due to poor drainage, or an extended period of rainfall late in the growing season (Goss 1949). The disease has become economically important to growers in many production areas since severe epidemics of pink rot can cause substantial losses to a potato crop resulting in decrease in yield and quality of processed products (G. A. Secor, *personal communication*).

Although most commonly grown cultivars demonstrate varying degrees of susceptibility to *P. erythroseptica* (Salas et al. 2003), no cultivars with adequate levels of resistance are available. Therefore, potato growers largely have relied on a single fungicide, metalaxyl/mefenoxam, for the past three decades to manage pink rot. Metalaxyl, a phenylamide fungicide, was introduced to control foliar diseases caused by oomycetes (Morton and Urech 1988) and does so by inhibiting r-RNA synthesis of species in Peronosporales (Davidse et al. 1983). An enantiomer of metalaxyl, mefenoxam (Ridomil Gold 48% EC and Ultra Flourish 25% EC) was released in 1997. Mefenoxam has increased activity against oomycetes as compared to metalaxyl (Nuninger et al. 1996) and is currently used commercially to control pink rot, with applications made at planting, hilling, and on foliage when the tubers are approximately 10 mm in diameter (Secor and Gudmestad 1999; Taylor et al. 2004; Wicks et al. 2000). Since the release of these compounds, variations in sensitivity to metalaxyl/mefenoxam have been reported among and within numerous species of *Phytophthora* (Coffey and Bower 1984; Coffey et al. 1984; Csinos and Bertrand 1994; Ferrin and Kabashima 1991; Goodwin and McGrath 1995; Goodwin et al. 1996; Hunger et al. 1982; Peters et al. 2003a; Sujikowski et al. 1995; Taylor et al. 2002a, 2004, 2006). The widespread development of metalxyl-resistance among *P. infestans* isolates was attributed to the fitness of metalaxyl-resistant isolates (Cohen and Coffey 1986). Metalaxyl-resistant isolates were found to be more fit than metalaxyl-sensitive isolates even under the absence of selection pressure of metalaxyl (Kadish and Cohen 1988). However, research proved that metalaxyl-resistance and fitness of the *P. infestans* oospore progeny were not linked with each other (Gisi and Cohen 1996). Resistance to metalaxyl also has been induced under in vitro conditions within *Phytophthora, Pythium,* and Peronosporales (Bruin and Edgington 1981, 1982).

In vitro sensitivity studies involving isolates recovered from potato tubers collected from storages in Minnesota, Wisconsin and Idaho in the late 1980s and early 1990s (Stack et al. 1993) found no evidence of metalaxyl resistance in the *P. erythroseptica* population. Metalaxyl/mefenoxam-resistant isolates of *P. erythroseptica* were detected first in Maine in 1993 (Lambert and Salas 1994), New York in 1994 (Goodwin and McGrath 1995), Idaho in 1998, Minnesota in 2000 (Taylor et al. 2002a), and subsequently in Colorado, Michigan, Wisconsin and North Dakota (G. A. Secor, *personal communication*). Mefenoxam resistance also has been reported to be present in the *P. erythroseptica* population in Oregon (P. B. Hamm, *personal communication*). An extensive survey of resistance in the North American population of *P. erythroseptica* demonstrated the presence of mefenoxam-resistant isolates, although nearly 79.0% of isolates examined remained sensitive to the fungicide (Taylor et al. 2002a). The emergence of potato pink rot as an economically important disease and the failure of mefenoxam to control pink rot in many areas of the United States (Taylor et al. 2004) can be attributed to an increase in occurrence of mefenoxam-resistant isolates of the pathogen (Taylor et al. 2002a), differences in mefenoxam sensitivity within the pathogen population, and the lack of disease resistant potato cultivars (Salas et al. 2003).

Due to the limited number of fungicides available for use in pink rot management and the associated risk of a complete shift in mefenoxam sensitivity among *P. erythroseptica* isolates, it is important to monitor the pathogen population in order to prolong fungicide use in pink rot management programs. Mefenoxam-resistant isolates of *P. erythroseptica* first were recovered in Minnesota in 2000 (Taylor et al. 2002a), representing 21.4% of the total number of isolates collected in Minnesota that year. The same survey failed to confirm the presence of mefenoxam resistance in the North Dakota population at that time. Previous research indicates that a shift in sensitivity may be occurring within pathogen population in commercial potato

fields in Minnesota and North Dakota (Taylor et al. 2002b). The objective of this study was to determine the current status of mefenoxam sensitivity in *P. erythroseptica* isolates recovered from these growing areas. The data generated in this study will contribute to pink rot management by determining the potential presence and frequency of mefenoxam-resistant populations in Minnesota and North Dakota, and allow for the modification of fungicide control programs as needed.

Materials and Methods

Collection of Tubers and Isolation of *P. erythroseptica*

Tubers with symptoms of pink rot were collected from fields and storage facilities from 2001 and 2006 through 2008 in Minnesota and North Dakota, respectively. The pathogen was isolated from infected tubers and these isolates were evaluated for in vitro sensitivity to mefenoxam. Sections of infected tuber tissue (~4×4 mm) were excised, placed on Petri dishes containing solid media comprised of 15% agar in distilled water amended with lactic acid (0.5 ml/liter) to suppress bacterial contamination, and incubated in darkness at 17–20°C for 3 days. Fungal colonies with mycelia resembling *P. erythroseptica* were selected and purified by hyphal tip isolations. Isolates were grown and maintained on 10% V8 juice agar medium (100 ml V8 juice, 15 gm of agar, and 900 ml distilled water) prior to performing mefenoxam sensitivity evaluations (Gudmestad et al. 2000; Taylor et al. 2002a, 2004). A substantial number of tubers showing symptoms of pink rot from North Dakota were obtained courtesy of Dr. Gary Secor (North Dakota State University, Fargo, ND).

In Vitro Assessment of Mefenoxam Sensitivity

Mefenoxam sensitivity was determined by growing *P. erythroseptica* isolates on 5% V8 agar plates containing 0 μg/ml, 0.01 μg/ml, 0.1 μg/ml, 1 μg/ml, 10 μg/ml, 100 μg/ml active ingredient of mefenoxam (Ridomil Gold EC; 48% a.i.). To achieve the desired concentrations, the fungicide was diluted in sterile deionized water and 10 ml of each mefenoxam solution was added to 5% V8 media (50 ml V8 juice filtered through four layers of cheesecloth, 940 ml distilled water, and 20 g agar) before autoclaving (Taylor et al. 2002a). The assay was performed by placing 5 mm agar plugs excised from the margins of actively expanding 4 to 5-day old cultures in 9 cm Petri plates containing amended and non-amended V8 agar medium. Isolate growth at each concentration was determined by measuring colony diameter in two perpendicular directions on each culture plate after 5–7 days of incubation at 22°C when the colonies growing on control media (without mefenoxam) were at least 6 cm in diameter. Radial growth measurements were averaged after the diameter of the mycelial plug was subtracted. The relative growth reduction for each concentration of mefenoxam was calculated using the formula [100-[growth with mefenoxam/growth in control plate] × 100]. The relative growth reduction at each fungicide concentration then was used to determine an EC_{50} value; the effective concentration at which a 50% reduction in fungal growth occurs as previously described (Taylor et al. 2002a) by fitting the data sets into the nonlinear Gompertz function. The Gompertz model composed of parameters in a sigmoidal function with the general formula (Draper and Smith 1981): $Y = \alpha^{*}\exp\{-\exp[\beta - (\gamma^{*}x)]$. Each of the parameters was subjected to monotonic transformation as required and was related to aspects of the sigmoidal curve (Ratkowsky 1983). As such, 'x' is the concentration of the fungicide, 'α', relates to the asymptote or maximum value, 'β' to the y intercept, and 'γ' is associated with the rate at which the response increases from the initial value (β) to the final value (α). Once obtained, data parameters were entered into the Gompertz function and the EC_{50} was estimated by solving the concentration at which a 50% reduction in growth occurred.

Experimental Design and Statistical Analysis

Petri plates containing isolates growing on media non-amended and amended with mefenoxam were arranged in a completely randomized design (CRD) with two replicates per isolate. The mefenoxam-sensitive isolate, PR-347 (EC_{50}=0.04 μg/ml), and the mefenoxam-resistant isolate, PE-89 (EC_{50}>100 μg/ml) (Taylor et al. 2002a), were included in every experiment as internal controls. Each isolate collected across years and location was evaluated twice for mefenoxam sensitivity using these methods. Further analysis was performed by categorizing *P. erythroseptica* isolates into sensitive (EC_{50}<1 μg/ml), intermediately resistant ($EC_{50}\geq 1 \leq 99.9$ μg/ml) and resistant ($EC_{50}\geq$100 μg/ml) populations (Gudmestad et al. 2000; Peters et al. 2001, 2003a; Taylor et al. 2002a, b, 2004). Similarly, fields were categorized into sensitive (only mefenoxam-sensitive isolates recovered), resistant (only mefenoxam-resistant isolates recovered), and mixed (mefenoxam-sensitive, -intermediately resistant and -resistant isolates recovered), and the frequency of each isolate and field category was calculated by year and location within year.

Logistic regression analyses were conducted on data from the 8 year survey conducted in Minnesota and from the 3 year survey conducted in North Dakota using LOGISTIC procedure in SAS version 9.2 (SAS Institute, Inc, Cary, NC) and model fit was evaluated using the Wald chi-square test (α=0.05). Logistic regression was used to evaluate the relationships between the frequencies of recovery of mefenoxam-resistant isolates by year. The response variable analyzed was for an increase or decrease in the frequency of resistant isolates

recovered and the frequency of fields from which only resistant isolates were recovered.

Results

A total of 1,772 *P. erythroseptica* isolates collected from 615 fields were obtained from diseased tubers submitted by potato growers and processors in Minnesota from 2001 through 2008 (Table 1). A majority of these samples were submitted from five growing areas across the state, including Park Rapids, Wadena, Parham, Pelican Rapids and Parkers Prairie. Substantial fluctuations in mefenoxam sensitivity were present among *P. erythroseptica* isolates collected from Minnesota during the survey period (Fig. 1). While the frequency of recovery of mefenoxam-sensitive isolates remained above 40.0% in all years of the survey, ranging from 92.3% in 2001 to 42.4% in 2008, the amount of variation was relatively high. Similarly, while the prevalence of mefenoxam resistance in *P. erythroseptica* was substantially lower than sensitive isolates, the frequency also fluctuated among years from 6.5% in 2001 to 55.1% in 2005. Finally, proportions of intermediately resistant isolates recovered remained low across all years, with little variation ranging from 1.2% in 2001 to 14.6% in 2008. A trend towards decreasing sensitivity and increasing resistance was observed across years of the survey with the sensitive:resistant ratio shifting from greater than 9:1 in 2001 to 1:1 in 2008. Logistic regression analysis demonstrated an overall significant yearly increase in the frequency of recovery of mefenoxam-resistant isolates across *P. erythroseptica* populations from 2001 to 2008 (χ^2 value=169.0, 7df, $P<0.001$). Trends in mefenoxam sensitivity data when classified by field category are similar to those reported among isolate categories (Fig. 2). The frequency of fields from which only mefenoxam-sensitive *P. erythroseptica* isolates were recovered decreased from 91.4% in 2001 to 27.3% in 2008. Conversely, the proportion of fields with a completely mefenoxam-resistant population of *P. erythroseptica* increased from 8.7% in 2001 to 49.2% in 2008. While the frequency of fields with a mixed population of mefenoxam-sensitive, -intermediately resistant and -resistant isolates was slightly higher in some years than the frequency of recovery of intermediately-resistant isolates, these frequencies remained relatively low across all years, ranging from zero in 2001 to 27.5% in 2005.

No consistent trend in the recovery of mefenoxam-resistant isolates was observed across five locations in Minnesota from 2005 to 2008; within some locations a trend across years was observed. Park Rapids was the only site at which the frequency of mefenoxam-resistant isolates recovered displayed an overall decreasing trend from 39.2% in 2006 to 16.7% in 2008 (Fig. 3). At Perham, an overall increasing trend was observed among the collection of -resistant isolates ranging from 5.5% to 37.5%. The trend observed in samples obtained from Wadena, Parkers Prairie and Pelican Rapids was very irregular. In Wadena, the frequency of recovery of mefenoxam-resistant isolates was less than 7% in 2005 and 2007, but greater than 35.0% in 2006 and 2008. At Parkers Prairie and Pelican Rapids the differences were not as great, ranging from 1.8% to 18.5% and from zero to 24.7%, respectively (Fig. 3).

A total of 293 *P. erythroseptica* isolates were collected over 3 years from 39 fields in six locations in North Dakota (Table 2). In contrast to the variability observed in the frequency of recovery of each *P. erythroseptica* isolate category observed in Minnesota, minor differences were observed across the 3 years of the survey in North Dakota (Fig. 4). Additionally, the prevalence of mefenoxam resistant *P. erythroseptica* isolates was much lower in North Dakota ranging from 6.7% to 15.4%. No isolates with intermediately-resistant reactions to mefenoxam were recovered from North Dakota, and consequently recovery frequencies of mefenoxam-sensitive isolates were high (84.6% to 93.3% in all years of the survey). As expected, the largest percentage of fields sampled in North Dakota contained *P. erythroseptica* populations that were entirely sensitive to mefenoxam (Fig. 5). Logistic regression analyses indicated that no significant differences existed among the 3 year isolate populations (χ^2 value=4.94, 2df, $P>0.085$) across samples received from North Dakota.

Table 1 Number of isolates recovered during the survey of mefenoxam resistance in *Phytophthora erythroseptica* populations over an 8 year period from 2001 to 2008 in Minnesota

Location	2001	2002	2003	2004	2005	2006	2007	2008	Total
Parkers Prairie	9	0	6	0	20	15	38	34	122
Park Rapids	80	45	25	25	90	144	59	329	797
Pelican Rapids	27	0	0	6	44	40	86	0	203
Perham	53	44	0	8	37	31	51	112	336
Wadena	1	0	0	1	9	45	3	97	156
Other	106	0	0	12	27	0	0	13	158
Total	276	89	31	52	227	275	237	585	1,772

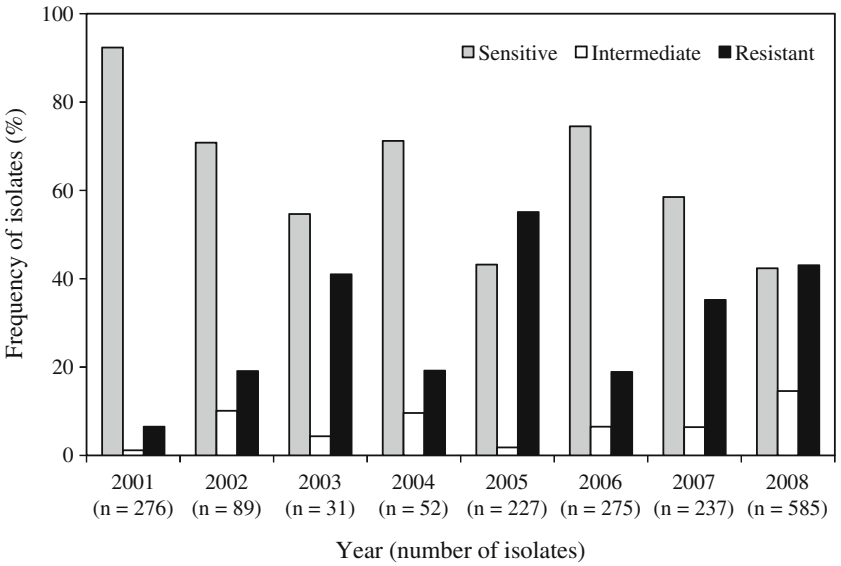

Fig. 1 Frequency of mefenoxam-sensitive (EC_{50}< 1.0 μg/ml), -intermediately resistant (EC_{50}≥1.0≤99.9 μg/ml) and mefenoxam-resistant (EC_{50}≥100.0 μg/ml) isolates of *Phytophthora erythroseptica* collected during a survey (2001 to 2008) of tubers showing symptoms of pink rot obtained from Minnesota

Interestingly, the frequency of fields with only mefenoxam-resistant isolates declined from 2006 with no fields having an entirely mefenoxam-resistant population detected in 2008. However, in all years of the survey, mefenoxam-resistant isolates were obtained from fields with mixed populations of mefenoxam-resistant and mefenoxam-sensitive isolates. The frequency of fields with mixed populations remained somewhat low across the 3 year survey.

Discussion

The present study provides a comprehensive overview of the status of mefenoxam resistance in the *P. erythroseptica* populations native to Minnesota and North Dakota. The Minnesota population has been monitored on a yearly basis for over a decade and the population in North Dakota has been screened annually since the discovery of mefenoxam resistance there in 2003. Metalaxyl/mefenoxam-resistant isolates initially were discovered in the year 2000 via routine screening for metalaxyl insensitivity of isolates obtained from potato fields in Minnesota (Taylor et al. 2002a). Prior to that, all isolates of *P. erythroseptica* collected in 1991 and 1992 (Stack et al. 1993; Taylor et al. 2002a) and between 1997 and 1999 (Taylor et al. 2002a) from potato tubers showing symptoms of pink rot in Minnesota were determined to be highly sensitive to metalaxyl. The information collected in these surveys indicates that while the incidence and severity of pink rot varied markedly from location to location, mefenoxam resistance continues to persist in the *P. erythroseptica* populations in the potato growing areas of these states

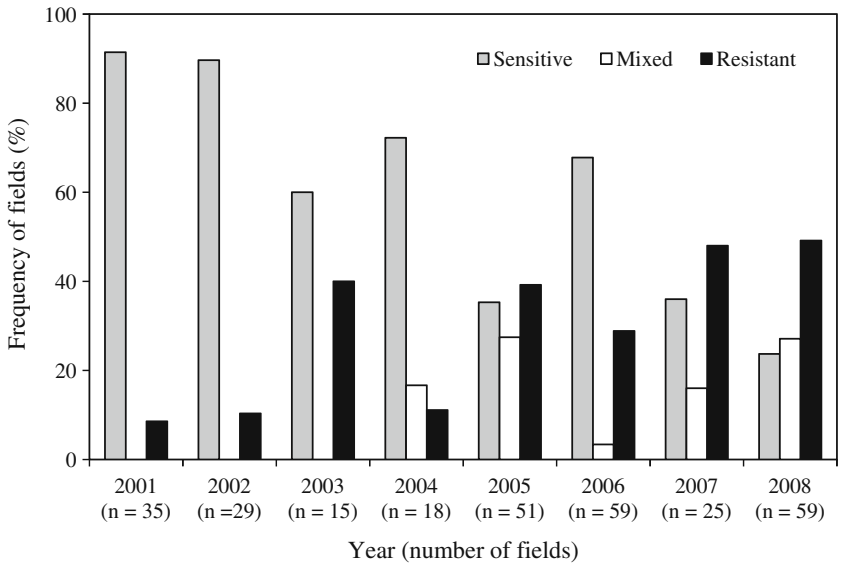

Fig. 2 Frequency of fields with mefenoxam-sensitive (EC_{50}< 1.0 μg/ml), -mixed (fields where both mefenoxam-sensitive, intermediately-resistant and -resistant isolates were recovered) and -resistant (EC_{50}≥ 100.0 μg/ml) isolate populations of *Phytophthora erythroseptica* during a survey (2001 to 2008) of tubers showing symptoms of pink rot in Minnesota

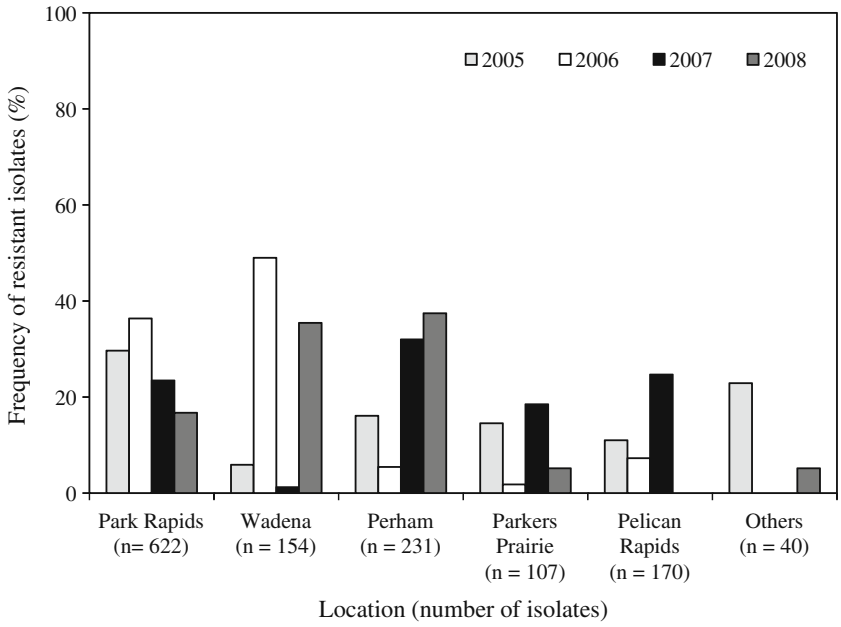

Fig. 3 Comparison of mefenoxam-resistant ($EC_{50}\geq$ 100.0 μg/ml) isolates of *Phytophthora erythroseptica* recovered at various locations during a survey (2005 to 2008) of tubers showing symptoms of pink rot obtained from Minnesota

and that fluctuation in the frequency of mefenoxam-resistant isolates occur over time.

Cropping history has been reported to influence the development and incidence of pink rot (Peters et al. 2003b, 2005b), therefore, the fluctuating frequency of mefenoxam resistance in *P. erythroseptica* isolates observed here is likely due, in part, to field rotations (crop rotation) by the growers. Most of the fields sampled in this study were on a 3 year rotation, that is, one potato crop every 3 years. This means that the potato fields sampled in 2008 also were planted to potato in 2005 and in 2002. It has been suggested that soil microflora and / or beneficial endophytic organisms may promote suppression of pink rot, resulting in significantly lower rates of infection in tubers from 3-year rotations inoculated with *P. erythroseptica* after harvest (Peters et al. 2003b, 2005b).

Fluctuations in frequency of mefenoxam-resistant isolates among years of the survey also may be caused by variation in levels of seed contamination. In the current study, samples of tubers showing symptoms of pink rot produced from a single seed source yielded 27 isolates of *P. erythroseptica* in 2005, all of which were found to be resistant to mefenoxam. It has been suggested that contaminated seed can introduce new genotypes / strains into a previously non-infested field or area. The introduction of new genotypes might lead to out-crossing, which has been found to play a significant role in the generation of genetic variation in other homothallic oomycetes such as *Aphanomyces euteiches* Drechs. (Shang et al. 2000). Genetic diversity among *P. erythrospetica* isolates has been attributed to the introduction of a small founding population

Table 2 Number of isolates recovered during the survey of mefenoxam resistance in *Phytophthora erythroseptica* over a 3 year period from 2006 to 2008 in North Dakota

Location	2006	2007	2008	Total
Dawson	29	135	0	164
Oakes	7	9	0	16
Linton	7	25	0	32
Tappen	1	0	24	25
Grand Forks	15	0	41	56
Total	59	169	65	293

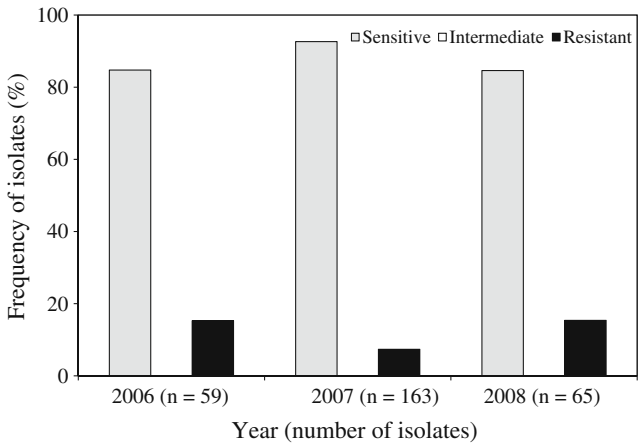

Fig. 4 Frequency of mefenoxam-sensitive ($EC_{50}<1.0$ μg/ml), and mefenoxam-resistant $EC_{50}\geq100.0$ μg/ml isolates of *Phytophthora erythroseptica* collected during a survey (2006 to 2008) of tubers showing symptoms of pink rot obtained from North Dakota. No intermediately mefenoxam-resistant isolates of *P. erythroseptica* were detected in North Dakota

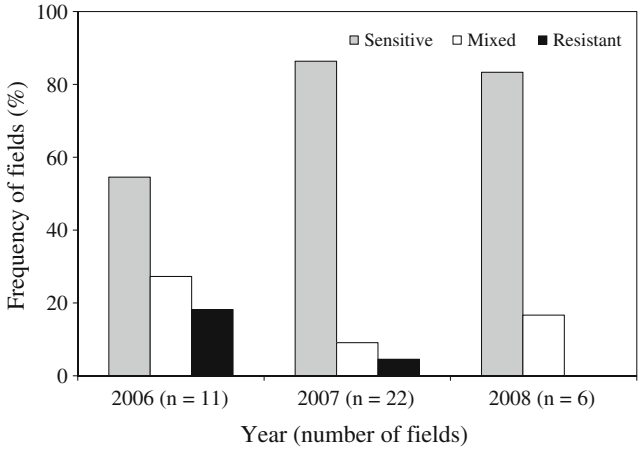

Fig. 5 Frequency of fields with mefenoxam-sensitive (EC_{50}<1.0 μg/ml), -mixed (fields where both mefenoxam-sensitive and -resistant isolates were recovered) and -resistant (EC_{50}≥100.0 μg/ml) isolate populations of *Phytophthora erythroseptica* during a survey (2006 to 2008) of tubers showing symptoms of pink rot in North Dakota

in North America (Peters et al. 2005a). Additionally, the pathogen may be moved from field to field on equipment or on healthy-appearing tubers during harvest and transportation. This becomes even more important when healthy tubers are wounded during harvest operations (Cunliffe et al. 1977) as infections may occur readily when *P. erythroseptica* infected tissue comes in contact with healthy tubers that have been wounded; thus acting as a source of infection in storage (Salas et al. 2003).

Previous studies indicated that mefenoxam resistance in *P. erythroseptica* appears to be in a state of flux in Minnesota. This was primarily attributed to the appearance of intermediately mefenoxam-resistant isolates and could be a major cause of the fluctuations of mefenoxam insensitivity observed year after year (Taylor et al. 2002a, 2006). The data reported here support these earlier studies, again suggesting that intermediately mefenoxam-resistant isolates are indicative of a transitional flux from a mefenoxam-sensitive population to a mefenoxam-resistant one. Indeed, *P. erythroseptica* isolates with an intermediate level of mefenoxam resistance ranged from 2.2% of the population in 2005 to 14.6% in 2008, two survey years in which many of the same fields were sampled because of 3 year crop rotations. Unfortunately, similar comparisons were not possible with survey fields of 2002 due to a lack of samples obtained from those areas.

Other work has demonstrated that the proportion of resistance in a pathogen population can change quickly and dramatically. In a study of the population dynamics of *P. erythroseptica* in Idaho soils, substantial shifts towards mefenoxam resistance in the native population were reported at GPS marked sites in an infested field (Taylor et al. 2002b). The composition of the population shifted from 92.3% mefenoxam-sensitive and 7.7% mefenoxam-intermediate in 1998 to 5.0% mefenoxam-intermediate and 95.0% mefenoxam-resistant just 2 years later at one site in the field. In a precursor to the present survey, the frequency of mefenoxam resistance in isolates collected from tubers grown in Minnesota increased from 0.0% in 1999 to 66.7% in 2000 (Taylor et al. 2002a). A gradual quantitative shift of intermediately-resistant populations of *P. erythroseptica* occurred towards mefenoxam-resistance under in vitro conditions (Abu-El Samen et al. 2005). In the first 2 years of the current study the frequency of fields containing mefenoxam-resistant populations was approximately 10% but by the third year of the study, that figure had increased to nearly 40%.

Results reported in recent studies suggest that mefenoxam-resistant isolates are more fit and therefore have an advantage over -sensitive isolates, particularly when placed under selection pressure offered by mefenoxam. In tuber challenge inoculation studies, mefenoxam-resistant isolates were more aggressive, causing more disease, than mefenoxam-sensitive isolates on non-mefenoxam-treated tubers and were significantly more aggressive on tubers treated with the fungicide (Taylor et al. 2006). Even isolates of *P. erythroseptica* with low intermediate resistance have been shown to be highly aggressive under the influence of mefenoxam, compared to other groups of isolates, indicating a competitive advantage and also suggesting that these isolates could be parasitically fit in nature (Taylor et al. 2006). Additionally, recent research noted that resistant isolates had greater growth rates and produced more oospores than mefenoxam-sensitive isolates in vitro. The combination of such factors contribute to the enhanced parasitic fitness associated with mefenoxam-intermediate and mefenoxam-resistant isolates (Porter et al. 2007).

Considerable spatial and temporal variation in mefenoxam sensitivity was observed in isolates collected during the course of the present survey. Regardless of the cause(s) of these fluctuations in mefenoxam sensitivity within the pathogen population, it is apparent that the general trend in Minnesota is that the proportion of fields containing mefenoxam-resistant *P. erythroseptica* populations has gradually increased over the past 10 years while the proportion of fields with mefenoxam-sensitive populations have shown a gradual decline. Also noted was a general increase in the proportion of recovery of mefenoxam-intermediate isolates as well as fields with mixed populations, suggesting that the population is in a transitional shift from sensitivity to resistance and that this change could occur very quickly (Taylor et al. 2002a, b, 2006).

P. erythroseptica isolates obtained from samples collected in North Dakota were predominantly sensitive to

mefenoxam and no intermediately-resistant isolates were recovered. However, the discovery of a substantial number of mefenoxam-resistant isolates indicates that resistance has reached detectable levels. Growers should be wary of this and monitor the composition of the *P. erythroseptica* population on a routine basis. Continued mefenoxam usage undoubtedly is providing selection pressure and accelerating these changes, therefore, alternative measures to control pink rot need to be considered.

Mefenoxam continues to provide adequate pink rot control in fields infested with a sensitive population of *P. erythroseptica*, particularly if applied in-furrow and currently most irrigated growers are practicing in furrow applications in Minnesota and North Dakota (Taylor et al. 2004; Wicks et al. 2000). This method of application also limits the proportion of the population exposed to the fungicide, potentially slowing the development of resistance in the population. Although mefenoxam should be effective in such situations, practices that maximize skin set and reduce the likelihood of wounding tubers during harvest, handling and storage are also of utmost importance. *P. erythroseptica* typically infects tubers through the stolon and eyes but is also an efficient wound pathogen (Salas et al. 2000). It has been suggested that resistance development may be related to the highly variable accumulation of mefenoxam in potato tubers with standard application rates of the fungicide (Barak et al. 1984). Fungicide concentrations at or near the periderm may be as much as twice that of the inner pulp, cortex or medullary tissues, indicating that protection offered by mefenoxam is restricted to the area just beneath the periderm. An increase in the efficacy of mefenoxam is possible if the fungicidal barrier present in the outer layers of the tissues is not disrupted or breached (Bruin et al. 1982; Taylor et al. 2004). If the periderm is disrupted the result is reduced-efficacy of mefenoxam (Taylor et al. 2004). These factors must be considered to preserve and maximize the level of control provided by this fungicide. Incorporating previously discussed cultural practices including limiting damage to tubers during harvest, handling, transportation, and storage as well as following agronomic practices traditionally employed to manage pink rot, such as, water management, allowing sufficient time for adequate periderm formation to occur before harvest and harvesting at tuber pulp temperatures lower than 21°C, should also impact the magnitude and composition of the pathogen population.

In light of the widespread occurrence of mefenoxam resistance in the North American population of *P. erythroseptica* (Taylor et al. 2002a) and the increased frequency of resistance noted in pathogen population in Minnesota, it is essential to consider alternative strategies to manage pink rot. While potato cultivars currently grown exhibit varying levels of susceptibility to *P. erythroseptica*, none are completely resistant to the pathogen (Peters and Sturz 2001; Salas et al. 2003). However, some genotypes including Atlantic, Butte, Irish Cobbler, Kasota and Russet Burbank, do possess moderate resistance (Goss 1949; Peters and Sturz 2001; Peters et al. 2004; Salas et al. 2003; Taylor et al. 2008). Additionally, three clonal lines highly resistant to pink rot and five other lines displaying moderate resistance have been developed (Thompson et al. 2007), demonstrating the potential of incorporating resistance into future cultivars. In the absence of the availability of current cultivars with high levels of resistance, crop rotation may provide some disease control. A three-year crop rotation of barley, red clover and potato resulted in less disease than two-year rotation of spring barley and potato (Peters et al. 2005b). Other than metalaxyl/mefenoxam, most fungicides have been generally ineffective in controlling pink rot. However one fungicide, phosphorous acid (phosphonate, phosphite), has been demonstrated to be an effective fungicide option for pink rot management and also for late blight and tuber blight caused by *P. infestans* (Johnson et al. 2004; Mayton et al. 2008; Miller et al. 2006; Wicks et al. 2000). Unfortunately, phosphorous acid is substantially more expensive than mefenoxam (Gudmestad et al. 2007) and has no effect on the other major soil-borne water rot, *Pythium* leak (Johnson et al. 2004). Future work should focus on the development of resistant cultivars and new, effective fungicides to manage this disease. Results reported here highlight the importance of routine monitoring of the pathogen population to maximize the efficacy of control practices. Any or all of the alternative management approaches potentially could be effective in reducing or eliminating mefenoxam resistance in field populations if it is discovered early and particularly if resistance is present in a very low percentage of the pathogen population.

Mefenoxam efficacy is tied to a multitude of factors including pathogen aggressiveness sensitivity of pathogen population to mefenoxam, rates and methods of mefenoxam application, cultivar susceptibility, soil type, water relations and half-life of the fungicide in the field. The information gathered from the studies discussed here improve the management of pink rot by providing details on the potential presence and frequency of mefenoxam-resistant populations in Minnesota and North Dakota and by allowing for the modification of fungicide control programs as needed. Management of pink rot of potato will continue to rely on integrated approaches, such as elimination of mefenoxam as a disease management option in fields which have a history of mefenoxam resistance and following longer crop rotations. The use of low-risk fungicides with a different mode of action, like phosphorous acid, will be needed in problem fields, in addition to use of less susceptible cultivars. Although our results pertain to the situations as they currently exist in the potato growing areas

of Minnesota and North Dakota, they should also be of interest to growers in other potato production regions.

Acknowledgements The authors thank Dean Peterson, Viviana Rivera and several growers of R. D. Offutt Company for collecting tuber samples. The authors also thank Dr. Anitha Chirumamilla for reviewing the manuscript. The authors acknowledge the financial support of the Minnesota Area II Potato Growers Association, Simplot, Lamb-Weston/RDO Frozen and Cavendish Farms.

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