MANAGEMENT OF POSTHARVEST DISEASES OF POTATO (*SOLANUM TUBEROSUM* L.)

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
For the degree of

MASTERS OF SCIENCE

Plant Pathology

2011

ABSTRACT

MANAGEMENT OF POSTHARVEST DISEASES OF POTATOES (*SOLANUM TUBEROSUM* L.)

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Postharvest diseases of potatoes such as late blight (*Phytophthora infestans*), *Fusarium* dry rot (*Fusarium sambucinum and* spp.), Pythium leak (*Pythium ultimum*) and Pink rot (*Phytophthora erythroseptica*), are responsible for significant economic losses in the potato industry. Management of these diseases using biocontrol fungicides and conventional fungicides was evaluated on cv. FL 1879 at 10^{o}C. Phosphorous acid, hydrogen peroxide and azoxystrobin applied in storage was moderately effective in controlling pink rot, Pythium leak and late blight pathogens compared to *Bacillus subtilis* and *Bacillus pumilus*. In-season application of phosphorous acid followed by bin loading applied phosphorous acid reduced late blight incidence, while field treatment with phosphorous acid or mefenoxam followed by storage treatment with phosphorous acid reduced pink rot and Pythium leak incidence. Field treatment with *B. subtilis* or mefenoxam followed by storage treatment with *B. subtilis*, the 3-way mixture of azoxystrobin, fludioxonil and difenoconazole or phosphorous reduced dry rot incidence. Eleven species of *Fusarium* were isolated from dry rot symptomatic seed potato tubers in Michigan. All the species were pathogenic to potato tubers with *F. sambucinum* being the most aggressive. *In vitro* tests showed that all isolates were sensitive to difenoconazole; only *F. sambucinum* isolates were insensitive to thiabendazole and both sensitive and insensitive-fludioxonil isolates of *F. sambucinum* and *F. oxysporum* were reported. Registration of new chemistries for control of Fusarium dry rot is necessary.

To my dear parents, Alexander and Paulina Gachango,
for their deep love and support.

Acknowledgements

I ascribe glory and honor to Almighty God, thus far he has seen me through. I thank my major professor, Dr. Kirk, and my committee members, Drs. Linda Hanson, Ray Hammerschmidt and Jay Hao for their guidance throughout the program. Much appreciation to the members of Kirk's lab for their numerous support. Also, I wish to thank the USDA-ARS, Michigan Green Project and the Michigan Potato Commission for funding this work. Last but not the least, I am grateful to my family and friends for their love and support throughout the program.

To any unmentioned persons who made this work a success, receive my appreciation.

May God bless you all!

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Literature Review

Importance of potato disease management

Potato (*Solanum tuberosum* L.*)* is ranked fourth in world's food crop production after wheat, maize, and rice (Bradshaw and Ramsay, 2009). Potato production has increased over the past years (Guenthner, 2010). However, disease incidence both in the field and storage remains the major limiting factor in a profitable potato production (Secor and Gudmestad, 1999). Potato production throughout the year is not feasible in North America, (Sonnewald, 2001) hence long-term storage is essential to maintain year-round delivery of fresh market and chip processing potatoes. Similarly, seed potato tubers are also stored for at least six months before they are shipped out for planting (Olsen, 2010). Therefore, good storage conditions are essential to maintain the quality of potatoes, which can be compromised through disease development and weight loss (Secor and Gudmestad, 1999). So far, 75 diseases and non-parasitic disorders have been reported to cause yield and quality loss in potato production areas of the northern United States (Zitter and Loria, 1986; Secor, 2009). Fungal pathogens predominate, causing a total of 35 diseases, while viruses cause 12, bacteria seven, and nematodes six, respectively (Secor, 2009).

In North America, diseases caused by fungal and fungal-like pathogens are the most detrimental to potato production. These diseases include late blight (*Phytophthora infestans*), dry rot (*Fusarium sambucinum* and spp.), pink rot (*Phytophthora erythroseptica*), Pythium leak (*Pythium ultimum*), and silver scurf (*Helminthosporium solani*) (Secor and Gudmestad, 1999). These pathogens are both soilborne and seedborne, hence the diseases have both field and storage stages (Powelson and Rowe, 2008). Infection of plants in the field occurs on underground stems, stolons, or roots as tubers develop (Powelson and Rowe, 2008). Tubers can be infected by pathogenic fungi, oomycetes, and bacteria through lenticels, eyes and wounds

inflicted during harvesting and loading into storage (Powelson and Rowe, 2008). The pathogens are also carried into storage either in soil adhering to tubers or as latent infection in tubers (Cullen *et al.*, 2005) . Once the tubers are stored, rapid suberization should be promoted through appropriate storage management (Knowles and Plissey, 2008).

The health of potato tubers in storage does not improve over time, but can be maintained by ensuring a proper storage environment (Knowles and Plissey, 2008). Once the tubers are harvested, they have to be cured at optimum temperature (10-13^{o}C), high humidity (95%), and good ventilation for two weeks (Powelson and Rowe, 2008). Later, the recommended holding temperatures are set depending on the ultimate use of the tubers. Potato tubers destined for seed are stored between 3.3 and 4.4^{o}C, fresh market potato tubers are stored between 3.3 and 10^{o}C, while those for chip processing and French fries are stored at 10 to 13^{o}C and 7 to 10^{o}C, respectively (Knowles and Plissey, 2008). The virulence of most pathogens increases with increase in temperature in storage (Kirk *et al.*, 2010). However, some pathogens have a lower cardinal profile. For instance, *P. infestans* has been reported to survive asymptomatically in potato seed tubers stored at 4^{o}C (Johnson and Cummings, 2009). The silver scurf pathogen, *Helminthosporium solani*, sporulated on seed tubers held at 4^{o}C and on processing tubers held at 10^{o}C (Secor and Gudmestad, 1999). Some dry rot pathogens, *Fusarium* spp. have been reported to cause infection at 5^{o}C, while most of the species have their optimum temperatures ranging from 10-15^{o}C, and others range from 25-30^{o}C (Daami-Remadi *et al.*, 2006a). Therefore, management of diseases on potato seed tubers during extended period of storage is critical.

Proper storage environment in combination with use of postharvest fungicides and disinfectants is important for managing losses during the storage period (Knowles and Plissey, 2008). Bin loading application of fungicides as tubers go into storage is a common practice to manage postharvest diseases (Knowles and Plissey, 2008). However, this practice is only successful if the tubers coming into storage are disease free, or have a very low of disease incidence. Loss estimates of up to 100% have been reported in storage both in developed and developing countries (Wale *et al.*, 2008) leading to insufficient planting material for the following season.

Potatoes are vegetatively propagated throughout the world by use of seed potato tubers (Secor and Rivera-Varas, 2004). Infected seed tubers in storage are a source of primary inoculum once the tubers are planted at warmer temperatures (Powelson and Rowe, 2008; Johnson and Cummings, 2009). Studies have shown that planting infected tubers results in disease development in the field (Johnson, 2010), and yet the best techniques to fully manage seedborne potato diseases are not practiced. Integrated disease management including use of resistant cultivars, certified seeds- free from diseases, crop rotation, nutrition management and use of chemicals is common in potato production (Secor and Gudmestad, 1999; Powelson and Rowe, 2008). However, chemical application in the field remains the key practice for controlling potato diseases and increasing tuber resistance against pathogens during storage (Secor and Gudmestad, 1999).

Chemical use to control potato diseases has been practiced for over a century (Fernández-Northcote *et al.*, 2000). The copper salts, Bordeaux mixture, invented in the 1880's, were the first effective compounds against late blight, and were considered the first generation of fungicides (Forbes and Landeo, 2006). In the 1940's, the second generation of fungicides, which

were organic chemicals were introduced. These products were dithiocarbamates and 1,2-bisdithiocarbamates, (Kaur and Mukerji, 2004) and included zineb, maneb, metiram, mancozeb, and propineb (Forbes and Landeo, 2006). Introduction of products with a broad spectrum, that could control *Phytophthora infestans* and fungal pathogens then followed. These products included chlorothalonil and phthalimides (captan and folpet) (Whisson, 2010).

The third-generation fungicides were more specific and penetrated the plant tissue (Waard *et al.*, 1993). These included cymoxanil (cyanoacetamide oximes), carbamates (prothiocarb and propamocarb), benzimidazoles (benomyl and thiabendazole; TBZ), phosphonates (fosetyl-Al), and carboxylic acid amides (mandipropamid and dimethomorp), and phenylamides (metalaxyl) (Waard *et al*., 1993; Whisson, 2010). Metalaxyl was the most effective in controlling late blight among the phenylamides (Schwinn and Margot, 1991), until metalaxyl- resistant isolates of *Phytophthora infestans* were developed. Benzimidazole fungicide, TBZ, has been used to control Fusarium dry rot for over 30yrs and has effectively reduced infection of seed potatoes (Leach and Nielsen, 1975). However, its effectiveness was reduced by development of TBZ-resistant isolates of *Fusarium sambucinum* (Hide *et al.*, 1992). The fourth generation of fungicides were later introduced, and consisted of products that are nonfungitoxic, but are able to interfere with fungal penetration, and trigger plant defense mechanisms (Waard *et al.*, 1993). Introduction of more fungicides and biofungicides has increased, but only limited research has been done to evaluate and compare their efficacy during postharvest management.

Late blight

Potato late blight caused by an oomycete, *P. infestans*, is a disease that affects foliage and tubers resulting in high yield losses (Kirk *et al.*, June 2004). Late blight is the most devastating

known potato disease worldwide (Fry and Goodwin, 1997; Stevenson, 2008). Occurrence of late blight has been the major limiting factor to potato production in North America (Guenthner *et al.*, 2001). The Midwest states have very conducive climatic conditions for late blight epidemics (Baker *et al.*, 2005) resulting in crop protection costs of up to $700/ha and crop losses up to $5,000/ha when intervention measures to control potato late blight are not successful (Guenthner *et al.*, 2001). Infection of potato tubers in the field is initiated most commonly by inoculum, i.e., sporangia and zoospores, produced on the plant foliage. Developing tubers can become blighted shortly after the pathogen is established on potato foliage (Hirst *et al.*, 1965). These same pathogen propagules continue the infection process on the tubers during storage causing significant losses.

Late Blight development

Phytophthora infestans, the causal agent of potato late blight, is an oomycete and exists as different genotypes. Several genotypes have commonly been found in the United States through history but sporadically appear and are quickly displaced (Stevenson, 2008) e.g. US-1 (no longer found), US-6, US-7, US-8, US-14 and US-22 (since 2008). US-1 and US-6 are the A1 mating type, while US-7, US-8, and US-14 genotypes are A2 mating type (Fry and Goodwin, 1997). In Michigan, the previously predominant genotype of *P. infestans* (US-1) was displaced by the new and more aggressive genotype (US-8), which is responsible for the foliar epidemics (Young *et al.*, 2009). As a result, late blight epidemics have increased leading to increase in yield and losses thus a reduction of grower's income along with high reliance on costly application of fungicides (Inglis *et al.*, 1996). Since 2009, US-8 has been rapidly displaced by US-22 (Kirk, personal communication).

The presence of both mating types may lead to production of oospores. They are resistant to freezing and other harsh conditions and can survive in plant debris or in the free soils becoming a source of inoculum for late blight epidemics (Yuen *et al.*, 2008). However, chances for sexual reproduction are limited because a single mating type dominates most populations. In Michigan both mating types (A1 and A2) have been discovered but there is no evidence of any sexual recombination (Young *et al.*, 2009). The same case applies to most potato production areas in the US, where oospores of *P. infestans* are rare, and infection of potato tubers in the field is initiated most commonly by sporangia and zoospores, produced on the plant foliage (Fig. 1); (Kirk *et al.*, June 2004). The sporangia produced on the foliage is washed off by rain or irrigation water into the soil and are able to germinate at temperatures between 6.7 and 12.7^{o}C (Stevenson *et al.*, 2008). The sporangia may also move in a water film down the stems and stolons, or through cracks in the soil (Johnson, 2010). Once the sporangia get into contact with the progeny tubers, infection takes place either through lenticels or the eyes and eventually the whole tuber becomes infected reducing the quality of the harvest and may lead to a total crop loss (Yuen *et al.*, 2008).

The other major source of inoculum in late blight epidemics is the use of infected seed tubers. In Michigan, potato seed tubers are stored from early September to early June at 4^{o}C (Heather, 2000). Studies have shown that *P. infestans* can survive in tubers during storage at temperatures as low as 4^{o}C in a latent form (Johnson and Cummings, 2009). These tubers are mostly asymptomatic and more often used as seed. The pathogen is then disseminated from one tuber others during cutting and handling leading to infection of the below and aboveground stem (Hirst and Stedman, 1960; Lambert *et al.*, 1998; Kirk *et al.*, 2009).

Chances of having *Phytophthora infestans* propagules in the soil are high due to left over infected tubers, cull piles and overwintering mycelium (Stevenson, 2008). Hence, the importance of modifying the previous chemical control practices to control both foliage and tuber blight of potatoes.

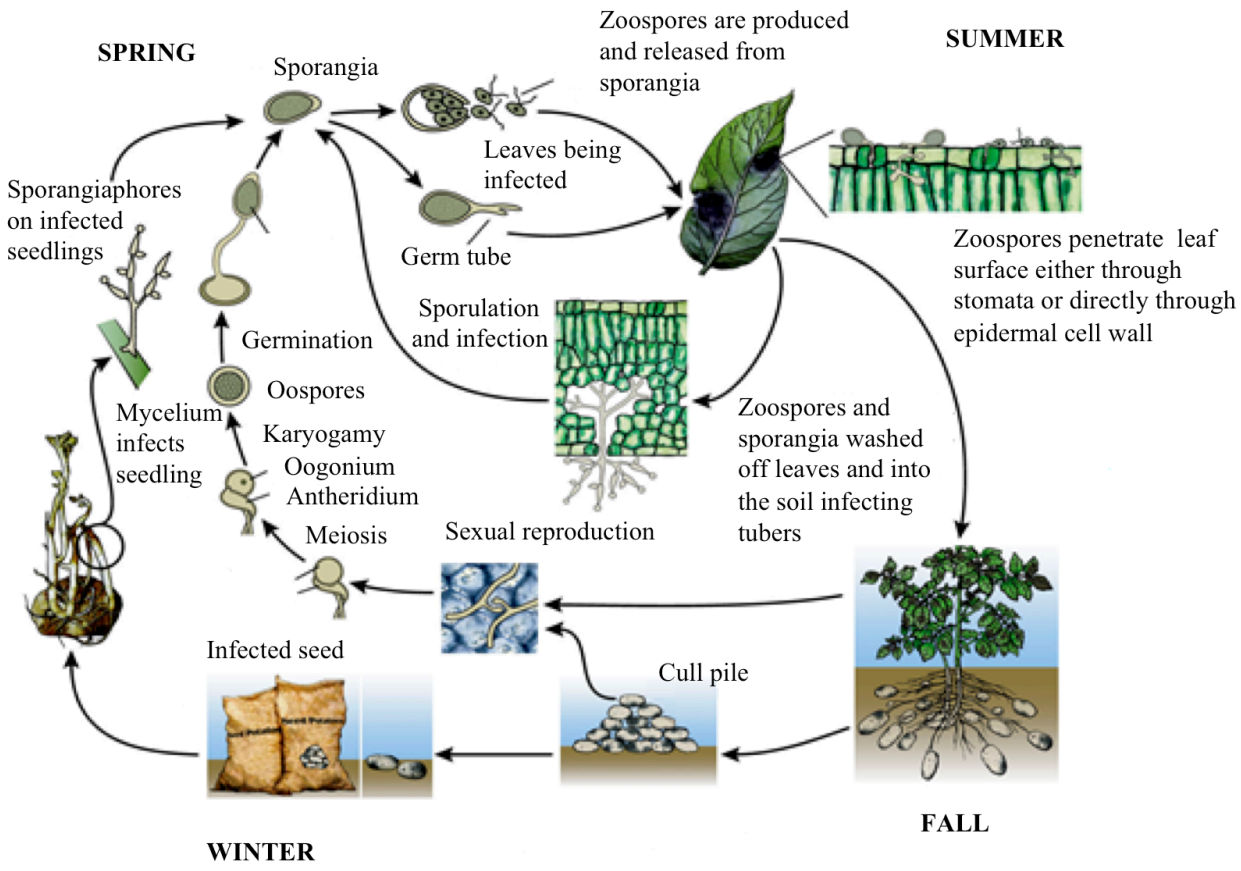

Figure 1 Life cycle of potato late blight pathogen, *Phytophthora infestans*. (Kirk *et al.*, 2004)

"For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis,"

Management of potato late blight

Since late blight affects potato in the field and in storage, management practices should be employed during the growing season as well as in storage (Secor and Gudmestad, 1999). Integration of cultural practices with chemical use is the most commonly used strategy to manage late blight, and aims at reducing direct contact of inoculum with tubers (Stevenson *et al.*, 2008). However there is still high reliance on use of chemical to control late blight development. Bordeaux mixture, a copper compound was the first fungicide to be used in control of late blight (Fernández-Northcote *et al.*, 2000). After Bordeaux mixture, dithiocarbamates and 1,2-bisdithiocarbamates were introduced between 1940 and 1960 (Kaur and Mukerji, 2004) followed by introduction of broad spectrum fungicides, chlorothalonil and phthalimides, (captan and folpet) (Whisson, 2010). More specific fungicides, which penetrated the plant tissue and controlled established infections were also introduced (Fernández-Northcote *et al.*, 2000). Among them was a phenylamide, metalaxyl, which effectively controlled potato late blight until the appearance of mefenoxam/ metalaxyl resistant *Phytophthora infestans* genotypes (Stevenson, 2008). Subsequent production and registration of other chemicals in the U.S followed to counteract the loss of metalaxyl. These were fungicides containing cymoxanil, dimethomorph and propamocarb hydrochloride. In addition, other products containing azoxystrobin, chlorothalonil, copper hydroxide, fenamidone, mancozeb, metiram, mandipropamid and triphenyltin hydroxide were developed (Stevenson, 2008).

Recommendation for reduced rates of fungicide applications with longer application intervals has been found to be the most economical strategy to control the disease in combination with the use of host resistance (Kirk *et al.*, 2001a). However, many growers believe that use of higher rates of fungicide application with fewer intervals throughout the growing season yield

the best control (Skelsey *et al.*, 2009). While this may be good for the contact fungicides, the effectiveness is highly dependent on the prevailing weather conditions (Fernández-Northcote *et al.*, 2000).In the northern US, majority of the cultivars grown are susceptible to *Phytophthora infestans* (Stevenson, 2008). Efforts in breeding have resulted into potato cultivars that have a high level of resistance against *P. infestans* (Kirk *et al.*, 2001b; Douches *et al.*, 2004) and so far one cultivar, Jacqueline Lee, has been developed in Michigan breeding program (Douches *et al.*, 2001). Other cultural practices like implementing soil covers and hilling are based on the ability to directly suppress inoculum or filter it out before it reaches the tubers and can be effective (Nyankanga *et al.*, 2008).

Pythium leak

Pythium leak, also known as watery rot or shell rot of potato tubers, is a disease caused by the soilborne oomycetes *Pythium* spp. (Salas and Secor, 2001). These species include *Pythium ultimum* Trow, *P. debaryanum* Hesse, and *P. aphanidermatum* (Edson) Fitzo (Salas and Secor, 2001; Platt and Peters, 2006). *Pythium ultimum* is considered the most common *Pythium* species causing Pythium leak of potato (Salas and Secor, 2001). It infects potato seed pieces, tubers in the field prior to harvest, at harvest, or after placing the tubers in storage facilities, causing severe yield losses (Secor and Gudmestad, 1999; Salas and Secor, 2001; Salas *et al.*, 2003).

Pythium ultimum is found in almost all potato growing areas, and is endemic to most soils (Salas and Secor, 2001). It is able to survive in the soil and infected plant tissue or debris for many years in the form of oospores. *Pythium ultimum* has a wide host range and has been reported to cause root rot and seedling damping off of many crops including peas, corn, carrots,

onions, beans, and cereals (Salas and Secor, 2001; Paulitz and Adams, 2003; Broders *et al.*, 2007).

Pythium leak development

Pythium ultimum cannot infect unwounded tubers because it cannot penetrate the periderm tissue (Taylor *et al.*, 2004). Occasionally, *P. ultimum* may enter the tuber through lenticels or the stem end but infection predominantly originates from wounds and thus tubers are more vulnerable to infection during harvesting, transport, and loading into storage facilities. Germinating sporangia or oospores enter the tubers through cut seed pieces (Powelson and Rowe, 2008) or wounds inflicted during harvesting, and a germ tube grows and invades the inner tissues, which results in tissue disintegration (Platt and Peters, 2006).

Potato cultivar, soil moisture level, and temperature affect the development of leak when tubers are still in the field. Seed tubers planted when the soils are near saturation and the soil temperatures are above 21^{o}C, readily get infected and become a soft watery mass resulting in delayed crop emergence and a non-uniform stand establishment (Powelson and Rowe, 2008). Similarly, wet conditions and high temperature during harvesting increase the chances of tuber infection (Salas and Secor, 2001) with increased disease severity reported when wetness duration and temperature are increased (Lui and Kushalappa, 2003).

Pythium leak infection is initiated in the field and the symptoms become severe in storage (Salas and Secor, 2001). The infected tubers become discolored, smoky gray to black in color (Fig. 2a), with further development of water-soaked lesions (Platt and Peters, 2006). Under favorable conditions in storage, the lesions expand and eventually become watery rots that are evident once the tuber is cut open (Fig. 2b). The rotten internal tissue becomes discolored (gray to brown) once exposed to air and a reddish brown to black boundary line delimits the rotten

zone. The completely rotted tubers exudate semi-liquid contents; when they are squeezed, an empty shell is left, thus the name shell rot (Powelson and Rowe, 2008).

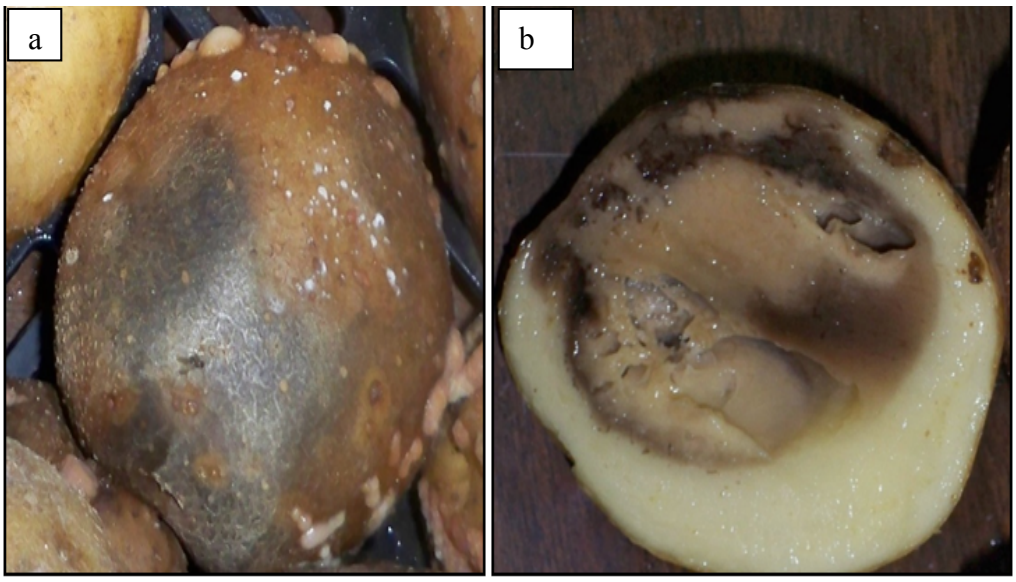

Figure 2 Tuber infected with Pythium leak discolored on the outer skin (a). The cut surface of infected tuber with the rot delimited by a brown boundary line (b).

Management of Pythium leak

Management of Pythium leak is basically designed to reduce any condition which favors infection and disease development. These include cultural practices such as field selection to avoid fields with a history of the disease or with poorly drained soils (Salas and Secor, 2001), and crop rotation practices with non-host crops (minimum of three years between potato crops) (Powelson and Rowe, 2008). Excessive irrigation should be avoided especially towards the end of the season to allow sufficient time for vine killing allowing a good skin set that is essential for reducing wounding during harvesting operations (Powelson and Rowe, 2008). Tubers should be harvested during cool dry conditions when the skin is fully set and the tuber pulp temperature is below 21^{O}C (Secor and Gudmestad, 1999; Salas and Secor, 2001). If the disease is detected in storage, the temperature should be lowered to12-15^{O}C, air circulation increased, and dehumidifiers employed immediately (Platt and Peters, 2006).

Seed tubers should be warmed to 10-13^{O}C before cutting them to reduce bruising during handling, promote rapid healing of cut surfaces, and enhance sprouting before planting (Secor and Johnson, 2008). A temperature above 13^{O}C should be avoided because it leads to excessive sprouting which has a negative effect on yield (Secor and Johnson, 2008), and also favors the deveolpment of seed piece decay (Powelson and Rowe, 2008). Seed treatment using fungicide has been effective in controlling seed decay caused by *Pythium ultimum* (Platt and Peters, 2006). A mixture of fludioxonil and mancozeb (Maxim^{TM} MZ) has been reported to effectively reduce seed piece decay prior to planting (Wharton *et al.*, 2007b). In addition, in-furrow application of fungicides during planting and foliar application at tuber initiation stage reduces the risk of infection of the progeny tubers (Platt and Peters, 2006). Mefenoxam and metalaxyl are

fungicides which effectively reduce Pythium leak of potato tubers. Over time, resistance to these fungicides has been reported in populations of *P. ultimum* in North America (Powelson and Rowe, 2008), but the population is still largely sensitive (Taylor *et al.*, 2002; Porter *et al.*, 2009). Foliar application of mefenoxam did not control leak, while in-furrow application at planting followed by sidedress application three weeks after planting showed a limited control (Taylor *et al.*, 2004).

Pink rot

Pink rot is a disease named for the pink coloration that develops on infected tissue once it has been cut and exposed to air. Pink rot is caused primarily by the oomycete *Phytophthora erythroseptica* Pethyb (Lambert and Salas, 2001). Other soilborne *Phytophthora* spp. which have been implicated in causing the disease include *Phytophthora crytogea* and *Phytophthora parasitica* (Grisham *et al.*, 1983). Pink rot is found worldwide in potato growing regions (Lambert and Salas, 2001). It was first reported in the US in Maine in 1938 (Bonde, 1938), and has spread to most potato production areas in North America (Taylor *et al.*, 2002; Peters *et al.*, 2005). Pink rot affects roots and tubers in the field as well as tubers in storage (Powelson and Rowe, 2008) and significant yield losses both pre and post harvest have been reported (Salas *et al.*, 2003).

Pink rot development

Phytophthora erythroseptica is endemic to most soils worldwide and can survive in soil for several years in the form of oospores (Lambert and Salas, 2001; Wharton and Kirk, 2007). When soils are near saturation, the oospores germinate into sporangia which later release zoospores. The zoospores move through the soil in the water film and infect tubers especially when tuber temperature is above 20^{o}C (Wharton and Kirk, 2007; Powelson and Rowe, 2008).

The pathogen infects the progeny tubers through lenticels and eyes but most of the infection occurs through diseased stolons (Lambert and Salas, 2001; Powelson and Rowe, 2008). Although pink rot is commonly noticed in storage, initial infections most likely occur in the field prior to harvest (Lennard, 1980). Harvested tubers can be contaminated with spores on the surface, which later enter the tuber through wounds inflicted during harvesting or through lenticels, causing further infection in storage (Powelson and Rowe, 2008). The result is tuber decay, which usually starts from the stem end and progresses through the tuber in a uniform manner, often with a nearly straight dark line between the healthy and the diseased portions of the tuber (Wharton and Kirk, 2007; Powelson and Rowe, 2008). The infected tissue may remain relatively firm and rubbery but attains a distinct pink color after exposure to air for 20 to 30 minutes (Fig. 3) (Taylor *et al.*, 2006; Wharton and Kirk, 2007; Powelson and Rowe, 2008). Tubers left in the field during harvesting may harbor oospores, which later become the initial inoculum for the new crop (Fig. 4). Cull piles damped near the fields are also sources of inoculum.

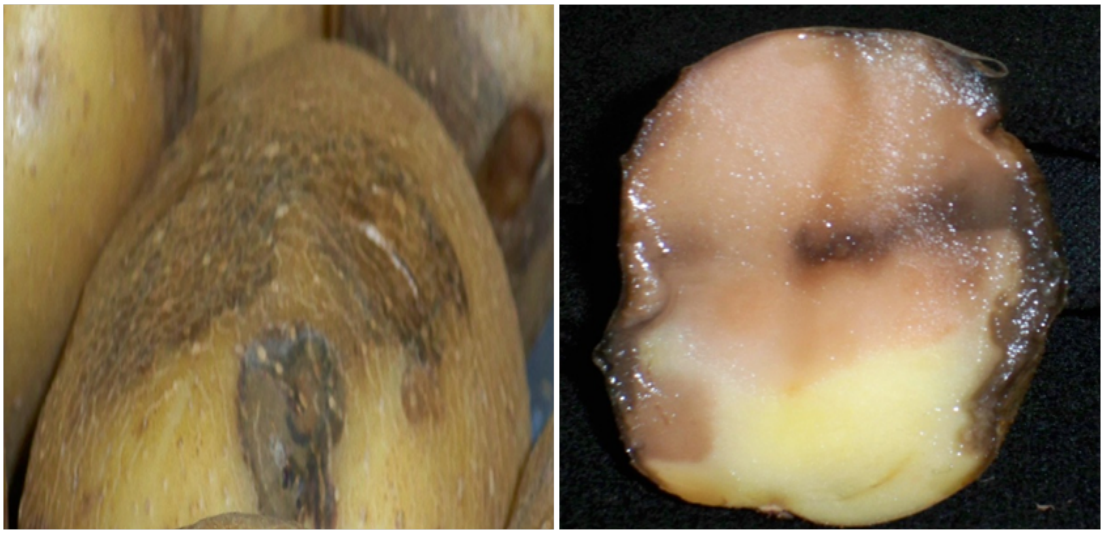

Figure 3 Tubers infected with pink rot, on the outer surface of the tuber (left). The cut surface of the infected tuber turns pink after exposure to air (right)

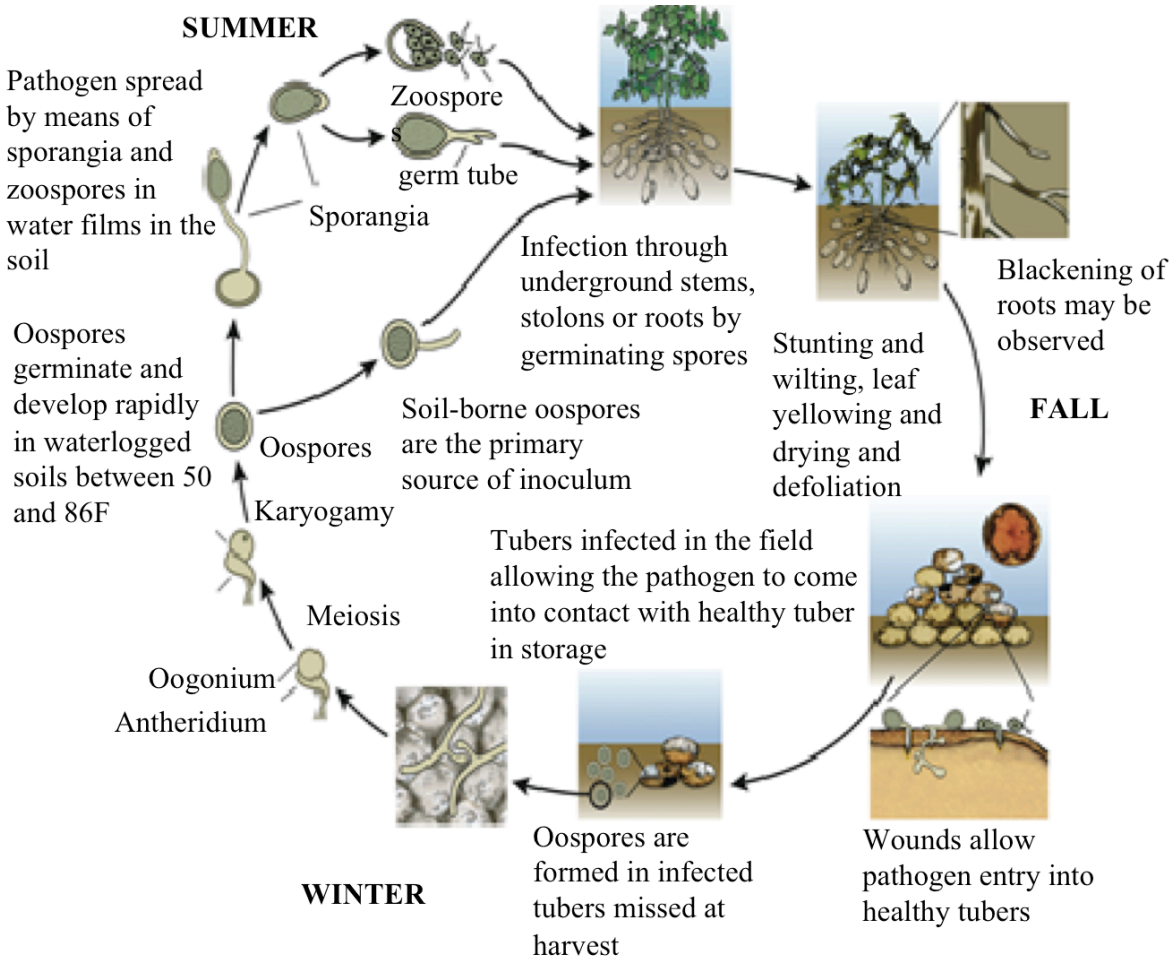

Figure 4 Life cycle of *Phytophthora erythroseptica*, the causal agent of pink rot diseases (Wharton and Kirk, 2007)

Management of pink rot

Pink rot infection is associated with high soil moisture content and extended periods of wetness late in the growing season. Therefore, good soil drainage towards the end of the growing season is imperative for management (Lambert and Salas, 2001). Other important practices include crop rotation, maximizing skin set, proper handling techniques during harvesting to avoid wounding the tubers, and harvesting when tuber pulp temperature is below 21^{o}C (Lambert and Salas, 2001; Powelson and Rowe, 2008). Integration of these techniques with utilization of host resistance can effectively control the disease. There are no cultivars resistant to pink rot in the US, but different levels of susceptibility have been reported among cultivars (Salas *et al.*, 2003). Therefore, potato growers continue to rely on application of metalaxyl/ mefenoxam (Taylor *et al.*, 2004) and more recently cyazofamid (Ranman) (Griffiths *et al.*, 2008). Metalaxyl effectively controlled pink rot in the 1990's. However, metalaxyl-resistant strains of *Phytophthora erythroseptica* were first reported in 1993 in Maine (Lambert and Salas, 1994) and have now been reported in other potato growing areas of North America (Taylor *et al.*, 2002). An enantiomer of metalaxyl, mefenoxam, released in 1997, has increased activity against the oomycetes. Although mefenoxam-resistant isolates of *P. erythroseptica* have been reported in North America, there is still a good percentage of sensitive isolates (Taylor *et al.*, 2002) and isolates with varying levels of resistance have been reported (Venkataramana *et al.*, 2010). Hence, mefenoxam is still being used to control pink rot with applications made at planting and on the foliage when the tubers are approximately 10 mm in diameter (Taylor *et al.*, 2007). Applications at planting time have been more effective in controlling pink rot than foliar application (Taylor *et al.*, 2004; Taylor *et al.*, 2006; Al-Mughrabi *et al.*, 2007). Alternative chemicals that have been evaluated for control of pink rot include hydrogen peroxide

(Oxidate^{TM}). When hydrogen peroxide was used *in vitro*, growth inhibition of *P. erythroseptica* was observed, indicating hydrogen peroxide could have a potential for controlling pink rot (Al-Mughrabi, 2006). Recently, a new fungicide, phosphorous acid (phosphonate, phosphite), was registered for control of pink rot and late blight (Zitter, 2010), and has demonstrated to be effective for pink rot management (Johnson *et al.*, 2004; Miller *et al.*, 2006; Mayton *et al.*, 2008).

Fusarium dry rot

Fusarium dry rot of potato (*Solanum tuberosum* L.) is a devastating postharvest disease worldwide and is caused by several *Fusarium* species (Boyd, 1972; Secor and Salas, 2001). It affects both tubers in storage and seed pieces in the field. Losses associated with dry rot have been estimated from 6 % to 25 %, and occasionally losses as high as 60% have been reported during long-term storage (Chelkowski, 1989; Secor and Salas, 2001). In addition to the damage inflicted on tubers, *Fusarium* species also produce mycotoxins harmful to humans and animals (Desjardins and Plattner, 1989; Desjardins *et al.*, 1993b). *Fusarium* is a ubiquitous pathogen in a wide variety of crops. Dry rot incidence has been reported to vary according to *Fusarium* species responsible (Peters *et al.*, 2008b). For instance, *Fusarium sambucinum* has been reported to have the ability to detoxify the phytoalexins produced by the potatoes thus increasing its virulence (Desjardins *et al.*, 1992). It is common to find a diverse *Fusarium* species composition within the same area (Hanson *et al.*, 1996), hence increasing chances of dry rot incidence.

***Fusarium* species implicated in causing potato dry rot**

Several *Fusarium* species are responsible for fungal dry rots of potato in storage and seed tuber decay after planting (Wharton *et al.*, 2005). Thirteen species have so far been implicated in causing dry rot worldwide (Hide *et al.*, 1992; Cullen *et al.*, 2005). Among them, eight species

have been reported in the northern United States (Hanson *et al.*, 1996). The most prevalent species are, *F. sambucinum* Fuckel (*Fusarium sulphureum* Schlechtend; teleomorph*: Gibberella pulicaris* (Fr.:Fr) Sacc.), *F. solani* (Mart.) Sacc. var. *coeruleum* (Lib. ex Sacc.) C. Booth (*F. coeruleum*; teleomorph: *Nectaria haematocca* Berk. & Broome) and *F. oxysporum* Schlechtend. Fr. (Hanson *et al.*, 1996). Other species reported in the northern United States that are less important in causing dry rot include, *F. avenaceum,* (Fr.) Sacc. (teleomorph: *G. avenaceum* R. J. Cook), *F. culmorum,* (W.G. Smith) Sacc. *F. acuminatum,* Ellis & Everh. *F. equiseti* (Corda) and *F. crockwellence* L.W. Bugess, P.E. Nelson & Ravenel. (*F. cerealis*) (Hanson *et al.*, 1996; Ocamb *et al.*, 2007). Most of these species were also recovered in the Pacific region of the United States, with *F. sambucinum* being the most prevalent (Ocamb *et al.*, 2007).

Recently, *Fusarium graminearum* was reported to cause dry rot in North Dakota (Ali *et al.*, 2005; Estrada Jr *et al.*, 2010) and accumulation of trichothecene mycotoxins within rotten tubers detected (Delgado *et al.*, 2010). In the U.K., *Fusarium coeruleum* (Libert) Sacc. was found to be prevalent (Hide *et al.*, 1992; Peters *et al.*, 2008a), while in Scotland *F. avenaceum* caused more dry rot compared to *F. solani* var. *coeruleum* (Cullen *et al.*, 2005; Choiseul *et al.*, 2006). In Michigan potato production, dry rot has been reported in most of the seed lots (Kirk and Wharton, 2008) and *F. sambucinum* was the predominant species affecting potato in storage and causing seed piece decay after planting (Lacy and Hammerschmidt, 1993). It was also reported that, rotting sprouts of the progeny tubers in Michigan was caused by *F. sambucinum* (Wharton *et al.*, 2006). However, the current composition of *Fusarium* species causing dry rot of potato seed tubers in Michigan is not known. Identifying the various *Fusarium* spp. responsible for dry rot is therefore important in designing a management scheme because different species respond differently especially to chemicals.

Morphological identification is usually the first step and the most difficult due to close resemblance of some species with the same origin (Leslie *et al.*, 2006), hence molecular identification through DNA sequencing and comparing with a known species is more precise (Geiser *et al.*, 2004). However, the combination of molecular and morphological characterization is recommended (Geiser *et al.*, 2004; Leslie *et al.*, 2006)

Fusarium dry rot development

Dry rot development is initiated by inoculum from infected seed tubers or infested soils (Secor and Salas, 2001). The pathogen survives from one season to the other in infected tubers, decaying plant tissue (Fig 5) or in the soil as chlamydospores or mycelium (Powelson and Rowe, 2008). *Fusarium* can infect potato tubers through wounds inflicted during harvesting or during seed handling and cutting (Glass *et al.*, 2001; Secor and Salas, 2001; Powelson and Rowe, 2008). In addition *F. oxysporum* and *F. solani* are able to infect potato crop in the field leading to wilting and root rotting which further results to stem-end rot of potato tubers (Theron and Holz, 1989; Mahdavi-Amiri *et al.*, 2009). Dispersal of dry rot pathogen could also be through infested soil adhering to the tuber surface during harvesting (Theron and Holz, 1991). These pathogens then invade the potato tuber through tissue injuries inflicted during lifting or grading. The infection requires a fresh, unsuberized wound, with suberization of wounds preventing the infection (O'Brien and Leach, 1983)

The initial symptom on the tuber surface is a shallow brown lesion, which later expands slowly and eventually becomes sunken and wrinkled. Necrotic areas shaded from light to dark chocolate brown or black characterize internal symptoms. This necrotic tissue is usually dry (hence the name dry rot) and may develop at an injury such as a cut or bruise (Fig 6). The pathogen enters the tuber, often rotting out the center. Rotted cavities may be lined with mycelia

and spores of various colors from yellow to white to pink (Wharton *et al.*, 2007b). In the field Fusarium dry rot in seed tubers can result in germination gaps or severely stunted, chlorotic, and necrotic stems as well as abnormal growth of roots and stolons (Wharton *et al.*, 2007a). Varying levels of aggressiveness among species and within isolates has been reported (Daami-Remadi *et al.*, 2006b), and this may have an implication on management especially if the predominant species happens to be the most virulent.

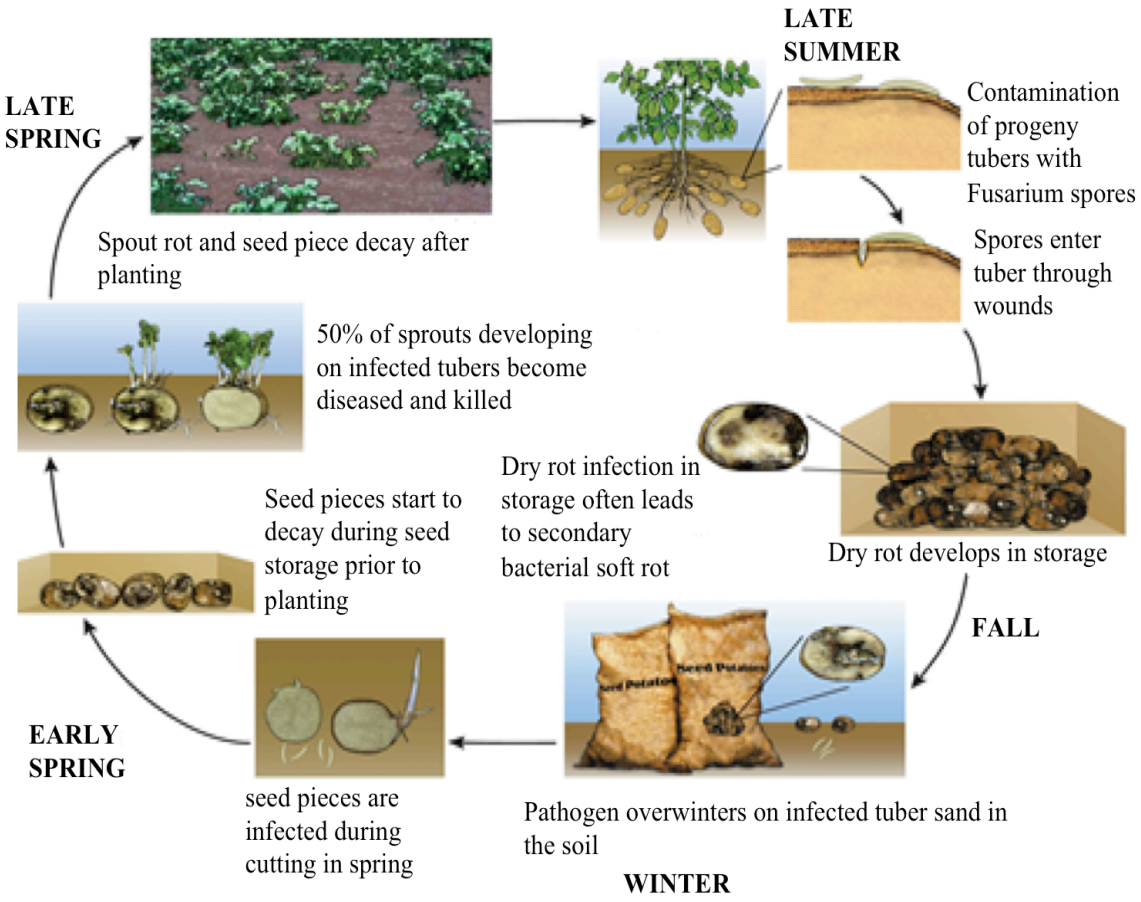

Figure 5 Disease cycle of Fusarium dry rot caused by *Fusarium sambucinum* (Wharton *et al.*, 2007a)

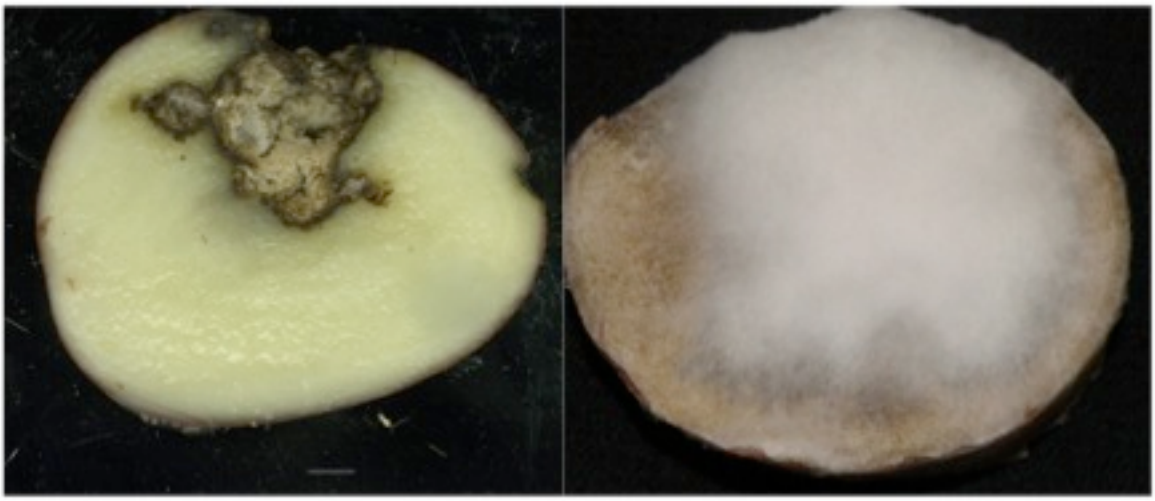

Figure 6 Potato tuber infected with Fusarium dry rot. On the left, the cavity is lined with yellow mycelium (*F. sambucinum*). On the right, infected tuber surface covered with pink to white mycelium (*F. graminearum*)

Management of Fusarium dry rot

Measures for controlling dry rot in storage are limited. There is no commercially grown potato cultivar that is resistant to dry rot in North America (Wharton *et al.*, 2007a). However, the level of susceptibility varies from one cultivar to the other and clones with a high level of resistance to *F. sambucinum* and *F. solani* have been reported (Leach and Webb, 1981). Use of long rotations effectively controls dry rot (Little and Bell, 2009). However, rotation with cereals and forage crops has resulted in an increase in dry rot incidence (Peters *et al.*, 2008b). Studies have shown that some of the *Fusarium* spp. affecting cereals (*F. graminearum* and *F. sporotrichioides*) and forage crops (*F. avenaceum* and *F. oxysporum*) are also pathogenic to potatoes (Peters *et al.*, 2008b). Indeed, it was recently reported that, *F. graminearum* the causal agent of head blight of wheat and barley, is the major dry rot pathogen in the north-central potato production regions of the US (Estrada Jr *et al.*, 2010). Thus, the need for alternative methods to manage dry rot.

There are two main opportunities in potato crop cycle for control of Fusarium dry rot. The first is postharvest control of dry rot in potato tubers intended for consumption or to control piece decay in seed aimed for the following season crop (Nolte *et al.*, 2003). The Second is control of seed piece decay and sprout infection prior to planting as this may act as the initial inoculum to the daughter tubers produced by the crop (Wharton *et al.*, 2005). Integrating storage technologies with physical methods and chemical treatments either at harvest or as tubers enter into storage or before planting could reduce the losses caused by *Fusarium* spp.

Dry rot has been managed primarily by reducing tuber bruising, providing conditions for rapid wound healing (Secor and Salas, 2001; Secor and Johnson, 2008) and by applying thiabendazole (TBZ), a benzimidazole fungicide as tubers enter into storage or before planting

(Hide *et al.*, 1992). However, isolates of *F. sambucinum* resistant to TBZ and other benzimidazoles were discovered in Europe in 1973 (Hide *et al.*, 1992) and in the United States in 1992 (Desjardins, 1995), thus rendering this chemical ineffective in controlling dry rot (Staub, 1991). Studies have shown varying responses of different isolates of *F. sambucinum* against TBZ with some isolates being resistant and others being sensitive (Desjardins *et al.*, 1993a). Characterization of fungal growth on a medium containing thiabendazole confirmed TBZ-resistant *Fusarium* species in the US (Hide *et al.*, 1992; Desjardins *et al.*, 1993a; Hanson *et al.*, 1996; Peters *et al.*, 2001). A mixture of TBZ, fenpiclonil (phenylpyrrole) and imazalil (Imidazole; Fungazil 100SL) was reported to effectively control *Fusarium* dry rot (Carnegie *et al.*, 1998). Imazalil alone has been used in Europe to control dry rot and reduction by 95% was reported when inoculated tubers were dipped into the fungicide (Cayley *et al.*, 1983), but imazalil applied as seed treatment did not effectively control dry rot in the daughter crop (Carnegie *et al.*, 1998).

The other fungicides registered for seed treatment against control of Fusarium dry rot in the United States are fludioxonil alone (Maxim™ Seed Potato, phenylpyrrole) or in combination with fludioxonil + mancozeb (Maxim® MZ, Syngenta Inc. Greensboro, NC, USA) (Zitter, 2010). Studies have shown that fludioxonil is able to reduce seedpiece decay as well as disease on sprouts (Wharton *et al.*, 2007b) which can help produce healthy progeny tubers. Fludioxonil has a single site mode of action hence a high probability of development of insensitive strains(Brent and Hollomon, 2007). Recently, fludioxonil-resistant strains of *Fusarium* spp. were reported in Canada and these include *F. sambucinum and F. coeruleum* (Peters *et al.*, 2008c). In Michigan seed tuber production, fludioxonil-resistant isolates of *F. sambucinum* and *F. oxysporum* have been reported (Gachango *et al.*, 2011). This has resulted to fewer alternatives of controlling

potato seedpiece decay caused by *F. sambucinum, F. oxysporum,* and *F. coeruleum*. However, sensitivity of other *Fusarium* spp. (causing potato seedpiece decay) to fludioxonil is unknown.

The high rates of resistance development by *Fusarium* spp. and the increased cost of chemicals have resulted in greater attention to use of biological control, alone or in combination with conventional crop protection products (Schisler *et al.*, 2000). A plant growth promoting rhizobacteria (PGPR), *Burkholderia cepacia* (formerly *Pseudomonas cepacia*) has been reported to be antagonistic to *F. sambucinum, F. oxysporum,* and *F. culmorum* responsible for dry rot of potatoes in storage (Recep *et al.*, 2009). *In vitro* testing of *Bacillus subtilis* (strain BA-140) indicated an antifungal activity against the *Fusarium* spp. but no control of disease development on tubers. Bacteria from the genera *Pseudomonas, Enterobacter,* and *Pantoea* have been reported to suppress Fusarium dry rot in whole tuber assay (Al-Mughrabi, 2010).

To counteract the loss of effectiveness caused by resistance towards TBZ and fludioxonil, additional registration of postharvest fungicides is needed and some have already been proposed; difenoconazole for managing decays caused by *Fusarium* species, azoxystrobin, and fludioxonil for potato and tuber crops decays respectively (Adaskaveg and Förster, 2010). There is also need to evaluate the sensitivity of *Fusarium* species other than *F. sambucinum* towards TBZ.

Potato diseases management strategies

The potato crop can be infected at any stage during growth and in storage and therefore management practices should aim at reducing the disease incidence both in the field and in storage. It is imperative to implement both pre- and post-harvest disease management practices. Cultural practices in combination with use of chemicals effectively control potato diseases (Gudmestad *et al.*, 2007). Cultural control practices in the field include the use of clean planting material, good soil drainage, avoiding over-irrigation, crop rotations, establishing good skin set

prior to harvesting, harvesting when the tuber pulp temperatures are optimal and proper handling of tubers as they are put in storage (Taylor *et al.*, 2004).

Seed tuber preparation

Planting of seed tubers with latent infection has been one of the major factors contributing to potato epidemics in potato growing areas (Johnson and Cummings, 2009). These tubers look healthy when removed from storage, but upon planting, pathogens within the tuber sporulate due to the favorable conditions in the soil (high moisture content and high temperatures compared to prior storage temperature) and cause infection of the stem and the progeny tubers (Zellner, 2006). Seed piece decay of potato is the major disease affecting potato seed tubers prior to planting or aboveground infection after planting and can be caused by *Fusarium* species*, P. infestans, Pythium* species*,* or bacterial pathogens. These pathogens can spread from one tuber to the other while cutting them in preparation for planting (Powelson and Rowe, 2008). Tubers infected with *P. infestans* have been reported to be the cause of late blight epidemics even though there are no visible symptoms on the tuber surface during planting (Keil *et al.*, 2008). To minimize the risk of seed piece decay, seed treatment with recommended fungicides together with good management while cutting the tubers should be implemented (Wharton *et al.*, 2007b). Potato seed treatment with mancozeb and fludioxonil has been effective against many pathogens since the development of resistance to TBZ by the *Fusarium* spp. (Powelson and Rowe, 2008).

Another method, which ensures that the tubers are free from diseases, is the use of host resistance. Breeding for resistance against late blight is of great concern especially in North America (Stevenson, 2008). Two cultivars, Jacqueline Lee (table stock) and Defender (light russeted long type) have successfully been released and shown high resistance to *P. infestans* genotype US-8 which is prevalent in North America (Stevenson *et al.*, 2007). Planting of disease

free tubers does not guarantee a healthy crop. Overwintering inoculum may cause infection through lenticels, buds or wounds once they come into contact with the tuber. Also, progeny tubers become infected when zoospores or sporangia of *P. infestans* are washed from the foliage into contact the soil (Porter *et al.*, 2006).

Management during planting

Cultural practices like adequate planting depth and hilling has been used to reduce the likelihood of tuber contact with pathogen spores like sporangia after being washed off from the leaves into the soil (Nyankanga *et al.*, 2008). In contrast, planting at shallow depths decreases the risks of tuber infection by *Fusarium* spp. and thus decreases the risk of un-uniform stand establishment (Hide and Lapwood, 1992). Site selection, delaying planting time to allow the soils to warm up, fertilizer and water management, and better integration of field operations contribute to production of healthy tubers. While all these practices may reduce infection in the field, there is need to increase tuber resistance to ensure tubers health and quality during storage. This can be done through in- season application of fungicides and biofungicides.

In-furrow application of fungicides/ biofungicides

Incorporating protective fungicides in the soil during planting has led to effective disease control especially against the oomycete pathogens (Al-Mughrabi and Peters, 2006). Fungicides applied in the soil aim at killing or inactivating the pathogen before tuber infection. These fungicides are supposed to remain on the soil surface as a barrier to tuber infection and should have a long half-life to ensure total protection (Porter *et al.*, 2006). A number of fungicides are registered for in-furrow application in the US to control potato diseases. These include mefenoxam/metalaxyl (Ridomil Gold^{TM}), phosphorous acid (Phostrol^{TM}) azoxystrobin (Amistar, Quadris) and mefenoxam + chlorothalonil (Flouronil^{TM}); (Zitter, 2010). Phosphorous

acid, mefenoxam and chlorothalonil are registered for control of pink rot and Pythium leak while azoxystrobin controls black scurf (*Rhizoctonia solani*), silver scurf (*Helminthosporium solani*) and Fusarium seed piece decay while applied on freshly cut seeds (Powelson and Rowe, 2008).

In-furrow application of mefenoxam at planting time is a common practice in managing oomycete pathogens on potatoes (Al-Mughrabi and Peters, 2006). It has been reported to effectively control sensitive isolates of *P. erythroseptica* and *P. ultimum* due to increased tuber resistance (Taylor *et al.*, 2007). Mefenoxam is a systemic fungicide with a single-mode of action (Stevenson, 2008) that inhibits ribosomal RNA polymerase enzyme (Fernández-Northcote *et al.*, 2000). It is able to penetrate the potato tuber and significant residues have been recovered 120 d after storage thus protecting the tubers (Bruin *et al.*, 1982). However, the intensive use of mefenoxam both in the field and storage has resulted in the development of insensitive isolates of *P. infestans* (Fernández-Northcote *et al.*, 2000)*, P. ultimum* and *P. erythroseptica* (Taylor *et al.*, 2002; Porter *et al.*, 2009). Nevertheless, there is still a population of sensitive isolates and studies have shown that mefenoxam applied in-furrow at planting followed by a side dress application can effectively reduce the incidence of pink rot compared to a foliar application (Taylor *et al.*, 2004). Similar studies have shown that in-furrow application of mefenoxam significantly controlled pink rot compared to phosphorous acid (Al-Mughrabi *et al.*, 2007).

In attempts to control potato tuber diseases, protectant fungicides registered for foliar application are being applied in the soil during planting because they are easily being washed off by rainfall (Fernández-Northcote *et al.*, 2000). For example, dithiocarbamates fungicides (Mancozeb^{TM} and Metiram^{TM}), which are commonly used to suppress foliar potato blight, but with less effect on tuber blight, gave good protection on tubers against *P. infestans* when applied to the soil (Porter *et al.*, 2009). This method does not work with all fungicides; for example,

copper fungicides did not protect the tubers and were associated with the formation of complexes with the soil (Finckh *et al.*, 2006). A new biofungicide, *Bacillus subtilis* strain QST 713 (Serenade Soil; AgraQuest) was recently registered for in-furrow application against *Fusarium, Pythium* and *Phytophthora* in potatoes, tomatoes and cucurbits (Anon, 2010).

Foliar application of fungicides

Application of fungicides on the foliage is the most common strategy practiced by growers in the western United States and Midwest to manage potato tuber diseases (Hamm *et al.*, 2008). Infection of the foliage leads to reduction in yield by reducing the photosynthetic area or killing vines prematurely. Foliage infection also has a direct effect on the health of the developing tubers. For instance, sporangia of *P. infestans* produced on the lower half of leaf canopy are easily washed off by rain or irrigation water (Stevenson *et al.*, 2008). The spores move down the stem and into stolons causing infection of the developing tubers. Conversely, sporangia produced on the upper leaf canopy are dispersed by wind for a distance and may initiate either foliar or tuber infection in the neighboring fields (Skelsey *et al.*, 2009). Therefore, foliar application of fungicides becomes imperative. Protectant fungicides registered for control of foliar potato diseases have less effect on tuber rots especially the dithiocarbamates (Fernández-Northcote *et al.*, 2000). Other foliar applied fungicides commonly used in the US to control seed borne and soilborne pathogens of potato are Phosphorous acid and mefenoxam (Powelson and Rowe, 2008). Mefenoxam, being a systemic fungicide, is translocated from the foliage to the tubers but its effectiveness is limited due to development of resistant isolates of the oomycete group (Fernández-Northcote *et al.*, 2000). Therefore, phosphorous acid is the most commonly used fungicide as a foliar application to control potato tuber rots caused by oomycetes (Cooke and Little, 2002; Johnson *et al.*, 2004; Mayton *et al.*, 2008). Phosphorous acid is a

systemic fungicide with both basipetal and acropetal movement, thus a foliar spray is translocated within the plant to the root system, controlling tuber rots (Brunings *et al.*, 2005).

Potato harvesting

Potato tuber harvesting is a rigorous exercise, which involves lifting, loading, transportation, and bin loading (Knowles and Plissey, 2008). To minimize tuber damage, these factors have to be considered: crop maturity, soil temperature, prevailing weather conditions, tuber pulp temperature and good harvesting and handling skills (Gottschalk and Ezekiel, 2006). Ideally, tuber harvesting is supposed to be done when the tubers are physiologically and chemically mature, as is indicated by a good skin set and optimal sugar/glucose level, respectively. This can be achieved by killing the vines prior to harvesting for at least 1-2 weeks, thus hastening maturity and skin set (Knowles and Plissey, 2008). The weather conditions have to be optimal (cool air during the night) and appropriate soil moisture content (typically between 60 and 75%) to carry the harvested potato and soil to the secondary conveyor on the harvester where the soil separates from the tubers (Pinhero *et al.*, 2009). This ensures the operation moves smoothly by allowing the harvester movement without clods and prevent damaging the tubers (Wustman, 2007). Damaging tubers lower the quality and quantity of the harvested crop. The U.S. industry incurs a cost of up to $7.5 million annually to reduce the impact of harvest damage to levels as low as 1% (Storey, 2007).

Tuber temperature is also of importance and should be optimal by harvesting time. Temperatures ranging from 10 to 18^{o}C are the best for tuber pulp (Knowles and Plissey, 2008), but in the case of fields with a high risk of pink rot, the tubers should be harvested when the pulp temperatures are 7-10^{o}C (Powelson and Rowe, 2008). Tuber hydration may also influence the level of bruising and this opens up the tuber for entry of many soilborne pathogens (Pinhero *et*

al., 2009). In addition, mechanical injury at harvest time and transit also cause quality loss as many fungal pathogens gain entry through wounds e.g., *Fusarium* spp. and *Pythium* spp. (Secor and Salas, 2001). To minimize the risk of infection once harvesting is completed, treatment with fungicide should be considered during the bin loading. Application of thiabendazole in the form of a mist along the conveyor as tubers are put to storage has been used against Fusarium dry rot (Powelson and Rowe, 2008). As mentioned (pg 27), resistance to TBZ has been a potential issue. A newly released fungicide, phosphorous acid is now being used on tubers as they enter storage and this has effectively controlled pink rot, late blight and Pythium leak (Powelson and Rowe, 2008).

Storage losses

Potato tubers are very prone to losses during storage and the losses are often specified as weight and quality losses (Pinhero *et al.*, 2009). The biochemical processes that result in weight and quality loss of the tubers in storage include, respiration, sprouting, incidence of pests and diseases, dehydration, changes in chemical composition of the tuber or damage by extreme temperatures (Shetty, 1996). The potato tuber is a living organism, and hence produces heat, moisture, and carbon dioxide during respiration (Lulai, 2001). While a portion of the average yearly losses are due to transpiration and respiration, the most serious economic loss is due to disease which has becoming a major concern to the potato industry (Olsen *et al.*, 2006).

The relative humidity has to be maintained at 90-95% throughout the storage period. Good air circulation within the potato pile should be enhanced to avoid build-up of carbon dioxide, which has been associated with development of soft rots. In the cases of tubers coming to storage having 1-2% of disease symptoms, then curing temperatures need to be lowered below 10^{o}C as soon as the tubers are put in storage (Knowles and Plissey, 2008).

Symptoms of infected tubers include; discoloration of the skin, discoloration of the tuber flesh, holes in skin and tuber flesh, rotting and odors arising from rotting tuber parts. The entire chain of producer, processor, retailer and consumer is faced with an undesirable product quality resulting in reduced demand and lower prices (Secor and Gudmestad, 1999). Since potato tubers do not gain quality during storage, there is a need to maintain their quality (Knowles and Plissey, 2008). Most of the tubers produced in the Northern USA are protected in the field against most diseases but this does not give assurance of disease free tubers in storage. Therefore, further protection of the tubers in storage may provide further insurance of a quality product especially for long-term storage.

Disease management in storage

The rationale behind management of potato storage diseases is the assumption that healthy tubers become exposed to pathogen inoculum in the field and during the harvest operation prior to storage. Once in storage, the pathogen can either proliferate or survive in the dormant phase until a favorable environment is encountered (Johnson and Cummings, 2009). Currently, the primary methods used to control potential storage diseases include, elimination of infected tubers prior to storage, ventilation and temperature and humidity manipulation (Knowles and Plissey, 2008). These practices in combination with postharvest fungicides, biofungicides or effective disinfectants are good means of preventing potato diseases in storage.

When used, the fungicides, biofungicides, and disinfectants are applied as low-pressure sprays as the tubers are conveyed into storage (Olsen *et al.*, 2003; Powelson and Rowe, 2008). The disinfectants work as surface sterilants and are not curative. The most commonly used disinfectants in the potato industry include chlorine dioxide (ClO_2) and mixtures of hydrogen peroxide and peroxyacetic acid. Hydrogen peroxide (H_2O_2; Oxidate^{TM}) is a broad- spectrum

disinfectant that is able to provide immediate control of pathogens. However, studies have shown that these disinfectants are not sufficient in controlling storage diseases (Olsen *et al.*, 2003; Miller *et al.*, 2006). Therefore, the use of fungicides and biofungicides becomes the most promising option in controlling postharvest diseases of potatoes.

A number of fungicides and biofungicides have been registered for postharvest use in the United States. These include phosphorous acid, thiabendazole, *Bacillus subtilis* (Serenade ASO & MAX), *Pseudomonas syringae* (Bio-Save 10LP) and *Bacillus pumilis* (Sonata); (Zitter, 2010). These fungicides and biofungicides work against a range of postharvest diseases of potato including, pink rot, Fusarium dry rot, late blight, silver scurf, early blight, and black scurf (Zitter, 2010). Although phosphorous acid is registered for post harvest use, a number of studies done are for in-season application (Johnson *et al.*, 2004). Studies using phosphorous acid as postharvest fungicides have shown potential efficacy of against late blight of tubers and pink rot (Miller *et al.*, 2006; Johnson, 2008). Phosphorous acid applied on potato tubers just after harvest and prior to storage significantly decreased disease development caused by *Phytophthora infestans* and *Phytophthora erythroseptica* (Miller *et al.*, 2006), and continues to show great potential in controlling *P. infestans* especially when the labeled rates are used (Johnson, 2008). Introduction of more fungicides and biofungicides has increased, but only limited research has been done to evaluate and compare their efficacy during postharvest management. The current study focused first on evaluation and comparison of the efficacy of fungicides and biofungicides in suppressing potato tuber diseases (specifically tuber late blight, Fusarium dry rot, pink rot, and Pythium leak) during storage. Second, the study evaluated the effect of combining in-season applied fungicides/ biofungicides and postharvest applied fungicide/ biofungicide on tuber protection against storage pathogens. Finally, the study focused on one of the most important

seed potato disease in Michigan, Fusarium dry rot, identifying the species responsible for dry rot and screening them for fungicide sensitivity.

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Chapter 1: Evaluation and Comparison of Biocontrol and Conventional Fungicides for Control of Postharvest Potato Tuber Diseases

Abstract

Two biocontrol fungicides (based on *Bacillus subtilis* and *Bacillus pumilus*) and three conventional fungicides (phosphorous acid, azoxystrobin and hydrogen peroxide) were evaluated in two storage trials over 2 years for efficacy in suppressing tuber infection caused by *Phytophthora infestans*, *Phytophthora erythroseptica*, *Pythium ultimum* and *Fusarium sambucinum*. A chip-processing cultivar, cv. FL 1879, stored at 10^{o}C was used for the two trials. Tubers were inoculated followed by treatment with the biofungicides and fungicides prior to storage. Disease incidence was assessed after 127 d in storage. Application of the biocontrol had limited control of the storage pathogens compared to the conventional fungicides. Phosphorous acid, hydrogen peroxide and azoxystrobin were moderately effective in controlling diseases caused by the oomycete pathogens. Although none of the products evaluated completely controlled the storage diseases, the conventional fungicides showed a higher potential for suppressing tuber infection in storage than the biocontrol fungicides. Use of these products should be integrated with other management strategies.

Key words: Biocontrol; Conventional fungicides; Potato; Storage diseases/ pathogens

1.1 Introduction

Potato growers aim to produce and harvest a healthy and high quality crop. However, there are several diseases that infect potatoes in storage thus compromising their quality and creating a potential economic loss (Secor and Gudmestad, 1999). Potatoes are susceptible to a variety of storage diseases including late blight caused by *Phytophthora infestans* (Mont.) de Bary, dry rot caused by *Fusarium sambucinum* Fuckel. and spp., Pythium leak caused by

Pythium ultimum Trow, pink rot caused by *Phytophthora erythroseptica* Pethbr., tuber soft rot caused by *Pectobacterium* spp., and silver scurf caused by *Helminthosporium solani* Durieu & Mont (Secor and Gudmestad, 1999). These pathogens are soilborne and seedborne, hence the diseases have both field and storage stages (Powelson and Rowe, 2008) with losses in storage being more severe. Infection in the field takes place through underground stems, stolons, or roots as tubers develop. Further infection takes place through the lenticels, eyes, and wounds inflicted during harvesting and in storage (Powelson and Rowe, 2008). The likelihood of infection by these pathogens is highly affected by the quality of the seed tubers, management practices during the growing period, harvesting and handling and storage conditions (Shetty, 1996). Currently, the primary control for these diseases in storage facilities include, elimination of infected tubers prior to storage and proper storage management with ventilation, temperature and humidity (Knowles and Plissey, 2008). There is a shortage of postharvest fungicides or effective disinfectant products to completely control these pathogens (Olsen *et al.*, 2003). The few compounds available for potato tuber treatment in storage include chlorine-based disinfectants such as, sodium hypochlorite, calcium hypochlorite and chlorine dioxide and mixtures of hydrogen peroxide and peroxyacetic acid (Afek *et al.*, 2001; Norikane *et al.*, 2001; Wharton *et al.*, 2007). Hydrogen peroxide (H_2O_2; Oxidate^{TM,} hydrogen dioxide 27%; inert ingredients 73%; BioSafe Systems, Glastonbury, CT) is a broad- spectrum disinfectant that is able to provide immediate control of storage pathogens (Norikane *et al.*, 2001; Olsen and Miller, 2005). It is environmentally friendly with its activities based on oxidation of fungi and bacteria, and has successfully been reported to control silver scurf (Afek *et al.*, 2001) and pink rot (Al-Mughrabi, 2006). However, use of disinfectants does not completely arrest the storage pathogens (Olsen *et*

al., 2003; Miller *et al.*, 2006), hence other postharvest products in combination with proper storage management should be practiced.

In recent years, several new biofungicides based on the biocontrol bacteria *Bacillus subtilis* (QST 713; Serenade; AgraQuest, Inc. CA) and *B. pumilis* (QST 2808; Sonata; AgraQuest, Inc. CA) have been registered for control of potato pathogens. These products are used for organic potato production to complement copper products and have successfully shown a reduction of foliar late blight disease development (Stephan *et al.*, 2005). *Bacillus subtilis* produces three groups of lipopeptides that together stop spores of plant pathogens from germinating, disrupt germ tube and mycelial growth, and inhibit attachment of the plant pathogen to the leaf surface (Marrone, 2002). Another product also registered for postharvest use is Bio-Save 10LP (*Pseudomonas syringae*: ESC-10), which has been reported to reduce Fusarium dry rot and silver scurf when applied to tubers prior to storage (Olsen and Miller, 2005; Hopkins and Hirnyck, 2008). So far no work has been to evaluate the effect of *B. subtilis* and *B. pumilus* in controlling postharvest diseases of potatoes in Michigan.

Phosphorous acid (Phostrol- mono and di-basic sodium, potassium and ammonium salts of phosphorous acid; Nufarm Americas, Inc., AGT-Division, Burr Ridge, IL) was recently registered for postharvest use in potato production (Powelson and Rowe, 2008). The United States Environmental Protection Agency (US-EPA) considers it as a systemic fungicide not a biochemical http://www.epa.gov/oppbppd1/biopesticides/ingredients/factsheets/factsheet_076416.htm - description. Phosphorous acid has both a direct and an indirect mode of action (Brunings *et al.*, 2005). Direct effects include inhibition of mycelial growth and inhibition of particular metabolic processes, and indirect effects include stimulation of the natural defense responses of the plant

(Guest and Bompeix, 1990). Phosphorous acid has been reported to effectively control tuber late blight and pink rot in storage (Olsen and Miller, 2005; Miller *et al.*, 2006; Johnson, 2008; Johnson, 2010).

Control of dry rot in storage has primarily been achieved through reducing tuber bruising, providing conditions for rapid wound healing (Secor and Johnson, 2008) and applying thiabendazole (TBZ; Mertect 340-F, Syngenta, Greensboro, NC), a benzimidazole fungicide as tubers are loaded into storage (Hide *et al.*, 1992). However, *F. sambucinum* resistant to TBZ and other benzimidazole fungicides were discovered in Europe in 1973 (Hide *et al.*, 1992) and in the US in 1992 (Desjardins, 1995), leading to reduced effectiveness in controlling dry rot (Staub, 1991). To counteract this loss, registration of other chemistries is imperative. Although azoxystrobin is registered for foliar application in potato fields, it has been proposed for registration as a postharvest fungicide to control tuber decay caused by *Fusarium* spp. (Adaskaveg and Förster, 2010).

Over the past three years these products have been evaluated for the control of pathogens under postharvest potato tuber storage conditions. Thus, studies were initiated to evaluate the efficacy of *Bacillus subtilis* and *B. pumilus* for the control of potato storage pathogens under post-harvest conditions. These products were compared with several commercial products including phosphorous acid, hydrogen dioxide and azoxystrobin (Quadris) under storage conditions.

1.2 Materials and Methods

1.2.1 Tuber preparation

Potato cultivar, cv. FL 1879, used for chip processing was used in both 2006 and 2007 trials. The tests were carried out at 10^{o}C, temperatures used in the potato industry; (49^{o}F) for

chip processing (Knowles and Plissey, 2008). Potatoes free from visible symptoms were selected and prepared for inoculation by grazing with a single light stroke with a wire brush, sufficient to abrade the skin of the tubers to a depth of 0.01 mm.

1.2.2 Inoculum

Isolates of *Phytophthora erythroseptica*, *Pythium ultimum*, and *Fusarium sambucinum* previously isolated from potato tubers in Michigan were grown on potato dextrose agar (PDA; Difco, Detroit, Michigan), while *Phytophthora infestans* was grown on rye media prepared by steaming rye seed (100 g L^{-1} of distilled water) for 1 h, with addition of 7.5 g sucrose, 0.05 g ß-sitosterol, and 1.5% agar to the resulting broth filtrate. The cultures were grown 10 days prior to preparation of inoculum solutions. Solutions containing sporangia of *P. infestans*, oospores/ sporangia of *P. erythroseptica*, oospores of *P. ultimum* and macroconidia of *F. sambucinum* were prepared and spore concentration adjusted to 10,000 per mL using a hemacytometer. Damaged tubers, (25/replicate/treatment; total 100 tubers/treatment) were sprayed with 10 mL of pathogen suspension, for a final dosage of about 0.1 mL/tuber. The inoculated tubers were stored at 20^{o}C for 24 h before treatment with the biocontrols or conventional fungicides (Table 1)

1.2.3 Treatments

Treatments applied to the potato tubers were 1) untreated and 2) treated with either the biocontrols [*Bacillus pumilus* (20.9 mL/100 kg potato tubers) or *B. subtilis* [(20.9 mL (high rate) or 10.4 mL (low rate)/100 kg potato tubers] or with conventional fungicides [phosphorus acid (83.5 mL/100 kg potato tubers), hydrogen peroxide (8.15mL/100 kg potato tubers) and azoxystrobin (1.96 mL/100 kg potato tubers]. All the treatments were applied as liquid in a water suspension with a single R&D XR11003VS spray nozzle at a rate of 1L/ton at 344.7 KPa onto the tuber surfaces, with an entire seed surface being coated. Two untreated controls, either

inoculated with one of the pathogens or non-inoculated were included in the trial for every treatment. Tubers were then incubated in the dark in plastic boxes for 127 d at 10^{o}C.

1.2.4 Disease Assessment

After incubation, all the tubers were cut in half longitudinally and disease incidence assessed visibly for each one as presence of signs or symptoms with late blight, Pythium leak, pink rot or Fusarium dry rot. Disease incidence was computed as percentage of tubers infected per replicate.

1.2.5 Data analysis

Data were tested for assumptions of normality and analyzed by analysis of variance platform in JMP (JMP © 2008. SAS Institute Inc., Cary, NC). Mean separation was performed using Tukey's honestly significant difference test when the F test was significant ($P< 0.05$) for a test factor. In both years, analysis of variance showed that there were significant differences between results from each year, so data from each year were analyzed separately.

Table 1.1 Products evaluated in the study including company code, FRAC group, active ingredient, formulation and manufacturer.

Product name/code FRAC [a] Group	Active ingredient	Formulation [b]	Manufacturer
Oxidate	Hydrogen dioxide	27SC	BioSafe Systems, LLC East Hartford, CT
Quadris (11)	Azoxystrobin	250 SC	Syngenta Crop Protection Inc., Greensboro, NC, USA
Serenade QST 713 (44)	*Bacillus subtilis*	1.34 SC	AgraQuest Inc. Davis, CA
Sonata QST 2808 (44)	*Bacillus pumilus*	1.38 SC	AgraQuest Inc. Davis, CA
Phostrol (34)	Phosphorous acid	53.6 SC	Syngenta Crop Protection Inc., Greensboro, NC, USA

[a] FRAC = Fungicide Resistance Action Committee; FRAC code- number and letters used to distinguish fungicide groups according to their cross resistance behavior (file://localhost/(http/::www.frac.info:frac:publication:anhang:FRAC Code List 2011-final.pdf)

[b] Formulation= products added to the active ingredient to change its physical characteristic and allow compatibility with the machinery; SC= Suspension concentrates

1.3 Results

1.3.1 Fusarium dry rot

There were significant ($p<0.05$) differences among treatments for dry rot incidence in 2006 and 2007 (Table 1.2). Higher disease levels were observed in 2007 than in 2006. However, a similar trend was observed in some of the treatments (Table 1.2). For instance, inoculated tubers treated with either *Bacillus subtilis* at high rate or *Bacillus pumilus* were did not differ ($p<0.05$) from the untreated inoculated checks in both years with respect to dry rot incidence. Dry rot incidence was also observed on the non-inoculated tubers but at a very low percentage (data not shown). Tubers treated with the biocontrols had a ($p<0.05$) higher dry rot incidence than those treated with the conventional fungicides (Table 1.2). In 2007, *B. subtilis* at low rate was the only treatment that differed ($p<0.05$) from the inoculated untreated check. However, its effect was not ($p<0.05$) different from that of *B. subtilis* (high rate), *B. pumilus*, phosphorous acid or hydrogen peroxide (Table 1.2).

1.3.2 Tuber blight

No tuber blight incidence was observed on non-inoculated tubers in 2006 and in 2007. Tuber blight incidence was lower in 2006 than in 2007 and all treatments differed ($p<0.05$) from the untreated check but did not differ ($p<0.05$) from each other with respect to tuber blight incidence (Table 1.3). In 2007, there were differences ($p<0.05$) among treatments; the untreated check and the azoxystrobin treatment had higher ($p<0.05$) tuber blight incidence compared to the other treatments. Treatment with hydrogen peroxide and *Bacillus subtilis* at high disease pressure (in 2007) effectively reduced tuber blight development (Table 1.3).

Table 1.2 Incidence of Fusarium dry rot on potato tubers, cv. FL1879, stored at 10^{o}C on for 120 days after treatment with bioconfungicides and biofungicides

Fungicides/ biofungicides Treatment and rates (mL/100kg tubers)[a]	Mean Incidence[b] (%)	
	2006	2007
Untreated	56.3 a[c]	93.8 a
Phosphorous acid (83.5)	10.0 cd	82.5 ab
Hydrogen peroxide (8.5)	12.5 bcd	76.3 ab
B. subtilis Low (10.4)	31.3 bc	75.0 b
B. subtilis High (20.9)	32.5 abc	86.3 ab
B. pumilus (20.9)	35.0 ab	92.5 ab
Azoxystrobin (1.96)	21.3 bcd	80.0 ab
Tukey's HSD ($p<0.05$)	24.25	17.54
Treatment Prob (F)	<0.0001	<0.0001

[a] Tubers were treated with the fungicides/biofungicides 24 h after inoculation. The rate were in mL of the product per 100 kg of potato tubers

[b] Incidence, expressed as a percentage was calculated as mean number of tubers showing dry rot symptoms relative to number of tubers per replicate X 100

[c] Numbers followed by the same letter within a column followed by the same letter are not significantly different at $p = 0.05$ (Tukey test)

Table 1.3 Incidence of tuber late blight on cv. FL1879 stored at 10°C on for 120 d after treatment with biocontrols and conventional fungicides

	Mean Incidence[b] (%)	
Fungicides/ biofungicides Treatment and rates (mL/100kg tubers)[a]	2006	2007
Untreated	22.5a[c]	97.5 a
Phosphorous acid (83.5)	3.8b	68.8 bc
Hydrogen peroxide (8.5)	0.0b	18.8 de
B. subtilis Low (10.4)	2.5b	33.8 d
B. subtilis High (20.9)	5.0b	1.3 e
B. pumilus (20.9)	6.3b	42.5 cd
Azoxystrobin (1.96)	0.0b	77.5 ab
Tukey's HSD ($p<0.05$)	6.86	26.98
Treatment Prob (F)	<0.0001	<0.0001

[a] Tubers were treated with the fungicides/ biofungicides 24 h after inoculation. The rate were in mL of the product per 100 kg of potato tubers

[b] Incidence, expressed as a percentage was calculated as mean number of tubers showing tuber late blight symptoms relative to number of tubers per replicate X 100

[c] Numbers followed by the same letter within a column followed by the same letter are not significantly different at $p = 0.05$ (Tukey test)

1.3.3 Pythium Leak

No Pythium leak incidence was observed on the non-inoculated tubers. In 2006, B*acillus subtilis* or *B. pumilus* treatments did not differ ($p<0.05$) from the untreated inoculated check (Table 1.4). However, in 2007 all treatments differed ($p<0.05$) from the untreated check, but did not differ from each other with respect to Pythium leak incidence. Treatment with azoxystrobin, phosphorous acid or hydrogen peroxide, provided moderately good control of Pythium leak compared to treatment with the biocontrols.

1.3.4 Pink rot

Pink rot development was observed only on the inoculated tubers (Table 1.5). Treatment with either *Bacillus subtilis* at low rate and *B. pumilus* did not differ ($p<0.05$) from the untreated check with respect to pink rot incidence in 2006. In 2007, all treatments differed ($p<0.05$) from to the untreated check, but not from each other with respect to pink rot incidence (Table 1.5). Treatment with azoxystrobin, phosphorous acid or hydrogen peroxide provided better control of pink rot incidence compared to treatment with the biocontrols.

Table 1. 4 Incidence of Pythium leak on potato tubers, (cv. FL1879), stored at 10^{o}C for 120 d after treatment with biocontrols and conventional fungicides

Fungicides/ biofungicides Treatment and rates (mL/100kg tubers)[a]	Mean Incidence[b] (%)	
	2006	2007
Untreated	35.0 a[c]	65.0 a
Phosphorous acid (83.5)	21.3 bc	1.3 b
Hydrogen peroxide (8.5)	13.8 cd	1.3 b
B. subtilis Low (10.4)	40.0 a	0.0 b
B. subtilis High (20.9)	27.5 ab	17.5 b
B. pumilus (20.9)	36.3 a	0.0 b
Azoxystrobin (1.96)	5.0 de	0.0 b
Tukey's HSD ($p<0.05$)	13.72	29.71
Treatment Prob (F)	<0.0001	<0.0001

[a] Tubers were treated with the fungicides/biofungicides 24 h after inoculation. The rate were in mL of the product per 100 kg of potato tubers

[b] Incidence, expressed as a percentage was calculated as mean number of tubers showing dry rot symptoms relative to number of tubers per replicate X 100

[c] Numbers followed by the same letter within a column followed by the same letter are not significantly different at $p = 0.05$ (Tukey test)

Table 1. 5 Incidence of pink rot on potato tubers, (cv. FL1879), stored at 10^{o}C for 120 days after treatment with biocontrols and conventional fungicides

Fungicides/ biofungicides Treatment and rates (mL/100kg tubers)[a]	Mean Incidence[b] (%)	
	2006	2007
Untreated	18.8 a[c]	20.0 a
Phosphorous acid (83.5)	3.8 cd	1.3 b
Hydrogen peroxide (8.5)	3.8 cd	5.0 b
B. subtilis Low (10.4)	20.0 a	5.0 b
B. subtilis High (20.9)	10.0 bc	2.5 b
B. pumilus (20.9)	12.5 ab	8.8 b
Azoxystrobin (1.96)	1.3 d	0.0 b
Tukey's HSD ($p<0.05$)	8.68	9.55
Treatment Prob (F)	<0.0001	<0.0001

[a] Tubers were treated with the fungicides/biofungicides 24 h after inoculation. The rate were in mL of the product per 100 kg of potato tubers

[b] Incidence, expressed as a percentage was calculated as mean number of tubers showing dry rot symptoms relative to number of tubers per replicate X 100

[c] Numbers followed by the same letter within a column followed by the same letter are not significantly different at $p = 0.05$ (Tukey test)

1.4 Discussion

Due to limited availability of postharvest products for control of potato storage pathogens, there is need to integrate all strategies to ensure tubers are free from risk of pathogen infection. The rationale behind postharvest application of fungicides is the assumption that healthy tubers are exposed to pathogens during harvesting operations and also in storage from soil adhering to the tubers or from infected tubers (Gudmestad *et al.*, 2007). Tubers become wounded during harvesting, transportation and bin loading operations thus increasing their susceptibility to pathogen infection (Knowles and Plissey, 2008). Our study was set up to mimic tuber bin loading in a situation where pathogens are present. The wounding of the tubers was more severe than what would occur in a normal situation in the field and the storage conditions provided were conducive for disease development. Therefore, postharvest application of fungicides to completely control storage disease development under these conditions was important. The variation in disease development between the two years could be attributed to the viability of the pathogens, since all other conditions remained the same during the experiments.

Application of the *Bacillus subtilis* and *B. pumilus* containing products had variable control of the storage pathogens depending on year, with variations according to the disease. For instance, treatment with *B. subtilis* at high rate had very little effect on reduction of Fusarium dry rot and Pythium leak compared to application of *B. subtilis* at low rate in 2006. This suggested that increasing the rate of *B. subtilis* did not improve efficacy in controlling storage pathogens and hence growers could use the lower rate in combination with other management strategies. *Bacillus subtilis* and *B. pumilus* are currently used in organic farming to supplement the copper compounds whose continued use has resulted to toxic build-up in the soil (Hopkins and Hirnyck, 2008). Indeed, *B. subtilis* was reported to reduce the development of foliar late blight while

applied up to 3 days before or just after inoculation, but was not as effective as the copper compounds (Stephan *et al.*, 2005). This finding partly agrees with our findings where *B. subtilis* at high rate fairly reduced the development of late blight. Other products could be used in addition to *B. subtilis* and *B. pumilus* for postharvest management to improve control of storage pathogens.

Application of phosphorous acid after tubers are harvested and at bin loading has been reported to effectively reduce the development of tuber late blight and pink rot once the tubers are exposed to the pathogens (Miller *et al.*, 2006). In our study, phosphorous acid effectively reduced pink rot incidence, Pythium leak and partially reduced late blight development under high disease pressure. However, phosphorous acid differed significantly from the inoculated untreated check and provided good control under low late blight pressure. These results indicate that phosphorous acid can be used to control tuber diseases caused by the oomycete pathogens, and are in agreement with other researchers (Cooke and Little, 2002; Johnson *et al.*, 2004).

Hydrogen peroxide, a disinfectant, has been shown to effectively suppress storage pathogens (Afek *et al.*, 2001; Al-Mughrabi, 2005; Al-Mughrabi, 2006). Despite the different application methods used, emitting hydrogen peroxide through a fogging system (Al-Mughrabi, 2005) or adding it into humidification water (Norikane *et al.*, 2001), hydrogen peroxide has had promising results in controlling storage pathogens. Our results indicated that hydrogen peroxide reduced the development of late blight, pink rot, Pythium leak, but had limited control of Fusarium dry rot under high disease pressure. According to Miller *et al.* (2006), hydrogen peroxide moderately controlled pink rot and late blight but the degree of control was subject to the duration between inoculation and time of application. Although the disinfectant was not applied immediately after inoculation as suggested by Miller *et al.*(2006), a significant reduction

of disease incidence and severity caused by the oomycete pathogens was attained using the method in this study.

Azoxystrobin is registered for foliar application in potato fields. However, it has been proposed for registration as a postharvest product for managing tuber decays caused by Fusarium species (Adaskaveg and Förster, 2010) since TBZ is the only registered postharvest fungicides for controlling Fusarium dry rot and is no longer effective in controlling dry rot caused by *F. sambucinum* (Ocamb *et al.*, 2007). Olsen and Miller (2005) reported that azoxystrobin could be used to reduce silver scurf in storage. Azoxystrobin provided limited control of Fusarium dry rot even under low disease pressure, but it effectively controlled Pythium leak and pink rot. The current study showed that none of the products evaluated provided complete control of the storage pathogens; however, they still have a high potential when used in an integrated management strategy for postharvest disease control in potatoes. These strategies include proper handling of tubers during harvesting, transportation, and bin loading, removal of all infected tubers prior to storage and maintenance of proper storage conditions (Knowles and Plissey, 2008). The biofungicides had limited control of storage diseases while compared to the fungicides.

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Chapter 2: Effects of In-season Applications Combined with Bin loading Applied Fungicides on Potato Tuber Disease Incidence Caused by Storage Pathogens

Abstract

The effects of in-season crop protection in combination with bin loading applied fungicides and biofungicides on tuber response against storage pathogens was evaluated. The in-season treatments included in-furrow and foliar application of mefenoxam and phosphorous acid and foliar application of and *Bacillus subtilis*. Bin loading treatments were phosphorous acid, *B. subtilis* and a 3-way mixture of azoxystrobin, fludioxonil and difenoconazole. These products were tested for control of tuber late blight (*Phytophthora infestans*), Fusarium dry rot (*Fusarium sambucinum* and *Fusarium* spp.), Pythium leak (*Pythium ultimum*) and pink rot (*Phytophthora erythroseptica*) under two storage temperatures on two cultivars, 10^{o}C (on cv. FL1879) and 4^{o}C (on cv. Goldrush). There was a significant interaction between field and storage treatment for Fusarium dry rot incidence and severity. The interaction between fields treated with *B. subtilis* or mefenoxam and storage treatment with *B. subtilis*, the 3-way mixture of azoxystrobin, fludioxonil and difenoconazole or phosphorous acid resulted in reduced dry rot incidence and severity. There was a significant interaction between field and storage treatments for tuber late blight incidence and severity. The combination of phosphorous acid in the field and in storage significantly reduced late blight development. The interaction between field treatment with *B. subtilis*, mefenoxam or phosphorous acid and storage treatment with phosphorous acid significantly reduced Pythium leak development. There was a significant interaction between field treatments and storage treatments for pink rot incidence. Tubers tested as inoculated checks from fields treated with mefenoxam or phosphorous had significantly lower pink rot incidence compared to those from the inoculated check from non-treated field. In-season crop protection

strategies, e.g. in-furrow and foliar applications combined with bin loading applied fungicides and biofungicides were viable options for controlling disease incidence caused by storage pathogens and hence should be adopted in potato production as integrated tools for storage disease management.

Key words: In-season, bin loading, fungicides/ biofungicides, storage-pathogens

2.1 Introduction

Potatoes (*Solanum tuberosum* L.) in storage are likely to lose quality and economic value due to diseases, thus control of storage pathogens has become increasingly important (Secor and Gudmestad, 1999). The major diseases in storage include tuber late blight caused by *Phytophthora infestans* (Mont.) de Bary, Fusarium dry rot caused by *Fusarium sambucinum* Fuckel and other *Fusarium* spp., Pythium leak caused by *Pythium ultimum* Trow and pink rot caused by *Phytophthora erythroseptica* Pethbr. (Powelson and Rowe, 2008). These pathogens can infect the tubers during the growing season (through underground stems, stolons, or roots), at harvest and in storage (Secor and Gudmestad, 1999) through the lenticels and the eyes or wounds inflicted during harvesting (Powelson and Rowe, 2008). The pathogens are also carried into storage either on soil adhering to the tuber or as latent infection on the tuber surface or inside the tuber. Significant losses in storage with estimates of up to 100% have been reported both in developed and developing countries when disease management is neglected (Wale *et al.*, 2008). In 2007, approximately 10% of potatoes produced in the United States (US) were unsold due to poor quality in storage caused by bruising, diseases, weight loss and sprouting (Guenthner, 2010).

Management of potato storage diseases can be achieved through integration of management practices at all stages of production. These practices include planting high quality seed tubers treated with fungicides, planting at the right depth, soil nutrient and water management and foliar application of fungicides and biofungicides during the growing season (Secor and Johnson, 2008). Use of fungicides has been the primary management practice in potato production (Hamm *et al.*, 2008). In-furrow application of systemic fungicides particularly with fungicides that are taken up by the roots and are able to move to the shoots, protect the crop from foliar diseases and insect attack allowing for healthy tuber development (Hamm *et al.*, 2008). Some of the fungicides registered in the US for in-furrow application to control potato diseases include mefenoxam (Ridomil Gold^{TM}), phosphorous acid (Phostrol^{TM}), azoxystrobin (Amistar^{TM}; Quadris^{TM}) and mefenoxam + chlorothalonil (Flouronil^{TM}; Ridomil Bravo^{TM}) (Zitter, 2010). Phosphorous acid, mefenoxam and chlorothalonil are registered for control of late blight, pink rot and Pythium leak while azoxystrobin controls black scurf caused by *Rhizoctonia solani* Kuhn, silver scurf caused by *Helminthosporium solani* Durieu & Mont and Fusarium seed piece decay when applied on freshly cut seed tubers (Powelson and Rowe, 2008).

In-furrow application of mefenoxam was reported to effectively control *Phytophthora erythroseptica* and *Pythium ultimum* compared to phosphorous acid (Al-Mughrabi *et al.*, 2007) and chlorothalonil (Platt *et al.*, 2004). Mefenoxam (or its parent isomer metalaxyl) is a systemic fungicide with a single-mode of action that inhibits ribosomal RNA polymerases enzyme (Fernández-Northcote *et al.*, 2000; Stevenson, 2008). It is able to penetrate the potato tuber and significant residue has been recovered in tubers from plants treated with mefenoxam in the field (Bruin *et al.*, 1982; Barak *et al.*, 1984). However, the intensive use of mefenoxam resulted in high levels of insensitive isolates of *Phytophthora infestans* (Fernández-Northcote *et al.*, 2000),

P. ultimum and *P. erythroseptica* (Taylor *et al.*, 2002; Porter *et al.*, 2009) in the field. Nevertheless, there is still a population of *P. ultimum* and *P. erythroseptica* that is sensitive to mefenoxam especially when applied in-furrow (Taylor *et al.*, 2004; Al-Mughrabi *et al.*, 2007). Mefenoxam is also registered for foliar applications and has been reported to prevent tuber blight development (Fernández-Northcote *et al.*, 2000).

To counteract the loss of effectiveness of mefenoxam against the oomycete pathogens, phosphorous acid was registered and is currently the most common foliar applied fungicide (Brunings *et al.*, 2005); its effectiveness in suppressing oomycete pathogens has been demonstrated (Cooke and Little, 2002; Johnson *et al.*, 2004; Mayton *et al.*, 2008). Phosphorous acid is a systemic fungicide with both basipetal and acropetal movement; thus a foliar spray is translocated within the plant to the root system and can control tuber rots (Brunings *et al.*, 2005). The mode of action is direct antifungal activity of phosphorous acid towards mycelial growth (Guest and Bompeix, 1990), and perhaps indirect by stimulation of plant defense (Guest and Bompeix, 1990; Brunings *et al.*, 2005; Lobato *et al.*, 2010). Phosphorous acid is also registered for postharvest use; when applied on potato tubers immediately after harvest and prior to storage, tuber late blight and pink rot development was greatly decreased (Miller *et al.*, 2006), and its use continues to show great potential especially when the labeled rates are used (Johnson, 2008).

Other fungicides and biofungicides registered for postharvest use include, thiabendazole (TBZ: Mertect^{TM}), *Bacillus subtilis* (Serenade^{TM} ASO & MAX), *Pseudomonas syringae* (Bio-Save 10LP) and *Bacillus pumilis* (Sonata^{TM}) (Zitter, 2010). These fungicides and biofungicides work against a range of postharvest diseases of potato including pink rot, Fusarium dry rot, late blight, silver scurf, early blight and black scurf. Resistance in populations of *Fusarium* and *Helminthosporium* species against TBZ has been reported (Kawchuk *et al.*, 1994). To counteract

the lack of effectiveness of TBZ, additional registration of postharvest fungicides is needed and some have already been proposed; difenoconazole, azoxystrobin and fludioxonil for managing decays caused by *Fusarium* species on potato and other tuber crops (Adaskaveg and Förster, 2010). Currently, a product of a 3-way mixture of azoxystrobin, fludioxonil and difenoconazole is also being tested by Syngenta for dry rot and silver scurf, but has not yet been registered (Kirk personal communication).

So far no study has been done to evaluate the effect of growing season tuber protection strategies, e.g. in-furrow and foliar applications of crop protectants, in combination with bin loading application of fungicides and biofungicides on tuber health during storage. Hence, the objective of this study was to determine the effects of these combinations on reduction of disease incidence caused by *Phytophthora infestans* (late blight), *Fusarium sambucinum* (Fusarium dry rot), *Pythium ultimum* (Pythium leak) and *Phytophthora erythroseptica* (pink rot).

2.2 Materials and Methods

2.2.1 Field Treatment

Two field trials were conducted at Michigan State University, Montcalm Research Farm, Entrican, MI, between 2008 and 2009. The 2008 trial was planted on May 15th, and the 2009 trial was planted on May 21st. The soil at Montcalm is a sandy loam. Four plots of 100 x 50 m were laid out in a randomized complete block design with each plot representing a field treatment (Table 2.1). Soils were plowed to a 20-cm depth in October following harvest of corn. Soils were prepared for planting with a mechanical cultivator in early May and fertilizer applied during final seedbed preparation before planting based on results of soil testing done in spring of

each year. Two potato cultivars, cv. FL1879, a chip processing cultivar and cv. Goldrush, a table-stock cultivar, were planted 12.5-cm deep in 0.9-m row spacing and 27.9-cm within rows. Nine rows of each variety were planted resulting in 18 rows per plot/ field treatment. Field treatment 1 consisted of in-furrow application at-planting of mefenoxam [(4.1mL/100 row meter); Syngenta Crop Protection, Inc. Greensboro, NC], followed by foliar applications of mefenoxam (2.24 kg/ha) at canopy closure (on June 26th in 2008 and on July 2nd in 2009) and repeated a further two times at 14-d intervals. Field treatment 2 consisted of an in-furrow at-planting application of phosphorous acid [(6.4 mL/100 row meter); Phostrol 53.6% SC; Nurfam Americas Inc. Houston, TX], followed by foliar applications of phosphorous acid (11.7 L/ha) at canopy closure (on June 26th in 2008 and on July 2nd in 2009) and repeated a further two times at 14-d intervals. Field treatment 3 consisted of foliar applications of *Bacillus subtilis*; Serenade ASO 1.34SC [(14 L/ha); AgraQuest Inc. Davis, CA] at canopy closure (on June 26th in 2008 and on July 2nd in 2009) and repeated a further two times at14-d intervals. The control plot was treated only with chlorothalonil (Bravo WS 6SC (1.75 L/ha; Syngenta) on a 7-d schedule initiated at canopy closure (on June 26th in 2008 and on July 2nd in 2009) for a total of seven applications prior to desiccation. Treatment plots 1 to 3 were also treated with chlorothalonil on a 7-d schedule (as described above) except when the experimental treatments were applied. Weeds were controlled by hilling and with metolachlor [(2.23L/ha); Dual 8E; Syngenta] during planting and sethoxydim [(1.75L/ha); Poast; BASF Ag Products, Research Triangle Park, NC] on July 10th in 2008 and July 15th in 2009. Insects were controlled with Imidacloprid [(1.4 L/ha); Admire 2F; Bayer CropScience, Triangle Park, NC)] at planting and on June 29th 2008 and July

6th 2009; Carbaryl [(1.4 kg/ha): Sevin 80S; Bayer] on July 11th and 25th in 2008 and on July 20th and August 3rd in 2009; and Endosulfan [(3.3 L/ha); Thiodan 3EC; Universal Crop Protection Alliance. LLC, Eagan MN]. Vines were killed on September 5th 2008 and September 29th 2009, with diquat dibromide [(1.17 L/ha); Reglone 2EC; Syngenta]. Plots, 9 rows per variety, were harvested on September 17th 2008 and on October 13th 2009. The harvested tubers were put in 450 kg aerated wooden boxes and labeled according to the field treatment. The tubers were transported to a curing facility, which was maintained at 10^{o}C, 90% relative humidity in the darkness for three weeks prior to the storage experiments.

Table 2.1 Methods and rates of application of fungicides and biofungicides in field experiments

Fields[a]	In-furrow application[b]	Rates mL/100 row meter[c]	Foliar application[d]	Rates L/ha; Kg/ha
1	Mefenoxam; Ridomil Gold 47.6% SC	4.1	Mefenoxam; Ridomil Gold Bravo	2.24
2	Phosphorous acid; Phostrol 53.6% SC	6.4	Phosphorous acid; Phostrol 53.6% SC	11.7
3	Untreated		*Bacillus subtilis*; Serenade ASO 1.34 SC	14
4	Untreated		Untreated	

NB: All fields were maintained with a chlorothalonil foliar application at 1 L/ha in 200 L water/ha

[a] Field (plot) represented a treatment: mefenoxam, phosphorous acid, *B. subtilis* or untreated

[b] Products used for in-furrow application at planting

[c] Rates used were the manufacturer's recommended rates

[d] Products used for foliar applications at 6 wks after planting (at canopy closure; June 26th in 2008 and July 2nd in 2009) with a further 2 applications at 14-d intervals

2.2.2 Storage experiment

2.2.2.1 Potato tubers preparation

The storage experiment was initiated on October 22nd for the 2008 trial and on November 10th for the 2009 trial. The cured potato tubers grown under the field treatments described above were used for storage experiments. Tubers free from any defect or visible disease symptoms were selected and washed by slowly passing them through a conveyor lined with nozzles, which sprayed water on the tubers thus washing off soil. The clean tubers were put in clean plastic crates and allowed to dry for 24 h. The tubers were then prepared for inoculation by grazing with a single light stroke with a wire brush, sufficient to abrade the skin of the tubers to a depth of 0.01 mm for 2008 trial. In 2009, only the tubers used for Fusarium dry rot trial were wounded as described for the 2008 trial, while the remaining tubers for late blight, Pythium leak and pink rot trial were soaked in the inoculum for 48 h. The tests were carried out at two storage temperatures used in the potato industry; 10^{o}C, (chip processing) and 4^{o}C (table stock). The cultivar used in the 10^{o}C was FL1879, a chip processing cultivar and cv. Goldrush at 4^{o}C, a table-stock russet-skinned cultivar.

2.2.2.2 Inoculum preparation and tuber inoculation

Storage pathogens previously isolated from potato tubers in Michigan were grown for 10 days prior to the preparation of inoculum. All pathogens were grown on potato dextrose agar (PDA; Difco, Detroit, Michigan) except for *Phytophthora infestans*, which was grown on rye media prepared by steaming rye seed (100 g/L of distilled water) for 1 h, with addition of 7.5 g sucrose, 0.05 g ß-sitosterol, and 1.5% agar to the resulting broth filtrate. Solutions containing of

sporangia of *P. infestans*, oospores/sporangia of *P. erythroseptica*, oospores of *P. ultimum* and macroconidia of *F. sambucinum* were prepared and spore concentrations adjusted to 1 x 10^3/mL by use of hemacytometer. Two untreated controls, either inoculated with the pathogen or a non-inoculated check were included in the trial for every treatment combination (field treatment x storage treatment). Tubers (25/replicate/treatment; total 100 tubers/treatment) were sprayed with 10 mL of *P. erythroseptica*, *F. sambucinum* or *P. infestans* suspension for a final dosage of about 0.1mL per tuber in 2008.

For the *Pythium ultimum* trial, inoculation was done by soaking the wounded tubers in the inoculum for 24 h prior to application of storage treatments. Based on past experience with failures using the aerosol technique for inoculating tubers with *P. ultimum* (unpublished data) the immersion technique was used and was described below. 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 tuber were soaked in the inoculum with or without prior wounding (Table 2.2).

In 2009, based on the optimization experiment results (Table 2.2), only tubers destined for the Fusarium dry rot trial were wounded while the remaining trials (late blight, pink rot and Pythium leak), the tubers were not wounded. The exposure time to the inoculum was increased to 48 h for all the trials to expose the tubers for a longer period and enhance disease development. After 48 h, tubers were removed from the inoculum and placed in plastic crates, 25 tubers per crate. The inoculated tubers were stored for 24 h at 20^{O}C before treatment with fungicides or biofungicides (Table 2.3). Fungicides were applied as liquid treatments in a water suspension with a single R&D XR11003VS spray nozzle at a rate of 1L/ton at 344.74 Kpa onto the tuber surfaces, with an entire seed surface being coated. Treated tubers were incubated in the

dark in plastic crates at 10^{o}C (cv. FL1879) or 4^{o}C (cv. Goldrush). In 2008, the Pythium trial was evaluated on January 7th 2009, after 60 d of storage, while the rest of the trials, pink rot, late blight and dry rot, were evaluated on March 9th, after 120 d in storage. In 2009, the evaluation for all the trials were done on March 10th, approximately 120 d of storage. Tubers were cut longitudinal into four slices and evaluated for presence of symptoms or signs. Tubers with symptoms or signs of the individual disease were counted and disease incidence determined (number of tubers with signs or symptoms/ number of tubers per rep * 100). Data were tested for assumptions of normality and analyzed using the analysis of variance platform (ANOVA) and the Tukey's HSD test in JMP (JMP © 2008. SAS Institute Inc., SAS Campus Drive, Cary, NC, USA 27513).

Table 2.2 Effects of exposure time to inoculum ($1X10^3$/mL) on potato tuber disease development after 60 d in storage at 10^oC

Exposure time (h)	Mean disease incidence (%)			
	Wounded	Not wounded		
	Pythium leak	Pythium leak	Late blight	Pink rot
4	8.3	-[a]	-	-
8	15.0	-	-	-
12	26.7	11.3	13.8	15.9
24	41.5	36.3	29.6	36.9
Tukey's HSD ($p<0.05$)	25.33	5.15	2.67	2.56

[a] = Data not available

Table 2.3 Storage treatment combination using fungicides and biofungicides

Treatments		Active ingredient	Formulation[a]	Rate mL/ 100kg[b]	Manufacturer
1	Non-inoculated check				
2	Inoculated Check				
3	Serenade ASO	*Bacillus subtilis*	1.34SC	20.9	AgraQuest, Inc. Davis CA
4	Phostrol	Phosphorous acid	53.6SC	83.5	NuFarm Americas, Inc. Burr Ridge, IL
5	A12705 (Quadris) +	Azoxystrobin	250SC	3.9	Syngenta Crop
	A9859A (Maxim) +	Fludioxonil	250SC	11.7	Protection, Inc.
	A8754B (Inspire)	Difenoconazole	250SC	1.96	Greensboro NC

[a] Formulation= products added to the active ingredient to change its physical characteristic and allow compatibility with the machinery; SC= Suspension concentrates

[b] Rate = mL of the product per 100 kg of potato tubers

2.3 Results

2.3.1 Fusarium dry rot

Analysis of variance showed that there were significant differences between results from each year for disease incidence at 10^{o}C on cv. FL 1879 and at 4^{o}C on cv. Goldrush, therefore data from each year was analyzed separately (Table 2.4). A lower level of dry rot incidence was observed in 2008 than in 2009. At 10^{o}C on cv. FL1879, the variable, field treatment, had a signifcant effect ($p=0.0068$ and $p=0.0163$) on dry rot incidence in 2008 and 2009, respectively (Table 2.5). Field treatment with phosphorous acid or mefenoxam significantly reduced dry rot incidence and differed from the untreated field, but not from field treated with *Bacillus subtilis* in 2008 and 2009. The variable, storage treatment, had a signifcant effect ($p<0.0001$) on dry rot incidence in 2008 and 2009 (Table 2.5). In 2008, storage treatment with phosphorous acid or *B. subtilis* had a significant effect on dry rot incidence compared to the inoculated check but did not differ from the the 3-way mixture of azoxystrobin, fludioxonil and difenoconazole. In 2009, storage treatment with phosphorous acid or the the 3-way mixture of azoxystrobin, fludioxonil and difenoconazole significantly reduced dry rot incidence compared to the inoculated check.

The interaction of field and storage treatments had a significant ($p=0.0005$, $p=0.0062$) effect on disease incidence in 2008 and 2009 respectively (Table 2.5). All inoculated checks from fields treated with phosphorous acid, *Bacillus subtilis* or mefenoxam resulted to significantly low dry rot incidence compared to the inoculated check from the untreated field in 2008 (Table 2.6). However, in 2009, all the inoculated checks from all field treatments did not differ significantly from each other with respect to dry rot incidence. The interaction of field treatment, *B. subtilis*, with storage treatment, phosphorous acid significantly reduced dry rot

incidence in 2009 compared to the interaction of untreated field and storage treatment with phosphorous (Table 2.6).

At 4^{o}C on cv. Goldrush, very low dry rot disease incidence was observed in 2008 compared to 2009 (Table 2.4). The variable, field, only had a significant effect on dry rot incidence in 2009 ($p=0.0197$) but not in 2008 ($p=0.4429$); (Table 2.5). Field treatment with mefenoxam had a significant effect on dry rot incidence while compared to the untreated field in 2009. The variable, storage, had a significant ($p=0.0002$, $p<0.0001$) effect on dry rot in 2008 and 2009, respectively (Table 2.5). Storage treatment, *Bacillus subtilis*, phosphorous acid or the the 3-way mixture of azoxystrobin, fludioxonil and difenoconazole significantly reduced dry rot incidence compared to the inoculated check in 2008 and 2009. The interaction between field and storage treatments had a significant ($p<0.0001$) effect on dry rot incidence in 2009 but not in 2008 ($p=0.1291$); (Table 2.5). The inoculated check from fields treated with mefenoxam or *B. subtilis* differed significantly from the inoculated checks from untreated field (Table 2.6).

Table 2.4 Main effects analyses of field and storage treatments on Fusarium dry rot incidence on tubers stored at 10^{o}C (cv. FL1879) and 4^{o}C (cv. Goldrush) as impacted by the year in which the experiments were carried out, 2008 and 2009

Variable Measured		F Ratio	Prob < F	Year of Trial 2008	2009	HSD
Incidence[a]	10^{o}C (cv. FL 1879)	723.0123	<0.0001	1.7b[c]	25.9a	16.05
	4^{o}C (cv. Goldrush)	33.3831	<0.0001	1.7b	10.7a	3.08

[a] Incidence was calculated as number of tubers showing Fusarium dry rot symptoms relative to the number of tubers per treatment

[c] Numbers followed by the same letter within a column are not significantly different at p = 0.05 (Tukey test)

Table 2.5 Main effects analyses of field and storage treatments and interactions between these variables on Fusarium dry rot incidence on tubers stored at 10°C (cv. FL1879) and at 4°C (cv. Goldrush) for 120 d in 2008 and 2009

		Incidence[a] (%)			
		10°C (cv. FL1879) Prob<F		4°C (cv. Goldrush) Prob<F	
Source of variation	Df[b]	2008	2009	2008	2009
Field[c]	3	0.0068	0.0163	0.4429	0.0197
Storage[d]	4	<0.0001	<0.0001	0.0002	<0.0001
Storage*Field	12	0.0005	0.0062	0.1291	<0.0001

[a] Incidence was calculated as number of tubers showing Fusarium dry rot symptoms relative to the number of tubers per treatment

[b] Df = degrees of freedom

[c] Field (plot) represented a treatment; phosphorous acid, mefenoxam, *Bacillus subtilis* and untreated control

[d] Storage treatment consisted of inoculated check, not-inoculated check, phosphorous acid, *B. subtilis*, and a 3-way mixture of azoxystrobin, fludioxonil and difenoconazole

Table 2.6 Effects of field and storage treatments and interactions on Fusarium dry rot incidence on tubers stored at 10°C (cv. FL1879) and 4°C for 120 d in 2008 and 2009

Variables[a]		Mean Incidence[b] (%)			
		10°C		4°C	
Field	Storage	2008	2009	2008	2009
Untreated control	Inoculated check	14.0 a[c]	74.0 a	5.0 a	58.0 a
	Not-inoculated check	0.0 d	0.0 f	0.0 a	0.0 d
	Phosphorous acid	2.0 bc	31.0 cd	3.0 a	5.0 d
	Azn + Fld +Dfz[d]	8.0 ab	28.0 cde	1.0 a	1.0 d
	Bacillus subtilis	4.0 bc	37.0 bc	3.0 a	4.0 d
Mefenoxam	Inoculated check	3.0 bc	59.0 a	3.0 a	30.0 c
	Not-inoculated check	0.0 d	0.0 f	0.0 a	0.0 d
	Phosphorous acid	3.0 bc	15.0 def	3.0 a	6.0 d
	Azn + Fld +Dfz	2.0 bc	17.0 cdef	2.0 a	4.0 d
	Bacillus subtilis	0.0 d	25.0 cde	0.0 a	2.0 d
Phosphorous acid	Inoculated check	7.0 bc	61.0 a	2.0 a	48.0 ab
	Not-inoculated check	0.0 d	0.0 f	0.0 a	0.0 d
	Phosphorous acid	1.0 cd	12.0 def	1.0 a	0.0 d
	Azn + Fld +Dfz	2.0 bc	19.0 cdef	1.0 a	0.0 d
	Bacillus subtilis	2.0 bc	27.0 cde	2.0 a	5.0 d
Bacillus subtilis	Inoculated check	4.0 bc	57.0 ab	4.0 a	43.0 bc
	Not-inoculated check	0.0 d	0.0 f	0.0 a	0.0 d
	Phosphorous acid	3.0 bc	9.0 ef	0.0 a	1.0 d
	Azn + Fld +Dfz	5.0 bc	24.0 cde	0.0 a	4.0 d
	Bacillus subtilis	1.0 cd	23.0 cde	4.0 a	3.0 d
Tukey's HSD ($p<0.05$)		6.38	20.43	5.24	13.43
Prob (F)		0.0005	0.0062	0.1291	<0.0001

NB: All fields were maintained with a chlorothalonil foliar application at 1 L/ha in 200 L water/ha

[a] Variable consisted of field treatment (phosphorous acid, mefenoxam, *Bacillus subtilis* and untreated control) and storage treatment (inoculated check, not-inoculated check, phosphorous acid *Bacillus subtilis* and a 3-way mixture of azoxystrobin, fludioxonil and difenoconazole) and their interactions

[b] Incidence was calculated as number of tubers showing Fusarium dry rot symptoms relative to the number of tubers per treatment

[c] Numbers followed by the same letter within a column are not significantly different at $p = 0.05$ (Tukey test)

[d] Azn = Azoxystrobin; Fld = Fludioxonil; Dfz= Difenoconazole

2.3.2 Tuber late blight

There were significant differences between late blight results from each year for disease incidence, therefore data from each year were analyzed separately (Table 2.7). Lower late blight incidence was observed in 2008 than in 2009. At 10^{o}C on cv. FL 1879, field treatment had a significant ($p<0.0001$) effect on late blight incidence in 2009 but not in 2008 ($p=0.2306$), with phosphorous acid significantly reducing late blight incidence in 2009. Storage treatment had a significant ($p<0.0001$) effect on late blight incidence in 2008 and in 2009 (Table 2.8). All the storage treatments, phosphorous acid, *Bacillus subtilis* and the 3-way mixture of azoxystrobin fludioxonil and difenoconazole had a significant effect on late blight incidence in 2008. However, in 2009, only phosphorous acid and *B. subtilis* had a significant effect on late blight incidence. The interaction of field and storage treatment had a significant ($p <0.0001$) effect on late blight incidence in 2008 and 2009 (Table 2.8). The inoculated checks from untreated field had significantly higher disease incidence compared to all the other interactions of field and storage in 2008. In 2009, the inoculated check from the field treated with phosphorous acid had a significantly lower late blight incidence compared to inoculated checks from the other fields (Table 2.9). The interaction of phosphorous acid in the field and with phosphorous acid in storage resulted to a significant reduction of late blight incidence in 2009.

At 4^{o}C on cv. Goldrush, late blight incidence was only observed in the 2009 trial (Table 2.8). Field treatment did not have a significant ($p = 0.2306$) effect on late blight incidence, but the storage treatment had a significant ($p<0.0001$) effect on late blight incidence. The variable field and the interaction of field and storage treatments did not have a significant effect on late blight incidence (Table 2.8). The inoculated check from the untreated field had significantly

higher late blight incidence compared to all the other interactions between field and storage treatments (Table 2.9).

Table 2.7 Main effects analyses of field and storage on disease incidence on potato tubers stored at 10^{o}C (cv. FL1879) and 4 ^{o}C (cv. Goldrush) as impacted by the year in which the experiments were carried out, 2008 and 2009

Disease incidence[a]		F Ratio	Prob < F	Year of Trial 2008	2009	HSD
Late blight	10^{o}C (FL 1879)	71.2484	<0.0001	1.6	26.2	5.76
	4^{o}C (Goldrush)	-[b]	-	-	-	-
Pythium leak	10^{o}C (FL 1879)	30.0125	<0.0001	33.5	17.0	3.96
	4^{o}C (Goldrush)	232.2981	<0.0001	45.5	3.6	5.43
Pink rot	10^{o}C (FL 1879)	94.5561	<0.0001	37.1	6.7	6.19
	4^{o}C (Goldrush)	31.9711	<0.0001	2.4	10.7	2.89

[a] Incidence was calculated as number of tubers showing late blight, Pythium leak or pink rot symptoms relative to the number of tubers per treatment

[b] = - Data not available

Table 2.8 Main effects analyses of field and storage treatments and interactions between these variables on disease incidence on potato tubers stored at 10°C (cv. FL1879) and at 4°C (cv. Goldrush) for 120 d in 2008 and 2009

			Incidence[a] (%)			
			10°C (cv. FL1879)		4°C (cv. Goldrush)	
			Prob<F		Prob<F	
Disease	Source of variation	df[d]	2008	2009	2008	2009
Late blight	Field[b]	3	0.2306	<0.0001	-[e]	0.1458
	Storage[c]	4	<0.0001	<0.0001	-	<0.0001
	Storage*Field	12	<0.0001	<0.0001	-	0.0514
Pythium leak	Field	3	<0.0001	0.4101	<0.0001	0.0012
	Storage	4	<0.0001	<0.0001	<0.0001	<0.0001
	Storage*Field	12	<0.0001	0.2104	<0.0001	<0.0001
Pink rot	Field	3	0.7687	<0.0001	0.0154	<0.0001
	Storage	4	<0.0001	<0.0001	<0.0001	<0.0001
	Storage*Field	12	<0.0001	<0.0001	<0.0001	<0.0001

[a] Incidence was calculated as number of tubers showing late blight, Pythium leak or pink rot symptoms relative to the number of tubers per treatment

[b] Field (plot) represented a treatment; phosphorous acid, mefenoxam, *Bacillus subtilis* and untreated control

[c] Storage treatment consisted of inoculated check, not-inoculated check, phosphorous acid, *B. subtilis*, and a 3-way mixture of azoxystrobin, fludioxonil and difenoconazole

[d] df = degrees of freedom

[e] = - Data not available

Table 2.9 Effects of field and storage treatments and interactions on late blight incidence on tubers stored at 10°C (cv. FL1879) and 4°C for 120 d in 2008 and 2009

Variables[a]		Mean Incidence[b] (%)		
		10°C		4°C
Field	Storage	2008	2009	2009
Untreated control	Inoculated check	11.0 a[c]	100.0 a	34.0 a
	Not-inoculated check	0.0 b	0.0 g	0.0 b
	Phosphorous acid	0.0 b	4.0 fg	0.0 b
	Azn + Fld +Dfz[d]	2.0 b	32.0 c	0.0 b
	Bacillus subtilis	1.0 b	8.0 efg	0.0 b
Mefenoxam	Inoculated check	4.0 b	100.0 a	12.0 b
	Not-inoculated check	0.0 b	0.0 g	0.0 b
	Phosphorous acid	0.0 b	9.0 efg	0.0 b
	Azn + Fld +Dfz	1.0 b	18.0 de	1.0 b
	Bacillus subtilis	1.0 b	10.0 g	0.0 b
Phosphorous acid	Inoculated check	4.0 b	83.0 b	5.0 b
	Not-inoculated check	0.0 b	0.0 g	0.0 b
	Phosphorous acid	0.0 b	2.0 g	0.0 b
	Azn + Fld +Dfz	0.0 b	6.0 efg	0.0 b
	Bacillus subtilis	0.0 b	7.0 efg	0.0 b
Bacillus subtilis	Inoculated check	3.0 b	97.0 a	15.0 ab
	Not-inoculated check	0.0 b	0.0 g	0.0 b
	Phosphorous acid	5.0 b	23.0 cd	0.0 b
	Azn + Fld +Dfz	0.0 b	16.0 def	0.0 b
	Bacillus subtilis	0.0 b	9.0 efg	0.0 b
Tukey's HSD ($p<0.05$)		5.33	13.91	21.11
Prob (F)		<0.0001	<0.0001	0.0514

NB: No disease incidence at 4°C on cv. Goldrush in 2008

[a] Variable consisted of field (phosphorous acid, mefenoxam, *Bacillus subtilis* and untreated control) and storage treatment (inoculated check, not-inoculated check, phosphorous acid *Bacillus subtilis* and a 3-way mixture of azoxystrobin, fludioxonil and difenoconazole) and their interactions

[b] Incidence was calculated as number of tubers showing late blight symptoms relative to the number of tubers per treatment

[c] Numbers followed by the same letter within a column are not significantly different at p = 0.05 (Tukey test)

[d] Azn = Azoxystrobin; Fld = Fludioxonil; Dfz= Difenoconazole

2.3.3 Pythium leak

There were significant differences between Pythium leak results from each year for disease incidence on tubers stored at 10ºC on cv. FL1879 and at 4^{O}C on cv. Goldrush; (Table 2.7), therefore data from each year were analyzed separately. A higher Pythium leak incidence was observed in 2008 than in 2009. At 10^{O}C on cv. FL 1879, the variables, field treatment had a significant ($p<0.0001$) effect on Pythium leak incidence in 2008 but not in 2009 ($p=0.4101$); (Table 2.8). Field treatment with phosphorous acid or mefenoxam significanly reduced Pythium leak incidence in 2008. The variable, storage treatment had a significant effect on Pythium leak incidence in 2008 and 2009. Storage treatment with phosphorous acid or *Bacillus subtilis* had a significant effect on Pythium leak incidence in 2008 and 2009. The interaction of field and storage treatment had a significant ($p<0.0001$) effect on Pythium leak in 2008 but not in 2009 ($p=0.2104$); (Table 2.10). Field treatment with mefenoxam, followed by storage treatment with phosphorous acid or field treatments with *B. subtilis* followed by storage treatment with phosphorous acid or the 3-way mixture of azoxystrobin, fludioxonil or difenoconazole, had a significant effect on Pythium leak incidence in 2008 (Table 2.14).

At 4^{O}C (cv. Goldrush), the variables field and storage, had a significant effect on Pythium leak incidence in 2008 and 2009 (Table 2.9). Field treatment with phosphorous acid had a significant effect on Pythium leak incidence in 2008. In 2009, field treatment with phosphorous acid or *Bacillus subtilis* had a significant effect on Pythium leak incidence. The variable storage treatment had a significant effect on Pythium leak incidence in 2008 and 2009. Storage treatment with phosphorous acid had a significant effect on Pythium leak incidence compared to the inoculated check in 2008. In 2009, storage treatment with phosphorous acid, *B. subtilis* or the 3-

way mixture of azoxystrobin, fludioxonil and difenoconazole had a significant effect on Pythium leak incidence compared to the inoculated check. The interaction of field and storage treatments has a significant ($p<0.0001$) effect on Pythium leak incidence in 2008 and 2009 (Table 2.8). Field treatment with mefenoxam, phosphorous acid or *B. subtilis* followed by storage treatment with phosphorous acid had a significant effect on Pythium leak incidence in 2008 (Table 2.10). Also interaction of field treatment with phosphorous acid or *B. subtilis* followed by storage treatment with the 3-way mixture of azoxystrobin, fludioxonil or difenoconazole, had a significant effect on Pythium leak incidence in 2008 (Table 2.10). In 2009, very low Pythium leak incidence was observed. The inoculated checks from fields treated with mefenoxam, phosphorous acid or *B. subtilis*, had a significant effect on Pythium leak incidence compared to the inoculated check from the untreated field (Table 2.10)

Table 2.10 Effects of field and storage treatments and interactions on Pythium leak incidence on tubers stored at 10^{o}C (cv. FL1879) and 4 ^{o}C for 120 d in 2008 and 2009

Variables[a]		Mean Incidence[b] (%)			
		10^{o}C		4^{o}C	
Field	Storage	2008	2009	2008	2009
Untreated control	Inoculated check	73.0 a[c]	97.0 a	75.0 a	47.0 a
	Not-inoculated check	0.0 f	0.0 c	0.0 j	0.0 c
	Phosphorous acid	53.0 ab	0.0 c	66.0 ab	0.0 c
	Azn + Fld +Dfz[d]	63.0 ab	0.0 c	46.2 bcde	0.0 c
	Bacillus subtilis	57.0 ab	24.0 bc	55.6 abc	1.0 bc
Mefenoxam	Inoculated check	57.0 ab	86.0 a	53.6 abcd	21.0 b
	Not-inoculated check	0.0 f	0.0 c	0.0 j	0.0 c
	Phosphorous acid	10.0 ef	0.0 c	34.8 cdef	0.0 c
	Azn + Fld +Dfz	52.0 ab	0.0 c	42.4 cdef	0.0 c
	Bacillus subtilis	47.0 ab	0.0 c	33.6 defg	0.0 c
Phosphorous acid	Inoculated check	45.0 ab	60.0 ab	31.0 efgh	1.0 bc
	Not-inoculated check	0.0 f	0.0 c	0.0 j	0.0 c
	Phosphorous acid	27.0 cd	0.0 c	4.2 ij	0.0 c
	Azn + Fld +Dfz	38.0 bc	0.0 c	14.2 ghij	0.0 c
	Bacillus subtilis	45.0 ab	0.0 c	9.8 hij	0.0 c
Bacillus subtilis	Inoculated check	74.0 a	73.0 a	48.0 bcde	2.0 bc
	Not-inoculated check	0.0 f	0.0 c	0.0 j	0.0 c
	Phosphorous acid	12.0 ef	0.0 c	23.2 fghi	0.0 c
	Azn + Fld +Dfz	17.0 def	0.0 c	22.4 fghi	0.0 c
	Bacillus subtilis	57.0 ab	0.0 c	42.0 cdef	0.0 c
Tukey's HSD ($p<0.05$)		30.45	41.40	19.37	20.30
Prob (F)		<0.0001	0.2104	<0.0001	<0.0001

[a] Variable consisted of field treatment (phosphorous acid, mefenoxam, *Bacillus subtilis* and untreated control) and storage treatment (inoculated check, not-inoculated check, phosphorous acid *Bacillus subtilis* and a 3-way mixture of azoxystrobin, fludioxonil and difenoconazole) and their interactions

[b] Incidence was calculated as number of tubers showing Pythium leak symptoms relative to the number of tubers per treatment

[c] Numbers followed by the same letter within a column are not significantly different at p = 0.05 (Tukey test)

[d] Azn = Azoxystrobin; Fld = Fludioxonil; Dfz= Difenoconazole

2.3.4 Pink rot

There were significant differences between pink rot results from each year for pink rot incidence, therefore data from each year were analyzed separately (Table 2.7). A lower pink rot incidence was observed in 2008 than in 2009. At 10^{o}C on cv. FL 1879, only the variables, storage treatment, had a significant ($p<0.0001$) effect on pink rot incidence 2008. However, in 2009, both variables, field and storage treatments, had a significant effect on pink rot incidence (Table 2.8). Field treatment with mefenoxam or phosphorous acid significantly reduced pink rot incidence compared to the untreated field in 2009. Storage treatment with phosphorous acid, *Bacillus subtilis*, or the 3-way mixture of azoxystrobin, fludioxonil and difenoconazole had a significant effect on pink rot incidence in 2008 and 2009. The interaction between field and storage treatment had a significant effect on pink rot incidence in 2008 and 2009 (Table 2.11). The inoculation checks from the untreated field and fields treated with phosphorous acid, *B. subtilis* or mefenoxam were not significantly different from one each other in 2008 (Table 2.11). The interaction of field treatment with phosphorous acid or mefenoxam and storage treatment with *B. subtilis* or the 3-way mixture of azoxystrobin, fludioxonil and difenoconazole had a significant effect on pink rot incidence in 2009 (Table 2.11). However, interaction of field treatemnt with mefenoxam, phosphoorus acid or *B. subtilis* and storage treatment with phosphorous acid, *B. subtilis* or the 3-way mixture of azoxystrobin, fludioxonil and difenoconazole did not significantly from each other for pink rot incidence in 2009 (Table 2.11).

At 4^{o}C (cv. Goldrush), only the variable storage treatment, had a significant effect on pink rot incidence 2008. However, in 2009, both variables, field and storage treatments had a signifcant effect on pink rot development. Storage treatment with phosphorous, *Bacillus subtilis*, or the 3-way mixture of azoxystrobin, fludioxonil and difenoconazole had a significant effect on

pink rot incidence in 2008 and 2009. Field treatment with mefenoxam or phosphorous acid had a significant effect on pink rot incidence in 2009. The interaction between field and storage had a significant effect on pink rot incidence in 2008 and 2009 (Table 2.8). Interaction of field treatment with mefenoxam or phosphorous acid and storage treatment with phosphorous acid or with the 3-way mixture of azoxystrobin, fludioxonil and difenoconazole had a significant effect on pink rot incidence in 2008 and 2009 (Table 2.11).

Table 2.11 Effects of field and storage treatments and interactions on pink rot incidence on tubers stored at 10°C (cv. FL1879) and 4 °C for 120 d in 2008 and 2009

Variables[a]		Mean Incidence[b] (%)			
		10°C		4°C	
Field	Storage	2008	2009	2008	2009
Untreated control	Inoculated check	16.0 a[c]	100.0 a	8.0 a	45.0 a
	Not-inoculated check	0.0 c	0.0 f	0.0 c	0.0 e
	Phosphorous acid	6.0 bc	39.0 bcd	4.0 abc	35.0 ab
	Azn + Fld +Dfz[d]	7.0 bc	55.0 b	2.0 abc	6.0 cde
	Bacillus subtilis	9.0 ab	50.0 bc	1.0 bc	27.0 abc
Mefenoxam	Inoculated check	10.0 ab	95.0 a	4.0 abc	5.0 de
	Not-inoculated check	0.0 c	0.0 f	0.0 c	0.0 e
	Phosphorous acid	8.0 ab	26.0 de	2.0 abc	0.0 e
	Azn + Fld +Dfz	5.0 bc	27.0 de	1.0 bc	0.0 e
	Bacillus subtilis	7.0 bc	11.0 ef	1.0 bc	0.0 e
Phosphorous acid	Inoculated check	10.0 ab	100.0 a	7.0 ab	2.0 e
	Not-inoculated check	0.0 c	0.0 f	0.0 c	0.0 e
	Phosphorous acid	8.0 ab	24.0 de	1.0 bc	0.0 e
	Azn + Fld +Dfz	9.0 ab	24.0 de	0.0 c	2.0 e
	Bacillus subtilis	5.0 bc	17.0 def	3.0 bc	2.0 e
Bacillus subtilis	Inoculated check	11.0 ab	94.0 a	6.0 abc	25.0 abc
	Not-inoculated check	0.0 c	0.0 f	0.0 c	0.0 e
	Phosphorous acid	6.0 bc	30.0 cde	3.0 abc	2.0 e
	Azn + Fld +Dfz	8.0 ab	19.0 def	3.0 abc	18.0 bcd
	Bacillus subtilis	8.0 ab	31.0 cde	2.0 abc	4.0 de
Tukey's HSD ($p<0.05$)		8.03	22.56	6.90	21.04
Prob (F)		<0.0001	<0.0001	<0.0001	<0.0001

[a] Variable consisted of field treatment (phosphorous acid, mefenoxam, *Bacillus subtilis* and untreated control) and storage treatment (inoculated check, not-inoculated check, phosphorous acid *Bacillus subtilis* and a 3-way mixture of azoxystrobin, fludioxonil and difenoconazole) and their interactions

[b] Incidence was calculated as number of tubers showing pink rot symptoms relative to the number of tubers per treatment

[c] Numbers followed by the same letter within a column are not significantly different at $p = 0.05$ (Tukey test)

[d] Azn = Azoxystrobin; Fld = Fludioxonil; Dfz= Difenoconazole

2.4 Discussion

Managing potato storage pathogens is primarily achieved through maintaining proper storage conditions and use of postharvest fungicides (Secor and Gudmestad, 1999). However, there is limited availability of postharvest products so crop protection during the growing season is important to increase tuber resistance against storage pathogens. Most of the studies carried out for managing potato storage pathogen focus either on in-season or postharvest crop protection but not the combination of the two. For instance, research has been done to evaluate in-season crop protection including in-furrow (Porter *et al.*, 2006; Al-Mughrabi *et al.*, 2007) and foliar (Johnson *et al.*, 2004; Platt *et al.*, 2004) application of fungicides to control potato tuber rots caused by oomycete pathogens. Other studies have focused on postharvest application of fungicides for control of tuber rots caused by oomycete pathogens (Miller *et al.*, 2006; Johnson, 2008), but no work has been done to evaluate the effects of combining in-season crop protection strategies with postharvest applied fungicide on tuber response to storage pathogens.

The results of our trials over the 2 years demonstrate that in-season crop protection strategies, e.g. in-furrow and foliar applications combined with bin loading applied fungicides and biofungicides is a viable option for increasing tuber protection against storage pathogens. The test utilized two storage temperatures, 10 and 4^{o}C, on two different cultivars to simulate storage rot development on tubers destined for chipping (cv. FL 1879) and table-stock or seed (cv. Goldrush), respectively. Tuber rot development measured as incidence generally increased with temperature. In 2008, very low disease development was observed at both temperatures on both cultivars for Fusarium dry rot, late blight and pink rot. Indeed, late blight developed only at 10^{o}C on cv. FL 1879, unlike in 2009 where it developed at both temperatures on both cultivars.

The failure for late blight development was unclear, although we attributed the low disease development in 2008 to the method of inoculation used.

Soaking wounded tubers in the inoculum as in the case of Pythium leak trial in 2008 resulted in high disease incidence compared to soaking unwounded tubers. Soaking wounded tubers may have delayed the wound healing process resulting in further infection of the tubers. Lulai (2001) reported that the wound healing process in potatoes requires a relative humidity of 90-95% and levels above this or presence of a film of water may delay the process due to restricted oxygen supply and cell enlargement leading to further infection. Spraying wounded tubers with the inoculum (*Fusarium sambucinum, Phytophthora infestans* or *P. erythroseptica*) in the 2008 trial resulted in less disease development than soaking the unwounded tubers in the inoculum for 48 h in the 2009 trial. This meant that although wounds were necessary for pathogen penetration, extended soaking of unwounded tubers in the inoculum led to severe disease (pink rot and late blight) development. Pythium leak developed on soaked unwounded tubers but at a very low level. *Pythium ultimum* has been said to cause tuber infection only through wounds (Salas and Secor, 2001) but our results indicated that it had a potential to directly infect intact tubers. Direct penetration could be attributed to enlargement of the lenticels, thus allowing infiltration of the pathogens (*P. infestans* and *P. erythroseptica*) into the tuber resulting to infection in (Lulai, 2001). It is therefore important to ensure that tubers are grown in well-drained soils, and there is no film of water on tubers during storage to avoid direct infection of tubers in the presence of pathogens. Potato tubers used for Fusarium dry rot trials were wounded as *Fusarium* spp. cannot infect intact tuber periderm or lenticels (Secor and Salas, 2001).

The interaction of field and bin loading applied fungicides and biofungicides significantly reduced the development of storage rots. For instance, in the Fusarium dry rot trial, the inoculated check from the untreated field had significantly higher disease incidence compared to the inoculated checks from fields treated with *B. subtilis* or mefenoxam. Although mefenoxam is registered for control of the oomycetes, there was a reduction of dry rot on tubers from the field treated with mefenoxam. Mefenoxam could have protected the tubers from infection by *F. sambucinum*. Barak *et al.* (1984) reported that tubers from a field treated with metalaxyl had a high resistance to tuber decay caused by *Fusarium sambucinum* and *F. culmorum* although these resistance decreased gradually with storage time. Field treatment with mefenoxam followed by bin loading application of either the 3-way mixture of azoxystrobin, fludioxonil and difenoconazole or phosphorous gave the best control of dry rot. The 3-way mixture of azoxystrobin, fludioxonil and difenoconazole has not been registered but is being tested by Syngenta for control of dry rot and silver scurf; it might become an alternative for controlling dry rot, since thiabendazole is no longer effective in controlling dry rot caused by *F. sambucinum* (Ocamb *et al.*, 2007), the most aggressive *Fusarium* spp. in Michigan (unpublished data). In addition, the recent discovery of fludioxonil-resistant *Fusarium* spp. in Canada (Peters *et al.*, 2008) and in Michigan (Gachango *et al.*, 2011) poses a challenge for controlling dry rot. To counteract the loss of TBZ, additional registration of postharvest fungicides is underway with difenoconazole already proposed for managing decays caused by *Fusarium* species; azoxystrobin and fludioxonil for potato and other tuber crops decay respectively (Adaskaveg and Förster, 2010). Despite phosphorous acid being registered for control of the oomycete pathogens (Brunings *et al.*, 2005), it reduced dry rot development on tubers from fields treated with mefenoxam, *B. subtilis* or phosphorous acid. This results agree with those of Lobato *et al.* (2010)

who found that phosphorous acid could reduce disease development caused by *Phytophthora infestans*, *Fusarium solani* and *Rhizoctonia solani*.

Application of phosphorous acid in the field and in storage effectively reduced tuber late blight incidence and severity, thus giving the best control for tuber late blight. Our results are in agreement with other researchers who either reported that in-season applications of phosphorous acid (Cooke and Little, 2002; Johnson *et al.*, 2004; Mayton *et al.*, 2008) or postharvest applications of phosphorous (Miller *et al.*, 2006) increased tuber protection against *P. infestans*. Introduction of phosphorous acid has counteracted the loss of effectiveness of mefenoxam in controlling late blight (Mayton *et al.*, 2008). This was evident in our study, where the field treated with phosphorous acid had significantly lower development of late blight compared to the field treated with mefenoxam. The biofungicide, *Bacillus subtilis,* had very limited activity against tuber late blight, although it is being used for organic potato production and has been reported to reduce foliar late blight development when tested on leaf discs (Stephan *et al.*, 2005).

Fields treated with mefenoxam, phosphorous acid, or *B. subtilis*, followed by bin loading treatments with either phosphorous acid or the 3-way mixture of azoxystrobin, fludioxonil and difenoconazole resulted in low disease with *Pythium ultimum* and *Phytophthora erythroseptica*. This indicates that mefenoxam, phosphorous acid and *B. subtilis* could be used in the field to increase tuber resistance against *P. ultimum* and *P. erythroseptica,* while phosphorous acid could be applied at bin loading to ensure infection is kept to a minimum. Although mefenoxam-insensitive isolates of *P. ultimum* and *P. erythroseptica* have been reported, mefenoxam continuous to be used in combination with other cultural practices to control pink rot and Pythium leak (Taylor *et al.*, 2007). However, to insure the effectiveness of mefenoxam, resistance-management strategies should be practiced (Taylor *et al.*, 2007). These strategies

include limiting the number of sprays to 2-4 consecutive applications per crop per year and use in early period of active plant growth and then switching to a non-phenylalamide product (Brent and Hollomon, 2007)

From this study we can therefore conclude that combination of in-season application and bin-loading application of fungicides and biofungicides effectively increases tuber protection against storage pathogens.

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Chapter 3: Identification of *Fusarium* spp. Responsible for Dry Rot of seed Potato Tubers in Michigan

Abstract

Fusarium dry rot of potato (*Solanum tuberosum* L.) is a postharvest disease caused by several *Fusarium* spp. In Michigan potato production, *F. sambucinum* was the predominant species according to a report in 1993. A survey was conducted between 2009 and 2010 to determine the current species responsible for dry rot of seed potato tubers in Michigan. A total of 370 samples of dry rot symptomatic tubers were collected and used for recovery of *Fusarium* species. Morphological characters were used for identification and species identity, which was confirmed by molecular techniques. A total of 228 *Fusarium* isolates were recovered, identified and classified into 11 species. *Fusarium oxysporum* was the most commonly isolated species comprising 27.4% of total *Fusarium* isolates. The second most commonly isolated species was the *F. equiseti* species complex comprising 21.0%. *Fusarium sambucinum* and *F. avenaceum* were third in prevalence, comprising 14.4 and 14.1%, respectively. The less prevalent *Fusarium* spp., within the range of 4-10%, included *F. cerealis (F. crockwellense)*, *F. solani,* and *F. acuminatum*. Other *Fusarium* species identified comprising of the isolates ≤3% included *F. sporotrichioides, F. torulosum, F. tricinctum*, and *F. graminearum*. This was the first time *F. torulosum* was reported from potato tubers in the United States. All the *Fusarium* species were pathogenic to potato tubers (cv. Red Norland) after incubation for 30 days at 10^{o}C and 4^{o}C. However, *F. sambucinum* was the most aggressive species. Presence of high proportions of different species may have implications for chemical management strategies for dry rot.

3.1 Introduction

Fusarium dry rot of potato (*Solanum tuberosum* L.) is a devastating postharvest disease worldwide and is caused by several *Fusarium* species (Boyd, 1972; Secor and Salas, 2001). Dry rot affects both tubers in storage and seed tuber pieces in the field (Wharton *et al.*, 2007). Losses associated with dry rot have been estimated from 6 to 25%, and occasionally losses as high as 60% have been reported during long term storage (Chelkowski, 1989; Secor and Salas, 2001). In addition to the damage inflicted on tubers, *Fusarium* species also produce toxins such as trichothecene, harmful to humans and animals (Desjardins and Plattner, 1989).

Fusarium is a ubiquitous pathogen in a wide variety of crops, potato being one of the major hosts. *Fusarium* is both seedborne and soilborne, thus the initial inoculum for disease development is either from infected seed tubers or infested soils (Secor and Gudmestad, 1999; Secor and Salas, 2001). Infection of potato tubers by dry rot pathogens occurs through wounds inflicted during harvesting, grading, cutting and handling of seed pieces (Glass *et al.*, 2001; Secor and Salas, 2001; Powelson and Rowe, 2008). The initial symptom on the tuber surface is a shallow brown lesion, which later expands slowly and eventually becomes sunken and wrinkled. Internal symptoms are characterized by dry necrotic areas shaded from light to dark chocolate brown or black, hence the name dry rot (Secor and Salas, 2001). In the field, Fusarium dry rot in seed tubers causes germination gaps or severely stunted, chlorotic and nectrotic stems as well as abnormal growth of roots and stolons (Wharton *et al.*, 2007). Dry rot incidence has been reported to vary according to the *Fusarium* species responsible (Desjardins *et al.*, 1992) and different levels of aggressiveness among species have been reported (Daami-Remadi *et al.*, 2006).

Thirteen species of *Fusarium* are implicated in fungal dry rots of potatoes worldwide (Hide *et al.*, 1992; Cullen *et al.*, 2005). Among them, eight species have been reported in the

northern United States (Hanson et al., 1996). The most prevalent species are *F. sambucinum* Fuckel (*Fusarium sulphureum* Schlechtend; teleomorph: *Gibberella pulicaris* (Fr.:Fr) Sacc.), *F. solani* (Mart.) Sacc. var. *coeruleum* (Lib. ex Sacc.) C. Booth (*F. coeruleum*; teleomorph: *Nectaria haematocca* Berk. & Broome), and *F. oxysporum* Schlechtend. Fr. (Hanson et al., 1996). Other species reported in the northern US, which are of less importance in causing dry rot include, *F. avenaceum,* (Fr.) Sacc. *F. culmorum,* (W.G. Smith), *F. acuminatum,* Ellis & Everh. *F. equiseti* (Corda), and *F. crockwellese* L.W. Bugess, P.E. Nelson & Ravenel. (*F. cerealis*) (Hanson et al., 1996; Ocamb et al., 2007). Most of these species were also recovered in the Pacific region of the US with *F. sambucinum* being the most prevalent (Ocamb *et al.*, 2007). Recently, *F. graminearum* was reported to be the prevalent *Fusarium* causing potato dry rot in North Dakota (Ali *et al.*, 2005; Estrada Jr *et al.*, 2010). In the UK, *F. coeruleum* (Libert) Sacc. has been found to be prevalent (Hide *et al.*, 1992; Peters *et al.*, 2008a), while in Scotland, *F. avenaceum* caused more dry rot compared to *F. solani* var. *coeruleum* (Cullen *et al.*, 2005; Choiseul *et al.*, 2006)

In Michigan potato production, dry rot has been reported in most of the seed lots (Kirk and Wharton, 2008). *Fusarium sambucinum* was the predominant species affecting seed potato in storage and causing seed piece decay after planting (Lacy and Hammerschmidt, 1993). In addition, Wharton *et al.* (2006) also reported that *F. sambucinum* was the causal agent of rotting sprouts of the progeny tubers in Michigan. Since the report of Lacy and Hammerschmidt (1993), there has been no assessment of *Fusarium* species composition responsible for dry rot in Michigan. Understanding the species composition is of importance in designing a management scheme. Management of dry rot has been achieved primarily by reducing tuber bruising, providing conditions for rapid wound healing (Secor and Johnson, 2008) and applying

thiabendazole (TBZ; Mertect 340-F, Syngenta, Greensboro, NC), a benzimidazole fungicide as tubers enter into storage (Hide et al., 1992; Hanson et al., 1996). However, *F. sambucinum* resistant to TBZ and other benzimidazole were discovered in Europe in 1973 (Hide *et al.*, 1992) and in the US in 1992 (Desjardins, 1995), thus leading to reduced effectiveness of this chemical in controlling dry rot (Staub, 1991). Nevertheless, studies have shown varying responses of different isolates of *F. sambucinum* against TBZ, with some isolates being resistant and others being sensitive (Desjardins *et al.*, 1993). Based on all of the above, the objective of the current study was to characterize the *Fusarium* species responsible for dry rot of seed potato tubers in Michigan.

3.2 Materials and methods

3.2.1 Isolation and identification

A total of 370 dry rot symptomatic tubers were collected from seed lots in the Michigan potato growing area in summer 2009 and 2010. Small pieces were cut from the margins of the necrotic region with a sterile scalpel, surface-disinfested in 0.5% sodium hypochlorite for 10 s, rinsed twice in sterile distilled water, and blotted with sterile filter paper. The tissue pieces were then plated on half-strength potato dextrose agar (PDA; Difco, Detroit, Michigan) amended with 0.5 g/L streptomycin sulfate. The Petri dishes were incubated at 23^{o}C for 5 to 7 d.

Cultures resembling *Fusarium* species were transferred onto water agar; hyphal tip transfer was done from the margin of actively growing isolates with a sterile probe and plated on carnation leaf agar (CLA) and half-strength PDA to generate pure cultures (Leslie *et al.*, 2006). The pure cultures were identified based on conidial morphology and production of chlamydospores, while those on PDA were identified based on colony pigmentation.

Identification to species was done morphologically as described by Leslie *et al.* (2006). To confirm species identity, lyophilized mycelium from pure cultures grown on PDA for 7 d were used for DNA extraction as described by Goodwin *et al.* (1992), followed by amplification and sequencing of the translation elongation factor (EF-1α) gene region (Geiser *et al.*, 2004). The Fusarium-ID (Geiser *et al.*, 2004) and the NCBI (GenBank) database were used to obtain the closest match to previously sequenced materials.

3.2.2 Pathogenicity Test

All isolates obtained were tested for pathogenicity on disease-free potato tubers, cv. Red Norland. The tubers were surface disinfested for 10 min in 0.5% sodium hypochlorite and rinsed twice in sterile water. Three replicate tubers per isolate were injected with 20 µL of a conidial suspension (ca. 10^6 conidia/ml) made from *Fusarium* cultures grown on PDA for 7 d, while control tubers were injected with 20 µL of sterile distilled water. The tubers were incubated in the dark for 30 days at 4^oC and at 10^oC. Tubers were cut in half from the point of inoculation and evaluated for development of lesions typical of potato dry rot: brown and dry decay lesions covered with colored mycelium towards the inner surface. Isolates that resulted in the development of lesions on the tuber were considered pathogenic. The fresh-cut tuber sections were placed on a glass 30 X 40 cm and 2-mm thick with the cut surface facing down. A ruler was placed on the lower side of the glass, which was used as a standard for calibration of the measurements during image analysis. The glass was transferred to a flatbed scanner (HP Scan-Jet 4c; Hewlett-Packard Co., Houston, TX) controlled by an IBM-compatible PC. A 486DX2-80 CPU (Intel Corp., Santa Clara, CA) and a RAM capacity of 32 MB adequate for the image processing. A scanner control software (DeskScan II version 2.4; Hewlett-Packard Co.) generated an image of the cut tuber surfaces against a black background (Niemira *et al.*, 1999).

The image files created with the scanner software were first loaded into Adobe Photoshop CS3 (Version 10.01, 2007, Adobe Systems Incorporated) where the lesions were selected and using the 'fill' tool, painted the lesion with a white color (Fig 3.1). The images were then loaded to the image analysis software (SigmaScan Pro 5, 1987-1999 SPSS© Inc., Chicago) to determine the area of the lesion as described by ONeal *et al.* (2002) with modifications. Using the image option, (from the toolbar) the distance (mm) and area (mm^2) were calibrated to convert pixels to a unit of measurement. A standard of known dimensions (a ruler) within the image was included for calibrating the pixel conversion. The measurement setting 'fill' was then adjusted to a threshold option so that the lesion was composed of a lighter color than the rest of the tuber surface. The lesion was then selected with the 'fill' measurement mode and the entire lesion covered with a color of choice. The area of the lesion was then calculated by selecting the measurement option. To reconfirm identity of the *Fusarium* isolates, the pathogens were re-isolated from all the symptomatic potato tubers.

Figure 3.1 Images of scanned tubers with the lesions selected and painted white. A ruler was also scanned to act as the standard of known dimensions

3.3 Results

3.3.1 Isolation and identification

Over the two-year survey, 228 *Fusarium* isolates were recovered and identified to 11 different species (Table. 3.1). *Fusarium oxysporum* was the most commonly isolated species comprising 28.8, and 25.9% out of the total *Fusarium* species isolated in 2009 and 2010, respectively (Table 3.1). The second most commonly isolated species were representatives from the *F. equiseti* species complex comprising 23.0 and 19.0% of the isolated *Fusarium* in 2009 and 2010, respectively. *Fusarium sambucinum* and *F. avenaceum* were third in prevalence, each comprising 14.4 and 14.2%, between 2009 and 2010, respectively. The less prevalent *Fusarium* spp. within the range of 4 - 10% included *F. cerealis (F. crockwellense)*, *F. solani* and *F. acuminatum*. Other *Fusarium* species identified but making up 3% or less of the isolate included *F. sporotrichioides, F. torulosum, F. tricinctum* and *F. graminearum*. The proportion of the recovered isolates per species remained markedly consistent over the 2-year period despite having a smaller sample size (110 potato tubers) in 2010 compared to 260 potato tubers in 2009. *Fusarium sporotrichioides* and *F. graminearum* were only recovered in one year each, 2009 and 2010, respectively.

Table 3.1 Relative frequencies (%) of Fusarium spp. isolated from symptomatic seed potato during 2009 to 2010

Fusarium species[a]	Relative frequency of isolated species[b]		
	2009	2010	Total
F. oxysporum	28.8	25.9	30.3
F. equiseti	23.0	19.0	19.3
F. sambucinum	14.9	13.8	13.6
F. avenaceum	11.1	17.2	13.6
F. solani	9.5	5.2	7.5
F. cerealis	6.3	5.2	6.1
F. acuminatum	4.1	6.9	4.4
F. torulosum	1.4	3.4	2.2
F. tricinctum	0.9	3.4	1.8
F. sporotrichioides	0.9	0.0	0.9
F. graminearum	0.0	1.7	0.4

[a] *Fusarium* species recovered from dry rot symptomatic seed tubers

[b] Relative frequencies were calculated as number of isolates per species relative to the total number of isolates recovered in each separate year

3.3.2 Pathogenicity test

All the isolates recovered were pathogenic to potato tubers at both 4^{o}C and 10^{o}C (Table 3.2). Test tubers developed typical potato dry rot symptoms consisting of a brown and dry decay (Fig. 3.1). No disease symptoms were observed on the control potato tubers. Differences in level of virulence among species were evident (Table 3.2). Overall, *F. sambucinum* was the most virulent species with significantly larger lesions compared to the other species. The rest of the species did not differ significantly from each other with respect to lesion size, and were not significantly different from the control. Difference in aggressiveness within species was only observed for *F. sambucinum*. Overall, lesion size (area) observed on tubers incubated at 10^{o}C was not significantly ($p<0.6774$) different from tubers stored at 4^{o}C indicating that the tested *Fusarium* spp. could infect both seed and commercial potatoes in storage. Re-isolation of the pathogen from the lesions resulted in the same pathogen as the tubers were inoculated with, thus completing the Koch's postulates.

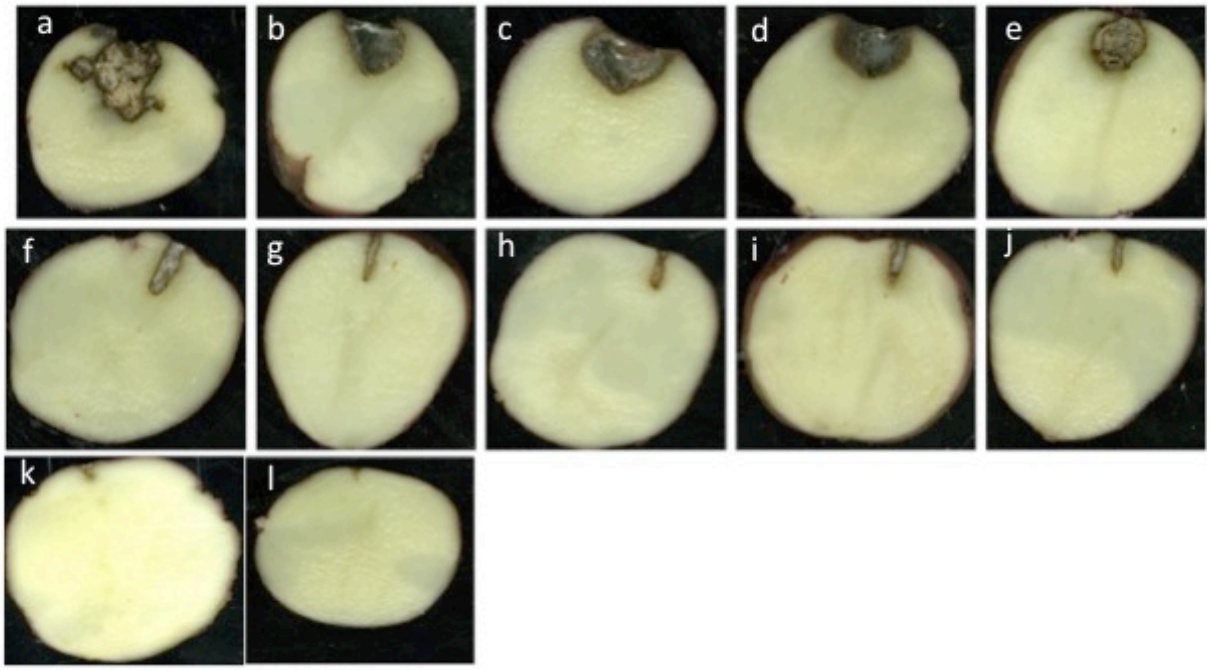

Figure 3.2 Virulence of Fusarium isolates on potato tubers (cv. Red Norland) inoculated with a) *F. sambucinum*, b) *F. avenaceum*, c) *F. tricinctum*, d) *F. acuminatum*, e) *F. cerealis*, f) *F. sporotrichioides*, g) *F. solani*, h) *F. equiseti*, i) *F. oxysporum*, j) *F. torulosum*, k) *F. graminearum* and the i) control respectively

Table 3.2 Virulence of *Fusarium* isolates on potato tubers (cv. Red Norland) based on the area of the lesion after 30-d incubation at $4^{o}C$ and $10^{o}C$

		Area of the lesion (mm^2)[c]			
	Number of	$4^{o}C$		$10^{o}C$	
Fusarium spp[a]	isolates[b]	Range	Mean	Range	Mean
F. sambucinum	31	12.9-589.9	234.2a[d]	6.9 -1186.8	250.6a
F. tricinctum	4	9.7-17.5	14.3b	27.6-85.2	47.3b
F. sporotrichioides	2	8.9-20.9	14.5b	17.6-46.7	33.4b
F. cerealis	17	13.0-20.9	17.2b	1.3-351.4	31.9b
F. torulosum	5	12.0-12.7	15.4b	5.4-64.8	30.2b
F. equiseti	43	8.1-31.1	17.6b	8.7-52.9	29.6b
F. solani	13	9.5-26.1	17.4b	7.5-64.8	29.0b
F. oxysporum	69	9.9-30.3	17.4b	8.2-89.4	28.6b
F. avenaceum	31	8.3-35.0	17.2b	3.0-194.8	19.8b
F. acuminatum	10	6.4-32.5	14.6b	4.2-29.5	11.3b
F. graminearum	1		1.6b		2.3b
Prob F ($p<0.05$)		<0.0001		<0.0001	

[a] *Fusarium* species isolated and recovered from dry rot symptomatic seed potato tubers

[b] Number of isolates per species tested for pathogenicity

[c] Mean area of the lesion from all isolates within a species- mean of 3 reps per isolate repeated once

[d] Numbers followed by the same letter within a column are not significantly different at $p = 0.05$ (Tukey test)

3.4 Discussion

Fusarium dry rot is one of the major diseases affecting potato seed production in Michigan (Wharton *et al.*, 2007). Occurrence of Fusarium dry rot has been reported in most of the seed lots in Michigan (Kirk and Wharton, 2008). The causal agent of Fusarium dry rot was for a long time reported to be *Fusarium sambucinum* in the northern United State (Secor and Salas, 2001) . However, other *Fusarium* species have been identified as pathogens of potato tubers. These include eight *Fusarium* spp. in the northern US (Hanson et al., 1996) and a total of 13 species worldwide (Hide *et al.*, 1992). In Michigan, the predominant species causing dry rot in storage and seed piece decay after planting was *F. sambucinum* according to a report in 1993 (Lacy and Hammerschmidt, 1993). However, during the current 2-year survey, 11 different *Fusarium* species were recovered. Use of molecular techniques confirmed the species identified using morphological characterization. A combination of morphological and molecular methods is preferable for identification of *Fusarium* spp. (Geiser *et al.*, 2004; Leslie *et al.*, 2006)

The species composition was consistent for most of the species during the 2-year survey, apart from *F. sporotrichioides* and *F. graminearum*, which each only were found of the two years. Only one isolate of *F. graminearum* was isolated and formed very small lesions when inoculated on healthy tubers. *Fusarium graminearum* could be categorized as a minor pathogen of potato in Michigan, although it has been reported to be the predominant dry rot pathogen in the north-central America (Estrada Jr *et al.*, 2010). The most frequently isolated species was *F. oxysporum* comprising 28.8 and 25.9% of total *Fusaria* isolated in 2009 and 2010, respectively. A high prevalence of *F. oxysporum* was also reported in the northern US (Hanson et al., 1996) and in the Columbia Basin of Oregon and Washington (Ocamb *et al.*, 2007). Literature also cites *F. oxysporum* as the most widely dispersed *Fusarium* species infecting a wide range of plants

(Leslie *et al.*, 2006). Its occurrence in high frequency could be as a result of rotation with crops such as corn and forage crops, which are also hosts of *F. oxysporum* (Leslie *et al.*, 1990; Peters *et al.*, 2008b).

All the isolates of *F. oxysporum* were pathogenic to potato tubers (cv. Red Norland), after incubation at 10^{O}C and 4^{O}C for 30 d, and re-isolation of the pathogen yielded *F. oxysporum*. This indicates that seed tubers can be infected during the storage period. The high prevalence of *F. oxysporum* poses a challenge to potato growers since *F. oxysporum* has been found also to cause wilting of potato plants (Secor and Salas, 2001; Mahdavi-Amiri *et al.*, 2009).

The *Fusarium equiseti* species complex was the second most commonly isolated species comprising 23.0 and 19.0% of the isolated *Fusarium* in 2009 and 2010, respectively. This species complex has not been reported in potato tubers in Michigan. However, Hanson *et al.* (1996) reported the occurrence of *F. equiseti* in potato tubers in the northern US, although at low frequency. Occurrence of *F. equiseti* at high frequency may cause a big challenge to seed potato production because all the isolates recovered were pathogenic. Also the literature states that *F. equiseti* is a potential human pathogen and is capable of producing mycotoxins (Leslie *et al.*, 2006) thus its high prevalence may be of concern to human safety. Although recovery of *F. equiseti* has been achieved through isolation from diseased plant tissue, with no completion of Koch's postulate (Leslie *et al.*, 2006), in the current study, koch's postulate was complete the using potato tubers.

Fusarium sambucinum and *F. avenaceum* were third and fourth in prevalence, each comprising about 14% of the isolates. Unlike the current study, *F. sambucinum* was the commonly isolated *Fusarium* spp. from either seed or table stock potatoes in the northeastern US (Hanson et al., 1996) and Columbia basin of Oregon and Washington (Ocamb *et al.*, 2007).

Although *F. sambucinum* was not the most prevalent species, it was the most virulent species causing significantly larger tuber lesions than all the other species regardless of the incubation temperature. Our results are in agreement with Ocamb *et al.* (2007) who reported that *F. sambucinum* resulted in relatively large areas of decay compared to *F. oxysporum* and *F. solani*. Several authors have reported that *F. sambucinum* is the major pathogen for potato in the northern US (Desjardins *et al.*, 1993; Secor and Salas, 2001). However, according to the current study there are at least other four *Fusarium* spp., which could be termed as major pathogen with respect to formation of lesions greater than 30 mm^2, although three of them were found at a low frequency (less than 4% over the 2 years). These species include *F. tricinctum*, *F. sporotrichioides*, *F. torulosum* and *F. cerealis*. This was the first time *F. torulosum* was reported as a potato pathogen in the US (Gachango *et al.*, 2011). However, *F. torulosum* was formerly identified as *F. sambucinum* but has been classified as a separate species on its own (Nirenberg, 1995).

Although *Fusarium avenaceum* is said to be a cereal pathogen (Leslie *et al.*, 2006), its prevalence was the same as that of *F. sambucinum*. In the northern potato regions of US, *F. avenaceum* has been less commonly reported (Hanson et al., 1996) while in UK, *F. avenaceum* has been recovered in high frequency (Cullen *et al.*, 2005; Peters *et al.*, 2008a). All isolates of *F. avenaceum* were pathogenic to potato tubers but the level of virulence was low with the area of the lesions from both temperatures at same magnitude. In contrast, Aprasad et al. (1997) found that *F. avenaceum* was slightly virulent at 5^oC than at 10^oC.

Fusarium culmorum, although previously reported in the northern US (Hanson et al., 1996) was not isolated in our current study. We can therefore conclude that, at least 11 species of *Fusarium* are responsible for dry rot of seed potato tubers although their prevalence varied.

Presence of high proportions of different species may have implications for chemical management strategies for dry rot since different species respond differently to chemicals. Other management practices like rotation may be impacted by the fact that some of the Fusarium species recovered have a wide host range. Host resistance may also be impacted, as potato tubers or plants will have different levels of resistance against the pathogen. It is therefore imperative to investigate *Fusarium* spp. composition on commercial tubers so that a management scheme can be established based on overall species composition in potato production in Michigan.

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Chapter 4: Baseline Sensitivity of Fungicides Against *Fusarium* Species Associated with Seed Potato Dry Rot in Michigan

Abstract

The development of thiabendazole (TBZ) and fludioxonil-insensitive isolates of *Fusarium* has instigated the need to determine the sensitivity levels of different *Fusarium* spp. to fungicides in order to find alternative options to manage Fusarium dry rot. *Fusarium* spp. (*F. sambucinum, F. oxysporum, F. equiseti, F. solani, F. avenaceum, F. acuminatum, F. torulosum, F. tricinctum, F. sporotrichioides, F. cerealis* and *F. graminearum*), causing dry rot in Michigan, were screened for sensitivity to TBZ, fludioxonil and difenoconazole. Effective concentration that inhibits fungal growth by 50% (EC_{50}) values was determined using spiral gradient dilution (SGD) and serial dilution plate (SDP) methods. All the *Fusarium* isolates were sensitive to difenoconazole with EC_{50} values less than 5 mg/L. All isolates of *F. sambucinum* were insensitive to TBZ with EC_{50} values greater than 100 mg/L, while the isolates of rest of the species were sensitive to TBZ with EC_{50} values less than 5 mg/L. Both fludioxonil-sensitive and insensitive isolates of *F. sambucinum* and *F. oxysporum* were identified, while the isolates of the other species were sensitive with EC_{50} values less than 5 mg/L. The fludioxonil-insensitive isolates had EC_{50} values greater than 100 mg/L. Difenoconazole has the potential to control dry rot. Thiabendazole can still be used to control *Fusarium* spp. causing dry rot other than *F. sambucinum*. There has been no compelling evidence to suggest that fludioxonil has failed to perform because of insensitivity of *F. sambucinum* and *F. oxysporum* to the fungicide. The occurrence of insensitive strains necessitates the development and registration of partner chemistries that can preempt any future concerns on lack of performance of products in use.

4.1 Introduction

Dry rot of seed potato tubers is an important postharvest disease caused by several *Fusarium* species worldwide (Secor and Salas, 2001). Thirteen species of *Fusarium* have been implicated in fungal dry rots of potatoes worldwide (Hide *et al.*, 1992; Cullen *et al.*, 2005) with eight species already reported in the northern United States (Hanson *et al.*, 1996). In Michigan, there are currently eleven known *Fusarium* species isolated from seed potato tubers; with *F. oxysporum* being predominant followed by *F. equiseti* and *F. sambucinum* (chapter 3). However, *F. sambucinum* is the most aggressive species compared to all the species recovered (Ocamb *et al.*, 2007).

Fusarium dry rot affects tubers in storage and seed piece after planting (Wharton *et al.*, 2007a). There are no commercially grown potato cultivars resistant to dry rot in North America although the level of tolerance varies in some cultivars and breeding lines (Leach and Webb, 1981). Measures for controlling dry rot in storage are limited. Fusarium dry rot can be controlled in two phases during the potato growth cycle. These phases include postharvest control of seedpiece decay and control of seedpiece decay prior to planting (Nolte *et al.*, 2003). Since *Fusarium* infects the tubers through wounds inflicted during harvesting (Secor and Salas, 2001), management has been achieved primarily by reducing tuber bruising, providing conditions for rapid wound healing (Secor and Johnson, 2008) and applying thiabendazole, a benzimidazole fungicide (TBZ; Mertect 340-F, Syngenta, Greensboro, NC) as tubers enter into storage (Hide *et al.*, 1992; Ocamb *et al.*, 2007). However, *F. sambucinum* resistant to TBZ and other benzimidazole were discovered in Europe in 1973 (Hide *et al.*, 1992) and in the US in 1992 (Desjardins *et al.*, 1993; Desjardins, 1995), thus reducing the effectiveness of TBZ in controlling dry rot (Staub, 1991; Ocamb *et al.*, 2007). Nevertheless, studies have shown varying responses

of different isolates of *F. sambucinum* against TBZ, with some isolates being resistant and others being sensitive (Desjardins *et al.*, 1993). Resistance to TBZ has also been reported in *Fusarium* spp. isolated from potato tubers including *F. sambucinum, F. oxysporum*, *F. solani* and *F. culmorum* (Hanson *et al.*, 1996; Ocamb *et al.*, 2007), *F. avenaceum, F. equiseti, F. sporotrichioides* (Ocamb *et al.*, 2007) and *F. acuminatum* (Hanson *et al.*, 1996). The resistance was defined as the ability of *Fusarium* isolates to grow on artificial media at a concentration of 5 mg/L of TBZ (Hanson *et al.*, 1996; Ocamb *et al.*, 2007). It is not known whether TBZ can effectively control other *Fusarium* species causing dry rot.

Control of seedpiece decay prior to planting is primarily achieved by seed treatment. Fludioxonil (Maxim^{TM} Seed Potato Protectant; Syngenta, Greensboro, NC) is among the few fungicides registered for seed treatment against *Fusarium* dry rot in the US (Zitter, 2010). Studies have shown that fludioxonil is able to reduce seedpiece decay as well as diseased sprouts (Wharton *et al.*, 2007b) that develop into unhealthy plants. Recently, fludioxonil-resistant strains of *Fusarium* spp. were reported in Canada and they include *F. sambucinum* and *F. coeruleum* (Peters *et al.*, 2008b). Fludioxonil-resistant isolates of *F. sambucinum* and *F. oxysporum* were reported in Michigan from a survey conducted between 2009-2010 (Gachango *et al.*, 2011). However, sensitivity to fludioxonil in other *Fusarium* spp. causing potato seedpiece decay is not known. Although there has been no compelling evidence to suggest that fludioxonil has failed to perform because of insensitivity to the fungicide, occurrence of insensitive isolates necessitates the development and registration of partner chemistries that can preempt any future concerns on lack of performance of products in use (Russell, 2003). To counteract the reduced effectiveness of TBZ and fludioxonil, additional registration of postharvest fungicides is needed and some have already been proposed; difenoconazole (Inspire$^{TM;}$ Seed Potato Protectant; Syngenta,

Greensboro, NC) for managing decays caused by *Fusarium* species, azoxystrobin and fludioxonil for potato and other tuber crops decays respectively (Adaskaveg and Förster, 2010).

Determination of baseline sensitivity level for new compounds and monitoring for sensitivity measures is important (Kuck and Gisi, 2008). Monitoring for early detection of resistance becomes feasible in field samples when a relatively high frequency of resistant isolates (<1%) is reached according to the Fungicide Resistance Action Committee (Brent and Hollomon, 2007). *In vitro* testing of the 50% effective concentration (EC_{50}), which is the fungicide concentration at which fungal mycelial growth or spore germination is inhibited by 50%, is a rapid technique used for monitoring shifts in sensitivity (Russell, 2003).

Thiabendazole-insensitivity has been characterized by fungal growth on PDA containing 5 mg of TBZ per liter (Hanson *et al.*, 1996; Ocamb *et al.*, 2007); we also looked used the same concentration (5 mg/L) to define insensitivity to TBZ. Difenoconazole baseline level has not been established, but other triazoles, prothioconazole and tebuconazole, have been tested against *Fusarium* spp. resulting in EC_{50} values ranging from 0.1-3.2 and 1.1-5.5 mg/L, respectively (Müllenborn *et al.*, 2008). Therefore, an estimate of fungal growth on PDA containing 5 mg of difenoconazole per liter could be taken as a benchmark for insensitivity. The objective of this study was to screen the *Fusarium* species causing dry rot of seed potato tubers in Michigan for sensitivity to TBZ, fludioxonil, and difenoconazole. The understanding of baseline sensitivity will aid in establishing a management scheme, as well as help in monitoring of any shift in sensitivity in the future.

4.2 Materials and methods

4.2.1 Fungal isolates

A total of 228 isolates representing 11 species of *Fusarium* previously isolated from seed potato tubers in Michigan during summer 2009 and 2010, were screened for sensitivity to difenoconazole, fludioxonil and thiabendazole (TBZ) using two screening methods as described below. These included 68 isolates of *F. oxysporum*, 44 isolates of *F. equiseti*, 31 isolates of *F. sambucinum,* 31 isolates of *F. avenaceum*, 18 isolates of *F. cerealis*, 14 isolates of *F. solani*, 11 isolates of *F. acuminatum*, four isolates of *F. tricinctum*, four isolates of *F. torulosum*, two isolates of *F. sporotrichioides*, and one isolate of *F. graminearum*. Two standard isolates, thiabendazole-resistant isolate of *F. sambucinum* (R-09271 -Desjardins YG-1 U7200A) and thiabendazole-sensitive isolate of *F. sambucinum* (R-00738, Cetas, R.C) were used for comparison in the thiabendazole-sensitivity assay. The cultures were grown on potato dextrose agar (PDA; Difco, Detroit, Michigan) for 7 d prior to the test.

4.2.2 Fungicides evaluated

Three fungicides were evaluated with each *Fusarium* isolate from the 11 species. These fungicides were formulated products in aqueous suspensions. The fungicides included thiabendazole (42.3% active ingredient, TBZ; Mertect; Syngenta Crop Protection Inc., Greensboro, NC, USA Syngenta) a benzimidazole; fludioxonil (0.5% active ingredient, 23.2% active ingredient Maxim; Syngenta Crop Protection) a phenyl-pyrrole and difenoconazole (Inspire; Syngenta Crop Protection) a sterol biosynthesis inhibitor. The molecular weights used

for calculation of stock concentrations were TBZ 201.2, fludioxonil 248.2 and difenoconazole 406.3.

4.2.3 Determination of EC_{50} values using the spiral gradient dilution method

The EC_{50} values were determined for all isolates. The spiral gradient dilution (SGD) method was used as described in detail by Förster *et al.* (2004). PDA (50 mL) was poured into Petri dishes (15 cm diameter) at least 24 h before fungicide solutions were applied. Stock concentrations for thiabendazole, fludioxonil and difenoconazole were made to10, 000 gm/L based on evaluation of several concentrations over a range of 0 to 1000 gm/L. A total of 50 µL of a fungicide solution was applied with a spiral plater (SGE^{TM}; Spiral Biotech, Inc. Norwood, MA) using the exponential deposition mode. The plates were incubated for 3 h to allow the fungicides to diffuse into the medium and form a gradient of concentrations along the radius of the plate (Table 4.2). Mycelial inoculum grown on PDA in 10 cm Petri dishes for 7 d was use to make conidial suspensions (10^6 conidia/ml) per isolate. Each plate was placed on the template provided with the SGE software, with the start of the spiral at the No. 1 plate position. Droplets of 10 µL of conidial suspension per isolate were spread across the radial lines in predetermined plate positions with a sterile plastic pestle. Three replicates per isolate were used for each fungicide. Controls consisted of PDA plates without fungicides to which conidial suspensions were applied. The plates were incubated at 25^{o}C for 3 days. Radial growth of the fungus per replicate was measured and the values averaged. The 3-day incubation option was used in the SGE software for calculation of the local concentrations where 50% growth inhibition was observed.

4.2.4 Determination of EC_{50} values using the serial dilution plate (SDP) method

PDA was amended with the fungicides mentioned above at a series of concentrations of 0, 0.1, 1, 10, or 100 ppm. Agar plugs (5 mm-diameter) were cut from pure cultures of all identified isolates and placed, mycelia-side down, on the amended agar and incubated for 5 d in darkness at 25^{O}C. Colony diameter (minus the diameter of the inoculation plug) was measured 5 d after initiation of the experiment with a caliper. Fungal growth was expressed as percentage inhibition compared to growth on the control plates (no fungicide). Three replicates for each treatment were used and the experiment was conducted twice. Data from repeated experiments were averaged and EC_{50} values for each isolate calculated by regression analysis of percentage of growth inhibition against the logarithmic value of fungicide concentration in excel.

Characterization of *Fusarium* isolates as insensitive to thiabendazole was based on fungal growth on PDA containing 5 mg of TBZ per liter (Hanson *et al.*, 1996; Ocamb *et al.*, 2007). For difenoconazole, an estimate of 5 mg of difenoconazole per litre was used to define insensitivity. This estimate was based on a previous studies using triazoles, prothioconazole and tebuconazole, against *Fusarium* spp., resulting in EC_{50} values ranging from 0.1-3.2 and 1.1-5.5 mg/L, respectively (Müllenborn *et al.*, 2008). Fludioxonil insensitivity was defined as lack of growth inhibition on PDA containing 100 mg of fludioxonil per litre (Peters *et al.*, 2008b).

4.2.4 Statistical analysis

All data analyses were conducted with the JMP program version 8.0 (SAS Institute Inc., Cary, North Carolina). Data for the repeated experiments were combined and mean EC_{50} values compared using one-way ANOVA and separated using Tukey's HSD.

4.3 Results

Application of fungicides using the spiral gradient dilution method resulted in a radial concentration of the fungicide, with the highest concentration at the smallest radius towards the center and the lowest concentration towards the edge of the agar plate. The spiral gradient method gave slightly lower EC_{50} values compared to the serial dilution plate method (Table 4.1), but the two methods were not significantly different ($p=0.7752$) and the values were the same order of magnitude.

4.3.1 Effects of thiabendazole on mycelia growth

Insensitivity to thiabendazole based on growth on PDA containing 5 mg of TBZ per liter was observed. All isolates of *Fusarium sambucinum* and the known TBZ- insensitive *F. sambucinum* isolate (R-09271 -Desjardins YG-1 U7200A), were insensitive to TBZ with EC_{50} values greater than 100 mg/L (data not shown). However, isolates from the rest of the species and the TBZ- sensitive *F. sambucinum* isolate (R-00738, Cetas, R.C) were sensitive to TBZ with EC_{50} values less than 5 mg/L (Table 4.2). A slightly higher EC_{50} values were obtained using the SDP method than using the SGD method, but results from both methods were the same order of magnitude.

4.3.2 Effects of fludioxonil on mycelial growth

Both sensitive and insensitive isolates of *F. sambucinum* and *F. oxysporum* were identified based on lack of growth inhibition on PDA containing 100 mg of fludioxonil per liter. The insensitive isolates for each species had EC_{50} values greater than 100 mg/L, and represented approximately 8.9% of the *F. sambucinum* and 20.4% of *F. oxysporum* isolated, respectively. All

isolates from the rest of the species were sensitive to fludioxonil, with EC_{50} values less than 5 mg/L (Table 4.2).

4.3.3 Effects of difenoconazole on mycelial growth

All the isolates from the 11 *Fusarium* spp. were sensitive to difenoconazole based on the arbitrary criterion EC_{50} values less than 5 mg/L for all the isolates using the SGD and SDP methods (Table 4.2). Isolates of *F. solani* and *F. equiseti* had slightly higher EC_{50} values compared to *F. torulosum*, *F. graminearum* and *F. sporotrichioides* although they were not significantly different. However, all the values were less than 5 mg/L, hence categorized as sensitive.

Table 4.1 Values of 50% effective concentration (EC_{50}) for inhibition of mycelial growth of *Fusarium* species recovered from dry rot symptomatic tubers as determined by spiral gradient dilution (SGD) method and serial dilution plate (SDP) method

				Mean EC_{50} values for inhibition of mycelia growth (mg/L)[a]			
		df	F (prob)	SGD[b]	Std error	SDP[c]	Std. error
Fungicide		2	<0.0001				
	Difenoconazole			1.4	0.249	1.6	0.203
	Fludioxonil			1.9	0.276	2.4	0.224
	Thiabendazole			2.3	0.249	2.8	0.203
Methods		1	0.7752				
Fungicide X Method		2	0.0615				

[a] EC_{50} is the effective concentration of the fungicide at which mycelial growth was inhibited by 50%.

[b] SGD= Spiral gradient dilution method

[c] SDP= Serial dilution plate method

Table 4. 2. Values of 50% effective concentration (EC_{50}) for inhibition of mycelial growth as determined by spiral gradient dilution (SGD) method and serial dilution method (SDP)

		EC_{50} for inhibition of mycelial growth (mg/L)[a]					
	Number of isolates	Thiabendazole		Fludioxonil		Difenoconazole	
Fusarium spp.		SGD[b]	SDP[c]	SGD	SDP	SGD	SDP
F. oxysporum	68	2.6 ab	2.6 bcd			1.7abc	1.6ab
F. equiseti	44	2.2 abc	1.8 cd	1.6ab	3.5a	2.3a	2.4ab
F. sambucinum	31					1.4bc	1.3ab
F. avenaceum	31	2.2 abc	3.6 abc	1.5b	2.7ab	0.8c	1.5ab
F. cerealis	18	2.4 ab	4.4 a	1.2b	1.9ab	1.9abc	1.1ab
F. solani	14	2.3 abc	3.1 abcd	2.4ab	2.9ab	2.2ab	3.1a
F. acuminatum	11	2.4 ab	4.0 a	1.7ab	1.3ab	1.1bc	1.9ab
F. tricinctum	4	2.9 a	2.3 bcd	2.9a	2.9ab	1.5abc	2.3ab
F. torulosum	4	1.6 bc	3.6 abc	1.6ab	1.5ab	0.9bc	0.6ab
F. sporotrichioides	2	1.1 c	1.6 d	2.6ab	2.8ab	0.8c	0.9ab
F. graminearum	1	1.0 c	1.9 cd	1.7ab	2.3ab	0.4c	0.9ab
R-00738[d]	1	2.6 ab	3.0 abcd				
Tukey's HSD ($p<0.05$)		1.30	1.52	0.81	2.79	0.96	2.53
prob (F)		<0.0023	<0.0001	<0.0064	<0.0046	<0.0001	<0.005

[a] EC_{50} is the effective concentration of the fungicide at which mycelial growth was inhibited by 50%. All values are means of two experiments, with three replicate Petri dishes per experiment

[b] SGD= Spiral gradient method

[c] SDP< Serial plated dilution method

[d] R-00738 = Thiabendazole-sensitive isolate of *F. sambucinum* (R-00738, Cetas, R.C)

4.4 Discussion

Determination of baseline sensitivity levels to fungicides for individual pathogens is important for monitoring studies to help early detection of changes in sensitivity in the field (Russell, 2003). The use of the spiral gradient dilution method for fungal pathogens to determine the effective concentration of a fungicide required to inhibit mycelia growth or spore germination by 50% (EC_{50}) has been proposed (Förster *et al.*, 2004). In the current study, we compared the spiral gradient dilution method with the traditional serial dilution plate method for determination of EC_{50} in *Fusarium* species that cause dry rot in seed potatoes in Michigan. Our results gave EC_{50} values in the same range for the two methods and the two methods were not significantly different. However, the serial dilution plate method resulted in a slightly higher range of EC_{50} values, thus the spiral gradient dilution method could be adopted for quick and easy determination if for a slightly more conservative estimate of baseline sensitivity levels. *In vitro* sensitivities for TBZ (Hanson *et al.*, 1996; Peters *et al.*, 2008a) and fludioxonil (Peters *et al.*, 2008b) have been previously reported for *Fusarium* spp causing potato dry rot. However, this is the first study to present baseline sensitivity of *Fusarium* species causing dry rot of potato tubers to a recently proposed postharvest fungicide, difenoconazole. Difenoconazole is a demethylation inhibitor systemic fungicide and has been proposed for registration against potato tuber decays caused by *Fusarium* spp. (Adaskaveg and Förster, 2010).

In Michigan, 11 species of *Fusarium* have been recovered from dry rot symptomatic seed potato tubers. All the isolates tested came from tubers that had not been previously exposed to difenoconazole. Fungal growth on PDA containing 5 mg of difenoconazole per liter was used as a benchmark for insensitivity. This estimate was based on a study testing other triazoles,

prothioconazole and tebuconazole, against *Fusarium* spp. that resulted to EC_{50} values ranging form 0.1-3.2 and 1.1-5.5 mg/L, respectively (Müllenborn *et al.*, 2008). The results indicated that all the isolates were sensitive to difenoconazole. Sensitivity of *Fusarium* spp. to difenoconazole varied among species with isolates although not significantly different with isolates of the *F. equiseti* species complex attaining a slightly higher $EC_{50.}$ This indicated that close monitoring of changes in sensitivity of the *F. equiseti* species complex to difenoconazole is important. While this study suggested that difenoconazole is an effective fungicide against *Fusarium* species from potato tubers, Allen *et al.* (2004) reported that difenoconazole had a limited effect on growth of *Fusarium* species isolated from pine seeds. This could mean that the host has an effect on the response of the *Fusarium* isolates to difenoconazole. Olaya *et al.* (2010) reported that difenoconazole was effective in reducing the growth of *Colletotrichum coccodes* and hence could be used for control of black dot of potatoes and therefore may have broad spectrum utility in potato production.

Fludioxonil is a protectant fungicide and has been reported to effectively reduce seed piece decay and sprout rot (Wharton *et al.*, 2007b). *In vitro* insensitivity to fludioxonil, characterized by no growth inhibition on PDA containing more than 100 mg fludioxonil per liter was reported on isolates for *F. sambucinum* and *F. coeruleum* (Peters *et al.*, 2008b) and *F. sambucinum* and *F. oxysporum* (Gachango *et al.*, 2011). Approximately 20% of *F. oxysporum* isolates and 9% of *F. sambucinum* isolates were insensitive to fludioxonil. All the other isolates from the other species recovered were sensitive to fludioxonil. All the isolates tested had not been previously exposed to fludioxonil, hence the discovery of insensitive isolates of *Fusarium* to fludioxonil may pose a challenge in controlling seed piece decay caused by *Fusarium* spp.

Thiabendazole-insensitivity has been characterized by fungal growth on PDA containing 5 mg of TBZ per liter (Hanson *et al.*, 1996; Ocamb *et al.*, 2007). Based on this classification, other *Fusarium* spp. insensitive to TBZ have been reported; they include *F. oxysporum*, *F. solani* and *F. culmorum* (Hanson *et al.*, 1996; Ocamb *et al.*, 2007), *F. avenaceum, F. equiseti, F. sporotrichioides* (Ocamb *et al.*, 2007) and *F. acuminatum* (Hanson *et al.*, 1996). This indicates that TBZ cannot provide sufficient protection against Fusarium dry rot caused by the aforementioned *Fusarium* spp. However, in the current study, only *F. sambucinum* was insensitive to TBZ, while the rest of the species were sensitive to TBZ. This indicated that TBZ could still be used to control Fusarium dry rot in Michigan seed production as long as the causal agent is not *F. sambucinum*. A frequent evaluation of *Fusarium* spp. composition in seed production in Michigan is necessary to justify continued use of TBZ. Since no other fungicide has been registered for postharvest use against Fusarium dry rot, cultural practices should not be neglected. These practices include harvesting when tuber skin has matured, proper handling of tubers during harvesting and transportation to avoid wounding and providing proper storage conditions that expedite the wound healing process (Secor and Salas, 2001).

The current study did not have compelling evidence to suggest that fludioxonil has failed to perform because of insensitivity to *Fusarium* spp., however, the occurrence of such insensitive strains necessitate the development and registration of partner chemistries that can preempt any future concerns on lack of performance of products in use (Russell, 2003).

General Conclusions

From the current work, we could therefore conclude that management of postharvest diseases of potatoes requires an integrated approach. The use of fungicides and biofungicides should be integrated with cultural practices for complete control of potato storage pathogens. In-

season protection crop protection strategies, e.g. in-furrow and foliar application of fungicides and biofungicides, together with good crop management during the growing season should be adopted to increase tuber resistance against storage pathogen. Frequent evaluation of *Fusarium* spp. populations and testing them for sensitivity levels towards fungicides is important for monitoring changes in sensitivity level. There is need to test the effectiveness of difenoconazole in controlling potato dry rot and seed piece decay in storage and after planting, respectively. Cultural practices, especially proper handling of tubers to avoid wounding should be emphasized since wounds are major sites of pathogen penetration.

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