# ASSESSING AND MANAGING METALAXYL-M RESISTANT STRAINS OF THE POTATO PINK ROT PATHOGEN *PHYTOPHTHORA ERYTHROSEPTICA* IN CANADA

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## ABSTRACT

*Phytophthora erythroseptica* Pethybr. is the causal agent of potato pink rot which results in a wet rot and complete breakdown of tubers leading to significant yield losses in field and storage settings. Traditionally, products containing metalaxyl and later its enantiomer metalaxyl-m have been used to manage *P. erythroseptica*. However, in recent years, metalaxyl-m resistant strains of *P. erythroseptica* have been recovered in the United States and Atlantic Canada. A national survey was conducted from 2013 to 2016 to assess the distribution of metalaxyl m-resistant populations in Canadian potato-producing regions. The survey recovered resistant isolates from Prince Edward Island, Nova Scotia, New Brunswick, Ontario, Manitoba, and Alberta. As a result of this survey, resistant isolates were identified for the first time in Ontario, Manitoba, and Alberta. Due to the increasing frequency of metalaxyl-m resistant populations there is a need for alternative management strategies. Therefore, registered and experimental fungicides were assessed for the capacity to control the pink rot pathogen in field and storage settings. The fungicides evaluated were metalaxyl-m, oxathiapiprolin, fluopicolide, phosphites, and a biological control agent *Bacillus subtilis*. The $EC_{50}$ values (the fungicide concentration inhibiting mycelial growth of the pathogen by 50% relative to the 0 μg $mL^{-1}$ control) for two strains (PE9913; metalaxyl-m sensitive and PE1204; metalaxyl-m resistant) of *P. erythroseptica* exposed to metalaxyl-m, oxathiapiprolin, fluopicolide, and phosphites were determined using an amended agar assay. The $EC_{50}$ values were 0.057 and 842.41 ppm, respectively, for the metalaxyl-m sensitive and metalaxyl-m resistant isolates grown on metalaxyl-m amended media. The $EC_{50}$ values of the metalaxyl-m sensitive and metalaxyl-m resistant isolates exposed to oxathiapiprolin, fluopicolide, or phosphite were <0.0001 ppm, 0.064 to 0.128, and 112.0 to 147.6 ppm,

respectively. Field trials were established from 2014 to 2016 to evaluate the fungicides for their efficacy in managing pink rot in the field. Significant control of pink rot was achieved by in-furrow treatments of oxathiapiprolin or fluopicolide, or phosphites applied foliarly. Some pink rot suppression was achieved following application of *Bacillus subtilis*, but of significantly smaller degree than provided by the other products. Storage trials were established in 2016 and 2017 to assess the capacity of these fungicides to inhibit *P. erythroseptica* infection of tubers inoculated with zoospores prior to storage. Significant control of pink rot was achieved with treatments of oxathiapiprolin, fluopicolide, phosphites, and to lesser extent *Bacillus subtilis* under storage conditions. The results of this study show that metalaxyl-m resistant populations of *P. erythroseptica* are increasing in frequency in Canadian potato-producing regions. However, management of these populations can be achieved with use of currently registered and experimental products in production settings.

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# INTRODUCTION

Potato production is part of the identity of Prince Edward Island. In 2016, PEI planted over 36 000 ha of potatoes which accounted for 25.7% of Canadian potato production. Globally, potato is a very import food staple. Ranked third in production after rice and wheat, potatoes provide a vital source of nutrition to millions of people. Potatoes are rich in nutrients, easy to prepare, versatile, easy to grow, and easy to store which accounts for their increasing popularity.

Like many other crops, potatoes are susceptible to a variety of pathogens and pests which reduce yield and lead to economic losses. One disease known as pink rot leads to significant yield losses in field and storage due to a wet rot of tubers. Pink rot is caused by the fungus-like oomycete *Phytophthora erythroseptica* Pethybr. and it has traditionally been managed with foliar and in-furrow applications of the fungicides metalaxyl and later its enantiomer metalaxyl-m (Al-Mughrabi *et al.,* 2007). In recent years, metalaxyl-m resistant strains of *P. erythroseptica* have evolved and have been recovered in the United States and Atlantic Canada (Peters *et al.,* 2001; Peters *et al.,* 2013). A two year survey of Canadian *P. erythroseptica* populations recovered metalaxyl-m resistant isolates in Atlantic Canada, Ontario, Manitoba, and Alberta (Crane, 2015). This has brought the continuing usefulness of metalaxyl-m into question. The recovery of metalaxyl-m resistant strains has resulted in the need for alternative management strategies to control pink rot in field and storage settings.

The objectives of this study were: to assess the distribution of metalaxyl-m resistant populations of *P. erythroseptica* via initiation of a national survey; and b) to conduct field and storage trials to assess the efficacy of experimental and registered products to inhibit pink rot development in field and in storage.

# LITERATURE REVIEW

## The Host: *Solanum tuberosum* L.

Potatoes are one of the most important horticultural crops grown in the world. Ranked third in total production behind rice and wheat, potato is an important staple in many regions due its ease of production, storage, nutrition, and versatility. Originating in the Andes mountains, many potato species were cultivated and domesticated by the indigenous peoples of South America approximately 12 000 years ago in the Lake Titicaca basin (Smith, 2011). These farmers developed many varieties that could be grown at different altitudes on the mountain slopes which provided them with a year-round food source. The farmers also developed methods to store the tubers and learned that potatoes could be propagated using the eyes of the tubers as well as seeds (Smith, 2011). By using the eyes of the tubers, the farmers could grow clones of the varieties they liked and by using the seeds they could develop new cultivars. Arguably the most important species that they domesticated is *Solanum tuberosum* L. which is now commercially grown globally with many varieties and is the most cultivated and consumed vegetable in the world (Smith, 2011).

Potato production in Canada is important to the Canadian economy. In 2016, 346,827 acres were planted with potatoes which yielded 105.1 million cwt (4.7 million tonnes) of tubers (Statistics Canada, 2016a; Statistics Canada, 2016b). In 2012, Canadian production netted a farm gate value of $1.12 billion. Potato production is of particular importance in Prince Edward Island which accounted for 24.45% of Canadian potato production in 2016 (Statistics Canada, 2016b). These tubers are grown for domestic consumption, seed propagation, processing, and global export and are a significant part of the agricultural industry. It is therefore of critical importance to protect this resource.

**The Genus *Phytophthora***

Potatoes are host to many pathogens and parasites that can cause various degrees of damage to plant tissues. The genus *Phytophthora* contains many plant pathogens and belongs to the order Peronosporales, class Oomycetes and Phylum Oomycota within the Kingdom Chromista (Martin *et al*., 2012). The genus name is derived from the Greek *phyto* (plant) and *phthora* (destroyer) (Erwin and Ribeiro, 1996). *Phytophthora* are behaviourally and morphologically similar to fungi in regards to nutrient absorption and mycelium production but biologically different due to differences in reproduction such as the production of oospores, a predominately diploid lifecycle, and the presence of a cell wall comprised of beta glucans rather than chitin (Palm and Rossman, 2006; Martin *et al*., 2014).

Traditionally, *Phytophthora* species are taxonomically identified based on the shape of the papilla of the sporangia, the form of the antheridium, its mode of reproduction, and the internal shape of the oogonium (Erwin and Ribeiro, 1996). The apical thickening of the sporangium is called the papilla and is also the structure that opens to allow for the release of zoospores. Sporangia can be papillate (conspicuous thickening), semipapillate, or nonpapillate (inconspicuous thickening) (Erwin and Ribeiro, 1996). The antheridium's (male gametangium) position relative to the oogonium (female gametangium) is used to determine the form of the antheridium. Amphigynous antheridia surround the oogonial stalk while paragynous antheridia are attached to the sides of oogonia (Erwin and Ribeiro, 1996). *Phytophthora* species are classified as homothallic or heterothallic based upon their method of reproduction. Heterothallic species require two mating types designated A1 and A2 to reproduce sexually while

homothallic species can reproduce by in-breeding (Cooke *et al*., 2000; Erwin and Ribeiro, 1996). The internal structure of the oogonium is determined by the shape of the oospore within it. Oogonia are plerotic when the oospore fills the oogonium or are aplerotic when the oospore doesn't fill the oogonium (Erwin and Ribeiro, 1996). The genus *Phytophthora* has been divided into six clades based upon morphological features (Waterhouse, 1963) and has since been divided into ten clades based upon nuclear and mitochondrial DNA sequence analyses (Blair *et al.,* 2008; Martin *et al*., 2014).

Molecular analyses of *Phytophthora* using conventional and real-time polymerase chain reaction (PCR) has been used in many phylogeny studies (Cooke *et al.,* 2000; Blair *et al.,* 2008; Martin *et al.,* 2014). Past studies separated species based on sequence variation at the internal transcribed spacer regions (ITS), nuclear ribosomal DNA, and cytochrome oxidase I and II of the mitochondrion. More recent studies have used multiple loci from nuclear and mitochondrial genomes to separate *Phytophthora* into the current ten clades (Blair *et al.,* 2008; Martin *et al.,* 2014).

**The Pink Rot Pathogen *Phytophthora erythroseptica***

*Phytophthora erythroseptica* Pethybr. is the causal agent of pink rot disease in potatoes. The pathogen was first described in 1913 in Ireland (Pethybridge, 1913) and is ubiquitous in potato producing regions globally. *Phytophthora erythroseptica* is a soilborne pathogen known as a water mould that is homothallic and reproduces asexually via sporangia that are multinucleated, hyaline (glassy, translucent), mainly ellipsoid-obpyriform in shape, thin walled, nonpapillate, and 39-47 x 25-29 µm in size (Erwin and Ribeiro, 1996). The sporangia can germinate directly or cleave to produce uninucleated

free-swimming zoospores that move via water films in soil. Zoospores are approximately 10 μm in diameter, biflagellate, reniform (kidney-shaped) to pyriform (pear-shaped), and have a deep longitudinal groove from which two flagella emerge (Erwin and Ribeiro, 1996). *Phytophthora erythroseptica* can also reproduce sexually via oogonia that are globose and 34-36 μm in diameter (Platt and Peters, 2006). Antheridia are strictly amphigynous, spherical to ovoid, and 14-16 x 11-13 μm in size. Oospores are aplerotic and 28-31 μm in diameter with walls about 3 μm thick (Platt and Peters, 2006). Oospores, sporangia, and zoospores are all infective stages of the *P. erythroseptica* lifecycle; however, infections are caused mainly by zoospores while it is believed that oospores are the primary source of overwintering pink rot inoculum in potato. *Phytophthora erythroseptica* belongs to clade 8 of the *Phytophthora* genus and is most closely related to *Phytophthora cryptogea* Pethybr. & Laff. which can also cause pink rot like symptoms in potatoes but less frequently (Erwin and Ribeiro, 1996). *Phytophthora erythroseptica* strains have been found to cause root rots, leaf spots, crown rots, fruit rots, wilt, and leaf and petiole blights on several plant species such as lilies, peas, raspberry, sugarcane, tomatoes, tulips, vetch, wild rice, and spinach (Platt and Peters, 2006).

**Pink Rot**

Pink rot is one of the most damaging potato-tuber diseases because harvested tubers can be superficially infected but appear healthy and later completely rot in storage leading to significant yield loss. The superficial infections can also remain dormant in storage and develop on seed tubers the following year after planting, providing inoculum

to introduce the pathogen to new areas. The pathogen can also transfer to healthy tubers in storage or during tuber handling (Platt, 2008).

Tuber infection usually occurs in wet, low-lying areas of a field. The pathogen is most damaging in water-logged soils with temperatures above 20°C, but the pathogen can also initiate infection at temperatures ranging from 5 to 33°C (optimum 20-27°C) (Al-Mughrabi *et al*., 2007; Platt, 2008). Infection occurs most often when wet conditions persist before harvest but infection can also occur shortly after planting during plant emergence (Platt, 2008). Oospores will germinate in soil in warm, wet conditions and produce mycelia and sporangiophores from which sporangia develop. Tuber infection occurs when the germinating oospores, sporangia, or zoospores form germ tubes and infect stolons or enter the host via open lenticels, wounds, and tuber eyes. In cases of extreme infection, the roots and basal stem can also become infected and lead to plant wilting and death. The primary source of infection in soil is by swimming zoospores that will encyst on the host and germinate to produce a germ tube that penetrates the host. Infection of the stolons allows for mycelia to infect daughter tubers and also leads to tuber decay. When infected tissue begins to breakdown, oospores that were formed in the tissue are released into the soil (Platt and Peters, 2006).

The exterior surfaces of infected tubers are irregularly shaped, and display dull brown lesions with dark skin tones around the eyes and lenticels (Platt, 2008). The infected tissues have a soft, wet, spongy, and rubbery texture. The infection of the internal tissue usually begins at the stem-end of the tuber and has a creamy to light brown colour that changed to a pink colour when exposed to air after 20-30 minutes, giving the disease its name (Platt, 2008). After turning pink, the tissue will eventually turn brown

and then black. The tissue will expel a clear, odourless liquid when squeezed. If left to develop, the infection will result in wet rot and complete tuber breakdown (Platt and Peters, 2006; Platt, 2008).

**Managing Pink Rot**

Methods of controlling pink rot include chemical and cultural controls. Crop rotations of three or four years have been shown to limit the disease (Peters *et al*., 2004a). Rotations with alternative crops such as clover, oats, or barley have been shown to significantly reduce the incidence of the disease. It was found that bacterial populations associated with clover roots (25 species of which are also commonly associated with potatoes) tended to reduce the susceptibility of potatoes to *P. erythroseptica* due to the bacteria's perseverance in the soil (Peters *et al*., 2004a). Crop rotations with non-host species can also allow for the reduction of pathogen propagule levels in the soil over time. To prevent pink rot development, it is recommended that producers use disease-free seed to limit the spread of the pathogen and plant their crop in warm, well-drained soils to improve plant growth (Platt, 2008). The use of potato cultivars with resistance to pink rot is recommended. However, many commercially available cultivars vary only slightly in their susceptibility to the disease (Platt and Peters, 2006).

Harvesting the tubers when the foliage is completely dead is recommended as it will encourage tuber skin development and reduce the incidence of disease. The tubers should also be harvested after the soil is dry and below 18°C which provides an inhospitable environment for spore germination (Peters *et al*., 2004b). Tuber damage during harvesting should also be minimized and the amount of soil adhering to the tubers

should be reduced to limit the spread of the pathogen. Grading out diseased tubers before storage helps to reduce storage rot, and harvesting suspect areas of field at the end the harvest season will also help to reduce the spread of the pathogen to healthy tubers (Platt and Peters, 2006).

**Metalaxyl and Metalaxyl-m**

Traditionally, metalaxyl has been used to manage *P. erythroseptica* infections. Metalaxyl (methyl 2-(N-(2-methoxyacetyl)-2,6-dimethylanilino)propanoate) is a systemic fungicide that can translocate throughout the plant and is used for the control of *Phytophthora*-based diseases (Al-Mughrabi, 2007). Metalaxyl [Fungicide Resistance Action Committee (FRAC), 4] is a phenylalamide fungicide and provides protection against some oomycete pathogens by selective inhibition of ribosomal RNA synthesis by affecting the activity of RNA polymerases. Due to its site-specificity, metalaxyl has a relatively high intrinsic risk of resistance development in target pathogens (Hu *et al*., 2008). Metalaxyl was first introduced in 1977 as a racemic mixture of R and S enantiomers and was used on a variety of plant species for the control of *Phytophthora* diseases. However, resistance to metalaxyl began to develop in *Phytophthora* species in the early 1990s, including *P. erythroseptica.* In 1997, metalaxyl-m, the biologically active R-enantiomer of metalaxyl, became commercially available. This isomer, also known as mefenoxam, has similar properties to metalaxyl but is used at lower rates. It can be applied as an in-furrow treatment at planting or as a foliar treatment during the growing season (Parra and Ristaino, 2001).

Resistance to metalaxyl-m developed in strains of *P. erythroseptica* in the early 2000s in the United States (Peters et al., 2001). In 2012, metalaxyl-m resistant strains were found in Prince Edward Island for the first time (Peters *et al.,* 2013) which gave rise to a national survey conducted in 2013 and 2014 to assess the distribution of metalaxyl-m resistant strains in Canada (Peters *et al.,* 2013). Metalaxyl-m resistant strains were recovered from Prince Edward Island, Nova Scotia, and New Brunswick and were identified for the first time in Ontario and Manitoba in 2013 and in Alberta in 2014 (Crane, 2015). This signified the need to continue to determine the distribution of metalaxyl-m resistant strains in Canadian potato growing regions and to develop and assess alternative management strategies as the effectiveness of metalaxyl-m came into question.

## Alternative Management Strategies

### Phostrol™

Other chemical treatments developed since the release of metalaxyl are registered, are waiting to be registered, or show promise to suppress and control pink rot. One class of fungicides that show promise in controlling water mould pathogens resistant to metalaxyl-m are phosphite-based fungicides (FRAC 33) such as Phostrol ™ (mono- and di-basic sodium, potassium, and ammonium salts of phosphorous acid; Engage Agro Corporation, Guelph, Ontario). Although phosphite-based fungicides were released commercially at approximately the same time as metalaxyl, phosphite-based products have only recently been thoroughly investigated as control agents for oomycete fungi. Phosphorous acid (phosphite or phosphonate) is a structurally simple compound ($H_3PO_3$)

but its mode of action appears to be complex and not well understood (Taylor *et al*., 2011). Studies have demonstrated that phosphorous acid affects the pathogen by inhibiting mycelial growth, suppressing sporulation and germination, and stimulating host plant defenses. Phosphorous acid is also translocated systemically within the plant, but unlike metalaxyl-m its biological activity against *Phytophthora* spp. is narrower, demonstrating low activity in some and high activity in others. However, previous studies show that phosphite-based fungicides show promise in controlling pink rot infections as both in-furrow and foliar treatments (Taylor *et al*., 2011). Currently Phostrol™ is registered for in-furrow, foliar, and post-harvest applications for the control of pink rot and late blight in potatoes.

**Serenade SOIL®**

Another fungicide that has recently become available is Serenade SOIL® (*Bacillus subtilis* QST 713; Bayer CropScience Inc., Calgary Alberta). Serenade SOIL® is a biological fungicide (FRAC 44) for fruit and vegetable crops and has a unique mode of action. These bacteria are ubiquitous in soils and many other habitats worldwide and are antagonistic towards many fungal plant pathogens. This antagonism can be achieved in many different ways including competition for nutrients, site exclusion, colonization, and attachment of the bacteria to the fungal pathogen. This strain of bacteria has also been shown to induce the plant's natural systemic resistance against bacterial pathogens. These bacteria can also stop pathogen spores from germinating, disrupt germ tube growth, and inhibit attachment of the pathogen to the plant (EPA, 2006). The bacteria produce a zone of protection around the seed potato and roots of the plant that expands as

the plant grows. This fungicide is exempted from maximum residue limits and does not require a delay between application and harvest (Kikkert and Rozdeba, 2014). Serenade SOIL® is currently registered for in-furrow applications on potatoes for the control of pink rot, Fusarium root rot, Pythium root rot, and Rhizoctonia black scurf, as well as a post-harvest treatment for the control of *Heliminthosporium solani* causing silver scurf of potatoes in storage.

**Presidio®**

One fungicide recently registered for pink rot management is Presidio® (Fluopicolide (2,6-dichloro-N-[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]methyl] benzamide); Valent Canada Inc., Guelph, Ontario). Fluopicolide (FRAC 43) is a mesosystemic fungicide that translocates toward stem tips via the xylem but does not translocate towards the roots (EPA, 2007). Fluopicolide has a new mode of action not found in other anti-oomycete fungicides. Fluopicolide induces delocalization of spectrin-like proteins of the pathogen which play a crucial role in membrane stability in fungi and oomycetes. When *Phytophthora infestans* (Mont.) de Bary hyphae were treated with fluopicolide, the spectrin-like proteins delocalized from the plasma membrane and were observed as spherical spots in the plasma membrane. Similar effects were observed in zoospores, which also exhibited swelling and cell lysis 15 to 20 minutes after treatment (Toquin *et al*., 2007). When isolates of *Phytophthora nicotianae var. nicotianae* (Breda de Haan) were grown on fluopicolide amended agar, mycelial growth, sporangial formation, and zoospore germination were all inhibited by 50% at concentrations of 0.09, 0.15, and 0.16 $\mu g\ mL^{-1}$, respectively (Qu *et al*., 2016).

**Orondis®**

Oxathiapiprolin (1-(4-{4-[(5RS)-5-(2,6-difluorophenyl)-4,5-dihydro-1,2-oxazol-3-yl]-1,3-thiazol-2-yl}-1-piperidyl)-2-[5-methyl-3-(trifluoromethyl)-1H-pyrazol-1-yl]ethanone) is a newly developed fungicide registered as Orondis® (Syngenta Canada Inc., Guelph, Ontario) in Canada for the control of oomycete pathogens *Phytophthora infestans* (late blight) and *Peronospora destructor* (downy mildew) in potatoes. Oxathiapiprolin is the first of the new piperidinyl thiazole isoxazoline class of fungicides (FRAC U15). Oxathiapiprolin's mode of action is through the inhibition of oxysterol binding proteins (OSBP) in oomycete pathogens. The function of OSBP in oomycetes is not clear, but they have low homology with other OSBP-related proteins of other eukaryotes which may account for the activity of oxathiapiprolin on oomycetes but not on other organisms (Miao *et al*., 2015). An in-vitro assay study of mefenoxam resistant *Phytophthora nicotianae* conducted to determine the effects of oxathiapiprolin found that mycelial growth, sporangia production, zoospore germination, and zoospore motility were all reduced by 50% at concentrations of 0.0039-0.0049, 0.00052-0.00081, 0.0035-0.0051, and 0.0055-0.0166 μg a.i. $mL^{-1}$, respectively (Bittner and Mila, 2014).

Like metalaxyl-m, the risk of resistance is assumed to be medium to high in target pathogens due to the single site mode of action of oxathiapiprolin. Miao et al., (2016) developed resistant strains of *Phytophthora capsici in vitro* using potato dextrose agar (PDA) media amended with 0.005 μg $mL^{-1}$ oxathiapiprolin. Sensitive isolates were plated onto the media and incubated for 15-20 days at 25°C in the dark. Plates exhibiting growth were covered with fresh amended PDA and grown for a further 15-20 days at 25°C in the dark. Afterward, surviving isolates were subcultured onto fungicide-free PDA media and

incubated. After 5 days, the isolates were subcultured and plated onto PDA media amended with 0.05, 0.1, or 0.5 µg $mL^{-1}$ oxathiapiprolin and incubated for 15-20 days. The selection process was repeated ten times until the resistant colonies exhibited normal mycelial growth rates on media amended with 0.05, 0.1, or 0.5 µg $mL^{-1}$ oxathiapiprolin (Miao *et al.,* 2015). Comparison of the PcORP1 genes in the sensitive and resistance isolates, which encode the oxysterol binding protein target of oxathiapiprolin, revealed that a heterozygous mutation caused the amino acid substitution G769W which was responsible for the observed resistance (Miao *et al*., 2016). This highlights the need for resistance management for this fungicide to ensure that its effectiveness will last for years to come.

**Identification of *Phytophthora erythroseptica* using PCR**

Identification of *P. erythroseptica* using conventional and real-time PCR has been based on the work of Tooley *et al.* (1997) and Cullen *et al.* (2007). DNA primers were designed to amplify the ITS regions of rRNA which is common in phylogeny research due to the high variability of ITS regions even amongst closely related species. The methods and primers developed by Tooley *et al.* and Cullen *et al.* have been used in other studies to identify the pathogen using PCR from plant and tuber tissue, and from soil using baiting techniques (Nanayakkara *et al*., 2009a; Nanayakkara *et al*., 2009b). Hairy nightshade (*Solanum sarrachoides* Sendt) and bitter nightshade (*Solanum dulcamara* L.) were used to detect the presence of *P. erythroseptica* in soils artificially inoculated with 20 000 or 50 000 zoospores $mL^{-1}$ (Nanayakkara *et al*., 2009a). However, the nightshade had to grow in the inoculated soil for a minimum of six days before the pathogen was

detectable with conventional or real-time PCR (Nanayakkara *et al*., 2009a). Recent molecular studies have identified *P. erythroseptica* using genes from nuclear and mitochondrial DNA. These studies have developed primers to amplify the genes such as Heat Shock Protein 90 and beta-tubulin amongst others to determine the phylogeny of *Phytophthora* (Blair *et al*., 2008). Currently, all molecular tests for *P. erythroseptica* require tissue infection and there are no methods to identify *P. erythroseptica* directly from soil due to the difficulty of obtaining microbial DNA that will support PCR (Nanayakkara *et al.,* 2009a). PCR has proved to be a useful method for identifying pathogens and has the potential to be developed further to aid in pest management systems before infection occurs.

**Research Objectives**

The objectives of this study were: A) to determine if the frequency of metalaxyl-m resistant populations of *P. erythroseptica* is increasing in Canadian potato growing regions; and B) to assess the efficacy of registered and experimental fungicide treatments to control *P. erythroseptica* in field, storage, and *in-vitro* conditions.

Chapter 1

**Characterization of the sensitivity of Canadian populations of *Phytophthora erythroseptica* Pethybr. to metalaxyl-m and the evaluation of alternative fungicides by inhibition of mycelial growth.**

## Abstract

*Phytophthora erythroseptica* Pethybr. is the causal agent of pink rot of potatoes which results in a wet rot and complete tuber breakdown. Traditionally, *P. erythroseptica* has been managed with metalaxyl and metalaxyl-m based products (Ridomil Gold®). However, in recent years, metalaxyl-m resistant isolates of *P. erythroseptica* have been recovered in Atlantic Canada. A national survey was conducted from 2013 to 2016 to determine the distribution of metalaxyl-m resistant populations in Canadian potato production regions and the $EC_{50}$ values (the fungicide concentration inhibiting mycelial growth of the pathogen by 50% relative to the 0 μg $mL^{-1}$ control) of strains exposed to metalaxyl-m, oxathiapiprolin, fluopicolide, and phosphite. This was determined using an amended agar study with both a metalaxyl-m sensitive isolate (9913) and a metalaxyl-m resistant isolate (1204) of *P. erythroseptica*. Metalaxyl-m resistant isolates were recovered from Prince Edward Island, Nova Scotia, New Brunswick, Ontario, Manitoba, and Alberta. The $EC_{50}$ values were 0.057 and 842.41 ppm for the metalaxyl-m sensitive and the metalaxyl-m resistant isolates grown on metalaxyl-m amended media. The $EC_{50}$ values of the metalaxyl-m sensitive and metalaxyl-m resistant isolates for oxathiapiprolin, fluopicolide, or phosphite were <0.0001 ppm, 0.064 to 0.128, and 112.0 to 147.6 ppm, respectively.

**Introduction**

Pink rot of potato is an important disease in potato-producing regions of Canada. Pink rot is caused by the oomycete pathogen *Phytophthora erythroseptica* Pethybr. and results in wet rot of tuber tissue and leads to significant yield losses in field and storage (Platt, 2008). Traditionally, *P. erythroseptica* has been managed with metalaxyl and later metalaxyl-m based products such as Ridomil Gold® (Al-Mughrabi, 2007). Metalaxyl-m is a phenylalamide fungicide that manages *Phytophthora* via selective inhibition of ribosomal RNA synthesis by affecting the activity of RNA polymerases. The site-specific mode of action has resulted in an intrinsically high risk of development of resistance to metalaxyl-m (Hu *et al.,* 2008). Resistance to metalaxyl-m began to appear in the late 1990s and early 2000s in the United States and resistant strains have been recovered in Atlantic Canada in 2012 (Peters *et al.,* 2013). This signifies a need to track the distribution of metalaxyl-m resistant strains in Canada and a need for alternative management strategies (Peters *et al.,* 2001).

Currently, there are some registered and experimental fungicides available to control pink rot of potato. Phosphite based products such as Phostrol™ (mono- and di-basic sodium, potassium, and ammonium salts of phosphorous acid; Engage Agro Corporation, Guelph, Ontario) have been shown in previous studies to control the pathogen by inhibiting mycelial growth, supressing sporulation and germination, and stimulating host defences (Taylor *et al*., 2011).

A newly registered fungicide is fluopicolide (2,6-dichloro-N-[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]methyl] benzamide) which is available as Presidio® (Valent Canada Inc., Guelph, Ontario) for the control of pink rot. The mode of action of

Presidio® is to induce delocalization of spectrin-like proteins of the pathogen. The spectrin-like proteins aid in membrane stability of the pathogen. When *Phytophthora infestans* hyphae were treated with fluopicolide, spectrin-like proteins were observed localizing in the plasma membranes and resulted in spherical spots on the membrane. Similar effects were observed in treated *P. infestans* zoospores which exhibited cell swelling and lysis 15 to 20 minutes after exposure (Toquin *et al*., 2007).

Oxathiapiprolin (1-(4-{4-[(5RS)-5-(2,6-difluorophenyl)-4,5-dihydro-1,2-oxazol-3-yl]-1,3-thiazol-2-yl}-1-piperidyl)-2-[5-methyl-3-(trifluoromethyl)-1H-pyrazol-1-yl]ethanone) is one of the active ingredients of Orondis® products (Syngenta Canada Inc., Guelph, Ontario). Oxathiapiprolin was developed for the control of oomycete pathogens and is the first of the new piperidinyl thiazole isoxazoline class of fungicides. Oxathiapiprolin inhibits oxysterol binding proteins of the pathogen. The role of oxysterol binding protein in oomycetes is not yet clear but it has low homology to oxysterol binding proteins of other eukaryotes which may account for the oomycete specificity of this fungicide (Miao *et al*., 2015). Previous studies have found that mycelial growth, sporangia production, and zoospore germination of *Phytophthora nicotianae* were all reduced by 50% or more at concentrations of 0.0166 µg a.i. $mL^{-1}$ or less (Bittner and Mila, 2014).

The purpose of this study was to determine the distribution of metalaxyl-m resistant strains of *P. erythroseptica* in Canadian potato growing regions through the use of a national survey; and to determine the concentration of registered and experimental fungicides for the control of pink rot that is needed to inhibit mycelial growth of *P. erythroseptica* by 50% relative to an inoculated control using an amended agar study.

## Methods

### National Survey

Tubers from harvested fields or warehouses exhibiting symptoms of pink rot were collected by industry, provincial, and federal agricultural organizations and submitted to the Charlottetown Research and Development Centre in Charlottetown (CRDC), Prince Edward Island. Tubers showing infection were cut in half longitudinally to reveal inner infected tissue. Small tissue samples (10 x 5 x 3 mm) were removed from the margin of the necrotic regions of the inner tissue with a sterile knife. The tissue samples were surface sterilized with 10% Javex® (0.6% sodium hypochlorite) solution for one minute, rinsed twice with sterile distilled water, and blotted dry on sterile filter paper (Whatman no. 4) and plated on 1.5% water agar in a Petri dish (90 mm x 15 mm). The Petri dish was incubated at room temperature for 3 days. Following incubation, hyphal tips were aseptically removed with a 5 mm cork borer from the margin of growth and plated onto clarified V8® medium to generate pure cultures. Clarified V8 medium was prepared by centrifuging 150 mL V8 juice at 6000 rpm for 20 minutes (17°C) and mixing the resulting supernatant with 1.5 g calcium carbonate, 20 g agar, and 850 mL distilled water. The mixture was heated and stirred for 45 minutes before autoclaving for 20 minutes to dissolve components.

### Metalaxyl-m Sensitivity Testing

Isolates of *P. erythroseptica* were characterized for metalaxyl-m sensitivity using an amended agar assay. Agar plugs (5 mm) were removed from 4-day-old cultures and transferred to Petri dishes (90 mm x 15 mm) containing clarified V8® medium amended

with 0, 1, 10, or 100 µg metalaxyl-m $mL^{-1}$ (Metalaxyl-m, Technical grade, Syngenta Crop Protection Canada, Inc. Guelph, Ontario, Canada). Metalaxyl-m was prepared as a 100 mg $mL^{-1}$ stock solution (910 µL Metalaxyl-m and 9090 µL Dimethyl Sulfoxide (DMSO)) and added to the molten agar after autoclaving (Table 1). Each isolate was grown on two replicate plates of each concentration for seven days at room temperature. Following incubation, two measurements of mycelial growth, perpendicular to each other (at 90° to each other through the center of the plug), for each replicate were taken using an electronic caliper. The means of the diameter of growth were calculated excluding the 5 mm diameter of the plug. The $EC_{50}$ values were calculated (the metalaxyl-m concentration inhibiting mycelial growth of the pathogen by 50% relative to the 0 µg metalaxyl-m $mL^{-1}$ control) and were used to classify each isolate into one of three categories of sensitivity: metalaxyl sensitive (MS, $EC_{50}$ < 1 µg $mL^{-1}$); metalaxyl moderately resistant (MMR, $EC_{50}$ 1-100 µg $mL^{-1}$); and metalaxyl-m highly resistant (MHR, $EC_{50}$ > 100 µg $mL^{-1}$) (Peters *et al*., 2001).

Table 1. Metalaxyl-m amended V8® agar plate preparation

| Plate Concentration of Metalaxyl-m | Volume of Media | Volume of DMSO | Volume of Stock Solution |
|---|---|---|---|
| 0 µg/mL | 1 L | 5000 µL | 0 µL |
| 1.0 µg/mL | 1 L | 4990 µL | 10 µL |
| 10 µg/mL | 1 L | 4900 µL | 100 µL |
| 100 µg/mL | 1 L | 4000 µL | 1000 µL |

**Legend**

DMSO - Dimethyl Sulfoxide
Stock Solution – Metalaxyl-m dissolved in DMSO as a 100 mg $mL^{-1}$ stock solution

**Fungicide Sensitivity testing**

A metalaxyl-m sensitive (PE9913) and a metalaxyl-m highly resistant strain (PE1204) of *P. erythroseptica* characterized previously for fungicide sensitivity were used as subjects in this amended agar assay. Agar plugs (5 mm diameter) were removed from 4-day-old cultures and plated onto Petri dishes containing clarified V8® medium amended with 0, 0.0001, 0.001, 0.01, 0.1, 1.0, 10.0, 100.0, or 1000.0 μg $mL^{-1}$ of fungicide. The fungicides used were metalaxyl-m (metalaxyl-m, 1098 mg $ml^{-1}$, Technical grade, Syngenta Crop Protection Canada, Inc. Guelph, Ontario, Canada), oxathiapiprolin (Orondis®; 101 mg $mL^{-1}$, Syngenta Canada Inc., Guelph, Ontario, Canada), fluopicolide (Presidio®; 395 mg $mL^{-1}$, Valent Canada Inc., Guelph, Ontario, Canada), and phosphite (Phostrol™; mono- and di-basic sodium, potassium, and ammonium salts of phosphorous acid; 536 mg $mL^{-1}$, Engage Agro Corporation, Guelph, Ontario, Canada). The fungicides were prepared as 100 μg $mL^{-1}$ and 100 mg $mL^{-1}$ stock solutions (Table 2 and 3) and added to molten agar after autoclaving. Each isolate was grown on four replicate plates of each concentration for seven days at room temperature and growth was measured as described previously, for evaluation of metalaxyl-m sensitivity in the Canadian survey collection. The $EC_{50}$ values were calculated by regression of the log of the chemical concentration against the corresponding probit of percent fungal inhibition (Zadoks and Schein, 1979). In cases where the probit line could not yield estimates, a simple linear trendline between the two concentrations bracketing 50% sensitivity was performed to estimate the $EC_{50}$ (Taylor *et al.,* 2002).

Table 2. Fungicide stock solution preparation. Solvent is sterile distilled water with exception of metalaxyl-m which was dissolved in dimethyl sulfoxide.

| Concentration | Fungicide | Fungicide Volume (µL) | Solvent Volume (µL) |
|---|---|---|---|
| **100 mg/mL** | Orondis® | 4950.00 | 50.00 |
| | Phostrol™ | 933.00 | 4067.00 |
| | Presidio® | 1266.00 | 3734.00 |
| | Metalaxyl-m | 455.00 | 4545.00 |
| **100 µL/mL** | Orondis® | 4.95 | 4995.05 |
| | Phostrol™ | 0.93 | 4999.07 |
| | Presidio® | 1.27 | 4.998.73 |
| | Metalaxyl-m | 0.46 | 4.995.54 |

Table 3. Fungicide plate preparation for 250 mL of media amended with Orondis®, Presidio®, Phostrol™, or metalaxyl-m. Solvent is sterile distilled water except plates with metalaxyl-m which was dissolved in dimethyl sulfoxide.

| Stock Solution Concentration | Plate Concentration (µg/mL) | Volume of Solvent (µL) | Volume of Stock Solution (µL) |
|---|---|---|---|
| | **0** | 1000 | 0 |
| **100 µg/mL** | **0.0001** | 999.75 | 0.25 |
| | **0.001** | 997.50 | 2.50 |
| | **0.01** | 975.00 | 25.00 |
| | **0.1** | 750.00 | 250.00 |
| **100 mg/mL** | **1.0** | 997.50 | 2.50 |
| | **10.0** | 975.00 | 25.00 |
| | **100.0** | 750.00 | 250.00 |
| | **1000.0** | 500.00 | 500.00 |

## Results

### National Survey

From 2013 to 2016, 85 samples of tubers with pink rot were submitted to the CRDC. From these samples, a collection of 345 isolates were obtained for metalaxyl-m sensitivity testing. The majority of isolates collected exhibited sensitivity to metalaxyl-m. However, resistant isolates were recovered from Prince Edward Island, New Brunswick, Nova Scotia, Ontario, Manitoba, and Alberta. The frequency of resistance was greater in eastern provinces and decreased westward (Table 4). As a result of this survey, metalaxyl-m resistant isolates were recovered for the first time in Ontario and Manitoba in 2013, and for the first time in Alberta in 2014.

Table 4. Summary of the number of samples and isolates collected from 2013 to 2016 across Canada and the percentage of the isolates exhibiting sensitivity or resistance to metalaxyl-m on amended V8® medium. Sensitivity is defined as metalaxyl sensitive (MS, $EC_{50}$ < 1 µg $mL^{-1}$); metalaxyl moderately resistant (MMR, $EC_{50}$ 1-100 µg $mL^{-1}$); and metalaxyl-m highly resistant (MHR, $EC_{50}$ > 100 µg $mL^{-1}$).

| | | | **Metalaxyl-m Sensitivity** | | |
|---|---|---|---|---|---|
| **Province** | **# Samples** | **# Isolates** | **% MS** | **% MMR** | **% MHR** |
| Prince Edward Island | 19 | 105 | 67.6 (71) | 10.5 (11) | 21.9 (23) |
| New Brunswick | 24 | 76 | 50 (38) | 21 (16) | 29 (22) |
| Nova Scotia | 1 | 3 | 0 (0) | 0 (0) | 100 (3) |
| Ontario | 5 | 25 | 52 (13) | 28 (7) | 20 (5) |
| Manitoba | 19 | 90 | 88.9 (80) | 0 (0) | 11.1 (10) |
| Alberta | 16 | 43 | 95 (41) | 5 (2) | 0 (0) |
| British Columbia | 1 | 3 | 100 (3) | 0 (0) | 0 (0) |
| Canada | 85 | 345 | 71.3 (246) | 10.4 (36) | 18.3 (63) |

(*) number of isolates

**Fungicide Sensitivity Testing**

The means of the diameter of growth were calculated for each isolate and were used to determine the $EC_{50}$ value for each fungicide by regression of the log of the chemical concentration against the corresponding probit of percent fungal inhibition. All $EC_{50}$ values were calculated using the probit method with the exception of metalaxyl-m and the metalaxyl-resistant isolate (PE1204) which was calculated using a linear trendline between the two concentrations bracketing 50% sensitivity.

The metalaxyl-m sensitive isolate (PE9913) had a lower $EC_{50}$ for metalaxyl-m than the metalaxyl-m highly resistant isolate (PE1204). The $EC_{50}$ values and the decrease of mycelial growth were similar for both tested isolates following exposure to fluopicolide, oxathiapiprolin, or phosphite (Figure 1). Oxathiapiprolin yielded the lowest $EC_{50}$ values of the chemicals tested. Fluopicolide resulted in 50% inhibition of mycelial growth at 0.06 and 0.12 ppm for isolated PE9913 and PE1204, respectively, while $EC_{50}$ values for PE9913 and PE1204 exposed to phosphite was 112 and 147.6 ppm, respectively (Table 5).

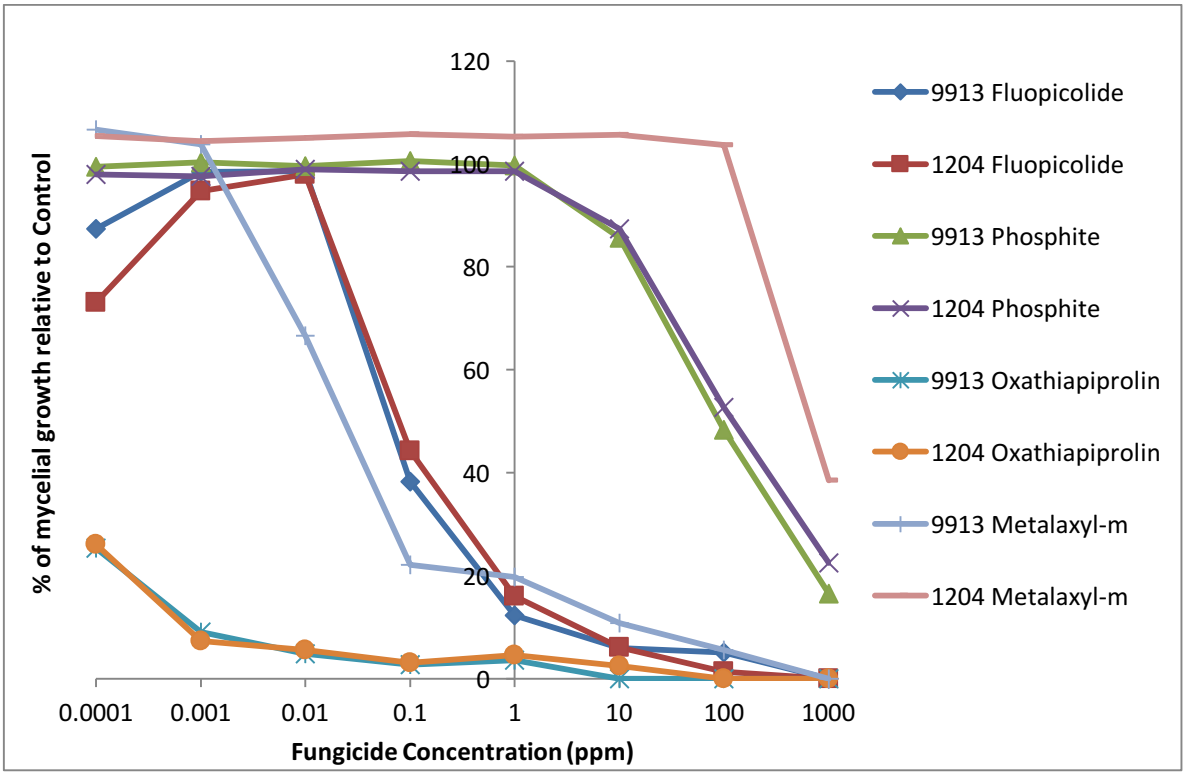


Figure 1. The percentage of mycelial growth relative to a 0 µg $mL^{-1}$ (ppm) control of metalaxyl-m sensitive (9913) and a metalaxyl-m resistant (1204) isolates of *Phytophthora erythroseptica* when grown on media amended with different concentrations of fluopicolide, phosphite, oxathiapiprolin, or metalaxyl-m.

Table 5. The $EC_{50}$ values (the fungicide concentration inhibiting mycelial growth of the pathogen by 50% relative to the 0 µg $mL^{-1}$ control) of metalaxyl-m sensitive (9913) and a metalaxyl-m resistant (1204) isolates of *Phytophthora erythroseptica* when grown on media amended with different concentrations of fluopicolide, phosphite, oxathiapiprolin, or metalaxyl-m. The $EC_{50}$ values were calculated by regression of the log of the chemical concentration against the corresponding probit of percent fungal inhibition or by a trendline between the two concentrations bracketing 50% sensitivity.

| Treatment | Isolate | Equation of line | $EC_{50}$ (µg/mL) (ppm) |
|---|---|---|---|
| **Metalaxyl-m** | 9913 | y = 0.6377x + 5.7951 | 0.05665 |
| | 1204 | y = 0.0723x - 10.906 | 842.4066 |
| **Oxathiapiprolin** | 9913 | y = 0.3561x + 7.2468 | < 0.0001 |
| | 1204 | y = 0.3089x + 7.0607 | < 0.0001 |
| **Fluopicolide** | 9913 | y = 0.7202x + 5.6426 | 0.12815 |
| | 1204 | y = 0.6832x + 5.8179 | 0.06351 |
| **Phosphite** | 9913 | y = 1.097x + 2.752 | 112.0018 |
| | 1204 | y = 1.035x + 2.755 | 147.5986 |

## Discussion

### National Survey

The tubers submitted to the national survey for metalaxyl-m sensitivity testing of infecting stains were primarily infected via the stolon. The disease began to develop at the stem end of the tubers and then progressed towards the bud end. In a few cases the tubers were recovered with infection occurring in a tuber eye or lenticel but these occurred with less frequency.

The results of the national survey have shown that metalaxyl-m resistance in the pink rot pathogen is found nationally in Canadian potato-growing regions. However, the frequency of resistance is greater in pathogen populations in eastern Canada than in western Canada. This may suggest an east to westward distribution of resistant strains of the pathogen. Metalaxyl-m resistant isolates have been recovered previously in New Brunswick in the late 1990s and Prince Edward Island in 2012 (Peters *et al.* 2001; Peters *et al.,* 2013). The *P. erythroseptica* population in Nova Scotia is not well represented in this study because we received only one sample over the four year period. This likely accounts for the population having isolates that are 100% metalaxyl-m resistant. This is similar to the results from British Columbia from which only one sample was received which also yielded only isolates with metalaxyl-m sensitivity. Metalaxyl-m resistant isolates were recovered for the first time ever in Ontario and Manitoba in 2013 and for the first time in Alberta in 2014.

The results of this study suggest that metalaxyl-m resistant populations of *P. erythroseptica* are increasing in frequency in Canada and therefore the effectiveness of metalaxyl-m based products as a method for the control of pink rot is decreasing. These

results signify a need for alternative management strategies to replace metalaxyl-m. This survey provides insight into the Canadian population structure of *P. erythroseptica* with regard to fungicide resistance. However, the frequency of strains may vary from what is reported in each region due to sample size, variation in sample location, regional incidences of disease due to seasonal growing conditions, and the reliance on samplers to retrieve and submit tuber samples for isolation and sensitivity testing.

**Fungicide Sensitivity Testing**

In the fungicide sensitivity testing study, the inhibition of mycelial growth relative to the 0 ppm control was used to determine the $EC_{50}$ values of two known pathogen isolates exposed to different fungicides. As anticipated, the metalaxyl-m sensitive isolate (PE9913) had a lower $EC_{50}$ value of 0.06 ppm than the metalaxyl-m highly resistant isolate (PE1204) did (842.41 ppm) when exposed to a range of concentrations of metalaxyl-m in agar media. Exposure of these same isolates to fluopicolide yielded $EC_{50}$ values between 0.06 and 0.12 ppm. These sensitivity results are similar to those cited by Zhang (2016), who reported an $EC_{50}$ response range of 0.08 ppm to 0.35 ppm among pathogen isolates. Inhibition of mycelial growth in agar with oxathiapiprolin was achieved at very low concentrations of the fungicide. The $EC_{50}$ for oxathiapiprolin was calculated to be less than 0.0001 ppm for both isolates of *P. erythroseptica.* In studies of mycelial inhibition of *P. nicotianae* by oxathiapiprolin, the $EC_{50}$ values were calculated to be 0.0039 to 0.0049 ppm (Bittner and Mila, 2014).

Amended agar media with phosphites yielded $EC_{50}$ values of 112 to 147.6 ppm for both isolates, which is higher than the $EC_{50}$ values obtained with exposure to

fluopicolide or oxathiapiprolin. However, phosphites have multiple modes of action that provide product efficacy against oomycetes in the field. This study focused on the inhibition of mycelial growth only. Phosphites have also been shown to suppress sporulation and germination, influencing membrane metabolism and phosphorylation reactions, as well as stimulating host plant defenses (Taylor *et al.,* 2011).

**Conclusion**

Metalaxyl-m resistant isolates of *P. erythroseptica* were recovered from Atlantic Canada, Ontario, Manitoba, and Alberta but occurred with greater frequency in eastern Canada. These results suggest that the efficacy of metalaxyl-m as a control agent of *P. erythroseptica* may be limited. However, fluopicolide and oxathiapiprolin based fungicides and to a lesser extent phosphites were shown to inhibit mycelial growth of the pathogen and may have potential as control agents of *P. erythroseptica* in potato production settings.

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Chapter 2

**Assessment of registered and experimental fungicides for control of *Phytophthora erythroseptica* Pethybr. in field and storage.**

## Abstract

Potatoes are threatened by many diseases that can lead to significant yield loss. *Phytophthora erythroseptica* Pethybr. is the causal agent of pink rot of potato which results in wet tuber rot and significant yield loss under field and storage conditions. Traditionally, *P. erythroseptica* has been managed with the fungicide metalaxyl-m, but in recent years metalaxyl-m resistant strains have evolved and spread to other potato growing regions in Canada. The purpose of this study was to evaluate alternative registered and experimental fungicides in field and storage settings for their capacity to manage metalaxyl-m resistant strains of the pathogen. Field trials were established from 2014 to 2016 to evaluate Orondis®, Presidio®, Serenade SOIL®, Ridomil Gold®, and Phostrol™ (applied foliarly or in-furrow) for their ability to prevent pink rot in daughter tubers following inoculation at planting. Significant control of pink rot was achieved with treatments of Orondis®, Presidio®, foliar Phostrol™, and to a lesser extent, Serenade SOIL®. Storage trials were established in 2016 and 2017 to assess Phostrol™, Orondis®, Presidio®, and Serenade SOIL® for their capacity to inhibit *P. erythroseptica* infection of tubers inoculated with *P. erythroseptica* zoospores prior to storage. Significant control of pink rot was achieved with treatments of Orondis®, Presidio®, Phostrol®, and to a lesser extent, Serenade SOIL® under storage conditions.

## Introduction

Potatoes are an important crop in Canada and around the world. Globally, potatoes are the third most produced crop after rice and wheat. In 2016, 346 827 acres were planted with potatoes which generated 105.1 million cwt of tubers and resulted in an annual farm gate value of $1.12 billion (Statistics Canada, 2016a, Statistics Canada 2016b). Like many economically important crops, potatoes are susceptible to many economically significant diseases.

Pink rot of potato is caused by *Phytophthora erythroseptica* Pethybr. and is ubiquitous in global potato producing regions. *Phytophthora erythroseptica* is a soilborne oomycete parasite that is known as a water mould that is homothallic and reproduces asexually via sporangia (Erwin and Ribeiro, 1996). The sporangia can germinate directly or cleave to produce uninucleated free-swimming zoospores that move via water films in soil. Oospores, sporangia, and zoospores are all infective stages of the *P. erythroseptica* lifecycle; however, infections are caused mainly by zoospores while it is believed that oospores are the primary source of overwintering inoculum.

Pink rot affects potato tubers and can lead to significant yield losses in field and storage. Tuber infection usually occurs in wet, low-lying areas of a field following planting or when wet conditions persist prior to harvest (Pratt, 2008). Tuber infection occurs when germinating oospores, sporangia, or zoospores form germ tubes or encyst on the stolons of the potato. Infection can also occur via open lenticels, wounds, and tuber eyes. Superficial infections of the tubers can also occur and result in disease development later in storage. Infections can remain dormant on seed tubers and act as a source of inoculum in non-infested fields.

Some cultural methods of control have been shown to reduce incidences of pink rot. Crop rotations of three or four years and rotations with alternative crops such as clover, oats, or barley are effective management strategies (Peters *et al*., 2004a). To prevent pink rot infections, it is recommended that producers use disease-free seed to limit the spread of the pathogen and plant their crop in warm, well-drained soils to improve plant growth and limit disease development (Platt, 2008). The use of potato cultivars with resistance to pink rot is recommended. However, many commercially available cultivars vary only slightly in their susceptibility to the disease (Platt and Peters, 2006). Tubers should be harvested when the foliage is completely dead as this will encourage tuber skin development and reduce the incidence of disease. Harvesting when soil is dry and below 18°C has been shown to provide an inhospitable environment for spore germination (Peters *et al*., 2004b). Tuber damage during harvesting should also be minimized and the amount of soil adhering to the tubers should be reduced to limit the spread of the pathogen. Grading out diseased tubers before storage will help to reduce storage rot and harvesting suspect areas of the field at the end the harvest season will help reduce the spread of the pathogen to healthy tubers in storage (Platt and Peters, 2006).

Chemical management of *P. erythroseptica* has traditionally been accomplished with metalaxyl and later with its enantiomer metalaxyl-m (Ridomil Gold®). Metalaxyl-m is a systemic phenylalamide fungicide that is translocated throughout the plant and provides protection against oomycete pathogens by affecting the activity of RNA polymerases, thereby providing selective inhibition of ribosomal RNA synthesis. Due to its site-specificity, metalaxyl-m has an intrinsically high risk of inducing resistance development in target pathogens (Hu *et al*., 2008). Resistance to metalaxyl-m in

populations of *P. erythroseptica* was observed in the United States in the early 2000s and later in Atlantic Canada in 2012 (Peters *et al*., 2001; Peters *et al.,* 2013).

Currently there are other fungicides registered in Canada for the management of pink rot of potato. Products such as Phostrol™, Serenade SOIL®, and Presidio® are all registered for the management of pink rot. Phostrol™ (mono- and di-basic sodium, potassium, and ammonium salts of phosphorous acid; Engage Agro Corporation, Guelph, Ontario) is a phosphite-based product that is registered for foliar and in-furrow application as well as post-harvest applications. Phosphites appear to have a complicated mode of action that is not well understood (Taylor *et al*., 2011). Studies have demonstrated that phosphorous acid affects the pathogen by inhibiting mycelial growth, suppressing sporulation and germination, and stimulating host plant defenses.

Serenade SOIL® (*Bacillus subtilis* QST 713; Bayer CropScience Inc., Calgary, Alberta) is a biological fungicide with a unique mode of action that is registered for field and post-harvest applications. These bacteria are ubiquitous in soils and many other habitats worldwide and are antagonistic towards many fungal plant pathogens. This antagonism can be achieved in many different ways including competition for nutrients, site exclusion, colonization, and attachment of the bacteria to the fungal pathogen. This strain of bacteria has also been shown to induce the plant's natural systemic resistance against bacterial pathogens. These bacteria can also stop pathogen spores from germinating, disrupt germ tube growth, and inhibit attachment of the pathogen to the plant (EPA, 2006).

Presidio® (Fluopicolide (2,6-dichloro-N-[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]methyl] benzamide); Valent Canada Inc., Guelph, Ontario) is a mesosystemic

fungicide that translocates toward stem tips via the xylem but does not translocate towards the roots (EPA, 2007). Fluopicolide has a new mode of action not found in other anti-oomycete fungicides. Fluopicolide induces delocalization of spectrin-like proteins of the pathogen, which play a crucial role in membrane stability in fungi and oomycetes (Toquin *et al*., 2007).

A new fungicide that is awaiting registration for pink rot management in Orondis® (oxathiapiprolin (1-(4-{4-[(5RS)-5-(2,6-difluorophenyl)-4,5-dihydro-1,2-oxazol-3-yl]-1,3-thiazol-2-yl}-1-piperidyl)-2-[5-methyl-3-(trifluoromethyl)-1H-pyrazol-1-yl]ethanone; Syngenta Canada Inc., Guelph, Ontario). Oxathiapiprolin is the first of the new piperidinyl thiazole isoxazoline class of fungicides (FRAC U15). Oxathiapiprolin's mode of action is through the inhibition of oxysterol binding proteins (OSBP) in oomycete pathogens. The function of OSBP in oomycetes is not clear but it has low homology with other OSBP-related proteins of other eukaryotes, which may account for the activity of oxathiapiprolin on oomycetes but not on other organisms (Miao *et al*., 2015).

The purpose of this study was to assess the efficacy of registered and experimental fungicide treatments to control metalaxyl-m sensitive and resistant isolates of *P. erythroseptica* in field and storage.

## Methods

### Field Trial

Field trials (11 x 95 m) were planted May 29, 2014; May 27, 2015; and May 25, 2016 at the Charlottetown Research and Development Centre (CRDC) research farm in Harrington, Prince Edward Island. The field plot design consisted of a randomized complete block with 64 test plots. The plots were 3.048 m long by 0.9144 m wide. The trial consisted of six chemical treatments, inoculated controls, and non-inoculated controls. The treatments were replicated four times. The test plots, except the non-inoculated control, were inoculated in-furrow with a slurry inoculum of metalaxyl-m sensitive (PE9913) or metalaxyl-m highly resistant (PE1204) strains of *P. erythroseptica.* In 2015 and 2016 the slurry inoculum was covered with 1 cm of soil prior to planting the seed pieces. The inoculum was prepared by blending 15 Petri dishes (90 mm x 15 mm) of 14-day-old cultures of *P. erythroseptica* on clarified V8® medium in 750 mL of water to form a thin slurry which generated enough inoculum for one test plot (Fitzpatrick-Peabody and Lambert, 2007). The non-inoculated control received slurry inoculum made from V8 agar only. The field was planted manually with ten seed pieces per plot with cv. Shepody at 30 cm spacing and fertilized at normal rates (N-P-K: 17-17-17). All plots were treated according to label rates with the insecticide imidacloprid (Admire PRO®, 1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine, Bayer CropScience, Calgary, Alberta, Canada) in-furrow at planting and the crop was also treated according to the label rate with Bravo® 500 (Chlorothalonil, Syngenta Crop Protection Canada, Inc., Guelph, Ontario, Canada) on a weekly schedule during the growing season to prevent late blight. The chemical treatments used in this study were applied in-furrow to

the seed pieces at planting except for one treatment of Phostrol™ which was applied as a foliar application. The in-furrow treatments included Serenade SOIL® (*Bacillus subtilis*; Bayer CropScience Inc., Calgary, Alberta, Canada) applied at 14 L $ha^{-1}$; Ridomil Gold 480SL® (metalaxyl-m; Syngenta Canada Inc., Guelph, Ontario, Canada) applied at 436 mL $ha^{-1}$; Orondis® (oxathiapiprolin; Syngenta Canada Inc., Guelph, Ontario, Canada) applied at 100 g ai (active ingredient) $ha^{-1}$; Phostrol™ (mono- and dibasic sodium, potassium, and ammonium phosphites; Engage Agro Corporation, Guelph, Ontario, Canada) applied at 11.6 L $ha^{-1}$; and Presidio® (fluopicolide; Valent Canada Inc., Guelph, Ontario, Canada) applied at 292 mL $ha^{-1}$. The foliar treatment of Phostrol™ was applied at label rates (11.6 L $ha^{-1}$) five times throughout the growing season. The plots receiving foliar Phostrol™ treatments were sprayed approximately every two weeks starting at tuber initiation. All chemical treatments summarized in Table 6 were applied using a backpack sprayer. Emergence data, stem count data, and tuber yield data were collected from all test plots in the field trial. The emergence data were collected for each treatment by counting the total number of emerged plants divided by the total number of seed pieces planted multiplied by 100. The stem count data were collected by counting the total number of primary stems for each emerged plant. The field trials were harvested with a single-row harvester October 16, 2014; October 14, 2015; and October 13, 2016. The number and weight of diseased and healthy tubers were recorded. Percentages of total tuber number and weight composed of diseased tubers and healthy tubers were calculated. Emergence, stem, and harvest data collected were subjected to analysis of variance (ANOVA). When a statistically significant treatment effect was found ($p < 0.05$) the Tukey's test was used to differentiate between means (GenStat 18th edition).

Table 6. Chemical treatment preparations for application to test plots. The test plots received 150 mL of designated chemical preparation.

| Chemical Name | Volume Water (mL) | Volume Chemical (mL) |
|---|---|---|
| Orondis® | 4791.12 | 8.88 |
| Ridomil Gold 480SL® | 4796.10 | 3.90 |
| Phostrol™ (in-furrow and foliar) | 4696.20 | 103.80 |
| Serenade SOIL® | 4674.72 | 125.28 |
| Presidio® | 4797.39 | 2.61 |

**Storage Trial**

The ability of four fungicides to inhibit *P. erythroseptica* infection prior to storage was assessed. The storage trial was designed as a randomized complete block with four replications that consisted of four fungicides applied at different rates, inoculated controls, and non-inoculated controls. Test plots of ten washed tubers (cv. Shepody), except the non-inoculated controls, were inoculated in plastic bags with 50 mL of 6000 zoospore $mL^{-1}$ inoculum of a metalaxyl-m sensitive (PE9913) or a metalaxyl-m highly resistant (PE1204) strain, shaken to distribute the inoculum, and left to incubate for one hour at room temperature. The non-inoculated control received 50 mL of sterile pond water (locally sourced). Following incubation, 8 mL of the assigned fungicide was applied to the tubers using a hand sprayer and the tubers were placed inside a paper bag (Table 7). Treatments included Phostrol™ (0.42 L in 2 L per metric tonne) and Serenade SOIL® (0.115 L in 2 L per metric tonne) applied at label rates as well as Presidio® (39.5% a.i.) and Orondis® (10.1% a.i.) applied at 1.0, and 2.0 times the label rate of the registered postharvest product Stadium® (32.5 mL in 2 L water per metric tonne) based upon concentration of active ingredient. In 2017, a rate of 0.5 times the rate of Stadium® was added for Presidio® and Orondis® treatments. The tubers were left to incubate at 15°C and 99% relative humidity for 2 weeks. The tubers were rated for percentage of external and internal necrosis and data were analyzed using ANOVA and Tukey's test (GenStat 18$^{th}$ edition).

Table 7. Fungicides prepared as 300 mL volumes according to their label rates or as modified label rates of Stadium (½X, 1X, or 2X) for the experimental products for application to potato tubers in storage trial.

| Product and Rate | Volume of Product (mL) | Volume of Water (mL) |
|---|---|---|
| Phostrol™ | 29.03 | 270.97 |
| Serenade SOIL® | 12.10 | 287.90 |
| Orondis® ½X | 1.12 | 298.88 |
| Orondis® 1X | 2.25 | 297.75 |
| Orondis® 2X | 4.49 | 295.51 |
| Presidio® ½X | 1.12 | 298.88 |
| Presidio® 1X | 2.25 | 297.75 |
| Presidio® 2X | 4.49 | 295.51 |

**Zoospore Inoculum**

The zoospore inoculum was prepared using a modified protocol of Al-Mughrabi *et al*., 2007. Clarified V8® broth was prepared by centrifuging Original V8® juice for 20 minutes at 6000 rpm and mixing 300 mL of the resulting supernatant with 700 mL distilled water and 1.25 g calcium carbonate. The mixture was heated while stirring without boiling for one hour and then allowed to cool to let the calcium carbonate precipitate from the mixture. The broth was decanted to remove the calcium carbonate and then dispensed in 225 mL aliquots into 500 mL Mason jars. The broth was autoclaved for 20 minutes and allowed to cool to room temperature before inoculating. Fifteen agar plugs (5 mm) taken from the margin of 3-day-old cultures of *P. erythroseptica* grown on clarified V8® medium were aseptically transferred to each Mason jar. The jars were incubated at room temperature in darkness for three days. Following incubation, each jar was individually decanted into a No. 140 soil screen (0.0041 inch opening) and the trapped mycelium washed twice with 150 mL sterile distilled water. The mycelium was returned to the Mason jar and 150 mL sterile pond water was added to the jar. Pond water was obtained locally from a pond with high content of tannins and organic acids and was sterilized by autoclaving at 121°C for 20 minutes. The jars were incubated for 24 hours at 18°C under continuous fluorescent light. After incubation, the pond water was decanted from each jar and replaced with 225 mL of fresh sterile pond water and the jars were incubated for a further 24 hours at 18°C under continuous fluorescent light. After this time period, the jars were placed in darkness at 4°C for 1.5 hours to provide a shock treatment to aid in zoospore differentiation. The jars were then incubated for two hours in darkness at room

temperature to encourage zoospore release (6000 zoospore $mL^{-1}$). The mycelium and agar plugs were filtered from the inoculum using a single layer of cheesecloth and the inoculum was consolidated into bottles. The bottles of inoculum were stored on ice packs in coolers to maintain zoospore motility and viability until the beginning of the experiment. Zoospores will remain viable for 6-8 hours if kept at 9°C.

## Results

### Field Trial

#### Number of Emerged Plants

The emergence of planted seed pieces was assessed for each growing season by counting the number emerged plants and dividing by the number of planted seed pieces then multiplying by 100 to determine the stand percentage for each treatment. In 2014, a lower percentage of plants emerged for all treatments as compared to 2015 and 2016. In 2014, the stand percentages of most treatments were not statistically different from the inoculated controls. In 2015, there were no significant differences among treatments with respect to stand emergence, and 92.5% emergence was the lowest recorded value. In 2016, fewer plants emerged than in 2015, but all treatments were not significantly different from the non-inoculated control except the treatment of Ridomil Gold® with PE1204 (Table 8).

Table 8. Summary of mean plant emergence during the 2014 to 2016 growing seasons presented as a percentage of stand emergence (the number of emerged plants out of the total number of seed pieces planted) of potatoes inoculated with a metalaxyl-m sensitive strain (9913) or a metalaxyl-m resistant strain (1204) of *Phytophthora erythroseptica* and treated with fungicide. Values in the same column sharing the same letter are not significantly different from each other ($P<0.05$, ANOVA, Tukey's test, GenStat 18th Edition).

| | | % Stand | | |
|---|---|---|---|---|
| **Isolate** | **Treatment** | **2014** | **2015** | **2016** |
| **9913** | Presidio® | 25[a] | 97.5[a] | 95[ab] |
| | Inoculated Control | 32.5[a] | 100[a] | 92.5[ab] |
| | In-furrow Phostrol™ | 35[a] | 100[a] | 100[b] |
| | Foliar Phostrol™ | 37.5[a] | 100[a] | 92.5[ab] |
| | Orondis® | 40[a] | 100[a] | 100[b] |
| | Serenade SOIL® | 45[ab] | 100[a] | 87.5[ab] |
| | Ridomil Gold® | 82.5[bc] | 100[a] | 100[b] |
| | Non-inoculated Control | 100[c] | 100[a] | 97.5[ab] |
| **1204** | Presidio® | 42.5[ab] | 97.5[a] | 92.5[ab] |
| | Inoculated Control | 42.5[ab] | 92.5[a] | 90[ab] |
| | In-furrow Phostrol™ | 47.5[ab] | 100[a] | 90[ab] |
| | Foliar Phostrol™ | 32.5[a] | 100[a] | 85[ab] |
| | Orondis® | 60[abc] | 95[a] | 97.5[ab] |
| | Serenade SOIL® | 35[a] | 97.5[a] | 87.5[ab] |
| | Ridomil Gold® | 55[ab] | 100[a] | 75[a] |
| | Non-inoculated Control | 100[c] | 100[a] | 97.5[ab] |

**Number of Stems**

The number of stems produced by each seed piece was assessed in the 2014 to 2016 growing seasons by counting the number of primary stems of each potato plant. There were no significant differences in the number of stems produced among treatments during all three growing seasons (Table 9).

Table 9. Summary of mean number of plant stems produced during the 2014 to 2016 growing seasons presented as the mean number of stems per potato seed piece inoculated with a metalaxyl-m sensitive strain (9913) or a metalaxyl-m resistant strain (1204) of *Phytophthora erythroseptica* and treated with fungicide. Values in the same column sharing the same letter are not significantly different from each other (P<0.05, ANOVA, Tukey's test, GenStat 18th Edition).

| | | # Stems | | |
|---|---|---|---|---|
| **Isolate** | **Treatment** | **2014** | **2015** | **2016** |
| **9913** | Presidio® | 3.9[a] | 2.6[a] | 2.5[a] |
| | Inoculated Control | 3.3[a] | 3.0[a] | 2.5[a] |
| | In-furrow Phostrol™ | 3.1[a] | 3.6[a] | 3.0[a] |
| | Foliar Phostrol™ | 3.6[a] | 3.6[a] | 2.7[a] |
| | Orondis® | 3.9[a] | 2.9[a] | 2.7[a] |
| | Serenade SOIL® | 3.7[a] | 3.2[a] | 2.6[a] |
| | Ridomil Gold® | 3.8[a] | 3.1[a] | 2.5[a] |
| | Non-inoculated Control | 3.4[a] | 3.2[a] | 2.6[a] |
| **1204** | Presidio® | 3.4[a] | 3.5[a] | 2.7[a] |
| | Inoculated Control | 4.2[a] | 3.2[a] | 2.6[a] |
| | In-furrow Phostrol™ | 3.9[a] | 3.5[a] | 2.4[a] |
| | Foliar Phostrol™ | 3.9[a] | 3.4[a] | 2.7[a] |
| | Orondis® | 3.5[a] | 3.5[a] | 2.6[a] |
| | Serenade SOIL® | 3.4[a] | 3.2[a] | 2.7[a] |
| | Ridomil Gold® | 4.1[a] | 3.5[a] | 2.7[a] |
| | Non-inoculated Control | 3.5[a] | 3.2[a] | 2.6[a] |

**Daughter Tubers Harvest**

The daughter tubers produced in the 2014 to 2016 field seasons were assessed for incidences of pink rot by the number and the mass of diseased tubers relative to the total number and mass of tubers from each treatment and presented as a percentage. The 2014 growing season resulted in the greatest number of infected daughter tubers of the three years of the experiments. In 2014, there was a statistically significant difference in the number of infected tubers among treatments relative to the inoculated control (Table 10). The 2015 and 2016 growing seasons yielded fewer diseased tubers than the 2014 growing season. There were no significant differences among treatments in the 2015 and 2016 seasons (Table 11 and 12).

Table 10. The mean percentage of harvested daughter tubers grown in 2014 that exhibited pink rot symptoms or were healthy (by count and by total mass). Seed tubers were inoculated with a metalaxyl-m sensitive strain (9913) or a metalaxyl-m resistant strain (1204) of *Phytophthora erythroseptica* and treated with fungicide. Values in the same column sharing the same letter are not significantly different from each other (P<0.05, ANOVA, Tukey's test, GenStat 18th Edition).

| Isolate | Treatment | % Healthy of total number | % Diseased of total number | % Healthy of total mass | % Diseased of total mass |
|---|---|---|---|---|---|
| **9913** | Inoculated Control | $73.8^{ab}$ | $26.2^{ab}$ | $82.9^{ab}$ | $17.1^{ab}$ |
| | Serenade SOIL® | $77.3^{ab}$ | $22.7^{ab}$ | $78.6^{ab}$ | $21.4^{ab}$ |
| | In-furrow Phostrol™ | $84.0^{ab}$ | $16.0^{ab}$ | $88.9^{ab}$ | $11.2^{ab}$ |
| | Orondis® | $97.8^{b}$ | $2.2^{a}$ | $98.1^{b}$ | $1.9^{a}$ |
| | Presidio® | $98.8^{b}$ | $1.2^{a}$ | $99.7^{b}$ | $0.4^{a}$ |
| | Foliar Phostrol™ | $100^{b}$ | $0^{a}$ | $100^{b}$ | $0^{a}$ |
| | Ridomil Gold® | $100^{b}$ | $0^{a}$ | $100^{b}$ | $0^{a}$ |
| | Non-inoculated | $100^{b}$ | $0^{a}$ | $100^{b}$ | $0^{a}$ |
| **1204** | Inoculated Control | $64.8^{a}$ | $35.2^{b}$ | $72.1^{a}$ | $27.9^{b}$ |
| | Serenade SOIL® | $83.6^{ab}$ | $16.4^{ab}$ | $88.3^{ab}$ | $11.7^{ab}$ |
| | In-furrow Phostrol™ | $76.2^{ab}$ | $23.8^{ab}$ | $79.8^{ab}$ | $20.2^{ab}$ |
| | Orondis® | $97.1^{b}$ | $2.9^{a}$ | $96.6^{ab}$ | $3.4^{ab}$ |
| | Presidio® | $100^{b}$ | $0^{a}$ | $100^{b}$ | $0^{a}$ |
| | Foliar Phostrol™ | $100^{b}$ | $0^{a}$ | $100^{b}$ | $0^{a}$ |
| | Ridomil Gold® | $71.7^{ab}$ | $28.3^{ab}$ | $76.5^{ab}$ | $23.5^{ab}$ |
| | Non-inoculated | $100^{b}$ | $0^{a}$ | $100^{b}$ | $0^{a}$ |

Table 11. The mean percentage of harvested daughter tubers grown in 2015 that exhibited pink rot symptoms or were healthy (by count and by total mass). Seed tubers were inoculated with a metalaxyl-m sensitive strain (9913) or a metalaxyl-m resistant strain (1204) of *Phytophthora erythroseptica* and treated with fungicide. Values in the same column sharing the same letter are not significantly different from each other (P<0.05, ANOVA, Tukey's test, GenStat 18th Edition).

| Isolate | Treatment | % Healthy of total number | % Diseased of total number | % Healthy of total mass | % Diseased of total mass |
|---|---|---|---|---|---|
| **9913** | Inoculated Control | 93.1[a] | 6.9[a] | 95.1[a] | 4.9[a] |
| | Serenade SOIL® | 96.5[a] | 3.5[a] | 97.8[a] | 2.2[a] |
| | In-furrow Phostrol™ | 97.5[a] | 2.5[a] | 98.3[a] | 1.7[a] |
| | Orondis® | 96.5[a] | 3.5[a] | 97.6[a] | 2.4[a] |
| | Presidio® | 98.7[a] | 1.3[a] | 98.2[a] | 1.8[a] |
| | Foliar Phostrol™ | 100[a] | 0[a] | 100[a] | 0[a] |
| | Ridomil Gold® | 100[a] | 0[a] | 100[a] | 0[a] |
| | Non-inoculated | 100[a] | 0[a] | 100[a] | 0[a] |
| **1204** | Inoculated Control | 92.0[a] | 8.0[a] | 95.1[a] | 4.9[a] |
| | Serenade SOIL® | 93.9[a] | 6.1[a] | 96.3[a] | 3.7[a] |
| | In-furrow Phostrol™ | 92.7[a] | 7.3[a] | 94.2[a] | 5.8[a] |
| | Orondis® | 97.5[a] | 2.5[a] | 97.8[a] | 2.2[a] |
| | Presidio® | 93.8[a] | 6.2[a] | 93.6[a] | 6.4[a] |
| | Foliar Phostrol™ | 100[a] | 0[a] | 100[a] | 0[a] |
| | Ridomil Gold® | 95.9[a] | 4.1[a] | 98.2[a] | 1.8[a] |
| | Non-inoculated | 100[a] | 0[a] | 100[a] | 0[a] |

Table 12. The mean percentage of harvested daughter tubers grown in 2016 that exhibited pink rot symptoms or were healthy (by count and by total mass). Seed tubers were inoculated with a metalaxyl-m sensitive strain (9913) or a metalaxyl-m resistant strain (1204) of *Phytophthora erythroseptica* and treated with fungicide. Values in the same column sharing the same letter are not significantly different from each other ($P<0.05$, ANOVA, Tukey's test, GenStat 18th Edition).

| Isolate | Treatment | % Healthy of total number | % Diseased of total number | % Healthy of total mass | % Diseased of total mass |
|---|---|---|---|---|---|
| **9913** | Inoculated Control | 96.9[a] | 3.1[a] | 97.4[a] | 2.6[a] |
| | Serenade SOIL® | 98.2[a] | 1.8[a] | 98.3[a] | 1.7[a] |
| | In-furrow Phostrol™ | 92.2[a] | 7.8[a] | 94.8[a] | 5.2[a] |
| | Orondis® | 100[a] | 0[a] | 100[a] | 0[a] |
| | Presidio® | 97.8[a] | 2.2[a] | 98.1[a] | 1.9[a] |
| | Foliar Phostrol™ | 100[a] | 0[a] | 100[a] | 0[a] |
| | Ridomil Gold® | 100[a] | 0[a] | 100[a] | 0[a] |
| | Non-inoculated | 100[a] | 0[a] | 100[a] | 0[a] |
| **1204** | Inoculated Control | 94.1[a] | 5.9[a] | 94.7[a] | 5.3[a] |
| | Serenade SOIL® | 96.1[a] | 3.9[a] | 96.5[a] | 3.5[a] |
| | In-furrow Phostrol™ | 97.5[a] | 2.5[a] | 98.6[a] | 1.4[a] |
| | Orondis® | 98.8[a] | 1.2[a] | 98.7[a] | 1.3[a] |
| | Presidio® | 98.9[a] | 1.1[a] | 99.4[a] | 0.6[a] |
| | Foliar Phostrol™ | 99.6[a] | 0.4[a] | 100[a] | 0[a] |
| | Ridomil Gold® | 94.1[a] | 5.9[a] | 96.8[a] | 3.2[a] |
| | Non-inoculated | 100[a] | 0[a] | 100[a] | 0[a] |

**Growing Season Weather Conditions**

The weather conditions at the CRDC research farm in Harrington, Prince Edward Island were very similar over the three growing seasons from 2014 to 2016. The field trials were planted on May 29, 2014, May 27, 2015, and May 25, 2016 and were harvested on October 16, 2014, October 14, 2015, and October 13, 2016 resulting in 141, 141, and 142 growing days, respectively. Monthly precipitation collected by Environment Canada at CRDC remained consistent with the exception of June 2015 which received more precipitation (Figure 2) (Environment Canada). The total rainfall during the 2014 growing season was approximately 22 mm less than 2015 and 2016 (Figure 3) (Environment Canada). All weather conditions presented are for the growing period of the specified year.

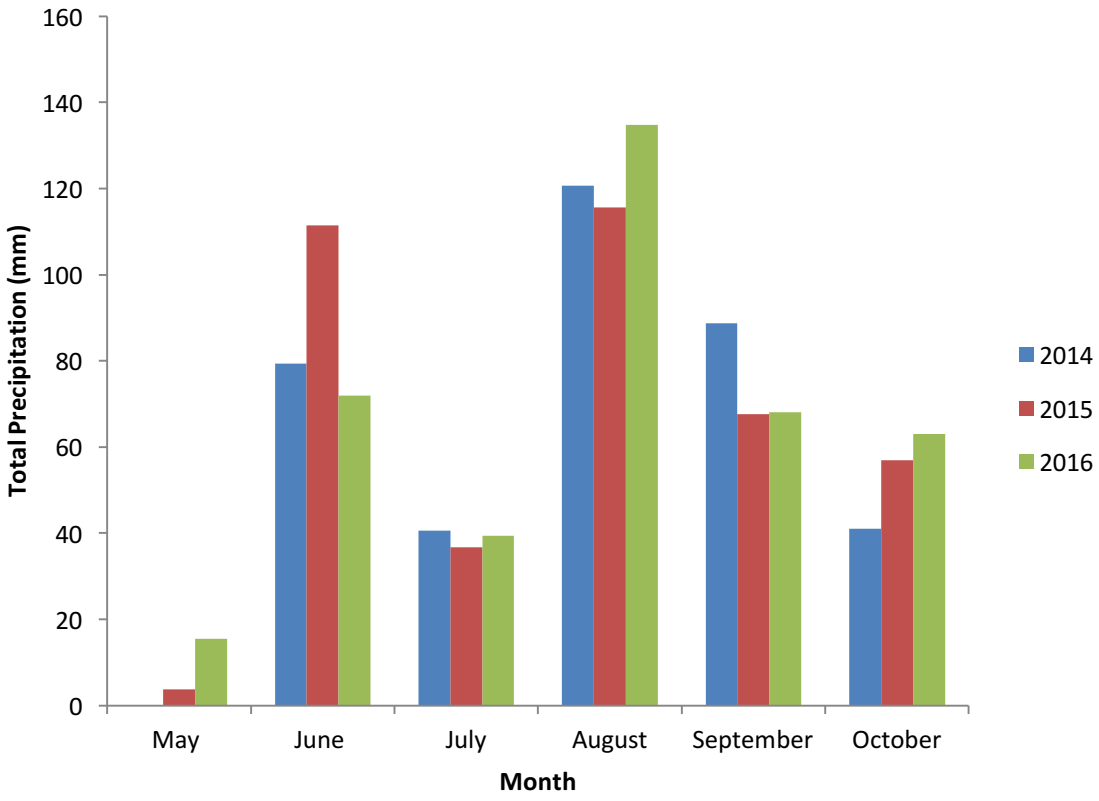


Figure 2. Total monthly precipitation at the Charlottetown Research and Development Centre research farm in Harrington, Prince Edward Island, Canada from May 29, 2014 to Oct 16, 2014, May 27, 2015 to October 14, 2015, and May 25, 2016 to October 13, 2016.

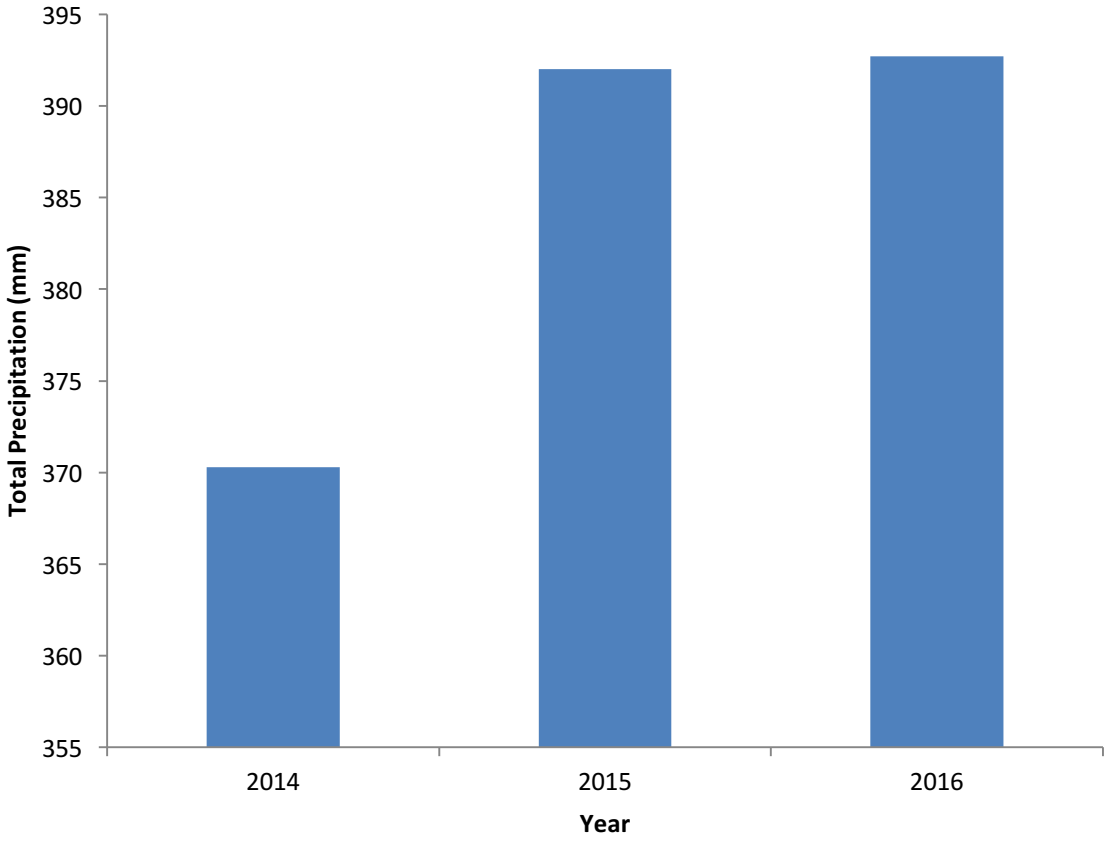


Figure 3. Total precipitation at the Charlottetown Research and Development Centre research farm in Harrington, Prince Edward Island, Canada from May 29, 2014 to Oct 16, 2014, May 27, 2015 to October 14, 2015, and May 25, 2016 to October 13, 2016 (Environment Canada).

## Storage Trial

### Rates of Infection

The tubers of the storage trial were visually rated for the number of tubers showing symptoms of infection with *P. erythroseptica* and for the percentage of necrosis of internal and external tuber tissue due to pink rot. In the 2016 storage trial, treatments of Orondis®, Phostrol™, or Presidio® resulted in rates of infection that were not significantly different from the non-inoculated controls. The Serenade SOIL® treatment was significantly different from all other treatments except the inoculated control. The treatment of Serenade SOIL® resulted in less tissue necrosis in infected tubers than the inoculated control. In 2017, the treatments of Orondis®, Phostrol™, and Presidio®, with the exception of Presidio® ½X, were not significantly different from the non-inoculated control. The treatment of Serenade SOIL® and Presidio® ½X were not significantly different from each other but had infection rates less than the inoculated control (Table 13).

Table 13. The mean percentage of tubers inoculated with *Phytophthora erythroseptica* zoospores and treated with fungicide under storage settings that developed pink rot and the percentage of necrosis of internal and external tuber tissue due to pink rot for the 2016 and 2017 storage trials. Treatments marked with '*' were not evaluated in 2016. Values in the same column sharing the same letter are not significantly different from each other ($P<0.05$, ANOVA, Tukey's test, GenStat 18th Edition).

| Treatment | 2016 | | | 2017 | | |
|---|---|---|---|---|---|---|
| | **Tubers Infected (%)** | **Surface Necrosis (%)** | **Internal Necrosis (%)** | **Tubers Infected (%)** | **Surface Necrosis (%)** | **Internal Necrosis (%)** |
| **Non-inoculated** | $0^{a}$ | $0^{a}$ | $0^{a}$ | $0^{a}$ | $0^{a}$ | $0^{a}$ |
| **Orondis® ½X** | * | * | * | $0^{a}$ | $0^{a}$ | $0^{a}$ |
| **Orondis®** | $0^{a}$ | $0^{a}$ | $0^{a}$ | $0^{a}$ | $0^{a}$ | $0^{a}$ |
| **Orondis® 2X** | $0^{a}$ | $0^{a}$ | $0^{a}$ | $0^{a}$ | $0^{a}$ | $0^{a}$ |
| **Phostrol™** | $0^{a}$ | $0^{a}$ | $0^{a}$ | $0^{a}$ | $0^{a}$ | $0^{a}$ |
| **Presidio® ½X** | * | * | * | $25.00^{b}$ | $9.00^{ab}$ | $8.5^{ab}$ |
| **Presidio®** | $1.25^{a}$ | $0.44^{a}$ | $0.44^{a}$ | $3.75^{a}$ | $1.44^{a}$ | $1.00^{a}$ |
| **Presidio® 2X** | $0^{a}$ | $0^{a}$ | $0^{a}$ | $10.00^{a}$ | $2.562^{a}$ | $2.38^{a}$ |
| **Serenade SOIL®** | $47.50^{b}$ | $27.44^{b}$ | $27.31^{b}$ | $26.25^{b}$ | $15.31^{b}$ | $15.56^{b}$ |
| **Inoculated** | $66.25^{b}$ | $48.50^{c}$ | $49.69^{c}$ | $82.50^{c}$ | $53.31^{c}$ | $56.00^{c}$ |

## Discussion

### Field Trial

The 2014 growing season yielded greater rates of infection than the 2015 and 2016 seasons as indicated by the decreased emergence rates and increased number of infected daughter tubers relative to 2015 and 2016. Growing conditions over the three years were similar, but the total precipitation in 2014 was less than the following years. This lower rate of precipitation is less favourable for pink rot development but 2014 had higher rates of infection in the field trial. This increased rate of infection is likely accounted for by the inoculation method used in 2014. This method resulted in the seed pieces being in direct contact with the inoculum slurry, which caused seed piece decay and likely accounted for the reduced emergence. Also, direct contact with the inoculum likely allowed for an enhanced opportunity for infection of the plants that did emerge, resulting in higher rates of disease in the daughter tubers. For these reasons, the inoculation protocol was modified in the 2015 and 2016 growing seasons to allow for less direct contact with the inoculum, so that better emergence was achieved. Pink rot is not commonly a cause for seed piece decay in production systems and we wanted to avoid loss of experimental plants by this somewhat artificial route.

Results of the field trial were consistent over the years of the project, even though disease levels in later years made treatment differences less apparent. As expected, the metalaxyl-m sensitive strain was controlled by treatments of Ridomil Gold®, whereas the metalaxyl-m resistant strain was not. Foliar applications of Phostrol™, and in-furrow applications of Orondis®, and Presidio® provided excellent control of pink rot for either strain used for inoculation in this study. Similarly, Zhang (2016) found that in-furrow

applications of Orondis® or Presidio® significantly decreased incidences of pink rot in harvested daughter tubers relative to inoculated controls. In-furrow applications of Serenade SOIL® at planting did not decrease incidences of pink rot in harvested daughter tubers compared to the inoculated controls (Zhang, 2016). Similarly, we found some limited effectiveness for disease control of in-furrow applications of Serenade SOIL® in this study.

In-furrow applications of Phostrol™ did not significantly reduce the incidence of pink rot in daughter tubers relative to the inoculated control. These findings are similar to those of Al-Mughrabi *et al.,* (2007) where no significant reduction in the incidence of pink rot was achieved when Phostrol™ was sprayed in-furrow at planting. In their study, they suggest that phosphites applied in-furrow were not taken up by the plants in sufficient quantities to provide disease suppression. By contrast, foliar applications of Phostrol™ provided complete suppression of pink rot in harvested tubers in this project. The foliar treatments were applied every two weeks starting at tuber initiation for a total of five applications over the growing season which may have allowed for sufficient accumulation of phosphites in the plant tissue to provide disease protection (Al-Mughrabi *et al.,* 2007).

**Storage Trial**

In 2016, significant control of pink rot caused by *P. erythroseptica* was achieved with treatments of Orondis®, Presidio®, or Phostrol™ applied to tubers entering storage. Due to the significant suppression of pink rot achieved in 2016, ½X rates were added to the 2017 trial. As in 2016, treatments of Phostrol™, Orondis®, or Presidio® (with the exception of ½X Presidio®) provided significant control of the pathogen in storage.

These results suggest that these fungicides provide effective post-harvest control of *P. erythroseptica* and are potential management strategies for controlling metalaxyl-m sensitive and resistant strains in storage. Currently, Phostrol™ is registered for post-harvest application in Canada but Orondis® and Presidio® are not.

The results of our in-vitro fungicide assays (Chapter 1) suggest that a lower post-harvest rate of application of oxathiapiprolin is likely possible. Studies have shown that *P. erythroseptica* has an $EC_{50}$ (the fungicide concentration inhibiting mycelial growth of the pathogen by 50% relative to the 0 µg $mL^{-1}$ control) of <0.0001 ppm for oxathiapiprolin while the lowest rate of oxathiapiprolin applied in this study was 377.07 ppm (Crane *et al.,* unpublished data). Future evaluation of lower chemical concentrations for post-harvest application as well as an evaluation of control of other oomycete pathogens in storage by oxathiapiprolin is warranted.

Previous studies have demonstrated control of *P. erythroseptica* using phosphite-based products like Phostrol™. Miller *et al.* (2006), found that tubers inoculated with *P. erythroseptica* zoospores and incubated for one hour before receiving applications of phosphite completely eliminated the incidence of pink rot. One study at the University of Maine, also found complete control of *P. erythroseptica* in storage on wounded tubers that were inoculated three hours prior to application of Phostrol™ (Johnston, 2008). Evaluations of fluopicolide have not been completed or published prior to this study. However, the results of this study suggest that fluopicolide provides significant control of the pathogen under storage conditions and is worth pursuing as a potential post-harvest treatment.

Post-harvest application of Serenade SOIL® provided statistically significant suppression of the pathogen relative to the inoculated control in 2017; however, it did not provide the same level of suppression as the chemical treatments. Serenade SOIL® is a biological fungicide and is currently registered for post-harvest application and is approved for use in organic production systems. Although Serenade SOIL® did not provide the same level of control as the chemical treatments; it did decrease the rate of infection of the tubers and decreased necrosis of the tuber tissue. Given that the conditions the tubers were stored in was favourable for the development of pink rot, the results of this study suggest that Serenade SOIL® may be a viable option for management of pink rot, especially in production systems with limited management options. In a study evaluating the control of pink rot in storage using *Bacillus subtilis*, researchers found a significant decrease in the incidence of pink rot in tubers treated with the bacteria compared to the inoculated control (Gachango *et al.,* 2012). However, as in this study, they found higher incidences of pink rot (31%) following biological control application than following application of chemical fungicide (Gachango *et al.,* 2012).

**Conclusion**

The management of metalaxyl-m resistant and sensitive strains of *P. erythroseptica* can be achieved in field and storage settings using currently registered and experimental fungicides. The results of these studies suggest that as the efficacy of metalaxyl-m based products decreases in potato growing regions, there are still viable options to control the pathogen in field settings and potential to develop these products into post-harvest products as well. Oxathiapiprolin, fluopicolide, and phosphite-based

products have potential to decrease incidences of pink rot; however, these products will have to be carefully managed with integrated pest management systems to reduce resistance development in *P. erythroseptica*. Serenade SOIL® provides less control of *P. erythroseptica* than the chemical treatments; however, it is a viable method of control for producers with limited pesticide options such as organic production systems.

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# GENERAL CONCLUSIONS

The results of this study have shown that metalaxyl-m resistant strains of *P. erythroseptica* are increasing in frequency in Canadian potato-producing regions. Metalaxyl-m resistant isolates were recovered from Prince Edward Island, Nova Scotia, New Brunswick, Ontario, Manitoba, and Alberta. However, resistance occurred with greater frequency in eastern Canada than western Canada.

Fortunately, the results of amended agar assay, field and storage trials have shown that there are alternative fungicides that can manage metalaxyl-m resistant strains of *P. erythroseptica*. The experimental fungicides evaluated in this study are now registered or are awaiting registration in Canada for control of pink rot in field settings, thanks in part, to the data generated in this project. Orondis®, Presidio®, and foliar applications of Phostrol™ were shown to be very effective in controlling pink rot and have potential to complement or replace metalaxyl-m based products in potato production systems. Currently, Orondis® and Presidio® are not registered for storage applications. However, the results of this study demonstrate the potential use of these products to inhibit pink rot development in storage and further work should be done to assess these products as a management strategy in storage settings. Serenade SOIL® provided pink rot suppression in field and storage settings but not to the extent of the chemical treatments. However, these results are useful in production systems with limited pesticide option such as in organic agriculture systems. Serenade SOIL® is a potential management option for controlling pink rot in field and storage settings when chemical applications are not an option.

As these products are adopted into potato production systems, careful management will be needed to help reduce the development of fungicide resistance in *P. erythroseptica* populations. Through the use of integrated pest management system, the efficacy of these products can be maintained into the future.

Future research on *P. erythroseptica* and pink rot has the potential to aid potato producers with their management decisions and help to mitigate yield loss due to disease. Research and development of molecular techniques to identify and quantify the pathogen rapidly from soil and plant is recommended. Also, the relationship between *P. erythroseptica* soil population density and disease development is not well understood and is a potential area of research. Through the use of quantitative molecular techniques and research into population densities, an economic threshold (the density of a pest at which a control treatment will provide an economic return) could be developed for *P. erythroseptica*. An economic threshold could be useful in potato production systems because it would help to determine if the risk of pink rot development justified specific applications of fungicides. This could help to reduce production costs, reduce chemical inputs into the environment, and could reduce the probability of resistance development due to decreased fungicide use.

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# APPENDIX ONE

A1.1 Metalaxyl sensitivity of *Phytophthora erythroseptica* isolates collected in 2013.

| Isolate | Province | Metalaxyl Sensitivity |
|---|---|---|
| PE1301-2-1 | Prince Edward Island | MS |
| PE1301-2-2 | Prince Edward Island | MS |
| PE1301-3-1 | Prince Edward Island | MS |
| PE1301-3-2 | Prince Edward Island | MS |
| PE1301-4-1 | Prince Edward Island | MS |
| PE1301-4-2 | Prince Edward Island | MS |
| PE1301-5-1 | Prince Edward Island | MS |
| PE1301-5-2 | Prince Edward Island | MS |
| PE1301-6-1 | Prince Edward Island | MS |
| PE1301-6-2 | Prince Edward Island | MS |
| PE1301-7-2 | Prince Edward Island | MS |
| PE1301-8-1 | Prince Edward Island | MS |
| PE1301-8-2 | Prince Edward Island | MS |
| PE1302-1-1 | Alberta | MS |
| PE1302-1-2 | Alberta | MS |
| PE1302-2-1 | Alberta | MS |
| PE1302-2-2 | Alberta | MS |
| PE1303-2-2 | Ontario | MHR |
| PE1303-3-1 | Ontario | MHR |
| PE1303-3-2 | Ontario | MHR |
| PE1303-5-1 | Ontario | MMR |
| PE1303-5-2 | Ontario | MMR |
| PE1303-7-1 | Ontario | MMR |
| PE1303-7-2 | Ontario | MMR |
| PE1304-3-1 | Manitoba | MS |
| PE1304-3-2 | Manitoba | MS |
| PE1304-4-1 | Manitoba | MS |
| PE1304-5-1 | Manitoba | MS |
| PE1304-5-2 | Manitoba | MS |
| PE1304-6-1 | Manitoba | MS |
| PE1304-7-2 | Manitoba | MS |
| PE1304-8-1 | Manitoba | MS |

Continued

A1.1. Continued

| Isolate | Province | Metalaxyl Sensitivity |
|---|---|---|
| PE1305-1-1 | Ontario | MS |
| PE1305-1-2 | Ontario | MS |
| PE1305-2-2 | Ontario | MS |
| PE1305-3-1 | Ontario | MS |
| PE1305-3-2 | Ontario | MS |
| PE1305-4-1 | Ontario | MS |
| PE1305-4-2 | Ontario | MS |
| PE1305-5-1 | Ontario | MS |
| PE1305-5-2 | Ontario | MS |
| | | |
| PE1306-3-1 | Manitoba | MS |
| PE1306-3-2 | Manitoba | MS |
| PE1306-4-1 | Manitoba | MS |
| PE1306-4-2 | Manitoba | MS |
| PE1306-8-1 | Manitoba | MS |
| PE1306-8-2 | Manitoba | MS |
| PE1306-9-1 | Manitoba | MS |
| PE1306-11-2 | Manitoba | MS |
| PE1306-12-1 | Manitoba | MS |
| PE1306-12-2 | Manitoba | MS |
| | | |
| PE1308-3-1 | Nova Scotia | MHR |
| PE1308-4-1 | Nova Scotia | MHR |
| PE1308-5-2 | Nova Scotia | MHR |
| | | |
| PE1309-1-1 | Ontario | MHR |
| PE1309-2-1 | Ontario | MMR |
| PE1309-2-2 | Ontario | MMR |
| PE1309-4-1 | Ontario | MS |
| PE1309-4-2 | Ontario | MS |
| | | |
| PE1310-2-1 | Manitoba | MS |
| PE1310-2-2 | Manitoba | MS |
| | | |
| PE1311-1-1 | Alberta | MS |
| PE1311-1-3 | Alberta | MS |

Continued

A1.1 continued

| Isolate | Province | Metalaxyl Sensitivity |
|---|---|---|
| PE1312-2-1 | Manitoba | MS |
| PE1312-2-2 | Manitoba | MS |
| PE1312-4-1 | Manitoba | MS |
| PE1312-4-2 | Manitoba | MS |
| PE1313-1-1 | Manitoba | MS |
| PE1313-4-1 | Manitoba | MS |
| PE1313-4-2 | Manitoba | MS |
| PE1314-1-2 | Ontario | MHR |
| PE1314-2-1 | Ontario | MMR |
| PE1316-1-1 | Alberta | MS |
| PE1316-2-2 | Alberta | MS |
| PE1316-3-1 | Alberta | MS |
| PE1316-3-2 | Alberta | MS |
| PE1316-4-1 | Alberta | MS |
| PE1317-2-1 | Alberta | MS |
| PE1320-1-1 | Manitoba | MS |
| PE1320-2-1 | Manitoba | MS |
| PE1320-2-2 | Manitoba | MS |
| PE1320-3-1 | Manitoba | MS |
| PE1320-3-2 | Manitoba | MS |
| PE1320-4-1 | Manitoba | MS |
| PE1320-4-2 | Manitoba | MS |
| PE1321-1-1 | Manitoba | MS |
| PE1321-1-2 | Manitoba | MS |
| PE1321-2-1 | Manitoba | MS |
| PE1321-2-2 | Manitoba | MS |
| PE1321-3-1 | Manitoba | MS |
| PE1321-3-2 | Manitoba | MS |
| PE1325-3-1 | Ontario | MS |
| PE1325-3-2 | Ontario | MS |

Continued

A1.1 continued

| Isolate | Province | Metalaxyl Sensitivity |
|---|---|---|
| PE1327-1-1 | Manitoba | MS |
| PE1327-1-2 | Manitoba | MS |
| PE1327-2-1 | Manitoba | MS |
| PE1327-2-2 | Manitoba | MS |
| PE1327-3-1 | Manitoba | MS |
| PE1327-3-2 | Manitoba | MS |
| | | |
| PE1328-1-1 | Manitoba | MS |
| PE1328-1-2 | Manitoba | MS |
| PE1328-2-1 | Manitoba | MS |
| PE1328-2-2 | Manitoba | MS |
| PE1328-3-1 | Manitoba | MS |
| PE1328-3-2 | Manitoba | MS |
| PE1328-4-1 | Manitoba | MS |
| PE1328-4-2 | Manitoba | MS |
| PE1328-5-1 | Manitoba | MS |
| PE1328-5-2 | Manitoba | |
| | | |
| PE1329-1-1 | Manitoba | MS |
| PE1329-1-2 | Manitoba | MS |
| PE1329-2-1 | Manitoba | MS |
| PE1329-2-2 | Manitoba | MS |
| PE1329-3-2 | Manitoba | MS |
| | | |
| PE1331-1-1 | Manitoba | MS |
| PE1331-1-2 | Manitoba | MS |
| PE1331-2-1 | Manitoba | MS |
| PE1331-2-2 | Manitoba | MS |
| PE1331-3-1 | Manitoba | MS |
| PE1331-3-2 | Manitoba | MS |
| | | |
| PE1332-1-1 | Manitoba | MS |
| PE1332-1-2 | Manitoba | MS |
| PE1332-1-3 | Manitoba | MS |
| | | |
| PE1333-2-2 | Manitoba | MS |
| PE1333-3-1 | Manitoba | MS |
| PE1333-3-2 | Manitoba | MS |

Continued

A1.1 Continued

| Isolate | Province | Metalaxyl Sensitivity |
|---|---|---|
| PE1334-1-1 | Manitoba | MHR |
| PE1334-1-2 | Manitoba | MHR |
| PE1334-2-1 | Manitoba | MHR |
| PE1334-2-2 | Manitoba | MHR |
| PE1334-3-1 | Manitoba | MHR |
| PE1334-3-2 | Manitoba | MHR |
| PE1335-1-1 | Manitoba | MS |
| PE1335-2-1 | Manitoba | MHR |
| PE1335-2-2 | Manitoba | MHR |
| PE1336 | Prince Edward Island | MMR |
| PE1337-1-2 | Prince Edward Island | MS |
| PE1337-3-1 | Prince Edward Island | MS |
| PE1337-3-2 | Prince Edward Island | MS |
| PE1338-1-1 | New Brunswick | MS |
| PE1338-1-2 | New Brunswick | MS |
| PE1338-2-1 | New Brunswick | MS |
| PE1338-2-2 | New Brunswick | MS |
| PE1338-3-2 | New Brunswick | MS |
| PE1338-4-2 | New Brunswick | MS |
| PE1339-2-2 | New Brunswick | MMR |
| PE1339-3-1 | New Brunswick | MHR |
| PE1339-3-2 | New Brunswick | MHR |
| PE1340-1-1 | New Brunswick | MS |
| PE1340-1-2 | New Brunswick | MMR |
| PE1342-1-1 | New Brunswick | MMR |
| PE1342-1-2 | New Brunswick | MHR |
| PE1342-2-2 | New Brunswick | MHR |
| PE1343-1-1 | New Brunswick | MMR |
| PE1343-2-1 | New Brunswick | MMR |
| PE1343-2-2 | New Brunswick | MMR |

Continued

A1.1 Continued

| Isolate | Province | Metalaxyl Sensitivity |
|---|---|---|
| PE1344-1-1 | New Brunswick | MS |
| PE1344-2-2 | New Brunswick | MMR |
| PE1344-3-2 | New Brunswick | MMR |
| PE1345-2-2 | New Brunswick | MS |
| PE1345-3-2 | New Brunswick | MHR |
| PE1346-1-1 | New Brunswick | MS |
| PE1346-1-2 | New Brunswick | MS |
| PE1346-2-2 | New Brunswick | MS |
| PE1347-1-2 | New Brunswick | MHR |
| PE1347-2-1 | New Brunswick | MHR |
| PE1347-3-1 | New Brunswick | MMR |
| PE1347-3-2 | New Brunswick | MMR |
| PE1348-2-1 | New Brunswick | MMR |
| PE1348-2-2 | New Brunswick | MMR |
| PE1348-3-1 | New Brunswick | MMR |
| PE1348-3-2 | New Brunswick | MMR |
| PE1349-1-1 | New Brunswick | MS |
| PE1349-1-2 | New Brunswick | MS |
| PE1349-2-1 | New Brunswick | MS |
| PE1349-2-2 | New Brunswick | MS |
| PE1350-1-1 | New Brunswick | MHR |
| PE1350-1-2 | New Brunswick | MHR |
| PE1350-2-1 | New Brunswick | MHR |
| PE1350-2-2 | New Brunswick | MHR |
| PE1351-1-2 | New Brunswick | MHR |
| PE1351-2-1 | New Brunswick | MHR |
| PE1351-2-2 | New Brunswick | MHR |

Continued

A1.1 Continued

| Isolate | Province | Metalaxyl Sensitivity |
|---|---|---|
| PE1352-2-2 | New Brunswick | MS |
| PE1353-1-2 | New Brunswick | MS |
| PE1353-2-1 | New Brunswick | MS |
| PE1353-3-1 | New Brunswick | MS |
| PE1356-1-2 | New Brunswick | MS |
| PE1356-2-1 | New Brunswick | MS |
| PE1356-3-1 | New Brunswick | MS |
| PE1356-3-2 | New Brunswick | MS |
| PE1357-3-2 | New Brunswick | MS |
| PE1360-1-1 | British Columbia | MS |
| PE1360-1-2 | British Columbia | MS |
| PE1360-2-1 | British Columbia | MS |

A1.2 Metalaxyl sensitivity of *Phytophthora erythroseptica* isolates collected in 2014.

| Isolate | Province | Metalaxyl Sensitivity |
|---|---|---|
| PE1401-1-1 | Prince Edward Island | MHR |
| PE1401-1-2 | Prince Edward Island | MHR |
| PE1401-2-1 | Prince Edward Island | MHR |
| PE1401-2-2 | Prince Edward Island | MHR |
| PE1401-3-1 | Prince Edward Island | MHR |
| PE1401-3-2 | Prince Edward Island | MHR |
| PE1401-4-1 | Prince Edward Island | MHR |
| PE1401-4-2 | Prince Edward Island | MHR |
| PE1401-5-1 | Prince Edward Island | MHR |
| PE1401-5-2 | Prince Edward Island | MHR |
| PE1401-6-1 | Prince Edward Island | MHR |
| PE1401-6-2 | Prince Edward Island | MHR |
| PE1401-7-1 | Prince Edward Island | MHR |
| PE1401-7-2 | Prince Edward Island | MHR |
| | | |
| PE1402-2-1 | Alberta | MS |
| PE1402-2-2 | Alberta | MS |
| PE1402-4-1 | Alberta | MS |
| PE1402-4-2 | Alberta | MS |
| PE1402-5-1 | Alberta | MS |
| | | |
| PE1403-1-2 | New Brunswick | MHR |
| PE1403-2-1 | New Brunswick | MHR |
| PE1403-2-2 | New Brunswick | MHR |
| PE1403-3-1 | New Brunswick | MS |
| PE1403-3-2 | New Brunswick | MS |
| | | |
| PE1404-1-1 | Prince Edward Island | MS |
| PE1404-1-2 | Prince Edward Island | MS |
| PE1404-2-1 | Prince Edward Island | MS |
| PE1404-2-2 | Prince Edward Island | MS |
| PE1404-3-1 | Prince Edward Island | MS |
| PE1404-3-2 | Prince Edward Island | MS |
| PE1404-4-1 | Prince Edward Island | MS |
| PE1404-4-2 | Prince Edward Island | MS |
| PE1404-5-1 | Prince Edward Island | MS |
| PE1404-5-2 | Prince Edward Island | MS |

Continued

A1.2 Continued

| Isolate | Province | Metalaxyl Sensitivity |
|---|---|---|
| PE1405-1-1 | Prince Edward Island | MS |
| PE1405-1-2 | Prince Edward Island | MS |
| PE1405-2-1 | Prince Edward Island | MS |
| PE1405-2-2 | Prince Edward Island | MS |
| PE1405-3-1 | Prince Edward Island | MS |
| PE1405-3-2 | Prince Edward Island | MS |
| PE1406-1-1 | Prince Edward Island | MHR |
| PE1406-1-2 | Prince Edward Island | MHR |
| PE1406-2-1 | Prince Edward Island | MHR |
| PE1406-2-2 | Prince Edward Island | MHR |
| PE1406-3-2 | Prince Edward Island | MHR |
| PE1406-4-1 | Prince Edward Island | MHR |
| PE1406-4-2 | Prince Edward Island | MHR |
| PE1406-5-1 | Prince Edward Island | MHR |
| PE1406-5-2 | Prince Edward Island | MHR |
| PE1407-1-1 | New Brunswick | MS |
| PE1407-1-2 | New Brunswick | MS |
| PE1407-2-1 | New Brunswick | MS |
| PE1407-2-2 | New Brunswick | MS |
| PE1407-3-1 | New Brunswick | MS |
| PE1407-3-2 | New Brunswick | MS |
| PE1408-1-1 | Prince Edward Island | MS |
| PE1408-2-1 | Prince Edward Island | MS |
| PE1408-3-1 | Prince Edward Island | MS |
| PE1408-4-1 | Prince Edward Island | MS |
| PE1409-1-1 | Prince Edward Island | MMR |
| PE1409-2-1 | Prince Edward Island | MS |
| PE1409-3-1 | Prince Edward Island | MMR |
| PE1409-4-1 | Prince Edward Island | MMR |
| PE1413 | Alberta | MS |
| PE1414-1-1 | Alberta | MS |

Continued

A1.2 Continued

| Isolate | Province | Metalaxyl Sensitivity |
|---|---|---|
| PE1414-2-1 | Alberta | MS |
| PE1414-3-1 | Alberta | MS |
| PE1414-4 | Alberta | MS |
| PE1415-4-1 | Manitoba | MS |
| PE1416-1-1 | Prince Edward Island | MMR |
| PE1416-2-1 | Prince Edward Island | MMR |
| PE1416-3-1 | Prince Edward Island | MMR |
| PE1417-1-1 | Prince Edward Island | MMR |
| PE1417-2-1 | Prince Edward Island | MMR |
| PE1417-3-1 | Prince Edward Island | MMR |
| PE1417-4-1 | Prince Edward Island | MMR |
| PE1418-1-1 | Alberta | MS |
| PE1418-2-1 | Alberta | MS |
| PE1418-3 | Alberta | MS |
| PE1421-1-1 | Prince Edward Island | MS |
| PE1421-2-1 | Prince Edward Island | MS |
| PE1421-3-1 | Prince Edward Island | MS |
| PE1421-4-1 | Prince Edward Island | MS |
| PE1422-1-1 | Prince Edward Island | MS |
| PE1422-2-1 | Prince Edward Island | MS |
| PE1422-3-1 | Prince Edward Island | MS |
| PE1422-4-1 | Prince Edward Island | MS |
| PE1423-1-1 | Prince Edward Island | MS |
| PE1423-2-1 | Prince Edward Island | MS |
| PE1423-3-1 | Prince Edward Island | MS |
| PE1423-4-1 | Prince Edward Island | MS |

Continued

A1.2 Continued

| Isolate | Province | Metalaxyl Sensitivity |
|---|---|---|
| PE1424-1-1 | Alberta | MS |
| PE1424-1-2 | Alberta | MS |
| PE1424-2-1 | Alberta | MS |
| PE1424-2-2 | Alberta | MS |
| PE1424-3-1 | Alberta | MS |
| PE1424-3-2 | Alberta | MS |
| PE1425-1-1 | Alberta | MMR |
| PE1425-1-2 | Alberta | MMR |
| PE1425-2-1 | Alberta | MS |
| PE1425-2-2 | Alberta | MS |
| PE1425-3 | Alberta | MS |
| PE1428-1 | Manitoba | MS |
| PE1428-2 | Manitoba | MS |
| PE1428-3 | Manitoba | MS |
| PE1429-2 | Manitoba | MS |
| PE1429-3 | Manitoba | MS |
| PE1434 | Alberta | MS |
| PE1435 | Alberta | MS |

A1.3 Metalaxyl sensitivity of *Phytophthora erythroseptica* isolates collected in 2015.

| Isolate | Province | Metalaxyl Sensitivity |
|---|---|---|
| PE1501-1-2 | New Brunswick | MHR |
| PE1501-2-1 | New Brunswick | MMR |
| PE1501-2-2 | New Brunswick | MMR |
| PE1501-3-2 | New Brunswick | MS |
| PE1502-2-1 | New Brunswick | MHR |
| PE1502-2-2 | New Brunswick | MHR |
| PE1507-1-1 | Manitoba | MHR |
| PE1507-1-2 | Manitoba | MHR |
| PE1508-1-1 | New Brunswick | MS |
| PE1508-2-2 | New Brunswick | MS |
| PE1508-3-1 | New Brunswick | MS |
| PE1508-3-2 | New Brunswick | MS |
| PE1512-1-1 | New Brunswick | MHR |
| PE1512-1-2 | New Brunswick | MHR |

A1.4 Metalaxyl sensitivity of *Phytophthora erythroseptica* isolates collected in 2016.

| Isolate | Province | Metalaxyl Sensitivity |
|---|---|---|
| PE1601-1-1 | Prince Edward Island | MS |
| PE1601-1-2 | Prince Edward Island | MS |
| PE1601-2-1 | Prince Edward Island | MS |
| PE1601-2-2 | Prince Edward Island | MS |
| PE1601-3-1 | Prince Edward Island | MS |
| PE1601-4-1 | Prince Edward Island | MS |
| PE1602-1 | Prince Edward Island | MS |
| PE1602-2 | Prince Edward Island | MS |
| PE1602-3 | Prince Edward Island | MS |
| PE1602-4 | Prince Edward Island | MS |
| PE1603-1 | Prince Edward Island | MS |
| PE1603-2 | Prince Edward Island | MS |
| PE1603-3 | Prince Edward Island | MS |
| PE1603-4 | Prince Edward Island | MS |
| PE1604-1 | Prince Edward Island | MS |
| PE1604-2 | Prince Edward Island | MS |
| PE1604-3 | Prince Edward Island | MS |
| PE1604-4 | Prince Edward Island | MS |
| PE1605 | Alberta | MS |
| PE1606 | Alberta | MS |
| PE1608-1-1 | Alberta | MS |
| PE1608-1-2 | Alberta | MS |
| PE1609-3-1 | Prince Edward Island | MS |
| PE1609-3-2 | Prince Edward Island | MS |
| PE1609-4-1 | Prince Edward Island | MS |
| PE1609-4-2 | Prince Edward Island | MS |
| PE1610-2 | Alberta | MS |

# APPENDIX TWO

A2.1 2014 field trial plot design and randomization where 'G' is a guard row between test plots, 9913 or 1204 is the isolate number of *Phytophthora erythroseptica* used, and treatments and plot numbers are listed for each test plot.

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| G | G | Healthy Plot 116 | G | 9913 Phostrol Foliar Plot 132 | G | G | 1204 Phostrol Foliar Plot 148 | G | 1204 Phostrol Furrow Plot 164 | G | G | Rep 4 |
| G | G | 9913 Presidio Plot 115 | G | 9913 Control Plot 131 | G | G | 1204 Control Plot 147 | G | 1204 Serenade Plot 163 | G | G | |
| G | G | 1204 Presidio Plot 114 | G | 9913 Phostrol Furrow Plot 130 | G | G | Healthy Plot 146 | G | 9913 Ridomil Gold Plot 162 | G | G | |
| G | G | 9913 Oxa Plot 113 | G | 1204 Oxa Plot 129 | G | G | 9913 Serenade Plot 145 | G | 1204 Ridomil Gold Plot 161 | G | G | |
| G | G | 1204 Phostrol Foliar Plot 112 | G | 9913 Ridomil Gold Plot 128 | G | G | 1204 Serenade Plot 144 | G | 1204 Phostrol Furrow Plot 160 | G | G | Rep 3 |
| G | G | 9913 Serenade Plot 111 | G | Healthy Plot 127 | G | G | 9913 Phostrol Furrow Plot 143 | G | 1204 Ridomil Gold Plot 159 | G | G | |
| G | G | 9913 Control Plot 110 | G | 1204 Presidio Plot 126 | G | G | 9913 Oxa Plot 142 | G | 1204 Oxa Plot 158 | G | G | |
| G | G | 9913 Phostrol Foliar Plot 109 | G | 9913 Presidio Plot 125 | G | G | 1204 Control Plot 141 | G | Healthy Plot 157 | G | G | |

Continued

A2.1 Continued

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| G | G | 9913 Ridomil Gold Plot 108 | G | 1204 Serenade Plot 124 | G | G | Healthy Plot 140 | G | 9913 Phostrol Furrow Plot 156 | G | G | Rep 2 |
| G | G | 9913 Phostrol Foliar Plot 107 | G | 9913 Serenade Plot 123 | G | G | 1204 Phostrol Furrow Plot 139 | G | 1204 Ridomil Gold Plot 155 | G | G | |
| G | G | 1204 Presidio Plot 106 | G | 1204 Phostrol Foliar Plot 122 | G | G | 1204 Oxa Plot 138 | G | Healthy Plot 154 | G | G | |
| G | G | 1204 Control Plot 105 | G | 9913 Oxa Plot 121 | G | G | 9913 Control Plot 137 | G | 9913 Presidio Plot 153 | G | G | |
| G | G | 1204 Phostrol Furrow Plot 104 | G | 9913 Phostrol Furrow Plot 120 | G | G | Healthy Plot 136 | G | 1204 Presidio Plot 152 | G | G | Rep 1 |
| G | G | 1204 Oxa Plot 103 | G | 1204 Serenade Plot 119 | G | G | 9913 Presidio Plot 135 | G | 9913 Serenade Plot 151 | G | G | |
| G | G | 9913 Control Plot 102 | G | Healthy Plot 118 | G | G | 9913 Ridomil Gold Plot 134 | G | 1204 Phostrol Foliar Plot 150 | G | G | |
| G | G | 1204 Control Plot 101 | G | 9913 Phostrol Foliar Plot 117 | G | G | 9913 Oxa Plot 133 | G | 1204 Ridomil Gold Plot 149 | G | G | |

A2.2 2015 field trial plot design and randomization where 'G' is a guard row between test plots, 9913 or 1204 is the isolate number of *Phytophthora erythroseptica* used, and treatments and plot numbers are listed for each test plot.

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| G | G | 1204 Serenade Plot 16 | G | 9913 Foliar Phostrol Plot 32 | G | G | 9913 Non-inoc Control Plot 48 | G | 9913 Oxa Plot 64 | G | G | Rep 4 |
| G | G | 9913 In-furrow Phostrol Plot 15 | G | 1204 Non-inoc Control Plot 31 | G | G | 1204 Inoc Control Plot 47 | G | 1204 Oxa Plot 63 | G | G | |
| G | G | 9913 Ridomil Gold Plot 14 | G | 1204 Ridomil Gold Plot 30 | G | G | 9913 Presidio Plot 46 | G | 9913 Serenade Plot 62 | G | G | |
| G | G | 1204 Presidio Plot 13 | G | 1204 In-furrow Phostrol Plot 29 | G | G | 9913 Inoc Control Plot 45 | G | 1204 Foliar Phostrol Plot 61 | G | G | |
| G | G | 1204 Serenade Plot 12 | G | 9913 Foliar Phostrol Plot 28 | G | G | 1204 Oxa Plot 44 | G | 9913 Presidio Plot 60 | G | G | Rep 3 |
| G | G | 1204 Ridomil Gold Plot 11 | G | 9913 Serenade Plot 27 | G | G | 9913 In-furrow Phostrol Plot 43 | G | 1204 In-furrow Phostrol Plot 59 | G | G | |
| G | G | 1204 Foliar Phostrol Plot 10 | G | 9913 Oxa Plot 26 | G | G | 9913 Non-inoc Control Plot 42 | G | 9913 Ridomil Gold Plot 58 | G | G | |
| G | G | 1204 Non-inoc Control Plot 9 | G | 1204 Presidio Plot 25 | G | G | 1204 Inoc Control Plot 41 | G | 9913 Inoc Control Plot 57 | G | G | |

Continued

A2.2 Continued

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| G | G | 1204 Inoc Control Plot 8 | G | 9913 In-furrow Phostrol Plot 24 | G | G | 9913 Non-inoc Control Plot 40 | G | 9913 Oxa Plot 56 | G | G | Rep 2 |
| G | G | 9913 Foliar Phostrol Plot 7 | G | 1204 In-furrow Phostrol Plot 23 | G | G | 9913 Presidio Plot 39 | G | 1204 Serenade Plot 55 | G | G | |
| G | G | 9913 Serenade Plot 6 | G | 9913 Ridomil Gold Plot 22 | G | G | 1204 Oxa Plot 38 | G | 1204 Non-inoc Control Plot 54 | G | G | |
| G | G | 1204 Presidio Plot 5 | G | 1204 Foliar Phostrol Plot 21 | G | G | 1204 Ridomil Gold Plot 37 | G | 9913 Inoc Control Plot 53 | G | G | |
| G | G | 9913 Serenade Plot 4 | G | 1204 In-furrow Phostrol Plot 20 | G | G | 9913 In-furrow Phostrol Plot 36 | G | 9913 Presidio Plot 52 | G | G | Rep 1 |
| G | G | 1204 Inoc Control Plot 3 | G | 9913 Oxa Plot 19 | G | G | 9913 Ridomil Gold Plot 35 | G | 9913 Foliar Phostrol Plot 51 | G | G | |
| G | G | 1204 Ridomil Gold Plot 2 | G | 1204 Foliar Phostrol Plot 18 | G | G | 1204 Non-inoc Control Plot 34 | G | 9913 Inoc Control Plot 50 | G | G | |
| G | G | 1204 Serenade Plot 1 | G | 1204 Presidio Plot 17 | G | G | 1204 Oxa Plot 33 | G | 9913 Non-inoc Control Plot 49 | G | G | |

A2.3 2016 field trial plot design and randomization where 'G' is a guard row between test plots, 9913 or 1204 is the isolate number of *Phytophthora erythroseptica* used, and treatments and plot numbers are listed for each test plot.

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| G | G | 9913 In-furrow Phostrol Plot 16 | G | 9913 Serenade Plot 32 | G | G | 1204 Presidio Plot 48 | G | 1204 Oxa Plot 64 | G | G | Rep 4 |
| G | G | 1204 In-furrow Phostrol Plot 15 | G | 1204 Non-inoc Control Plot 31 | G | G | 9913 Presidio Plot 47 | G | 1204 Inoc Control Plot 63 | G | G | |
| G | G | 9913 Inoc Control Plot 14 | G | 9913 Foliar Phostrol Plot 30 | G | G | 1204 Serenade Plot 46 | G | 1204 Ridomil Gold Plot 62 | G | G | |
| G | G | 9913 Oxa Plot 13 | G | 9913 Non-inoc Control Plot 29 | G | G | 1204 Foliar Phostrol Plot 45 | G | 9913 Ridomil Gold Plot 61 | G | G | |
| G | G | 9913 Non-inoc Control Plot 12 | G | 1204 Inoc Control Plot 28 | G | G | 9913 In-furrow Phostrol Plot 44 | G | 9913 Foliar Phostrol Plot 60 | G | G | Rep 3 |
| G | G | 1204 In-furrow Phostrol Plot 11 | G | 1204 Foliar Phostrol Plot 27 | G | G | 1204 Oxa Plot 43 | G | 9913 Inoc Control Plot 59 | G | G | |
| G | G | 9913 Presidio Plot 10 | G | 9913 Serenade Plot 26 | G | G | 1204 Presidio Plot 42 | G | 9913 Ridomil Gold Plot 58 | G | G | |
| G | G | 9913 Oxa Plot 9 | G | 1204 Serenade Plot 25 | G | G | 1204 Non-inoc Control Plot 41 | G | 1204 Ridomil Gold Plot 57 | G | G | |

Continued

A2.3 Continued

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| G | G | 1204 Serenade Plot 8 | G | 9913 Serenade Plot 24 | G | G | 1204 Oxa Plot 40 | G | 1204 Non-inoc Control Plot 56 | G | G | Rep 2 |
| G | G | 1204 Foliar Phostrol Plot 7 | G | 9913 Inoc Control Plot 23 | G | G | 9913 Oxa Plot 39 | G | 9913 In-furrow Phostrol Plot 55 | G | G | |
| G | G | 1204 Presidio Plot 6 | G | 1204 Ridomil Gold Plot 22 | G | G | 9913 Presidio Plot 38 | G | 9913 Non-inoc Control Plot 54 | G | G | |
| G | G | 9913 Ridomil Gold Plot 5 | G | 1204 Inoc Control Plot 21 | G | G | 1204 In-furrow Phostrol Plot 37 | G | 9913 Foliar Phostrol Plot 53 | G | G | |
| G | G | 1204 Inoc Control Plot 4 | G | 9913 Foliar Phostrol Plot 20 | G | G | 9913 Presidio Plot 36 | G | 9913 Inoc Control Plot 52 | G | G | Rep 1 |
| G | G | 9913 Ridomil Gold Plot 3 | G | 9913 Serenade Plot 19 | G | G | 1204 Non-inoc Control Plot 35 | G | 1204 Oxa Plot 51 | G | G | |
| G | G | 1204 In-furrow Phostrol Plot 2 | G | 1204 Foliar Phostrol Plot 18 | G | G | 9913 Non-inoc Control Plot 34 | G | 1204 Serenade Plot 50 | G | G | |
| G | G | 9913 In-furrow Phostrol Plot 1 | G | 9913 Oxa Plot 17 | G | G | 1204 Presidio Plot 33 | G | 1204 Ridomil Gold Plot 49 | G | G | |