

Project Title: Effective Pink Rot Disease Control and Management of Mefenoxam Resistance in *Phytophthora erythroseptica*

Submitted to MN Area II Potato Growers

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Research Objectives:

1. Determine the prevalence of mefenoxam-resistance in the *P. erythroseptica* population in Minnesota.
2. Determine the impact of an alternative fungicide, phosphorous acid, on the management of mefenoxam resistance.
3. Determine if phosphorous acid provides residual control of pink rot in storage that is not currently provided by mefenoxam.

Procedures:

Pink rot survey. *P. erythroseptica* isolates will be collected by transferring small pieces of infected tissue, approximately 25 mm³ in size, to culture dishes containing water agar amended with ampicillin (100 µg/ml) and incubated in the dark at 17 to 20°C for 3 to 5 days. Colonies with mycelia resembling that of *P. erythroseptica* will be selected and purified by hyphal tipping.

Mefenoxam sensitivity testing. Mefenoxam (Ridomil Gold 4EC) sensitivity will be determined using an in vitro screening method. Tests will be conducted on modified V8 juice agar amended with fungicide in a 10-fold dilution series ranging from 0.01 to 100 µg/ml and control plates not amended with mefenoxam. A 5-mm-diameter disk containing mycelium and agar from the margin of actively growing colonies of 4- to 6-day-old cultures will be positioned in the center of a culture dish. Isolate growth will be determined by measuring colony diameters in two perpendicular directions after 6 days of incubation in the dark at 20 ± 1°C. Measurements were averaged, the diameter of the mycelial plug will be subtracted, and relative growth reduction for each rate of fungicide will be calculated as follows: $(100 - [\text{growth with fungicide}/\text{growth in control plate}] \times 100)$. The EC₅₀ relative to the control will be estimated by plotting the percentage inhibition against the log-scale of fungicide concentration.

Field plots and mefenoxam application. Fungicide application trials will be conducted under center pivot irrigation over two consecutive growing seasons. Fungicide treatments will be established each year to provide different levels of pink rot control in treated versus non-treated tubers (Table 1). At planting, a 50:50 blend of mefenoxam sensitive and insensitive isolates of the pink rot pathogen will be applied in the seed piece zone. Fungicide treatments will be applied at the recommended label rate. Mefenoxam (Ridomil Gold 4EC or Ultrafluorish) as an in-furrow application of 200 g a.i./ha at planting followed by an additional side-dress application of 100 g a.i./ha 21 days later (Table 1). This split application of mefenoxam at these rates previously has been demonstrated to provide the highest level of pink rot control (Taylor et al., 2004). Another mefenoxam treatment will be two foliar applications of 100 g a.i./ha when tubers are approximately 10 mm in diameter and 14 days later. One, two and three phosphorous acid

(Phostrol) treatments will all be made at a rate of 11.65 L/ha (Table 1). No in furrow treatments will be used since these have been demonstrated to be ineffective in controlling pink rot (Johnson, et al., 2004). The foliar phosphorous acid treatments will be applied when tubers are 10mm in diameter and 14 days later (2 applications) and the same treatment regime with a third application 14 days after the second application (total of three foliar applications). An additional phosphorous acid treatment will include a post-harvest application simulating tubers going into storage. Two treatments of cyazofamid (Ranman) will be used in this experiment (Table 1). The first will be an in furrow, at planting application at a rate of 450 mL/ha. The second treatment will be an in furrow treatment of 450 mL/ha followed by a sidedress application of 225 mL/ha.

Disease evaluations at harvest. Pink rot tubers will be obtained at harvest from all non-treated and all fungicide (2 treatments each of mefenoxam, 4 phosphorous acid and 2 cyazofamid) treated plots. These pink rot infected tubers will be taken to the laboratory and isolations for *P. erythroseptica* will be performed. All isolates obtained will be maintained on a treatment X replication basis and tested for their sensitivity to mefenoxam based on the methods previously described. The purpose of this portion of the proposed research is to determine the effect of non-mefenoxam fungicides on the mefenoxam sensitive and insensitive populations of *P. erythroseptica*.

Post-harvest pink rot inoculations. Plants will be killed by mechanical flailing 2 to 3 weeks prior to maturity to insure the availability of a sufficient quantity of tubers of the desired size and adequate skin set. After harvest, tubers were stored for 2 weeks at 15°C and 90% relative humidity to facilitate wound healing. However, because levels of mefenoxam in tubers will decline over time, test tubers used in this study were stored at 10°C for no longer than 4 months prior to testing. We do not know the length of residual control for phosphorous acid, but the experiments conducted here will provide that information and determine if this fungicide provides control of pink rot beyond harvest.

The level of residual, post-harvest control of pink rot will be determined using challenge inoculations conducted at 30 day intervals after harvest. Residual pink rot control studies will focus on the phosphorous acid treatments and comparing this to the known residual control provided by mefenoxam. We will not test the residual control potential of cyazofamid, since it is not a systemic fungicide (Table 1). Wounded and non-wounded tubers will be placed in plastic moist chamber boxes and inoculated with 10 µl of the zoospore suspension of *P. erythroseptica*. Inoculated tubers will be covered with four layers of paper towels moistened to saturation with deionized water. The chamber boxes will be sealed to establish high humidity to promote infection and incubated in the dark at ambient temperature at 20 to 22°C for 10 days.

Disease assessment. Inoculated tubers will be removed from the moist chambers and infection will be determined by cutting each tuber in half through the axis from the sites of inoculation on the apical bud end to the basal stem end. Split tubers will be covered with moist paper towels and incubated at ambient temperatures of 20 to 24°C for approximately 30 min to enhance the development of the discoloration diagnostic of pink rot. Infected tubers will be counted and disease incidence calculated as (number of diseased tubers/number of inoculated tubers) × 100. To determine pink rot severity, the maximum width of rot (W) and the depth (D) of rot from the inoculation point will be measured and penetration (P) of rot was calculated as $P = (W/2 + [D -$

5)]/2. Disease incidence will be transformed to percent disease control using the formula $([\text{disease incidence of untreated control} - \text{disease incidence of treatment}]/\text{disease incidence of untreated control}) \times 100$.

Results:

Pink rot survey. The incidence of pink rot in Minnesota in 2009 was at an all time low over the nine years of the survey (Figures 1 & 2). Some of this can be attributed to the environmental conditions near the end of the growing season when tuber infections by *P. erythroseptica* take place, but it is also likely due to the increase use of phosphorous acid to control the disease. Phosphorous acid controls mefenoxam-sensitive and resistant populations of the pink rot pathogen (see below) and its increased use in the state has obviously reduced pink rot disease pressure. Pink rot disease pressure is also very low in North Dakota (Figures 3 & 4).

Management of pink rot with phosphorous acid. The incidence of pink rot in field plots conducted in a grower field in Park Rapids, MN was low, at 2.5%, in non-treated control plots (Table 1). The low incidence of pink rot in this field with historically high disease pressure corroborates the observation of low pink rot incidence in Minnesota discussed above. Nonetheless, significant reductions were observed in field plots treated with in furrow followed by sidedress applications of mefenoxam as well as in furrow followed by foliar applications of mefenoxam (Table 1). Two and three applications of phosphorous acid applied to the foliage were also very effective in significantly reducing pink rot, however, a single application of phosphorous acid was insufficient to provide effective disease control. Interestingly, in contrast to results obtained in previous years, post-harvest applications of phosphorous acid did not significantly reduce pink rot (Table 1). This is likely due to the length of time between harvest and post-harvest applications of phosphorous acid took place (10 days) which allowed the pink rot pathogen to gain entry through wounds made at harvest.

Residual control of pink rot with phosphorous acid. Results obtained in 2007 suggested that foliar and post-harvest applications of phosphorous acid provided excellent residual control of pink rot in storage, up to 150 days after harvest. Those data also clearly demonstrated that phosphorous acid control mefenoxam-resistant and mefenoxam-sensitive populations of *P. erythroseptica* with equal efficacy. To date, we have conducted a 91 DAH assessment which continues to demonstrate excellent residual control of pink rot using two and three foliar applications of phosphorous acid (Table 2, Figure 5). We will continue to challenge inoculate tubers from these trials to determine if phosphorous acid treatments provide residual pink rot control well into the storage period.

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Table 1. Percentage tuber rot among treatments evaluated at harvest and 25 to 27 days after harvest (DAH). Mean separation based on Fisher's protected least significant difference (LSD) test ($P = 0.05$).

Treatment	Rate	Application Timing	Percent Tuber Rot	
			At Harvest	25 to 27 DAH
3301 Non-treated	-	-	2.5	0.65
3302 Ridomil 4EC	6.1 oz / a	in-furrow	1.9	0.68
3303 Ridomil 4EC	12.2 oz / a	in-furrow	1.7	0.35
3304 Ridomil 4EC	6.1 oz / a	in-furrow	0.9	0.45
Ridomil 4EC	6.1 oz / a	sidedress		
3305 Ridomil 4EC	6.1 oz / a	in-furrow	0.8	0.41
Ridomil MZ	2.0 lb / a	tuber set		
3306 Ridomil MZ	2.0 lb / a	tuber set	1.3	0.69
Ridomil MZ	2.0 lb / a	tuber set + 14 days		
3307 Phostrol	10.0 pt / a	tuber set	1.2	0.37
3308 Phostrol	10.0 pt / a	tuber set	0.9	0.04
Phostrol	10.0 pt / a	tuber set + 14 days		
3309 Phostrol	10.0 pt / a	tuber set	0.4	0.01
Phostrol	10.0 pt / a	tuber set + 14 days		
Phostrol	10.0 pt / a	tuber set + 28 days		
3310 Phostrol	12.8 fl oz / ton	10 days post-harvest	2.8	0.41
LSD $P = 0.05$			1.4	NS

Table 2. Percentage tuber rot among treatments challenge inoculated with a mefenoxam resistant and sensitive isolate of *Phytophthora erythroseptica* at 29, 64, 78, and 91 days after harvest (DAH). Mean separation based on Fisher's protected least significant difference (LSD) test ($P = 0.05$).

Treatment	Rate	Application Timing	<i>P. erythroseptica</i> isolate	<i>P. erythroseptica</i> challenge inoculation (% incidence)			
				29 DAH	64 DAH	78 DAH	91 DAH
Non-treated	-	-	Mefenoxam Resistant	40.0	15.0	42.5	22.5
Ridomil 4EC	6.1 oz / a	in-furrow	Mefenoxam Resistant	30.0	17.5	32.5	20.0
Ridomil 4EC	12.2 oz / a	in-furrow	Mefenoxam Resistant	37.5	25.0	35.0	20.0
Ridomil 4EC	6.1 oz / a	in-furrow	Mefenoxam Resistant	30.0	27.5	25.0	15.0
Ridomil 4EC	6.1 oz / a	sidedress	Mefenoxam Resistant	30.0	27.5	25.0	15.0
Ridomil 4EC	6.1 oz / a	in-furrow	Mefenoxam Resistant	30.0	35.0	30.0	27.5
Ridomil MZ	2.0 lb / a	tuber set	Mefenoxam Resistant	30.0	35.0	30.0	27.5
Ridomil MZ	2.0 lb / a	tuber set	Mefenoxam Resistant	37.5	15.0	37.5	15.0
Ridomil MZ	2.0 lb / a	tuber set + 14 days	Mefenoxam Resistant	37.5	15.0	37.5	15.0
Phostrol	10.0 pt / a	tuber set	Mefenoxam Resistant	35.0	10.0	20.0	12.5
Phostrol	10.0 pt / a	tuber set	Mefenoxam Resistant	7.5	2.5	2.5	2.5
Phostrol	10.0 pt / a	tuber set + 14 days	Mefenoxam Resistant	7.5	2.5	2.5	2.5
Phostrol	10.0 pt / a	tuber set	Mefenoxam Resistant	2.5	2.5	7.5	10.0
Phostrol	10.0 pt / a	tuber set + 14 days	Mefenoxam Resistant	2.5	2.5	7.5	10.0
Phostrol	10.0 pt / a	tuber set + 28 days	Mefenoxam Resistant	2.5	2.5	7.5	10.0
Phostrol	12.8 fl oz / ton	10 days post-harvest	Mefenoxam Resistant	0.0	0.0	0.0	0.0
LSD _{P = 0.06}				21.7	13.4	19.8	16.6
Non-treated	-	-	Mefenoxam Sensitive	32.5	15.0	17.5	40.0
Ridomil 4EC	6.1 oz / a	in-furrow	Mefenoxam Sensitive	5.0	0.0	10.0	7.5
Ridomil 4EC	12.2 oz / a	in-furrow	Mefenoxam Sensitive	7.5	2.5	5.0	7.5
Ridomil 4EC	6.1 oz / a	in-furrow	Mefenoxam Sensitive	0.0	0.0	2.5	7.5
Ridomil 4EC	6.1 oz / a	sidedress	Mefenoxam Sensitive	0.0	0.0	2.5	7.5
Ridomil 4EC	6.1 oz / a	in-furrow	Mefenoxam Sensitive	5.0	7.5	10.0	7.5
Ridomil MZ	2.0 lb / a	tuber set	Mefenoxam Sensitive	5.0	7.5	10.0	7.5
Ridomil MZ	2.0 lb / a	tuber set	Mefenoxam Sensitive	0.0	5.0	7.5	5.0
Ridomil MZ	2.0 lb / a	tuber set + 14 days	Mefenoxam Sensitive	0.0	5.0	7.5	5.0
Phostrol	10.0 pt / a	tuber set	Mefenoxam Sensitive	25.0	20.0	7.5	17.5
Phostrol	10.0 pt / a	tuber set	Mefenoxam Sensitive	7.5	2.5	5.0	0.0
Phostrol	10.0 pt / a	tuber set + 14 days	Mefenoxam Sensitive	7.5	2.5	5.0	0.0
Phostrol	10.0 pt / a	tuber set	Mefenoxam Sensitive	2.5	0.0	0.0	0.0
Phostrol	10.0 pt / a	tuber set + 14 days	Mefenoxam Sensitive	2.5	0.0	0.0	0.0
Phostrol	10.0 pt / a	tuber set + 28 days	Mefenoxam Sensitive	2.5	0.0	0.0	0.0
Phostrol	12.8 fl oz / ton	10 days post-harvest	Mefenoxam Sensitive	0.0	0.0	0.0	0.0
LSD _{P = 0.06}				15.0	8.8	8.5	13.1

Table 2. Con't).							
Treatment	Rate	Application Timing	<i>P. erythroseptica</i> isolate	<i>P. erythroseptica</i> challenge inoculation (% incidence)			
				29 DAH	64 DAH	78 DAH	91 DAH
Non-treated	-	-		36.3	15.0	30.0	31.3
Ridomil 4EC	6.1 oz / a	in-furrow		17.5	8.8	21.3	13.8
Ridomil 4EC	12.2 oz / a	in-furrow		22.5	13.8	20.0	13.8
Ridomil 4EC	6.1 oz / a	in-furrow		15.0	13.8	13.8	11.3
Ridomil 4EC	6.1 oz / a	sidedress					
Ridomil 4EC	6.1 oz / a	in-furrow		17.5	21.3	20.0	17.5
Ridomil MZ	2.0 lb / a	tuber set					
Ridomil MZ	2.0 lb / a	tuber set		18.8	10.0	22.5	10.0
Ridomil MZ	2.0 lb / a	tuber set + 14 days					
Phostrol	10.0 pt / a	tuber set		31.3	15.0	13.8	15.0
Phostrol	10.0 pt / a	tuber set		7.5	2.5	3.8	1.3
Phostrol	10.0 pt / a	tuber set + 14 days					
Phostrol	10.0 pt / a	tuber set		2.5	1.3	3.8	5.0
Phostrol	10.0 pt / a	tuber set + 14 days					
Phostrol	10.0 pt / a	tuber set + 28 days					
Phostrol	12.8 fl oz / ton	10 days post-harvest		0.0	0.0	0.0	0.0
LSD _{P = 0.06}				13.4	8.0	10.3	10.4
			Mefenoxam Resistant	25.3	15.0	23.3	14.5
			Mefenoxam Sensitive	8.5	5.3	6.5	9.3
LSD _{P = 0.06}				6.0	3.6	4.6	4.7

Note: Interaction of main effects of treatment and mefenoxam resistance were significant for all inoculation dates ($P = 0.05$)

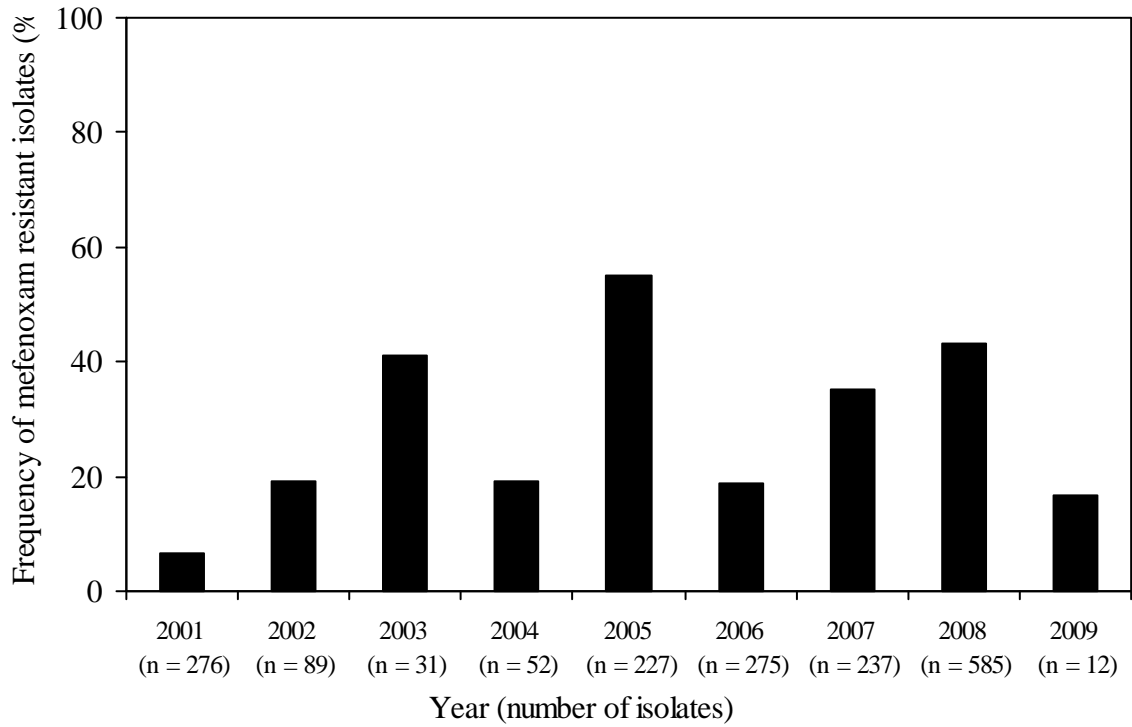


Figure 1. Frequency of mefenoxam resistance in *Phytophthora erythroseptica* in Minnesota from 2001 to 2009.

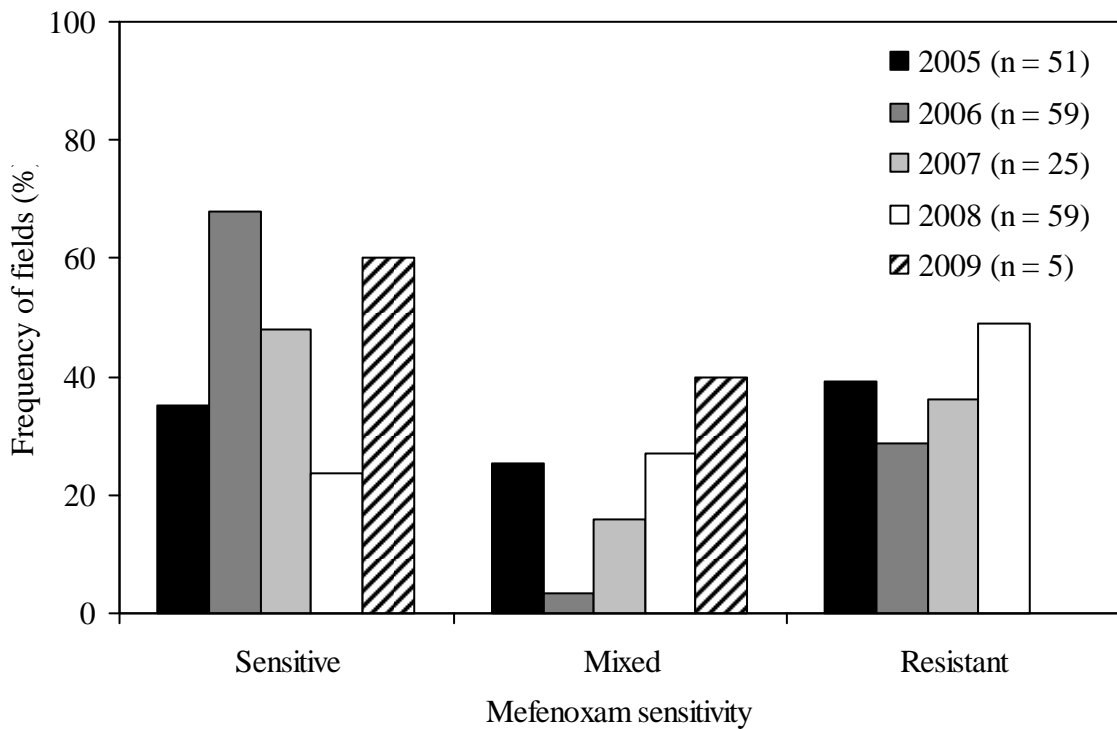


Figure 2. Frequency of potato fields with mefenoxam sensitive, resistant or mixed populations of *Phytophthora erythroseptica* in Minnesota from 2005 to 2009. Number of fields in the survey each year given parenthetically.

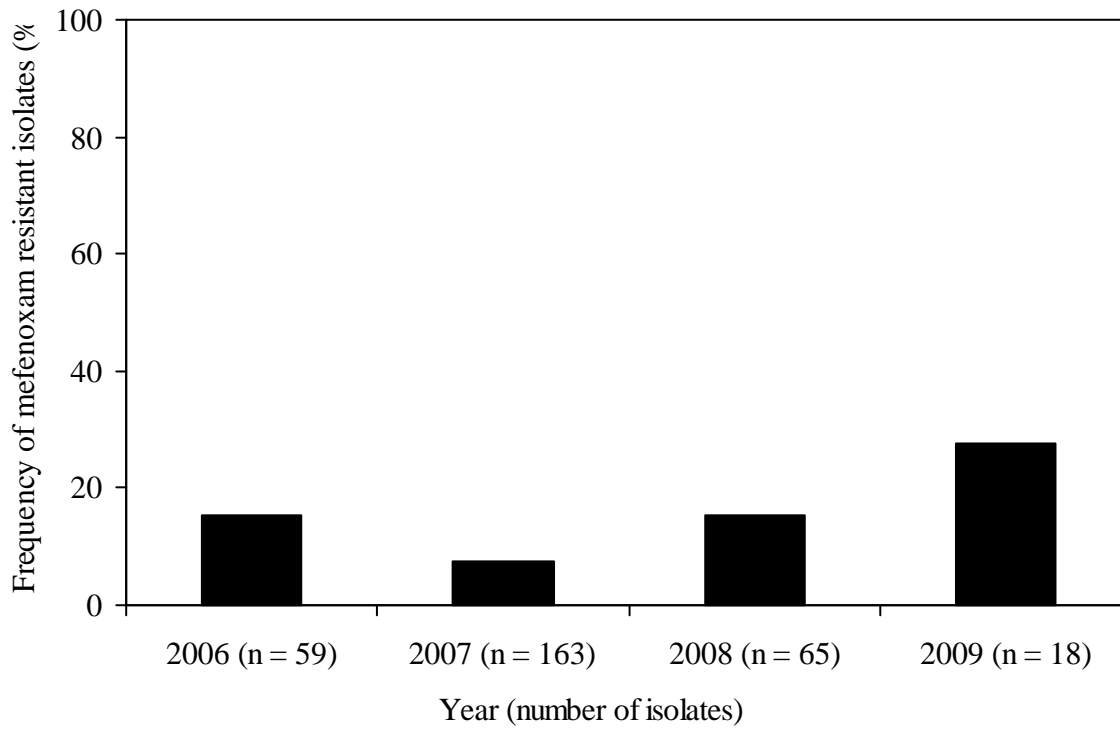


Figure 3. Frequency of mefenoxam resistance in *Phytophthora erythroseptica* in North Dakota from 2006 to 2009.

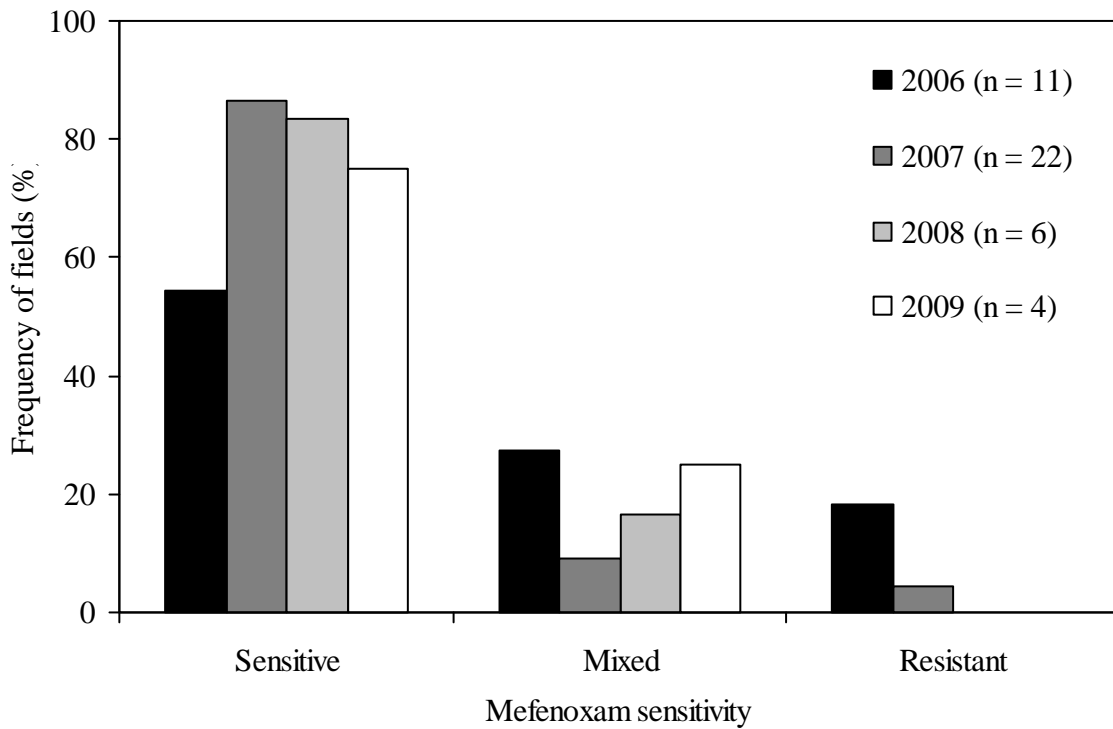


Figure 4. Frequency of potato fields with mefenoxam sensitive, resistant or mixed populations of *Phytophthora erythroseptica* in North Dakota from 2006 to 2009.

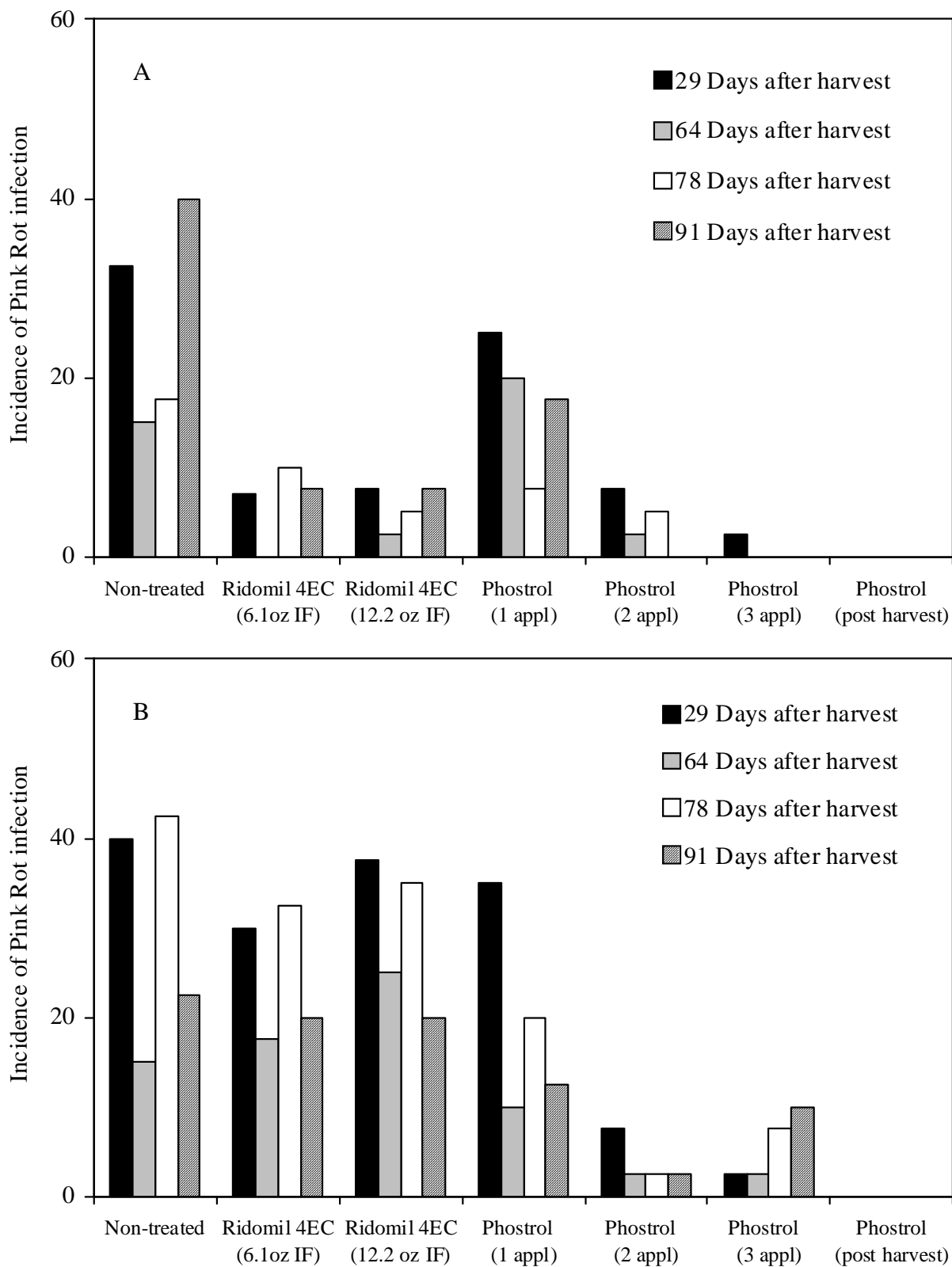


Figure 5. Incidence of pink rot caused by mefenoxam sensitive (A) and resistant (B) isolates of *Phytophthora erythroseptica* in potato tubers treated with mefenoxam or phosphorus acid.