INTERACTION OF POTATO TUBERS WITH *PHYTOPHTHORA INFESTANS* AND MANAGEMENT OPTIONS FOR SEED-BORNE LATE BLIGHT

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ABSTRACT

INTERACTION OF POTATO TUBERS WITH *PHYTOPHTHORA INFESTANS* AND MANAGEMENT OPTIONS FOR SEED-BORNE LATE BLIGHT

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Potato tubers differ in susceptibility to *Phytophthora infestans* and several techniques have been used to determine the degree of tuber susceptibility. A comparison of different tuber inoculation techniques based on internal or external placement of inoculum within or on tubers across different cultivars of potato and genotypes of *P. infestans* was carried out to evaluate the efficiency of inoculation. Internal or external placement of inoculum such as direct injection of sporangial suspension and mycelia colonized agar plug or the soaking method (SM) were the most effective ($p \leq 0.05$) in terms of causing tuber late blight. A separate study was conducted to evaluate the interaction between the infection processes of *P. infestans* and the periderm of tubers of three cultivars of potato differing in skin set development as influenced by harvest date. Mature and immature Russet Norkotah required higher torque to excoriate the periderm indicating improved skin-set compared to the other two (Snowden and Dark Red Norland) cultivars tested. Overall, immature cultivars were more susceptible compared to mature cultivars. Field trials were conducted to evaluate the effects of several different fungicides and biofungicides applied alone or mixed as seed treatments, in-furrow at planting, or in combination (seed + in-furrow treatment) for control of seed-borne *P. infestans*. The application of fenamidone and fungicides containing mancozeb such as fenamidone + mancozeb, mandipropamid + mancozeb or cymoxanil + mancozeb to potato seed pieces were more effective and improved final plant stand and rate of emergence ($p \leq 0.05$) in comparison to other treatments tested.

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CHAPTER 1: LITERATURE REVIEW

1.1 THE IMPORTANCE OF THE POTATO

The potato (*Solanum tuberosum* L.*)* is ranked fourth in importance in the world's food crop production, with a total production of 375 million Mt, after corn (1018 million Mt), rice (741 million Mt) and wheat (716 million Mt; Fiers et al. 2012; FAOSTAT 2014). The potato is the top non-grain food commodity providing a good source of carbohydrates, proteins, vitamins, and minerals (Neiderhauser 1993; Bradshaw and Ramsay 2009). The potato is believed to have been cultivated originally in the Andes mountain range of South America, what is present-day southern Peru and northwestern Bolivia, between 8000 and 5000 BC (Hawkes and Ortega 1993; Bradshaw and Ramsay 2009). The crop has since been adopted for food and starch production around the world. The potato was first introduced to Europe by the Spanish in the 16th century (Hawkes and Ortega 1993) and later became a major staple food for Europeans in the 19th century (Salaman 1949; Glendinning 1983). Currently potatoes are produced in more than 100 countries worldwide in many different environments and consumed as a staple food with average world consumption of about 32.6 kg per capita year^{-1} (CIP 2010; FAOSTAT 2014).

Potatoes are largely propagated by vegetative horticultural techniques throughout the world mainly by use of seed potato tubers (Secor and Rivera-Varas 2004). In recent years, potato production has increased worldwide because of the efficiency of vegetative propagation of potato plants and the nutritive value of the crop and largely due to expansion in countries in Europe and Asia (Roest and Bokelmann 1976; CIP 2010; Fiers et al. 2012). The net return of potato is about three or four times greater compared to other major food crops in terms of nutritive value per unit land or water use, respectively (Singh and Shekhawat 1999). By 2013, Europe and Asia contributed 30.4% and 49.7% of total production for the world (FAOSTAT 2014). The top five

potato producing countries in 2013 were China (89 million Mt), India (45 million Mt), Russia (30 million Mt), Ukraine (22 million Mt) and the United States (US; 20 million Mt; FAOSTAT 2013). In 2014, 429,411 ha of potatoes were planted in the US with a total production of 22 million Mt (USDA NASS 2014).

Diseases are a major constraint to sustainable and profitable potato production (Secor and Gudmestad 1999; Powelson and Rowe 2008; Kirk and Wharton 2014). Potatoes are a suitable host for a long list of pathogens (Stevenson et al. 2001; Kirk and Wharton 2014) and insect pests (Alyokhin et al. 2012). At least 75 diseases and non-parasitic disorders are known to negatively affect the yield and quality in potato production areas of the northern United States (Zitter and Loria 1986). Among these diseases, 60 are considered major diseases of potato in the US (Secor 2004). Fungal and oomycete pathogens cause a total of 35 diseases of potato (Secor 2004; Kirk and Wharton 2014). Among the fungal and oomycete pathogens, *Phytophthora infestans*, the causal organism of potato late blight, is a major threat to potato production in North America causing significant economic loss (Fry and Goodwin 1997b; Haverkort et al. 2008; Hu et al. 2012). Economic loss globally due to late blight from yield loss and cost of disease management has been estimated to exceed $6.7 billion annually (Haverkort et al. 2008). Thus, *P. infestans* is an important pathogen which needs to be controlled for the profitable potato production.

Michigan is the major producer of potatoes for chip processing in the US. In Michigan, 17,199 ha of potatoes were planted with a total production of 786,000 Mt in 2014 (USDA NASS, 2014). Typical cultivars grown commercially in Michigan include Russet Norkotah (a processing cultivar, usually frozen), Snowden (a chipping cultivar) and Dark Red Norland (a table stock cultivar) (Johansen et al., 1959, Johansen et al., 1988; Peloquin et al., 1994; Douches et al., 1996). Several diseases have impacted potato production in Michigan, with potato late blight

being the most important (Kirk per comm.). The upper Great Lakes region of the US can sporadically have conducive climatic conditions for potato late blight epidemics (Baker et al. 2005). Climatic conditions such as high relative humidity, frequent rainfall, and moderate temperatures (10-25°C) are ideal for the spread of potato late blight (Baker et al. 2005; Baker and Kirk 2007; Baker et al. 2014a, b; Baker et al. 2015).

1.2 *PHYTOPHTHORA INFESTANS*

Phytophthora infestans (Mont. de Bary) is a fungus-like organism in the phylum oomycota and sometimes referred to as a water mold (Van West and Vleeshouwers 2004). Oomycetes are eukaryotic plant pathogens classified in the kingdom Chromista (Cavalier-Smith 1986; Hawksworth et al. 1995; Rossman 2006). The oomycetes are most closely related to heterokont, biflagellate, golden-brown algae (Cavalier-Smith 1986; Hawksworth et al. 1995; Rossman 2006). The cell wall composition of oomycetes includes cellulose and beta glucans but does not include chitin (Rossman 2006). Oomycetes produce non-septate hyphae and are diploid (Randall et al. 2005).

Phytophthora infestans is heterothallic with two distinct mating types designated as A1 and A2 (Ko 1978; Shaw 1985). Sexual reproduction may occur if opposite mating types make contact to form gametangia (antheridia and oogonia) and consequently form an oospore (Judelson 1997; Ko 1988). The oospore is a sexual spore that rarely occurs in nature because it needs A1 and A2 mating types in the ratio of 1:1 (Drenth et al. 1995; Cohen et al. 1997). The oospore has a double cell wall (Hegnauer and Hohl 1978) that helps to resist freezing and other detrimental conditions in the soil or in plant debris (Drenth et al. 1995). Oospores can survive for more than four years in plant debris or free in soils to become a source of inoculum for late blight epidemics (Lehtinen and Hannukkala 2004). However, chances for sexual reproduction

are limited because single mating types usually predominate in most populations with the exception of the Toluca valley of Mexico where both mating types are found in a 1:1 ratio and in some parts in the Netherlands (Frinking et al. 1987). In Michigan both mating types (A1 and A2) have been found with no evidence of any sexual recombination (Young et al. 2009). Similarly, recently both mating types have been concurrently reported in Wisconsin as different genotypes with no evidence of sexual recombination (Seidle et al. 2013). It has been reported that at least one genotype US-8, can produce oospores through a homothallic process referred to as selfing (Smart et al. 1998). In most potato production areas in the US, oospores of *P. infestans* are rare, and infection of potato tubers in the field is initiated most commonly by asexual spores, sporangia and zoospores, that develop from lesions on stems and foliage of seedlings produced from infected tubers (Goodwin et al. 1994).

The sporangium of *Phytophthora infestans* is semi-papillate, lemon shaped, caducous and dehiscent (Hirst and Stedman 1960). Sporangia develop on sporangiophores, and can be carried to susceptible hosts on air currents (Aylor et al. 2001). Sporangia can infect host tissue by directly producing a germ tube at temperatures from 15 to 25°C and may indirectly infect tissue by producing zoospores at lower temperatures with optima between 10 to 15°C (Melhus 1915) however this may vary with isolates of different genotypes (Mizubuti and Fry 1998). Mizubuti and Fry (1998) reported optima for indirect infection of potato tissue between 4 to 12°C. Hyphae generated from sporangia or zoospores can penetrate potato tissue through lenticels, eyes, stomata, wounded or immature tissue (Lacey 1967; Adams 1975a; Lapwood 1977).

Sporangia also can germinate and produce 8-12 biflagellate zoospores when there is free water available such as on leaf or tuber surfaces or in the soil when the temperature is between 4 and 12°C (Mizubuti and Fry 1998) or 15°C (Melhus 1915). Zoospores released from the

sporangia encyst and germinate on the host (Grenville-Briggs et al. 2005). Lesions develop from hyphae within infected tissues and the pathogen can sporulate producing sporangiophores and more sporangia that then dislodge, spread and infect other plants. Epidemics can proceed exponentially on host tissue and cause extensive damage to the infected plants as the disease cycle is polycyclic in nature with an optimum of about 5 to 7 days from germination to production of secondary sporangia (Kamoun and Smart 2005). Under the conditions described above sporangia may release motile biflagellate zoospores that can swim in a water film down the stems and stolons (Bain et al. 1997; Kirk et al. 2004). When sporangia or the zoospores come into contact with progeny tubers infection may occur through lenticels or the eyes (Lacey 1967; Kirk and Wharton 2014). The infected tuber can become blighted in the field, thus reducing yield or potentially can cause rot problems in storage (James et al. 1972; Lambert et al. 1998; Kirk et al. 1999). *P. infestans* can survive in tubers from season to season to complete the disease cycle (Fig 1.1) as described by Kirk et al. (2004).

Several different genotypes of *P. infestans* have been reported in the US with different degrees of aggressiveness (Fry and Goodwin 1997a,b; Goodwin et al. 1998; Hu et al. 2012; Danies et. al. 2013; Rojas et al. 2014). The common genotypes found in the US since 1995 are designated US-1, US-6, US-23 and US-24 (A1 mating type), US-7, US-8, US-14 and US-22 (A2 mating type) (Fry and Goodwin 1977; Hu et al. 2012). The most recent genotypes found in the US in potato crops are US-8, US-22, US-23 and US-24 (Hu et al. 2012; Danies et al. 2013). The appearance of more aggressive genotypes of *P. infestans* compared to the genotypes in the early 1990s in North America increased the potential risk for successive late blight epidemics (Kirk et al. 2009).

Historically, displacement of genotypes of *P. infestans* has occurred regardless of the degree of aggressiveness (Hu et al. 2012; Kirk and Wharton 2014). For instance, the US-8 genotype that was predominant in 1990's in the US (Goodwin et al. 1998) was displaced by the less aggressive US-22 genotype (Rojas et al. 2014). In Michigan, the previously predominant genotype of *P. infestans* (US-1) before 1990's was displaced by the new and more aggressive genotype (US-8) in the 1990's, which was displaced by the slightly less aggressive US-22 in 2009 and recently displaced by US-23 which also is less aggressive than US-8 and US-22 (Young et al. 2009; Rojas et al. 2014 and Kirk pers. comm.). The newly emerged genotypes US-23 and US-24 are less virulent compared to the US-8 and US-22 genotypes (Danies et al. 2013).

The phenylamides, which include metalaxyl are a highly active class of fungicides and effective against oomycetes (Schwinn and Staub 1987). During the mid to latter part of the 20th century the phenylamides were developed and metalaxyl was the most effective in controlling late blight (Schwinn and Margot 1991), until metalaxyl-resistant isolates of *P. infestans* were discovered (Goodwin et al. 1998). The changes in the genetic structure of populations of *P. infestans* in the US has impacted disease management strategies because of the variation in sensitivity of the newly emerged genotypes to fungicides such as metalaxyl (Hu et al. 2012; Danies et al. 2013). Thus, late blight epidemics caused by newly emerged genotypes of *P. infestans* have increased the reliance of growers on alternative fungicides such as mancozeb, cymoxanil, dithane or penncozeb to metalaxyl (Inglis et al. 1996).

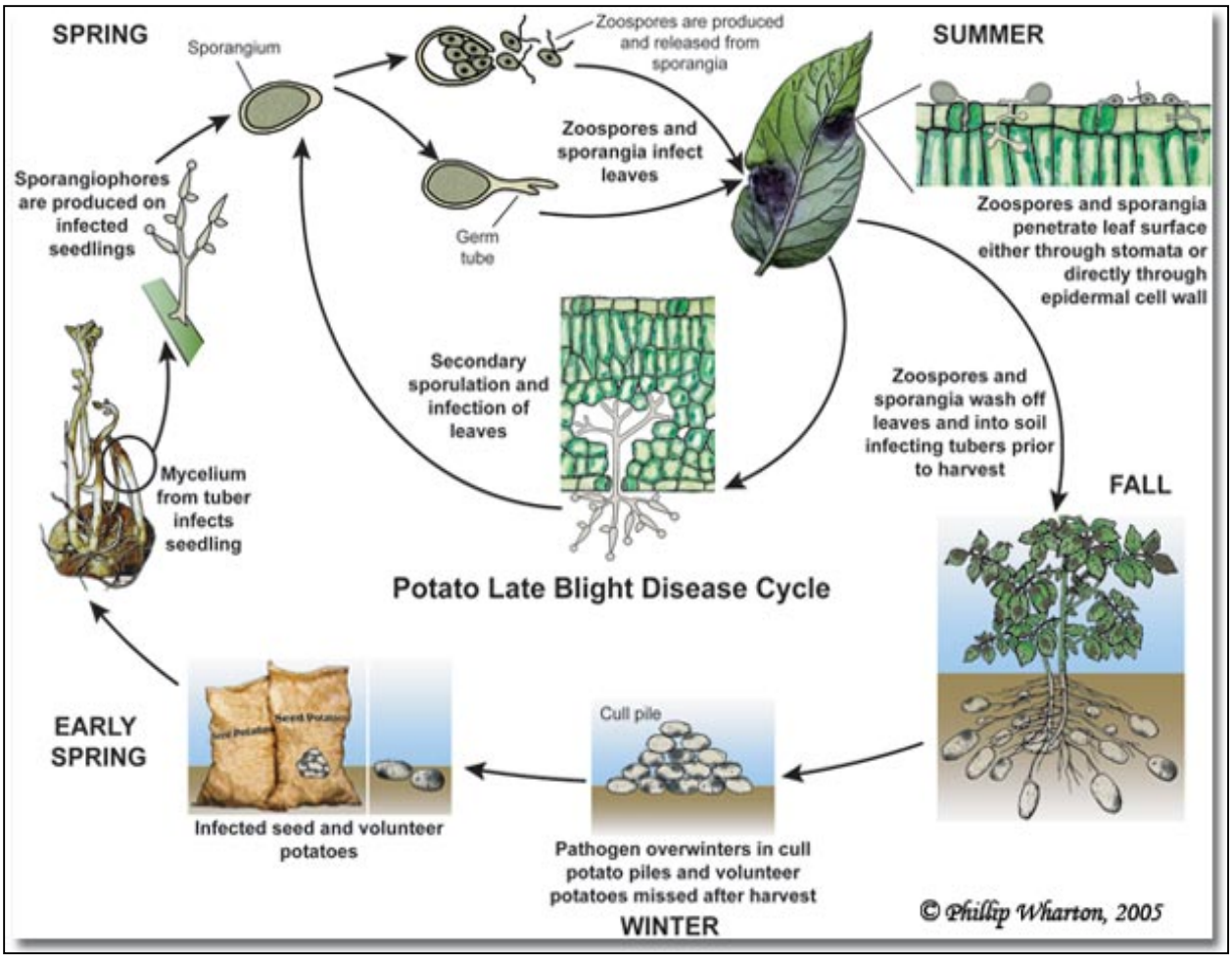

Figure 1.1: Disease cycle of the potato late blight pathogen, *Phytophthora infestans*, on potato in North America.

1.3 IMPORTANCE OF TUBER BLIGHT

Seed tubers are the main means of vegetative propagation of potato crops and are vital in potato production. Infected seed tubers, infected tubers in cull piles of discarded low quality potatoes near fields, infected volunteer tubers and subsequently shoots from plants, and rarely oospores, have been reported as primary sources of inoculum for the initiation of potato late blight epidemics (Bonde and Schultz 1943; Zwankhuizen et al. 1998; Kirk 2003; Fernández-Pavía et al. 2004; Johnson and Cummings 2009; Kirk et al. 2010). During an epidemic, tubers become infected when sporangia from foliage and blighted vines are washed into the soil by precipitation and irrigation events (Andrivon 1995; Bain et al. 1997; Porter et al. 2005). Tubers can become blighted shortly after the disease is established on the foliage (Hirst et al. 1965) and participate in the disease cycle (Fig.1.1, Kirk et al. 2004).

Potato tubers are stored at different temperatures based on the intended use (Knowles and Plissey 2007). Potato seed tubers are stored between 3.3 and 4.4°C, fresh market tubers stored between 3.3 and 10°C, chip processing tubers between 10 and 13°C, and tubers for French fries between 7 and 10°C (Knowles and Plissey 2007). Studies have shown latent infection of potato seed tubers by *P. infestans* during storage at temperatures at approximately 4°C (Johnson and Cumings 2009) and survival of *P. infestans* at 3.3°C (Kirk 2003a, b). While there is survival of the pathogen the disease severity of tuber infection by *P. infestans* varies depending upon temperature (Kirk 2003b; Johnson and Cummings 2009). Tuber late blight can result in tuber rotting both in the field and later in storage either in tubers intended for seed or for consumption (Bonde and Schultz 1943; Johnson and Cummings 2009; Johnson 2010). Seed tubers infected with *P. infestans* may rot after planting in the field, or survive and initiate new epidemics of potato late blight (Doster et al. 1989; Inglis et al. 1996; Stevenson et al. 2007; Olanya et al.

2009). As a result of tuber infection, movement of contaminated seed tubers have been implicated in the spread of both novel and common genotypes of *P. infestans* from one location to another (Fry et al. 1993). Therefore, tuber infection is very important process in the survival and dissemination of *P. infestans* and initiation of potato late blight epidemic.

The epidemiology of the foliar phase of the disease is correlated to infection in the tuber phase and vice versa (Fairclough et al. 1997; Cooke et al. 2011). The transmission of *P. infestans* from infected seed tubers into developing sprouts and consequently to the foliage further supports the importance and role of infected seed tubers in late blight epidemics as discussed above (Johnson and Cumming 2009). Tubers with latent infection are easily overlooked during potato seed cutting and handling operations (Johnson and Cummings 2009). During the potato planting season, seed tubers in the storage are gradually warmed at 2°C per day for a week prior to planting (Secor and Johnson 2008). *P. infestans* remains dormant at 3°C and increase in the temperature of infected seed tubers facilitates pathogen colonization of tuber tissue and sporulation (Gigot et al. 2009). The pathogen may be disseminated by direct contact of mycelia or sporangia from one tuber to the other during potato seed cutting and handling and may lead to infection of below and aboveground stems after planting the infected seed tubers (Hirst and Stedmann 1960; Lambert et al. 1998; Kirk et al. 2009). Because of the importance of infected seed tubers in potato late blight epidemic, seed tubers could be treated with effective fungicides before planting along with other cultural management strategies such as delaying planting after treatment to let the cut seed tuber heal or suberize (Wharton et al. 2012).

During harvest, not all tubers are collected from the field and some tubers may be left in the field at different soil depths (Perombelon 1975; Boydston et al. 2006). The tubers on the surface and up to 5 cm below the soil may be killed due to soil freezing in the northern Midwest

region of the US and cold places where snow occurs frequently during winter (Perombelon 1975; Boydston et al. 2006). However, under winter conditions when snow cover is extensive the soil may not freeze and tubers at 10 cm below the soil surface may survive (Boydston et al. 2006) and produce volunteer plants on which the pathogen may develop and grow and produce sporangia that can serve as a source of inoculum in the following spring (Perombelon 1975; Boydston et al. 2006) such as Midwest region of the US (e.g. http://www.lateblight.org/volunteer-risk.php).

1.4 RESISTANCE OF POTATO PERIDERM AGAINST TUBER BLIGHT

Potato periderm is a significant barrier to the loss of water and solutes from internal tissue (Lulai and Corsini 1998) as well as infection by various pathogens (Lulai and Orr 1994). Periderm consists of the phellem, (suberized cells); the phellogen, (a single layer of meristematic cells that give rise to phellem and the phelloderm); and phelloderm (the inner persistent living cells produced by the phellogen which connect the periderm to the underlying cortical, or storage tissue; Reeve et al. 1969; Sabba and Lulai 2002). In addition to the physical barriers waxes with fungicidal properties found on the suberized tuber surface are believed to aid in the prevention of tuber infection by pathogens (Kolattukudy 1984). *P. infestans* cannot infect through an intact periderm of matured tubers (Lacey 1967) although the lenticels and eyes of even mature tubers may be breached by the pathogen (Lacey 1967; Davila 1964; Rojas et al. 2014). While mature lenticels can be breached, the susceptibility of lenticels to *P. infestans* appears to be maximal when tubers are immature before lenticel suberization (Tyner et al. 1997). The immature tubers are considered to be more susceptible than mature tubers to late blight infection by *P. infestans* because of lack of lenticel suberization.

Lenticels are porous tissue consisting of cells with large intercellular spaces, usually scattered all over the tuber surface as minute openings and are involved in gaseous exchange during tuber respiration (Adams 1975a). The number of lenticels found on a mature tuber range from an average of 1 to 3 per 1.0 cm² (Burton 1989). However the number and size of lenticels per unit area of tuber can be influenced by soil type and the prevailing weather conditions during growth (Rastovski et al. 1981). According to Adams (1975a), dry soil conditions promote suberin deposition on the walls of the lenticel-filling cells that result in the closing of the lenticels. A physical barrier of suberized cells may form at the base of the lenticel after harvest, retarding water loss from the interior tissue (Lulai and Corsini 1998). In addition of the above, suberization of lenticels occurs as tubers mature (Tyner et al. 1997) however under moist storage condition lenticels can open to facilitate gas exchange between the tuber tissue and the ambient environment, a factor that can contribute to invasion of the tuber by pathogens (Lulai and Orr 1994).

Excoriation of potato periderm is a "skinning injury resulting from fracture of the radial phellogen cell wall linking the phellem to the phelloderm" (Lulai and Freeman 2001). Excoriation and bruising of tubers during harvest make them vulnerable to dehydration and disease later in storage because of the loss of periderm (Lulai and Orr 1995; Lulai and Corsini 1998). Wounded potatoes contribute to storage loss due to shrinkage due to dehydration (Lulai and Corsini 1998) and rot which in the US can exceed 1.3 million t/year in loss from wounded potatoes (Sabba et al. 2007). Matured tubers with improved skin-set compared to immature tuber contribute to improved storability (Sabba et al 2007). Immature tubers are more susceptible to excoriation than mature tubers (Lulai and Freeman 2001). Immature tubers have periderm with a thin radial cell wall of the phellogen layer, which breaks up easily during harvest (Lulai and

Freeman 2001). Upon periderm maturation, the phellogen becomes inactive and radial cell walls thicken and strengthen the periderm against excoriation and bruising (Lulai and Freeman 2001).

The sprout meristem (eye or bud), lenticels, stolen and wounds on the tuber are the sites for pathogen entry (Davila 1964; Rojas et al. 2014). The successful colonization of the eyes of potato tubers depends on the aggressiveness of *P. infestans* genotypes, for instance, isolates of the US-8 genotype colonized eyes on tubers with higher incidence than isolates of the US-22 genotype (Rojas et al. 2014). While the different genotypes of *P. infestans* can colonize the eyes, lenticels or the wounds, they may differ in aggressiveness (Kirk et al. 2009, 2010; Hu et al. 2012; Danies et al. 2013; Rojas et al. 2014). The aggressiveness of these genotypes also vary depending upon the cultivars of potatoes with different level of resistance (Rojas et al. 2014) so the recently found genotypes US-22, US-23 and US-24 need to be tested against different cultivars of potatoes to assess these interactions.

1.5 CHEMICAL CONTROL OF POTATO LATE BLIGHT

Integrated disease management strategies are practiced for controlling late blight (Fry et al. 1977; Cooke et al. 2011; Kirk and Wharton 2014). As a part of this growers rely heavily on fungicides to combat the disease (Basu et al. 2003; Fry 1997). Other integrated disease management strategies include the use of certified late blight-free seed, elimination of primary sources of inoculum such as infected tubers in cull piles and volunteer, use of disease resistant cultivars and applications of protective fungicides (Fry et al. 1977; Mundt et al. 2002; Basu et al. 2003; Nyankanga et al. 2004; Cooke et al. 2011). Although certified seed is recommended as a control measure (Frost et al. 2013), it does not guarantee that *P. infestans*-infected seed tubers will not be present in a seed lot (Stevenson 1993; Platt et al. 1999; Nolte et al. 2003). Because of

the potential infestation, the seed tubers should be treated with fungicides to control seed-borne late blight as an additonal protective measure.

Appropriate rates of fungicide applications with managed application intervals in combination with the use of host resistance, have been found to be the most economical strategy to control potato late blight (Kirk et al. 2001b). The use of higher rates of fungicide application with fewer intervals than used in the past throughout the growing season may result in the best control of potato late blight (Skelsey et al. 2009). In order to develop effective control programs minimization of the impact of inoculum at all stages during crop development must be achieved (Kirk et al. 1999; Kirk et al. 2001b; Benbrook et al. 2002; Stein and Kirk 2002). Thus inoculum in the seed-tuber cannot be overlooked.

Fungicides continued to be developed through the 20th century and new effective fungicides for control of late blight were developed such as dithiocarbamates, specifically ethylene bisdithiocarbamates, (Kaur and Mukerji 2004). Ethylene bisdithiocarbamates includes zineb, maneb, metiram, mancozeb, and propineb (Forbes and Landeo 2006). During the mid-to latter part of the 20th century the phenylamides were developed, of which metalaxyl was the most effective in controlling late blight (Schwinn and Margot 1991) until metalaxyl-resistant isolates of *P. infestans* emerged (Goodwin et al. 1996). Populations of metalaxyl-resistant *P. infestans* were found in Ireland soon after the metalaxyl was introduced in 1980s (Dowley and O'Sullivan 1985). After the failure of metalaxyl many new products were introduced for control of late blight such as cymoxanil (cyanoacetamide oximes), carbamates (prothiocarb and propamocarb), phosphonates (fosetyl-Al), and carboxylic acid amides (mandipropamid and dimethomorph discussed below) (Waard et al. 1993; Blum et al. 2010).

Cymoxanil is a systemic fungicide in the group of cyanoacetamide oximes (Waard et al. 1993; Blum et al. 2010) and is used to control different oomycete-caused diseases in vegetables including late blight on potato foliage and potato seed pieces (Powelson and Inglis 1999). A combination of cymoxanil plus mancozeb was reported as effective for the control of seed-borne late blight and increased emergence in inoculated treated potato seed tubers in multiple studies (Lambert et al. 1998; Kirk et al. 1999; Powelson and Inglis 1999).

Another available fungicide is mancozeb which is in the group of dithiocarbamates and 1, 2-bisdithiocarbamates. These are broad-spectrum fungicides (Russell 2005) used to control foliar late blight. These fungicides are recommended for seed treatment (Thompson 1993).

Fenamidone is another fungicide for the control of potato late blight and several fungal and oomycete pathogens in a wide variety of crops (Stevenson 2008). Fenamidone is a quinone outside inhibitor (QoI) product and is reported to have broad-spectrum activity (Stevenson 2008). Fenamidone was one of many fungicides registered to control metalaxyl-resistant genotypes of *P. infestans* (Stevenson 2008). Mancozeb and fenamidone were reported to reduce the infection of progeny tubers when applied to the soil (Porter et al. 2006).

Mandipropamid is a fungicide from the mandelamide group with translaminar and protectant properties, that is effective against foliar and stem blight of potato (Rekanovic et al. 2010).

Other fungicides registered for control of seed-borne late blight include mixture of flutolanil + mancozeb and fludioxonil + mancozeb which are registered as seed treatments to control Fusarium dry rot, and Rhizoctonia stem canker and black scurf of potatoes (Wharton et al. 2007a, b). However, these products have not been systematically tested as seed treatments for the control of seed-borne late blight caused by recently found *P. infestans* genotypes. US-8 was

already tested to a few of the above mentioned fungicides such as fludioxonil + mancozeb (Wharton et al. 2007a). These recently found genotypes differ among each other in terms aggressiveness and metalaxyl sensitivity. As the populations of metalaxyl-resistant *P. infestans* were found in Ireland soon after the metalaxyl was introduced in 1980s (Dowley and O'Sullivan 1985), it is prudent to test fungicides with new genotypes before using them in the field to control the potato late blight caused by these genotypes. It also is necessary to monitor fungicide sensitivity of the new emerging pathogens in advance to reduce the risk of economic loss.

To manage seed-borne late blight and other seed-borne diseases a strategy of mixing different products could be employed (Gisi et al. 1985; Gisi 1996) as fungicides used in combinations have been shown to provide better disease control efficacy than when used independently (Platt 1985; Caldiz et al. 2007). Fungicides with different modes of action used in combination have been reported to reduce cost, save labor and provide better control of some diseases (Gisi et al. 1985; Gisi 1996). The benefit of combining fungicides with two or three ingredients that have different modes of action may be an improvement in disease control and could delay the development of resistance in pathogen populations to high-risk systemic fungicides (Samoucha and Gisi 1987; Baider and Cohen 2003).

Due to the importance of seed tubers as a source of late blight as cull piles (Kirk 2003) and potato volunteers (Nyankanga et al. 2004), seed tubers should be treated with effective fungicides as a component of integrated late blight management (Bassler et al. 2002) or because of the possibility of latent infection of seed tubers by *P. infestans* (Johnson and Cummings 2009).

Over the past 15 years there has been an increased introduction of many fungicides and biofungicides for the control of potato diseases (Gullino et al. 2000; Wharton et al. 2012). Most

have been tested against foliar diseases with limited research to evaluate and compare their efficacy when applied as seed treatments or as in-furrow treatments (Gachango et al. 2012; Kirk et al. 2013). Some biofungicides have been tested against the more recently found genotypes of *P. infestans* but there is still limited information on the efficacy of such products in potato cropping situations (Gachango et al. 2012). Some recently introduced biocontrol products include those based on the bacterium *Bacillus subtilis* (Serenade Max; AgraQuest Inc., Davis, CA), and the fungi *Trichoderma* spp. (T-22 Planter Box; Bioworks Inc., Victor, NY) and been tested as seed treatments to control late blight (*P. infestans*, US-8 genotype; Wharton et al. 2012), but these products have not been tested against isolates of genotypes of *P. infestans* US-22 and US-23 as in-furrow treatments. The efficacy of biological control agents and foliar fungicides may vary when applied as seed or in-furrow treatments. So, further testing is appropriate.

1.6 OBJECTIVES

The potato tuber is a key component in potato production and its health is vital for economic production globally. Many potato pathogens interact with the tuber either when the tuber is planted as seed or when stored. *Phytophthora infestans*, the cause of potato late blight survives between seasons in tubers intended for seed, in cull piles or as volunteer tubers in the field from previous seasons. Late blight epidemics are initially started from sporangia that become airborne from lesions in immature potato plants that have developed from infected seed tubers. Potato tubers differ in susceptibility to *P. infestans* and several techniques have been used to determine the degree of tuber susceptibility.

The specific objectives of this work were therefore to initiate a comparison of different tuber inoculation techniques based on internal and external placement of inoculum (within or on

the tuber) across different cultivars of potato and genotypes of *P. infestans*. This testing was carried out to evaluate the efficiency of inoculation. Internal inoculation was accomplished by direct injection of sporangial suspension (DI) or the placement of an infested agar plug into the tuber (AP). These two methods were targeted to determine the resistance responses in medullar and cortex tissues and could be used, for example, in breeding programs to test breeding lines against isolates of different genotypes of *P. infestans.* Three external tuber inoculation methods were accomplished to produce late blight in tubers. The methods included a soaking method (SM) that entailed soaking tubers in water suspensions of sporangia of *P. infestans*, placement of inoculum soaked filter paper over individual tuber eyes (meristem buds) and spraying the inoculum over the tuber surface. These methods were evaluated to test the resistance of periderm, eyes and of lenticels of tubers as these are potentially exposed to inoculum.

A separate objective was to evaluate the interaction between the infection processes of *P. infestans* and the periderm of tubers to assess the late blight severity in the different cultivars of potato differing in skin set development as influenced by harvest date.

Potato seed tubers contaminated with *P. infestans* are a primary source of inoculum and can initiate potato late blight epidemics. Latently infected potato seed tubers are easily overlooked during the potato seed cutting and handling operation. Therefore, a further objective of this project was to conduct field trials to evaluate the effects of different fungicides and biofungicides applied as seed treatments or in-furrow at planting, or in combination for control of seed-borne *P. infestans*.

CHAPTER 2: COMPARISON OF INOCULATION TECHNIQUES WITH *PHYTOPHTHORA INFESTANS* ON POTATO TUBERS

ABSTRACT

Potato tubers differ in susceptibility to *Phytophthora infestans*, the cause of late blight. Several different techniques have been used in the past to determine the degree of tuber susceptibility. However, there are no standardizations of inoculation techniques to test tuber blight responses. This is an issue because results may vary with the type of inoculation technique used. In the current study, five different potato tuber inoculation techniques were assessed for their efficiency to cause tuber blight. The techniques included two inoculation methods that involved placement of inoculum within the tuber tissue a) sub-periderm injection of *P. infestans* zoospore/sporangial suspensions (DI, direct injection) or b) insertion of *P. infestans*-colonized agar plugs into a tuber surface wound (AP, agar plug); and three that utilized external inoculation methods involving placement of inoculum on the undisturbed tuber surface; c) soaking tubers in *P. infestans* zoospore/sporangial suspensions (SM, soaking method), d) placing *P. infestans* inoculum-saturated filter paper over meristematic organs (eyes), (FP, filter paper) or e) spraying the tuber surface with *P. infestans* zoospore/sporangial suspensions (SP, spray). Tests were carried out using four isolates each of recently identified genotypes of *P. infestans* US-8, US-22, US-23 and US-24 and three commercial potato cultivars, Russet Norkotah, Snowden and Dark Red Norland. The DI and AP methods caused significantly higher ($p \leq 0.05$) tuber late blight severity than the SM, FP and SP methods. Among the external inoculation techniques, the SM method produced significantly higher ($p \leq 0.05$) tuber late blight severity. The FP and SP methods produced the least tuber late blight. Overall, results indicated that internal inoculation techniques caused greater tuber blight severity compared to external inoculation techniques. Cv.

Russet Norkotah was significantly ($p \leq 0.05$) more susceptible than cvs. Snowden or Dark Red Norland in these studies. Isolates of *P. infestans* genotypes US-8 and US-22 were significantly more aggressive compared to isolates of genotypes US-23 and US-24, indicating that the isolates of these genotypes may cause less tuber rot damage compared to the two genotypes that were predominant in Michigan from 1995 to 2012 on the cultivars used in this study.

2.1 INTRODUCTION

Phytophthora infestans is a highly destructive plant pathogen that causes late blight in potato (*Solanum tuberosum*), tomato (*Solanum lycopersicum*) and other Solanaceous crops (Fry and Goodwin 1997b). The pathogen was the cause of the Irish potato famine in the mid-19th century (Andrivon 1995) and also was reported in North America starting in New York and Philadelphia in 1843 (Stevens 1933). Since then it has been documented as a major constraint to potato production worldwide (Fry and Goodwin 1997a,b). Despite the efforts of plant breeders to produce resistant germplasm (Corsini et al. 1999; Douches et al. 2001), the availability of effective fungicides (Guenthner et al. 2001) and decision support systems (DSS, Baker et al. 2014b) to monitor and manage the disease, numerous challenges still remain for the management of tuber blight (Olanya et al. 2009).

Prior to 1980, only A1 lineage of *P. infestans* caused the late blight epidemic worldwide (Fry and Goodwin 1997b). But recently *P. infestans* exists in both A1 and A2 mating types which contribute in the genetic diversity through sexual recombination subsequently producing new genotypes (Goodwin et al. 1995; Kalischuk et al. 2012). New clonal lineages of *P. infestans* displaced the US-1 genotype, which had prevailed globally, at least since methods to distinguish among genotypes existed in the US and other countries in the early 1990s (Goodwin et al. 1995). The US-8 clonal lineage was the predominant genotype in the US from the mid-1990s to 2009

(Fry and Goodwin 1997 a,b). Starting on 2009, a significant genetic change in the population of *P. infestans* occurred in the eastern US with the appearance of new isolates with unique genotypes and epidemiological characteristics (Hu et al. 2012). The new genotypes, US-22, US-23, and US-24, represents both mating types A2, A1, and A1, respectively (Hu et al. 2012; Danies et al. 2013) and have been reported as sensitive to metalaxyl which is a systemic fungicide that was effective to control late blight until the insensitive genotypes emerged (Davidse et al. 1981).

Different cultivars of potato tubers vary in susceptibility to *P. infestans* and many inoculation techniques have been used in a number of studies to determine the effect of variables such as temperature, relative humidity, growing season fungicide application and potato cultivar on the degree of tuber susceptibility (Johnson and Cummings 2009; Kirk et al. 2009; Rojas et al. 2014). However, no studies have been conducted to compare the efficiency of the inoculation techniques. This is important because the disease severity of tubers may vary depending upon the type of inoculation techniques used (Granke et al. 2012). Spore suspensions applied to sliced tubers have been widely used (Lapwood and McKee 1961) to test the resistance of cultivars against *P. infestans*. Direct inoculation of potato tubers was found to be very effective to produce tuber blight by Niemira et al. (1999), Kirk et al. (2001a, c) and Rojas et al. (2014) in a range of cultivars of potato. Inoculation was conducted by wounding tubers using a cork borer followed by removal of a plug from the surface of the test tuber at the apical end and placing a plug of *P. infestans* grown on agar into the cavity made with the cork borer and then covering the wound with petroleum jelly to prevent desiccation (Niemira et al. 1999; Kirk et al. 2001c; Rojas et al. 2014). Scratching and scrubbing to mechanically bruise tuber periderm (Toxopeus 1958) prior to inoculation has also been documented as effective to test the resistance in potato controlled by

major genes (Toxopeus 1958). Injection of potato tubers near the apical meristem with an inoculum suspension zoospores of *P. infestans* was successfully used to produce tuber blight by Kirk et al. (2001a). In another study, tubers were punctured at the bud end, and each injected with homogenized suspensions of individual isolates of *P. infestans* followed by covering the wound with Parafilm (Lambert and Currier 1997).

Point inoculations of tubers using pipette tips, inoculation of mechanically bruised tubers, or a standard slice inoculation method were used to compare the effectiveness of different methods of inoculation for screening for bacterial soft rot resistance in potato (Lojkowska 1994). Similar techniques could also be applied for tuber late blight as *P. infestans* also requires a tuber wound to penetrate the periderm. However lenticels and meristematic areas (potato eyes or buds) also can be penetrated by *P. infestans* (Rojas et al. 2014). These inoculation techniques could be utilized to test variables such as resistance of medullar storage tissue against *P. infestans*.

Localized inoculation techniques to determine the susceptibility of potato tubers to *P. infestans* by placing inoculum-saturated filter paper discs on selected sites on the tubers (eyes, lenticels, intact periderm, and wounds) have been used to test the susceptibility of potato tubers to infection by *P. infestans* (Lacey 1967). Another method used was whole healthy tubers inoculated by an atomized sporangial suspension for a study of the effect of registered potato seed piece fungicides on seed-borne *P. infestans* (Inglis et al. 1999; Flier et al. 2001) or for the assessment of laboratory methods for evaluating the resistance of potato tubers to late blight (Dorrance and Inglis 1998). Tubers dipped in a spore suspension of *P. infestans* (Bonde et al. 1940) or sprayed with an inoculum suspension, developed significant tuber late blight (Adams 1975b). Single eyes of tubers were inoculated with inoculum-saturated filter paper, placed in mist chambers and incubated in cold storage facilities to study the latent infection of potato seed

tubers by *P. infestans* which resulted in successful infection by *P. infestans* (Johnson and Cummings 2009).

Comparison and selection of appropriate inoculation techniques is therefore vital in that the same research conducted using different inoculation technique may produce inconsistent results. External inoculation techniques may have influenced by the combined resistance provided by lenticels, periderm, the outer cortical cells and medullar storage tissue, whereas internal inoculation techniques would be affected only by the resistance component of medullar storage tissue, which may respond differently to the pathogen protection offered by the mechanical barrier of the skin (Pathak and Clark 1987; Flier et al. 2007; Nyankanga et al. 2008). There is a lack of understanding whether the new genotypes (US-22, US-23 and US-24) differ in aggressiveness in relation to the inoculation techniques in these three commercial cultivars (Snowden, Russet Norkotah and Dark Red Norland) of potato commonly grown in MI.

The specific objectives of this study were to initiate a comparison of different tuber inoculation techniques based on internal or external placement of inoculum on tubers across different cultivars of potato and genotypes of *P. infestans* to evaluate the efficiency of inoculation.

2.2 MATERIALS AND METHODS:

2.2.1 ISOLATES AND INOCULUM PREPARATION

The study used four isolates of each genotypes of *Phytophthora infestans* 1) US-8 (phenylamide-insensitive, A2 mating type, isolated from potato during the 2006 growing season in Michigan by Kirk's lab); 2) US-22 (phenylamide-sensitive, A2 mating type, isolated from potato during the 2010 growing season in Michigan by Kirk's lab); 3) US-23 (phenylamide-sensitive, A1 mating type, isolated from tomato during the 2011 growing season in Wisconsin by

Gevens's lab); 4) US-24 (phenylamide-sensitive, A1 mating type, isolated from potato during the 2011 growing season in Wisconsin by Gevens's lab). The genotyping, metalaxyl sensitivity and mating type of these isolates were determined using methods developed by Goodwin et al. (1996) and Hu et al. (2012). Isolates of the US-8 and US-22 genotypes were collected and characterized by Dr. William Kirk's lab and those of US-23 and US-24 kindly provided by Dr. Amanda Gevens, University of Wisconsin Madison.

Cultures of *P. infestans* were prepared on rye B agar (Caten and Jinks 1967), amended with antibiotics (20mg/L rifampicin, TCI America, Portland, OR; 100 mg/L ampicillin, EMD Chemicals, Inc. San Diego, CA; and 20mg/L nystatin, Sigma-Aldrich Corporation, St. Louis, MO) to control bacterial contamination, for 14 d in the dark at 18°C prior to inoculation of potato tubers. For sporangial inoculation methods, plates were flooded with 50 ml of sterile distilled water and the sporangia were dislodged from the mycelia by gently scrapping using a sterile L-shaped glass rod. The concentration of the sporangial suspension was estimated using counting chamber (Makler). The initial concentration was adjusted to approximately 2 x 10^5 sporangia ml^{-1} by adding sterile distilled water and diluted as mentioned in each inoculation technique. The suspension was placed at 10°C for 2 hours to release zoospores (Judelson and Roberts 2002). For plug inoculation method, a cylindrical core of mycelium/sporangia plus agar of about 5 x 4 mm (length x diameter) was prepared using a cork borer.

2.2.2 INOCULATION TECHNIQUES

The inoculation techniques included two internal techniques (that involved placement of inoculum within the tuber tissue), 1) a sub-periderm injection of a zoospore/sporangial suspension of mixed isolates of P. infestans [approximately 20 sporangia (estimated as mentioned above) per inoculation; DI] or 2) insertion of colonized agar plugs (generated from cultures of mixed isolates of P. infestans) into a wound made by a sterile cork borer near apical eyes about 1 cm deep in the periderm and sealed by petroleum jelly (AP).

In addition three external inoculation techniques (that involved placement of inoculum on the surface of the tuber) included 1) soaking tubers in a zoospore/sporangial suspension of mixed isolates of *P. infestans* (2 x 10^5 sporangia/ml suspension) for 24 h (SM); 2) placing inoculum-saturated filter paper (Sterile Whatman filter paper, 10 X 10 mm^2) generated from a suspension of mixed isolates of *P. infestans* over an individual apical eye and covering with parafinn film (Parafilm Covra), (FP); or 3) spraying the tuber surface with a zoospore/sporangial suspension from a suspension of mixed isolates of *P. infestans* (2 x 10^5 sporangia/ml; SP). Tubers were stored at approximately 3°C in the dark for about 4 or 6 months at 3°C until used in the study.

Before being inoculated, tubers were washed in running tap water to remove soil from the tuber surface. Tubers were disinfected by soaking them in a 0.6% sodium hypochlorite suspension for 10 min (Clorox) and rinsed with sterile distilled water. Tubers were dried in a controlled environment chamber (Environmental Growth Chambers, Chagrin Falls, Ohio) with continuous airflow at 18°C in dry air (30% relative humidity) for four hours prior to inoculation. Tubers were inoculated as described below. After inoculation tubers were placed in plastic boxes (35 x 22 x 20 cm; Akro N.S.T, Akro-Mils, Akron, OH) with fitted plastic lids and lined with moist paper towels (prepared soaking in 100 ml sterile water) to prevent desiccation of the

inoculum suspension and tubers. Tubers were incubated in environmental growth chambers (1.8 m^3 volume; Environmental Growth Chambers, Chagrin Falls, OH) at 15°C and 90% relative humidity for 30 d.

a. Direct Injection method (DI); the tubers were washed and disinfested and inoculated by a sub-peridermal injection of a sporangia suspension of mixed isolates of *P. infestans*. About 20 sporangia were delivered per inoculation with a hypodermic syringe and needle (20 gauge) at the apical end of the tuber about 1 cm from the dominant sprout to a maximum depth of 1 cm. Control tubers were injected with 25 μl sterile distilled water. Tubers were incubated as described above.

b. Agar Plug method (AP); a single plug of tuber tissue was removed from the tuber by puncturing the surface with a 4 mm diameter sterile cork borer at the apical end of the tuber about 1 cm from the dominant sprout to a maximum depth of 1 cm. The wound was plugged with a cylindrical core of mycelium/sporangia plus agar (cultures prepared as above) about 5 x 4 mm (length x diameter) and the tuber tissue plug was replaced. The wound was sealed with petroleum jelly. Each AP inoculation contained about 2 x 10^5 sporangia/ml of mixed isolates of *P. infestans* per plug as determined from an inoculum suspension prepared from a set of test plugs and estimated using a counting chamber. A sterile rye B agar plug was placed in the cavity of the control tubers. Tubers were incubated as described above.

c. Soaking method (SM); tubers were inoculated by immersing in the inoculum suspension of mixed isolates of *P. infestans* (as above) for 24 h. Soaking of tubers was adapted from results of an optimization experiment to determine the period of time it took for infection to take place when tubers were soaked in an inoculum solution with or without prior wounding (unpublished data). After 24 h tubers were removed from the inoculum suspension and placed in plastic boxes

as described above. The control tubers were immersed in sterile distilled water for 24 h. Tubers were incubated as above.

d. Filter-paper method (FP); 0.05 ml of inoculum of mixed isolates of *P. infestans* containing 2 x 10^5 sporangia ml^{-1} was applied with a micropipette to a single 10-by-10-mm square of sterile filter paper (Whatman no. 2, Sigma-Aldrich, MO). The inoculum-saturated filter paper square was placed over a single eye on each test tuber. Tubers used as not-inoculated controls received a sterile filter paper square saturated with sterile distilled water. Tubers were incubated as described above.

e. Spray method (SP); the disinfected tubers were sprayed using an atomizer sprayer with 6 mL of a suspension of mixed isolates of *P. infestans* 2 x 10^5 zoospores/sporangia per ml for a final dosage of about 0.1 mL per tuber. Control tubers were sparayed with sterile distilled water. Tubers were incubated as described above.

2.2.3 EXPERIMENTAL DESIGN AND HOST MATERIAL

Tubers of three cultivars of potato (Russet Norkotah, Dark Red Norland and Snowden) that had been stored in a cold storage for 4 (2012) or 6 months (2013) at 3°C and 95% relative humidity in the dark were used in the study. Tubers of all three cultivars (Snowden, Russet Norkotah and Dark Red Norland) used in the study were previously reported to be susceptible to late blight (Bradeen et al. 2009; Johansen et al. 1988; Kirk et al. 2009). Tubers were stored in mesh bags (Etsy Inc., Brooklyn, NY) within the boxes. In all experiments, five tubers were placed in each of 4 replicate bags unless otherwise mentioned. Boxes were arranged in a complete randomized design and each treatment was replicated four times. Sample size per single replicate was five tubers.

2.2.4 DETERMINATION OF TUBER LATE BLIGHT RESPONSE

The experiment was conducted from February to April in 2012 and 2013. Tubers were dormant prior to inoculation. None of the tubers were sprouted prior to inoculation. A standard digital image analysis technique (Niemira et al. 1999; Kirk et al. 2001a) was used to assess tuber tissue symptoms. Tuber late blight development rates within tubers in relation to storage temperature are known from previous experiments (Gachango et al. 2012) and a single sampling date was selected about 30 days after inoculation (DAI) to assess the tuber tissue symptoms. Tubers were cut in 3 serial sections approximately 25% (apical), 50% (middle) and 75% (basal) the length of the tuber from apical end (Fig 2.1) and scanned using a flatbed scanner (HP ScanJet 4c, Hewlett-Packard Co., Houston, TX) (example: typical images shown in Fig 2.2). This technique resulted in 15 estimates of tuber tissue infection and 60 per treatment per genotypes per cultivar.

The image files created with the scanner were loaded into image analysis software (SigmaScan ver. 3.0, Jandel Scientific, San Rafael, CA). The area selection cut-off threshold was set to 10 light intensity units (LIU), allowing the software to exclude all parts of the image darker than 10 LIU, e.g. the black background. The average reflective intensity (ARI) of all the pixels within the image gave a measurement of severity of the disease in the tuber tissue of each sample (Niemira et al. 1999; Kirk et al. 2001a).

The ARI was measured for three sections. The amount of late blight symptomatic tissue per tuber was expressed as a single value (Mean ARI) calculated as the average ARI of the three sections. The presence of *P. infestans* in sample tubers was confirmed by isolating mycelia from a randomly selected sample of the five tubers and completing Koch's Postulates. The mycelia from symptomatic tubers were transferred to rye B selective media (CIP Protocol, International

Potato Center), thus pure cultures of *P. infestans* were maintained by hyphal tipping. Potato tuber slices (cultivars used in the experiment) and seven stage leaves were inoculated using the inoculum suspensions from pure cultures and incubated as above to confirm that the infection was by *P. infestans*. The symptoms of late blight in tuber, reddish tan discoloration of tissue (Kirk et al. 2004) and leaves, water-soaked necrotic lesion (Kirk et al. 2004) were observed and sporulating structures were examined under a microscope (Olympus BX41, Olympus America Inc., Waltham, MA) to confirm the presence of *P. infestans*.

The severity of tuber tissue symptoms was expressed relative to the ARI of the control tubers for each cultivar. The Relative ARI [(RARI (%)] was calculated as follows:

$$\text{RARI (\%)} = \left(1 - \frac{\text{mean ARI treatment}}{\text{mean ARI control}}\right) \text{X } 100\%$$

RARI (%) has a minimum value of 0% (no symptoms) and a maximum value of 100% (black tuber surface) (Niemira et al. 1999)

2.2.5 DATA ANALYSIS:

All data were subjected to analysis of variance (ANOVA) using the statistical analysis software package JMP version 10.0 (SAS Institute, Cary, NC). Mean separation tests were conducted when the main effects were significant at $p \leq 0.05$ with Least Square Means Tukey's honestly significant difference (HSD) test at $p \leq 0.05$. Treatment effects were determined by a three-way factorial ANOVA, where the main effects were inoculation technique, cultivar and *P. infestans* genotype.

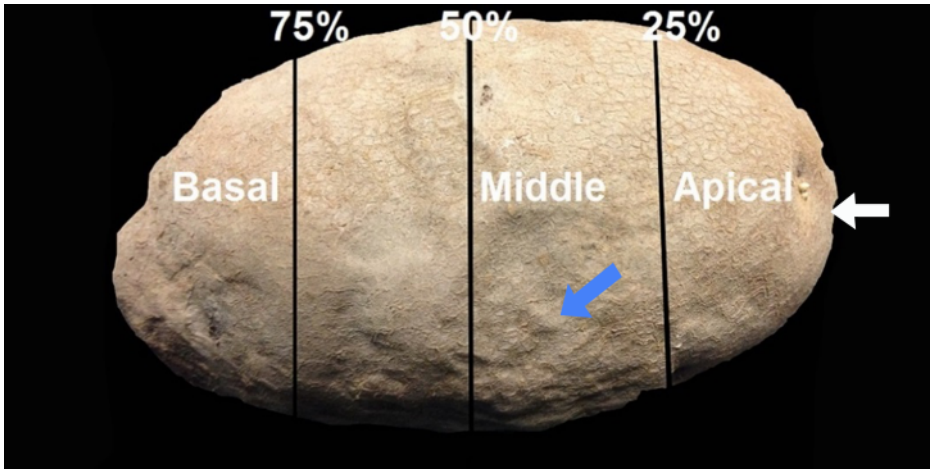

Figure 2.1: Potato tuber showing the different cross sections of the tuber that were cut (apical, middle and basal) at about 25, 50 and 75% distal from the apex of the tuber and used for scanning to determine Relative Average Reflective Intensity (RARI %). The isolate used in this example was the US-22 genotype of *Phytophthora infestans* using the agar plug method. (Legend: blue arrow showing the symptoms on tuber expressed by slightly depressed brown to purplish areas on skin; white arrow showing the point of inoculation).

Figure 2.2: Potato tuber (cv. Snowden) expressing late blight, cut open 25% distal from the apex of the tuber to expose the internal darkening of tissue (US-22 genotype of *Phytophthora infestans*).

2.3 RESULTS:

2.3.1 EVALUATION OF DIFFERENT INOCULATION TECHNIQUES ON TUBER SUSCEPTIBILITY TO *PHYTOPHTHORA INFESTANS*

The range in response of the three potato cultivars to *Phytophthora infestans* when inoculated with five different inoculation techniques was not significantly different over the two experiments in 2012 (A and B) determined by t-test and data were therefore combined (Table 2.1). However, the results of experiments in 2012 (experiment A and B combined) and in 2013 (experiment C) were significantly different and data were therefore analyzed separately (Table 2.2). Tubers showed significant tuber late blight within 30 DAI (Table 2.3) in all experiments and treatments (Figs 2.3 and 2.4). Late blight did not develop in control tubers (non-inoculated) of any cultivar. Therefore these data were not included in the statistical analyses but were used to calculate Relative Average Reflective Intensity [RARI (%)]. Significantly greater RARI (%) values were observed in the two 2012 experiments compared with the 2013 experiment except in tubers inoculated using the SM method (Table 2.3). The RARI (%) of the SM method was higher in 2013 compared to 2012 (Table 2.3). However the results from both years were similar in that the internal inoculation methods resulted in higher RARI (%) than external inoculation methods (Table 2.3) ($p \leq 0.05$)

Internal inoculation (either the DI or the AP methods) had significantly higher tissue discoloration (severity) across all three cultivars inoculated with isolates of the four different genotypes of *P. infestans* compared to the external inoculation methods (Figs. 2.3a and 2.3b). There was no significant difference in tuber late blight development between the DI and the AP methods in terms of RARI (%) except in the 2012 experiment (combined) (Table 2.3). The DI method caused higher RARI (%) in the 2012 experiments compared to the AP method.

Table 2.1: Effect of five different inoculation techniques (Direct injection, Agar plug, Soaking, Filter-paper and Spray) on late blight severity [Relative Average Reflective Intensity {RARI (%)}] in three cultivars of potato tubers (Snowden, Russet Norkotah and Dark Red Norland) inoculated with isolates of four different genotypes of *Phytophthora infestans* (US-8, US-22, US-23 and US-24) and stored at 15°C and 90% relative humidity for 30 d in the dark in three different experiments (A, B in 2012 and C in 2013)

Experiments	Disease Severity[a] [RARI (%)]
Experiment A (2012)	13.7 a[b]
Experiment B (2012)	13.1 a
Experiment C (2013)	8.7 b

[a] Disease severity measured in terms of tuber tissue darkening; score expressed as Relative Average Reflective Intensity [RARI (%)]; RARI (%) = [1-Mean $ARI_{treatment}$ / Mean $ARI_{control}$] * 100; RARI (%) has a minimum value of 0% (no darkening) and maximum value of 100% (completely blackened). The numbers are derived from the mean RARI (%) of three surfaces cut latitudinally 25, 50 and 75% distal from the apex of the tuber. [n= 1200 tubers (total 3600 tuber pieces].

[b] Values followed by the same letters are not significantly different at $p \leq 0.05$ for comparison of disease severity RARI (%) of three experiments by LS Means Tukey's Honestly Significant Difference test

Table 2.2: Main effects analyses of the comparisons among five different inoculation techniques (Direct injection, Agar plug, Soaking, Filter-paper and Spray) using isolates of four different genotypes of *Phytophthora infestans* (US-8, US-22, US-23 and US-24) and three cultivars of potato (Snowden, Russet Norkotah and Dark Red Norland) and interactions among these variables on late blight severity on potato tubers from the 2012 and 2013 experiments

	p value	
Source of Variation	Disease Severity[a] [RARI (%)]	
	2012	2013
Inoculation method	<0.0001	<0.0001
Cultivar	<0.0001	<0.0001
Genotype	<0.0001	<0.0001
Cultivar*Genotype	0.0001	<0.0001
Inoculation method*Cultivar	<0.0001	<0.0001
Inoculation method*Genotype	<0.0001	<0.0001
Inoculation method*Cultivar*Genotype	<0.0001	<0.0001

[a] Disease severity measured in terms of tuber tissue darkening; score expressed as Relative Average Reflective Intensity [RARI (%)]; RARI (%) = [1-Mean $ARI_{treatment}$ / Mean $ARI_{control}$] * 100; RARI (%) has a minimum value of 0% (no darkening) and maximum value of 100% (completely blackened). The numbers are derived from the mean RARI (%) of three surfaces cut latitudinally 25, 50 and 75% distal from the apex of the tuber.

Table 2.3: Effect of five different inoculation techniques (Direct injection, Agar plug, Soaking, Filter-paper and Spray) on late blight severity [Relative Average Reflective Intensity {RARI (%)}] using isolates of four different genotypes of *Phytophthora infestans* (US-8, US-22, US-23 and US-24) and across the three cultivars of potato (Snowden, Russet Norkotah and Dark Red Norland)

Inoculation methods	Disease Severity [RARI (%[a])]	
	2012	2013
Injection (DI)	21.6 b[b]	14.8 a
Plug (AP)	31.0 a	14.0 a
Soaking (SM)	5.2 c	8.9 b
Filter-paper (FP)	5.2 c	3.0 c
Spray (SP)	3.3 d	3.0 c

[a] Disease severity measured in terms of tuber tissue darkening; score expressed as Relative Average Reflective Intensity [RARI (%)]; RARI (%) = [1-Mean $ARI_{treatment}$ / Mean $ARI_{control}$] * 100; RARI (%) has a minimum value of 0% (no darkening) and maximum value of 100% (completely blackened). The numbers are derived from the mean RARI (%) of three surfaces cut latitudinally 25, 50 and 75% distal from the apex of the tuber [n= 80 tubers (total 240 tuber pieces].

[b] Values followed by the same letters are not significantly different at $p \leq 0.05$ for comparison of disease severity RARI (%) of inoculation methods per year by LS Means Tukey's Honestly Significant Difference test.

Among the external inoculation methods (SM, FP and SP), the SM method resulted in the most tuber tissue discoloration and therefore the highest RARI (%) in the 2013 experiment. There was no significant difference between the SM and FP methods for RARI (%) in the 2012 experiments (Table 2.3). Although there was no significant difference between the FP and SP methods in the 2013 experiments in terms of late blight severity in tubers, the SP method caused the least amount of tuber infection and discoloration when compared with other inoculation methods in 2012 (Table 2.3). Significant differences among the inoculation techniques in the different cultivars in relation to the effect of different genotypes of *P. infestans* are detailed in Figs 2.3a and 2.3b for the 2012 (combined) and 2013 experiments.

The four *P. infestans* genotypes were significantly different from each other in terms of late blight severity measured as RARI (%) value in the 2012 experiments, however the *P. infestans* genotypes US-23 and US-24 in the 2013 experiment were not significantly different from each other (Table 2.4).

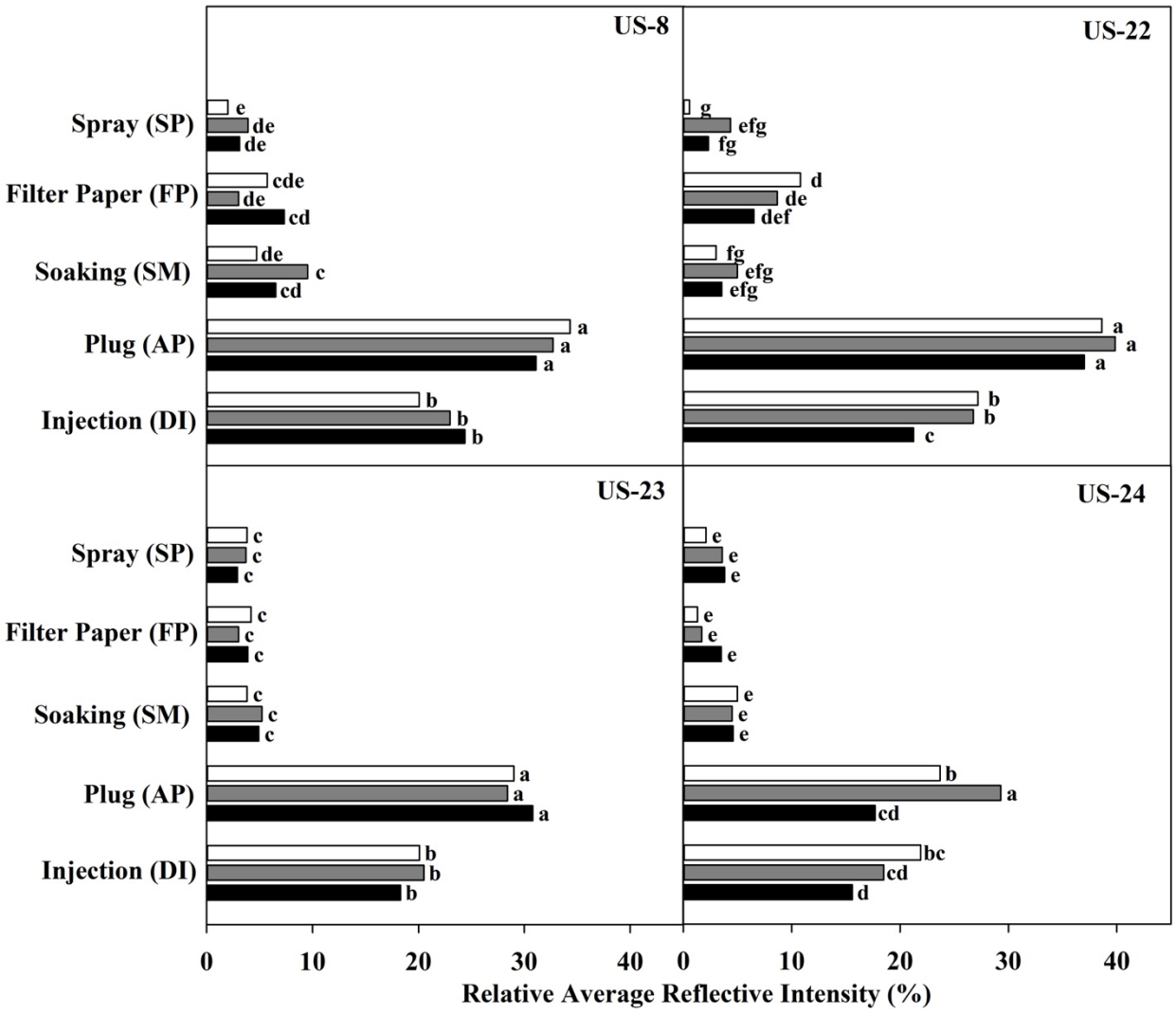

Figure 2.3: Efficacy of five different inoculation techniques (Direct injection, Agar plug, Soaking, Filter-paper and Spray) on late blight severity [RARI (%)] in potato tubers inoculated with isolates of four different genotypes of *Phytophthora infestans* (US-8, US-22, US-23 and US-24) and across the three cultivars of potato (Snowden, Russet Norkotah and Dark Red Norland) in the 2012 experiment. [Each bar is a mean of RARI (%) from 60 tuber pieces]. Columns annotated with same letter are not significantly different at $p \leq 0.05$ by Least Square Means Tukey's Honestly Significant Difference test. (Legend: white bar = cv. Snowden, dark grey bar = Russet Norkotah and black bar = Dark Red Norland).

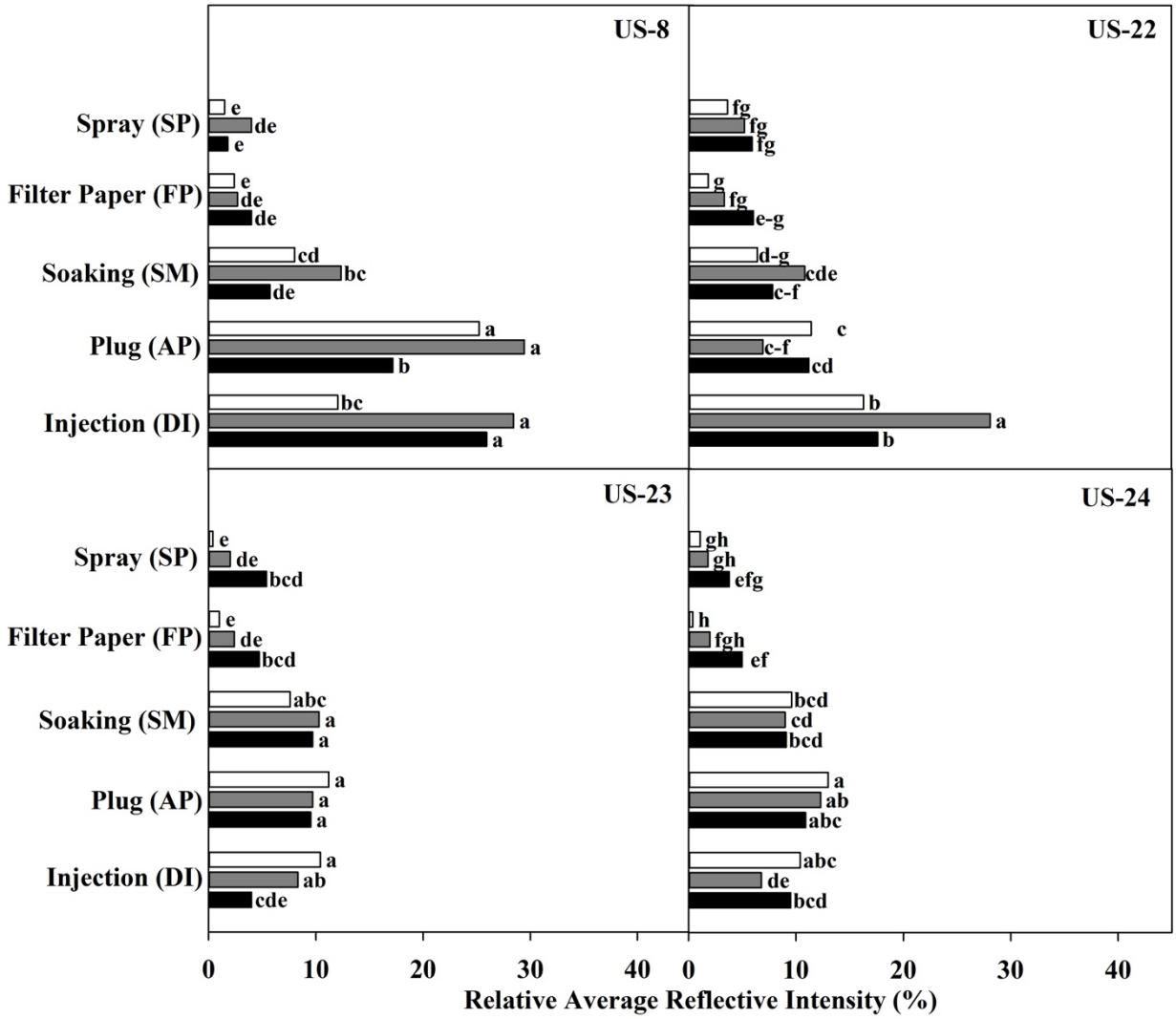

Figure 2.4: Efficacy of five different inoculation techniques (Direct injection, Agar plug, Soaking, Filter-paper and Spray) on late blight severity [RARI (%)] in potato tubers inoculated with isolates of four different genotypes of *Phytophthora infestans* (US-8, US-22, US-23 and US-24) and across the three cultivars of potato (Snowden, Russet Norkotah and Dark Red Norland) in the 2013 experiment. [Each bar is a mean of RARI (%) from 60 tuber pieces]. Columns annotated with same letter are not significantly different at $p \leq 0.05$ by Least Square Means Tukey's Honestly Significant Difference test. (Legend: white bar = cv. Snowden, dark grey bar = Russet Norkotah and black bar = Dark Red Norland).

Table 2.4: Effect of isolates of four different genotypes of *Phytophthora infestans* (US-8, US-22, US-23 and US-24) on the development of potato tuber late blight severity [Relative Average Reflective Intensity {RARI (%)}]. Potato tubers (Snowden, Russet Norkotah and Dark Red Norland) were inoculated then stored at 15°C and 90% relative humidity for 30 d in the dark in 2012 or 2013

Genotypes[a]	RARI (%)[b]	
	2012	2013
US-8	14.0 b[c]	12.0 a
US-22	16.4 a	9.5 b
US-23	12.2 c	6.4 c
US-24	10.4 d	6.9 c

[a] Different genotype of *Phytophthora infestans* classified according to Goodwin et al. (1995) and Hu et al. (2012).

[b] Disease severity measured in terms of tuber tissue darkening; score expressed as Relative Average Reflective Intensity [RARI (%)]; RARI (%) = [1-Mean $ARI_{treatment}$ / Mean $ARI_{control}$] * 100; RARI % has a minimum value of 0% (no darkening) and maximum value of 100% (completely blackened). The numbers are derived from the mean average reflective intensity of three surfaces cut latitudinally 25, 50 and 75% distal from the apex of tuber. [n= 100 tubers (total 300 tuber pieces].

[c] Values followed by the same letters are not significantly different at $p \leq 0.05$ by Least Square Means Tukey's Honestly Significant Difference test for comparison of mean disease severity [RARI (%)] values of different genotypes of *Phytophthora infestans.*

The cultivars had significant differences in the amount of potato tuber tissue discoloration measured as RARI (%) (Figs 2.3a and 2.3b). When the responses of the cultivars were analyzed in relation to the effect of different isolates of *P. infestans* genotypes, there were significant differences among the cultivars with respect to RARI (%) within different genotypes of *P. infestans* for two genotypes, US-22 and US-24, in 2012; and for three genotypes, US-8, US-22 and US-24, in 2013 (Table 2.5). The three cultivars were not significantly different from each other in terms of tuber rot severity [RARI (%)] in 2012 except Dark Red Norland inoculated with US-22 and US-24. In 2013, the three cultivars were not significantly different from each other except Russet inoculated with US-8, Snowden inoculated with US-22 and Russet Norkotah and Dark Red Norland inoculated with US-24 (Table 2.5). Overall, RARI (%) values of all cultivars were higher in the 2012 experiments [RARI (%) range; 9.0 to 17.6%] compared with the 2013 experiments except RN inoculated with US-8 [RARI (%) range; 6.1 to 15.3; Table 2.5]. In both years tubers of different cultivars inoculated with isolates of US-8 and US-22 genotypes of *P. infestans* had significantly higher late blight severity compared to those inoculated with isolates of US-23 and US-24 (Table 2.4).

Table 2.5: Response of three cultivars of potato (Snowden, Russet Norkotah and Dark Red Norland) inoculated using internal or external inoculation techniques with isolates of four different genotypes of *Phytophthora infestans* (US-8, US-22, US-23 and US-24). Response is expressed in terms of Relative Average Reflective Intensity [RARI (%)] in experiments conducted in 2012 and 2013

Cultivars	RARI (%)[a]			
	Genotypes of *Phytophthora infestans*[b]			
	US-8	US-22	US-23	US-24
2012				
Snowden	13.3	16.7 a[c]	12.0	10.8 a
Russet Norkotah	14.4	17.6 a	12.2	11.5 a
Dark Red Norland	14.4	14.8 b	12.2	9.0 b
	NSD[d]		NSD	
2013				
Snowden	9.8 b	7.9 b	6.1	6.9 ab
Russet Norkotah	15.3 a	10.9 a	6.5	6.4 b
Dark Red Norland	10.9 b	9.7 a	6.7	7.6 a
			NSD[d]	

[a] Disease severity measured in terms of tuber tissue darkening; score expressed as Relative Average Reflective Intensity [RARI (%)]; RARI% = [1-Mean $ARI_{treatment}$ / Mean $ARI_{control}$] * 100; RARI (%) has a minimum value of 0% (no darkening) and maximum value of 100% (completely blackened). The numbers are derived from the mean RARI (%) of three surfaces cut latitudinally 25, 50 and 75% distal from the apex of the tuber [n= 20 tubers (total 60 tuber pieces].

[b] Different genotypes of *Phytophthora infestans* classified according to Goodwin et al. (1995) and Hu et al. (2012).

[c] Values followed by the same letters are not significantly different at $p \leq 0.05$ for comparison of mean RARI (%) of different cultivars within different genotypes of *Phytophthora infestans* by Least Square Means Tukey's Honestly Significant Difference test.

[d] NSD = No significant difference at $p < 0.05$.

2.4 DISCUSSION

Many studies have been conducted on the interactions between *Phytophthora infestans* and potato tubers as the impact on freshly harvested table stock tubers, tubers in storage and seed tubers is potentially devastating (Colon et al. 1995; Johnson et al. 1997; Kirk et al. 1999, 2001c). The current study was undertaken to compare the efficiency of different inoculation techniques to cause tuber late blight.

Different inoculation techniques previously have been used to test variables such as cultivar resistance and effect of temperature and fungicides in relation to a range cultivars and genotypes of *P. infestans* (Lacey 1967; Inglis et al. 1999; Johnson and Cummings 2009). Potential advantages (such as avoidance of secondary infection) of one inoculation technique over another such as internal versus external inoculation, each has limitations. For instance, wounding of periderm to inoculate may alter the resistance reaction to *P. infestans* (Davila 1964; Peters 1999). Periderm acts as a physical barrier to pathogens (Pathak and Clark 1987; Lulai and Orr 1994; Lulai and Corsini 1998) and also contains significant amount of pathogen inhibitory compounds such as phenols (Farkas and Kiraly 1962) and steroid alkaloids (Allen and Kuc 1968).

Potato cultivars/advanced breeding lines (ABLs) should be tested with the new and old genotypes of *P. infestans* but effect of different inoculation technique on level of late blight severity has not been studied. A comparison of five different inoculation techniques across three different cultivars of potato and four genotypes of *P. infestans* was thus justified to evaluate the inoculation techniques to determine if differences in tuber blight development could be determined between the techniques.

The main focus of the study was to evaluate the efficiency of different inoculation techniques in terms of disease severity using internal inoculation (wounding or injection through the periderm) and external inoculation (non-wounding of periderm). Inoculation of tubers with the direct injection (DI) and agar plug (AP) methods involved the wounding or bypassing the periderm and were focused to see variation in tuber blight severity compared to external or non-invasive inoculation techniques. Each cultivar used in the study was reported by breeders or pathologists to be susceptible to *P. infestans* (Johansen et al. 1988; Bradeen et al. 2009; Kirk et al. 2009).

Potato periderm acts as a physical barrier to several pathogens (Pathak and Clark 1987; Lulai and Orr 1994; Lulai and Corsini 1998) including *P. infestans* (Pathak and Clarke 1987; Flier et al. 1998; Flier et al. 2001). In addition, potato periderm also contains antimicrobial compounds which also play role in defense against *P. infestans* such as phenols and steroid alkaloids (Farkas and Kiraly 1962; Allen and Kuc 1968). Inoculation of tubers without wounding of the periderm essentially focused on the resistance response of periderm but may have been confounded by simple differences in tuber maturity (Lacey 1967; Lapwood 1977; Bjor 1987; Grinberger et al. 1995). Tuber maturity is difficult to standardize and variation in response was somewhat expected in the tubers with different physiological ages (Bjor 1987). In our study the tubers used in 2013 were 3-4 weeks older than the tubers used in 2012, so there was slight variation in tuber susceptibility between these years.

A range of disease severity was found between different experiments of the trials. In 2012 experiments A and B, the cultivars used were from the same growing season and the inoculum of *P. infestans* was freshly isolated from potato and tomato, supporting that less variation was observed within repetitions of experiments when there was minimal variation

between tubers and inoculum used. It has been reported that *P. infestans* tends to lose virulence if continuously sub-cultured on artificial media (Hodgson and Sharma 1967).

Despite the variation in disease severity between 2012 and 2013 experiments, the internal inoculation techniques (DI and AP) had significantly higher tuber blight severity compared to the external inoculation techniques (FP, SM and SP) in both years. Although the periderm is a natural barrier (Lulai and Orr 1994), breeders may be more interested in the resistance of medullar tissue because this component is marked by reduced hyphal growth and sporulation of *P. infestans* (Pathak and Clarke 1987; Flier et al. 2001) and this can be evaluated precisely using internal inoculation methods. There was slight variation in results between the two years, in the DI and AP techniques. These techniques were consistently produced more severe tuber blight results than the three other techniques tested. The variation in the results may have been due to the virulence of the isolates of *P. infestans* (Hodgson and Sharma 1967) as freshly isolated isolates were used in 2012 and repeatedly (two or three times) sub-cultured isolates were used in 2013.

Johnson and Cummings (2009) used a filter-paper technique to study the latent infection of potato seed tubers by *P. infestans* during long-term cold storage. The limitation of inoculation techniques such as the external placement of inoculum (non-wounding) as in the study by Johnson and Cummings might argue for the internal inoculation technique. Thus, alternative inoculation (external inoculation) techniques need further evaluation. Although contradictory, there is also a drawback of the internal inoculation methods in that there is increased potential for secondary infection by pectolytic bacteria and *Fusarium* spp. after inoculation with *P. infestans* (Kadish et al. 1992; Gigot 2009) and this should not be overlooked because *P. infestans* is a weak competitor against these pathogens (Kadish 1992; de Boer 2008). Such secondary infection

events may be avoided by employing strict sterile technique and disinfestation of tubers. However, in our study the incidence of dry rot and soft rot were not observed.

Among the three external inoculation techniques tested significantly higher disease severity was observed using the SM method in 2013 followed by the FP and SP methods. The results using the FP method were different from the result of Johnson and Cumming's (2009) study where higher disease incidence and severity were reported compared to our result in different cultivars inoculated with different genotypes of *P. infestans*. However, as reported by Granke et al. (2012) in *Phytophthora capsici* the cultivars, strain of pathogen, environmental conditions in the chamber and storage period may have affected the results. Using the FP method, the pathogen was limited to entry through a single eye on the tuber. Although late blight did develop in these tubers, inoculation of a single eye limited the degree of disease development in the tuber within the incubation period because this method had only one site for pathogen entry. Had the incubation period been extended it is likely the tuber tissue would have been extensively colonized (Gigot et al. 2009), which is true for all the external inoculation techniques. In this experiment, the apical bud was inoculated. However, eyes are not uniformly susceptible over the tuber (Lacey 1967) and buds in the center of the tuber (in the mid-region) are reported to be more susceptible than other buds on the tubers (Lacey 1967).

In many studies the SP method has been documented as effective method to inoculate the potato tubers (Lacy 1967; Adams 1975b; Dorrance and Inglis 1998) but it did not give as high disease severity as the internal inoculation methods used in the current study. Differences in results between past and current studies may be impacted by the use of tubers at even marginally different maturity and growth stages (Lacey 1967). Environmental conditions or the incubation facilities may also impact the tuber infection severity (Parker et al. 1992; Stewart et al. 1994) as

the moisture conditions maintained at the site of inoculation in mist chambers and microenvironments imposed by moistened paper towels or soaked filter paper may differ, where mist chamber maintained humidity might not be the same as with wet towels used in different studies. Tubers inoculated with the SP method exploited all the lenticels and eyes (Nyankanga et al. 2008) that may have contributed to disease development over the incubation period. Though lenticels were numerous and occupy more of the surface area of tuber than eyes at an average 1 to 3 per cm^2 (Burton 1989), infection was significantly lower compared to the DI, AP and SM inoculation methods. Lenticels in tubers are documented to suberize during maturation and develop a layer of cork meristem and became more resistant to pathogen penetration with age (Lacey 1967; Adams 1975b). This cork meristem layer, limits the chances of *P. infestans* infection through lenticels in mature tubers. Although, the lenticels might have suberized, eyes were available as a site of entry for *P. infestans*.

Among the external inoculation methods, the SM method ensured all the lenticels and eyes of tubers were exposed to the sporangia/zoosporangia continuously for 24 h. Therefore, there was a greater potential for sporangia/zoosporangia to infect through the eyes of tubers than in the FP method. A disadvantage of the extended exposure of tubers to an inoculum suspension is that it creates an anaerobic condition which is suitable for pectolytic bacterial infection and also reduce defensive capacity of *P. infestans* (Perombelon et al. 1975), this agreed with the results of a preliminary trial to assess the optimum period for inoculation using SM. Bacteria rotted most of the tubers within 3 weeks after soaking them for 24 to 48 h (unpublished).

Variation between internal and external inoculation techniques can be attributed to more than one factor. For instance, tubers can remain dormant for up to 6 to 7 months when stored at 3°C (Davidson 1958) and periderm and lenticels of tubers appear to be more resistant with time

in storage (Lacey 1967; Adams 1975b). Conversely, the resistance level of both cortical and medullar tissues tends to decline with tuber storage (Bhatia and Young 1985; Pathak and Clark 1987). The variation in resistance even in the same tuber with age might be due to accumulation of reducing and non-reducing sugars as well as stress-related metabolites (Bhatia and Young 1985). The variable resistance might be one of the reasons for low disease severity in all external inoculation methods compared to internal inoculation methods in 2013. Direct wounding increases disease severity by avoiding the intact periderm and brings the pathogen in direct contact with the underlying tuber tissue (Bjor 1987). Inoculation of wounded tubers ensured the presence of pathogen in the medullar tissue because the outer layer of cortex was reported to be very resistant to colonization by *P. infestans* in some potato cultivars (Pathak and Clarke 1987). In the external inoculation techniques tested, the pathogen needed to overcome the physical barrier as provided by periderm (Pathak and Clarke 1987; Lulai and Orr 1994) and chemical barriers (Lulai and Corsini 1998) that might be in the periderm as well as in the internal tissues.

All four genotypes of *P. infestans* used in the study were recently found in the US (Hu et al. 2012). The genotype US-8 has been previously reported to be more aggressive compared to US-22, US-23 and US-24 (Kirk et al. 2009; Danies et al. 2013; Rojas et al. 2014). However, in this study US-22 was found to be more aggressive in terms of disease severity than US-8. Rojas et al. (2014) reported that US-22 was slightly less aggressive compared to US-8 in potato but more aggressive in tomato. The variation in the aggressiveness of these genotypes in this study was unknown.

Variation in degree of susceptibility on the cultivars was noted depending upon the inoculation techniques with different genotypes. In the current study, the disease severity on cultivars varied with the SM and AP methods across aggressive genotypes (US-8 and US-22),

and the DI method across all genotypes at least in one year. Granke et al. (2012) reported that isolates of *Phytophthora capsici* (n=13692) vary in pathogenicity from non-pathogenic to virulent due to differences in storage conditions, inoculation techniques, environmental conditions, or host type.

This research agreed with previous studies that different inoculation techniques could be used to evaluate the degree of tuber susceptibility to *P. infestans*. In conclusion, the DI and AP methods were more effective than other methods in terms of disease severity and can be used to evaluate the susceptibility of tuber medullar tissue to *P. infestans*. The SM method was an excellent technique to evaluate the resistance levels of periderm and of lenticels of tubers as all are potentially exposed to inoculum (Gachango et al. 2012). More than a single eye could be inoculated to increase the efficiency of inoculation with the FP method. Inoculating multiple eyes increased the disease severity compared to single eye inoculation (unpublished). The SP method could be used to inoculate immature tubers when the lenticels are still "open" compared to "closed" lenticels on mature tubers (Tyner et al. 1997). In future, research could be conducted to see the interaction of tuber resistance of different advanced breeding lines/transgenic lines to the newly emerged and highly aggressive genotypes of *P. infestans* using both internal and external methods of inoculation depending on tuber maturity. Different tuber maturity level of breeding lines should be tested to assess whether the resistance was by the periderm or the medullar tissues because the resistance level of either periderm or medulla tissue tends to change over time. Internal or external inoculation technique might under or over estimate the resistance level of cultivars when periderm or medulla tissue is excluded in the assay (Nyankanga et al. 2008).

The results of this research can be employed to establish standardized protocols for testing the different variables such as testing breeding lines or fungicide treatments in storage. For instance, the AP and DI methods could be used for testing breeding lines in high throughput screening using multiple genotypes of *P. infestans*. The SM method is ideal for testing the efficacy of fungicides for tuber protection in storage (Gachango et al. 2012).

CHAPTER 3: SUSCEPTIBILITY OF IMMATURE AND MATURE POTATO TUBERS TO DIFFERENT GENOTYPES OF *PHYTOPHTHORA INFESTANS*

ABSTRACT

Mature potato tuber periderm is a significant morphological barrier that reduces infection by various pathogens. However immature periderm of potato tubers may be susceptible to skinning injury and consequently vulnerable to infection. The importance of the maturity of tuber periderm to prevention of infection by isolates of four genotypes of *Phytophthora infestans* (US-8, US-22, US-23 and US-24) was evaluated using three cultivars of potato [Snowden, Russet Norkotah (RN) and Dark Red Norland (DRN)] harvested at two different tuber maturity stages. Resistance of the periderm to physical injury was determined using a skin-set measuring device (Halderson Periderm Shear Tester). The development of the periderm was further evaluated utilizing histochemical techniques. Sample tubers were immersed in suspensions of *P. infestans* (sporangia and zoospores) for 24 h to determine the susceptibility of potato tubers to infection at the two different maturity stages. Immature and mature RN required the highest torque ($p \leq 0.05$) to remove the periderm (273.5 and 450.7 mN m respectively in 2012; and 298.3 and 398.7 mN m in 2013) in comparison to Snowden (208.0 and 316.4 mN m in 2012; and 235.5 and 299.5 mN m in 2013) and DRN (223.3 and 304.1 mN m in 2012; and 212.0 and 296.1 mN m in 2013). Immature tubers compared to mature tubers of all three cultivars were more susceptible ($p \leq 0.05$) to infection in both years. Immature RN and DRN in 2012 and immature DRN in 2013 were the most susceptible cultivars to infection by *P. infestans* in terms of disease severity of potato tuber. Isolates of *P. infestans* genotypes US-22 and US-8 caused more tuber rot, and thus were more aggressive isolates compared to US-23 and US-24. These results agreed with previous studies that immature potato tubers were more susceptible to genotypes of *P. infestans* than

mature potato tubers. Isolates of more recently found genotypes of *P. infestans* still impose a threat in potato production regardless of their aggressiveness or the maturity of the potato tuber. The findings of this study were in agreement with the practice of vine desiccation to minimize infection by *P. infestans* during harvest.

3.1 INTRODUCTION

Potato periderm is a significant barrier to the loss of water and solutes from internal tissue (Lulai and Orr 1994) as well as infection by various pathogens (Lacey 1967; Lulai and Corsini 1998). Bacterial and fungal pathogens can infect the potato tuber through the wounded periderm and a competent native periderm reduces infection (Lulai 2001). *P. infestans* cannot infect through the intact periderm of matured tubers (Lacey 1967) although the lenticels and eyes (meristematic areas of tubers) of even mature tubers may be breached (Davila 1964; Lacey 1967; Kirk and Wharton 2014; Rojas et al. 2014). The susceptibility of lenticels to penetration by mycelium of *P. infestans* appears to be maximal when tubers are immature before lenticel suberization (Lacey 1967; Tyner et al. 1997). Lenticels become more resistant to the pathogen with maturity (Lacey 1967; Adams 1975a, b). The resistance of the potato tuber against pathogens such as *P. infestans* is the result of different components present in tubers including those in the medulla, periderm, outer cortical cells, lenticels, or eyes individually or together as complexes (Pathak and Clarke 1987; Lulai and Orr 1994; Flier et al. 1998; Lulai and Corsini 1998; Flier et al. 2001; Flier et al. 2007; Nyankanga et al. 2008; Olanya et al. 2009; Nyankanga et al. 2011).

Excoriation of potato periderm is a "skinning injury resulting from fracture of the radial phellogen cell wall linking the phellem (skin) to the phelloderm" (Lulai and Freeman 2001). Excoriation and bruising of tubers during harvest make them vulnerable to dehydration (Lulai

and Corsini 1998) and disease later in storage (Lulai and Orr 1995; Sabba et al. 2007). Wounded potatoes contribute to storage loss due to shrinkage and rot which can exceed 1.3 million t/year in the US (Agricultural Statistic Board 2004). Mature tubers with good skin set contribute to improved storability (Sabba et al. 2007) as immature tubers are more susceptible to excoriation than mature tubers (Lulai and Freeman 2001). Upon periderm maturation, the phellogen becomes inactive resulting in the development of resistant radial cell walls that strengthen the periderm against excoriation (Lulai and Freeman 2001). The mature tuber has a higher level of resistance to physical bruising, which provides protection against mechanical injuries during harvesting, handling and in storage (Lulai and Freeman 2001; Bussan et al. 2009).

"Skin-set" is a term used in potato production that describes the resistance of the periderm against excoriation (Lulai 2007; Sabba et al. 2007). Tubers with "improved skin-set" prevent the entry of pathogens through the periderm (Lulai 2007) limiting the chances of infection to entry through natural openings like eyes and lenticels (Lacey 1967). It is important to maximize skin-set in the field prior to harvest in order to obtain the "best skin finish out of store" (Adams 1975a, b). This is commonly achieved by practicing vine desiccation before harvesting the crop (Halderson and Henning 1993; Lulai and Orr 1993; Kempenaar and Struik 2007). Soil types that minimize abrasion such as those with high water holding capacity and low sand content are optimal for skin-set (Sabba and Bussan 2012). However, there is limited information on optimal soil types for good skin finish and potato crops are often grown in sandy soils that may promote abrasion (Sabba and Bussan 2012). The practice of vine desiccation has become widely accepted for many reasons including prevention of oversized tubers, limitation of spread of potato virus, late blight management and facilitating vine-free harvesting, but most

importantly to hasten the development of the periderm of potato tubers (Murphy 1968; Haderlie et al. 1989).

In the current study, the maturity and susceptibility of the periderm in three cultivars of potato harvested at two different maturity stages was assessed against isolates of four different genotypes of *P. infestans*. The cultivars selected in the study were Snowden (chipping cultivar), Russet Norkotah [(RN), processing cultivar, usually frozen], and Dark Red Norland [(DRN) table stock, Johansen et al. 1959; Johansen et al. 1988; Peloquin et al. 1994; Douches et al. 1996)]. All three cultivars selected have different skin characteristics such as thickness, color, and skin smoothness (Johansen et al. 1959; Johansen et al. 1988; Peloquin et al. 1994). Tubers of all three cultivars used in the study were reported by breeders or pathologists as susceptible to late blight (Johansen et al. 1988; Bradeen et al. 2009; Kirk et al. 2009).

Since 2009 there have been significant genetic changes in the population of *Phytophthora infestans* in the eastern US with the appearance of new isolates with unique genotypes and epidemiological characteristics (Hu et al. 2012). These new genotypes differ in terms of mating type, to the fungicide metalaxyl and aggressiveness (Danies et al. 2013; Rojas et al. 2014). There is lack of understanding whether these genotypes differ in aggressiveness in relation to the maturity stage of tuber of these three commercial cultivars of potato commonly grown in Michigan. Therefore, the purpose of this study was to evaluate the interaction between the infection processes of recently found genotypes of *P. infestans* and the periderm of three different cultivars of potato differing in skin-set as influenced by harvest date. The specific objectives were:

a) Evaluate the skin-set and periderm development of potato tubers of three commercial cultivars harvested at two different maturity stages; immature (on the day of vine desiccation) and mature (14 days after vine desiccation).

b) Evaluate the response of tubers of three commercial cultivars at two different maturity stages to isolates of varying genotypes of *Phytophthora infestans*.

3.2 MATERIALS AND METHODS

3.2.1 FIELD PREPARATION, PLANTING, AND MAINTENANCE

Potato cultivars 'Snowden', 'Russet Norkotah' (RN) and 'Dark Red Norland' (DRN) obtained from the Michigan State University breeding program (Dr. Dave Douches) were selected for the study. To evaluate the effect of tuber maturity stage on periderm development, resistance to excoriation, and response to late blight infection, seed tubers were planted at the Clarksville Research Center, Michigan State University, Clarksville, MI (Capac loam soil; 42.8733, -85.2604 deg; elevation 272.8 m) on Jun 13 and 6 in 2012 and 2013, respectively. Two-row, 6 m long plots (ca. 25 cm between plants to give a target population of 50 plants at 86.4 cm row spacing) were used. The two-row beds were separated by a 1.5 m unplanted row. Fertilizer was drilled into plots before planting, formulated according to results of soil tests.

Additional nitrogen (final N 31.4 kg/ha) was applied to the growing crop with irrigation 45 days after planting (DAP) for a total of 336 kg N/ha. Weeds were controlled by cultivation, hilling, and with S-metolachlor (Dual II Magnum; Syngenta Crop Protection, NC) at 2.24 L/ha 10 DAP and sethoxydim (Poast; BASF Corporation, TX) at 1.75 L/ha 58 DAP. Insects were controlled with imidacloprid (Admire Pro 2F: Bayer CropScience, TX) at 3.4 mL/100 row-m at-planting, and two applications of beta-cyfluthrin (Baythroid XL; Bayer CropScience, TX) 116.9

mL/ha at 60 and 90 DAP or as needed based on commercial potato pest recommendations. All sprays were applied with a tractor mounted spray boom (R&D Sprayers, Opelousas, LA) delivering 233.8 L/ha (551.6 kPa) and using three XR11003VS nozzles per row. Plots were irrigated to supplement precipitation to about 0.63 cm/ha/4 d period with overhead irrigation.

Meteorological variables were measured with a weather station (Campbell Scientific Inc., Logan UT) located at the farm from Jun 13 or 6 to final harvest Sep 25 or 18 in 2012 or 2013, respectively. Meteorological data were provided by the Michigan Automated Weather Network (MAWN, Enviroweather, Clarksville, MI).

3.2.2 EVALUATION OF SKIN-SET

Periderm maturity was described in terms of periderm resistance to bruising/skinning (Pavlista 2002) and was measured with a Halderson periderm shear tester (Snap On Tools, Kansas City, KS) initially developed by Halderson and Henning (1993) as modified by Lulai and Orr (1993). The protocol kindly provided by Lulai (pers. comm.) was followed to measure skin-set. The tubers were harvested manually to reduce wounding or bruising on the same day of the skin-set measurement. Immediately after tubers were harvested, they were transferred to paper bags (Sysco Corporation, Houston, TX) and stored in 35 x 22 x 20 cm^3 plastic boxes (Akro N.S.T, Akro-Mils, Akron, Ohio). On the same day tubers were taken to the lab and washed under running distilled water to remove soil from the tuber surface. Tubers were air-dried at 18°C for 2 hours in the controlled environment chamber before measurements were taken.

The basic steps for the skin set determination were similar to those described by Pavlista (2002) and protocol provided by Lulai (pers. comm). A rubber tip (1.5 cm^2) of the skin-set testing device was glued to 320-grit wetable sandpaper (Taber Industries, N. Tonawanda, NY) to increase the grip on the native periderm. Tubers were placed on a flat bench and supported with

one hand when the skin-set testing device was placed on top of the three areas at approximately 25% (apical), 50% (middle) and 75% (basal) distal from the apical end of each of the tubers tested. The device was pressed downward on an electronic pan balance until pressure was equal to 11.3 kg mass on top of an area of 1.5cm^2 of the tuber periderm, and the point was marked to calibrate the device. The marked point was the guide to apply pressure consistently to all the test tubers. Three readings were recorded and averaged from each tuber. Skin-set was measured from tubers harvested on the day of vine desiccation and 14 days after vine desiccation [90 days after planting (DAP) and 105 DAP, respectively].

3.2.3 LATE BLIGHT SUSCEPTIBILITY OF DIFFERENT CULTIVARS AT TWO MATURITY STAGES

Tubers of all three cultivars used in the study were reported by breeders or pathologists as susceptible to late blight (Johansen et al. 1988; Bradeen et al. 2009; Kirk et al. 2009). The immature and mature tubers were harvested either on the same day or 14 d after vine desiccation (90 DAP and 105 DAP, respectively). Tubers were cleaned under running distilled water and disinfested in 0.6% sodium hypochlorite (Clorox) for 5 min. After disinfestation, tubers were washed twice with sterile distilled water and air dried for 2 h at 18°C in controlled environment chamber (Environmental Growth Chambers, Chagrin Falls, Ohio).

The four isolates of each genotypes of *P. infestans* were prepared on rye B agar (Caten and Jinks 1967) amended with antibiotics (20mg/L rifampicin, TCI America, Portland, OR; 100 mg/L ampicillin, EMD Chemicals, Inc. San Diego, CA and 20mg/L nystatin, Sigma-Aldrich Corporation, St. Louis, MO) to control bacterial contamination. The culture plates were kept in the dark for 14 d at 18°C. Plates were flooded with 50 ml of sterile distilled water and the sporangia were dislodged from the mycelia by gently scrapping using a sterile L-shaped glass

rod. The concentration of the sporangial suspension was estimated using a Makler counting chamber. The initial concentration was adjusted to approximately 2 x 10^5 sporangia ml^{-1} by adding sterile distilled water. The suspension was placed at 10°C for 2 hours to release zoospores (Judelson and Roberts 2002). Tubers were inoculated by immersing in the *P. infestans* inoculum for 24 h. The control tubers were immersed in sterile distilled water for 24 h. Soaking of tubers was adapted from results of an optimization experiment to determine the period of time it took for infection to take place when tubers were soaked in an inoculum solution with or without prior wounding (unpublished).

After 24 h tubers were removed from the inoculum and placed in plastic boxes lined with two layers of moist paper towel (Wypall, all purpose blue; Kimberly-Clark Professional, Roswell, GA) prepared using sterile distilled water (50ml per paper towel) and incubated in a growth chamber (Environmental Growth Chambers, Chagrin Falls, Ohio) at 15°C and 90% relative humidity for 30 d in the dark. After 30 days tubers were cut in 3 serial sections approximately at apical, middle and basal of the length of the tuber from the apical end and the cut surface was scanned using a flatbed scanner (HP ScanJet 4c, Hewlett-Packard Co., Houston, TX.

The image files created with the scanner were loaded into image analysis software (SigmaScan ver. 3.0, Jandel Scientific, San Rafael, CA). The area selection cut-off threshold was set to 10 light intensity units (LIU), allowing the software to exclude all parts of the image darker than 10 LIU, e.g. the black background. The average reflective intensity (ARI) of all the pixels within the image gave a measurement of severity of the disease in the tuber tissue of each sample (Niemira et al. 1999; Kirk et al. 2001a).

The ARI was measured in three sections. The amount of late blight symptomatic tissue per tuber was expressed as a single value (Mean ARI) calculated as the average ARI of three sections.

The Relative ARI [RARI (%)] was calculated as follows:

$$\text{RARI (\%)} = \left(1 - \frac{\text{mean ARI treatment}}{\text{mean ARI control}}\right) \text{X } 100\%$$

RARI (%) has a minimum value of 0% (no symptoms) and a maximum value of 100% (black tuber surface).

3.2.4 DATA ANALYSIS

Data for all experiments were analyzed using the statistical analysis software package JMP (version 10 SAS Institute Inc., Cary, NC). Data were subjected to analysis of variance (ANOVA), and a mean separation test was conducted using Least Square Means Tukey's Honestly Significant Difference at $p \leq 0.05$. Treatment effects were determined by a three-way factorial ANOVA, where the main effects corresponded to cultivars, genotypes of *Phytophthora infestans* and maturity stages.

3.3 RESULTS

3.3.1 EVALUATION OF SKIN-SET

Susceptibility of the cultivars to excoriation was significantly different for tubers harvested at the two maturity stages over the two years of the experiment, so results were analyzed separately (Table 3.1). The results from both years were similar in that mature tubers (harvested 14 d after vine desiccation) required significantly higher torque to excoriate the periderm compared to immature tubers (harvested on the day of vine desiccation, Fig 3.1). Mature and immature RN required the highest torque ($p \leq 0.05$) to excoriate the skin compared

to Snowden and DRN in both years (Fig 3.1). Immature DRN required higher torque compared to Snowden in 2012, however the result was reversed in 2013 (Fig 3.1). There were no significant differences between mature Snowden and DRN in either year (Fig 3.1).

Table 3.1: Main effects analyses on torque required with a Halderson periderm shear tester to excoriate the periderm of three different cultivars of potato (Snowden, Russet Norkotah and Dark Red Norland) harvested at two maturity stages (on the same day or 14 days after vine desiccation) and interactions between the sources of variation

Source of Variation	*p* value	
	Torque required to excoriate the periderm[a]	
	2012	2013
Maturity stage	<0.0001	<0.0001
Cultivar	<0.0001	<0.0001
Maturity stage * Cultivar	0.0496	<0.0001

[a] Torsional force [milliNewton meters (mN m)] required to excoriate the periderm of the tuber

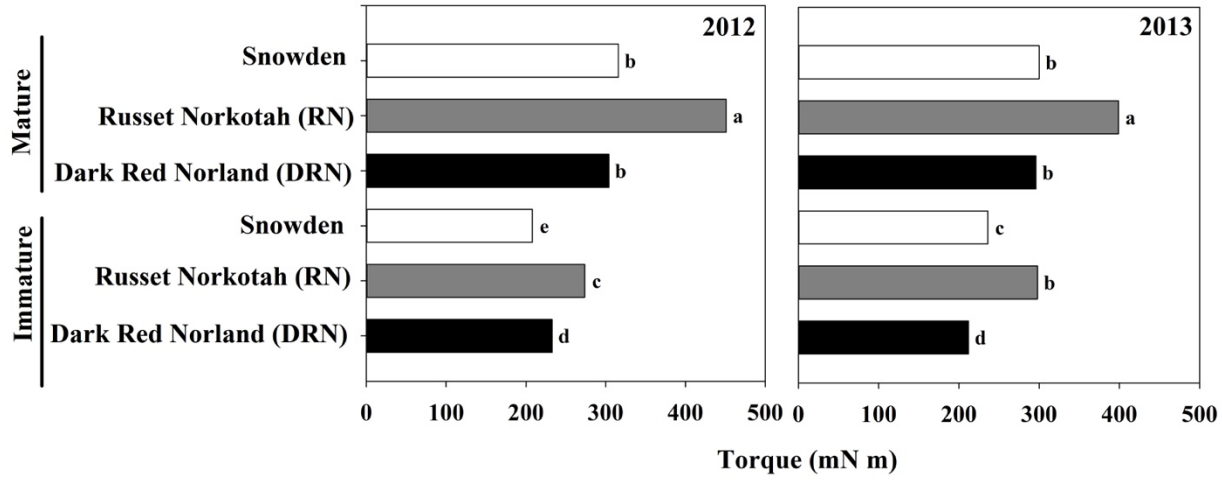

Figure 3.1: Response of three different cultivars of potato (Snowden, Russet Norkotah and Dark Red Norland) at two maturity stages (harvested on the same day or 14 days after vine desiccation) for periderm excoriation by a Halderson Periderm shear tester in 2012 and 2013. Values followed by the same letters in each year are not significantly different at $p \leq 0.05$ (Least Square Means Tukey's Honestly Significant Difference). [Each bar is a mean of torque from 20 tubers].

3.3.2 LATE BLIGHT SUSCEPTIBILITY OF TUBERS OF THREE POTATO CULTIVARS AT TWO MATURITY STAGES

Weather was conducive for normal potato production in both years and there was a wet period before harvest in 2013. Average daily air temperature, average daily relative humidity (%), average daily soil temperature at a 10 cm depth and average daily soil moisture at a 10 cm depth (% of field capacity) for each month and precipitation over the same period is shown in appendix C.

The range in susceptibility of the two different maturity stage tubers of three potato cultivars to isolates of four different genotypes of *Phytophthora infestans* was significantly different over the two experiments in 2012 and 2013 (Tables 3.2), thus experiments were analyzed separately. Tubers representative of the two maturity stages inoculated with isolates of four genotypes of *P. infestans* and stored at 15°C in the dark resulted in significant tuber late blight symptoms 30 days after inoculation (DAI, Table 3.3). Late blight did not develop in not-inoculated control tubers of any cultivar. Therefore these data were not included in the statistical analyses but were used to calculate RARI (%). Significantly greater RARI (%) for the different cultivars were observed in the 2013 experiment compared with the 2012 experiment except in mature tubers of RN inoculated with isolates of the US-22 genotype of *P. infestans* and immature tubers of Snowden inoculated with isolates of the US-23 genotype. The results from both years were similar in that immature tubers had higher late blight severity measured as RARI (%) than mature tubers (Table 3.3).

Among the immature tubers, RN and DRN had the higher RARI (%) than Snowden ($p \leq 0.05$). Mature tubers of DRN had the highest RARI (%). Mature tubers of Snowden and RN were not significantly different from each other in terms of tuber blight severity. Significant

differences between the two maturity stage tubers in the three cultivars in relation to the effect of isolates of four genotypes of *P. infestans* are detailed in Table 3.3.

Table 3.2: Main effect analyses of two maturity stages of potato tubers (harvested on the same day or 14 days after vine desiccation), four genotypes of *Phytophthora infestans* (US-8, US-22, US-23 and US-24) and three potato cultivars (Snowden, Russet Norkotah and Dark Red Norland) and interactions between these variables on late blight severity on tubers stored at 15°C for 30 d in the dark in 2012 and 2013 experiments

Source of variation	*p-value* RARI %[a]
Year	<.0001
Maturity Stage	<.0001
Cultivar	<.0001
Genotype of *P. infestans*	<.0001
Year*Maturity Stage	<.0001
Year*Cultivar	<.0001
Year*Genotypes of *P. infestans*	<.0001
Maturity Stage*Cultivar	<.0001
Maturity Stage*Genotypes of *P. infestans*	<.0001
Cultivar*Genotypes of *P. infestans*	<.0001
Year*Maturity Stage*Cultivar	<.0001
Year*Maturity Stage*Genotypes of *P. infestans*	<.0001
Year*Cultivar*Genotypes of *P. infestans*	<.0001
Maturity Stage*Cultivar*Genotypes of *P. infestans*	<.0001
Year*Maturity Stage*Cultivar*Genotypes of *P. infestans*	<.0001

[a] Disease severity measured in terms of tuber tissue darkening; score expressed as % Relative Average Reflective Intensity [RARI (%)]; RARI % = [1-(Mean $ARI_{treatment}$ / Mean $ARI_{control}$)] * 100 %; RARI (%) has a minimum value of 0% (no darkening) and maximum value of 100% (cut tuber surface blackened)

Table 3.3: Effect of two maturity stages (harvested on the same day or 14 days after vine desiccation) and three potato cultivars (Snowden, Russet Norkotah or Dark Red Norland) on late blight severity [measured as Relative Average Reflective Intensity {RARI (%)}] in tubers stored at 18°C and 90% relative humidity for 30 d in the dark after inoculation with isolates of one of four genotypes of *Phytophthora infestans* (US-8, US-22, US-23 or US-24)

		RARI (%)[a] Genotypes of *Phytophthora infestans*[b]							
Year	Cultivar	US-8		US-22		US-23		US-24	
2012									
Immature[c]	Snowden	7.3	a[d]	8.5	c	10.9	b	7.8	b
	Russet Norkotah	8.2	a	13.7	a	7.3	c	9.8	a
	Dark Red Norland	7.8	a	10.7	b	12.3	a	8.4	b
Mature	Snowden	2.3	b	4.1	d	1.8	d	2.0	d
	Russet Norkotah	1.6	b	13.3	a	2.4	d	4.0	c
	Dark Red Norland	1.8	b	2.0	e	1.5	d	2.7	d
2013									
Immature	Snowden	20.3	b	14.2	c	1.0	c	8.4	cd
	Russet Norkotah	31.2	a	31.0	a	12.8	b	15.1	a
	Dark Red Norland	31.1	a	19.6	b	18.3	a	13.5	ab
Mature	Snowden	3.4	d	3.8	d	2.2	c	3.6	e
	Russet Norkotah	3.0	d	1.6	d	3.3	c	10.0	bc
	Dark Red Norland	10.1	c	11.2	c	3.7	c	4.7	de

[a] Disease severity measured in terms of tuber tissue darkening; score expressed as % Relative Average Reflective Intensity [RARI (%)] = [1-Mean $ARI_{treatment}$ / Mean $ARI_{control}$] * 100; RARI (%) has a minimum value of 0% (no darkening) and maximum value of 100% (cut tuber surface blackened). The numbers are derived from the mean average reflective intensity of three surfaces of inoculated tubers cut latitudinally at 25, 50 and 75% distal from the apical end (n=20 tubers per maturity stage per cultivar per genotype of *Phytophthora infestans*)

[b] Different genotypes of *Phytophthora infestans* classified according to Goodwin et al. (1995) and Hu et al. (2012)

[c] "Immature" tubers harvested on the day of vine desiccation; "mature" tubers harvested 14 days after vine desiccation

[d] Values followed by the same letters are not significantly different at $p \leq 0.05$ by Least Square Means Tukey's Honestly Significant Difference

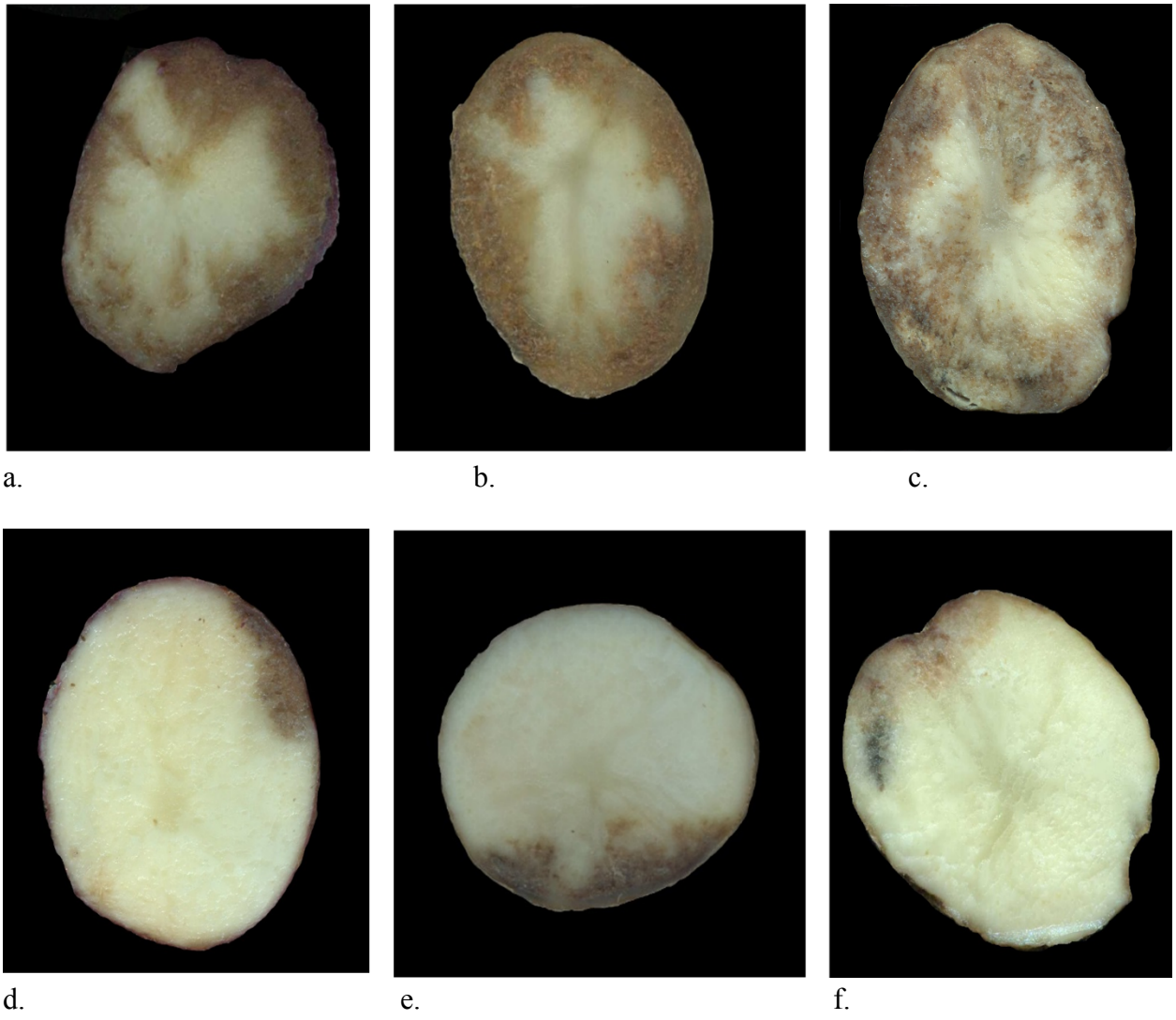

Figure 3.2*:* Tubers at two different maturity stages [top row are immature (on the day of vine desiccation) and bottom row mature (14 days after vine desiccation)] were inoculated with an isolate of the US-22 genotype of *Phytophthora infestans* using immersion of tubers in an inoculum suspension of sporangia and zoospores at 2 x 10^5 sporangia/ml. Cultivars a. and d. = Dark Red Norland [Disease severity measured in terms of relative average reflective intensity {RARI (%)} 20.2 and 3.4], b. and e. = Russet Norkotah [RARI (%) 28.0 and 5.3] an c. and f. = Snowden [RARI (%) 27.6 and 4.2, left to right]. All the sections are 25% distal from the apical end. All images were recorded 30 days after inoculation.

3.4 DISCUSSION:

The skin-set measurements obtained from immature (harvested on the day of vine desiccation) and mature tubers (harvested 14 d after vine desiccation) in each of the two years agreed with earlier reports that mature tubers had significantly increased periderm maturation compared to immature tubers. For example, Lulai (1993) reported that "skin-set" was improved as periderm matured after vine desiccation. Thus, the vine desiccation before harvest improves the skin set and minimizes the skin bruising and excoriation.

A range of periderm resistance in terms of skin-set across the three cultivars tested, was found between the two experiments necessitated a split analysis. As discussed by Lulai (1993), variation in periderm strength of varying cultivars at different maturity stages was consistently observed in this experiment. The "immature" and "mature" periderm of cultivar RN required significantly higher torque to excoriate in both years compared to the periderm of cultivars DRN and Snowden. The torque values required to excoriate the periderm in mature tubers of RN, DRN and Snowden were higher than 400 mN m, 300 mN m and 300 mN m, respectively in both years. Pavlista (2002) reported that Snowden harvested after 2 weeks had torque values below 300 mN m and were not ready for harvesting. In another study by Lulai and Orr (1993), cultivars Norchip and Russet Burbank required more than 450 and 550 mN m torque to excoriate the periderm in tubers harvested 14 days after vine desiccation and the torque increased slightly when the tubers were tested 21 days after vine desiccation. Basically the findings of the current study are in agreement with these studies in that skin-set was improved when harvested after 2-3 weeks of vine desiccation. The slight differences in the results between the current study and these studies may have been due to the cultivars, soil and environmental conditions which can impact periderm maturation (Lulai and Orr 1993; Sabba and Bussan 2012). In the current study

the mature tubers of the DRN and Snowden cultivars were not significantly different in terms of torque required to excoriate the periderm. However the torque required to excoriate the immature periderm of cultivars DRN and Snowden were significantly different and the reason behind this difference between mature and immature tubers was unclear. One of the possible reason for the lower torque required to excoriate the periderm of DRN compared to Snowden may be the red skinned cultivars develop weaker skin-set compared to russet cultivars (Lulai and Orr 1993). In 2012, DRN required higher torque than Snowden but in 2013 lower torque was required to excoriate the periderm. Overall, the torque required to excoriate the periderm of mature tubers of all cultivars was lower in 2013. Periderm strength may have been decreased due to the differing soil moisture levels in 2012 and 2013. A heavy rainfall occurred one week before harvesting of mature tubers in 2013. High soil moisture has been reported to increase the periderm thickness in some cultivars (Tyner et al. 1997), however high relative humidity has been reported to have a negative effect on skin-set on Norchip and Norland cultivars (Lulai and Orr 1993).

Immature periderm has a meristematically active phellogen layer which becomes inactive in mature periderm (Lulai and Freeman 2001). It has been reported that the susceptibility to excoriation and bruising of the periderm of potato tubers is merely due to the separation of phellem from the phellogen layer rather than the thickness of the periderm (Lulai and Freeman 2001). However, the thickness of the periderm within the cultivar indicates the state of periderm maturity. As the periderm reaches a critical thickness, the cell walls of the phellogen thicken and strengthen, resulting in resistance to excoriation (Lulai and Freeman 2001). In the current study, matured tubers had improved skin-set as determined by the Halderson periderm shear tester. The thicker periderm of mature tubers compared to immature potato tubers indicated the effect of vine desiccation on periderm development and maturation over 14 days after vine desiccation

which was in agreement with the results of Pavlista (2002), Sabba et al. (2007) and Sabba and Bussan (2012) who reported the effect of timing of vine desiccation on skin-set. These studies reported an improved skin-set on the tubers harvested after vine desiccation.

The practice of vine desiccation 14 d before harvest allowed the tubers to increase periderm thickness and phellem layers which contributed to skin-set as defined by Sabba et al. (2007). Skin-set and periderm maturity is followed by the suberization of phellem cells, accompanied by a secondary thickening of cell walls driven by the activity of peroxidases (Lulai 2007). The suberized phellem cells have a closely packed arrangement that acts as a barrier to solute movement and infection (Lulai 2007). In the current study, the skin-set was assessed in terms of maturity stages and it was found that sufficient skin-set to resist against the excoriation was developed in tubers after 14 d after vine desiccation in the three cultivars tested. In previous studies improved skin-set was dependent upon the period between vine desiccation and harvest, which was usually two to three weeks (Stark and Love 2003). The environmental conditions, soil type (Sabba and Bussan 2012), relative humidity (Lulai and Orr 1993) and method of desiccation (Pavlista 2002) affect skin-set, so results may vary with location. Having established differences in skin set, it was incumbent on the researchers to determine if these would influence susceptibility to infection of the immature and mature tubers to a pathogen such as *Phytophthora infestans*.

The main focus of the study was to compare the susceptibility of three cultivars of potato at two maturity stages in relation to the skin-set to *P. infestans*. The differences in tuber late blight in immature and mature tubers may be attributed to more than one factor, including the differences in periderm development, lenticel resistance and tuber age (Lacey 1967; Walmsley-Woodward and Lewis 1975; Bjor 1987; Lapwood 1997). Lenticel resistance and tuber age were

not measured in this study. Tubers had more severe late blight in 2013 than in 2012, particularly in the immature tubers might be due to wetter soil in 2013 than in 2012 during the tuber-maturing period, as wetter soil has been reported to contribute to a delay of periderm maturation (Lulai and Orr 1993) and higher tuber late blight severity (Lacey 1967). Waterlogging is thought to increase netting, skin dullness and cause raised lenticels (Allen and Scott 2001). In the current study there was no waterlogging even after a high rainfall in 2013. The raised lenticels are more susceptible to infection by *P. infestans* (Adams and Stevenson 1990). Lenticels in tubers suberize with cork meristem and become more resistant to pathogens with age (Lacey 1967; Adams 1975a, b), however newly proliferated lenticels may be susceptible to pathogen entry (Tyner 1997). In this study, in 2013 the soil was still wet during harvest and lenticels were proliferated in all three cultivars, which may have enhanced late blight in the mature tubers of these cultivars in 2013.

Variation in disease severity in tubers among the three cultivars inoculated with isolates of four genotypes of *P. infestans* was observed in this study. The variation may have been due to differences between cultivars in maturity period in immature (harvested on the day of vine desiccation) and mature potato tubers (harvested after 14 days of vine desiccation) and periderm maturity affecting their susceptibility to *P. infestans*. RN is a medium to late maturing cultivar but sets tubers early in the season (Johansen et al. 1988), DRN is early to medium (Salas et al. 2003) and Snowden is medium to late in maturity (Peloquin et al. 1994). It has been reported that red-skinned cultivars tend to develop poorer skin-set compared to russet cultivars (Lulai and Orr 1993). However, in this study RN (russet cultivar) was as susceptible as DRN (red skinned cultivar) even though RN had thicker periderm with a higher torque value required to excoriate the periderm than DRN.

The resistance level to infection by pathogens of lenticels increases with tuber maturity, however the level remains the same over time for the eyes (Lacey 1967) and more eyes were present in RN tubers compared to DRN (data unpublished). This factor alone might account for the similarity in the late blight susceptibility measured in the cvs. RN and DRN.

The differences between isolates of genotypes of *P. infestans* in levels of tuber infection in the field depends on several factors such as the amount of rain, shedding of rain by potato foliage and movement of spores from lesions, soil moisture and the resistance of the tuber itself (Lapwood 1977), although tuber resistance is the major factor (Lapwood 1977; Olanya et al. 2009; Nyankanga et al. 2011; Kirk and Wharton 2014). In this study the variation between cultivars and years might be due to differences in maturity period of each cultivar and the prevailing weather and soil conditions in the two years (Sabba and Bussan 2012).

Variation in response of immature versus mature potato tubers to different genotypes of *P. infestans* was observed in both years. Isolates of all genotypes of *P. infestans* were capable of infecting the tubers at both maturity stages. The difference between the immature and mature tubers was in the degree of severity. Immature tubers were severely affected ($p \leq 0.05$). The isolates of the US-8 and US-22 genotypes of *P. infestans* were more aggressive and caused greater tuber blight damage ($p \leq 0.05$) compared to isolates of the other two genotypes of *P. infestans*. Hu et al. (2012), Danies et al. (2013) and Rojas et al. (2014) reported US-8 to be more aggressive followed by US-22 compared to other genotypes such as US-23 and US-24. In the current study US-8 was more aggressive in 2012 than US-22 and in 2013 the opposite occurred. Variability on aggressiveness of isolates of genotypes of *P. infestans* has been documented in the previous studies (Kirk et al. 2001c; Kuhl et al. 2007; Kirk et al. 2009; Young et al. 2009; Kirk et al. 2010; Danies et al. 2013; Rojas et al. 2014)

In conclusion, this study agreed with prior work that skin-set was related to susceptibility of tubers to *P. infestans*. When tubers were harvested 14 days after vine desiccation they were less susceptible to isolates of all genotypes of *P. infestans* tested than the tubers harvested prior to vine desiccation. Generally, isolates of genotypes of *P. infestans* are known to infect immature tubers more readily than mature tubers (Lacey 1967). Under the field condition isolates of these genotypes of *P. infestans* may infect the immature tubers during the growing season or contaminate the tubers from infected vines during harvest and elevate potato tuber loss in the storage. In addition, tubers can be infected later during handling and in storage (Powelson et al. 2002; Lambert et al. 1998). The infection of potato tubers in the field can be minimized by the practice of vine desiccation (Johnson et al. 1997). It has been reported that mechanical vine desiccation may increase the problem of some pathogen such as *Rhizoctonia solani, Pectobacterium* spp. and *Phoma exigua* var. *foveata* (Muddler at al. 1992). Currently, most growers desiccate vines of potatoes using chemical desiccants. Vine desiccation prior to harvest reduces the risk of infection of tubers indirectly by reducing sporangia number and enhancing periderm development (Johnson et al. 1997; Lulai and Orr 2001) and this study suggests that the periderm was not set in immature tubers which would make them more susceptible to late blight. The quality of tubers in storage depends upon the quality of tubers during harvest. The quality of potato tubers is maintained in a storage by harvesting healthy tubers with improved skin-set which can be achieved by vine desiccation.

CHAPTER 4: SEED AND IN-FURROW TREATMENTS FOR CONTROL OF SEED-BORNE *PHYTOPHTHORA INFESTANS*

ABSTRACT

The potato tuber is a key component in potato production and its health is vital for economic production globally. Potato seed tubers contaminated with *Phytophthora infestans* are a primary source of inoculum and can initiate potato late blight epidemics. Latently infected potato seed tubers are easily overlooked during the potato seed cutting and handling operation. Thus, the seed tubers should be treated with fungicides or biofungicides before or at planting. Field trials were conducted to evaluate the effects of several different fungicides and biofungicides applied alone or mixed as seed treatments, in-furrow at planting, or in combination (seed + in-furrow treatment) for control of seed-borne *P. infestans*. In the controlled environment trials, fungicides used as seed treatments were evaluated for control of seed-borne late blight. The number of emerged plants was recorded over a 16 days or 6-week period after planting to estimate final plant stand (%) and the relative area under emergence progress curve (RAUEPC) in controlled environment or field trials respectively. In controlled environment both rates of mandipropamid, fenamidone and cymoxanil applied in combination with mancozeb increased percent of final plant stand (72.5 to 92.5 and 82.5 to 100% in 2012 and 2014 respectively) and RAUEPC (32.5 to 40.2 and 44.0 to 59.5 in 2012 and 2014 respectively) value in comparison to other fungicide treatments tested. The application of fenamidone and fungicides containing mancozeb such as fenamidone + mancozeb, mandipropamid + mancozeb or cymoxanil + mancozeb to potato seed pieces were more effective and improved final plant stand and rate of emergence ($p \leq 0.05$) in comparison to other treatments tested in the field trials. Early management of seed tuber such as fungicide treatment prior to or at planting can be useful to

control seed-borne *P. infestans* and break the disease cycle and to reduce the early foliar treatments.

4.1 INTRODUCTION:

Potato late blight, caused by the oomycete *Phytophthora infestans* (Mont. de Bary), is a major constraint in potato (*Solanum tuberosum* L.) production worldwide (Fry and Goodwin 1997a; Stevenson et al. 2007; Kirk and Wharton 2014). Potato late blight can cause significant losses in the field and storage (Guenthner et al. 2001) and cost of control and loss has been estimated globally to exceed $6.7 billion annually (Haverkort et al. 2008). Infected seed tubers, infected tubers in cull piles, infected volunteer plants from tubers and rarely oospores (in the soil) have been reported as primary sources of inoculum for initiation of epidemics (Bonde and Schultz 1943; Zwankhuizen et al. 1998; Kirk 2003b; Fernández-Pavía et al. 2004; Johnson and Cummings 2009). During a late blight epidemic, tubers can become infected within 4-5 days after the pathogen is established on the foliage (Hirst et al. 1965) when inoculum from blighted vines is washed into the soil by precipitation and irrigation events (Andrivon 1995; Bain et al. 1997; Porter et al. 2005) leading to severe losses in storage (Nyankanga et al. 2007). Due to this grower desiccate the vines 3-4 weeks prior to harvest to reduce late blight tuber rot in storage (Stevenson et al. 2007).

Potato seed tubers contaminated with *P. infestans* have been reported as a major cause in initiating the disease at field level (Inglis et al. 1996; Johnson and Cuming 2009; Kirk et al. 2009). Potato seed tubers infected by *P. infestans* can play a role in long-term dispersion and introduction of new genotypes of *P. infestans* (Abad and Abad 1997; Nyankanga et al. 2011). In recent years the population of *P. infestans* has dramatically changed with the appearance of isolates with unique genotypes and epidemiological characteristics (Hu et al. 2012). The new

genotypes of *P. infestans* differ in phenotypic characteristics such as aggressiveness, epidemiological characteristics, mating type and sensitivity to the fungicide metalaxyl (Hu et al. 2012; Danies et al. 2013). For example, genotypes US-8 and US-22 are A2, and US-23 and US-24 are A1 mating types (Hu et al. 2012).

Metalaxyl is a systemic fungicide which was effective in controlling late blight (Schwinn and Margot 1991) until insensitive genotypes appeared (Goodwin et al. 1998). Isolates of *P. infestans* genotype US-8 are insensitive to metalaxyl, US-24 intermediate in sensitivity and US-22 and US-23 are sensitive (Hu et al. 2012; Danies et al. 2013). Isolates of *P. infestans* genotype US-22 have been reported as more aggressive than isolates of genotypes US-23 and US-24 (Danies et al. 2013) and slightly less aggressive than isolates of genotype US-8 (Rojas et al. 2014).

Infected potato tubers may rot in the field or later in storage, affecting both tubers intended for seed and for consumption (Johnson and Cummings 2009; Kirk et al. 2009; Olanya et al. 2009). They also can rot after planting, or may initiate a late blight epidemic if the seed tubers survive and the pathogen is transmitted to the developing shoot and then to foliage (Kirk et al. 1999; Johnson 2010; Kirk and Wharton 2014). In severe cases, isolates of aggressive genotypes of *P. infestans* such as US-8 can either kill the developing sprouts outright, resulting in non-emergence, or delay emergence, which is usually expressed as poor and uneven plant stand with weakened plants (Kirk et al. 2001c). The more aggressive genotypes may produce limited primary inoculum because as infected tubers are killed prior to emergence (Kirk et al. 2009; Kirk et al. 2010). Tubers infected with less aggressive genotypes of *P. infestans* may emerge and produce lesions on seedlings before the seed piece can decay completely contributing to the production of primary inoculum which may lead to the initiation of a late

blight epidemic (Powelson et al. 2002). Potato tubers infested with *P. infestans* are prone to additional infections such as soft rot and dry rot caused by *Pectobacterium* spp. and *Fusarium* spp., respectively (Secor and Salas 2001).

Due to the importance of seed tubers as a source of inoculum, fungicide treatment of seed tubers cannot be overlooked for control of seed-borne late blight (Bassler et al. 2002). Fungicide treatment in storage may not be effective due to the latent infection of tubers by *P. infestans* (Johnson and Cummings 2009) and contamination of healthy potato tubers from infected tubers during handling and seed cutting operations (Lambert et al. 1998). Therefore, it is important to treat seed tubers with effective fungicides or biological control agents (BCA) prior to or at planting to protect the seed tuber and the emerging sprout. This could possibly reduce the need of early foliar fungicide sprays (Wharton et al. 2012).

Although integrated disease management is the most reliable strategy for controlling late blight (Fry et al. 1977; Basu et al. 2003), growers rely heavily on commercially available fungicides to combat the disease (Fry 1997; Basu et al. 2003). Various fungicide products are available that are effective against potato late blight. Some of them are listed below. Cymoxanil is a locally systemic fungicide used to control different oomycete-caused diseases in vegetables including late blight on potato foliage and potato seed pieces (Powelson and Inglis 1999). Mancozeb is a broad-spectrum fungicide used to control foliar late blight and is recommended as a seed treatment (Thompson 1993). Fenamidone and azoxystrobin [quinone outside inhibitors (QoI)] are broad-spectrum foliar fungicides for the control of potato late blight and several fungal and oomycete-caused diseases in a wide variety of crops (Stevenson 2008). Fenamidone is one of many fungicides registered to control metalaxyl-resistant genotypes of *P. infestans*

(Stevenson 2008). Similarly, mandipropamid is a fungicide from the mandelamide group, effective against foliar and stem blight of potato (Rekanovic et al. 2010).

Fludioxonil with mancozeb is a broad-spectrum fungicide mixture effective for seed and soil-borne diseases of potato such as dry rot and late blight (Wharton et al. 2007). Flutolanil with mancozeb is broad-spectrum potato seed-piece treatment to control *Rhizoctonia solani* (Wharton et al. 2007).

Biological control agents (BCA) are living microorganisms, which can provide disease protection essentially through production of antibiotics, competition for nutrients with pathogens and induce resistance (Daayf et al. 2003). Several BCA based on bacteria and fungi have been tested for the control of soil and seed borne diseases such as late blight (McBeath and Kirk 2000; Sadfi et al. 2002; Wharton et al. 2012).

Fungicides used in combinations may provide better disease control than when used independently (Platt 1985 and Caldiz et al. 2007). From previous studies fungicides with different modes of action used in combinations have been reported to reduce cost, save labor and provide better control of diseases than when used alone (Gisi et al. 1985; Gisi 1996). Another benefit of combining fungicides with two or three ingredients with different modes of action is that they might exhibit synergism (Samoucha and Gisi 1987) and could delay the development of resistance to high-risk systemic fungicides (Samoucha and Gisi 1987; Baider and Cohen 2003).

The objectives of this study were to evaluate different fungicides and biological control agents for the management of seed-borne late blight caused by different genotypes (US-8, US-22, US-23 and US-24) of *P. infestans* to determine a) effectiveness of different fungicides/biological control agents to control seed-borne *P. infestans* applied as seed treatments

or in-furrow during planting and b) to determine the impact of fungicides applied alone or in combinations on plant stand and rate of emergence of *P. infestans*-inoculated potato seed tubers.

4.2 MATERIALS AND METHODS

4.2.1 PHYTOPHTHORA INFESTANS INOCULUM PREPARATION

Four isolates of each genotype of *Phytophthora infestans* (US-8, US-22, US-23 and US-24) recovered from potato or tomato using a single spore isolation technique were used as detailed in Table 4.1. Some of the isolates of the US-23 and US-24 genotypes were kindly provided by Dr. Gevens's University of Wisconsin. The US-8 and US-22 genotypes were obtained from Dr. Kirk lab stored in long term storage at 4°C on Rye A media (Michigan State University). The *P. infestans* isolates used in the study had genotypic and phenotypic differences including sensitivity to the commonly used metalaxyl fungicide (Danies et al. 2013) as described in Table 4.1. Rye B media (Caten and Jinks 1967), amended with antibiotics (20mg/L rifampicin, TCI America, Portland, OR; 100 mg/L ampicillin, EMD Chemicals, Inc. San Diego, CA; and 20mg/L nystatin, Sigma-Aldrich Corporation, St. Louis, MO) to control bacterial contamination was used for culturing isolates. Isolates were sub-cultured by transferring a mycelial disc (5x5x5 mm^3) onto the rye B media from pure culture plates. The plates were incubated at 18°C in the dark for two weeks to promote growth then transferred to light for two days to encourage sporulation (Niemira et al. 1999). Plates were flooded with 50 ml of sterile distilled water and the sporangia were dislodged from the mycelia by gently scrapping using a sterile L-shaped glass rod. The concentration of the inoculum was estimated using a Makler counting chamber. The initial concentration was adjusted to approximately 2 x 10^5 sporangia ml^{-1} using sterile distilled water. The suspension was placed at 10°C for 2 h to release zoospores (Judelson and Roberts 2002).

Table 4.1: Characteristics of isolates of genotypes of *Phytophthora infestans* used in the inoculation of potato seed tubers for the study of seed and in-furrow chemical and biological treatments for the control of seed-borne late blight

Genotype (No. of isolates)	Metalaxyl sensitivity[a]	Mating type	Isolates collected Year, Location[b] and Host	Location and Year tested	
				Field	Greenhouse
US-8 (4)	Insensitive	A_2	2008, Michigan from potato	Not used	2012-2014
US-22 (4)	Sensitive	A_2	2012, Michigan from potato	2011-2013	2012-2014
US-23 (4)	Sensitive	A_1	2011, Wisconsin from tomato	2014	2012-2014
US-24 (4)	Sensitive/ Intermediate	A_1	2011, Wisconsin from tomato	Not used	2012-2014

[a]Response of the isolates to the fungicide metalaxyl

[b]Isolates of Phytophthora infestans were collected from Michigan and Wisconsin and characterized by the programs of Dr. William Kirk and Dr. Amanda Gevens, respectively.

4.2.2 POTATO SEED TUBER INOCULATION AND TREATMENT WITH FUNGICIDES

Potato seed tubers of the cv. Snowden [susceptible to late blight (Kirk et al. 2001c)], stored in a cold storage for 4 or 6 months at 3°C and 95% relative humidity in the dark were used in all the greenhouse and field experiments in each year. The tubers remained dormant throughout this period (Davidson 1958). All greenhouse and field trials were conducted in February/March or May/June in each year. Tubers were removed from storage, warmed from 3°C, in 2°C increments, every 2 days, up to 12°C over a period of 10 days in the dark in a controlled environment chamber (Environmental Growth Chambers, Chagrin Falls, Ohio) with forced air ventilation at 5950 l min^{-1} (Wharton et al. 2012). Tubers free from disease symptoms were selected for the experiments. The potato seed tubers were sprouted at approximately 18°C and 16 h light cycle controlled environment chamber, which is a visible indication of break of dormancy and initiation of germination, before potato seed cutting. Potato tubers were cut into two or three sections (based on the size of potato) longitudinally ensuring the presence of sprouts on each seed piece. The cut seed pieces were immersed in a mixture of sporangia and zoospores of *P. infestans* (as described above) for 30 minutes then left in stackable-vented plastic crates (38L) at approximately 18°C to remove excess water for 1 h prior to treating with fungicides or BCA. All calculations of amount of product required were determined using ARM software (Version 8 or 9, Gylling Data Management Inc., Brookings, SD).

Dust formulations were measured using an electronic pan balance (PCE Americas Inc., Palm Beach, FL), added to inoculated seed pieces in paper bags, and shaken for 2 min to ensure uniform coating of the fungicide on the seed. Fungicides applied as liquid treatments were applied in a water suspension at a rate of 2.08 ml H_2O per kg by spraying onto the exposed seed tuber surfaces using a single nozzle R & D sprayer (R & D sprayer XR11003VS, Bellspray, Inc.,

Opelousas, LA) at 206 kPA, with the entire seed surface being coated. In-furrow applications were delivered at 0.94 ml/m^2 in an 18 cm band using a single nozzle (R & D sprayer) at 206 kPA.

The potato seed pieces were planted 24 h after treatment in 2011 and 2014 and on the same day as treatment in 2012 and 2013. Rain delayed planting for 15 h in 2013. Twelve different fungicides (sole or mixed formulation of fungicides) with various concentrations were used in the study as indicated in Table 4.2. All treatments were repeated across years at least once in greenhouse tests except that fenamidone, fenamidone + mancozeb and azoxystrobin were used only once in the field experiments. Treatment regimes applied to the seed pieces were a) not-inoculated, b) inoculated, and c) inoculated and treated with fungicides either after inoculation or in-furrow. The number of samples of seed pieces for each treatment consisted of 320 that were further split into groups of 50 for 6 replications for the field experiment experiments. The number of samples of seed pieces for each treatment consisted of 20 for 4 replications of the greenhouse experiments.

A sample of 20 tubers per treatment was collected for determination of inoculation efficiency in a controlled environment chamber (in-furrow regimes excluded unless a seed treatment also was applied). The sample was split into 4 replications of 5 tubers and placed into 2.3 kg capacity plastic mesh bags (Etsy Inc., Brooklyn, NY). The tubers in the bags were incubated in 35 x 22 x 20 cm^3 plastic boxes (Akro N.S.T, Akro-Mils, Akron, OH) lined with two layers of paper towels (Wypall, all purpose blue; Kimberly-Clark Professional, Roswell, GA) moistened with 100 ml of sterile water at 15°C in an environmental control chamber for 30 days in the dark. After 30 days, disease incidence was evaluated by counting the number of seed

pieces with late blight symptoms, identified as reddish tan discoloration of tuber tissue (Kirk et al. 2004).

Table 4.2: Products evaluated in the study of potato seed and in-furrow treatments for the control of seed-borne potato *Phytophthora infestans* including Fungicide Resistance Action Committee (FRAC) code, active ingredient, formulation, rate and manufacturer

Product name	Active ingredient (FRAC[a] Code)	Formulation[b]	Rate[c]	Manufacturer
Cruiser Maxx Potato	Thiamethoxam (4A) Fludioxonil (12)	28EC	0.14 l/ton	Syngenta Crop Protection Inc.
Curzate	Cymoxanil (40)	60DF	0.63 kg/ton	Dupont
Maxim	Fludioxonil (12)	4FS	0.052 kg/ton	Syngenta Crop Protection Inc.
Maxim MZ	Fludioxonil (12) + mancozeb (M3)	6.2DP	5 kg/ton	Syngenta Crop Protection Inc.
Moncoat MZ	Flutolanil (7) + mancozeb (M3)	7.5DP	7.5 kg/ton	Nichino America Inc.
Nubark MZ[c]	Mancozeb (M3)	6DS	10 kg/ton	Wilbur-Ellis Company
Quadris	Azoxystrobin (11)	2.08FL	5.8 ml/100 m	Syngenta Crop Protection Inc.
Reason	Fenamidone (11)	500SC	0.1 l/ton	Bayer CropScience LP
Revus	Mandipropamid (40)	250SC	0.2 or 0.4l/ton	Syngenta Crop Protection Inc.
Ridomil Gold	Mefenoxam (4)	4SL	4.1 ml/100 m	Syngenta Crop Protection Inc.
Serenade soil	*Bacillus subtilis* strain QST 713 (44)	1.34SC	86.7 ml/100 m	Bayer CropScience LP
Tenet	*Trichoderma asperellum* + *T. gamsii* (NC)	4WP	0.014, 0.021 or 0.028 kg/100 m	Gowan Company USA, Inc.

[a] FRAC = Fungicide Resistance Action Committee; FRAC code- number and letters used to distinguish fungicide groups according to their cross resistance behavior.
(FRAC Code List, 2015. Fungicides sorted by mode of action (including FRAC Code numbering.)

[b] Formulation= products added to the active ingredient to change its physical characteristic and allow compatibility with the machinery where EC = Emulsifiable Concentrate, DF = Dry Flowable, FS = Flowable Suspension, DP = Dustable Powder, DS = Soluble Dust, FL = Flowable, SC = Suspension Concentrate, SL = Soluble Concentrate and WP = Wettable Powder.

[c] Rate applied as seed treatment per ton and as in-furrow treatment per 100 m row (products with multiple rates have 2 or more treatments in the experiments).

4.2.3 EVALUATION OF PLANT EMERGENCE AND RELATIVE AREA UNDER EMERGENCE PROGRESS CURVE IN FIELD AND GREENHOUSE CONDITIONS

Potato seed tubers inoculated with *Phytophthora infestans* and treated with fungicides or BCA listed in Table 4.2 or controls were planted at the Michigan State University Clarksville Research Center, Clarksville, MI (Capac loam soil, 42.8733, -85.2604 deg, elevation 272.8 m) on Jun 6, 13, 6 and May 28 in 2011, 2012, 2013 and 2014, respectively. The potato tubers were planted in two-row with 86.4 cm row spacing by 8 m long plots with ca. 25 cm between plants to give a target population of 50 plants per plot. Plots were replicated six times in a randomized complete block design. The two-row beds were separated by a 1.5 m unplanted row.

In each year, nitrogen fertilizer (Urea) was drilled into plots before planting, based on soil test results. Additional nitrogen (final N 31.4 kg/ha) was applied to the growing crop with irrigation about 45 days after planting (DAP) for a total of 336 kg N per ha. Weeds were controlled by cultivation, hilling, and with S-metolachlor (Dual II Magnum; Syngenta Crop Protection, Greensboro, NC) at 2.24 L per ha 10 DAP and sethoxydim (Poast; BASF Corporation, Florham Park, NJ) at 1.75 L per ha about 55 DAP. Insects were controlled with imidacloprid (Admire Pro 2F: Bayer CropScience) at 3.4 mL per 100 m row at planting, and two applications of Beta-cyfluthrin (Baythroid XL; Bayer CropScience, Lubbock, TX) at 116.9 mL per ha at about 60 and 90 DAP, or based on commercial potato pest recommendations. All sprays were applied with a tractor mounted spray boom (R & D Sprayers, Opelousas, LA) delivering 233.8 L per ha (551.6 kPa) and using three XR11003VS nozzles per row. Plots were irrigated to supplement precipitation to about 0.63 cm per ha per 4 days' period with overhead irrigation.

In greenhouse experiments 5 tubers per treatment with four replications were planted in a randomized complete block design at Michigan State University. Each tuber was planted in

general all purpose mix (Suremix perlite growing media, Michigan Grower Products, Inc, Galesburg, MI) in a 15 x 15 x15 cm^3 plastic container (HC companies, Middlefield, OH) in the greenhouse under 16 h duration light cycle and at approximately 22°C. The containers were irrigated lightly at approximately 100 ml per pot on a three-day interval. The experiment was repeated three times.

4.2.4 DATA COLLECTION AND ANALYSIS

The number of emerged plants was recorded at two-day intervals for the first 10 days and weekly after that for a 40-day period after planting in all field experiments. In the greenhouse experiments the number of plants was recorded every two days over a 16-day period after planting. The rate of emergence was calculated initially as the area under the emergence progress curve [AUEPC; max = 100, (Kirk et al. 2001a,c)]. From this, the Relative Area Under the Emergence Progress Curve (RAUEPC) was calculated by a modification of the method used to calculate the Relative Area Under the Disease Progress Curve [RAUDPC (Madden and Hughes 1995)]. The RAUEPC was calculated using the equation,

$$\text{RAUEPC} = \frac{\sum(t_{i+1}-t_i)*(\frac{E_{i+1}+E_i)}{2}}{T_{total}*100};$$

Where t is the time in days after planting, E is the percentage of plant emergence and T is the time in days from planting to final emergence count.

All data were subjected to analysis of variance (ANOVA) using the statistical analysis software package JMP version 10.0 (SAS Institute, Cary, NC). Mean separation tests were conducted with the Least Square Means Tukey's honestly significant difference (HSD) test at $p \leq 0.05$ (field data) and Student's t at $p \leq 0.05$ (greenhouse data). Meteorological variables were

measured with a weather station located at the farm from the date of planting to harvest (Campbell Scientific, Logan, UT).

4.3 RESULTS

4.3.1 EVALUATION OF PLANT EMERGENCE IN THE GREENHOUSE

Late blight developed in the seed pieces in all treatments except the not-inoculated not-treated check. Few treatments achieved 100% plant stand, including the not-inoculated not-treated check in some years. There were significant differences in the potato seed tuber emergence and final plant stand for plants inoculated with the four different genotypes of *P. infestans* (Table 4.3) and data were therefore analyzed separately. Statistical analysis of the data from 2012 and 2014 showed that there were significant differences between results from years so the data from the two years were analyzed separately.

Final plant stand (16 DAP) ranged from 0 (inoculated check inoculated with the isolates of the US-23 genotype in 2012) to 100% (not-inoculated not-treated check and US-22-inoculated tubers treated with fenamidone in 2014) with the not-inoculated check significantly higher than the inoculated check and both rates of mandipropamid in both years and fludioxonil + mancozeb in 2014 for all four genotypes. Both rates of mandipropamid, fenamidone and cymoxanil applied in combination with mancozeb increased percent of final plant stand and RAUEPC value in comparison to other fungicide treatments (Tables 4.4 and 4.5) in both years with the exception of tubers inoculated with US-22 and US-24 and treated with cymoxanil + mancozeb in terms of plant stand and RAUEPC values, respectively.

In 2014 seed tubers that were inoculated with isolates of any of the genotypes of *P. infestans* and treated with fenamidone and fenamidone + mancozeb had 90 to 100% plant stand and 44 to 50.3 RAUEPC and were not significantly different from the not-inoculated not-treated

check (97.5 to 100% final plant stand and 41.6 to 46.9 RAUEPC). Inoculated seed tubers and treated with mancozeb, fludioxonil + mancozeb or flutolanil + mancozeb had varying responses in terms of percent of final plant stand and RAUEPC (Tables 4.4 and 4.5). Both rates of mandipropamid had poor percent of final plant stand in both years and were significantly different from both the inoculated not-treated and the not-inoculated not-treated controls. These two products had mixed performances in terms of RAUEPC. All the treatments had significantly higher plant stands compared to the not-treated inoculated check.

The response of potato seed tubers inoculated with four different genotypes of *P. infestans* and treated with 10 or 12 different fungicide treatments including the controls in the final plant stand % and RAUEPC are illustrated in Tables 4.4 or 4.5 and Fig 4.1 for both years.

Table 4.3: Summary of analysis of variance for the main effects of 12 fungicide seed treatments on potato seed tubers (cultivar Snowden) inoculated with isolates of four genotypes of *Phytophthora infestans* (US-8, US-22, US-23 or US-24) on final plant stand (%) and relative area under the emergence progress curve (RAUEPC; maximum = 100) (two sets of data combined in each year)

Source	*p* value			
	Final Plant Stand[a] %		RAUEPC[b] (0-100)	
	2012	2014	2012	2014
Treatment	<0.0001	<0.0001	<0.0001	<0.0001
Genotypes	0.0367	0.0013	0.0096	0.003
Treatment*Genotypes	0.0045	0.0008	0.0012	0.0154

[a] Final plant stand (%) is calculated as the number of emerged plants out of total seed pieces planted expressed as a percentage.

[b] RAUEPC (Relative area under the emergence progress curve) is the rate of emergence from planting to 16 days after planting counted at two-day interval.

Table 4.4: The effect of fungicide seed treatments on potato seed tubers (cultivar Snowden) inoculated with isolates of four different genotypes of *Phytophthora infestans* (US-8, US-22, US-23 or US-24) on percentage final plant stand. Experiments were conducted over two years in the greenhouse (2012 and 2014) (two sets of data combined in each year)

Treatment (rate per ton)	Final Plant Stand[a] % US-8[b]		US-22		US-23		US-24	
2012								
Not-inoculated not-treated check.....	95.0	a[c]	90.0	a	85.0	a	92.5	a
Mandipropamid 250SC (0.4 L) + mancozeb 6DS (10 kg).....	92.5	a	85.0	abc	90.0	a	90.0	a
Mandipropamid 250SC (0.2 L) + Mancozeb 6DS (10 kg).....	85.0	a	80.0	abc	82.5	ab	90.0	a
Cymoxanil 60DF (0.63 kg) + mancozeb 6DS (10 kg).....	80.0	a	75.0	abc	80.0	ab	82.5	a
Flutolanil + mancozeb 7.5 DP (7.5 kg)	35.0	c	57.5	c	65.0	bcd	35.0	c
Mancozeb 6DS (10 kg).....	57.5	b	62.5	bc	72.5	abc	80.0	a
Fludioxonil + mancozeb 6.2DP (5 kg)	57.5	b	72.5	abc	85.0	a	60.0	b
Mandipropamid 250SC (0.2 L).....	52.5	bc	32.5	d	52.5	d	42.5	bc
Mandipropamid 250SC (0.4 L).....	45.0	bc	30.0	d	55.0	cd	40.0	c
Inoculated not-treated check.....	10.0	d	5.0	e	0.0	e	2.5	d
2014								
Not-inoculated not-treated check.....	97.5	a	100.0	a	97.5	a	97.5	a
Fenamidone 500SC (0.1 L) + mancozeb 6DS (10 kg).....	97.5	a	90.0	ab	92.5	ab	92.5	a
Mandipropamid 250SC (0.4 L) + mancozeb 6DS (10 kg).....	97.5	a	90.0	ab	97.5	a	97.5	a
Mandipropamid 250SC (0.2 L) + mancozeb 6DS (10 kg).....	95.0	a	90.0	ab	85.0	abc	90.0	ab
Fenamidone 500SC (0.1 L).....	92.5	ab	100.0	a	97.5	a	97.5	a
Cymoxanil 60DF (0.63 kg) + mancozeb 6DS (10 kg).....	90.0	ab	77.5	b	97.5	a	82.5	abc
Flutolanil + mancozeb 7.5 DP (7.5 kg)	90.0	ab	80.0	b	87.5	abc	55.0	d
Mancozeb 6DS (10 kg).....	77.5	bc	80.0	b	92.5	ab	75.0	bc
Fludioxonil + mancozeb 6.2DP (5 kg)	72.5	c	75.0	b	80.0	bcd	70.0	cd
Mandipropamid 250SC (0.4 L).....	70.0	c	80.0	b	67.5	d	92.5	a
Mandipropamid 250SC (0.2 L).....	67.5	c	75.0	b	77.5	cd	70.0	cd
Inoculated not-treated check.....	7.5	d	5.0	c	10.0	e	7.5	e

[a] Final plant stand (%) was calculated as the number of emerged plants out of total seed pieces planted expressed as a percentage.

[b] Genotype designations of *Phytophthora infestans* used to inoculate the potato seed pieces classified according to Goodwin et al. (1995) and Hu et al. (2012).

[c] Values followed by the same letter in each year in a column are not significantly different at $p \leq 0.05$ by Least Square Means Student' t test. Each value is the average of four replications with five plants per replication.

Table 4.5: The effect of fungicide seed treatments on potato seed tubers (cultivar Snowden) inoculated with isolates of four different genotypes of *Phytophthora infestans* (US-8, US-22, US-23 or US-24) on Relative Area Under the Emergence Progress Curve (RAUEPC; maximum = 100). Experiments were conducted over two years in the greenhouse (from 2012 to 2014) (two sets of data combined in each year)

	RAUEPC[a] (0-100)							
Treatment (rate per ton)	US-8[b]		US-22		US-23		US-24	
2012								
Not-inoculated not-treated check.....	42.0	a[c]	40.8	a	34.0	a	40.5	ab
Mandipropamid 250SC (0.4 L) + mancozeb 6DS (10 kg).....	40.2	a	39.7	a	30.0	ab	40.5	ab
Mandipropamid 250SC (0.2 L) + Mancozeb 6DS (10 kg).....	33.9	ab	34.2	ab	30.0	ab	38.3	ab
Cymoxanil 60DF (0.63 kg) + mancozeb 6DS (10 kg).....	32.0	abc	30.2	abc	31.1	ab	29.3	bc
Flutolanil + mancozeb 7.5 DP (7.5 kg)..	12.6	de	20.9	cd	20.4	bc	10.3	ef
Mancozeb 6DS (10 kg).....	19.9	cd	22.5	bcd	27.3	abc	31.8	abc
Fludioxonil + mancozeb 6.2DP (5 kg)..	23.4	bcd	31.8	abc	31.3	ab	23.0	cd
Mandipropamid 250SC (0.2 L).....	16.5	d	7.0	e	17.5	c	17.1	de
Mandipropamid 250SC (0.4 L).....	18.0	d	11.3	de	15.9	c	12.0	def
Inoculated not-treated check.....	2.2	e	1.6	e	0.0	d	0.5	f
2014								
Not-inoculated not-treated check.....	46.9	a-d	41.6	ab	46.9	abc	42.1	ab
Fenamidone 500SC (0.1 L) + mancozeb 6DS (10 kg).....	49.7	abc	45.1	ab	44.0	a-d	45.5	ab
Mandipropamid 250SC (0.4 L) + mancozeb 6DS (10 kg).....	59.5	a	48.4	ab	54.7	a	50.1	a
Mandipropamid 250SC (0.2 L) + mancozeb 6DS (10 kg).....	54.2	ab	48.5	ab	47.1	abc	43.5	ab
Fenamidone 500SC (0.1 L).....	45.3	bcd	49.2	a	50.3	abc	44.7	ab
Cymoxanil 60DF (0.63 kg) + mancozeb 6DS (10 kg).....	40.8	bcd	35.1	b	45.2	a-d	30.2	cd
Flutolanil + mancozeb 7.5 DP (7.5 kg)..	51.0	ab	38.7	ab	46.1	a-d	26.8	d
Mancozeb 6DS (10 kg).....	34.9	d	42.6	ab	52.7	ab	35.4	bcd
Fludioxonil + mancozeb 6.2DP (5 kg)..	44.8	bcd	37.2	ab	40.5	cd	39.5	abc
Mandipropamid 250SC (0.4 L).....	36.2	cd	39.7	ab	34.6	d	48.7	a
Mandipropamid 250SC (0.2 L).....	34.6	d	37.2	ab	41.0	bcd	37.6	bcd
Inoculated not-treated check.....	5.5	e	2.4	c	4.3	e	2.9	e

[a] RAUEPC (Relative Area Under Emergence Progress Curve) is the rate of emergence from planting to 16 days after planting counted every two-day interval.

[b] Genotype designations of *Phytophthora infestans* used to inoculate the potato seed pieces classified according to Goodwin et al. (1995) and Hu et al. (2012).

[c] Values followed by the same letter in each year in a column are not significantly different at $p \leq 0.05$ by Least Square Means Student' t test. Each value is the average for four replications of 5 tubers per replication.

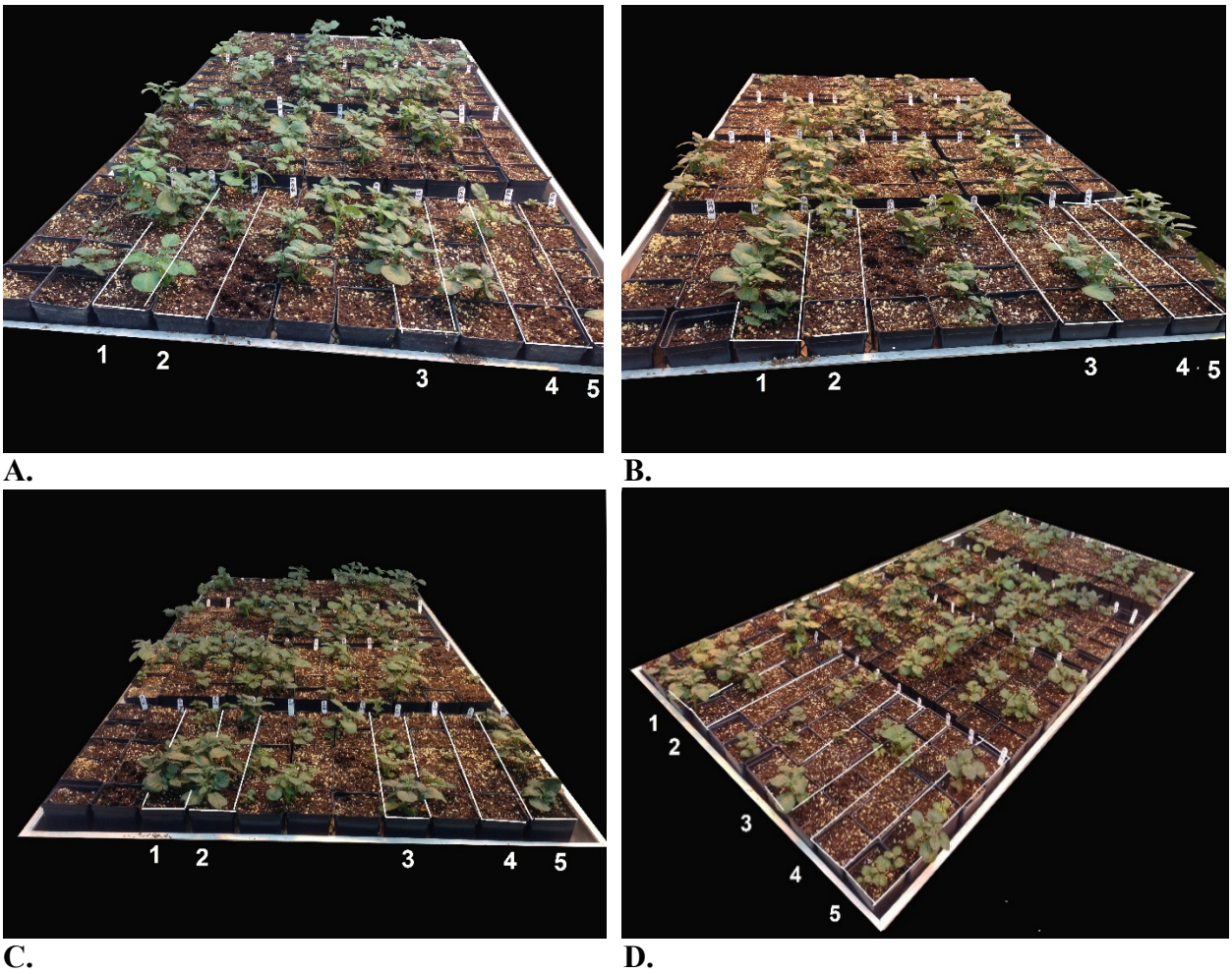

Figure 4.1: Emergence of potato plants (cultivar Snowden) from seed pieces inoculated with isolates of four different genotypes of *Phytophthora infestans* (A. US-8, B. US-22, C. US-23 or D. US-24). The seed pieces previously had been treated with one of 12 seed treatment products of which five are shown in this plate 1) Fenamidone, 2) Mandipropamid + Mancozeb, 3) Fenamidone + Mancozeb, 4) Inoculated – Control and 5) Mancozeb treatment (all highlighted for illustrations of effective treatments).

4.3.2 EVALUATION OF PLANT EMERGENCE IN THE FIELD

Weather was conducive for normal potato production (2012-2014). Average daily air temperature, average daily relative humidity (%), average daily soil temperature at a 10 cm depth and average daily soil moisture at a 10 cm depth (% of field capacity) for each month and precipitation over the same period are shown in appendix C.

The responses of potato seed tubers inoculated with *P. infestans* and treated with 20 different fungicide treatments for plant emergence and final plant stand percentage were evaluated for four different years (Tables 4.7 and 4.8). Each trial was analyzed separately because treatments were not the same over the four years although most treatments were repeated at least once, with the exception of fenamidone, fenamidone + mancozeb and azoxystrobin.

Late blight developed consistently in the trials in each year (Table 4.6) and affected plant stand in all treatments except the not-inoculated not-treated check (Tables 4.7 and 4.8). The inoculation efficiency tested in the controlled environment chamber supported the evidence in the field as all the replications developed late blight (Table 4.6). None of the treatments achieved 100% plant stand including the not-inoculated not-treated check in most of the years. Responses of potato tubers treated with mancozeb (2011-12), flutolanil + mancozeb (2011), mandipropamid (0.2L per ton) + mancozeb (2012) and fenamidone + mancozeb (2014) relative to the not-inoculated not-treated check indicated that these treatments enhanced emergence each year except in 2013 (Tables 4.7 and 4.8). At the final plant stand evaluation majority of the treatments had significantly greater plant stand than the inoculated check except the BCA in 2013 and 2014; both rates of mandipropamid, flutolanil + mancozeb, mefenoxam, fludioxonil + azoxystrobin in 2013; and flutolanil and thiamethoxam + fludioxonil in 2014 (Tables 4.7 and 4.8).

4.3.2.1 EVALUATION OF PLANT EMERGENCE IN 2011

All the treatments increased plant stand in comparison to the inoculated check treatment (final plant stand check = 28%). The mancozeb alone, cymoxanil + mancozeb and flutolanil + mancozeb treatments were not significantly different from the not-treated not-inoculated check (final plant stand = 87.7%, Table 4.7). These three treatments were significantly different from the inoculated not-treated check for RAUEPC (RAUEPC inoculated not-treated check = 21.2, Table 4.8). The mefenoxam, thiamethoxam + fludioxonil and thiamethoxam + fludioxonil + mandipropamid (both single and double rates) treatments did not increase the plant emergence of inoculated seed tubers and were significantly different compared to the not-inoculated not-treated check. The RAUEPC indicated that treatments mefenoxam, thiamethoxam + fludioxonil and thiamethoxam + fludioxonil + mandipropamid (single rate) were not significantly different from the inoculated not-treated check (RAUEPC inoculated not-treated check = 10.2, Table 4.8). Late blight symptoms did not appear on stems or foliage in any treatment during the assessment period. Seed treatments and in-furrow applications of fungicides showed no phytotoxicity to leaves or stems based on visual observation.

4.3.2.2 EVALUATION OF PLANT EMERGENCE IN 2012

Treatments such as mancozeb, flutolanil + mancozeb and a single rate (0.2 l per ton) of mandipropamid + mancozeb enhanced the rate of emergence of inoculated treated potato seed tubers compared to the not-inoculated not-treated check (Table 4.7). At the final plant stand evaluation all treatments had significantly greater plant stand than the inoculated check treatment for final plant stand (29.8%). and RAUEPC (14.7, Table 4.8). The mancozeb, flutolanil + mancozeb, both rates of mandipropamid + mancozeb and cymoxanil + mancozeb increased the final plant stand of inoculated seed tubers and the RAUEPC and were not significantly different

from each other or the not-treated not-inoculated check final plant stand (76.0%) and RAUEPC (41.3, Tables 4.7 and 4.8). Both rates of mandipropamid did not increase the final plant stand or the RAUEPC values. The fludioxonil + mancozeb and mefenoxam treatment increased the final plant stand but were not significantly different from the inoculated not-treated check. The combination of fludioxonil + mancozeb and mefenoxam fungicides and both rates of mandipropamid were significantly different from other treatments such as mancozeb, single rate of mandipropamid + mancozeb and flutolanil + mancozeb, in terms of plant stand and rate of emergence. Late blight symptoms did not appear on stems or foliage in any treatment during the assessment period. Seed treatments and in-furrow applications of fungicides showed no phytotoxicity on leaves or stem based on visual observation.

4.3.2.3 EVALUATION OF PLANT EMERGENCE IN 2013

No treatments achieved 100% plant stand including the not-inoculated not-treated check (Table 4.7). The not-inoculated not-treated check had significantly greater plant stand (79.7%) than any of the treatments (Table 4.7). At the final plant stand evaluation mancozeb, both single and double rates of mandipropamid + mancozeb and fludioxonil + mancozeb-treated seed tubers had significantly greater plant stand than the inoculated not-treated check. None of the BCA increased the final plant stand or RAUEPC (Tables 4.7 and 4.8). The analysis of the RAUEPC values indicated that the not-inoculated not-treated check, mancozeb, both single and double rates of mandipropamid + mancozeb, and fludioxonil + mancozeb were significantly different from the inoculated not-treated check (RAUEPC = 2.5, Table 4.8). Late blight signs and symptoms appeared on stems of 3 plants (Fig 4.2) in two treatments including on the inoculated not-treated check about 5 weeks after planting. Before emergence, 92-96% of the tubers had rotted in inoculated check and BCA treatments and those three symptomatic plants emerged

from the inoculated check (one) and *Trichoderma asperellum* + *T. gamsii* 4WP (21 g) treatment (two).

4.3.2.4 EVALUATION OF PLANT EMERGENCE IN 2014

None of the treatments achieved 100% plant stand, including the not-inoculated not-treated check (final plant stand = 83.0%). The fenamidone + mancozeb (final plant stand = 88.3%) treatment enhanced ($p \leq 0.05$) emergence rate compared to the not-inoculated not-treated check (Table 4.7). At the final evaluation of plant stand all treatments except the three rates of *Trichoderma asperellum* + *T. gamsii*, *Bacillus subtilis* (QST 713), flutolanil + mancozeb and thiamethoxam + fludioxonil had significantly greater plant stand than the not treated inoculated check. The RAUEPC analysis indicated that all the treatments except the three rates of *Trichoderma asperellum* + *T. gamsii*, *Bacillus subtilis* (QST 713), flutolanil + mancozeb, azoxystrobin and thiamethoxam + fludioxonil were significantly different from the not-treated inoculated check (RAUEPC = 16.0, Table 4.8). Late blight symptoms did not appear on stems or foliage in any treatment at the time of final plant stand count.

Table 4.6: Effect of fungicide seed treatments on potato seed tubers (cultivar Snowden) inoculated with genotypes of *Phytophthora infestans* US-22 in 2012 and 2013 or US-23 in 2014 on disease incidence over three years (2012-2014). Tubers were incubated in a controlled environment chamber at 15°C in the dark for 30 days

	Disease Incidence[a] %					
Treatment (rate per 100 m row or rate per ton)	2012		2013		2014	
Inoculated not-treated check.....	70	a[b]	95	a	80	a
Not-inoculated not-treated check.....	0	c	0	c	0	f
Mancozeb 6DS (10 kg).....	25	bc	30	bc	30	cde
Mandipropamid 250SC (0.2 L).....	45	ab	45	abc	35	cde
Mandipropamid 250SC (0.4 L).....	40	ab	45	abc	35	cde
Mandipropamid 250SC (0.2 L) + mancozeb 6DS (10 kg).....	15	bc	60	ab	30	cde
Mandipropamid 250SC (0.4 L) + mancozeb 6DS (10 kg).....	35	abc	65	ab	20	def
Flutolanil + mancozeb 7.5 DP (7.5 kg).....	20	bc	55	ab	45	bcd
Fludioxonil + mancozeb 6.2DP (5 kg).....	30	bc	70	ab	40	bcd
Cymoxanil 60DF (0.63 kg) + mancozeb 6DS (10 kg).....	20	bc	NA		20	def
Thiamethoxam + fludioxonil 420FS (0.14 L)....	NA[c]		NA		65	ab
Thiamethoxam + fludioxonil 420FS (0.14 L) + mandipropamid 250SC (0.2 L).....	NA		NA		50	bc
Thiamethoxam + fludioxonil 420FS (0.14 L) + mandipropamid 250SC (0.4 L).....	NA		NA		30	cde
Fludioxonil 4FS (0.052 kg) + azoxystrobin 2.08FL (5.8 ml).....	NA		40	bc	45	bcd
Fenamidone 500SC (0.1 L).....	NA		NA		25	c-f
Fenamidone 500SC (0.1 L) + mancozeb 6DS (10 kg).....	NA		NA		10	ef

[a] Disease incidence (%) was calculated as the ratio of tubers developing late blight symptoms (reddish tan discoloration of tuber tissue) to the total tubers used in the treatment. 20 potato seed pieces were assessed for each treatment (only seed treatment were included, in-furrow treatments were dropped for this assessment)

[b] Values followed by the same letter in each year are not significantly different at $p \leq 0.05$ by Least Square Means Tukey's Honestly Significant Difference

[c] NA = Not available (treatments were not tested in that year)

(Note: the inoculated check was inoculated in *Phytophthora infestans* inoculum for 30 min, not-inoculated not-treated check was cut and planted in the field on the same day)

Table 4.7: Effect of fungicide seed and in-furrow treatments on plant stand on potato (cultivar Snowden) inoculated with isolates of genotypes of *Phytophthora infestans*, US-22 in 2011-2013 or US-23 in 2014 in the field over four years (2011-2014)

Treatment (rate per 100 m row or rate per ton) + (Application method)	Final Plant Stand[a] %							
	2011		2012		2013		2014	
Inoculated not-treated check.....	28.0	e[c]	29.8	f	4.0	d	30.0	g
Not-inoculated not-treated check.....	87.7	ab	76.0	abc	79.7	a	83.0	ab
Mancozeb 6DS (10 kg) (A[b]).....	91.3	a	86.1	a	39.7	b	69.3	a-f
Mandipropamid 250SC (0.2 L) (A).....	NA[d]		49.7	d	7.0	d	72.3	a-e
Mandipropamid 250SC (0.4 L) (A).....	NA		44.1	de	4.7	d	73.3	abc
Mandipropamid 250SC (0.2 L) + mancozeb 6DS (10 kg) (A).....	NA		81.9	ab	38.7	b	82.0	ab
Mandipropamid 250SC (0.4 L) + mancozeb 6DS (10 kg) (A).....	NA		70.1	bc	26.7	bc	83.7	ab
Flutolanil + mancozeb 7.5 DP (7.5 kg) (A).....	80.3	b	84.8	a	19.3	cd	46.3	fg
Fludioxonil + mancozeb 6.2DP (5 kg) (A).....	NA		67.0	c	25.7	bc	78.0	ab
Mefenoxam 4SL (4.1 ml) (B).....	39.3	d	66.4	c	11.0	cd	62.0	b-f
Cymoxanil 60DF (0.63 kg) + mancozeb 6DS (10 kg) (A).....	89.0	ab	75.4	abc	NA		81.3	ab
Thiamethoxam + fludioxonil 420FS (0.14 L) (A).....	40.7	d	NA		NA		49.7	c-g
Thiamethoxam + fludioxonil 420FS (0.14 L) + mandipropamid 250SC (0.2 L) (A)...	43.7	d	NA		NA		71.7	a-e
Thiamethoxam + fludioxonil 420FS (0.14 L) + mandipropamid 250SC (0.4 L) (A)...	63.0	c	NA		NA		71.0	a-e
Fludioxonil 4FS (0.052 kg) (A) + azoxystrobin 2.08FL (5.8 ml) (B).....	NA		NA		11.0	cd	71.3	a-e
Bacillus subtilis QST 713 1.34 SC (85.4 ml) (B).....	NA		NA		6.7	d	32.3	g
Trichoderma asperellum + *T. gamsii* 4WP (14 g) (B).....	NA		NA		7.7	d	48.0	efg
Trichoderma asperellum + *T. gamsii* 4WP (21 g) (B).....	NA		NA		5.0	d	48.7	d-g
Trichoderma asperellum + *T. gamsii* 4WP (28 g) (B).....	NA		NA		6.7	d	49.0	c-g
Azoxystrobin 2.08FL (5.8 ml) (B).....	NA		NA		NA		60.0	b-f
Fenamidone 500SC (0.1 L) (A).....	NA		NA		NA		73.0	a-d
Fenamidone 500SC (0.1 L) + mancozeb 6DS (10 kg) (A).....	NA		NA		NA		88.3	a

[a] Final plant stand (%) was calculated as the number of emerged plants out of total seed pieces planted (n = 50) expressed as a percentage from planting to 40 days after planting.

table 4.7 continued…

[b] Application methods A = seed treatment (liquid formulations at 2.08 ml H_2O per kg); A= seed treatment (dry formulation); B = in-furrow application (liquid formulations at 0.4 L H_2O per 100 m row in a 17 cm band using a single XR11003VS nozzle R & D sprayer at 206 kPA; B = in-furrow application (dry formulation broadcast in-furrow)
[c] Values followed by the same letter in each year in the column are not significantly different at $p \leq 0.05$ by Least Square Means Tukey's Honestly Significant Difference. Each number is the average of 300 seed pieces
[d] NA = Not Available (Treatment not tested in that year)
(Note inoculated not-treated check immersed in inoculum suspension of *Phytophthora infestans*; not-inoculated not-treated check; both treatments were not treated with fungicides)

Table 4.8: Effect of 20 fungicide seed and in-furrow treatments on relative area under emergence progress curve values (RAUEPC; 0 - 100) on potato (cultivar Snowden) inoculated with one of two genotypes of *Phytophthora infestans* US-22 in 2011-2013 or US-23 in 2011-2013 or US-23 in 2014 in the field over four years (2011-2014)

Treatment (rate per 100 m row or rate per ton) + (Application method)	RAUEPC[a] (0-100) 2011		2012		2013		2014	
Inoculated not-treated check.....	10.2	c[c]	14.7	d	2.5	e	16.0	fg
Not-inoculated not-treated check.....	21.2	b	41.3	ab	68.8	a	40.4	abc
Mancozeb 6DS (10 kg) (A[b]).....	35.5	a	46.3	a	30.9	b	38.8	a-d
Mandipropamid 250SC (0.2 L) (A).....	NA[d]		26.1	c	5.0	e	39.8	abc
Mandipropamid 250SC (0.4 L) (A).....	NA		24.4	c	3.6	e	38.5	a-d
Mandipropamid 250SC (0.2 L) + mancozeb 6DS (10 kg) (A).....	NA		42.3	ab	32.0	b	43.0	ab
Mandipropamid 250SC (0.4 L) + mancozeb 6DS (10 kg) (A).....	NA		35.5	b	22.1	bc	45.3	ab
Flutolanil + mancozeb 7.5 DP (7.5 kg) (A).....	30.9	a	47.6	a	14.6	cde	24.5	d-g
Fludioxonil + mancozeb 6.2DP (5 kg) (A).....	NA		34.4	b	21.9	bcd	38.9	a-d
Mefenoxam 4SL (4.1 ml) (B).....	13.4	c	35.7	b	8.7	de	32.1	b-e
Cymoxanil 60DF (0.63 kg) + mancozeb 6DS (10 kg) (A).....	35.4	a	41.7	ab	NA		44.9	ab
Thiamethoxam + fludioxonil 420FS (0.14 L)(A).....	12.2	c	NA		NA		26.2	c-g
Thiamethoxam + fludioxonil 420FS (0.14 L) + mandipropamid 250SC (0.2 L) (A).....	13.2	c	NA		NA		36.7	a-e
Thiamethoxam + fludioxonil 420FS (0.14 L) + mandipropamid 250SC (0.4 L) (A).....	21.0	b	NA		NA		36.0	a-e
Fludioxonil 4FS (0.052) kg + azoxystrobin 2.08FL (5.8 ml) (B).....	NA		NA		9.5	de	36.3	a-e
Bacillus subtilis QST 713 1.34 SC (85.4 ml) (B).....	NA		NA		5.0	e	15.3	g
Trichoderma asperellum + *T. gamsii* 4WP (14 g) (B).....	NA		NA		5.8	e	23.6	efg
Trichoderma asperellum + *T. gamsii* 4WP (21 g) (B).....	NA		NA		5.4	e	24.6	d-g
Trichoderma asperellum + *T. gamsii* 4WP (28 g) (B).....	NA		NA		3.6	e	25.1	d-g
Azoxystrobin 2.08FL (5.8 ml) (B).....	NA		NA		NA		30.6	b-f
Fenamidone 500SC (0.1 L) (A).....	NA		NA		NA		40.2	abc
Fenamidone 500SC (0.1 L) + mancozeb 6DS (10 kg) (A).....	NA		NA		NA		47.6	a

table 4.8 continued…

[a] RAUEPC (Relative Area Under Emergence Progress Curve) is the rate of emergence from planting to the 40 days after planting counted every two-day interval for 10 days and every week after 10 days

[b]Application methods A = seed treatment (liquid formulations at 2.08 ml H_2O per kg); A= seed treatment (dry formulation); B = in-furrow application (liquid formulations at 0.4 L H_2O per100 m in a 17 cm band using a single XR11003VS nozzle R & D sprayer at 206 kPA; B = in-furrow application (dry formulation)

[c] Values followed by the same letter are not significantly different at $p \leq 0.05$ by Least Square Means Tukey Honestly Significant Difference

[d] NA = Not Available (Treatment not tested in that year)

(Note inoculated not-treated check immersed in inoculum suspension of *Phytophthora infestans*; not-inoculated not-treated check had healthy seed tubers; both treatments were not treated with fungicides)

A. **B.**

Figure 4.2: A. Potato tuber expressing late blight symptoms (reddish tan discoloration of tuber tissue) and developing sprout. B. Potato plant with symptoms of late blight in the seed tuber (green arrow in B) and main stem at the point of attachment of the seed tuber, and a lesion on the stem about midway between seed tuber and lower leaf (blue arrow in B).

4.4 DISCUSSION

Healthy potato seed tubers are of paramount importance in commercial potato crop production because seed tubers infested by *Phytophthora infestans* have been reported as a major cause in initiating late blight epidemics in the field (Inglis et al.1996). Planting infected seed tubers results in reduced plant stands due to rapid decay of seed pieces and delay in emergence of symptomatic plants (Powelson et al. 2002; Kirk et al. 2009). The ability of *P. infestans* for latent infection of potato seed tubers in long term cold storage (Johnson and Cummings 2009) and the chance of emergence of symptomatic plants from infected seed tubers (Van der Zaag 1956; Johnson 2010) necessitate management strategies under high risk situations with conducive environmental conditions, as in the current study. Given the potential risk of the initiation of a late blight epidemic from infected seed tubers, which has been reported to be less than 2% (Van Der Zaag 1956; Van Der Plank 2012) it is prudent to treat potato seed tubers with fungicides.

Potato late blight is a polycyclic disease and secondary cycles complete within 4-5 days, therefore secondary inoculum can increase exponentially (Kamoun and Smart 2005). So even a single late blight-infected plant emerged from an infected potato seed tuber can cause a late blight epidemic, resulting in damage to the whole field within 2-3 weeks (Fry and Goodwin 1997a) and crop failures may result that limit profitable potato production (Fry and Goodwin 1997b; Stevenson et al. 2007; Kirk and Wharton 2014). Therefore, seed-borne late blight should be controlled using effective fungicides along with good cultural practices from planting to storage for healthy and quality output.

In recent years the population of *P. infestans* has dramatically changed with the appearance of new isolates with unique genotypes and epidemiological characteristics (Hu et al.

2012). The new genotypes of *P. infestans* differ in phenotypic characteristics such as mating type and metalaxyl sensitivity (Hu et al. 2012; Danies et al. 2013). The changes in the genetic structure of the *P. infestans* population in Michigan and in the US necessitate the evaluation of currently used and commercially available fungicides and biological control agents for the control of potato seed-borne late blight epidemics. Some of the fungicides may be combined to exploit the additive benefits of fungicides (Gisi 1996). In the current study some fungicides such as mandipropamid or fenamidone were combined with mancozeb to see better results than used alone. The main aim of this study was to evaluate the efficacy of commercially available fungicides and biological control agents for the control of potato seed-borne *P. infestans* applied alone or in combination.

Potato seed pieces and in-furrow treatments of fungicides and biological control agents are commonly used in commercial potato production to control seed-borne late blight (Wharton et al. 2012). Some fungicides used in combinations can provide better disease control than when used independently (Platt 1985; Caldiz et al. 2007). In the current study, fenamidone + mancozeb (tested once in the field), single and double rates of mandipropamid + mancozeb and cymoxanil + mancozeb significantly increased plant stand and the RAUEPC both in the field and greenhouse experiments except in 2013. These treatments consistently reduced seed-borne late blight in greenhouse and field experiments and maintained equal or higher final plant stand compared to the not-inoculated not-treated check. These results are in agreement with previous studies in which mancozeb alone and fenamidone were reported to reduce the infection of progeny tubers when applied to the soil (Porter et al. 2006). In the current study, a combination of fenamidone + mancozeb was effective at controlling late blight in the potato seed pieces.

A combination of cymoxanil + mancozeb was effective in controlling late blight applied to potato seed tubers and increased plant stand and the RAUEPC compared to the inoculated check and other fungicides such as fludioxonil, fludioxonil + MZ, and tested rates of mandipropamid. Synergy of cymoxanil + mancozeb has been reported when used to control both foliar and potato tuber blight (Samoucha et al. 1987, 1988; Evenhuis et al. 1996; Powelson and Inglis 1999). Powelson and Inglis (1999) treated potato seed tubers prior to inoculation to test the efficacy of cymoxanil + mancozeb as a protective seed treatment with isolates of different genotypes of *P. infestans* (US-8 and US-11). In the current study all the treatments were applied after inoculation of potato seed tubers. Cymoxanil + mancozeb protected the potato seed tubers against tuber blight applied prior or after inoculation regardless of the different genotypes of *P. infestans* in greenhouse and field situations in agreement with Powelson and Inglis (1999).

Flutolanil + mancozeb and fludioxonil + mancozeb were commercially available products and are currently recommended as seed treatments for Fusarium dry rot, Rhizoctonia stem canker, and black scurf of potatoes (Wharton et al. 2007a,b). These fungicides were tested to see whether these were effective than mancozeb used alone or not. Flutolanil + mancozeb and fludioxonil + mancozeb were less effective than other treatments tested in the current study for the control of seed-borne late blight both in greenhouse and field experiments. Flutolanil + mancozeb was more effective in 2011-12 than in 2013-14 in the field and in 2014 than 2012 in greenhouse experiments. The variation in results in the field might have been due to differences in response to the isolates of the different genotypes used in the field, however this hypothesis was not supported by greenhouse results. This variation in the activity of flutolanil + mancozeb to control seed-borne late blight had not been previously reported. Fludioxonil + mancozeb has

been reported as an effective fungicide to control late blight in seed tubers by Wharton et al. (2012) but in our study it was inconsistent.

The BCA tested, *Trichoderma asperellum* + *T. gamsii* and *Bacillus subtilis* QST 713, did not increase plant stand or the RAUEPC from seed tubers inoculated with either of two genotypes (US-22 or US-23 in 2013 or 2014, respectively) of *P. infestans* when used as in-furrow treatments. This is in contrast to previous trials using different *Trichoderma* species such as *Trichoderma atroaviride* as a seed treatment which showed that the fungus was as effective as mancozeb to control seed-borne late blight (Mcbeath and Kirk 2000). However, the species of fungus and genotypes of *P. infestans* used in the study of McBeath and Kirk (2000) were different from our study. Also in contrast to our result, in a previous study, *B. subtilis* QST 713 and *T. harzianum* applied alone as seed treatments were effective in reducing seed piece decay after inoculation of seed pieces with *P. infestans* (US-8) treated up to 10 days before planting (Wharton et al. 2012). This indicates that these BCA may be more effective as seed treatments than as in-furrow treatments. BCA such as *Bacillus cereus* have been reported to colonize the cut seed surface up to 61 days after planting (Sadfi et al. 2002). However, the BCA used in the current study are different from previous studies and different BCA have different mechanisms to control the pathogens such as induced resistance, competition for nutrients, and secretion of inhibitory compounds (Guetsky et al. 2002). The soil and the weather also may make a differences in the performance of BCA because the efficacy of many BCA have been evaluated under certain conditions, so disease control may not be the same in other conditions (Larkin et al. 1998). As BCA are living organisms these can be more susceptible to environmental conditions such as temperature, humidity and pH than synthetic chemicals (Barbercheck 1992). Some possible reasons for the inconsistent performance of BCA reported here are loss of ecological

competence due to *in vitro* culture, not-target pathogen interference, variable root colonization by bacteria and loss of rhizosphere competence traits (Weller 1988).

The results of the current study agree with the need for effective fungicides for seed-borne potato late blight control especially when inoculum is plentiful and as late blight epidemics become more commonplace then it likely that the frequency of contaminated seed lots will increase. The curative application of fungicides may not completely control potato tuber blight (Powelson and Inglis 1999). Similar results to Powelson and Inglis (1999) were also observed by Inglis et al. (1999) on the tubers inoculated 5-7 days prior to the application of thiophanate-methyl + mancozeb. Inglis et al. (1999) reported that the performance of fungicides against *P. infestans* during seed cutting and handling operations of potato seed piece may be more beneficial than if used after the establishment of pathogen. Almost all the tubers rotted before emergence in the inoculated check (4% plant stand) in the 2013 field experiment. Similarly, extensive tuber rot in the environmental control chamber experiments supported the results of the field experiments in 2013. This indicated that potato seed pieces and sprouts were not protected before emergence when fungicides were applied, which highlights the need for fungicide application prior to or at planting.

Seed cutting promotes invasion by soft rotting bacteria (Case et al. 1988) and also fungal dry rot pathogens (Secor and Salas 2001) and some loss in final plant stand of not-inoculated not-treated check was to be expected. The inoculated check had lower plant stands and higher disease incidence than the not-inoculated controls and fungicide treatments in all experiments. None of the treatments had 100% plant stand in all experiments after seed was cut and planted on the same day or 24 hours after treatment. Wharton et al. (2012) explained that due to insufficient time to heal under optimal conditions (18°C and 95% RH with forced ventilation at 5950 l min

per min), seed pieces cut and planted on the same day were susceptible to seed piece decay caused by *Fusarium* spp. that occurred naturally in the field. Emergence increased when seed was cut and planted after 24 hours of fungicide treatment in our study in 2011 and 2014 rather than on the same day as occurred in 2012 and 2013.

Fludioxonil + azoxystrobin or thiamethoxam + fludioxonil + both rates of mandipropamid were effective to control seed-borne late blight in 2014 compared to the inoculated check and were not significantly different from the not-inoculated check. In the current study, mandipropamid used alone was ineffective compared to both rates of mandipropamid + mancozeb, cymoxanil + mancozeb or mancozeb against seed-borne late blight except in the 2014 field experiment. In 2014 US-23 of *P. infestans* was used for the inoculation, while US-22 was used in previous years. The variation in response of fungicides to different genotypes may have ben affected by the aggressiveness of these genotypes. US-22 has been reported to be more aggressive than US-23 (Hu et al. 2012; Danies et al. 2013) although in the context of tuber blight Kirk et al. (2009, 2010) and Rojas et al. (2014) reported that the aggressiveness of the isolates depends upon the cultivar of potato.

Mandipropamid combined with mancozeb was effective in the greenhouse but did not significantly increase emergence when compared to mancozeb alone in the field and therefore should not be applied alone for the control of seed-borne potato late blight. However, mandipropamid has been reported to control foliar late blight effectively (Rekanovic et al. 2010).

Treating potato seed with fungicides has several advantages compared to not-treated potato seed tubers. Excess seed after planting disposed close to potato production fields may result in the production of secondary inoculum of late blight (Inglis et al. 1999). Treated seed pieces may prevent the transmission of *P. infestans* from such activities (Inglis et al. 1999).

Furthermore, not-treated cut seed pieces may be contaminated with secondary infection of soft rot or dry rot caused by *Pectobacterium* spp. or *Fusarium* spp., respectively, in the field after planting resulting in poor emergence (Wharton et al. 2014). Seed tubers treated with fungicides with no or poor efficacy may facilitate the emergence of late blight symptomatic plants (Inglis et al. 1999). Also the fungicides with no efficacy against Fusarium dry rot may not prevent the rotting of tubers because some commercially available tubers always have certain level of Fusarium dry rot (Leach 1985).

Seed tubers may become contaminated by *P. infestans* during handling and seed cutting processes (Lambert et al. 1998). Lambert et al. (1998) reported that pathogen transmission from infected seed tubers to healthy cut seed tubers occurs within 8 hours of contact and sporulation of already infected tubers in 24 hours. A fungicide application right after cutting may prevent such contamination (Lambert et al. 1998). Treated seed tubers can be re-stored with good management practices until planting in case meteorological conditions prevail that prevent planting (Wharton et al. 2012). Thus, pathogen transmission from infected seed pieces to healthy tubers can be prevented until planting. Early management of seed tuber such as fungicide treatment prior to or at planting can be useful to break the disease cycle and to reduce the early foliar treatments.

None of the fungicides used as seed treatment guarantee the prevention of foliar infection from other sources of inoculum. The economic benefit from these fungicide applications may outweigh the risk of applying no fungicide treatments if there is a high risk of a potato late blight epidemic.

APPENDICES

FIRST REPORT OF LATE BLIGHT CAUSED BY *PHYTOPHTHORA INFESTANS* CLONAL LINEAGE US-23 VARIANT ON POTATO IN MICHIGAN, USA

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Keywords: *Solanum tuberosum*, *Solanum lycopersicum*, genotype, late blight epidemic

Phytophthora infestans (Mont.) de Bary, the cause of late blight of potato (*Solanum tuberosum*) and tomato (*S. lycopersicum*), is an important disease in the United States. The population structure of *P. infestans* is dynamic (Goodwin et al., 1998; Hu et al., 2012). The US-8 clonal lineage was the predominant genotype and displaced the US-7 genotype in the United States during 1994 (Goodwin et al., 1998). Similarly, during 2009-2010, the dominant US-8 genotype was displaced by new genotypes of *P. infestans* in the eastern United States (Hu et al., 2012). The new genotypes US-22, US-23, and US-24, which are sensitive to metalaxyl and represent mating types A2, A1, and A1, respectively (Danies et al., 2013; Hu et al., 2012). From 2009 to 2012, only the US-8 and US-22 genotypes had been documented in Michigan (Rojas et al., 2014). In 2013 and 2014, late blight appeared in July and August on tomato and potato crops (cv. Snowden, Russet Norkotah) in Allegan, Clinton, Gratiot, Ingham, Ionia, Isabella, Mecosta, Montcalm, Saginaw, St. Joseph and and Washtenaw counties. Infected foliage (five samples from each county) was processed to characterize the collected isolates. Five sections from the leading edge of lesions were excised with a sterilized scalpel and placed on potato tuber slices (‘Dark Red Norland’). Tuber slices were incubated in moist chamber for 5 days at 18°C in the

dark. Sporulating lesions were transferred onto pea agar medium (160 g peas, 5 g sucrose, 15 g agar, 700 ml distilled water) amended with 50 mg/ml vancomycin. Ten pure cultures were obtained for each of 5 isolates per county by hyphal tipping. Cellulose acetate electrophoresis was conducted to determine the Gpi allozyme genotype of all isolates (Goodwin et al., 1995). The allozyme banding patterns were 100/100 at the Gpi locus (Fig 1), consistent with the US-23 genotype (Danies et al., 2013; Hu et al., 2012). Genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Germantown, MD) from 55 pure cultures representing county of origin, and SSR analysis was performed. Microsatellite markers Pi02, Pi4B, Pi63, PiG11, and D13 were used. Pi02, Pi4B, and Pi63 had alleles of 162/164, 213/217, and 270/279 bp in size, respectively, which is consistent with the reference US-23 genotype (Danies et al., 2013). However, heterozygosity was detected at locus D13 in all isolates of Michigan with allele size of 134/210 bp and an additional allele of 140/155/176 bp at locus PiG11. This is in contrast to the standard US-23 genotype (homozygous alleles 134/134 at locus D13 and two alleles 140/155 at locus PiG11). These allelic changes indicate the isolates may be variants of the US-23 lineage with all other phenotypic characteristics similar to the reference US-23 isolates. The Michigan isolates were sensitive to metalaxyl both in-vitro on rye A agar amended with metalaxyl at <0.1 ppm (Fig 2), and in-vivo on Ridomil treated foliage tests at <0.1 ppm (Danies et al., 2013; Hu et al., 2012). Mating type assays confirmed the pathogen to be the A1 mating type. In the 2009 and 2010 US-23 was the predominant genotype responsible for late blight epidemics in the eastern United States, but to our knowledge this is the first report of this genotype recovered in Michigan. This US-23 variant genotype displaced the previously predominant US-22 genotype of *P. infestans*, significantly changing the pathogen population structure in Michigan. The source of US-23 variant genotype remains unknown, although transportation of infected potato tubers or tomato

crop may be responsible for the widespread dissemination of this genotype *of P. infestans* in Michigan.

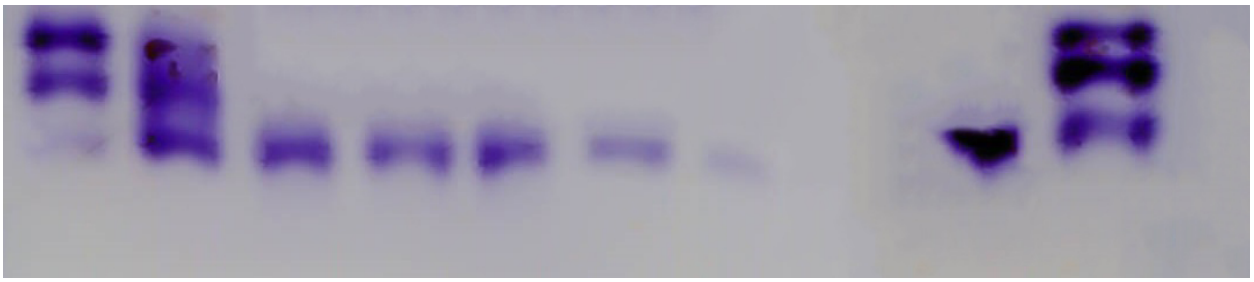
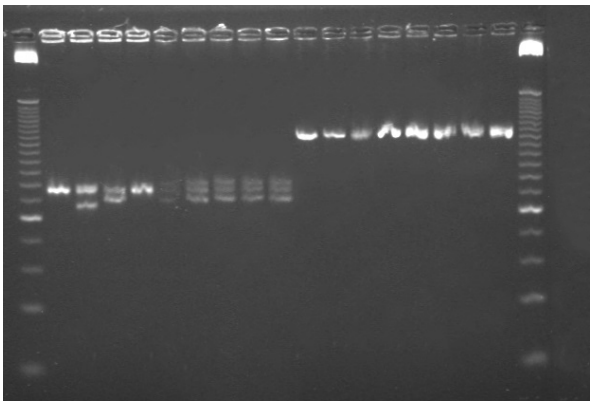


Figure A1: Cellulose acetate electrophoresis gel showing allozyme banding patterns of isolates of *Phytophthora infestans*. In fig from left to right US-22, US-24 (standard isolates), sample 1, sample 2, sample 3, sample 4, sample 5 (isolates collected from Allegan, Montcalm, St. Joseph, Ingham and Mecosta counties, MI in 2013 or 2014), US-23 and US-22 (standard isolates).

A.



B.

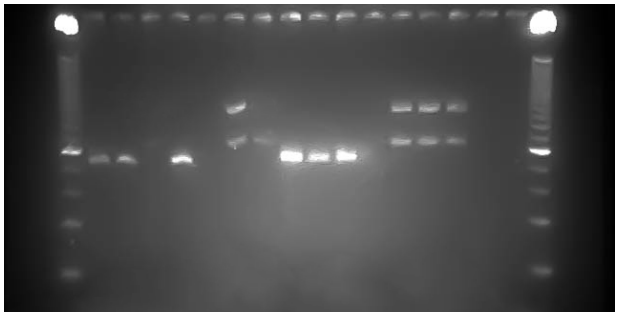


Figure A2: Allelic diversity observed at three loci (G11 and Pi63 in A and D13 in B) in the total population of *Phytophthora infestans* in Michigan. In fig A, from left to right US-8, US-22, US-23, US-24, sample 1, 2, 3, 4, 5 at G11 locus and US-8, US-22, US-23, US-24 and sample 1, 2, 3, and 4 at Pi63 locus. In fig B, from left to right US-8, US-22, US-23, US-24, sample 1 US-23, US-8, US-22, US-23, US-24, sample 2, sample 3 and sample 4. US-8, 22, 23 and 24 were standard isolates and sample 1, 2, 3, 4 and 5 were samples collected from Allegan, Montcalm, St. Joseph, Ingham and Mecosta counties. Ladder used in the gel was 25 base pairs.

A. **B.**

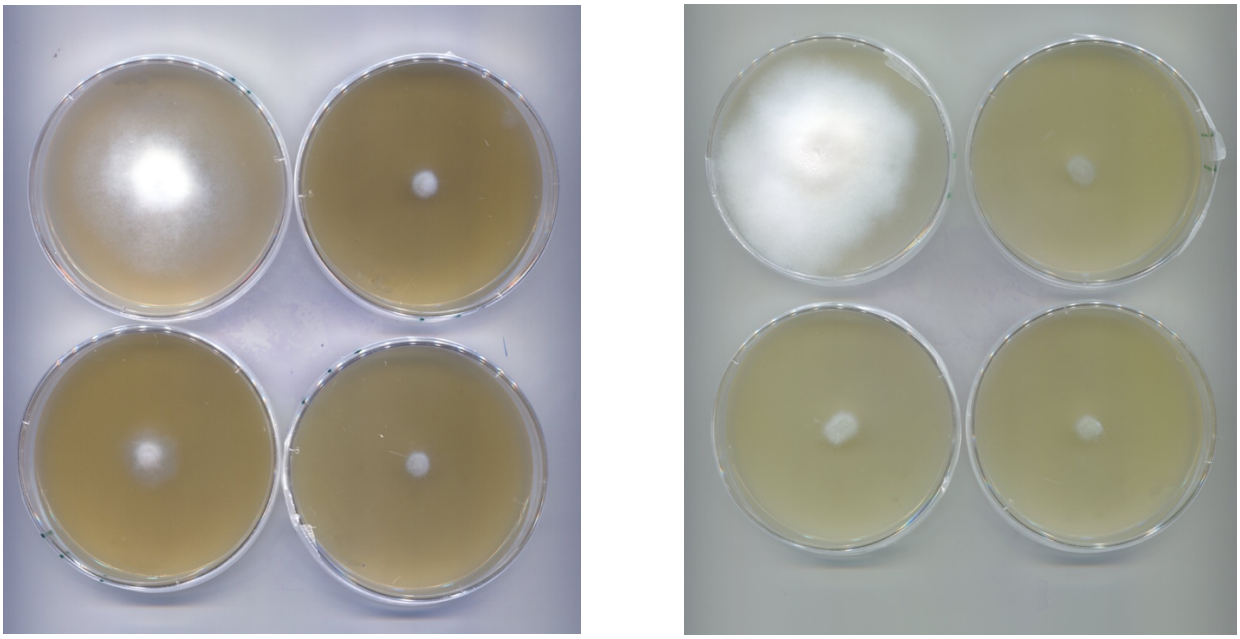


Figure A3: Response of two isolates of *Phytophthora infestans* (A standard US-23 isolate and B isolate collected from Allegan county) to four different concentrations (0, 0.1, 5 and 100 ppm) of Ridomil (active ingredient mefenoxam). In fig A and B from left to right top row 0 and 5 ppm; and bottom row 0.1 and 100 ppm.

HISTOLOGICAL ANALYSES OF POTATO TUBER PERIDERM OF TWO MATURITY STAGES

INTRODUCTION

Potato periderm is a significant barrier to the loss of water and solutes from internal tissue (Lulai and Orr 1994) as well as infection by various pathogens (Lacey 1967; Lulai and Corsini 1998). Periderm consists of the phellem, (suberized cells), phellogen, (a single layer of meristematic cells that give rise to phellem) and phelloderm (the inner persistent living cells produced by phellogen which connects the periderm to the underlying cortical, or storage tissue, Reeve et al. 1969; Sabba and Lulai 2002). The main aim of the study was to assess the development of periderm in immature and mature potato tubers.

MATERIALS AND METHODS

Potato tubers were planted, harvested and cleaned as described in chapter 3, 10 tubers from each cultivar were selected, based on no visible excoriation, to measure the width of the periderm. Periderm disks measuring 1.0 x 0.5 cm (diameter x depth) were obtained by cutting the periderm with sterile sharp knife from the three sections of the tuber of three cultivars. Each periderm disk was fixed by dipping in formal acetic alcohol (FAA, 10% formalin, 50% ethanol, 5% glacial acetic acid and 35% distilled water) for 2 h (Sabba and Lulai 2002) before being embedded in wax (Zeller 1989). All the periderm disks from different sections of the tubers were embedded in the wax serially (apical, middle and basal sections). The blocks were sectioned using a manual rotatory microtome (RM2235, Leica Biosystem, Nussloch, Germany) set to cut 10 μm wide tissue sections. The tissues were mounted on a microscope slide and stained using hematoxylin and eosin stain (Fisher et al. 2008). The number of layers of the phellem and the

width of the periderm were measured using standard light microscopy (Olympus BX41, Olympus America Inc., Center Valley, PA) and QCapture Pro7 software calibrated with ocular ruler (QImaging Corporation, Burnaby, BC, Canada). In addition to the light micrograph histological analyses the mature tubers were observed with a scanning electronic microscope (SEM, Tescan USA Inc., Warrendale, PA). A stub appropriate for the particular SEM being used was selected. The stub was stuck with a convenient double-stick, electrically-conducive carbon tape. Periderm disks ($1cm^3$) were prepared and mounted on carbon tape attached to the stub and observed under SEM.

RESULTS

Hematoxylin stain imparted the blue color and eosin imparted the yellowish brown color in the periderm sections (Fisher et al. 2008). The phellem, phellogen and the phelloderm layers of periderm could be clearly distinguished from the parenchyma underlying the periderm characterized by difference in the color and size of cells. There were no significant differences between results from 2012 and 2013 for the development of periderm in either number of layer of phellem or the thickness of periderm subjected to t-test, so the results were combined and analyzed. Significant differences in the number of layers of phellem and thickness of periderm in tubers of three cultivars (Russet Norkotah, Snowden and Dark Red Norland) at two maturity stages (tubers harvested on the day of vine desiccation and 14 days after vine desiccation) were noticed (Figs B1-B3). Results generally showed an increase of 1-5 layers of phellem in mature periderm compared to the immature periderm of all cultivars (Table B2). The average number of phellem layers was 6, 11 and 7 in immature tubers and these increased to 7, 15 and 8 for mature tubers in DRN, RN and Snowden, respectively (Table B2). A significant change was observed in the periderm thickness of mature tubers compared to immature tubers (Table B3). The average

thickness of the periderm was 117.7, 135.3 and 168.0 µm in immature versus 136.0, 159.8 and 200.7 µm in mature tubers, in DRN, RN and Snowden, respectively (Table B3). Mature DRN was not significantly different from immature Snowden in terms of both the number of phellem layers and the periderm thickness (Table B2 and B3). Overall, RN had a highest number of phellem layers ($p \leq 0.05$) and greatest thickness of periderm ($p \leq 0.05$) compared to the periderms of DRN and Snowden. In addition to the histological analyses, the periderms (n=20) of mature DRN tuber was observed under a scanning electronic microscope (SEM) (Tescan USA, Warrendale, PA) (Fig. B4) and width of phellem was measured (Fig. B5). The thickness of the phellem of mature DRN tuber was 19.1 µm (Fig B5).

Table B1: Main effects analyses of tubers of three different cultivars (Snowden, Russet Norkotah and Dark Red Norland) of potato and two maturity stages (harvested on the day or 14 days after vine desiccation) and interactions between these variables on number of phellem layer and thickness of periderm

	p-value	
Source of Variation	Layer of phellem[a]	Thickness of periderm[b]
Maturity stage	<0.0001	<0.0001
Cultivar	<0.0001	<0.0001
Year	0.2868	0.06351
Maturity stage * Cultivar	0.0003	<0.0001

[a] The layer of phellem is the number of suberized cells arranged in columnar rows in the periderm

[b] Thickness of periderm measured in μm is the total thickness including the phellem layer, phellogen and phelloderm

A B

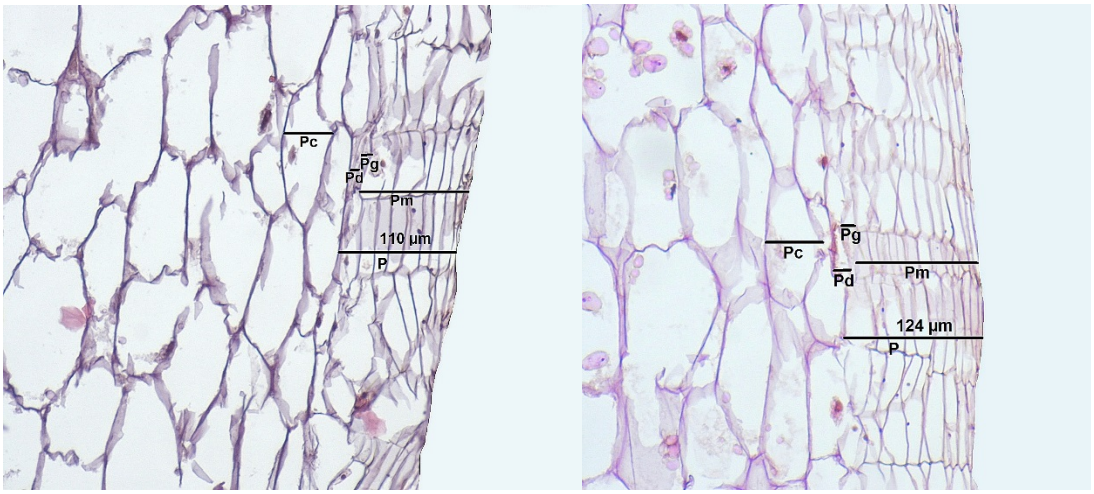


Figure B1: Hematoxylin and eosin staining of the immature, harvested on the day of vine desiccation (A) and mature, harvested 14 days after vine desiccation (B) potato tuber section of Dark Red Norland (DRN); Pc = parenchyma underlying periderm, Pd = phelloderm, Pg = Phellogen, Pm = Phellem and P = Periderm.

A B

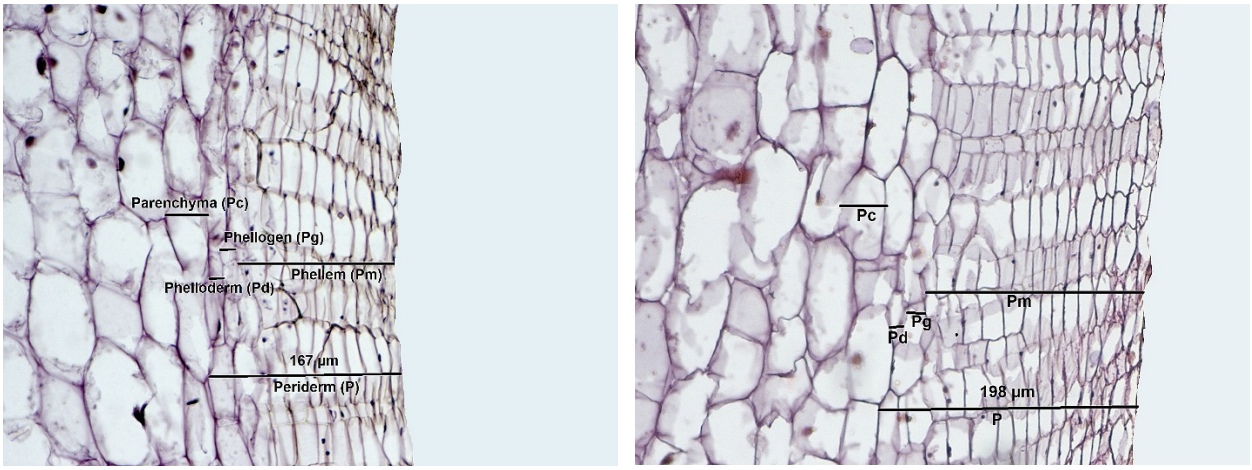


Figure B2: Hematoxylin and eosin staining of the immature, harvested on the day of vine desiccation (A) and mature, harvested 14 days after vine desiccation (B) potato tuber section of Russet Norkotah (RN); Pc = parenchyma underlying periderm, Pd = phelloderm, Pg = Phellogen, Pm = Phellem and P = Periderm.

A B

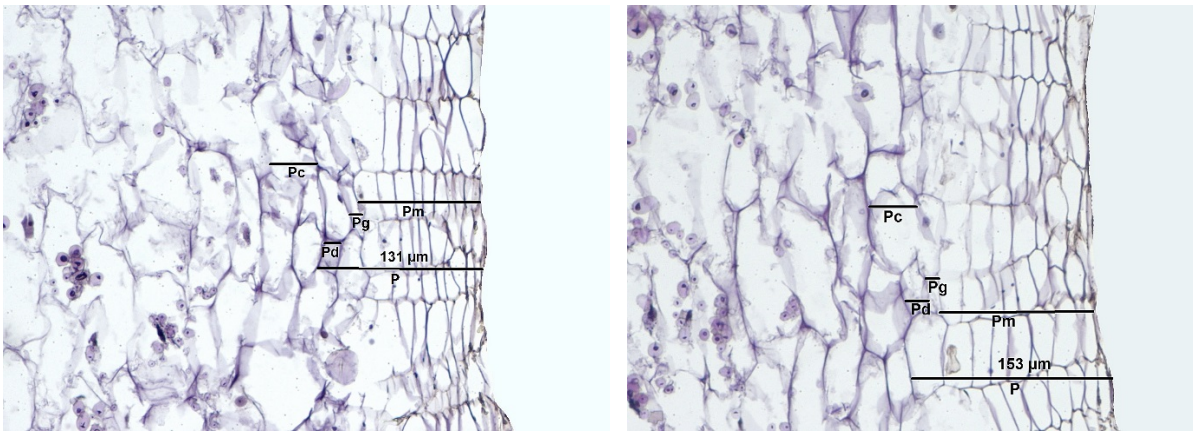


Figure B3: Hematoxylin and eosin staining of the immature, harvested on the day of vine desiccation (A) and mature, harvested 14 days after vine desiccation (B) potato tuber sections of Snowden; Pc = parenchyma underlying periderm, Pd = phelloderm, Pg = Phellogen, Pm = Phellem and P = Periderm.

Table B2: Effect of two maturity stages (harvested on the day of vine desiccation and 14 days after vine desiccation) of tubers of three potato cultivars (Russet Norkotah, Snowden and Dark Red Norland) on increase in number of phellem layers of the periderm as determined by histological analyses

Maturity Stage	Number of phellem layers[a]		
	Russet Norkotah (RN)	Snowden	Dark Red Norland (DRN)
Mature[b]	15 a[c]	8 c	7 d
Immature	11 b	7 d	6 e

[a] Number of phellem layers counted across the periderm for three sections from 20 tubers
[b] "Immature" tubers were harvested on the day of vine desiccation and "mature" tubers were harvested 14 d after vine desiccation.
[c] Values followed by the same letters are not significantly different at $p \leq 0.05$ by Least Square Means Tukey's Honestly Significant Difference.

Table B3: Effect of two maturity stages (harvested on the day of vine desiccation and 14 days after vine desiccation) of tubers of three potato cultivars (Russet Norkotah, Snowden and Dark Red Norland) on the thickness of the periderm as determined by histological analyses

Maturity Stage	Thickness of periderm[a] (μm)		
	Russet Norkotah (RN)	Snowden	Dark Red Norland (DRN)
Mature[b]	200.7 a[c]	159.8 c	136.0 d
Immature	168.0 b	135.3 d	117.7 e

[a] Thickness of periderm measured across the periderm (phellem, phellogen and phelloderm) for three sections from 20 tubers

[b] "Immature" tubers were harvested on the day of vine desiccation; "mature" tubers harvested at 14 d before vine desiccation

[c] Values followed by the same letters are not significantly different at $p \leq 0.05$ by Least Square Means Tukey's Honestly Significant Difference.

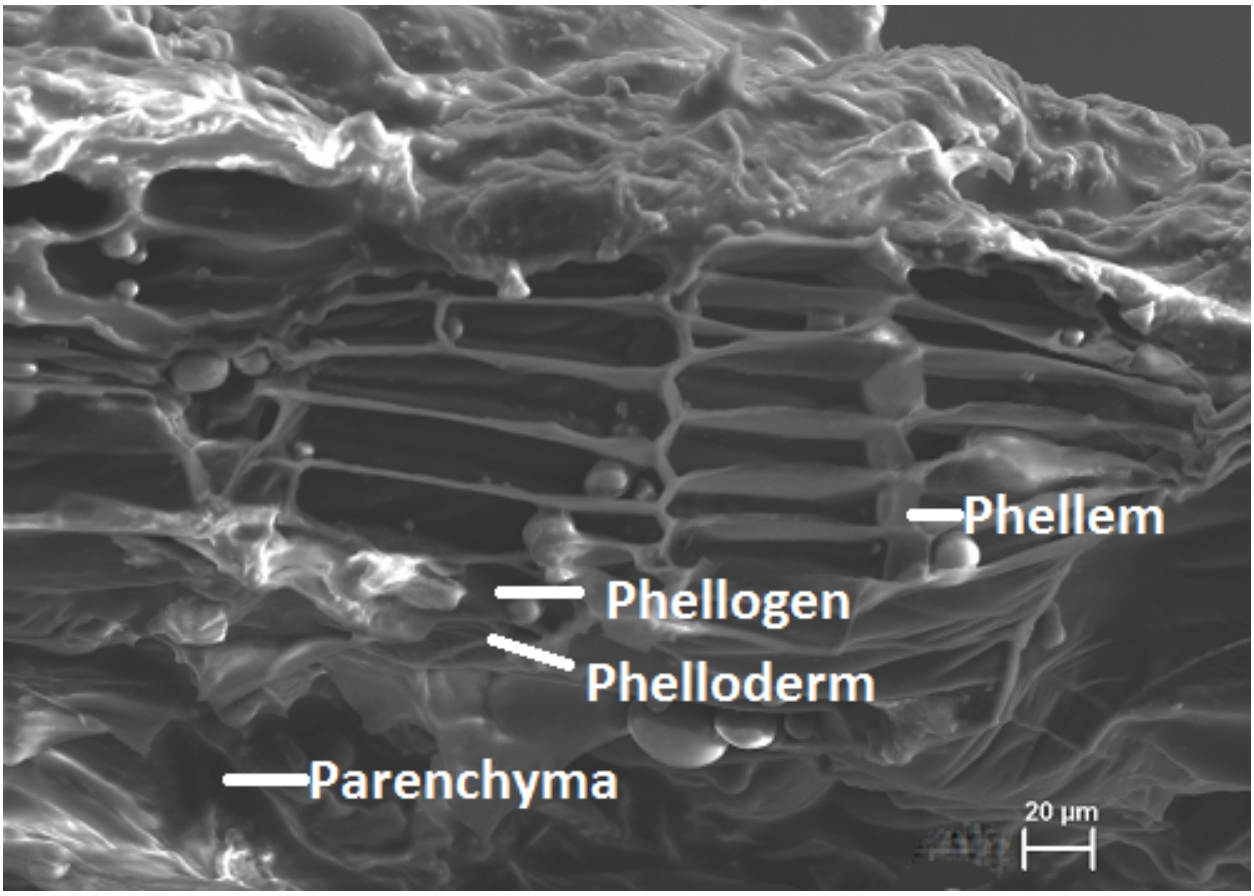


Figure B4: Periderm of mature Dark Red Norland (DRN) potato tuber examined by scanning electronic microscopy showing different periderm sections (phellem, phellogen, phelloderm) and parenchyma underlying the periderm. Bar = 20 µm.

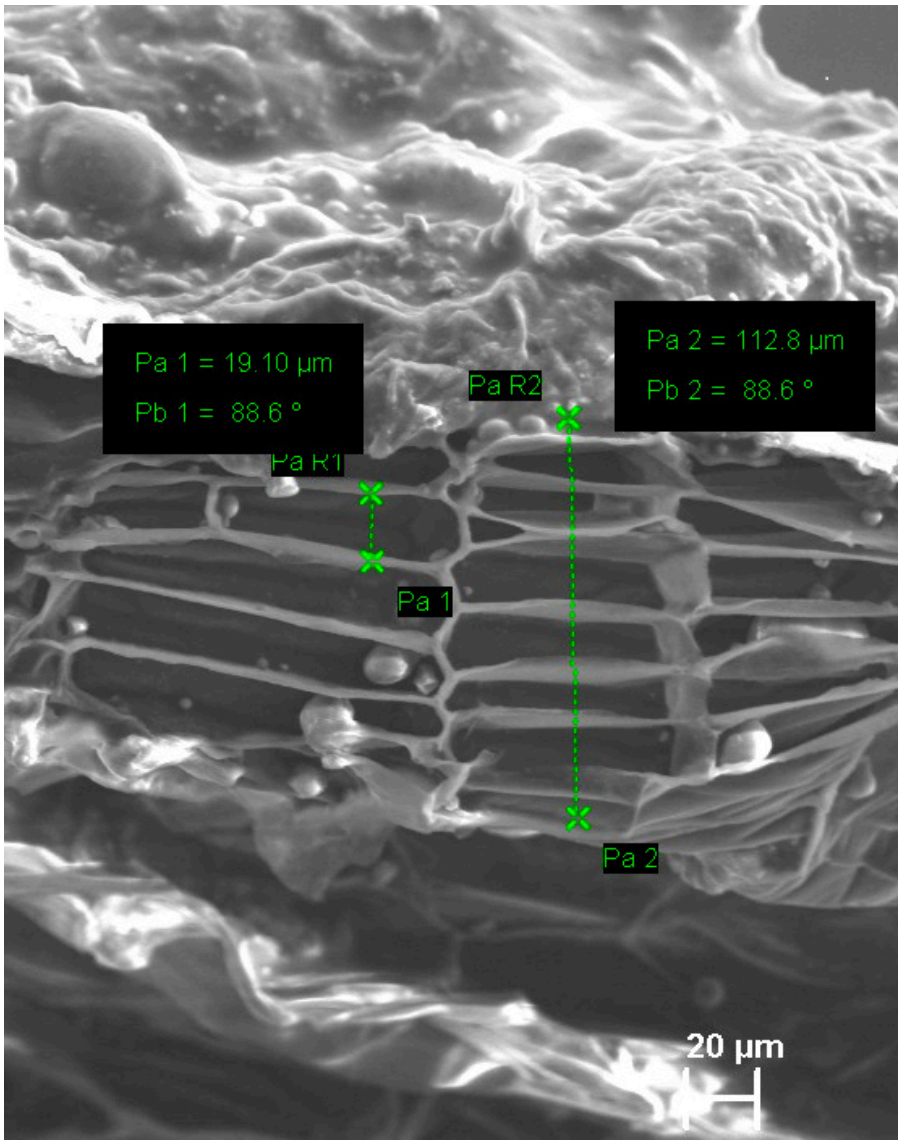


Figure B5: Periderm of mature Dark Red Norland (DRN) potato tuber examined by scanning electronic microscopy with measurement of single layer of phellem and periderm (Pa 1= measurement, Pb 1, Pb 2 = position of the sample, Pa R1, Pa R2 = measurement 1 and 2).

METEREOLOGICAL CONDITION DURING POTATO GROWING SEASON (2011-2014)

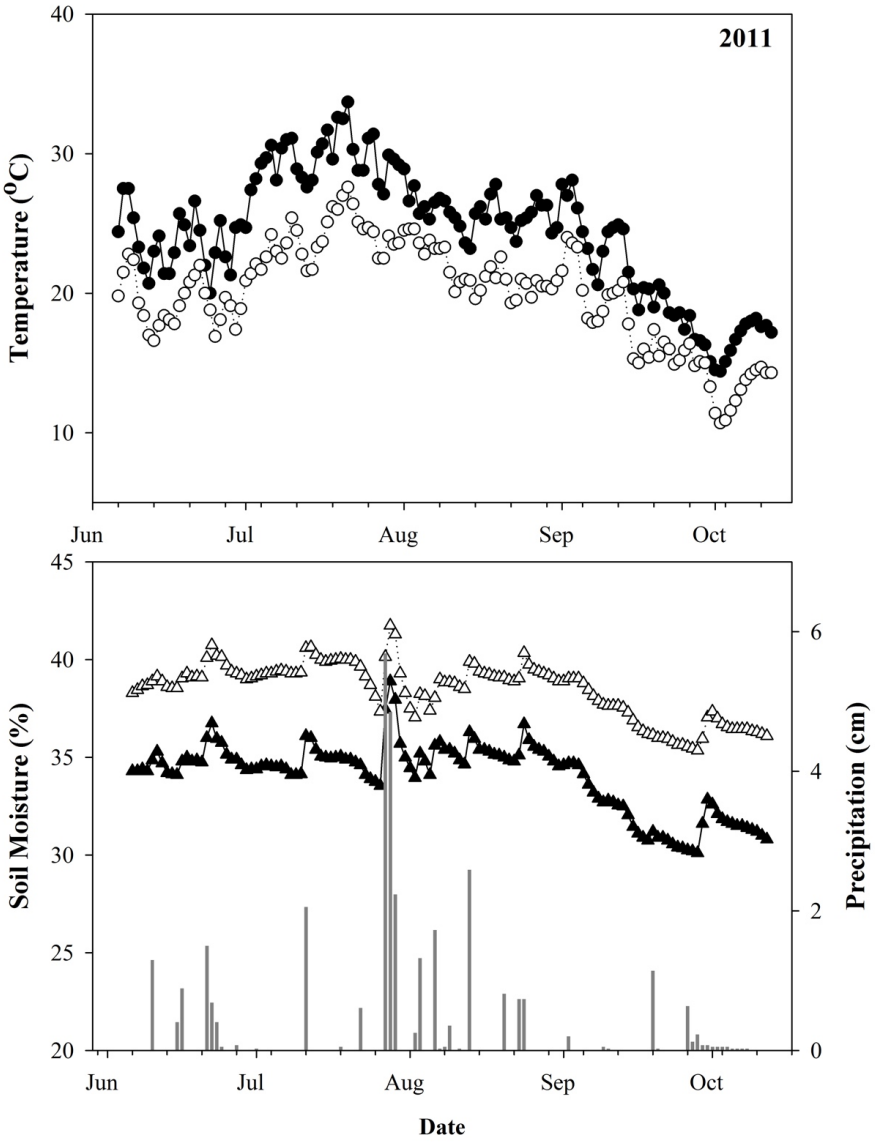


Figure C1: Summary of the meteorological data at the Clarksville Research Center, Michigan State University, Clarksville, MI, 2011. Upper graph shows the minimum (open circle) and maximum (black circle) soil temperature (°C) at a 10 cm depth each day throughout the growing season, from Jun 6 (planting) to Oct 12 (harvest). Lower graph shows the average soil moisture (%) at a 5 cm depth (open triangle) and 10 cm depth (solid triangle) collected from four moisture probes and the amount of precipitation (cm) (grey vertical bars) received each day throughout the growing season. Supplemental irrigation not included.

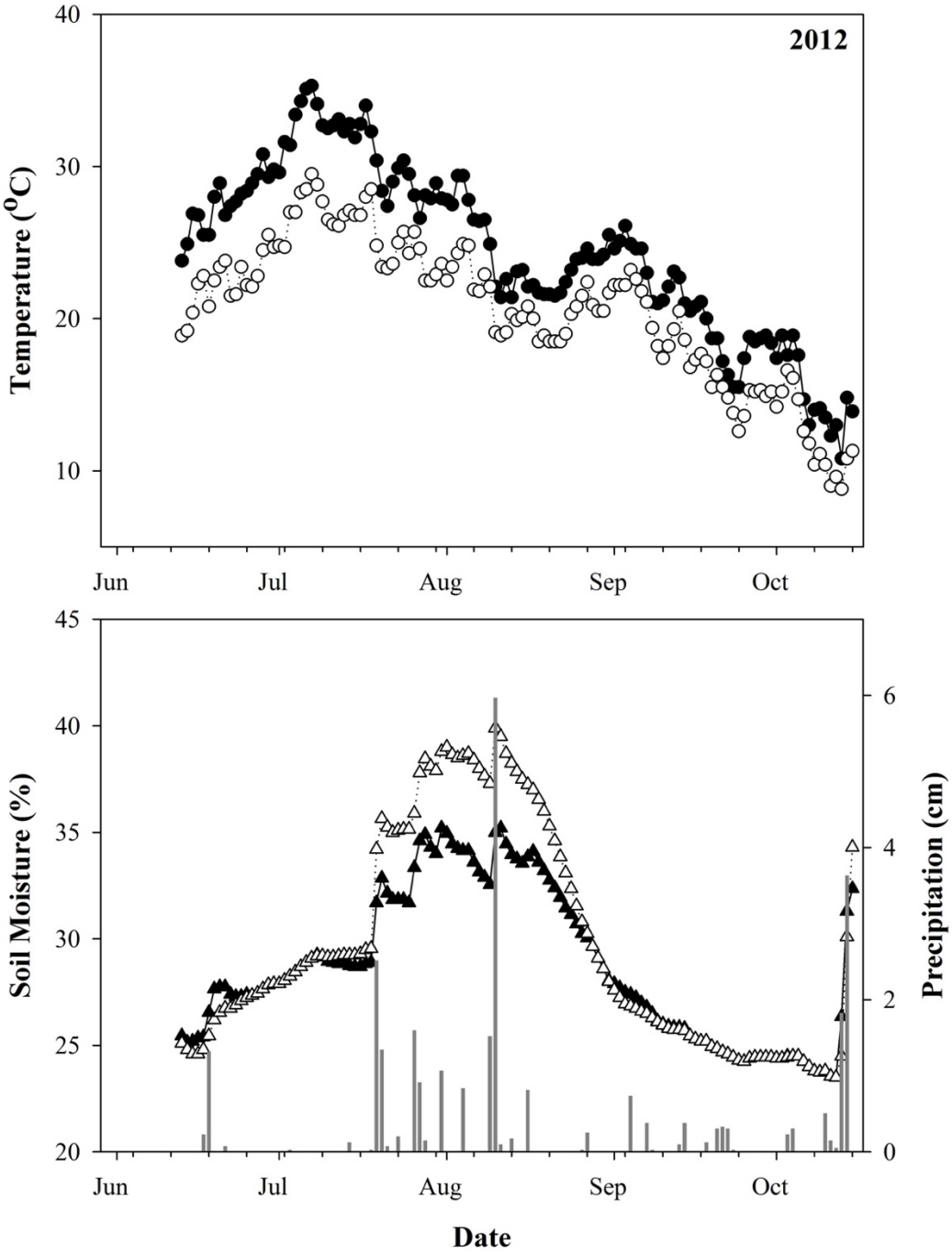


Figure C2: Summary of the meteorological data at the Clarksville Research Center, Michigan State University, Clarksville, MI, 2012. Upper graph shows the minimum (open circle) and maximum (black circle) soil temperature (°C) at a 10 cm depth each day throughout the growing season, from Jun 13 (planting) to Oct 16 (harvest). Lower graph shows the average soil moisture (%) at a 5 cm depth (open triangle) and 10 cm depth (solid triangle) collected from four moisture probes and the amount of precipitation (cm) (grey vertical bars) received each day throughout the growing season. Supplemental irrigation not included.

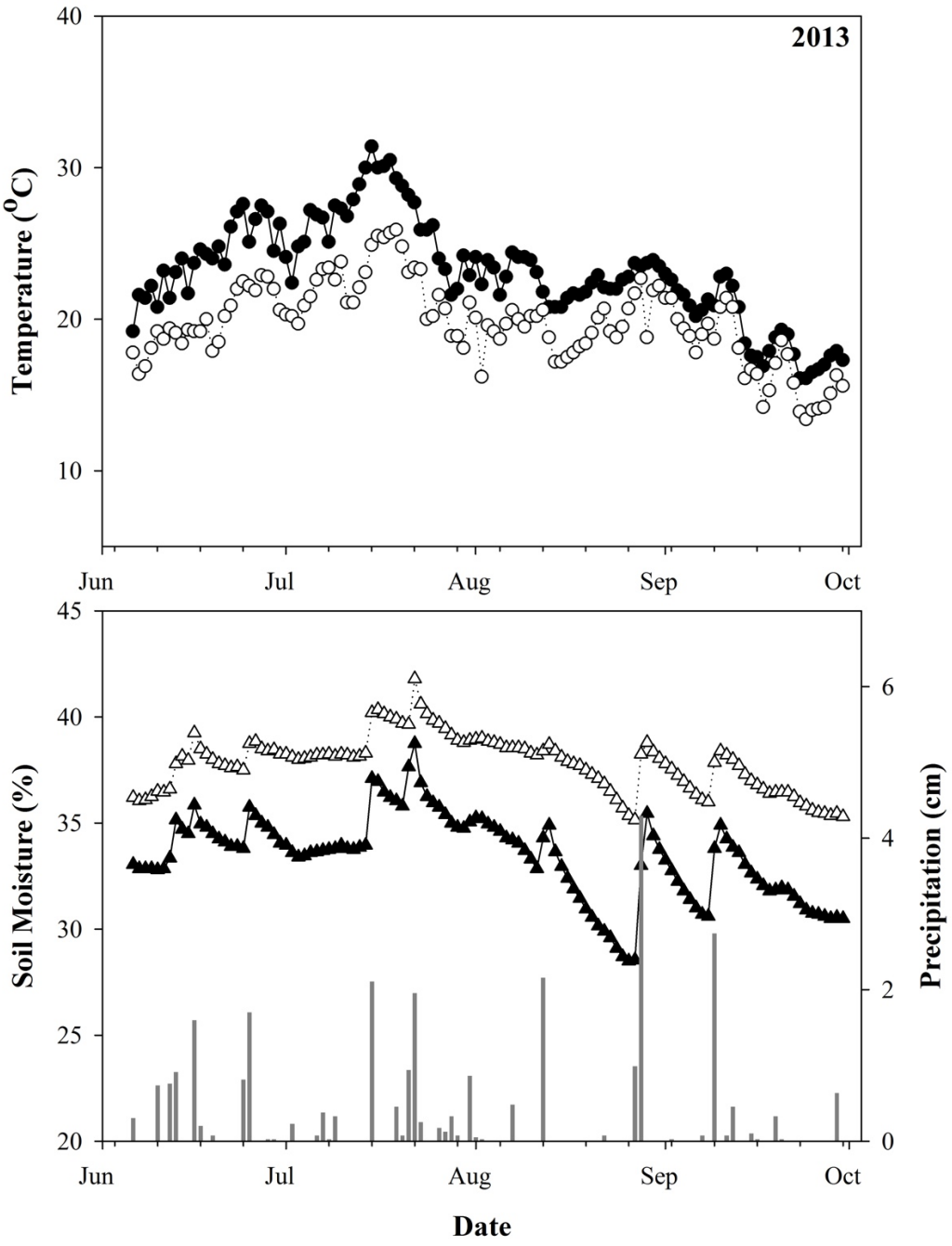


Figure C3: Summary of the meteorological data at the Clarksville Research Center, Michigan State University, Clarksville, MI, 2013. Upper graph shows the minimum (open circle) and maximum (black circle) soil temperature (°C) at a 10 cm depth each day throughout the growing season, from Jun 6 (planting) to Sept 30 (harvest). Lower graph shows the average soil moisture (%) at a 5 cm depth (open triangle) and 10 cm depth (solid triangle) collected from four moisture probes and the amount of precipitation (cm) (grey vertical bars) received each day throughout the growing season. Supplemental irrigation not included.

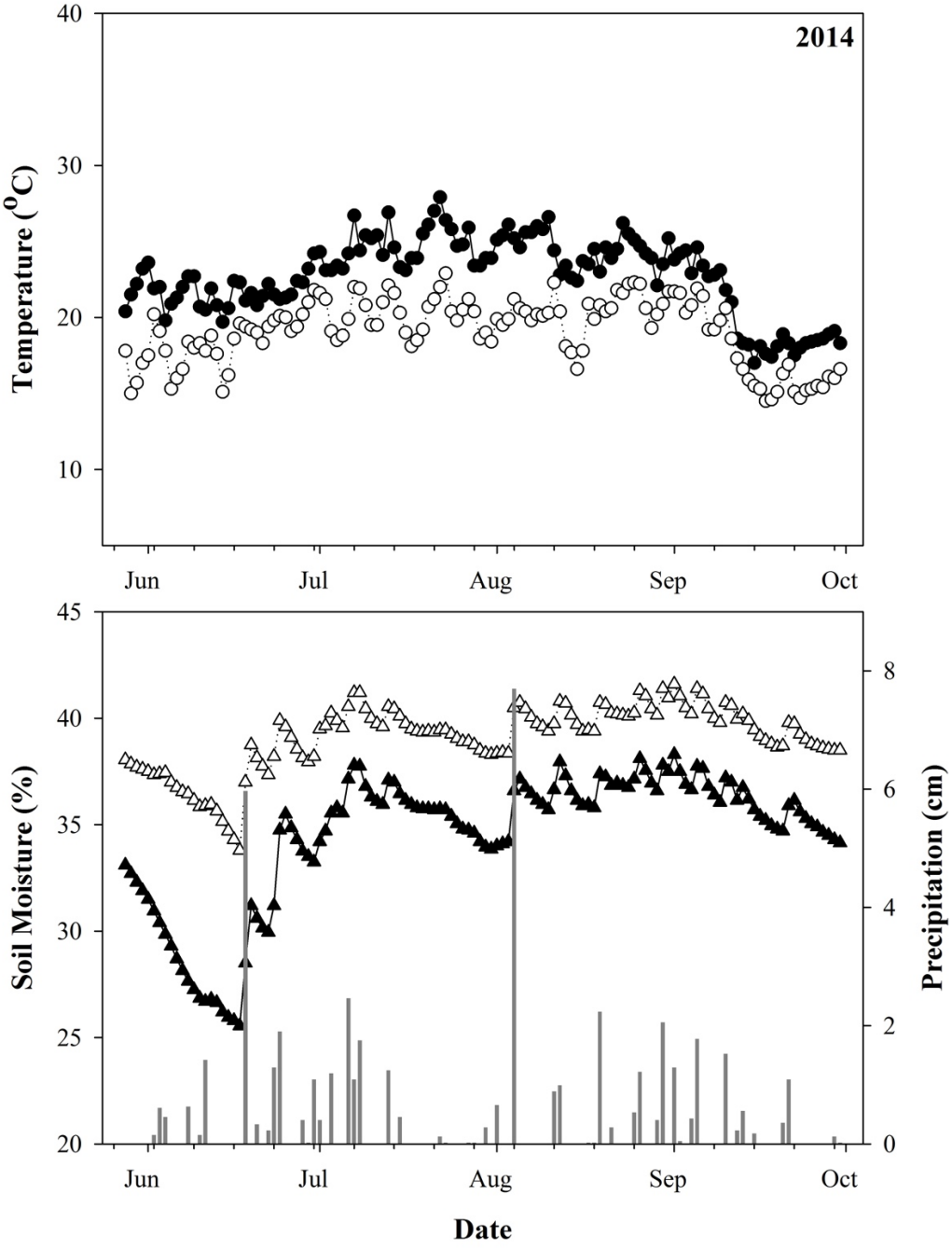


Figure C4: Summary of the meteorological data at the Clarksville Research Center, Michigan State University, Clarksville, MI, 2014. Upper graph shows the minimum (open circle) and maximum (black circle) soil temperature (°C) at a 10 cm depth each day throughout the growing season, from May 28 (planting) to Sept 30 (harvest). Lower graph shows the average soil moisture (%) at a 5 cm depth (open triangle) and 10 cm depth (solid triangle) collected from four moisture probes and the amount of precipitation (cm) (grey vertical bars) received each day throughout the growing season. Supplemental irrigation not included.

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