MANAGEMENT OPTIONS FOR CONTROL OF FUSARIUM DRY ROT (*FUSARIUM* SPP.) AND POTATO COMMON SCAB (*STREPTOMYCES* SPP.) OF POTATO (*SOLANUM TUBEROSUM* L.) IN MICHIGAN

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ABSTRACT

MANAGEMENT OPTIONS FOR CONTROL OF FUSARIUM DRY ROT (*FUSARIUM* SPP.) AND POTATO COMMON SCAB (*STREPTOMYCES* SPP.) OF POTATO (*SOLANUM TUBEROSUM* L.) IN MICHIGAN

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Potato production systems have long been plagued by recurrent and persistent soil-borne diseases, including Fusarium dry rot (*Fusarium* spp.) and potato common scab (*Streptomyces* spp.). Eleven *Fusarium* spp. were isolated from symptomatic commercially grown potato tubers in Michigan. All species were pathogenic when inoculated onto potato tubers with isolates of *F. sambucinum*, *F. avenaceum*, and *F. acuminatum* consistently being the most aggressive. *In vitro* tests showed that some isolates *of Fusarium* spp. were insensitive to azoxystrobin, fludioxonil, difenoconazole, and thiabendazole. Insensitivity of *F. incarnatum/equiseti*, *F. oxysporum,* and *F. solani* to difenoconazole is a first report in North America. A field trial was conducted to evaluate the effects of fungicide and biofungicide seed treatments applied to potato seed pieces, in-furrow, or in combination for control of soil-borne *F. sambucinum*. The application of mancozeb to potato seed pieces was most effective and improved final plant stand, rate of emergence (RAUEPC), and total potato yields in comparison to many of the other treatments. Field trials were conducted to investigate the influence of cultivar, sulfur, cultural practices, and crop protection strategies on potato common scab control. Overall, no management strategies were completely effective in controlling common scab. Some field treatments reduced the severity of common scab, however no treatment reduced the severity to an acceptable level required by commercial potato growers or processors.

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CHAPTER 1: IMPORTANCE OF POTATO

1.1 INTRODUCTION

Globally, potato (*Solanum tuberosum L.*) is the fourth most important food crop after maize (*Zea mays* L.), rice (*Oryza sativa* L.), and wheat (*Triticum aesitvum* L.), and is the top non-grain food commodity (Bradshaw and Ramsay, 2009; Fiers et al., 2012). Potato production has increased worldwide since the early 1990's, largely due to expansion in countries such as Asia and Europe (Fiers et al., 2012). Potato production in the North Central region of the US accounts for approximatley 23% of the total US production at an estimated value of $716 million (USDA NASS, 2011). However, diseases remain the major limiting factor in sustainable and profitable potato production (Secor and Gudmestad, 1999; Powelson and Rowe, 2008). At least 60 major diseases are known to negatively affect potato production in the USA (Secor, 2009). Several of these diseases are caused by soil-borne pathogens. Soil-borne potato diseases can be separated into two distinct groups; diseases that affect crop development and those affecting tuber quality (Gudmestad et al., 2007).

In Michigan, potato production throughout the year is not possible due to the long winter months (Sonnewald, 2001). As a result, long-term storage is essential to maintain a year round supply of high quality potato tubers for fresh market and chip processing (Secor and Gudmestad, 1999). Seed potato tubers are also stored for up to six months before they are shipped or planted (Bussan and Olsen, 2010). Appropriate storage conditions, such as temperature and relative humidity, are needed to maintain high quality potatoes and maximize potato seed performance (Olsen, 2010). Management strategies can be implemented to minimize disease development during this storage period (Secor and Gudmestad, 1999).

Following harvest, the storage period can be divided into three phases (Kleinkopf, 2010). First, potato tubers undergo a curing period that allows for suberization and maturation of the potato tuber that helps the periderm to set, stimulate wound healing, reduce respiration and water loss, and retard pathogens including rots (Kleinkopf, 2010). Optimal conditions during the curing phase consist of slowly reducing storage temperature (0.5-1°C per day) to 10-13°C, maintaining 95% relative humidity, and air ventilation (appropriate for end use of the tubers) for a period of one to two weeks (Powelson and Rowe, 2007). The longer second phase consists of the holding period, lasting two to 12 months, in which the temperature recommendations are set based on the intended use of the tubers. Recommendations for seed potato tubers are to be stored between 3.3 and 4.4°C, fresh market tubers stored between 3.3 and 10°C, chip processing tubers between 10 and 13°C, and French fry destined tubers between 7 and 10°C (Knowles and Plissey, 2007). The final phase is most important for seed tubers to reduce bruising and consists of a warming period until the potatoes reach approximately 10°C, prior to removing tubers from storage (Kleinkopf, 2010). The health of potato tubers in storage does not improve over time, but can be maintained by maintaining proper storage environments (Hellevang, 1993).

As in all crops, the occurrence and severity of any potato disease is a function of the interaction between the host, pathogen, and environment (Schumann and D'Arcy, 2006). An integrated pest management strategy is often needed to control or suppress disease (Horsfield et al., 2010). These management strategies to prevent or reduce disease incidence and severity include a combination of host resistance (Douches et al., 1996), disease prediction models (Baker and Kirk, 2007; Wharton et al., 2008), crop protection measures with fungicides and biofungicides (Olanya et al., 2009; Wharton and Kirk, 2014), and cultural practices such as management of inoculum sources (Gudmestad et al., 2007) and soil amendments (Lazarovits et

al., 2001; Goicoechea, 2009). Management strategies to control potato diseases include applications of fungicides to potato tubers prior to storage and at planting, in-furrow applications of fungicides (Al-Mughrabi et al., 2007; Kirk et al., 2013), and the development of machinery for potato handling and storage (Finckh, 2008).

Potato production systems have long been plagued by recurrent and persistent soil-borne diseases, including Fusarium dry rot [(*Fusarium* spp.); Manns, 1911; Small, 1944; Secor and Gudmestad, 1999; Wharton et al., 2007c] and potato common scab [(*Streptomyces* spp.); Millard, 1923; Muncie et al., 1944; Larkin and Griffin, 2007; Dees and Wanner, 2012]. These pathogens have resting spores that can survive in the soil for extensive periods in the absence of known hosts (Bruehl, 1987), which confounds successful management strategies. Typically soil-borne diseases of potatoes impact root health, plant health and vigor, tuber quality, and marketable yield (Gudmestad et al., 2007).

1.2 FUSARIUM DRY ROT

Fusarium dry rot is one of the most important postharvest diseases of potato worldwide and is caused by several species of *Fusarium* (Boyd, 1997; Secor and Salas, 2001). *Fusarium* spp. can be devastating pathogens affecting seed and commercial tubers in storage (Leach and Webb, 1981; Hide and Cayley, 1985; Choiseul et al., 2006; Wharton et al., 2007b; Gachango et al., 2012a). Fusarium dry rot of seed tubers can reduce crop establishment by affecting the development of potato sprouts, resulting in poor emergence and reduced plant stands with weakened plants (Leach and Nielsen, 1975; Leach, 1985; Wharton et al., 2006; 2007b). Storage losses associated with Fusarium dry rot have been estimated to range from 6 to 25%, and occasionally losses as great as 60% have been reported (Secor and Salas, 2001; Estrada et al., 2010). In the US, losses attributed to dry rot in storage are estimated from $100 to 200 million

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annually (unpublished data from the USDA, Schisler; 1815 N. University Street Peoria, IL, 61604-3902). Fusarium dry rot is also important to human health as certain species can produce mycotoxins, specifically diacetoxyscirpenol and other trichothecene toxins, which can pose serious health problems to people and animals (Desjardins and Plattner 1989; Desjardins and Proctor, 2007).

Fusarium spp. are common in most soils where potatoes are grown and can survive as chlamydospores free in the soil for very long periods of time (Sitton and Cook, 1981; Leslie and Summerell, 2006; Wharton et al., 2007b). These pathogens have a broad host range, although tuber rotting *Fusarium* spp. are generally host-specific and often do not infect other plants species (Secor and Salas, 2001); however the converse does not hold (Gachango et al., 2011a). Isolates of *F. graminearum* (isolated from potato tubers with dry rot, wheat kernels with scab, and sugarbeet tap roots with decay) were pathogenic with no significant differences in aggressiveness to potato tubers, regardless of original host (Estrada et al., 2010). Contaminated seed tubers can be significant sources of initial inoculum, consequentially resulting in infested soils (Secor and Gudmestad, 1999; Cullen et al., 2005).

Infection of potato tubers by *Fusarium* spp. generally occurs through wounds or bruising to the periderm during harvesting, grading, loading, cutting, and general handling of potato tubers (Secor and Salas, 2001; Powelson and Rowe, 2007). Initial symptoms of Fusarium dry rot appear as shallow dark depressions on the surface of the tuber, which can expand and become wrinkled in concentric rings as the underlying dead tissue desiccates (Secor and Salas, 2001; Wharton et al., 2007b). Internal tuber symptoms of Fusarium dry rot are characterized by dry necrotic areas varying from light to dark brown or black (Fig. 1.1). Rotted tissue often develops

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cavities lined with mycelium with variation in pigmentation from yellow to white to pink (Fig. 1.1).

Currently there are at least 13 known *Fusarium* spp. responsible for causing potato dry rot worldwide (Hide et al., 1992; Secor and Salas, 2001; Cullen et al., 2005), and 11 of these species have been reported in Michigan (Gachango et al., 2012a). The most prevalent species infecting potatoes are *F. sambucinum* Fuckel (synonym *F. sulphureum* Schltdl.), *F. solani* (Mart.) Sacc. var. *coeruleum* (Lib. ex Sacc.) C. Booth (synonym *F. coeruleum*), and *F. oxysporum* Schltdl. Fr. (Hanson et al., 1996; Gachango et al., 2012a). Other species reported in the northern US include *F. avenaceum* (Fr.) Sacc., *F. culmorum* (W.G. Sm.) Sacc., *F. acuminatum* Ellis & Everh., *F. incarnatum/ equiseti* (Corda)/(Roberge) Sacc., *F. crookwellense* L.W. Bugess, P.E. Nelson & Ravenel (*F. cerealis*), *F. graminearum* Schwabe, *F. proliferatum* (Matsushima) Nirenburge, *F. sporotrichioides* Sherb., *F. torulosum* (Berk. & Curt.) Nirenberg, and *F. tricinctum* (Corda), (Hanson et al., 1996; Ocamb et al., 2007; Gachango et al., 2012a).

The most prevalent species reported in the north-eastern US by Hanson et al. (1996), were *F. sambucinum*, *F. solani*, and *F. oxysporum*. In addition, Lacy and Hammerschmidt (1993) reported that of these species, *F. sambucinum* was the predominant species affecting potato tubers in storage and caused seed piece decay after planting and that *F. sambucinum* was the most aggressive of these species. Similarly, the most prevalent *Fusarium* spp. that caused Fusarium dry rot in a study by Ocamb et al. (2007) was *F. sambucinum*. In two reports from Michigan, the predominant species affecting seed potato tubers in storage in 2006 and 2013 was *F. sambucinum*, which caused seed piece decay and rotted sprouts after planting, leading to replanting of fields due to poor stand emergence (Wharton et al., 2007b; Kirk and Wharton, 2008; Merlington and Kirk, 2013). In North Dakota, *F. graminearum* was recently reported to be

the most prevalent species causing potato dry rot (Estrada et al., 2010). *Fusarium coeruleum* (AKA *F. solani*) is considered the most important species of concern in the United Kingdom (Cullen et al., 2006; Peters et al., 2008a). Most of the species reported to cause potato dry rot have been recovered in the Pacific region of the USA (Ocamb et al., 2007) and in Michigan potato seed lots (Gachango et al., 2012a).

In a more recent survey conducted on Michigan potato seed stocks, a more diverse species of *Fusarium* was identified, with *F. oxysporum* reported to be the species recovered most frequently from dry rot symptomatic tubers and that *F. sambucinum* was the most virulent on potato tubers (Gachango et. al., 2012a). Additionally, Gachango et al. (2011a) identified *F. torulosum* causing dry rot of potato tubers, a first report in the US. Many studies have determined that the prevalence and virulence of *Fusarium* species differ based on field location and cropping history (Hanson et al., 1996; Choiseul et al., 2006; Peters et al., 2008a; Gachango et al., 2012a).

In Michigan, seed potatoes are produced in the Upper and Lower Peninsulas, but potato seed is also imported from other areas of the US (MPIC, 2012). Seed lots are inspected and evaluated for Fusarium dry rot and other diseases according to the US seed certification standards. States can adopt the basic seed standards imposed by United States Department of Agriculture (USDA) or establish more rigorous requirements (MPIC, 2012). The limited seed tuber generation program in Michigan was adopted in 2002 and currently utilizes the tissue culture system. The Michigan Department of Agriculture and Rural Development (MDARD) in partnership with the Michigan Seed Potato Association (MSPA, Gaylord, MI) requires seed lots to be tested and certified according to agreed standards (MPIC, 2012). Winter testing of all seed lots entered for certification is done from Dec to Feb in a warm climatic region e.g. FL or HI,

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US. The seed lots are evaluated by MSPA seed inspectors. The seed lots that pass this stage achieve "blue tag" certification (U.S. Standards for Seed Potato Grades Regulation number 628) from the MSPA and MDARD (MPIC, 2012).

There are no known commercially grown potato cultivars resistant to dry rot in North America, although the level of susceptibility varies between cultivars (Secor and Salas, 2001; Wharton et al., 2007b). Potato seed lots in Michigan (>50%) are affected by Fusarium dry rot to varying degrees (Kirk et al., 2013). Commercial potato growers receive seed as whole tubers, which can be used for commercial production. In addition, early generation seed lots are used to produce the next generation of seed.

Tubers are cut into seed pieces and can be treated with fungicides prior to planting for potato dry rot control (Wharton et al., 2007b). Cutting seed tubers can potentially facilitate or enhance the transmission of pathogens from one tuber to another as in the case of *Phytophthora infestans* [potato late blight (Lambert et al., 1998; Kirk et al., 1999)]; *Clavibacter michiganensis* spp. *sepdonica* [bacterial ring rot (Franc, 1999)]; and *Pectobacterium carotovora* [bacterial soft rot (Czajkowski et al., 2011)]. Potentially, *Fusarium* spp., which can lead to potato dry rot infection and development in the field or in storage, could be transmitted between tubers and are also exacerbated by poor re-storage after cutting (Wharton and Kirk, 2014).

There are two main opportunities during the potato crop cycle for Fusarium spp. to infect potato tubers spring and fall (Fig. 1.2). Consequently, Fusarium dry rot can be controlled during these infection periods and include control of seed-piece decay prior to and at planting and postharvest control of potato dry rot in storage facilities (Secor and Salas, 2001; Nolte et al., 2003; Wharton et al., 2007b). Potato seed pieces infected with dry rot can act as initial inoculum to the daughter tubers produced by the crop (Wharton et al., 2005). Postharvest management of

potato dry rot can be achieved by implementing practices that avoid tuber injury, reduce tuber bruising, and provide conditions for rapid wound healing (Secor and Salas, 2001; Secor and Johnson, 2008). One of the most important strategies for controlling potato diseases, including dry rot, in storage facilities includes elimination of infected tubers prior to storage along with proper storage management (Knowles and Plissey, 2007; Powelson and Rowe, 2007).

Management strategies for controlling potato dry rot in storage facilities and the field are limited. In addition to the methods mentioned above, Fusarium dry rot in storage on both stored commercial tubers and seed tubers can be controlled by postharvest applications of fungicides during storage loading (Hide and Cayley, 1980; 1985; Hanson et al., 1996; Wharton et al., 2007b; Gachango et al., 2012b). Fusarium dry rot on potato seed pieces can be controlled by applying fungicide seed treatments prior to planting or at planting (Ayers and Robinson, 1956; Nolte et al., 2003; Wharton and Kirk, 2014). However, some *Fusarium* isolates have shown varying responses to fungicides that are applied post-harvest globally and in Michigan (Desjardins et al., 1995; Hanson et al., 1996; Gachango et al., 2012a).

Control of Fusarium dry rot in storage has been achieved primarily by postharvest applications of thiabendazole (TBZ or Mertec 340F™; Syngenta Crop Protection, Greensboro, NC) as the tubers enter into storage, although TBZ-resistant strains compromise the efficacy of dry rot control (Hide et al., 1992; Hanson et al., 1996; Ocamb et al., 2007; Gachango et al., 2012a). Isolates of *F. sambucinum* resistant to TBZ were first identified in Europe in 1973 (Hide et al., 1992) and later identified in the US in 1992 (Desjardins et al., 1993). Many strains of *F. sambucinum* are known to be resistant to TBZ and other benzimidazoles globally (Hide et al., 1992; Ocamb et al., 2007), while all *F. sambucinum* samples were resistant to TBZ in a survey conducted on commercial potato seed tubers in Michigan, US in 2009 and 2010 (Gachango et.

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al., 2012a). Resistance to TBZ has also been reported for most of the *Fusarium* spp. implicated in causing potato dry rot. Hanson et al. (1996) reported isolates of *F. sambucinum, F. oxysporum, F. solani, F. acuminatum,* and *F. culmorum* with resistance to TBZ. Ocamb et al. (2007) detected TBZ-resistant isolates of *F. sambucinum, F. oxysporum, F. solani, F. avenaceum, F. equiseti, F. sporotrichioides,* and *F. culmorum.*

Fungicide resistance has reduced the efficacy of TBZ in controlling *F. sambucinum*, but the chemical may be useful where TBZ-sensitive isolates still occur. Fenpiclonil (phenylpyrrole) and a mixture of TBZ and imazalil (Imidazole; Fungazil 100 SL) were reported to effectively control Fusarium dry rot between 1989 and 1994 (Carnegie et al., 1998). Postharvest application of fungicide mixtures may help prevent the development of TBZ resistant *Fusarium* isolates where TBZ-sensitive isolates still occur (Bojanowski et al., 2013). Imazalil alone has shown to be effective against dry rot when applied as a postharvest treatment (Hide and Cayley, 1985), but more recent studies concluded it was ineffective against some *Fusarium* isolates (Carnegie et al., 1998).

Fludioxonil (Maxim™; Syngenta Crop Protection), also a phenylpyrrole, is a fungicide registered for potato seed treatment against Fusarium dry rot in the US (Wharton et al., 2007b; Zitter, 2010). Fludioxonil can be used alone or in combination with other active ingredients, such as mancozeb (Maxim MZ™; Syngenta Crop Protection) to control Fusarium dry rot (Wharton et al., 2007c). Fludioxonil can reduce seed piece decay as well as the incidence of diseased sprouts that develop into infected plants (Wharton et al., 2007b). However, fludioxonil-resistant strains of *F. sambucinum*, *F. oxysporum*, and *F. coeruleum* were reported in Canada (Peters et al., 2008a; Peters et al., 2008b) and in Michigan (Gachango et al., 2011b). This has resulted in fewer strategies for controlling potato seed piece decay and sprout rot caused by *Fusarium* spp.

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(Gachango et al., 2012a). Fludioxonil has a single site mode of action, interfering with the osmotic signal transduction pathway, leading to a high probability of development of resistant strains (Brent and Hollomon, 2007).

Azoxystrobin (Quadris™; Syngenta Crop Protection) has been used to control soil-borne diseases, including Fusarium seed piece decay when applied on freshly cut seed tubers (Powelson and Rowe, 2007), although no assessment has been made on the efficacy of azoxystrobin for Fusarium dry rot control in Michigan. Azoxystrobin has a single site mode of action, inhibiting mitochondrial respiration, which increases the risk for the development of pathogen resistance (Brent and Hollomon, 2007). Isolates of *Alternaria solani*, the cause of early blight, with reduced sensitivity or complete resistance to azoxystrobin and similar chemistries have been identified (Pasche and Gudmestad, 2008; Rosenzweig et al., 2008; Belcher et al., 2010; Fairchild et al., 2013). To counteract the reduced effectiveness of TBZ and, potentially, of fludioxonil associated with resistant strains of pathogens, new registrations of efficacious postharvest fungicides with differing mechanisms of action (FRAC groups) are needed (Brent and Hollomon, 2007).

Difenoconazole (Inspire™; Syngenta Crop Protection), a sterol biosynthesis inhibitor, was introduced into the US for control of Fusarium dry rot and other potato diseases in 2010 (Adaskaveg and Förster, 2010; Gachango et al., 2012a). Furthermore, a 3-way mixture of difenoconazole, azoxystrobin, and fludioxonil was recently registered for managing decay caused by *Fusarium* species on potato and other tuber crops (Adaskaveg and Förster, 2010). This 3-way mixture (Stadium™; Syngenta Crop Protection) has been registered in Michigan for potato dry rot and silver scurf management in storage (Kirk et al., 2013). The mixture of azoxystrobin, fludioxonil, and difenoconazole provided adequate potato dry rot control and

significantly reduced dry rot incidence compared to inoculated control potato tubers (Kirk et al. 2013). Integrating storage technologies with physical and chemical management strategies at harvest or as tubers are loaded into storage or before planting could reduce losses caused by *Fusarium* spp.
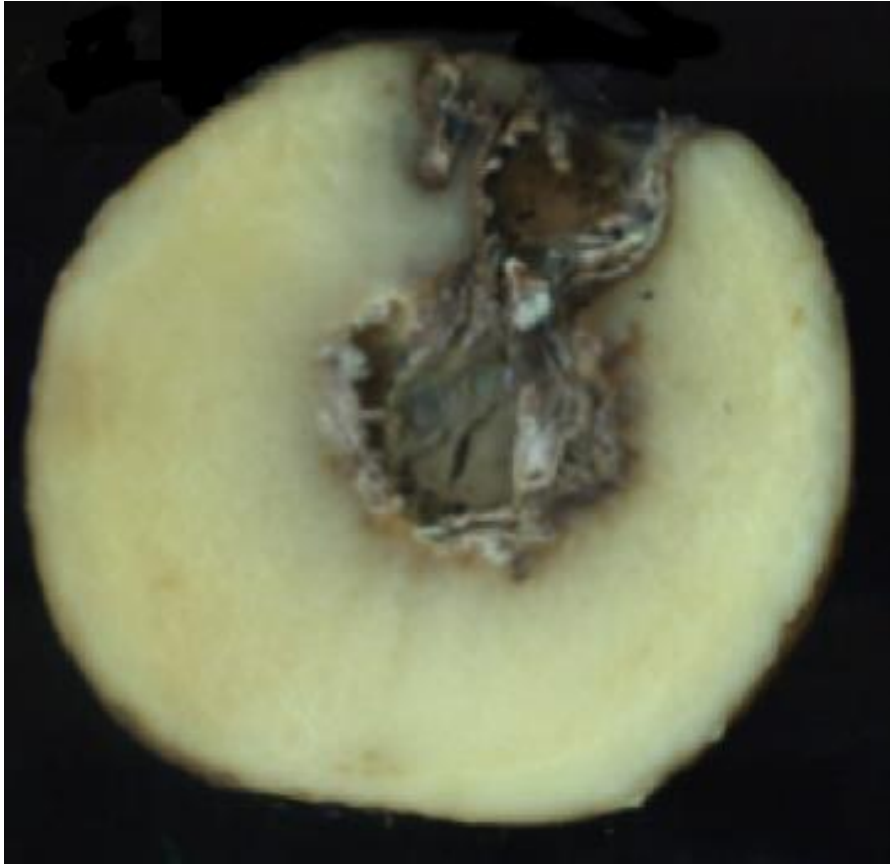
Figure 1.1 Potato tubers expressing Fusarium dry rot, cut open longitudinally across the point of inoculation to expose the internal dry rot lesion.
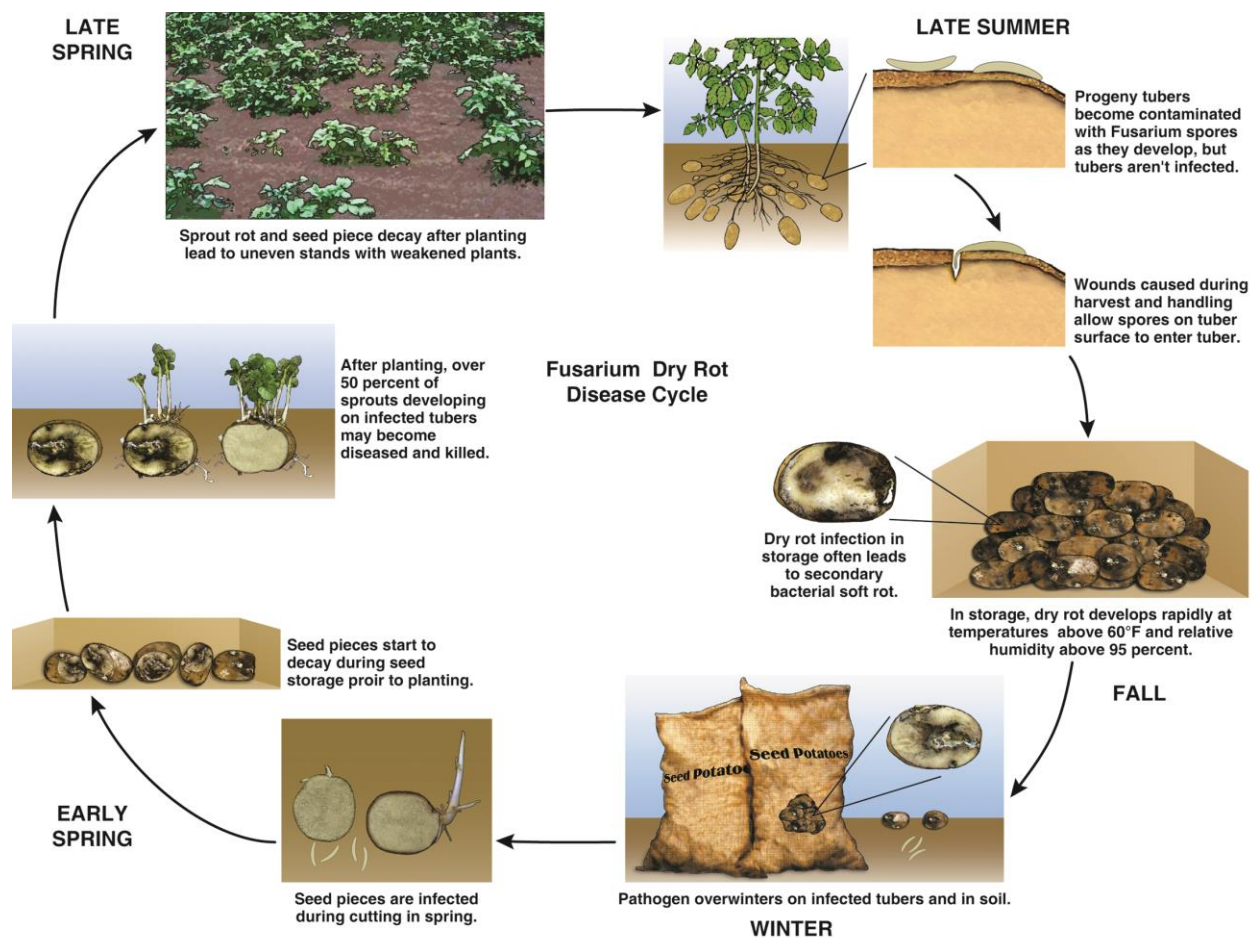
Figure 1.2 The disease cycle of *Fusarium sambucinum* and species, causal agent of Fusarium dry rot (Wharton et al., 2007).

1.3 POTATO COMMON SCAB

Potato common scab (PCS) in North America can be caused by several species of *Streptomyces*, including *S. scabies*, *S. acidiscabies*, *S. europaeiscabiei*, *S. turgidiscabies*, and *S. stelliscabiei* (Lambert and Loria, 1989a; 1989b; Wanner, 2006; 2009; Dees and Wanner, 2012). There are hundreds of species of *Streptomyces* described in the literature, but only about ten of these are pathogenic and cause PCS worldwide (Lambert and Loria, 1989a; 1989b; Loria et al., 1997; Hao et al., 2009; Dees and Wanner, 2012). These different strains can co-occur in fields and in the same scab lesion (Lehtonen et al., 2004; Hiltunen et al., 2009). Many *Streptomyces* spp. are actually beneficial microbes in the soil and some species can aid in controlling PCS and other potato pathogens (Lorang et al., 1995; Lui et al., 1996; Loria et al., 2006; Hao et al., 2009). The genus *Streptomyces* is also well-known for its ability to produce medicinally important antibiotics (Chater, 2006).

PCS is a serious, recurrent, and important soil-borne disease of the potato (*Solanum tuberosum* L.) globally (Loria, 2001), particularly in Michigan (Wharton et al., 2007a). There are very few estimated losses due to PCS available, but economic losses of potatoes in Canada, due to PCS, were estimated to be between 15.3 and 17.3 million Canadian dollars in 2002 (Hill and Lazarovitz, 2005). PCS affects the cosmetic quality of the potato tuber and ultimately the market value of the crop (Loria, 2001, Loria et al., 2006). Economic losses are greatest for tubers intended for table stock, although significant losses have been reported for processing cultivars (Hill and Lazarovitz, 2005; Loria et al., 2006; Wanner, 2009).

PCS has been reported to be related to delayed emergence of the potato plants and a reduction in potato yield (Hiltunen et al., 2009). Typically PCS impacts tuber quality and marketable yield, but in seed PCS can cause poor plant vigor or even death (Loria, 2001; Loria et

al., 2006; Wanner, 2009). Common scab is also a disease on other root crops, including, radish (*Raphanus sativus* L.), parsnip (*Pastinaco sativa* L.) turnip (*Brassica rapa* L.), beet (*Beta vulgaris* L.), and carrot (*Daucus carota* L.) (Lambert, 1991; Goyer and Beaulieu, 1997; Loria et al., 1997). Significant losses have been reported for most root crops.

The symptoms of PCS are present on the surface of the potato tuber, but can be variable. Scab lesions start out as small brownish spots on the potato tuber surface, which expand into water-soaked lesions within a few weeks after infection (Loria, 2001; Wharton et al., 2007a; Naher et al., 2013). The disease is characterized by the formation of corky lesions on the tuber surface, which can be categorized into at least three symptomatic lesion types, including superficial, raised, or pitted (Loria et al., 1997); (Fig. 1.3). Sometimes superficial lesions are also referred to as russeting, particularly on round white tubers, as the appearance resembles that of a russet tuber skin (Fig. 1.3-d). Symptoms are generally noticed late in the growing season or at harvest, although infection occurs during early tuber development and growth. Once the pathogen penetrates through several layers of cells of a young tuber, it is able to derive its nutrient needs from the dead cell material it has infected (Loria et al., 1997; 2006; Dees and Wanner, 2012). Scab lesions can be categorized further into discrete or coalescing. To further classify disease severity, an index of surface area infected using these categories can be used (Wanner et al., 2014).

Incidence and severity of PCS vary based on location, from year to year, cultivar to cultivar, and within fields (Goyer et al., 1996; Wanner, 2006). It is unclear as to what factors, strains, or species determine the type or severity of scab symptoms (Loria et al., 1997). The variability and severity of the disease is of importance to Michigan and USA, where environmental conditions are favorable and often conducive for PCS (Loria, 2001; Wharton et

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al., 2007a). These conditions are typically warm, dry seasons, with high soil temperatures and variable rainfall (Loria, 2001). Reasons for the variability are not well understood, although many hypotheses have been described, including environmental conditions, aggressiveness of the *Streptomyces* strains, and differences in cultivar susceptibility (Loria, 2001).

Streptomyces scabies, the predominant species causing PCS, is a filamentous bacterium with branched mycelium (Lambert and Loria, 1989b; Wanner, 2009). Sporogenous hyphae develop into corkscrew-like spiral chains with cross walls that eventually constrict and break off into individual spores (Loria et al., 1997). The pathogen is an efficient saprophyte that can overwinter in soil organic matter, on the surface of tubers, and on crop residues for over a decade (Fig. 1.4); (Loria et al., 1997; Wharton et al., 2007a; Hao et al., 2009; Dees and Wanner, 2012). Most potato soils have a resident population of *Streptomyces* spp. (Janssen, 2006), which can increase with each succeeding potato crop (Larkin et al., 2010). The population can be reduced by rotation with non-host crops, but this practice does not eliminate the disease because the pathogen can reproduce on organic matter (Vruggink, 1976; Loria et al., 2006; Larkin et al., 2011; Wharton et al., 2007a). Spores can persist in the soil for many years, and can germinate and infect in the presence of a suitable host (Fig. 1.4); (Loria et al., 2006). The pathogen can be spread from one location to another by rain, soil transfer, and on seed tubers (Wharton et al., 2007a). Infection of the potato tuber by *Streptomyces* spp. occurs primarily through the lenticels and wounds (Lapwood and Hering, 1970; Adams 1975; Wanner, 2007; Khatri et al., 2011). Therefore, tubers are most susceptible during the six-week period encompassing tuber initiation and growth (Adams 1975; Adams and Lapwood, 1978; Khatri et al., 2011).

Management of PCS is one of the most important challenges in potato production worldwide and strategies are often initiated in the fall prior to planting the potato crop the

following spring. Essentially, fall strategies focus on creating an environment unfavorable for *Streptomyces* spp. and subsequent infection and disease development. Different management techniques often provide inconsistent PCS control (Dees and Wanner; 2012). Scientists still struggle and have little understanding of the exact conditions or factors that contribute to the differences and variation of disease seen in the field.

Using tolerant cultivars has been the most cost effective and most reliable tool for PCS control, but availability of resistant germplasm is limited (Douches et al., 2009; Wanner and Haynes, 2009). The mechanism of cultivar resistance to PCS is not well understood, which complicates effective and successful breeding. Furthermore, resistant cultivars are not immune to the disease and can become diseased especially when conditions are conducive and inoculum is plentiful (Loria et al., 1997; Loria, 2001). Although there are no cultivars that are immune to pathogenic *Streptomyces* spp., resistant cultivars can be grown to minimize the rate of infection (Hiltunen et al., 2005; Lambert et al., 2006; Haynes et al., 2007). A few cultivars have been identified to have relatively good levels of tolerance to PCS including cvs. 'Superior', 'Russet Burbank', and 'Pike' (Dees and Wanner, 2012; Douches et al., 2012; 2013).

Cultural practices or management techniques are often implemented for control of PCS, but results are inconsistent. Acidic soils, with a level below 5.2 pH can significantly reduce the incidence and severity of PCS (Loria, 2001; Powelson and Rowe, 2007). Reducing soil pH to around 5.2 can create crop rotation problems and also negatively influence plant nutrient availability (Loria et al., 1997). Furthermore, this management strategy can fail because *S. acidiscabies* can survive and cause PCS symptoms at a pH as low as 4.5 (Lindholm et al., 1997). Achieving a lower pH can be accomplished in many different ways. One successful approach has been the addition of sulfur to reduce soil pH. Historically, sulfur has been used for PCS control,

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(Martin 1920), but the mechanism is not well known or understood (Pavlista, 2005). Few experiments have been conducted on the influence of sulfur, however in Michigan; Hammerschmidt et al. (1986) concluded the addition of 125 kg/ha of ammonium sulfate (AS) reduced common scab when incorporated into the potato hill.

Traditional management strategies focus on maintaining soil moisture levels near field capacity two to six weeks during tuber initiation to inhibit infection (Lapwood, et al. 1973; Loria, 2001; Powelson and Rowe, 2007). Maintaining soil moisture at field capacity is difficult and can enhance infection risk by other potato pathogens (Powelson and Rowe, 2007). Overall, this strategy has been fairly successful, although some reports show inconsistent results (Lapwood et al., 1973). Soil amendments including biological agents, manure, lime, and cover crops have produced inconsistent results in controlling PCS (Lazarovits, 2001, 2004; Conn and Lazarovits, 2007; Lazarovits et al., 2008; Wanner et al., 2014).

Chemical control and antimicrobial compounds can be used as a management strategy for PCS control, but have shown variable success (Wilson et al., 1998; Dees and Wanner, 2012). Chemical fumigation is one of the best options to control soil-borne plant pathogens of potato including PCS, but varying levels of success have been reported (Davis, 1976; Hutchinson, 2005; Jordan et al., 2011). There have been several chemical treatments used in an attempt to control PCS, but many resulted in injury to the plant. Pentachloronitrobenzene (PCNB), under the trade name Blocker™ (Amvac Chemical Corporation), for example has resulted in reduced disease incidence is some experiments (Davis et al., 1974; Davis, 1976; Hutchinson, 2005; Jordan et al., 2011). However, results have been inconsistent and PCNB can have a detrimental impact on the potato plant at high concentrations (22.4 kg/A) by reducing tuber size or yield (Wharton et al., 2007a).

Chloropicrin (Pic Plus; TriEst Ag Group Inc.) has had some success in reducing PCS, (Jordan et al., 2011), but the applications are required at relatively high soil temperature preceding planting (8°C) and with a 30-day post-application planting restriction interval, which makes application difficult in Michigan (Wharton et al., 2007a). Synthetic auxins, including 2-4-D (Agri Star; Albaugh Inc.) at extremely high doses (1.0 mM) can reduce the severity of PCS (Tegg et al. 2008). The use of commercially available antagonistic *Streptomyces* spp. and other biocontrol approaches [*Bacillus subtilis* (Serenade Soil™; Bayer Cropscience)] have been shown to decrease the amount of pathogenic *S. scabies* present in the soil and reduce common scab on harvested tubers in some studies (Schmiedeknecht et al., 1998; Han et al., 2005; Hiltunen et al., 2009; Wanner et al., 2014).

Tillage and cultivation practices are essential for preparation of the seedbeds to maximize potato quality and yields (Powelson and Rowe, 2007). Soil physical and chemical properties, moisture and temperature, root growth, and pathogen vectors are all influenced by tillage practices, and consequently pathogen virulence, diversity and host susceptibility are likewise influenced (Sumner et al., 1981). Tillage practices can increase or decrease incidence and severity of potato diseases, depending on the disease of interest and the environment (Gudmestad et al., 2007). The impact of tillage on plant disease development has been highly variable, depending on the specific regional crop-pathogen-environment interactions (Sumner et al., 1981).

Chisel plowing, as opposed to the traditional tillage practice of moldboard plowing, is the most commonly accepted practice that is used for conservation tillage in potato (Peters et al., 2004). Generally the chisel plow provides less soil inversion and pulverizing than the moldboard plow (Dickey et al., 1986). Moldboard plowing has been shown to have positive effects in the

management of some soil-borne potato diseases including potato early die, relative to conventional tillage (Gudmestad et al., 2007). Leach et al. (1993) showed that chisel compared to moldboard plowing resulted in a reduction of the incidence and severity of stem and stolon lesions caused by *Rhizoctonia solani* on the potato. Few studies have looked at the effect of tillage practices on incidence and severity of PCS. Peters et al. (2004) reported the severity of PCS was not influenced by tillage practices or rotation, although the severity was low in all years of that study.
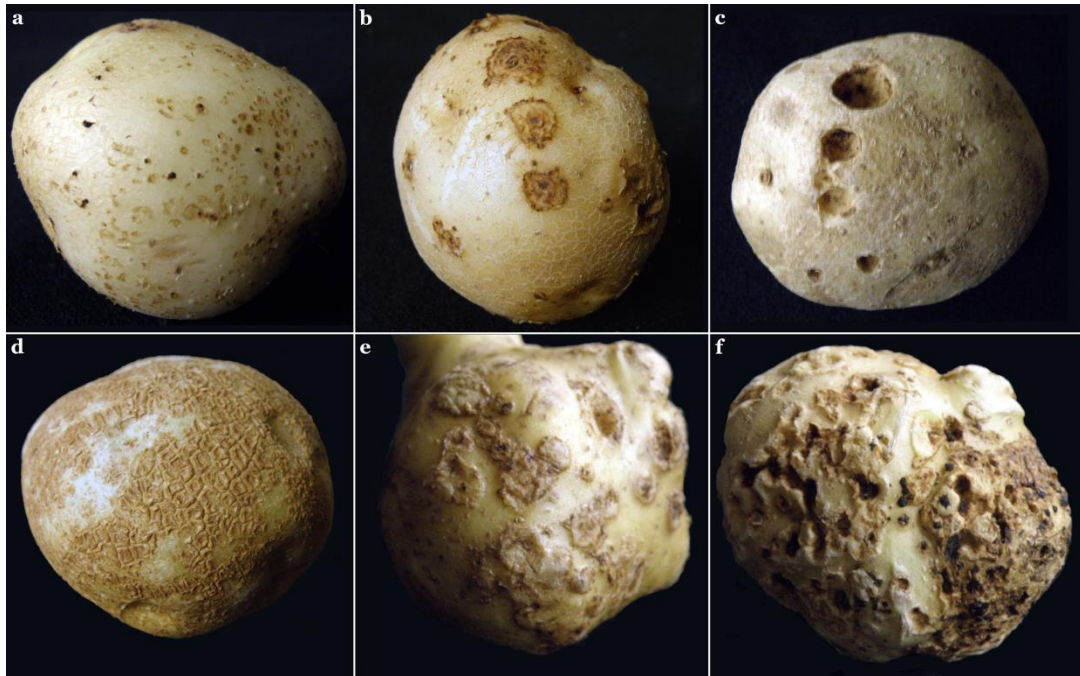
Figure 1.3 The different lesion types of common potato scab caused by *Streptomyces scabies*. (a) Superficial discrete lesions formed on the surface of the tuber; (b) Raised discrete lesions; (c) Pitted discrete lesions varying in depth from 3-5 mm; (d) Coalescing superficial lesions, often referred to as russeting, as when they cover the entire surface of the tuber the appearance resembles that of the skin of a russet tuber; (e) Raised coalescing lesions; (f) Pitted coalescing lesions.

SPRING

Spores dispersed by wind, rain splash and movement of contaminated soil on farm machinery.

Sporogenous hyphae break into spores.

SUMMER

Germinating spores infect through natural openings (lenticels) and wounds.

Spores can infect developing tubers in soil with-in a wide range of temperatures – from 50° to 80°F. Scab is greatly suppressed in soils with a pH below 5.2.

Sporogenous hyphae develop cross walls.

Common Scab of Potato

Mycelia grow through up to three peridermal cell layers, causing cell death. The pathogen then feeds on them.

The pathogen secretes a compound that promotes the formation of a corky layer.

Sporogenous hypha

Vegetative mycelium

In spring, vegetative mycelia develop specialized sporogenous spiral hyphae.

The corky layer pushes the infected area outward.

As the potato periderm breaks, a scab forms.

Pathogen overwinters in the soil and on infected tubers missed at harvest.

As the first cork layer is penetrated, a new one forms below, repeating the cycle and resulting in the development of large scab lesions.

WINTER

FALL

Figure 1.4 The disease cycle of the common scab pathogen, *Streptomyces scabies* (Wharton et al., 2007).

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CHAPTER 2: IDENTIFICATION OF *FUSARIUM* SPP. CAUSING DRY ROT OF POTATO TUBERS IN MICHIGAN'S COMMERCIAL POTATO PRODUCTION

ABSTRACT

Fusarium dry rot is an important postharvest disease of potato worldwide and is caused by several *Fusarium* spp. These *Fusarium* spp. can be devastating pathogens affecting both seed tubers in the field and tubers in storage. A survey of potato tubers from Michigan commercial production storage facilities was carried out from 2011-2012 to determine the *Fusarium* species responsible for dry rot. Isolates resembling *Fusarium* associated with tuber dry rot symptoms were identified to species, and pathogenicity and virulence were determined on two cultivars each year. Symptomatic tubers (n = 972) were collected from 32 commercial potato lots, from which 730 isolates of *Fusarium* species were recovered and identified to 11 species. *Fusarium oxysporum* was the most commonly isolated species (67.3%), followed by *F. incarnatum/equiseti* (13.6%), *F. solani* (5.8%), *F. sambucinum* (5.7%), *F. proliferatum* (3.2%) and *F. acuminatum* (1.8%). Less prevalent species, present at ≤1%, included *F. sporotrichioides*, *F. avenaceum*, *F. redolens*, *F. graminearum*, and *F. crookwellense*. Pathogenicity was evaluated on potato cvs. 'Snowden' and 'MSQ440-2' in 2011 and cvs. 'Atlantic' and 'Russet Norkotah' in 2012. Representative isolates of all species were pathogenic when inoculated onto potato tubers. Cultivars 'Snowden' and 'Atlantic' were significantly more susceptible than 'MSQ440-2' and 'Russet Norkotah' based on percent area of symptomatic tuber tissue. Isolates of *F. sambucinum*, *F. avenaceum*, and *F. acuminatum* were consistently the most aggressive, with minor differences in cultivar and year.

2.1 INTRODUCTION

Fusarium dry rot of potato (*Solanum tuberosum* L.) is a devastating and economically important postharvest disease worldwide (Boyd, 1997; Secor and Salas, 2001; Wharton et al., 2007b; Gachango et al., 2011a). Dry rot affects both tubers in storage and seed tuber pieces in the field (Choiseul et al., 2006; Wharton et al., 2007b). Crop losses associated with Fusarium dry rot have been estimated to range from 6 to 25%, and occasionally storage losses as great as 60% have been reported (Secor and Salas, 2001).

Fusarium spp. are common in most soils where potatoes are grown and can survive as spores free in the soil or in plant material and are resistant to breakdown (Leach, 1985; Alabouvette, 1990; Cotton and Munkvold, 1998). Contaminated seed tubers can be significant sources of initial inoculum, resulting in infested soils (Secor and Gudmestad, 1999; Cullen et al. 2005). Infection of potato tubers by *Fusarium* spp. occurs through wounds or bruising to the periderm during harvesting, grading, loading, cutting, and handling of potato tubers (Secor and Salas, 2001; Powelson and Rowe, 2007). Initial symptoms of Fusarium dry rot appear as shallow dark depressions on the surface of the tuber, which can expand and become sunken and wrinkled (Secor and Salas, 2001; Wharton et al., 2007b; Peters et al., 2008c). Internal tuber symptoms are characterized by dry necrotic areas varying from light to dark brown or black. Cavities below the dry-rotted areas are often lined with mycelium which can vary in color.

Currently there are at least 13 known *Fusarium* spp. implicated in causing potato dry rot worldwide (Hide et al., 1992; Secor and Salas, 2001; Cullen et al., 2005), and 11 of these species have been reported in Michigan, US (Gachango et al., 2012a). The relative frequency and severity of these *Fusarium* spp. are often dependent on the crop location and history (Peters et al., 2008a). In Michigan potato production, *F. sambucinum* is considered the predominant

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species affecting potatoes in storage and causing seed-piece decay after planting (Wharton et al., 2007b; Gachango et al., 2012a; Kirk et al., 2013). The most prevalent species reported in the north-eastern US by Hanson et al. (1996), were *F. sambucinum*, *F. solani*, and *F. oxysporum*. Most of the *Fusarium* spp. known to cause dry rot were recovered from the Pacific region of the US with *F. sambucinum* the most prevalent (Ocamb et al., 2007). The most important species in the United Kingdom is *F. coeruleum* [*F. solani*, (Cullen et al., 2006; Peters et al., 2008a)]. In North Dakota, *F. graminearum* was recently reported to be the most prevalent and important species causing potato dry rot (Estrada et al., 2010).

Most of the species reported to cause potato dry rot have been recovered in Michigan potato seed lots (Gachango et al., 2012a). In Michigan potato production, dry rot has been reported in most seed lots (Kirk and Wharton, 2008). Infected seed tubers can reduce crop establishment by affecting the development of potato sprouts, resulting in poor emergence and reduced plant stands with weakened plants (Choiseul et al., 2001; Wharton et al., 2006; 2007b). In severe cases, seed pieces may rot completely before planting. In two reports from Michigan, the predominant species affecting seed potato tubers in storage in 2006 and 2008 was *F. sambucinum*, which caused seed piece decay and rotted sprouts after planting (Wharton et al., 2007b; Kirk and Wharton, 2008). In 2013, a severe instance of dry rot seed piece decay caused by *F. sambucinum* lead to replanting of fields due to poor stand emergence (Merlington and Kirk, 2013).

In a more recent survey conducted on Michigan (MI) potato seed stocks in 2008 and 2009, a more diverse variety of *Fusarium* spp. was identified, with *F. oxysporum* reported to be the species recovered most frequently from tubers and *F. sambucinum* the most aggressive species (Gachango et. al., 2012a). Additionally, Gachango et al. (2011a) identified *F. torulosum*

causing dry rot of potato tubers in the US for the first time. Many studies have determined that the prevalence and virulence of *Fusarium* species differ among locations, cultivars, and storage types (Hanson et al., 1996; Choiseul et al., 2006; Peters et al., 2008a; Gachango et al., 2012a).

There are no known commercially grown cultivars resistant to dry rot in North America, although the level of susceptibility varies between cultivars (Secor and Salas, 2001; Kirk and Wharton, 2008). Potato seed lots in Michigan (>50%) are affected by Fusarium dry rot to varying degrees (Kirk et al., 2013). Differences in cultivar susceptibility have been attributed in part to the species of *Fusarium* (Yilma et al., 2012). Cultivars may be resistant to one *Fusarium* spp., but susceptible to another species (Bojanowski et al., 2012).

Commercial potato growers receive seed as whole tubers, which can be used for commercial production. In addition, early generation seed lots are used to produce the next generation of seed. Tubers are generally cut into seed pieces, from which *Fusarium* spp. could potentially be transmitted between tubers and lead to potato dry rot infection and development in the field or in storage. The damage is exacerbated by poor re-storage after cutting (Wharton et al., 2005; Wharton and Kirk, 2014). Fusarium dry rot on potato seed pieces can be controlled by applying fungicide seed treatments prior to planting or at planting (Ayers and Robinson, 1956; Nolte et al., 2003; Wharton et al., 2007c; Wharton and Kirk, 2014).

Postharvest management of potato dry rot can be achieved by avoiding tuber injury, reducing tuber bruising, and providing conditions for rapid wound healing (Secor and Salas, 2001; Secor and Johnson, 2008). Additional control of Fusarium dry rot in storage can be by postharvest applications of fungicides during storage loading (Hide and Cayley, 1980; 1985; Wharton et al., 2007b; Gachango et al., 2012a; 2012b). Field treatment strategies combined with storage applied fungicides and biofungicides can be effective options for reducing dry rot

incidence (Kirk et al., 2013). However, some *Fusarium* isolates have shown varying responses to fungicides that are applied post-harvest globally (Desjardins et al., 1995; Hanson et al., 1996; Gachango et al., 2012a). Understanding the *Fusarium* species composition in potato dry rot is important in deciding chemical and cultural management strategies.

Gachango et al. (2012) characterized the *Fusarium* spp. causing dry rot of seed potato tubers in Michigan. However, there had been no systematic assessment of the *Fusarium* spp. responsible for causing potato dry rot in Michigan commercial potato storages. The objective of this study was to characterize *Fusarium* spp. responsible for dry rot of potato tubers produced in Michigan commercial potato production and determine the aggressiveness of these species on two potato cultivars. The hypothesis is that this study will identify a more diverse array of *Fusarium* spp. responsible for causing dry rot than has been reported previously in MI. Furthermore, it is hypothesized this study will reveal differences in cultivar susceptibility that will be dependent on the species of *Fusarium*.

2.2 MATERIALS AND METHODS

2.2.1 POTATO TUBER COLLECTION

Dry rot symptomatic potato tubers (40-50/storage) were collected from Michigan (MI) commercial potato production facilities in the fall of 2011 and 2012. In 2011, nine cultivars were sampled from a total of 13 fields from seven farms in six counties. In 2012, 11 cultivars were sampled from a total of 19 fields from 11 farms in nine counties (Fig 2.1). The tuber samples included nine publicly available cultivars: 'Atlantic', 'Dark Red Norland', 'Goldrush', 'MI Purple', 'Norwis', 'Pike', 'Russet Burbank', 'Russet Silverton', and 'Snowden', as well as eight proprietary cultivars. All symptomatic tubers had shallow, sunken, and/or wrinkled necrotic

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areas with light tan to dark brown internal lesions (Fig. 1.1) with variable mycelial pigmentation from yellow to white to pink. Tubers were stored at 3°C in the dark at 90% relative humidity in a temperature-controlled walk-in refrigerator (Chrysler and Koppin Co., Detroit, MI) for 2 wk following collection.
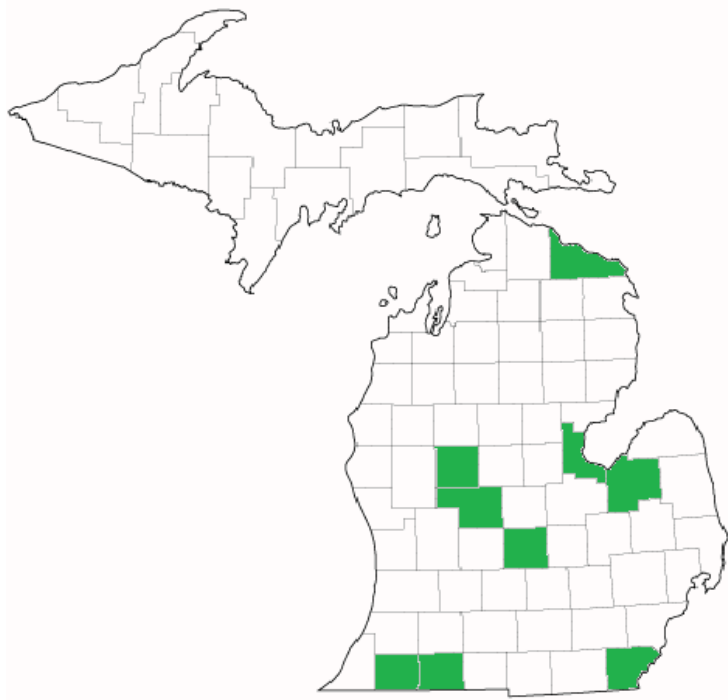
Figure 2.1 Map of Michigan highlighting the counties from which potato tubers were collected for the 2011 and 2012 survey of the *Fusarium* spp. causing potato dry rot in Michigan.

2.2.2 PATHOGEN ISOLATION AND IDENTIFICATION

Sections (0.5 cm in diameter, six pieces per tuber) were cut from the margins of necrotic or symptomatic tissue with a sterile scalpel, surface disinfested in 0.6% sodium hypochlorite for 10 s, rinsed twice in sterile distilled water, and dried on sterile filter paper. The tissue sections were plated on half-strength potato dextrose agar (PDA; Difco Laboratories, Detroit, MI) amended with 0.5 g/L of streptomycin sulfate. Petri dishes were incubated at 23°C in the dark for 5 to 7 d. Putative *Fusarium* isolates were transferred onto water agar (WA), and hyphal tips from the margin of actively growing cultures were removed with a sterile scalpel and plated to carnation leaf agar (CLA) and full strength PDA to generate pure cultures. Preliminary identification of the *Fusarium* isolates was based on conidial and colony morphology and pigmentation on CLA and PDA, respectively (Leslie et al., 2006; Gachango et al., 2012a).

The identity of *Fusarium* spp. was confirmed through DNA extraction using the high salt method (Aljanabi and Martinez, 1997), followed by amplification of the translation elongation factor [EF-1α, (Geiser et al., 2004)]. DNA sequencing was performed by the Genomics Technology Support Facility (Michigan State University, East Lansing, MI). The Fusarium-ID (Geiser et al., 2004) and the National Center for Biotechnology Information (GenBank) databases were used to obtain the closest match to previously sequenced *Fusarium* isolates (lowest e-value, maximum coverage, and greatest identity %). Isolates were included in the survey if they had a 95% or greater identity match to a previously sequenced *Fusarium* isolate.

2.2.3 FUSARIUM INOCULATION, PATHOGENICITY, AND VIRULENCE

Disease-free potato tubers cvs. 'Snowden' and 'MSQ440-2' were used for pathogenicity and virulence testing in 2011 and cvs. 'Atlantic' and 'Russet Norkotah' in 2012. Tubers were surface-disinfested for 10 min in 0.6% sodium hypochlorite and rinsed twice in sterile water.

These cultivars were chosen based on availability ('MSQ440-2'), but were generally representative of potato cultivars grown in commercial potato production. Three tubers of each cultivar were wounded at the apical end to a depth of 4-10 mm with a 4 mm diameter cork-borer. Tubers were inoculated by inserting an mycelial-infested agar plug (4 mm diameter) from a 7 d-old *Fusarium* culture grown on PDA into the wound and incubating the tubers in a temperature-controlled environment chamber, 1.8 m^3 volume (Environmental Growth Chambers, Chagrin Falls Ohio, USA) in the dark at 15°C for 30 d. Wounds were covered with petroleum jelly following inoculation to reduce the risk of incursion of bacterial soft rot. All identified *Fusarium* isolates were tested and the experiment was repeated. Control tubers were inoculated by inserting a sterile PDA plug. After 30 d, tubers were cut longitudinally across the point of inoculation and evaluated for presence of symptoms or signs that are typical of potato dry rot. Isolates that caused light tan to dark brown areas (Fig. 1.1) on all three replicate tubers of each cultivar were considered pathogenic. To complete Koch's postulates, the pathogen was re-isolated from infected areas of the tuber (as described above) and compared morphologically to the original isolate used for inoculation. The isolate was then inoculated onto a disease free tuber and a comparison of the symptoms to those of the original diseased tuber was used to confirm pathogenicity. Isolation from the control tubers was also performed to verify the potato tubers were disease-free and not infected by *Fusarium*.

To determine the virulence of the *Fusarium* isolates, areas of the infected potato tubers were measured using a method adapted from Niemira et al., (1999) as described by Gachango et al. (2012a). Freshly-cut tuber sections were placed on a piece of glass (30 cm x 40 cm x 2 mm) with the cut surface facing down. The glass was transferred to a flatbed scanner (Epson Perfection V30; Epson America, Inc., Long Beach CA) for image processing. The scanner

software (Epson Scan Version 3.50A; Epson America, Inc.) generated images of the cut tuber surfaces against a dark background (Niemira et al., 1999). A ruler was placed on the scanner to provide a scale during the image analysis. The image files created with the scanner software were loaded into Adobe Photoshop CS6 (Version 13.01.1, 2012, Adobe Systems Inc.) and the lesions were selected and painted white using the 'fill' tool. The images were then loaded into image analysis software (SigmaScan Pro 5, 1987-1999 SPSS© Inc., Chicago) to determine the area of the lesion as described by Gachango et al., (2012a). Using the image option, the length and width were measured and calibrated to convert image pixels to a dimension (mm) and area (mm^2) unit of measurement. The ruler within the image provided a standard unit of measurement 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 compared to the rest of the tuber surface. The area of the lesion was calculated by selecting the 'fill' measurement mode under the measurement option according to the SigmaScan manufacture's protocol.

2.2.4 DATA COLLECTION AND ANALYSIS

All data were subjected to analysis of variance (ANOVA) using JMP Version 10.0 (SAS Inc., Cary, NC). Because variances were homogeneous for each experiment and no significant differences were measured between the experimental repeats, the data were combined and analysed statistically using ANOVA. Means separation was conducted using Tukey's honestly significantly difference (HSD) test.

2.3 RESULTS

2.3.1 POTATO COLLECTION; PATHOGEN ISOLATION AND IDENTIFICATION

Symptomatic tubers (n = 972) were collected from 32 commercial potato lots in MI, from which 730 isolates of *Fusarium* spp. were recovered and identified to 11 species during this two-year survey (Table 2.1; Fig. 2.2). A total of 378 isolates in 2011 and 352 isolates in 2012 were recovered and identified to species. The *F. oxysporum* species complex was the most commonly isolated, comprising 73.4 and 61.1% of the total number of isolates collected in 2011 and 2012, respectively. The total number of isolates (relative frequency) for the *F. oxysporum* species complex in 2011 and 2012 was (67.3%), followed by the *F. equiseti* species complex (13.6%), the *F. solani* species complex (5.8%), the *F. sambucinum* species complex (5.7%), *F. proliferatum* (3.2%) and *F. acuminatum* (1.8%). Less prevalent species present at ≤1% included *F. sporotrichioides*, *F. avenaceum*, *F. redolens*, *F. graminearum*, and *F. crookwellense* (Table 2.1). The total number of tubers sampled and the total number of isolates recovered remained similar over the 2-year study. More species were recovered in the second year of the survey, with the addition of *F. proliferatum*, *F. graminearum*, and *F. crookwellense*. Furthermore, in a few instances multiple *Fusarium* spp. were recovered from the same potato tuber during this study.

Table 2.1 Relative frequencies (%) of *Fusarium* spp. isolated from symptomatic potato tubers collected in MI commercial potato production facilities in 2011 and 2012.

Fusarium spp.[a]	Relative frequency (%) of isolated[b] species		
	2011	2012	Total
Fusarium oxysporum	73.4	61.1	67.3
F. incarnatum/ equiseti	16.8	10.3	13.6
F. sambucinum	3.1	8.3	5.7
F. solani	5.2	6.4	5.8
F. avenaceum	0.3	0.9	0.6
F. proliferatum	0.0	6.4	3.2
F. acuminatum	0.5	3.1	1.8
F. sporotrichioides	0.5	0.9	0.7
F. redolens	0.3	0.6	0.5
F. graminearum	0.0	1.1	0.6
F. crookwellense	0.0	0.9	0.5
Total number of isolates	378	352	730
Total number of tubers sampled	482	490	972

[a] *Fusarium* spp. isolated and identified from dry rot symptomatic potato tubers from MI commercial production

[b] Relative frequency = percentage of isolates of a given species relative to the total number of isolates recovered in 2011 and 2012 (378 and 352 isolates), respectively.
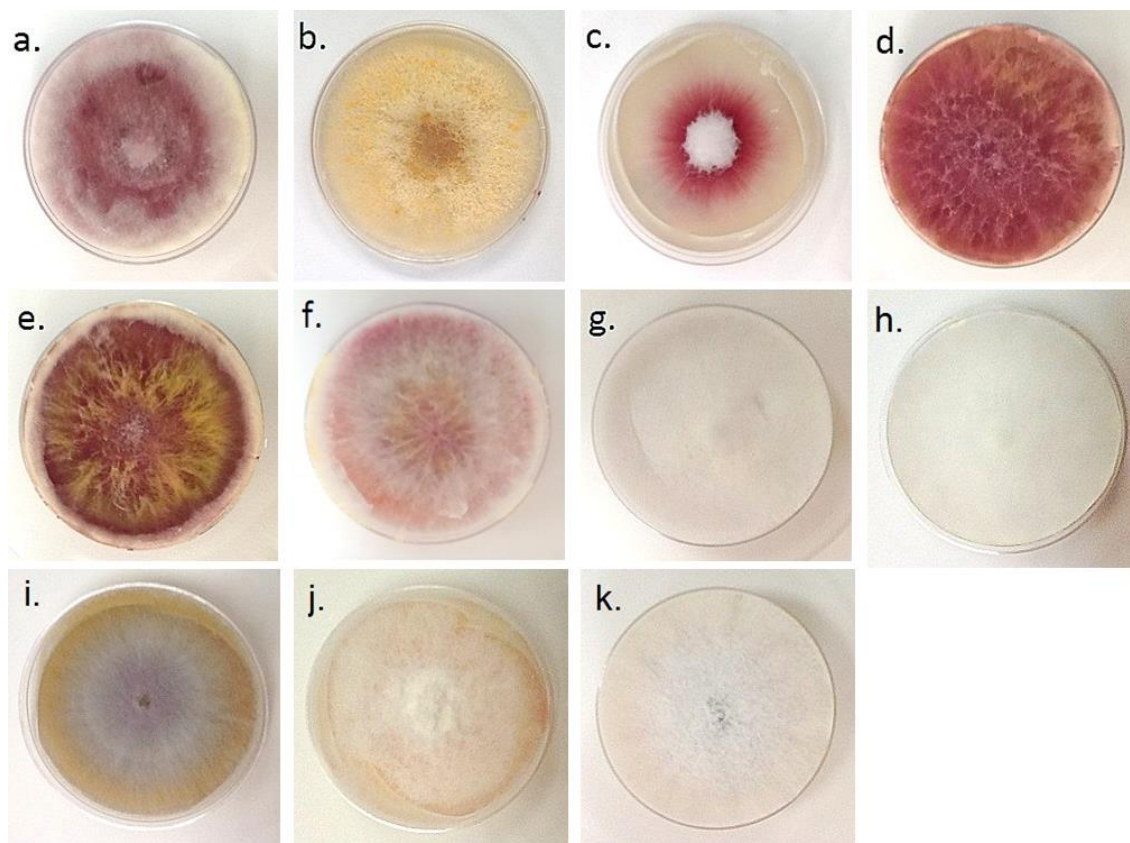
Figure 2.2 *Fusarium* spp. causing potato dry rot in MI commercial potato production as determined in 2011 and 2012. (a) *F. avenaceum*; (b) *F. sambucinum*; (c) *F. acuminatum*; (d) *F. graminearum;* (e) *F. crookwellense*; (f) *F. sporotrichioides*; (g) *F. redolens*; (h) *F. oxysporum;* (i) *F. solani*; (j) *F. incarnatum/equiseti*; (k) *F. proliferatum.*

Representative isolates of all species were pathogenic when inoculated onto potato tubers. All potato tubers inoculated with the *Fusarium* isolates developed potato dry rot symptoms similar to those on potato tubers collected and used for the original isolation and identification. These symptoms on the periderm consisted of shallow lesions or depressions at the point of inoculation, with internal necrotic areas in shades of brown with dry rot decay. Furthermore, no disease symptoms were observed on the control potato tubers. Differences in virulence or aggressiveness among species were evident based on visual observations after the tubers were cut open (Fig. 2.3 and 2.4).

In 2011, *F. sambucinum* was the most aggressive species and had a significantly higher percentage of infection ($p < 0.05$) compared to all other species on potato tubers cvs. 'Snowden' and 'MSQ440-2' (Table 2.2). The rest of the species recovered during this year of testing were not significantly different from each other in terms of lesion size, on both cultivars tested at $p < 0.05$.

In 2012, *F. acuminatum*, *F. avenaceum* and *F. sambucinum* had the highest overall percent dry rot lesion size on cv. 'Russet Norkotah' and were not significantly different ($p < 0.05$). *Fusarium avenaceum* was the most aggressive species with significantly higher overall percent dry rot infection on cv. 'Atlantic' ($p < 0.05$). *Fusarium sambucinum* was the second most aggressive species ($p < 0.05$; Table 2.3). The remainder of the *Fusarium* spp. recovered during 2012 ranged from an average 5.0 to 17.7% dry rot symptoms on potato tuber cv. 'Atlantic' and 2.9 to 8.0% on cv. 'Russet Norkotah'.

Cultivars 'Snowden' and 'Atlantic' were significantly more susceptible than 'MSQ440-2' and 'Russet Norkotah' based on percent area of infected tuber tissue for 2011 and 2012,

respectively at $p < 0.05$ (Table 2.4). Isolates of *F. sambucinum*, *F. avenaceum*, and *F. acuminatum* were among the most aggressive species, but differed with cultivar and year. Re-isolation of the pathogen from the infected potato tubers resulted in the same *Fusarium* spp. as the tubers were initially inoculated with, based on morphological similarities of the original cultures used to inoculate.
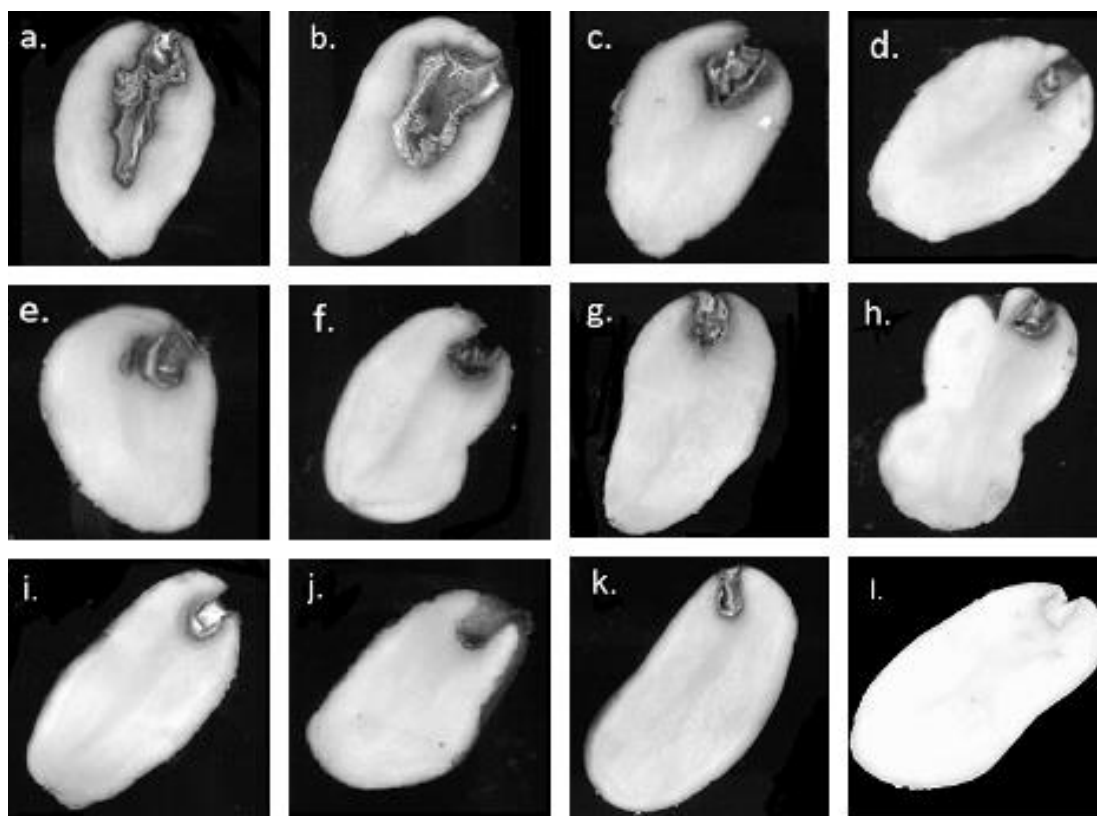
Figure 2.3 Response of potato tubers (cv. 'Russet Norkotah') to different *Fusarium* isolates collected from potato dry rot in MI commercial storage. Potatoes were inoculated with PDA agar plugs containing mycelium of (a) *F. avenaceum*; (b) *F. sambucinum*; (c) *F. acuminatum*; (d) *F. graminearum*; (e) *F. crookwellense*; (f) *F. sporotrichioides*; (g) *F. redolens*; (h) *F. oxysporum*; (i) *F. solani;* (j) *F. incarnatum/ equiseti*; (k) *F. proliferatum*; (l) Control, inoculated with sterile PDA agar plug.
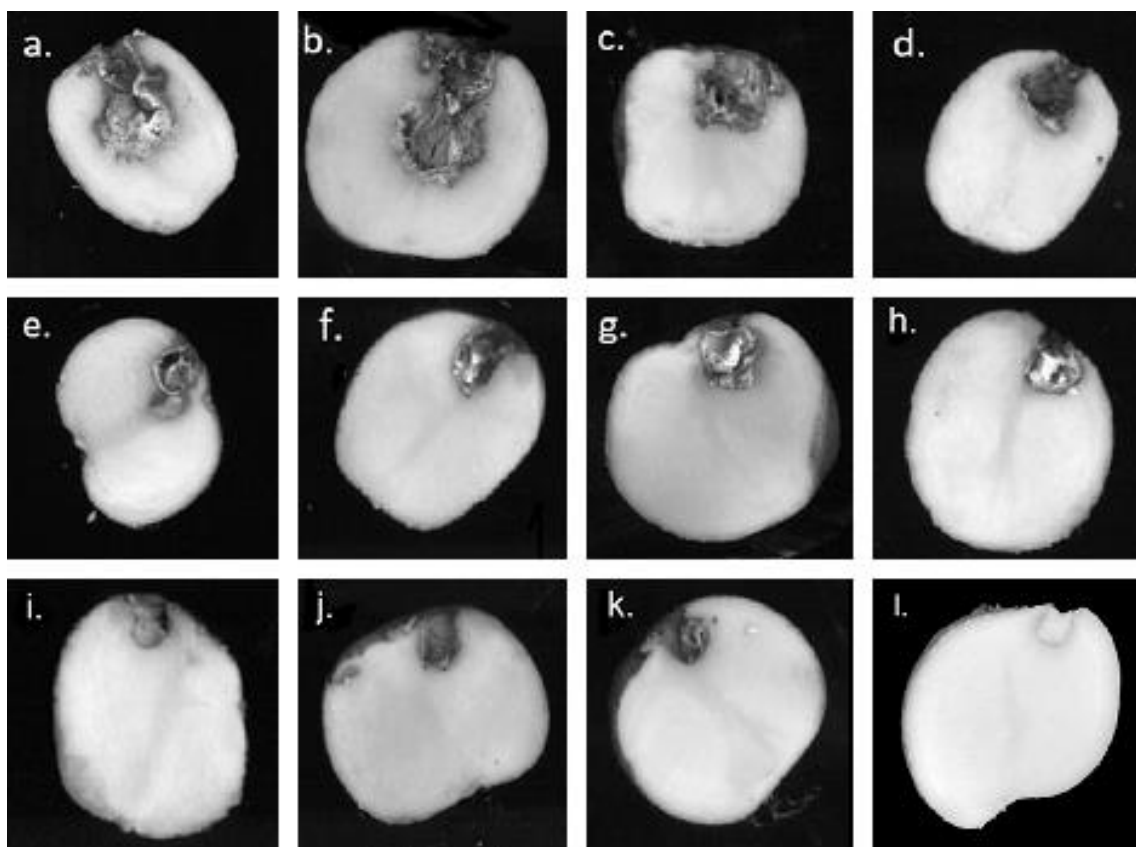
Figure 2.4 Response of potato tubers (cv. 'Atlantic') to different *Fusarium* isolates collected from potato dry rot in MI commercial storage. Potatoes were inoculated with PDA agar plugs containing mycelium (a) *F. avenaceum*; (b) *F. sambucinum*; (c) *F. acuminatum*; (d) *F. graminearum*; (e) *F. crookwellense*; (f) *F. sporotrichioides*; (g) *F. redolens*; (h) *F. oxysporum*; (i) *F. solani;* (j) *F. incarnatum/ equiseti*; (k) *F. proliferatum*; (l) Control, inoculated with sterile PDA agar plug.

Table 2.2 Virulence of *Fusarium* spp. isolates collected in 2011 from Michigan commercial storage on potato tubers (cvs. 'Snowden' and 'MSQ440-2').

Fusarium spp.[a]	Number of isolates[a]	Tuber dry rot (% symptomatic area)[c]			
		'Snowden'		'MSQ440-2'	
		Range	Mean	Range	Mean
F. sambucinum	12	2.8 - 51.1	20.7 a[d]	1.3 - 40.8	8.9 a
F. sporotrichioides	2	1.2 - 5.0	3.1 b	1.4 - 3.4	2.4 b
F. redolens	1	2.8	2.8 b	1.7	1.7 b
F. oxysporum	67	0.5 - 22.5	3.8 b	0.2 - 17.8	3.5 b
F. solani	9	2.4 - 18.1	4.1 b	1.2 - 16.3	3.9 b
F. incarnatum /equiseti	19	0.4 - 27.8	5.8 b	0.9 - `9.1	4.6 b

[a] *Fusarium* spp. isolated and identified from dry rot symptomatic potato tubers from MI commercial production.

[b] Total number of isolates per species tested for pathogenicity and virulence.

[c] Range and mean % area of tuber dry rot symptomatic area for all isolates within a species (mean of three replicates per isolate) and the experiment conducted twice. Data were pooled, because there were no significant differences between the two experiments.

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

Table 2.3 Virulence of *Fusarium* spp. isolates collected in 2012 from Michigan commercial storage on potato tubers (cvs. 'Atlantic' and 'Russet Norkotah').

Fusarium spp.[a]	Number of isolates[a]	'Atlantic' Range			Mean	'Russet Norkotah' Range			Mean
F. avenaceum	3	7.3	-	61.9	30.7 a[d]	3.1	-	47.2	14.7 ab
F. sambucinum	28	2.4	-	67.2	23.2 b	2.6	-	90.8	14.6 a
F. acuminatum	10	2.8	-	79.1	17.7 c	1.4	-	68.3	18.5 a
F. graminearum	4	2.3	-	29.9	12.4 c-e	1.2	-	39.5	7.3 cd
F. crookwellense	3	8.5	-	15.7	11.0 c-f	3.9	-	12.4	8.0 b-d
F. sporotrichioides	3	5.2	-	21.1	9.4 d-f	2.8	-	8.2	5.0 cd
F. redolens	2	6.3	-	11.1	8.7 d-f	2.5	-	6.8	4.7 d
F. oxysporum	148	0.9	-	61.5	7.4 d-f	0.7	-	37.8	5.0 d
F. solani	17	1.9	-	28.9	6.7 ef	0.9	-	9.3	3.8 d
F. incarnatum /equiseti	32	1.1	-	25.6	6.3 ef	0.7	-	20.1	4.3 d
F. proliferatum	5	0.7	-	5.9	5.0 f	0.8	-	3.4	2.9 d

[a] *Fusarium* spp. isolated and identified from dry rot symptomatic potato tubers from MI commercial production.

[b] Total number of isolates per species tested for pathogenicity and virulence.

[c] Range and mean % area of tuber dry rot symptomatic area for all isolates within a species (mean of three replicates per isolate) and the experiment conducted twice. Data were pooled, because there were no significant differences between the two experiments.

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

Table 2.4 Main effect of inoculation of *Fusarium* spp. isolates collected in 2011 and 2012, respectively on susceptibility of tubers of potato cultivars 'Snowden' and 'MSQ440-2' (2011) and 'Atlantic' and 'Russet Norkotah' (2012).

Cultivar	Mean dry rot symptomatic area (%)[a]
2011	
'Snowden'	8.60 a[b]
'MSQ440-2'	5.43 b
2012	
'Atlantic'	12.15 a
'Russet Norkotah'	8.04 b

[a] Mean % area of tuber dry rot symptomatic area for all *Fusarium* isolates on each cultivar (mean of three replicates per isolate) and the experiment conducted twice. Data were pooled, because there were no significant differences between the two experiments.

[b] Numbers followed by the same letter within a column are not significantly different at P = 0.05 by Tukey honestly significantly different (HSD) test.

2.4 DISCUSSION

Throughout the two-year survey, *Fusarium* spp. were the principle fungi isolated from tuber dry rot lesions, and few non-*Fusarium* saprophytic fungi were isolated in both years (although pathogenic *Alternaria* were sometimes isolated). A total of 11 *Fusarium* spp. were recovered throughout the duration of this study. The current findings along with the study conducted on Michigan potato seed stocks (Gachango et al., 2012a), identified a total of 13 *Fusarium* spp. responsible for causing potato dry rot in Michigan commercial potato production areas, comprised of seed, tablestock, and processing potatoes. The current study identified *F. proliferatum* and *F. redolens* on processing tubers while Gachango et al. (2011a; 2012a) identified *F. torulosum* and *F. tricinctum* as pathogens causing dry rot on seed tubers and these species were unique to processing and seed tubers, respectively in these studies. The presence and absence of these species does not exclude them from being present on reciprocal sources. The remaining nine *Fusarium* spp. were recovered in both studies. *Fusarium culmorum* was previously reported in the north-eastern US (Hanson et al., 1996), although this species was not isolated in this study or from the prior seed potato tuber study (Gachango et al., 2012a).

Fusarium oxysporum was recovered most frequently from dry rot symptomatic potato tubers collected in Michigan during 2011 and 2012, which is consistent with previous findings conducted on potato seed stocks from Michigan in 2009 and 2010 (Gachango et al. 2012a). The relative frequency of *F. oxysporum* was much higher in this study of potato tubers produced in Michigan commercial potato fields (73.4 and 61.1%), compared to the Michigan potato seed stock study (28.8 and 25.9%, Gachango et al., 2012a). These findings were similar to the report by Hanson et al. (1996), who found a high proportion of *F. oxysporum* causing potato dry rot in the northern US. Ocamb et al. (2007) also identified *F. oxysporum* as one of the predominant

species isolated from dry rot infected potato tubers from the Columbian Basin of Oregon and Washington.

Fusarium oxysporum is considered one of the most widely dispersed species of the *Fusarium* genus with a broad host range and is able to infect many plant families (Leslie et al., 2006). Pathogenic *F. oxysporum* isolates are not restricted to causing diseases on the potato (Venter et al., 1992; Garcia et al., 2011) and can infect plants through roots and leaves (Manici and Cerato, 1994). Furthermore, *F. oxysporum* is an efficient saprophyte that can persist indefinitely by feeding on dead or decaying organic matter (Leslie et al., 2006), possibly contributing to the high relative frequency found in this study.

The species composition was moderately consistent throughout the 2-year study. *Fusarium oxysporum*, *F. incarnatum/equiseti*, *F. sambucinum*, and *F. solani* were the most frequently isolated species, with an average relative frequency for the two years at 67.3, 13.6, 5.7, and 5.8%, respectively. All four of these species are referred to as *Fusarium* species complexes because each consists of more than one phylogenetically distinct species (Geiser et al., 2004; Leslie et al., 2006). Therefore, it is logical that these species made up of the majority of isolates recovered in this study. These results are similar to the seed tuber study, but Gachango et al. (2012a) recovered a relatively high total frequency of *F. avenaceum* at 13.6%. *Fusarium oxysporum, F. solani, F. avenaceum*, and *F. sambucinum* have been associated with wilting of potato plants in the field, so field infection may contribute to the higher proportion of these *Fusarium* spp. relative to the other species (Mahdavi-Amiri et al., 2009; Secor and Salas, 2001).

The *F. incarnatum/equiseti* species complex was the second most commonly isolated species representing 16.8 and 10.3% for 2011 and 2012, respectively, for an average relative frequency of 13.6 for both years. These findings are similar to those of Gachango et al. (2012a),

who identified 23.0 and 19.0% (21.0% on average) of isolated species recovered in 2010 and 2011, respectively. This species may pose a concern for human safety as this species can produce mycotoxins and can be allergenic or have estrogenic effects (Leslie et al., 2006). The relative frequency of *F. incarnatum/equiseti* was higher in this study compared to the survey conducted by Hanson et al. (1996), who reported this species comprised around 1.0% of the total *Fusarium* isolates collected. This species was originally found on only tablestock and processing samples (Hanson et al., 1996), however it was later identified on seed potato tubers (Gachango et al., 2012a).

Fusarium solani* and *F. sambucinum* were the third and fourth most prevalent species, comprising a total of 5.8 and 5.7% respectively. These findings are similar to the study conducted on potato seed tubers, although Gachango et al., (2012a) recovered a higher frequency of *F. sambucinum* isolates and averaged 13.6% over the two years of the survey, compared to the current study. Furthermore, Hanson et al., (1996) identified *F. sambucinum* as the most prevalent species at 35.5%, throughout north-eastern US. *Fusarium sambucinum* was also reported to be one of the most commonly isolated species in the Columbia basin of Oregon and Washington (Ocamb et al., 2007). This species also poses a concern for human safety as this species can produce mycotoxins and can be allergenic (Leslie et al., 2006). *Fusarium solani* comprised around 15% of the *Fusarium* spp. recovered in the Columbian Basin of Oregon and Washington (Ocamb et al., 2007) and a total 7.5% in the Michigan seed potato study (Gachango et al., 2012a).

Fusarium proliferatum* was only isolated from potato tubers in 2012, representing 6.4% of the species recovered that year, which was a first report of this species causing potato dry rot in Michigan (Merlington et al., 2013). The identification of *F. proliferatum* causing potato dry

rot was previously reported in the Columbia Basin of Oregon and Washington, and was recovered at a low frequency and considered a minor pathogen of the potato (Ocamb et al. 2007), similar to the isolates recovered in Michigan (Merlington et al., 2013). The reason for *F. proliferatum* being recovered only in the second year may be related to the rotational crop and represented a sporadic infection.

Four isolates of *F. graminearum* were recovered during this study. The aggressiveness of *F. graminearum* in terms of dry rot lesion size was variable. Three out of four *F. graminearum* isolates would be considered minor pathogens of potato in Michigan, and was similar to the seed tuber study (Gachango et al., 2012a), although one isolate caused around 30 and 40% dry rot symptoms on cvs. 'Atlantic' and 'Russet Norkotah', respectively. *Fusarium graminearum* was identified as a pathogen of potato in the US in 2005 (Ali et al., 2005) and is now considered the predominant pathogen causing dry rot in the north-central US (Estrada Jr. et al., 2010). Potatoes, wheat, barley, and sugarbeet are frequently used in crop rotations in this region, which may contribute to a higher proportion of *F. graminearum* isolates recovered because this species has been shown to be crops specific (Estrada Jr. et al., 2010). The remainder of the *Fusarium* spp. recovered during this study may be considered as minor pathogens in MI in terms of the total number of isolates recovered, comprising a total of 24 of the 730 isolates. These included *F. avenaceum*, *F. acuminatum*, *F. crookwellense*, *F. redolens*, and *F. sporotrichioides. Fusarium avenaceum* is reported at higher frequencies in Scandinavia and United Kingdom potato stocks (Seppanen, 1981, Cullen et al., 2004, Choiseul et al., 2007, Peters et al., 2008a)

Although, identification of some *Fusarium* spp. can be differentiated by morphological characteristics, additional analysis such as amplification of the translation elongation factor (EF-1α), followed by DNA sequencing, can be conducted to differentiate certain species (Geiser et

al., 2004). Advancements in molecular techniques and data bases such as FUSARIUM-ID and GenBank, might contribute to the identification of more diverse species within the Fusarium dry rot complex. A combination of morphological and molecular methods is most reliable for the identification of some *Fusarium* spp. (Geiser et al., 2004; Leslie et al., 2006).

All *Fusarium* isolates recovered in this study were pathogenic to all potato cultivars tested, similar to the potato seed tuber study (Gachango et al., 2012a). Isolates of *F. sambucinum* were the most aggressive species isolated from potato seed tubers (Gachango et al., 2012a), while in this study*, F. sambucinum*, *F. avenaceum*, and *F. acuminatum* were the most aggressive species isolated from commercial potato storage facilities. These *Fusarium* spp. were not the most frequently isolated species, but caused significantly larger lesions compared to other species. This report agrees with the study conducted by Lacy and Hammerschmidt (1993), which also identified *F. sambucinum* as the species causing the most severe dry rot symptoms in storage and seed decay after planting in MI. Ocamb et al. (2007) found *F. sambucinum* to be the most virulent and resulted in larger dry rot lesions compared to other species. *Fusarium sambucinum* is thought to be the major potato dry rot pathogen in the northern US (Hanson et al., 1996; Secor and Salas, 2001), although several other species including *F. tricinctum*, *F. sporotrichioides*, *F. torulosum*, and *F. crookwellense* (*F. cerealis*) could be termed as major potato dry rot pathogens in Michigan with respect to lesion size (Gachango et al., 2012a).

The total average area of infected tuber dry rot tissue for *F. oxysporum* isolates was relatively low at 7.4 and 5.0 % on cvs. 'Atlantic' and 'Russet Norkotah', respectively. *Fusarium oxysporum* may be considered a weak pathogen in terms of aggressiveness however; some isolates were as aggressive as *F. sambucinum*, *F. avenaceum*, and *F. acuminatum*, causing 51.5

and 37.8% dry rot on cvs. 'Atlantic' and 'Russet Norkotah', respectively. *Fusarium oxysporum* consist of a species complex, so some of these strains could be major pathogens.

Cultural management practices such as crop rotation may impact the proportion and number of *Fusarium* spp. affecting potatoes. The wide host range of some of these species may contribute to greater species diversification. Based on this diversity, controlling Fusarium dry rot in commercial potato production should integrate several control methods. Further investigations are needed to investigate how *Fusarium* spp. composition relates to certain integrated pest management schemes in commercial potato production. Identification of management strategies to reduce the proportion of *Fusarium* spp. that are considered major dry rot pathogens are needed. One of the major control options for potato dry rot includes the application of fungicides as pre-planting seed treatments or as pre-storage treatments. There are limited products available for either control option and efficacy varies greatly (Gachango et al., 2012b; Kirk et al., 2013; Wharton and Kirk, 2014). In addition, resistance in the Fusarium dry rot populations to some fungicides has been reported (Hanson et al., 1996; Ocamb et al., 2007; Peters et al., 2008b; Gachango et al., 2012a). The following chapters investigate the risk of the development of resistance and fungicide efficacy.

CHAPTER 3: BASELINE SENSITIVITY OF *FUSARIUM* SPP. ASSOCIATED WITH POTATO DRY ROT IN MICHIGAN TO FUNGICIDES

ABSTRACT

At least 13 *Fusarium* spp. have been identified as causal agents responsible for potato dry rot in Michigan. Due to the development of thiabendazole (TBZ) and fludioxonil-resistant *Fusarium* isolates, it is essential to identify effective fungicides for potato dry rot control in commercial potato fields and storages. The 11 *Fusarium* spp. recovered in this study (*F. oxysporum*, *F. equiseti*, *F. solani*, *F. sambucinum*, *F. proliferatum*, *F. acuminatum*, *F. sporotrichioides*, *F. avenaceum*, *F. redolens*, *F. graminearum*, and *F. crookwellense*) were screened for sensitivity to azoxystrobin, fludioxonil, difenoconazole, and TBZ (active ingredients of fungicides used for potato dry rot management). The effective fungicide concentration that caused 50% inhibition of mycelial growth (EC_{50}) compared to the control was determined using the spiral gradient dilution (SGD) method. The serial dilution plate (SDP) method was used to verify *Fusarium* isolates resistant to difenoconazole. In 2011, all isolates of *Fusarium* spp. were sensitive to thiabendazole ($EC_{50}<5$ mg/L), except isolates of *F. sambucinum* and *F. solani* ($EC_{50}> 5$ mg/L), most isolates were sensitive to fludioxonil ($EC_{50}< 100$ mg/L) and difenoconazole ($EC_{50}< 5$ mg/L), and few were sensitive to azoxystrobin ($EC_{50}< 10$ mg/L). In 2012, all *Fusarium* spp. were sensitive to thiabendazole, except isolates of *F. sambucinum, F. solani*, and *F. oxysporum*. Most isolates of *Fusarium* spp. were sensitive to fludioxonil, some were sensitive to azoxystrobin, and the majority were sensitive to difenoconazole, except for 8.3, 3.6, and 15.4% of the isolates of *F. incarnatum/equiseti*, *F. oxysporum*, and *F. solani*, respectively. Furthermore, four isolates grew on PDA containing 20 mg/L difenoconazole. Mixed resistance to the fungicides tested was also observed for isolates of *F. sambucinum, F.*

solani, F. oxysporum, and *F. incarnatum/ equiseti*, although no isolates were resistant to any 3 or 4-way fungicide combinations.

3.1 INTRODUCTION

Fusarium dry rot is one of the most important diseases of potato and is of worldwide importance (Secor and Salas, 2001; Wharton et al., 2007). Currently there are at least 13 known *Fusarium* spp. responsible for causing potato dry rot worldwide (Cullen et al., 2005; Hide et al., 1992: Secor and Salas, 2001). *Fusarium sambucinum* remains one of the most important and aggressive species causing dry rot in the US (Hanson et al., 1996; Ocamb et al., 2007) and Michigan (Gachango et al., 2012a). In Michigan, 13 known *Fusarium* spp. have been isolated from seed and commercially grown potato tubers, with *F. oxysporum* the predominant species in both potato tuber stocks (Chapter 1). Gachango et al., (2012a) identified 11 *Fusarium* spp. responsible for dry rot of seed potato tubers in Michigan, while the survey of species responsible for dry rot from commercial production areas identified an additional two species (Chapter 1). These recent findings with the addition of those of Hanson et al., (1996), suggests there are up to 14 *Fusarium* spp. responsible for causing potato dry rot in the northeast US (Gachango et al., 2011a; 2012a).

Fusarium spp. can be devastating pathogens affecting both tubers in storage and seed tubers in the field (Choiseul et al., 2006; Wharton et al., 2007b). Management strategies for controlling potato dry rot in storage facilities and the field are limited. No commercially grown cultivars are resistant to dry rot in North America, although the level of tolerance varies between cultivars (Secor and Salas, 2001; Kirk et al., 2013). Progeny tubers can become contaminated with *Fusarium* spores as they develop throughout the growing season. Infection of potato tubers by *Fusarium* generally doesn't occur until the periderm is wounded during harvesting, grading,

loading, cutting, and handling (Secor and Salas, 2001; Powelson and Rowe, 2007). Dry rot can be controlled in two phases during the potato growth cycle. These phases include control of seedpiece decay prior to or at planting and postharvest control in storage facilities (Secor and Salas, 2001; Nolte et al., 2003; Wharton et al., 2007b). Since many *Fusarium* spp. can cause infection when the potato skin is ruptured (Boyd, 1972), postharvest management of potato dry rot is primarily achieved by implementing practices that avoid tuber injury, reduce tuber bruising, and provide conditions for rapid wound healing (Secor and Salas, 2001; Secor and Johnson, 2008).

Fusarium dry rot on seed potato pieces can be controlled by applying fungicide seed treatments prior to planting or at planting (Nolte et al., 2003; Wharton et al., 2007b). Control of Fusarium dry rot in storage has been primarily controlled by postharvest applications of thiabendazole (TBZ, Mertec 340F™; Syngenta Crop Protection, Greensboro, NC) as the tubers enter into storage (Secor and Salas, 2001), although TBZ-resistant strains compromise the efficacy of dry rot control (Hanson et al., 1996; Ocamb et al., 2007; Gachango et al., 2012a). TBZ-resistant *F. sambucinum* isolates were first identified in Europe in 1973 (Hide et al., 1992) and later identified in the US in 1992 (Desjardins et al., 1993). Many strains of *F. sambucinum* are known to be resistant to TBZ and other benzamidazoles (Hide et al., 1992; Ocamb et al., 2007), and all *F. sambucinum* samples were resistant to TBZ in a survey conducted on commercial potato seed tubers in Michigan (Gachango et. al., 2012a). Resistance to TBZ has also been reported for isolates of most of the *Fusarium* spp. implicated in causing potato dry rot. These species include *F. sambucinum, F. oxysporum, F. solani, F. acuminatum, F. culmorum, F. avenaceum, F. equiseti, F. sporotrichioides,* and *F. culmorum* (Hanson et al., 1996; Ocamb et al., 2007). Resistance to TBZ was defined as the ability of *Fusarium* to grow on artificial media

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containing a concentration of 5 mg/L of TBZ (Hide et al., 1992; Hanson et al., 1996; Ocamb et al., 2007).

Fludioxonil (Maxim™; Syngenta Crop Protection) is a fungicide registered for potato seed treatment against Fusarium seed-piece decay in the US (Wharton et al., 2007b; Zitter, 2010). Fludioxonil can be used alone or in combination with other active ingredients, such as mancozeb (Maxim MZ™; Syngenta Crop Protection) to control Fusarium dry rot. Fludioxonil can reduce seed piece decay as well as the incidence of diseased sprouts that develop into unhealthy plants (Wharton et al., 2007b). However, fludioxonil-resistant strains of *F. sambucinum*, *F. oxysporum*, and *F. coeruleum* were reported in Michigan (Gachango et al., 2011b) and Canada (Peters et al., 2008a; Peters et al., 2008b), resulting in fewer strategies for controlling potato seed piece decay and sprout rot caused by *Fusarium* spp. (Gachango et al., 2012a).

Azoxystrobin (Quadris™; Syngenta Crop Protection) has been used to control soil-borne diseases, including Fusarium seed piece decay, when applied on freshly cut seed tubers (Powelson and Rowe, 2007), although no assessment has been made on the efficacy of azoxystrobin for Fusarium dry rot control in Michigan. To counteract the reduced effectiveness of TBZ and possibly fludioxonil, difenoconazole (Inspire™; Syngenta Crop Protection) was introduced into North America for control of Fusarium dry rot and other potato diseases (Adaskaveg and Förster, 2010). Difenoconazole is a broad spectrum fungicide that has been developed as a postharvest fungicide for stored potatoes to control dry rot (Olaya et al., 2010). Furthermore, a 3-way mixture of difenoconazole, azoxystrobin, and fludioxonil was recently registered for managing decay caused by *Fusarium* species on potato and other tuber crops (Adaskaveg and Förster, 2010; Kirk et al., 2013). This 3-way mixture (Stadium™; Syngenta

Crop Protection) has been registered for potato dry rot management in storage (Kirk et al., 2013). This mixture of azoxystrobin, fludioxonil, and difenoconazole has been shown to be effective in controlling dry rot (Kirk et al., 2013).

In vitro testing to determine the effective concentration of a fungicide to inhibit mycelial growth or spore germination by 50% (EC_{50}), is a rapid technique used to monitor shifts in sensitivity and to determine resistant isolates (Russell, 2003; Förster et al., 2004). The EC_{50} values can be determined by the spiral gradient dilution method (SGD) or the serial dilution plate method (SDP) (Förster et al., 2004; Gachango et al., 2012a). Studies have shown the two methods to determine the EC_{50} values are not significantly different and should be comparable (Förster et al., 2004, Gachango et al., 2012a).

The objectives of this study were to determine the baseline sensitivity of the *Fusarium* isolates collected from Michigan commercial potato production to TBZ, fludioxonil, difenoconazole, and azoxystrobin. It is important to know which species are causing dry rot and their sensitivity to commercially available fungicides. Understanding and monitoring the sensitivity of *Fusarium* spp. to commonly used fungicides in commercial potato production is critical for effective Fusarium dry rot management.

3.2 MATERIALS AND METHODS

3.2.1 *FUSARIUM ISOLATES*

Isolates identified as *Fusarium* species (Chapter 1) were maintained on potato dextrose agar (PDA; Difco Laboratories, Detroit, MI) and carnation leaf agar (CLA) throughout the study to test their sensitivity to fungicides. Pure cultures of each *Fusarium* isolate were sub-cultured on three petri-dishes following the determination of *Fusarium* spp. *Fusarium* isolates were

incubated in the dark at 23°C and sub-cultured on PDA in a Petri-dish and agar slants every 2-3 months as needed. Furthermore, a mycelial plug (4-mm diameter x 4-mm depth) was transferred from a 7-d old *Fusarium* culture grown on PDA and transferred into 50% glycerol and stored at 20°C for long-term storage and preservation, although these cultures prepared by this technique were not used. Additional long-term storage of *Fusarium* isolates was prepared by harvesting approximately 0.2 g of mycelium and carnation leaves from a 7-14 d-old *Fusarium* culture grown on CLA. The mycelium was transferred into a sterile 2-mL screw-cap vial, lyophilized, and incubated at -20°C in a 1-L Drierite (Hammond Drierite Co., Xenia, OH) glass jar containing 0.25 kg of the chemical desiccant Drierite (anhydrous calcium sulfate).

3.2.2 *FUNGICIDES EVALUATED AND THRESHOLDS FOR INSENSITIVITY*

The EC_{50} value, which is the concentration of fungicide that inhibits colony diameter of the fungus on PDA by 50%, was determined for all the identified *Fusarium* isolates. In 2011, all fungicides were obtained from Syngenta (Syngenta Crop Protection Inc., Greensboro, NC) and stock fungicide concentrations of azoxystrobin (22.9% a.i., Quadris™), thiabendazole (TBZ) (42.3% a.i., Mertec™), fludioxonil (0.5% a.i., Maxim™), and difenoconazole (23.2% a.i., Inspire™) were diluted to 10,000 mg/L with sterile distilled water. In 2012, analytical standard grades of azoxystrobin, TBZ, fludioxonil, and difenoconazole were purchased from Sigma-Aldridge (Sigma-Aldrich, St Louis, MO, USA) and a 10,000 mg/L stock solution was prepared in acetone (10 mg a.i./1.0 mL acetone). The stock concentration of 10,000 mg/L was chosen based on prior evaluations of several concentrations. Salicylhydroxamic acid (SHAM, Sigma-Aldrich) was dissolved in acetone at the concentration of 100 mg/L and plated with azoxystrobin to block the alternate oxidation pathway (Olaya et al., 1998).

Azoxystrobin insensitivity was defined as the effective concentration of the fungicide that inhibited 50% of the fungal growth $EC_{50} > 10$ mg/L (Olaya and Holm, 2001; Stevenson et al., 2004). Fludioxonil insensitivity was defined as $EC_{50} > 100$ mg/L (Peters et al., 2008b; Gachango et al., 2011b). TBZ and difenoconazole insensitivity were defined as $EC_{50} > 5$ mg/L (Hanson et al., 1996; Olaya et al., 2010; Gachango et al., 2012a; Müllenborn et al., 2008). Each isolate was classified as sensitive or resistant based on these criteria for each fungicide.

3.2.3 FUNGICIDE SENSITIVITY IN VITRO ASSAY USING THE SPIRAL GRADIENT DILUTION (SGD) METHOD

The EC_{50} value was determined for all the identified *Fusarium* isolates. The spiral fungicide gradient dilution (SGD) method was used to estimate the EC_{50} value, as described by Förster et al. (2004). PDA (50-mL) was poured into each 15 cm diameter Petri dish, to a depth of 4 mm, 24-h before applying the fungicide solution. A spiral plater (SGE[TM]; Spiral Biotech, Inc. Norwood, MA) applied 54.3-µL of each fungicide solution spirally using the exponential deposition mode. The plates were incubated for 12 h at 20°C to allow the fungicides to diffuse into the agar medium and form a concentration gradient from a high concentration at the center of the spiral to a lower concentration around the perimeter.

Fusarium cultures were grown on PDA in 60-mm Petri dishes for 7 d and used to make conidial suspensions (10^6 conidia/mL water) for each isolate. The mycelia and conidia were scraped from the surface of aseptic pure cultures and the suspension filtered through a double layer of cheesecloth and adjusted to a concentration of 10^6 conidia/mL water, determined with a hemocytometer. Each Petri dish was placed on the template provided by SGE, as described by Gachango et al., (2012a). Droplets (totalling 10-µl) of the appropriate conidial suspension were spread across the radial lines in predetermined plate positions using a sterile plastic pestle. Three replicates per isolate were used for each fungicide and the assays were repeated. Controls

treatments consisted of spreading the conidial suspension on PDA plates amended with water in 2011 and acetone in 2012. Following incubation at 25°C for 3-d, radial growth of the fungus was measured and the three replicates were averaged. In the SGE software, the 1-d incubation option was used for calculation of the local concentrations of fungicides where 50% growth inhibition was observed compared to the control plate. The EC_{50} values were determined from the SGE software for each isolate by entering the ER (end radius) and TER (tail end radius) values, respectively. The local fungicide concentration is then automatically displayed as the EC (ending concentration) and the TEC (tail ending concentration).

Plates of PDA were prepared and amended with difenoconazole concentrations of 0.0, 10.0, 20.0 and 100.0 mg/L as described by Gachango et al. (2012a) to confirm the sensitivity of the 10 *Fusarium* isolates to difenoconazole. Conidial suspensions (10^6 conidia/mL) were prepared as described above. The conidial suspension (10-µl) was placed in the center of the amended agar medium and incubated in the dark at 25°C for 7-d. Three plates for each fungicide concentration were used and the experiment was repeated.

3.2.4 DATA COLLECTION AND ANALYSIS

Data were tested for assumptions of normality and analyzed using the analysis of variance platform using JMP Version 10.0 (SAS Inc., Cary, NC). Significant mean separations were conducted using Tukey's honestly significant difference (HSD) test.

3.3 RESULTS

3.3.1 FUNGICIDE EFFICACY

In 2011, the mean EC_{50} for the *Fusarium* spp. ranged from 0.9-50.8, 1.9-32.2, 0.4-64.3, and 0.3-11.0 mg/L for thiabendazole, fludioxonil, azoxystrobin, and difenoconazole, respectively

(Table 3.1). Fungicide EC_{50} ranges were reported for each *Fusarium* spp. (Table 3.2). All isolates of *Fusarium* spp. were sensitive to thiabendazole (EC_{50} <5 mg/L), except 75% of the *F. sambucinum* isolates and 16.6% of the *F. solani* isolates (EC_{50} > 5 mg/L). 20.0, 17.2, and 33.3% of isolates of *Fusarium incarnatum/equiseti*, *F. oxysporum*, and *F. solani* were resistant to fludioxonil (EC_{50} < 100 mg/L), respectively. Few *Fusarium* isolates were sensitive to azoxystrobin (EC_{50} < 10 mg/L), but most were sensitive to difenoconazole, except three *F. oxysporum* isolates and one isolate each of *F. solani* and *F. sambucinum* (EC_{50} < 5 mg/L); (Table 3.1, 3.2).

Mixed resistance to the fungicides tested was also observed in 2011 (Table 3.3). Insensitivity to more than one fungicide was observed for isolates of *F. sambucinum, F. solani, F. oxysporum,* and *F. incarnatum/ equiseti,* except for the fungicide combination of fludioxonil and thiabendazole (Table 3.3). Furthermore, no isolates were resistant to any 3 or 4-way fungicide combinations, including difenoconazole, azoxystrobin, and fludioxonil combinations.

In 2012, the mean EC_{50} for the *Fusarium* spp. ranged from 0.5-30.7, 0.6-32.6, 0.3-23.9, and 0.4-10.6 mg/L for thiabendazole, fludioxonil, azoxystrobin, and difenoconazole, respectively (Table 3.1). In 2012, the majority of isolates were sensitive to thiabendazole, except for 80.0% of the *F. sambucinum* isolates, 23.1% of the *F. solani* isolates, and 1.8% of the *F. oxysporum* isolates. Fungicide EC_{50} ranges were reported for each *Fusarium* spp. (Tables 3.4, 3.5). Most *Fusarium* isolates were sensitive to fludioxonil, except 16.6, 6.4, 8.0 and 46.2% of the isolates of *F. incarnatum/equiseti*, *F. oxysporum*, *F. sambucinum*, and *F. solani*, respectively. Some *Fusarium* isolates were sensitive to azoxystrobin, and the majority of the isolates were sensitive to difenoconazole, except for 8.3, 3.6, and 15.4% of the isolates of *F. incarnatum/equiseti*, *F. oxysporum*, and *F. solani*, respectively. Ten *Fusarium* isolates showed reduced sensitivity to

difenoconazole using the SDP method, which constituted a first report of reduced sensitivity of *Fusarium* spp. to difenoconazole in the USA. Additional testing on PDA-amended media showed four isolates were able to grow at 20 mg/L of difenoconazole, although no isolates were able to grow at 100mg/L.

Mixed resistance to the fungicides tested was also observed in 2012 (Table, 3.6). Insensitivity to more than one fungicide was observed for isolates of *F. sambucinum, F. solani, F. oxysporum,* and *F. incarnatum/ equiseti,* except for the fungicide combination of difenoconazole and thiabendazole (Table 3.6). Furthermore, no isolates were resistant to any 3 or 4-way combinations, including difenoconazole, azoxystrobin, and fludioxonil combinations.

Table 3.1 Characterization of the effective fungicide concentration that caused 50% inhibition of mycelial growth (EC_{50}) for multiple isolates of five species of *Fusarium* to four fungicides (azoxystrobin, difenoconazole, fludioxonil, and thiabendazole) determined by the spiral gradient dilution (SGD) method in 2011.

Fusarium spp.[a]	Number of isolates	Mean EC_{50} for inhibition of mycelial growth (mg/L)[b]			
		Azoxystrobin	Difenoconazole	Fludioxonil	TBZ[c]
F. acuminatum	2	0.4	0.3	1.9	0.9
F. incar/equi[d]	5	17.6	0.6	32.2	1.0
F. oxysporum	29	47.4	7.8	20.9	1.2
F. sambucinum	4	64.3	2.1	10.7	50.8
F. solani	6	50.7	11.0	42.1	4.4

[a] *Fusarium* spp. isolated and identified from dry rot symptomatic potato tubers from MI commercial production.

[b] EC_{50} is the effective concentration of the fungicides that inhibit mycelial growth by 50% compared to the control plate with no fungicides. Values represent means of two experiments, with three replicates per experiment.

[c] TBZ = Thiabendazole.

[d] *F. incar/equi* = *F. incarnatum/equiseti* complex.

Table 3.2 Range of the effective fungicide concentration that caused 50% inhibition of mycelial growth (EC$_{50}$) of multiple isolates of five species of *Fusarium* to four fungicides (azoxystrobin, difenoconazole, fludioxonil, and thiabendazole) determined by the spiral gradient dilution (SDG) method in 2011. N = total number of isolates resistant to the fungicide for each *Fusarium* spp.

Fusarium spp.[a]	Azn[d]: N[c]				Dfz[d]: N				Fld[d]: N				TBZ[d]: N			
		EC$_{50}$ range for inhibition of mycelial growth (mg/L)[b]: N[c] = Total number of resistant isolates														
F. acuminatum	0.3	-	0.5	0	0.3	-	0.3	0	0.5	-	3.2	0	0.6	-	1.2	0
F. incar/equi[e]	14.9	-	19.0	5	0.3	-	1.4	0	14.1	-	108.7	1	1.0	-	1.1	0
F. oxysporum	0.3	-	64.3	11	0.3	-	63.7	3	0.5	-	108.7	5	0.5	-	4.6	0
F. sambucinum	64.3	-	64.3	4	0.4	-	5.6	1	0.4	-	22.5	0	7.0	-	65.4	3
F. solani	0.3	-	64.2	5	0.3	-	63.7	1	3.2	-	108.7	2	0.5	-	20.2	1

[a] *Fusarium* spp. isolated and identified from dry rot symptomatic potato tubers from MI commercial production.

[b] EC$_{50}$ range is the minimum and maximum effective concentration of the fungicides that inhibit mycelial growth by 50% compared to the control plate with no fungicides. Values represent means of two experiments, with three replicates per experiment.

[c] N = total number of isolates resistant to the fungicide for each *Fusarium* spp.

[d] Azn = Azoxystrobin; Dfz = Difenoconazole; Fld = Fludioxonil; TBZ = Thiabendazole.

[e] *F. incar/equi* = *F. incarnatum/equiseti* complex.

Table 3.3 Percent sensitivity of multiple isolates of five species of *Fusarium* to four fungicides (azoxystrobin, difenoconazole, fludioxonil, and thiabendazole), determined by calculating the effective fungicide concentration that caused 50% inhibition of mycelial growth (EC_{50}) determined by the spiral gradient dilution method (SGD) in 2011. No *Fusarium* spp. were resistant to any three or four-way fungicide combination.

Fusarium spp.[a]	Relative frequency of resistant *Fusarium*[b] isolates to multiple fungicides					
	Fld[c] + Azn	Fld + TBZ[c]	Fld + Dfz	Dfz[c] + Azn	Dfz + TBZ	Azn[c] + TBZ
F. acuminatum	0.0	0.0	0.0	0.0	0.0	0.0
F. incar/equi[d]	0.0	0.0	0.0	0.0	0.0	0.0
F. oxysporum	13.8	0.0	3.4	10.3	0.0	0.0
F. sambucinum	0.0	0.0	0.0	25.0	25.0	75.0
F. solani	33.3	0.0	0.0	16.7	0.0	0.0

[a] *Fusarium* spp. isolated and identified from dry rot symptomatic potato tubers from MI commercial production.

[b] Relative frequency = percentage of isolates of a given species resistant to multiple fungicides relative to the total number of isolates recovered.

[c] Azn = Azoxystrobin; Dfz = Difenoconazole; Fld = Fludioxonil; TBZ = Thiabendazole.

[d] *F. incar/equi* = *F. incarnatum/equiseti* complex.

Table 3.4 Characterization of the effective fungicide concentration that caused 50% inhibition of mycelial growth (EC$_{50}$) for multiple isolates of 11 species of *Fusarium* to four fungicides (azoxystrobin, difenoconazole, fludioxonil, and thiabendazole) determined by the spiral gradient dilution (SDG) method in 2012.

Fusarium spp.[a]	Number of isolates	Mean EC$_{50}$ for inhibition of mycelial growth (mg/L)[b]			
		Azoxystrobin	Difenoconazole	Fludioxonil	TBZ[c]
F. acuminatum	4	0.5	0.4	1.8	1.1
F. avenaceum	2	2.1	0.8	1.5	2.1
F. crookwellense	1	0.3	4.8	1.7	1.1
F. graminearum	1	0.3	0.8	1.7	1.3
F. incar/equi[d]	24	15.9	2.6	7.2	0.7
F. oxysporum	110	7.2	2.8	32.6	1.4
F. proliferatum	3	0.4	0.3	6.2	1.1
F. redolens	2	0.7	0.8	0.6	0.7
F. sambucinum	25	13.2	0.4	8.4	30.7
F. solani	13	23.9	10.6	30.4	2.7
F. sporotrichioides	1	0.3	0.8	0.4	0.5

[a] *Fusarium* spp. isolated and identified from dry rot symptomatic potato tubers from MI commercial production.

[b] EC$_{50}$ is the effective concentration of the fungicides that inhibit mycelial growth by 50% compared to the control plate with no fungicides. Values represent means of two experiments, with three replicates per experiment.

[c] TBZ = Thiabendazole.

[d] *F. incar/equi = F. incarnatum/equiseti* complex.

Table 3.5 Range of the effective fungicide concentration that caused 50% inhibition of mycelial growth (EC$_{50}$) of multiple isolates of 11 species of *Fusarium* to four fungicides (azoxystrobin, difenoconazole, fludioxonil, and thiabendazole) determined by the spiral gradient dilution (SDG) method in 2012. N = total number of isolates resistant to the fungicide for each *Fusarium* spp.

Fusarium spp.[a]	Azn[d]: N				Dfz[d]: N				Fld[d]: N				TBZ[d]: N			
	EC50 range for inhibition of mycelial growth (mg/L)[b]: N[c] = Total number of resistant isolates															
F. acuminatum	0.3	-	1.5	0	0.3	-	0.7	0	0.5	-	5.9	0	0.5	-	3.4	0
F. avenaceum	0.3	-	3.3	0	0.3	-	0.9	0	0.4	-	2.9	0	1.5	-	2.4	0
F. crookwellense	0.3	-	0.3	0	4.4	-	4.9	0	1.5	-	1.9	0	1.1	-	1.1	0
F. graminearum	0.3	-	0.3	0	0.8	-	0.8	0	1.7	-	1.7	0	1.3	-	1.3	0
F. incar/equi[e]	0.3	-	64.3	8	0.3	-	63.7	2	0.4	-	108.7	4	0.5	-	1.7	0
F. oxysporum	0.4	-	64.3	19	0.3	-	63.7	4	1.2	-	108.7	7	0.5	-	64.4	2
F. proliferatum	0.3	-	0.7	0	0.3	-	0.4	0	5.9	-	6.5	0	0.7	-	1.7	0
F. redolens	0.5	-	1.3	0	0.7	-	0.9	0	0.4	-	0.7	0	0.5	-	0.7	0
F. sambucinum	0.3	-	64.3	14	0.4	-	0.4	0	0.4	-	108.7	2	0.5	-	65.4	20
F. solani	0.3	-	64.2	9	0.3	-	63.7	2	0.4	-	108.7	6	0.5	-	27.1	3
F. sporotrichioides	0.3	-	0.3	0	0.5	-	1.3	0	0.4	-	0.4	0	0.5	-	0.5	0

[a] *Fusarium* spp. isolated and identified from dry rot symptomatic potato tubers from MI commercial production.

[b] EC$_{50}$ range is the minimum and maximum effective concentration of the fungicides that inhibit mycelial growth by 50% compared to the control plate with no fungicides. Values represent means of two experiments, with three replicates per experiment.

[c] N = total number of isolates resistant to the fungicide for each *Fusarium* spp.

[d] Azn = Azoxystrobin; Dfz = Difenoconazole; Fld = Fludioxonil; TBZ = Thiabendazole.

[e] *F. incar/equi = F. incarnatum/equiseti* complex.

Table 3.6 Percent of multiple isolates of 11 species of *Fusarium* to four fungicides (azoxystrobin, difenoconazole, fludioxonil, and thiabendazole), determined by calculating the effective fungicide concentration that caused 50% inhibition of mycelial growth (EC_{50}) determined by the spiral gradient dilution method (SGD) in 2012. No *Fusarium* spp. were resistant to any three or four-way fungicide combination.

Fusarium spp.[a]	Relative frequency of resistant *Fusarium*[b] isolates to multiple fungicides					
	Fld[c] + Azn	Fld + TBZ[c]	Fld + Dfz	Dfz[c] + Azn	Dfz + TBZ	Azn[c] + TBZ
F. acuminatum	0.0	0.0	0.0	0.0	0.0	0.0
F. avenaceum	0.0	0.0	0.0	0.0	0.0	0.0
F. crookwellense	0.0	0.0	0.0	0.0	0.0	0.0
F. graminearum	0.0	0.0	0.0	0.0	0.0	0.0
F. incar/equi[d]	0.0	0.0	0.0	4.2	0.0	0.0
F. oxysporum	5.5	0.9	4.5	0.9	0.0	0.9
F. proliferatum	0.0	0.0	0.0	0.0	0.0	0.0
F. redolens	0.0	0.0	0.0	0.0	0.0	0.0
F. sambucinum	0.0	8.0	0.0	0.0	0.0	36.0
F. solani	7.7	0.0	0.0	7.7	0.0	15.4
F. sporotrichioides	0.0	0.0	0.0	0.0	0.0	0.0

[a] *Fusarium* spp. isolated and identified from dry rot symptomatic potato tubers from MI commercial production.

[b] Relative frequency = percentage of isolates of a given species resistant to multiple fungicides relative to the total number of isolates recovered.

[c] Azn = Azoxystrobin; Dfz = Difenoconazole; Fld = Fludioxonil; TBZ = Thiabendazole.

[d] *F. incar/equi = F. incarnatum/equiseti* complex.

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3.4 DISCUSSION

Baseline sensitivity of targeted pathogens to particular fungicides is a tool used to monitor fungicide resistance management strategies (Russell, 2003). Sampling the population of *Fusarium* spp. causing potato dry rot (Chapter 2) and determining baseline sensitivity levels for standard fungicides registered for disease management can help detect changes in sensitivity of pathogens to different fungicides used for potato dry rot control in the field or storage. *In vitro* testing to determine the effective concentration of the fungicide to inhibit fungal mycelial growth or spore germination by 50% (EC_{50}) is a rapid technique used to monitor shifts in sensitivity and to determine resistance in pathogen isolates (Russell, 2003; Förster et al., 1997). EC_{50} values can be determined by the spiral gradient dilution method (SGD) or the serial dilution plate method (SDP, Förster et al., 1997). Historically, the SDP method has been widely used in determining fungicide sensitivity however the SGD method, that was introduced more recently, is becoming used more frequently (Kanetis et al., 2008; Wharton et al., 2012; Fairchild et al., 2013). Studies have shown that the two methods to are not significantly different for determining EC_{50} values (Förster et al., 2004; Kanetis et al., 2008; Gachango et al., 2012a). Furthermore, Gachango et al. (2012a) determined the SDP method resulted in slightly higher EC_{50} values in comparison to the SGD method which may be considered marginally more conservative. Therefore, the SGD method was used for calculating EC_{50} values in this study because it is more rapid and cost effective (Förster et al., 2004).

Azoxystrobin, a member of the strobilurin class of fungicides, has a single site mode of action, leading to a high probability for a pathogen to develop resistance (Brent and Hollomon, 2007). Strobilurins are often an essential part of many crop protection programs due to their broad protection against a wide range of fungal and oomycete diseases (Barlett et al., 2002;

Rosenzweig et al., 2008). Field resistance to strobilurins have been well documented in many pathosystems, including early blight of potato, caused by *Alternaria solani* (Barlett et al., 2002; Pasche et al., 2004; Pasche and Gudmestad, 2008; Rosenzweig et al., 2008; Belcher et al., 2010; Fairchild et al., 2013). Azoxystrobin has been used to control soil-borne diseases, including Fusarium seed piece decay, when applied to freshly cut seed tubers (Powelson and Rowe, 2008). No assessment has been made on the efficacy of azoxystrobin for Fusarium dry rot control in Michigan until this study. Azoxystrobin insensitivity was defined as $EC_{50} > 10$ mg/L in this study and has been used in other studies (Olaya and Holm, 2001; Stevenson et al., 2004). In this study, resistance to azoxystrobin was identified in isolates of *F. incarnatum/equiseti*, *F. oxysporum*, *F. sambucinum*, and *F. solani*. Since these *Fusarium* species constitute the majority of isolates collected during this study, it would not be recommended to use azoxystrobin to control potato dry rot in Michigan. However, assessments of the efficacy of azoxystrobin agaisnt other soil-borne diseases of potato are well documented and it's continued use is justified.

Fludioxonil is a protectant fungicide registered for potato seed treatment against Fusarium dry rot in the USA (Wharton et al., 2007b; Zitter, 2010). Fludioxonil can reduce seed piece decay as well as the incidence of diseased sprouts that develop into infected plants (Wharton et al., 2008). However, insensitivity to fludioxonil has been reported for isolates of *F. sambucinum*, *F. oxysporum*, and *F. coeruleum* in Michigan and Canada (Peters et al., 2008a; Peters et al., 2008b; Gachango et al., 2011b). Fludioxonil also has a single site mode of action, thus leading to a high probability of development of resistant strains (Brent and Hollomon, 2007).

In this study 17.2, 8.6, 42.1, and 6.9 % of *F. incarnatum/equiseti*, *F. oxysporum*, *F. solani*, and *F. sambucinum* were resistant to fludioxonil, respectively. Fludioxonil insensitivity

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was defined as $EC_{50} > 100$ mg/L (Peters et al., 2008b; Gachango et al., 2011b). Fludioxonil was effective at controlling all other *Fusarium* spp. These results are somewhat similar to that of Gachango et al., (2012a), who identified approximately 20% of *F. oxysporum* and 9% of *F. sambucinum* isolates as resistant to fludioxonil. The differences in the relative frequency of *F. oxysporum* resistant isolates in these two studies may be attributed to the larger number of isolates collected in this study. The isolates collected in this study may have been previously exposed to fludioxonil. Overall, the results from this study do not suggest that fludioxonil has failed to adequately control potato dry rot. The majority of the isolates collected during this study were sensitive to fludioxonil, however the continued use of this chemical could select for isolates within the population resistant to fludioxonil. The resistant strains found in this study and by Gachango et al., (2012a) demonstrate the need for the development and registration of additional fungicides with alternative modes of action or use of other methods for resistance management.

Thiabendazole-resistant strains continue to compromise the efficacy of this fungicide for dry rot control (Hanson et al., 1996; Ocamb et al., 2007; Gachango et al., 2012a). Many strains of *F. sambucinum* are known to be resistant to TBZ and other benzimidazoles, characterized by growth on PDA containing > 5 mg/L (Hide et al., 1992; Hanson et al., 1996; Ocamb et al., 2007; Gachango et al., 2012a). Thiabendazole insensitivity was defined as $EC_{50} > 5$ mg/L in the current study. Resistance to TBZ has also been reported for most of the *Fusarium* spp. implicated in causing potato dry rot. Hanson et al. (1996) reported isolates of *F. sambucinum, F. oxysporum, F. solani, F. acuminatum,* and *F. culmorum* resistant to TBZ from the northeastern US. Ocamb et al., (2007) detected TBZ-resistant isolates of *F. sambucinum, F. oxysporum, F. solani, F. avenaceum, F. equiseti, F. sporotrichioides,* and *F. culmorum* from the northwestern US. In a more recent survey conducted on commercial potato seed tubers in Michigan, all *F. sambucinum*

isolates were resistant to TBZ, while the other 10 *Fusarium* spp. were sensitive (Gachango et. al., 2012a).

In this study, the majority of *F. sambucinum* isolates (79.3%) were resistant to TBZ, which is consistent with the majority of past studies in the US in which a high proportion of *F. sambucinum* isolates resistant to TBZ were identified (Hanson et al., 1996; Gachango et al., 2012a). The proportion of *F. sambucinum* isolates resistant to TBZ in this study was lower compared to the other recent studies in the US (Gachango et al., 2012a), but higher than studies in the United Kingdom (68%) (Hide et al., 1992) and Canada (71%) (Ocamb et al., 2007). Other *Fusarium* spp. resistant to TBZ in the current study included isolates of *F. oxysporum* (1.4%) and *F. solani* (21.1%). Due to the reduced efficacy of TBZ in the US since the early 1990's (Desjardins et al., 1993) the use of TBZ in commercial potato production in Michigan has been minimal and but used successfully in combination with fungicides with other modes of action as a mixture (Daami-Remadi et al., 2010).

The introduction of azoxystrobin, fludioxonil and difenoconazole could be considered as an expansion of the available fungicides for control of Fusarium dry rot and other potato diseases (Adaskaveg and Förster, 2010). Difenoconazole insensitivity was defined as $EC_{50} > 5$ mg/L in this and other studies (Müllenborn et al., 2008; Gachango et al., 2012a). This fungicide threshold for insensitivity was chosen based on other triazoles, including prothioconazole and tebuconazole, which resulted in EC_{50} values ranging from 0.1-3.2 and 1.1-5.5 mg/L, respectively (Müllenborn et al., 2008). In the current study 6.9, 5.0, 3.4, 15.7% of the *F. incarnatum/equiseti*, *F. oxysporum*, *F. sambucinum*, and *F. solani* isolates were resistant to difenoconazole, respectively, based on the $(EC_{50}) > 5$ mg/L threshold. All other *Fusarium* spp. were sensitive to difenoconazole. Ten *Fusarium* isolates were tested against difenoconazole, using the SDP

method, to determine the maximum fungicide concentration in amended media on which these isolates would grow. The results of this testing indicated that four isolates (two *F. oxysporum*, one *F. solani*, and one *F. incarnatum/equiseti*) were able to grow on PDA containing 20 mg/L of difenoconazole. These results are similar to Allen et al., (2004), who reported that difenoconazole had a limited effect on growth of *Fusarium* spp. isolated from pine seeds. According to these results, difenoconazole should be used with caution for potato dry rot control. However, it has been shown to control other soil-borne potato diseases like black dot [*Colletotrichum coccodes* (Olaya et al., 2010)], so it could possibly be used for control of other pathogens in commercial potato production.

Mixed resistance to two fungicides was observed in this study, although the fungicides were not combined and tested. A *Fusarium* isolate resistant to two fungicides in independent tests was reported as resistant to those two fungicides and termed as exhibiting mixed resistance in this survey. Ten *F. oxysporum* and three *F. solani* isolates were resistant to fludioxonil and azoxystrobin. One *F. oxysporum* and two *F. sambucinum* isolates were resistant to fludioxonil and TBZ. Six *F. oxysporum* isolates were resistant to fludioxonil and difenoconazole. One *F. oxysporum*, *F. sambucinum* and *F. incarnatum/equiseti*, and two *F. solani* isolates were resistant to difenoconazole and azoxystrobin. One isolate of *F. sambucinum* was resistant to difenoconazole and TBZ and this combination was identified only in 2011. Lastly, one *F. oxysporum*, 12 *F. sambucinum*, and two *F. solani* isolates were resistant to azoxystrobin and TBZ.

A mixture of difenoconazole, azoxystrobin, and fludioxonil (Stadium®) was recently registered for managing decay caused by *Fusarium* spp. on potato and other tuber crops. (Adaskaveg and Förster, 2010). This mixture of azoxystrobin, fludioxonil, and difenoconazole

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provided adequate potato dry rot control and significantly reduced dry rot incidence compared to inoculated control potato tubers (Kirk et al. 2013). The current study did not test the efficacy of the 3-way mixture of difenoconazole, azoxystrobin, and fludioxonil, but no *Fusarium* isolates were resistant to all three active ingredients in this study, thus supporting the conclusion that this mixture of fungicides would be expected to be effective in controlling potato dry rot. No *Fusarium* isolate was resistant to any three or four-way combinations in 2011 or 2012.

Infection of potato tubers by most *Fusarium* spp. generally occurs through wounds or bruising to the periderm during harvesting, grading, loading, cutting, and handling of potato tubers (Secor and Salas, 2001; Powelson and Rowe, 2008). Therefore, postharvest management of potato dry rot should be emphasized by implementing practices that avoid tuber injury, reduce tuber bruising, and provide conditions for rapid wound healing (Secor and Salas, 2001; Secor and Johnson, 2008). Due to the reduced effectiveness of fungicides tested during this study, combining such cultural control methods with alternative fungicides and biofungicides need to be explored.

The use of fungicides is an important tool for managing potato diseases and maintaining a healthy crop. Proper disease management strategies are needed to reduce the risk of developing fungicide resistance (Brent and Hollomon, 2007). Understanding the importance of Fungicide Resistance Action Committee (FRAC) codes (Brent and Hollomon, 2007) and following label recommendations are vital in fungicide resistance management. Evaluating changes in sensitivity within a population such as of the *Fusarium* spp. examined in the current study towards fungicides is essential for monitoring pathogen resistance. Although fungicides are often needed to control Fusarium dry rot and seedpiece decay, the importance of cultural practices should not be overlooked.

CHAPTER 4: SEED AND IN-FURROW TREATMENTS FOR CONTROL

OF SEED-BORNE *FUSARIUM SAMBUCINUM*

ABSTRACT

Potato dry rot of seed tuber pieces can be a devastating disease that can reduce crop establishment by damaging or killing developing potato sprouts, resulting in poor emergence and reduced plant stands with weakened plants. Fungicides and biofungicides are commonly used in commercial potato production for management of soil-borne potato diseases, including Fusarium dry rot. The effects of fungicidal and biofungicidal seed treatments (14 total treatments) applied to potato seed pieces, in-furrow, or in combination were evaluated for control of soil-borne *F. sambucinum*. Potato emergence (%) was evaluated 20, 27, and 32 days after planting and total yield was determined for each treatment in a field experiment. Final plant stand and RAUEPC were significantly increased by all treatments that included the application of mancozeb to the seed pieces, regardless of additional treatments. There were no statistical differences between mancozeb applied alone compared to the combination of mancozeb with in-furrow fungicide or biofungicide treatments. Mancozeb treatments (RAUEPC = 37.8 to 46.3) were not significantly different from the non-inoculated control (RAUEPC = 42.5). Furthermore, the majority of the mancozeb seed treatments significantly increased US-1 and total potato yields and most were not significantly different from the non-inoculated check. There were a few exceptions, including treatments of *Trichoderma asperellum* + *T. gamsii* applied in-furrow, which had variable effects on RAUEPC, but increased total yield in most of the treatments.

4.1 INTRODUCTION

Seed and soil-borne diseases, such as Fusarium dry rot of potato, present significant constraints to potato production in the US (Boyd, 1972; Hanson et al., 1996; Secor and Salas, 2001; Wharton et al., 2007c). *Fusarium sambucinum* Fuckel (synonym *F. sulphureum* Schltdl.)

is the predominant species affecting potato in storage and causing seed piece decay after planting and is one of the most aggressive *Fusarium* spp. (Lacy and Hammerschmidt, 1993; Hanson et al., 1996; Wharton et al., 2007b; Gachango et al., 2012a; Kirk et al., 2013). Dry rot of seed tubers can reduce crop establishment by affecting the development of potato sprouts, resulting in poor emergence and reduced plant stands with weakened plants (Leach and Nielsen, 1975; Leach, 1985; Wharton et al., 2006; 2007b). Seed tuber pieces infected with Fusarium dry rot can also become infected with bacterial pathogens such as *Pectobacterium* spp., causing soft rot decay, which can quickly destroy seed pieces (Secor and Salas, 2001). According to two reports from Michigan, *F. sambucinum* affected seed potato tubers in storage in 2006 and 2013 and caused seed piece decay and rotted sprouts after planting, leading to replanting of fields due to poor stand emergence (Wharton et al., 2007b; Kirk and Wharton, 2008; Merlington and Kirk, 2013).

Dry rot development is initiated by inoculum from infected seed tubers or infested soils (Secor and Salas, 2001). The pathogen is able to survive on potato tubers, plant tissue and debris, or as resistant chlamydospores in the soil for long periods of time (Powelson and Rowe, 2008). Progeny tubers can become contaminated with *Fusarium* spores as they develop throughout the growing season (Cullen et al., 2005; Wharton et al., 2005). There are two main opportunities during the potato crop cycle for *Fusarium* spp. to infect potato tubers, spring and fall (Nolte et al., 2003). Fall management strategies focus on postharvest control of dry rot in potato tubers intended for consumption or of tubers intended for seed for growing the following field season (Nolte et al., 2003). Postharvest practices that avoid tuber injury, reduce tuber bruising, and provide conditions for rapid wound healing can help minimize dry rot problems (Secor and Salas, 2001; Secor and Johnson, 2008). Spring management strategies rely on controlling seed

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tuber piece decay and sprout infection, prior to or after planting (Wharton et al., 2005; 2007c; Wharton and Kirk, 2014).

There are differences in the susceptibility of commercially grown potato cultivars to dry rot, although none are resistant to the disease (Secor and Salas, 2001; Wharton et al., 2007b). Some level of Fusarium dry rot is almost always present in commercially available seed (Kirk et al., 2013). Potato seed tubers are maintained in storage at 3°C, which is about the temperature at which *F. sambucinum* is relatively dormant, resulting in minimal dry rot development (Lui and Kushalappa, 2002; Wharton et al., 2007c; Kirk et al., 2013). During the pre-planting phase of potato production, seed tubers are warmed to about 12°C and then cut into seed pieces prior to planting (Wharton et al., 2007b; Wharton and Kirk, 2014). Cutting seed tubers may enhance the transmission of pathogens between tubers, such as *Fusarium* spp., which can lead to potato dry rot infection and development in the field or in storage (Wharton and Kirk, 2014). Tubers infected with *F. sambucinum* are particularly susceptible to the development of seed piece decay during this phase (Wharton et al., 2007c). In severe cases, seed pieces may rot completely before planting.

Management strategies for controlling Fusarium dry rot in storage and in the field are limited, partly due to fungicide resistant *Fusarium* strains (Chapter 3). Several products have provided adequate control of seed and soil-borne potato diseases including Fusarium dry rot (Wharton et al., 2007b; Gachango et al., 2012b). The most common type of fungicide application for dry rot control in the field are seed treatments applied on cut or whole potato seed tubers or in-furrow at planting (Wharton and Kirk, 2014).

Azoxystrobin (Quadris™; Syngenta Crop Protection) can be sprayed in-furrow, at the time of emergence, or at hilling/cultivation to control soil-borne and foliar potato diseases (Vea

and Palmer, 2013). Azoxystrobin has been shown to be effective in controlling soil-borne diseases, including Fusarium seed piece decay, when applied on freshly cut seed tubers (Powelson and Rowe, 2008). However, azoxystrobin-resistant *Fusarium* isolates may compromise effective dry rot control in Michigan (Chapter 3).

Fludioxonil [Maxim™ (registered on vegetable crops); Syngenta Crop Protection], mancozeb (Nubark Mancozeb™; Wilbur-Ellis Co.), or a combination of products, such as fludioxonil + mancozeb (Maxim MZ™; Syngenta Crop Protection) have shown to be effective in controlling Fusarium dry rot when applied to potato tubers up to 10 days prior to planting (Wharton et al., 2007c; Zitter, 2010). Similarly, fludioxonil [Medallion™; (registered on turf grass and ornamental crops); Syngenta Crop Protection] is registered for controlling Fusarium stem and root rot, caused by *Fusarium* spp. However, fludioxonil-resistant strains of *F. sambucinum* were reported in Michigan (Gachango et al., 2011b) and Canada, and could compromise effective dry rot control (Peters et al., 2008a; Peters et al., 2008b).

Difenoconazole (Inspire™; Syngenta Crop Protection), was introduced into the US for control of Fusarium dry rot and other potato diseases (Adaskaveg and Förster, 2010), although it may not be effective against some *Fusarium* isolates (Chapter 3). Some fungicides and biofungicides are also labelled for controlling foliar potato diseases following emergence or during cultivation, including fluopyram + pyrimethanil (Luna Tranquility™; Bayer Cropscience) and penthiopyrad (Vertisan™; Dupont Crop Protection).

Biofungicides such as *Bacillus subtilis* (Serenade Soil™; Bayer Cropscience) or *Trichoderma asperellum + T. gamsii* (Tenet™, Sipcam Agro USA, Inc.) have shown variable success at controlling soil-borne and foliar potato diseases in the field (Wharton and Kirk, 2014). Potato tubers treated with *B. subtilis* following harvest had limited control of Fusarium dry rot in

comparison to conventional fungicides (Gachango et al., 2012b). Kirk et al. (2013) reported there was a significant interaction between field and storage treatments of *B. subtilis* in reducing Fusarium dry rot when seed tubers were inoculated with *F. sambucinum*. Thus there is potential for use of biofungicides to control *Fusarium* spp., but isolates have shown to vary in responses (Schisler et al., 1997; 2000; Slininger et al., 2003).

Systemic fungicides are taken up by the roots of the potato plants after in-furrow application and are able to move systemically throughout the plant to promote healthy tuber development and contribute to protection against foliar diseases (Hamm et al., 2008). A combination of fungicides and application timing or methods can be an effective part of integrated pest management. Field treatment strategies combined with storage applied fungicides or biofungicides has shown to be effective in controlling Fusarium dry rot (Kirk et al., 2013)

To complement the research to determine the baseline sensitivity of *Fusarium* spp. associated with potato dry rot in Michigan, a field experiment was established in 2013 to examine the effects of seed and in-furrow biofungicide and fungicide treatments for control of soil-borne *F. sambucinum*. It was hypothesized that mancozeb would be effective for controlling dry rot of seed tuber pieces because of its broad spectrum disease control. Secondly, mancozeb with additional applications of fungicides and biofungicides might increase potato emergence and enhance potato yield. Lastly, it was hypothesised that azoxystrobin, fludioxonil, and difenoconazole might enhance control of Fusarium dry rot of seed potato tubers. Although understanding and monitoring the sensitivity of *Fusarium spp.* to commonly used fungicides in commercial potato production at the field level is important it is also critical to determine chemical efficacy for effective Fusarium dry rot control.

4.2 MATERIALS AND METHODS

4.2.1 *FUSARIUM INOCULUM PREPARATION*

Fusarium cultures (n=15) were selected at random from *F. sambucinum* isolates grown from single spore selections (Chapter 2). The cultures were grown on potato dextrose agar (PDA; Difco, Detroit, Michigan) for 7-d at 20°C. Inoculum was produced in bulk by growing *F. sambucinum* on millet seed (1.3 kg), which was placed in Sterilizable Airflow Spawn Bags (57 by 21 by 12 cm; Fungi Perfecti LLC, Shelton, WA). The millet seed was soaked with 500 mL of sterile distilled water in the spawn bags. After 24-h soaking time, the excess water was removed from the spawn bags which were then sealed with an impulse sealer (AIE-300; American International Electric Inc., Whittier, CA), and autoclaved twice at 115°C for 90 minutes each. The medium was removed from the autoclave and allowed to cool overnight (12-h). Two 5-mm-diameter plugs from each of the 15 *F. sambucinum* isolates were added to each of the millet bags (totalling 30 mycelial plugs per millet bag). The bags were resealed with the impulse sealer, mixed by hand-shaking, and incubated for 28-d at 20°C.

4.2.2 *FIELD PREPARATION, PLANTING, AND MAINTENANCE*

Soils were plowed using a chisel plow (Brillion 5-shank) to a 30.5-cm depth during Oct, 2012. The soil was prepared with a mechanical cultivator (John Deere 1010) for planting in Apr, 2013. The field plots were marked out, rows opened up with a hill opener, and millet seed infected with *F. sambucinum* was broadcast over the center two rows of each plot at a rate of 280 g of *F. sambucinum*-infested millet seed inoculum per 6.1-m of row length. Non-inoculated control plots did not receive any *F. sambucinum* inoculum.

Potato seed tubers cv. 'Snowden' were prepared by cutting and treating with fungicidal seed treatments two days prior to planting. Dust formulation seed treatments were measured and

added to cut potato seed pieces in a cement mixer (seed-treater) and mixed for 2-min to ensure even spread of the fungicide. Liquid seed treatments were applied in water suspension at a rate of 1 L/ton onto the exposed seed tuber surfaces in the seed treater. In-furrow at-planting applications were delivered in 9.35 L water/ha in a 17.8-cm band using a single XR11003VS nozzle (Lechler Inc., IL) at 206.8 KPa. The products evaluated in this study are summarized in Table 4.1. The inoculated control consisted of applying no treatment to the potato seed tubers or in-furrow at-planting.

The field trial was conducted at the Clarksville Research Center (CRS), Michigan State University, Clarksville, MI (sandy loam soil). Single-cut potato seed tubers (above) were planted on 30 May 2013 into two-row by 6.1-m plots (ca. 25.4-cm between plants) replicated four times in a randomized complete block design. Potato seed tubers were covered with soil and rows closed with a row closer. The target population was 50 plants at 86-cm between row spacing and a 1.5-m non-planted alley separated the two-row beds of each treatment.

Fertilizer was drilled into plots before planting, formulated based on soil tests results. Additional nitrogen (final N 31.4 kg/ha) was applied to the growing crop with irrigation 45 days after planting (DAP) for a total of 336 kg N/ha. Weeds were controlled by cultivation, hilling, and with S-metolachlor (Dual II Magnum; Syngenta Crop Protection) at 2.24 L/ha 10 DAP and (Poast; BASF Corporation) at 1.75 L/ha 58 DAP. Insects were controlled with imidacloprid (Admire Pro 2F: Bayer CropScience) at 3.4 mL/100 row-m at-planting, and two applications of Beta-cyfluthrin (Baythroid XL; Bayer CropScience) at 116.9 mL/ha at 60 and 90 DAP, or as needed based on commercial potato pest recommendations. Propamocarb-HCL (Previcur N 6SC; Bayer CropScience) was applied at 0.82 L/ha on a seven-day interval, for a total of four applications, starting one day after inoculation of adjacent plots with *Phytophthora infestans*, to

prevent spread of potato late blight. For the weeks when Previcur was not applied, potato late blight and general foliar diseases were managed with weekly applications of chlorothalonil (Bravo WS; Syngenta Crop Protection) at 1.75 L/ha starting at early canopy closure. Vines were killed with diquat dibromide (Reglone 2EC; Syngenta Crop Protection Inc.) applied at 1.17 L/ha on 15 Sep 2013. All sprays (above) were applied with a tractor mounted spray boom (R&D Inc. LA) delivering 233.8 L/ha (551.6 kPa) and using three XR11003VS nozzles per row. Plots were irrigated to supplement precipitation to about 0.63 cm/ha/4 d period with overhead irrigation. Plots were harvested on 7 Oct, 2013 and individual treatments were weighed and graded.

Meteorological variables were measured with a Campbell weather station (Campbell Scientific Inc., Logan UT) located at the farm from 30 May to harvest 7 Oct. Weather data were provided by the Michigan Automated Weather Network (MAWN).

4.2.3 DISEASE RATING

Plant stand was rated 21, 27 and 33 days after planting (DAP) and relative rate of emergence was calculated as the Relative Area Under the Emergence Progress Curve using the formula:

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

where t is the time in days after planting, E is the percentage of plant emergence, and T is the total number of days (Kirk et al., 2001).

4.2.4 DATA COLLECTION AND ANALYSIS

All data were subjected to analysis of variance (ANOVA) using JMP Version 10.0 (SAS Inc., Cary, NC). Means separation was conducted using Tukey's honestly significantly difference (HSD) test.

Table 4.1 Products evaluated for effect on Fusarium dry rot of potato caused by *F. sambucinum* in the study including product name, active ingredient, formulation and manufacturer.

Product name	Active ingredient	Formulation[a]	Manufacturer
Nubark mancozeb	Mancozeb	DS	Wilbur-Ellis Co., San Francisco, CA
Quadris	Azoxystrobin	FL	Syngenta Crop Protection Inc., Greensboro, NC
Inspire	Difenoconazole	SC	Syngenta Crop Protection Inc., Greensboro, NC
Medallion	Fludioxonil[d]	WP	Syngenta Crop Protection Inc., Greensboro, NC
Maxim	Fludioxonil[e]	FS	Syngenta Crop Protection Inc., Greensboro, NC
Luna Tranquility	Fluopyram + pyrimethanil	SC	Bayer Cropscience, Raleigh, NC
Serenade Soil	*Bacillus subtilis*	SC	Bayer Cropscience, Raleigh, NC
Tenet	*Trichoderma gamsii + T. asperellum*	WP	Sipcam Agro USA Inc., Durham, NC
Vertisan	Penthiopyrad	EC	DuPont Crop Protection, Wilmington, DE

[a] Formulation = product types when including products added to the active ingredient to change its physical characteristics and allow compatibility for machinery application. DS = Soluble dust; FL = Flowable liquid; SC = Suspension concentrate; WP = Wettable powders; FS = Flowable concentrate for seed treatment; EC = Emulsifiable concentrates.
[d] Fludioxonil = Medallion
[e] Fludioxonil = Maxim 4FS

4.3 RESULTS

4.3.1 METEOROLOGICAL VARIABLES

Average daily air temperature (^{o}C) at CRC for each month Jun to Oct was 18.6, 20.8, 19.5, 15.4 and 16.7 and the number of days with maximum temperature >32.2^{o}C was 0, 4, 0 and 0 (Jun, Jul, Aug, Sep, Oct, respectively (Fig. 4.1). Average daily relative humidity (%) for each month over the same period was 64.8, 71.4, 72.1, 72.7 and 74.7%. Average daily soil temperature at 10 cm depth for each month over the same period was 21.3, 24.3, 19.8, 17.8 and 17.5^{o}C (Fig. 4.1). Average daily soil moisture at 10-cm depth (% of field capacity) for each month was 37.4, 39.2, 37.8, 36.6 and 36.3% (Fig. 4.1). Precipitation over the same period for was 7.9, 8.4, 8.1, 4.5 and 4.3 cm (Fig. 4.1). Plots were irrigated to supplement precipitation to about 0.6 cm/ha/4 day period with overhead sprinkle irrigation. Supplemental irrigation was not included in the soil moisture or precipitation summary because the Campbell weather stations soil moisture probes and rain gauge were located next to the field experiment.
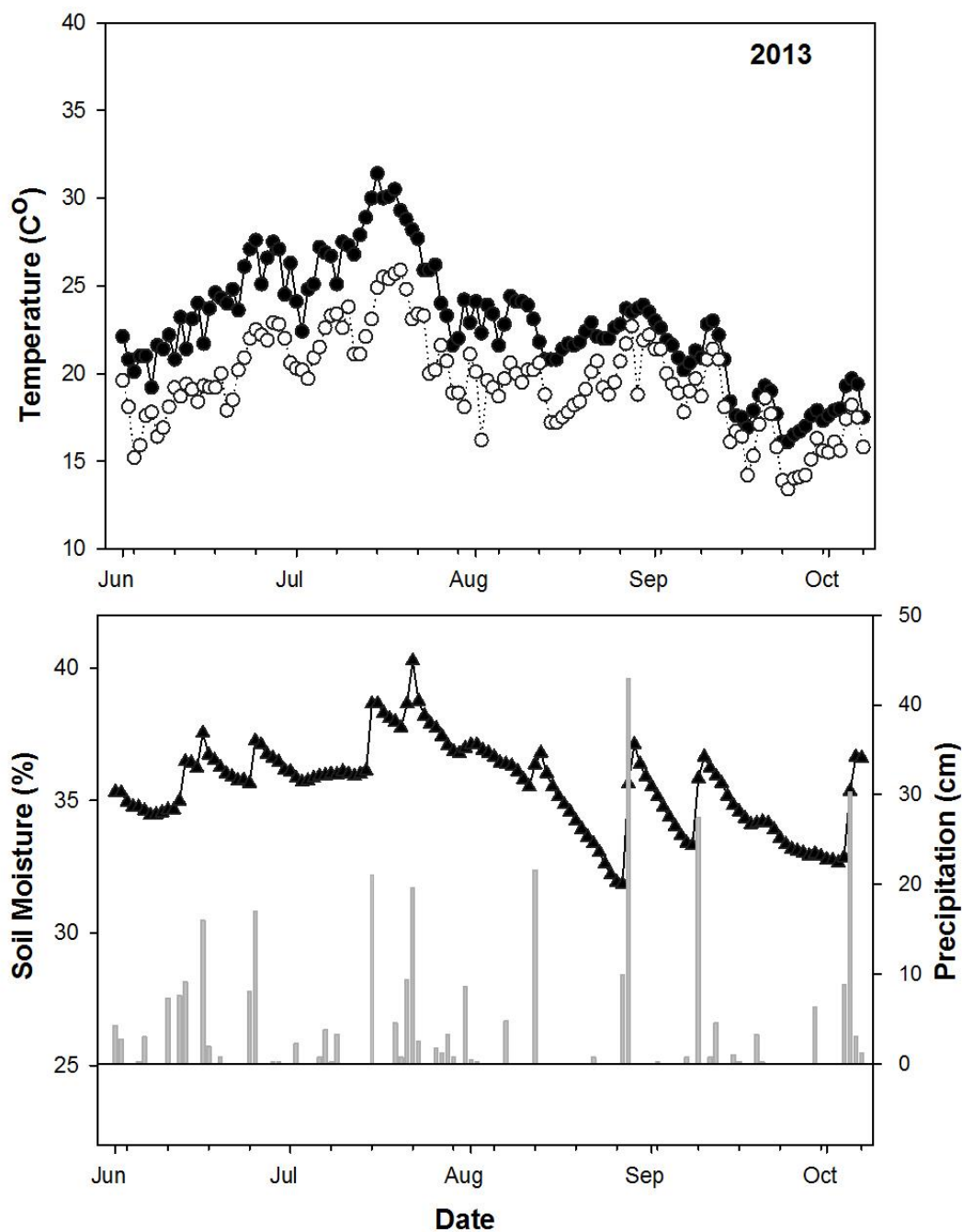
Figure 4.1 Summary of the 2013 meteorological data at the Clarksville Research Center, Michigan State University, Clarksville, MI. Top graph shows the minimum (open circle) and maximum (black circle) soil temperature (^{o}C) at a 10 cm depth for each day throughout the growing season, from 30 May (planting) to 7 Oct (harvest). Bottom graph shows the average (black triangle) soil moisture (%) collected from four soil moisture probes and the amount (grey vertical bar) of precipitation (cm) received each day throughout the growing season. Supplemental irrigation is not included.

Final plant stand (32 DAP) ranged from 34.5 (*B. subtilis* 85 mL/100 row-m in-furrow application) to 90.0% (untreated non-inoculated control) with the non-inoculated control significantly higher in comparison to all of the inoculated treatments (Table 4.2). Many of the treatments with mancozeb seed-piece application increased final plant stand in comparison to the in-furrow application of biofungicide treatments. The biological-based treatments and fungicides without the mancozeb component performed poorly and were not statistically different in final plant stand 32 DAP when compared to the untreated inoculated check, with the exception of treatments including *T. asperellum + T. gamsii* (21 g/100 row-m) applied in-furrow. Treatments with difenoconazole, fludioxonil, penthiopyrad, and azoxystrobin applied to seed piece, in-furrow, or in combination also were not statistically different in final plant stand 32 DAP when compared to the untreated inoculated check (Table 4.2). The seed piece application of fludioxonil and in-furrow *B. subtilis* application had a significantly lower final plant stand 32 DAP compared to the untreated inoculated check.

No treatments had a relative rate of emergence (RAUEPC; max = 100) significantly greater than the untreated non-inoculated control (42.5). The treatments with mancozeb seed-piece applications increased RAUEPC (average 41.0) and were not statistically different from the untreated non-inoculated control. The biological based treatments and fungicides without the mancozeb component were not statistically different in RAUEPC when compared to the untreated inoculated check, with the exception of *T. asperellum + T. gamsii* (21 g/100 row-m) applied in-furrow (Table 4.2).

Treatments with final stem number greater than 5.0 were significantly higher in comparison to the untreated control (3.6 stems/plant). US-1 and total potato yield ranged from

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11.9 to 39.9 t/ha and 16.5 to 48.0 t/ha, respectively. Treatments with total yield greater than 30.4 t/ha had significantly higher yield than the untreated inoculated control (25.0 t/ha, Table 4.3). Most treatments with mancozeb seed-piece applications increased total yield compared to treatments without the mancozeb component. Seed treatments showed no phytotoxicity symptoms. *Fusarium sambucinum* was reisolated from infected seed-pieces and decaying sprouts to confirm it was the causal agent.

Table 4.2 Effects of seed and in-furrow chemical and biofungicide treatments on potato (cv. Snowden) emergence and plant stand in a field infested with *Fusarium sambucinum* propagules.

Treatment (A)= rate/1000 kg potato seed[a] (B)= foliar rate/100 row-m	Emergence (%) 20 DAP		27 DAP		32 DAP		RAUEPC[b] Max = 100 (0 – 32 DAP)	
Mancozeb 1.04 kg (A)…………………..	52.2	ab[c]	59.5	bcd	68.0	bc	37.8	ab
Mancozeb 1.04 kg (A); Azoxystrobin 5.8 mL (B)……………......	60.2	a	72.3	ab	66.0	bcd	44.0	a
Mancozeb 1.04 kg (A); Difenoconazole 4.66 mL (B)…………...	55.1	a	68.1	abc	63.5	bcd	40.7	a
Mancozeb 1.04 kg (A); Fludioxonil[d] 4.46 g (B)…………….........	55.8	a	68.7	abc	71.5	b	41.9	a
Mancozeb 1.04 kg (A); Fluopyram + pyrimethanil 5.34 mL (B)...	57.4	a	62.3	bc	67.5	bc	40.6	a
B. subtilis 85.0 mL (B)………................	17.4	e	24.5	h	34.5	h	14.7	f
Fludioxonil[e] 5.2 mL (A)……..……….....	17.9	e	29.4	gh	39.5	gh	16.2	ef
T. gamsii + T. asperellum 14 g (B)……..	34.1	cd	42.0	f	56.0	de	26.4	cd
T. gamsii + T. asperellum 21 g (B)……..	36.3	bcd	56.5	cde	68.5	bc	31.2	bc
T. gamsii + T. asperellum 28 g (B)……..	23.4	de	45.4	ef	46.5	efg	22.2	c-f
Fludioxonil[e] 5.2 mL (A); *T. gamsii + T. asperellum* 28 g (B)….......	28.9	de	49.0	def	57.5	cde	25.7	cd
Penthiopyrad 10.7 mL (B)……………...	24.4	de	41.0	fg	44.0	fgh	21.5	def
Fludioxonil[e] 5.2 mL (A); Azoxystrobin 5.8 mL (B)………………..	28.4	de	43.5	f	49.5	efg	24.2	cde
Difenoconazole 2.9 mL (B)…………..…	25.6	de	41.9	fg	46.5	efg	22.1	c-f
Untreated Check (inoculated)………..…	26.4	de	44.4	ef	51.5	ef	23.4	c-f
Untreated Check (not-inoculated)……...	47.5	abc	80.8	a	90.0	a	42.5	a

[a] Application dates: A = 15 May (liquid formulations for seed piece application at 1.0 L/t); B = 17 May (in-furrow).

[b] RAUEPC = Relative area under the emergence progress curve measured from planting to 31 days after planting.

[b] Values followed by the same letter are not significantly different at $p = 0.05$ (Fishers LSD).

[d] Fludioxonil = Medallion

[e] Fludioxonil = Maxim 4FS

Table 4.3 Effects of seed and in-furrow chemical and biofungicide treatments on US-1 and total potato yield infested with *F. sambucinum* propagules.

Treatment (A); rate/100 kg potato seed[a] (B); foliar rate/100 row-m	Yield (t/ha) US-1		Total	
Mancozeb 1.04 kg (A)………………....	33.7	bc[b]	41.2	b
Mancozeb 1.04 kg (A); Azoxystrobin 5.8 mL (B)………………..	39.9	a	48.0	a
Mancozeb 1.04 kg (A); Difenoconazole 4.66 mL (B)……..............	33.0	bcd	40.2	bc
Mancozeb 1.04 kg (A); Fludioxonil[d] 4.46 g (B)………………….	36.7	ab	44.0	ab
Mancozeb 1.04 kg (A); Fluopyram + pyrimethanil 5.34 mL (B)...	26.8	ef	33.0	de
B. subtilis 85.0 mL (B)………………....	15.9	hi	20.6	gh
Fludioxonil[e] 5.2 mL (A)……………….......	11.9	i	16.5	h
T. gamsii + T. asperellum 14 g (B)……..	30.8	cde	37.7	bcd
T. gamsii + T. asperellum 21 g (B)……..	33.2	bcd	39.0	bcd
T. gamsii + T. asperellum 28 g (B)……..	26.4	ef	32.8	de
Fludioxonil[e] 5.2 mL (A); *T. gamsii + T. asperellum* 28 g (B)….......	30.7	cde	38.4	bcd
Penthiopyrad 10.7 mL (B)………………...	23.5	fg	28.8	ef
Fludioxonil[e] 5.2 mL (A); Azoxystrobin 5.8 mL (B)…………………	17.9	h	22.5	fgh
Difenoconazole 2.9 mL (B)…………….…	28.0	def	34.4	cde
Untreated Check (inoculated)………..….	18.8	gh	25.0	fg
Untreated Check (not-inoculated)………..	36.9	ab	44.4	ab

[a] Application dates: A = 15 May (liquid formulations for seed piece application at 1.0 L/t); B = 17 May (in-furrow).
[b] Values followed by the same letter are not significantly different at $p = 0.05$ (Fishers LSD).
[d] Fludioxonil = Medallion
[e] Fludioxonil = Maxim 4FS

4.4 DISCUSSION

Potato seed-piece and in-furrow treatments of fungicides and biofungicides are commonly used in commercial potato production to control soil-borne diseases such as black dot (*Colletotrichum coccodes*), (Ingram et al., 2011), black scurf and stem canker (*Rhizoctonia solani*) (Nolte et al., 2003), silver scurf (*Helminthosporium solani*) (Geary et al., 2007) and potato dry rot (*Fusarium* spp.) (Wharton et al., 2007b). The ability of pathogens to persist in soils necessitates the need for management strategies under high-risk situations with conducive environmental conditions, as in this study.

Multiple isolates of *F. sambucinum* were used for the inoculum in the current study and were representative of isolates recovered from Michigan commercial potato production. Multiple strains of *F. sambucinum* were identified to vary in their sensitivity to commercially available fungicides (Chapter 3). Few management strategies exist for controlling Fusarium seed piece decay, partly due to *Fusarium* strains resistant to commonly used fungicides (Gachango et al., 2012a).

The results of this study demonstrate the need for effective fungicides for potato dry rot control under high inoculum levels. The non-inoculated control had significantly higher percent emergence 32 DAP in comparison to all of the inoculated treatments or untreated inoculated check. The non-inoculated control did not receive any *F. sambucinum* inoculum, and was thus expected to have the highest percent emergence, as was the case in this study. Additionally, the untreated, inoculated control did not have any potato seed piece treatment and had one of the lowest percent emergences as expected.

Although the untreated inoculated control had a low percent emergence compared to the non-inoculated control, the in-furrow application of *B. subtilis* and the fludioxonil seed treatment

had a significantly lower percent emergence 32 DAP. These results indicate that potato sprouts were not protected and became infected and killed prior to emerging from the soil. Reasons for the poor performance of the *B. subtilis* treatment compared to the non-treated, inoculated control are unknown. This product may have created a conducive environment for other pathogens, like *Pectobacterium* spp., the cause of bacterial soft rot (Czajkowski et al., 2011), although this was not measured in this trial.

Seed-piece applications that included mancozeb were very effective and increased final plant stand 32 DAP regardless of the secondary fungicide or biofungicide treatments. This indicates that mancozeb seed treatments prevented seed piece decay and possibly protected developing sprouts from becoming infected and killed prior to emerging from the soil. Many if the fungicide and biological-based treatments and fungicides without the mancozeb component performed poorly and were not statistically different in final plant stand 32 DAP when compared to the untreated inoculated check, with the exception of one of the *T. asperellum* + *T. gamsii* treatment and the fluopyram + pyrimethanil treatment, applied in-furrow. The reason that the lowest and highest rate of *T. asperellum* + *T. gamsii* (14 and 28 g/ 100 row-m, respectively) performed poorly and the middle rate (21 g/ 100 row-m) performed well is unknown.

Difenoconazole, penthiopyrad, and azoxystrobin treatments were also not statistically different in final plant stand 32 DAP when compared to the untreated inoculated check. These fungicides are used in commercial potato production for control of soil-borne diseases (Gudmestad et al., 2007), but were found to be ineffective at controlling seed or soil-borne *F. sambucinum* in this study. These findings were different than some of the past studies that found fludioxonil and azoxystrobin to be effective in controlling Fusarium dry rot (Bains et al., 2001; Daami-Remadi et al., 2006). However, some of the *F. sambucinum* isolates used in this study

were insensitive to difenoconazole, fludioxonil, and azoxystrobin (Chapter 3), which may help explain the poor performance of these products in this field trial. Furthermore, difenoconazole, fludioxonil, and azoxystrobin resistant *F. sambucinum* isolates were discovered fairly recently, which might explain why the chemicals were effective in those prior studies, as resistance might not have been present or as prevalent.

Similar to percent emergence, there were significant differences in the relative rate of emergence (RAUEPC) with the different treatments. No treatments had RAUEPC indices significantly greater than the untreated non-inoculated control (42.5), as expected because this treatment did not receive any *F. sambucinum* inoculum. Seed-piece applications containing mancozeb had significantly higher RAUEPC indices in comparison to all *Fusarium* inoculated treatments, with the exception of the *Trichoderma asperellum + T. gamsii* (21 g/ 100 row-m) and the fluopyram + pyrimethanil treatment, applied in-furrow. The biological based treatments and fungicides without the mancozeb component performed poorly and were not statistically different in terms of RAUEPC values when compared to the untreated inoculated check. The seed piece application of fludioxonil and the in-furrow application of *B. subtilis*, penthiopyrad, and difenoconazole had one of the lowest RAUEPC indices and were not statistically different from the inoculated control. These fungicides and biofungicides would not be recommended in Michigan for control of Fusarium seed piece decay based on this study. Mancozeb applied to the tuber seed-piece has been shown to be effective in controlling *Fusarium* in previous reports (Leach and Nielsen, 1975; Wharton et al., 2007b; 2007c), which is consistent with the results of the current study.

Although mancozeb seed-piece applications increased RAUEPC in all treatments compared to the inoculated check, total yields did not always reflect this. Mancozeb + fluopyram

+ pyrimethanil had one of the highest RAUEPC, but performed poorly in terms of total yield. Furthermore, the *T. asperellum + T. gamsii* treatments were not statistically different in terms of RAUEPC compared to the inoculated control, but total yield was significantly higher than the inoculated untreated control. Most of the treatments with the mancozeb component had the highest yield in comparison to treatments comprising the same products applied alone. Some of the treatments (Table 4.3) with a combination of mancozeb and other products had a higher total yield than the mancozeb only treatment, although only one was significantly higher. This indicates that some of the additional components had additive properties on potato tuber growth and development other than preventing sprout and tuber rot.

In a case study from Michigan, a high incidence of Fusarium dry rot in seed potato tubers was observed (Merlington and Kirk, 2013). As a result, the commercial potato grower applied fludioxonil in-furrow to help reduce seed piece decay and rotted sprouts after planting. Poor emergence and uneven plant stands lead to replanting these fields (Merlington and Kirk, 2013). The causal agent was identified as *F. sambucinum*, in which all isolates collected from this field were identified to be resistant to fludioxonil (unpublished data). The poor performance of fludioxonil in this case study was similar to the field trial conducted using *F. sambucinum* isolates from MI in the current study. As a result, fludioxonil would not be recommended for controlling *F. sambucinum* in MI commercial potato production.

The result from the current field trial and the case study mentioned above demonstrates the importance of an integrated approach in managing Fusarium dry rot. In-season crop protection strategies combined with other cultural management strategies may be needed to manage Fusarium dry rot of potato seed-tubers in the field. Control and management of Fusarium dry rot in potatoes relies on cultural practices such as crop rotation, use of disease-free

seed, minimizing wounds and injuries during harvesting, and promoting wound healing of stored potatoes, (Secor and Gudmestad, 1999). Since cultural practices alone are not always sufficient to effectively control this disease, crop protection strategies are needed. Although fungicide seed treatments such as mancozeb are expensive, these results indicate that applying mancozeb-containing fungicides to seed tubers before planting can provide effective control of soil-borne *F. sambucinum*. Further research is needed to investigate if mancozeb seed treatments are as effective at controlling seed-borne *F. sambucinum.* The economic benefit from fungicide applications may outweigh the risk of applying no fungicide seed treatment if there is a high *F. sambucinum* inoculum density that may result in poor emergence and reduced total yields, as in this study.

CHAPTER 5: THE INFLUENCE OF SULFUR, CULTURAL PRACTICES, AND CROP PROTECTION STRATEGIES ON POTATO COMMON SCAB (PCS) CONTROL

ABSTRACT

Potato common scab (PCS) caused by *Streptomyces* spp. is one of the most important diseases of potato worldwide, and can be particularly severe in some fields in Michigan (MI). PCS degrades the cosmetic quality of the potato tuber and ultimately decreases the market value of the crop. Incidence and severity of PCS vary based on location, from year to year, and cultivar to cultivar. Management of PCS is one of the most important challenges gorwers are facing in potato production. Many control strategies have been proposed and practiced, but can be inconsistent depending on the field characteristics, cultivars, environmental conditions and inoculum levels. The purpose of this study was to investigate the influence of sulfur, cultural practices to enhance aeration of soil, and some crop protection options for control of PCS. The addition of elemental sulfur or ammonium sulfate had no effect on the overall incidence and severity of PCS or total yield in most trials. Different tillage practices (minimal disturbance, chisel plow, and moldboard plow) had variable effects on the overall severity of PCS, depending on year and location. Cultivars 'Dark Red Norland' and 'Snowden' had less overall PCS severity indices compared to 'Russet Norkotah', although significant in only one of the experiments. In a chemical trial, chloropicrin was effective in reducing the incidence and severity of PCS in one of the two years compared to the not-treated check. Other chemical or biological treatments had minimal but variable effects on PCS severity. Environmental conditions were conducive to PCS during these experiments. No single management strategy was effective in reducing PCS to acceptable levels required by commercial processors. Management of PCS requires an integrated

approach that combines the use of host resistance, cultural management strategies, and possibly chemical control.

5.1 INTRODUCTION

Potato production is driven by consumer and processor demand for high quality potatoes (Keijbets, 2008), which is impeded by soil-borne diseases, such as potato common scab (PCS) (Loria, 2001; Gudmestad et al., 2007). In North America, several *Streptomyces* spp., including *S. scabies*, *S. acidiscabies*, *S. europaeiscabiei*, *S. turgidiscabies*, and *S. stelliscabiei* have been identified as causal agents (Dees and Wanner, 2012). PCS is an annual production concern for commercial potato growers (Loria et al., 1997), and has been identified as a high priority by some regional potato commodity groups such as the Michigan Potato Industry Commission. PCS affects the cosmetic quality of the potato tuber and ultimately reduces the marketability of the crop (Loria et al., 2006; Wanner, 2009). *Streptomyces scabies* has been well documented as causing PCS (Loria, 2001; Wanner, 2005; Loria et al., 1997; 2006; Wharton et al., 2007a). Several hundred *Streptomyces* spp. have been identified, while only about 10 of these species are considered plant pathogens (Dees and Wanner, 2012). Many non-pathogenic *Streptomyces* spp. produce secondary metabolites in the soil, such as antibiotics, which can aid in controlling PCS (Hiltunen et al., 2009; Wanner et al., 2014) and other soil-borne diseases, such as Rhizoctonia crown and root rot of sugarbeet (*Rhizoctonia solani*), Fusarium damping-off of sugarbeet (*Fusarium oxysporum*), and Verticillium wilt of potatoes (*Verticillium* spp.); (Sabaratnam and Traquair, 2002; Minuto et al., 2006).

PCS is a recurrent, persistent, and important soil-borne disease of the potato (*Solanum tuberosum* L.) globally (Bruehl, 1987; Loria, 2001), and can be particularly severe in some fields in MI (Wharton et al., 2007a). There are no data available for PCS losses in the US, but

economic losses of potatoes in Canada were estimated to be between 15.3 and 17.3 million Canadian dollars in 2002 (Hill and Lazarovitz, 2005). Economic losses are greatest for tubers intended for table stock, although significant losses have been reported for processing varieties (Wharton et al., 2007a).

The symptoms of PCS are present on the surface of the potato tuber and can be variable (Loria, 2001; Loria et al., 1997). Scab lesions start out as small brownish spots on the potato tuber surface, which expand into water-soaked lesions within a few weeks after infection (Loria, 2001; Wharton et al., 2007a; Naher et al., 2013). Common scab is characterized by the formation of corky lesions on the tuber surface, which can be categorized into at least three symptomatic lesion types, superficial, raised, or pitted (Loria et al., 1997). Symptoms are generally noticed late in the growing season or at harvest, although infection occurs during early tuber development and growth (Loria, 2001). Symptoms become most noticeable late in the growing season when tubers are fully expanded or at harvest. Scab lesions can be categorized further into discrete or coalesced, which affect extensive areas of individual tubers (Wanner, 2006). To further classify disease severity, an index using these categories can be used, with tubers placed into classes based on lesion type; and percentage surface area covered with PCS, for example using the Merz scale, with classes $0 - 6$ (Mertz, 2000) as described by Wanner et al. (2014).

Incidence and severity of PCS vary based on location, year, cultivar, and within fields (Goyer et al., 1996; Lehtonen et al., 2004). It is unclear what factors, strains, or species determine the type or severity of scab symptoms (Loria et al., 1997). The variability and severity of the disease is of importance to the potato industry in MI, where environmental conditions are often conducive for PCS (Loria, 2001; Wharton et al., 2007a). These conditions consist of warm, dry seasons, with high soil temperatures and variable rainfall that permits rapid soil drying

especially during the early development and growth of tubers (Loria, 2001). Reasons for the variability in severity and symptoms are not well understood, although many hypotheses have been described, including environmental conditions, aggressiveness of the *Streptomyces* strains, and differences in cultivar susceptibility (Loria, 2001).

Streptomyces spp. are efficient saprophytes that can overwinter in the soil, on potato tubers, and crop residues for over a decade (Wharton et al., 2007a; Dees and Wanner, 2012). Most potato soils have a resident population of *Streptomyces* spp., which can increase with each succeeding host crop (Wanner, 2006; 2007; Hao et al., 2009). The population can be reduced by rotation with non-host crops, but this practice does not eliminate the disease because the pathogenic species can reproduce on soil organic matter, the surface of tubers, and crop residues for over a decade (Fig. 1.4); (Loria et al., 1997; Wharton et al., 2007a; Dees and Wanner, 2012). Spores can persist in the soil for many years, and can germinate and infect in the presence of a suitable host (Loria et al., 2006). The pathogen can spread from one location to another by the transfer of soil and on seed tubers (Wharton et al., 2007a). Infection of the potato tuber by *Streptomyces* spp. occurs primarily through the lenticels and wounds (Wanner, 2007). Tubers are most susceptible during the early period of development and growth of tubers encompassing initiation through early maturation.

Different management strategies often provide inconsistent or inadequate results when relating to PCS incidence and severity (Wanner, 2007). Scientists still have little understanding of the exact conditions or factors that contribute to the differences and variation of disease symptoms (Dees and Wanner, 2012). Using tolerant cultivars has been the most effective and most reliable tool for PCS control (Hiltunen et al., 2005; Lambert et al., 2006; Dees and Wanner,

2012). However, tolerant cultivars are not immune to PCS and can become diseased when inoculum is plentiful and conditions are conducive (Loria et al., 1997; Loria, 2001).

Cultural practices or management techniques are often implemented for control of PCS, but results are inconsistent (Dees and Wanner, 2012). Acidic soils, with pH levels below 5.2 can significantly reduce the incidence and severity of PCS (Loria, 2001). Reducing soil pH to around 5.2 has been used for disease management (Loria, 2001; Loria et al., 1997), but can create problems for acid sensitive rotation crops, such as barley (Locci, 1994). Furthermore, this management strategy can fail because *S. acidiscabies* thrives in soils with pH <5.0 and can cause PCS disease under such conditions (Lindholm et al., 1997). Achieving a lower pH can be accomplished in various ways. One successful approach has been the addition of sulfur to reduce soil pH below the optimal range for pathogenic *Streptomyces* species. Historically, sulfur has been used for PCS control (Martin 1920), but the mechanism is not well known or understood (Pavlista, 2005). Few experiments have been conducted on the influence of sulfur, however in MI, Hammerschmidt et al. (1986) concluded the addition of 125 kg/ha of ammonium sulfate (AS) reduced common scab when incorporated into the potato hill.

Irrigation has been used traditionally as a management strategy since the early 1920's (Lapwood et al., 1973; Wharton et al., 2007a). Maintaining soil moisture levels near field capacity during the two to six weeks during tuber initiation can inhibit infection (Loria, 2001). However, maintaining soil moisture at high levels is problematic in regions where precipitation is erratic and irrigation is not available. In addition, saturated soils can enhance infection risk by other potato pathogens (Powelson and Rowe, 2007). Overall, this strategy has been fairly successful, although some studies indicated inconsistency (Lapwood et al., 1973; Adams and Lapwood, 1978; Larkin et al., 2011).

Chemical control can be used as a management strategy for PCS control, but has shown variable success (Wilson et al., 1998; Dees and Wanner, 2012). Chemical fumigation is one of the best options to control soil-borne plant pathogens of potato including PCS, but varying levels of success have been reported (Davis, 1976; Jordan et al., 2011; Dees and Wanner, 2012). The soil fumigant pentachloronitrobenzene (PCNB), under the trade name Blocker™ (Amvac Chemical Corporation) has resulted in reduced disease incidence is some experiments (Davis et al., 1974; Davis, 1976; Hutchinson, 2005; Jordan et al., 2011). However, results have been inconsistent and PCNB can have a detrimental impact on the potato plant at high concentrations by reducing tuber size or yield (Wharton et al., 2007a). Chloropicrin (Pic Plus; TriEst Ag Group Inc.) has had some success in reducing PCS, but the applications are required at relatively high soil temperatures preceding planting (8°C) and with a 30-day post-application planting restriction interval makes application difficult in MI (Wharton et al., 2007a).

The use of commercially available antagonistic *Streptomyces* spp. and other biocontrol approaches [*Bacillus subtilis* (Serenade Soil™; Bayer Cropscience)] have been shown to decrease the amount of pathogenic *S. scabies* present in the soil and reduce common scab on harvested tubers in some studies (Schmiedeknecht et al., 1998; Han et al., 2005; Hiltunen et al., 2009; Wanner et al., 2014).

Tillage practices are essential for preparation of the seedbeds to maximize potato quality and yields (Powelson and Rowe, 2007). Soil physical and chemical properties, moisture and temperature, root growth, and pathogen vectors are all influenced by tillage practice, and consequently pathogen virulence, diversity and host susceptibility are likewise influenced (Sumner et al., 1981). Tillage practices can increase or decrease incidence and severity of potato diseases, depending on the disease of interest and the environment (Gudmestad et al., 2007). The

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impact of tillage on plant disease development has been highly variable, depending on the specific interactions between the crop, pathogen, and environnement (Sumner et al., 1981).

Chisel plowing, as opposed to the traditional tillage practice of moldboard plowing, is the most commonly accepted practice that is used for conservation tillage in potato (Peters et al., 2004). Generally the chisel plow provides less soil inversion and pulverizing than the moldboard plow (Dickey et al., 1986). Moldboard plowing, relative to conventional tillage, has been shown to reduce incidence and severity of some soil-borne potato diseases including potato early die (Gudmestad et al., 2007). Leach et al. (1993) showed that chisel compared to moldboard plowing reduced incidence and severity of stem lesions caused by *Rhizoctonia solani* in potato. Few studies have looked at the effect of tillage practices on incidence and severity of PCS. Peters et al. (2004) reported the severity of PCS was not influenced by tillage practices or rotation, although disease incidence was low in all years of the study.

Field experiments were established in 2012 and 2013 to:

1) Investigate how different tillage practices (minimal disturbance, chisel plow, and moldboard plow) influence the incidence and severity of PCS and soil oxygen (%) levels as a physical indicator of the effect of tillage.

2) Evaluate the effects of applying elemental sulfur [ES; 0 and 448 kg ES/ha; (done in 2012) and 0, 224, and 448 kg ES/ha; (done in 2013)] in the fall, prior to the potato field season, for its influence on the incidence and severity of PCS and to determine if different tillage practices [chisel plow and moldboard plow (done in 2012)] and [minimal disturbance, chisel plow, and moldboard plow (done in 2013)] influence incidence and severity of PCS; and investigate if there were interactions between ES and tillage practices (done in 2013):

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3) Evaluate the interactions of potato cultivars with different susceptibility to PCS ['Dark Red Norland'; (least susceptible), 'Russet Norkotah' and 'Snowden'; (moderately susceptible)] on incidence and severity of PCS with different tillage practices (minimal disturbance, chisel plow, and moldboard plow on incidence and severity of PCS).

4) Evaluate the effects of ammonium sulfate (AS) (0, 140, and 280 kg/ha) applied in the spring, prior to tuber initiation, on incidence and severity of PCS.

5) Evaluate the efficacy of crop protection programs on the incidence and severity of PCS.

It was hypothesized that applications of ES or AS would result in reduced incidence and severity of PCS. Secondly, moldboard tillage would reduce scab severity, by inverting the soil and possibly burying PCS inoculum or aerating the soil. Thirdly, 'Dark Red Norland' would have significantly less PCS compared to 'Russet Norkotah' and 'Snowden'. Lastly, the interactions of the factors listed above would reduce PCS incidence and severity.

5.2 MATERIALS AND METHODS

5.2.1 METEOROLOGICAL VARIABLES

Meteorological variables were measured with a Campbell weather station (Campbell Scientific Inc., Logan, UT) located near the field plots at the Montcalm Research Center (MRC), Michigan State University, Entrican, MI; 42.3526, -85.1761 deg; elevation 290-m and at the Clarksville Research Center (CRC), Michigan State University, Clarksville, MI; 42.8733, -85.2604 deg; elevation 273. The Enviroweather Michigan Automated Weather Network (MAWN) provided meteorological data such as soil temperature and soil moisture at a 15 cm depth. Plots were irrigated to supplement precipitation to about 0.63 cm/ha/4 day period with overhead sprinkle irrigation. Supplemental irrigation was not included in the soil moisture or

precipitation summary because the Campbell weather stations soil moisture probes and rain gauge were located adjacent to the field plot area.

5.2.2 INTERACTION BETWEEN SOIL TILLAGE PRACTICES AND APPLICATION OF ELEMENTAL SULFUR ON PCS

A field trial was conducted in 2011-2012, planted with potato cv. 'Snowden' at the MRC on 8 May, 2012 into four-row by 15.3-m plots (ca. 25.4-cm between plants at 86.4-cm row spacing) replicated four times in a split-plot randomized block design within the split. Treatment plots were laid out in the fall, 2011 (prior to the field season and any fertilizer application) and seven soil samples were taken from each treatment plot with a soil sampling probe (25-cm depth) and combined for a total of 16 separate composite soil samples. Soils at this location were sampled on 28 Sep, 2011 and consisted of a fine sandy loam with an average pH of 5.9; P, 249 µg/g; K, 148 µg/g; Mg, 58 µg/g; Ca, 406 µg/g; CEC 4.1 meq/100 g. The soil samples were combined and used as a baseline indication of the soil characteristics at MRC only in 2011. Soil pH test were performed on all 16 soil samples.

The split (tillage treatments) was done in the fall, 3 Oct, 2011 and consisted of a moldboard plow (John Deere 3-bottom) to 30.5-cm depth along the width of each replication and a chisel plow (Brillion 5-shank) to 25.4-cm depth along the width of each replication for a total plot length of 61-m. Elemental sulfur (Tiger 90; Tiger-Sul Products Co.) was applied after tillage at rates of 0 or 448 kg ES/ha. Fertilizer formulated according to soil tests results was drilled into plots before planting. The soil sampling procedure was repeated, as described above, after hilling, following tuber initiation around 45 days after planting (DAP). Additional nitrogen (final N 31.4 kg/ha) was applied to the growing crop with irrigation 46 DAP for a total of 336 kg N/ha. Potato was the preceding crop in all field studies.

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Weeds were controlled by cultivation, hilling, and with S-metolachlor (Dual II Magnum; Syngenta Crop Protection) at 2.24 L/ha 10 DAP and sethoxydim (Poast; BASF Corporation) at 1.75 L/ha 58 DAP. Insects were controlled with imidacloprid (1.46 L/ha; Admire Pro 2F: Bayer CropScience) at planting, and two applications of Beta-cyfluthrin (Baythroid XL; Bayer CropScience) at 116.9 mL/ha 60 and 90 DAP. Potato late blight and general foliar diseases were managed with weekly applications of chlorothalonil (Bravo WS; Syngenta Crop Protection) at 1.75 L/ha starting at early canopy closure and continued until vine desiccation. Vines were desiccated with diquat dibromide (Reglone 2EC; Syngenta Crop Protection Inc.) at 1.17 L/ha on 1 Sep. Maintenance treatments were applied with a tractor mounted spray boom (R&D Inc. LA) delivering 233.8 L/ha (551.6 KPa) and using three XR11003VS nozzles per row.

Plots (2 x 15.2-m plots) were harvested on 19 Sep, 2012 18 days after desiccation (121 DAP). Tubers were washed and assessed for PCS incidence and severity two weeks after harvest. Severity of PCS was measured as an index by rating 50 randomly selected tubers from each treatment and replication. The rating system classes were classified based on a $0 - 6$ scale, falling into classes based on lesion type; 0 = no scab symptoms, 1 = superficial discrete, 2 = coalescing superficial, 3 = raised discrete, 4 = raised coalescing, 5 = pitted discrete, and 6 = pitted coalescing (Wanner et al., 2014). Tubers were further classified into (%) surface area covered with PCS using the Merz scale, with classes $0 - 6$: 0 = no scab, $1 = 0.1 - 2.0\%$, $2 = 2.1 - 5.0\%$, $3 = 5.1 - 10\%$, $4 = 10.1 - 25\%$, $5 = 25.1 - 50.0\%$, and $6 = > 50.0\%$ (Mertz, 2000). The number of tubers in each lesion class was multiplied by the % surface area class. The result was then multiplied by a constant (21) to express the severity index as a percentage index from 1-100. These combined rating scales provide a qualitative and quantitative measure of PCS incidence and severity (Wanner et al., 2014).

The second location for the field trial was planted with potato cv. 'Snowden', at CRC on 12 May, 2012, as described above. A minimal disturbance treatment was added to this trial by using a disc plow (John Deere 210) to a 2.5-cm depth along the width of each treatment. Tillage treatments were implemented, soil sampling, and all maintenance treatments were carried out as described on the same dates as described above. Soils at this location were sampled, as described above on 18 Sep, 2011 and tillage treatments were implemented on 27 Sept, 2011. The soil was a sandy loam with an average pH of 6.6; P, 123 µg/ g; K, 132 µg/ g; Mg, 65 µg/ g; Ca, 653 µg/ g; CEC 9.8 meq/ 100 g. The soil samples were combined and used as a baseline indication of the soil characteristics at CRC only in 2011. Plots (2 x 15.2-m rows) were harvested on 15 Sep, 2012 following plant desiccation (126 DAP) and PCS was measured as described above. Soils were intensively sampled at MRC and CRC and a regular field soil test with the addition of CEC was performed by the Michigan State University Soil and Plant Nutrient Laboratory.

Following the results from the preliminary experiments in 2012, a field trial was planted with potato cv. 'Snowden' at the MRC on 16 May, 2013 into two-row by 9.1-m plots (ca. 25.4-cm between plants at 86.4-cm row spacing) replicated four times within a complete randomized block design, for a total plot length of 82.3-m. The trial was repeated at CRC, and planted on 17 May, 2013. Tillage treatments (minimal disturbance, chisel plow, and moldboard plow) were implemented 4 Oct, 2012 at MRC and 6 Oct, 2012 at CRC and ES (Tiger 90) was applied after tillage at rates of 0, 224, and 448 kg ES/ha, as described above. Additional fertilizer was applied, and weeds, insects and diseases were controlled as described above. Plots (2 x 9.1-m rows) were harvested on 18 Sep, 2013 at MRC and 25 Sep, 2013 at CRC following plant desiccation (125 DAP and 131 DAP, respectively), and PCS was measured as described above.

Following planting of the field trials (effects of soil tillage practices in 2012 and interaction between soil tillage practices and application of elemental sulfur on PCS in 2013), a Campbell scientific weather station (Campbell Scientific Inc.) was placed within the field plot at CRC. Soil oxygen content (%) was measured hourly using 12 SO-110 soil oxygen sensors (Apogee Instruments, Inc., Logan, Utah) attached to a CR10-X data logger (Campbell Scientific Inc.). Soil oxygen sensors were placed in the potato hill (following 70% plant emergence), below the potato plant, in the area where potato tubers are initiated (around 15-cm depth) 2-h after hilling. To place the soil oxygen sensor where tubers would develop, a garden trowel was used to break up the soil without pruning roots or stolons and the soil near the potato seed piece was removed by hand. A total of four soil oxygen sensors per tillage treatment (two sensors in one tillage treatment plot and two sensors in the adjacent plot) were placed into the potato hill with caution to minimize disturbance. The sensors were covered with soil and the potato hill was reshaped to normal form.

5.2.4 INTERACTION BETWEEN SOIL TILLAGE PRACTICES AND CULTIVARS VARYING IN SUSCEPTIBILITY TO PCS

A field trial was planted with three potato cvs. 'Dark Red Norland', 'Russet Norkotah', and 'Snowden' at MRC on 16 May, 2013 into two-row by 9.1-m plots (ca. 22.9-cm between plants at 86.4-cm row spacing) replicated four times within a complete randomized block design. The trial was repeated at CRC and planted on 17 May, 2013. Tillage treatments (minimal disturbance, chisel plow, and moldboard plow) were implemented as described above. Fertilizer was applied and weeds, insects and diseases were controlled as described above. Plots (2 x 9.1-m

rows) were harvested on 18 Sep, 2013 at MRC and 25 Sep, 2013 at CRC following plant desiccation (125 DAP and 131 DAP, respectively). PCS was measured as described above.

5.2.5 EFFECTS OF AMMONIUM SULFATE ON PCS

A field trial was planted with potato cv. 'Snowden' on 16 May, 2012 at CRC. Potato seed was planted into two-row by 15.2-m plots (ca. 22.9-cm between plants at 86.4-cm row spacing) replicated four times within a complete randomized block design. Treatments consisted of applying ammonium sulfate (AS; 21-0-0-24) at rates of 0, 140, and 280 kg/ha before tuber initiation on 21 Jun, 2012, 35 DAP. Seven soil samples for each treatment plot were taken after planting, with a soil sampling probe (25-cm depth), and combined from each treatment plot for a total of 36 composite soil samples. The soil sampling procedure was repeated 15-d following the application of AS, 50 DAP. Total nitrogen (N) was balanced throughout the treatments during the same period as the application of AS by applying equal units of N using urea (46-0-0). Following the application of AS and N, the fertilizers were incorporated into the soil with overhead irrigation for 2-h. Additional N was applied to the growing crop with irrigation around 55 DAP for a total of 336 kg N/ha for all treatments. Weeds, insects and diseases were controlled as described above. Plots (2 x 15.2-m rows) were harvested on 20 Sep, 2012 following plant desiccation and PCS was measured as described above (127 DAP). Soil pH was measured as described above.

5.2.6 EFFICACY OF CROP PROTECTION PROGRAMS ON PCS

A field trial to determine the efficacy of chloropicrin (PicPlus 85.5AP) and oxamyl (Vydate 3.77SL) was established on 13 Oct, 2011 at MRC. Soil treatments were applied using a tractor-mounted soil injection system calibrated to deliver 78.5, 109.8, 152.4, and 183.8 kg/ha chloropicrin into prepared seedbeds four-row plots (22.9-cm by 21.3-m at 86.4-cm row spacing)

on 13 Oct, 2011. The seed beds were replicated four times in a randomized complete block design. Potato seed pieces cv. 'FL 1879' were planted on 8 May, 2012. Oxamyl was applied as an in-furrow at planting treatment at 4.9 L/ha on 8 May, 2012 followed by an application at hilling (2.5 L/ha) on 12 Jun, 2012. Fertilizer was applied and weeds, insects and foliar diseases were controlled as described above. Plots (center 2 rows x 21.3-m) were harvested on 24 Oct, 2012 following plant desiccation and PCS was measured as described above (169 DAP).

An additional field trial to determine the efficacy of some fungicides, bactericides and biofungicides was planted with potato cv. 'Snowden' on 24 May, 2012 at MRC. Potato seed pieces were planted into two-row by 4.6-m plots (ca. 22.9-cm between plants at 86.4-cm row spacing) replicated four times within a complete randomized block design. In-furrow at planting applications of *Bacillus subtilis* (Serenade Soil 1.34SC, Bayer CropSience), *Streptomyces lydicus* (Actinogrow 0.0371 WP, Sipcam Corp.), and pentachloronitrobenzene (PCNB, Amvac Corp.) were delivered in 9.35 L water/ha in a 17.8 cm band using a single XR11003VS nozzle (Lechler Inc., IL) at 206.8 KPa. Additional nitrogen was applied and weeds, insects and foliar diseases were controlled as described above. Plots (2 row x 4.6-m) were harvested on 5 Oct, 2012 following plant desiccation and PCS was measured as described above (134 DAP).

5.3 RESULTS

5.3.1 METEOROLOGICAL VARIABLES

Average daily air temperatures at CRC in 2012 were 16.1, 20.8, 24.2, 20.1, 15.6 and 9.6°C (May, Jun, Jul, Aug, Sep, Oct, respectively) and the number of days with maximum temperature >32.2°C was 1, 2, 15, 3, and 0. Average daily soil temperature at 10-cm depth over the same period was 16.8, 22.3, 25.6, 20.5, 16.8, and 10.4°C (Fig. 5.1-A). Over the same period,

average daily soil moisture (% of field capacity at 10-cm depth) was 19.9, 14.3, 9.9, 13.0, 10.1 and 14.6% and precipitation was 2.5, 2.5, 9.2, 8.4, 1.9 and 13.7 cm (Fig. 5.1-C). Supplemental irrigation was not included in any of the precipitation or soil moisture summaries.

Average daily air temperature at CRC in 2013 was 18.6, 20.8, 19.5, 15.4 and 16.7^{o}C (Jun, Jul, Aug, Sep, Oct, respectively) and the number of days with maximum temperature >32.2^{o}C was 0, 4, 0, 0, and 0. Average daily relative humidity (%) over the same period was 64.8, 71.4, 72.1, 72.7 and 74.7%. Average daily soil temperature at 10-cm depth was 21.3, 24.3, 19.8, 17.8 and 17.5^{o}C (Fig. 5.1-B). Average daily soil moisture (% of field capacity) at 10-cm depth over the same period was 37.4, 39.2, 37.8, 36.6 and 36.3% and precipitation was 7.9, 8.4, 8.1, 4.5 and 4.3 cm (Fig. 5.1-D).

Average daily air temperature at MRC in 2012 was 15.6, 20.8, 24.2, 20.1, 15.6 and 9.6 ^{o}C and the number of dates with maximum temperature >32.2^{o}C was 1, 2, 15, 3, 0 and 0 (May, Jun, Jul, Aug, Sept, Oct, respectively). Average daily soil temperature at 10-cm depth was 16.8, 22.3, 78.1, 25.6, 16.8, and 10.4 (Fig. 5.2-A). Average daily soil moisture at 10-cm depth (% of field capacity) over the same period was 19.9, 14.3, 9.9, 13.0, 10.1 and 14.6 and precipitation was 2.5, 2.5, 9.2, 8.4, 1.9 and 13.7 cm (Fig. 5.2-C)

Average daily air temperature at MRC in 2013 was 16.7, 18.9, 20.8, 19.6, and 15.8^{o}C and the number of days with maximum temperature >32.2^{o}C was 0, 0, 3, 0 and 0 (May, Jun, Jul, Aug, and Sep, respectively). Average daily relative humidity (%) over the same period was 71.4, 70.7, 72.6, 72.0 and 74.1%. Average daily soil temperature at 10-cm depth over the same period was 18.2, 22.6, 26.7, and 24.7 (Fig. 5.2-B). Average daily soil moisture at 10-cm depth (% of field capacity) over the same period was 30.9, 35.4, 37.8, 36.9 and 35.9% and 19.8^{o}C and precipitation was 9.8, 5.7, 3.4, 12.6, and 3.4 cm (Fig. 5.2-D).
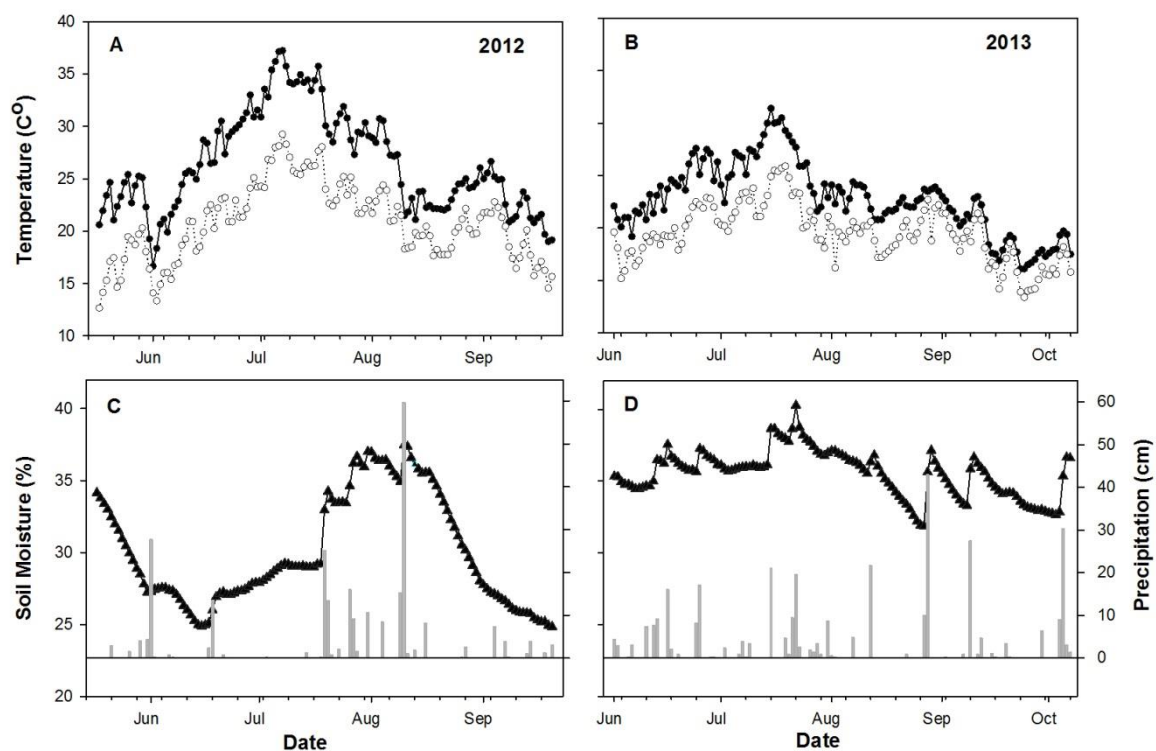
Figure 5.1 Summary of the 2012 and 2013 meteorological data at the Clarksville Research Center, Michigan State University, Clarksville, MI. Top graphs show the minimum (open circle) and maximum (black circle) soil temperature (^{o}C), at a 10-cm depth for each day throughout the growing season (A = 2012 and B = 2013). Bottom graphs show the average (black triangle) soil moisture (%) collected from four soil moisture probes and the amount (grey vertical bar) of precipitation (cm) received each day throughout the growing season (C = 2012 and D = 2013). Supplemental irrigation is not included.
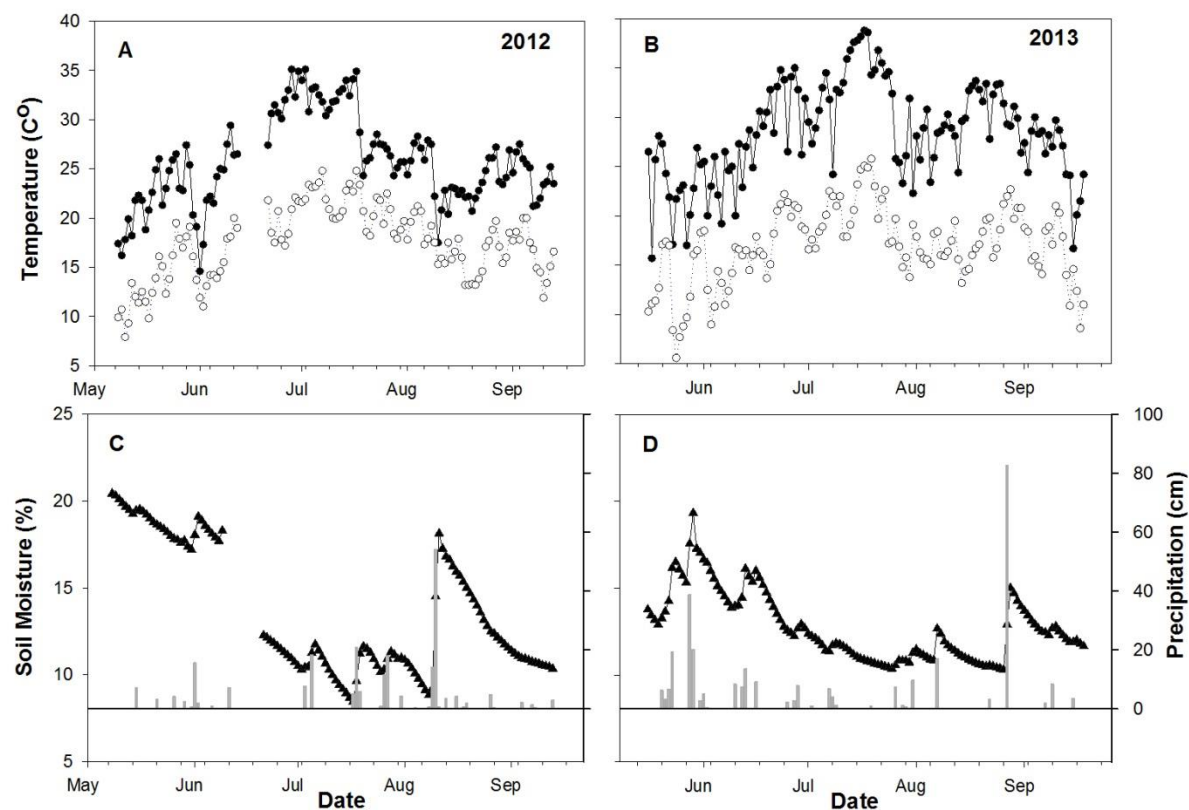
Figure 5.2 Summary of the 2012 and 2013 meteorological data at the Montcalm Research Center, Michigan State University, Entrican, MI. Top graphs show the minimum (open circle) and maximum (black circle) soil temperature ^{o}C, at a 10-cm depth for each day throughout the growing season (A= 2012 and B= 2013). Bottom graphs show the average (black triangle) soil moisture (%) collected from four soil moisture probes and the amount (grey vertical bar) of precipitation (cm) received each day throughout the growing season (C= 2012 and D= 2013). Supplemental irrigation is not included. The gaps in the 2012 graphs are because meteorological variables were not collected for that period.

In the preliminary field trial at MRC in 2012 there were no significant differences between tillage treatments and ES on overall scab incidence or severity ($p > 0.05$). PCS was severe in this trial and the total incidence was in excess of 97%. However, upon analyzing the lesion classes individually, significant differences in PCS severity were identified within the severity group 6 (SG − 6; pitted coalescing lesion) classification (Fig. 1.3-f); (Table 5.1). The chisel plow + ES treatment had a significantly lower percent incidence and scab index for the SG − 6 class compared to all other treatments. Application of ES produced inconsistent results for soil pH, but did reduce the pH in the ES treatment plots, with the exception of two replications. The average pH of the site was 6.2 and averaged 6.1 following the application of ES (the pH will normally become more alkaline during the growing season). The plants growing in the moldboard treated plots remained healthy for a longer period of time compared to the chisel treatment based on visual assessment of the average percent necrotic tissue in the canopy. The total amount of necrotic tissue averaged 85 and 95% for the chisel treatment and 45 and 50% for the moldboard treatment on 25 Sept and 9 Aug, respectively. Total yield ranged from 24.2 to 32.0 t/ha and was not significantly different among treatments (Table 5.1).

In the other preliminary field trial at CRC in 2012, there were no significant differences between tillage treatments on overall scab incidence and no significant effect on total yield ($p > 0.05$; Table 5.2). Total yield ranged from 18.5 to 21.0 t/ha. PCS was severe in this trial and overall incidence was 100% for all treatments. The moldboard plow treatment significantly reduced the overall scab severity compared to the chisel plow and minimal disturbance treatments. Upon analyzing the lesion classes individually, the moldboard plow treatment

significantly reduced PCS incidence and severity for the lesion type class SG − 6 class compared to the chisel plow and minimal disturbance treatment.

At MRC in 2013 there were no significant differences between tillage and ES treatments on overall scab incidence or severity or within individual lesion type class SG − 6 ($p > 0.05$; Table 5.3). PCS was severe in this trial and the incidence was 96% or greater for all treatments with overall severity ranging from 11.8 to 13.1. The moldboard plow treatments had significantly higher total yield (29.1 t/ha) compared to the minimal disturbance treatment (20.9 t/ha), and the chisel plow treatments (25.1 t/ha) were not statistically different from the other two tillage methods (Table 5.3). Total yield ranged from 22.4 to 28.0 t/ha in the sulfur treatments and there were no significant differences among treatments (Table 5.3). There were no significant interactions between ES and tillage practices. The pH ranged from 6.4 to 7.6 before the application of ES and ranged from 6.0 to 7.2 following the application of ES. Average pH was 6.8, 6.7, and 6.5 for 0, 224, and 448 kg ES/ha treatments, respectively.

At CRC, 2013, there were no significant differences between plowing treatments or ES treatments on overall scab incidence or severity or within individual lesion type class SG − 6 ($p > 0.05$; Table 5.4). There were no significant interactions between ES and tillage practices. PCS was severe in this trial and the incidence was 98% or greater for all treatments with overall severity ranging from 11.9 to 13.1. Total yield ranged from 20.9 to 28.029 t/ha with no significant differences between tillage or ES treatments (Table 5.4). The pH ranged from 6.2 to 7.1 before the application of ES and ranged from 6.0 to 7.0 following the application of ES. Average pH was 6.8, 6.6, and 6.5 for 0, 224, and 448 kg ES/ha treatments, respectively.

Table 5.1 Effects of soil tillage practices (moldboard and chisel plow) and elemental sulfur (ES; Tiger 90; 0 and 448 kg ES/ha) on the incidence and severity of potato common scab and total potato yield at the Montcalm Research Center, Michigan State University, Entrican, MI in 2012.

Treatment	Common scab incidence and severity index			
	Incidence SG6[a] (%)	Scab Index SG6[b] (0-100)	Scab Index Overall[c]	Total Yield (t/ha)
Moldboard – Sulfur…...	51.8 a[d]	38.3 a	17.3 ns[e]	29.1 ns
Moldboard + Sulfur.......	45.5 ab	28.3 ab	15.8	24.2
Chisel – Sulfur…...........	34.0 b	27.8 a	14.6	25.8
Chisel + Sulfur……….	36.0 a	23.8 b	14.4	32.0

[a] Severity of common scab was measured as an index calculated by counting the number of tubers (n = 50) falling in class 0:0= 0%; 1:1 to 1:6; 2:1 to 2:6; 3.1 to 3:6; 4.1 to 4:6; 5.1 to 5:6; and 6.1 to 6:6 where the first number is the type of lesion (0= no lesions; 1= superficial discrete; 2= coalescing superficial; 3= raised discrete; 4= raised coalescing; 5= pitted discrete; 6= pitted coalescing surface area of tuber covered with tuber lesions (surface and pitted) and the second number is surface area affected (1= 1 lesion to 2%; 2= 2.1-5%; 3= 5.1-10%; 4= 10.1-25%; 5= 25.1%-50%; 6, > 50% surface area). These incidence data are for Scab Severity Group 6 only.

[b] Severity index data are for Scab Severity Group 6 only.

[c] Weighted Severity index data are for Scab Severity Groups 1 through 6; each severity index 1 through 6 was multiplied by 1, 2, 3, 4, 5 and 6, respectively then divided by a constant (21) to express the severity data as an index from 1–100.

[d] Values followed by the same letter are not significantly different at $p = 0.05$ (Honest Significant Difference; Tukey Multiple Comparison).

[e] ns= no significant differnces among treatment means.

Table 5.2 Effects of soil tillage practices (moldboard plow, chisel plow, and minimal disturbance) on the incidence and severity of potato common scab and total potato yield at the Clarksville Research Center, Michigan State University, Clarksville, MI in 2012.

Treatment	Common scab incidence and severity index			
	Incidence SG6[a] (%)	Scab Index SG6[b] (0-100)	Scab Index Overall[c]	Total Yield (t/ha)
Moldboard……….…....	56.0 b[d]	39.6 b	10.9 b	21.0 ns[e]
Chisel…….....…..…….	80.5 a	65.3 a	12.7 a	18.5
Minimal disturbance….	82.0 a	64.6 a	12.6 a	19.4

[a] Severity of common scab was measured as an index calculated by counting the number of tubers (n = 50) falling in class 0:0= 0%; 1:1 to 1:6; 2:1 to 2:6; 3.1 to 3:6; 4.1 to 4:6; 5.1 to 5:6; and 6.1 to 6:6 where the first number is the type of lesion (0= no lesions; 1= superficial discrete; 2= coalescing superficial; 3= raised discrete; 4= raised coalescing; 5= pitted discrete; 6= pitted coalescing surface area of tuber covered with tuber lesions (surface and pitted) and the second number is surface area affected (1= 1 lesion to 2%; 2= 2.1-5%; 3= 5.1-10%; 4= 10.1-25%; 5= 25.1%-50%; 6, > 50% surface area). These incidence data are for Scab Severity Group 6 only.
[b] Severity index data are for Scab Severity Group 6 only.
[c] Weighted Severity index data are for Scab Severity Groups 1 through 6; each severity index 1 through 6 was multiplied by 1, 2, 3, 4, 5 and 6, respectively then divided by a constant (21) to express the severity data as an index from 1–100.
[d] Values followed by the same letter are not significantly different at $p = 0.05$ (Honest Significant Difference; Tukey Multiple Comparison).
[e] ns= no significant differnces among treatment means.

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Table 5.3 Effects of soil tillage practices (moldboard plow, chisel plow, and minimal disturbance) and elemental sulfur (ES; Tiger 90; 0, 224, and 448 kg ES/ha) on the incidence and severity of potato common scab and total potato yield at the Montcalm Research Center, Michigan State University, Entrican, MI in 2013.

| Treatment | Common scab incidence and severity index | | | |
	Incidence SG6[a] (%)	Scab Index SG6[b] (0-100)	Scab Index Overall[c]	Total Yield (t/ha)
Chisel…………………	75.1 ns[d]	63.5 ns	13.1 ns	25.1 ab[e]
Moldboard……….……	70.3	55.2	11.8	29.1 a
Minimal disturbance.....	79.5	65.4	12.9	20.9 b
0 kg Sulfur……..…..…	76.0 ns	62.3 ns	12.5 ns	22.4 ns
224 kg Sulfur…………	73.2	60.9	12.9	24.7
448 kg Sulfur…….…...	75.7	60.8	12.5	28.0

[a] Severity of common scab was measured as an index calculated by counting the number of tubers (n = 50) falling in class 0:0= 0%; 1:1 to 1:6; 2:1 to 2:6; 3.1 to 3:6; 4.1 to 4:6; 5.1 to 5:6; and 6.1 to 6:6 where the first number is the type of lesion (0= no lesions; 1= superficial discrete; 2= coalescing superficial; 3= raised discrete; 4= raised coalescing; 5= pitted discrete; 6= pitted coalescing surface area of tuber covered with tuber lesions (surface and pitted) and the second number is surface area affected (1= 1 lesion to 2%; 2= 2.1-5%; 3= 5.1-10%; 4= 10.1-25%; 5= 25.1%-50%; 6, > 50% surface area). These incidence data are for Scab Severity Group 6 only.
[b] Severity index data are for Scab Severity Group 6 only.
[c] Weighted Severity index data are for Scab Severity Groups 1 through 6; each severity index 1 through 6 was multiplied by 1, 2, 3, 4, 5 and 6, respectively then divided by a constant (21) to express the severity data as an index from 1–100.
[d] ns= no significant differnces among treatment means.
[e] Values followed by the same letter are not significantly different at $p = 0.05$ (Honest Significant Difference; Tukey Multiple Comparison).

Table 5.4 Effects of tillage type (moldboard plow, chisel plow, and minimal disturbance) and elemental sulfur (ES; Tiger 90; 0, 224, and 448 kg ES/ha) on the incidence and severity of potato common scab and total potato yield at the Clarksville Horticultural Experiment Station, Michigan State University, Clarksville, MI in 2013.

| Treatment | Common scab incidence and severity index | | | |
	Incidence SG6[a] (%)	Scab Index SG6[b] (0-100)	Scab Index Overall[c]	Total Yield (t/ha)
Chisel…………….……	83.0 ns[d]	68.1 ns	13.1 ns	19.7 ns
Moldboard……….……	84.4	66.4	12.5	15.7
Minimal disturbance.....	76.5	57.2	11.9	15.4
0 kg Sulfur……….……	80.5	65.4	12.8	18.4
224 kg Sulfur…………	82.8	64.0	12.4	17.2
448 kg Sulfur………....	80.5	52.3	12.3	15.2

[a] Severity of common scab was measured as an index calculated by counting the number of tubers (n = 50) falling in class 0:0= 0%; 1:1 to 1:6; 2:1 to 2:6; 3.1 to 3:6; 4.1 to 4:6; 5.1 to 5:6; and 6.1 to 6:6 where the first number is the type of lesion (0= no lesions; 1= superficial discrete; 2= coalescing superficial; 3= raised discrete; 4= raised coalescing; 5= pitted discrete; 6= pitted coalescing surface area of tuber covered with tuber lesions (surface and pitted) and the second number is surface area affected (1= 1 lesion to 2%; 2= 2.1-5%; 3= 5.1-10%; 4= 10.1-25%; 5= 25.1%-50%; 6, > 50% surface area). These incidence data are for Scab Severity Group 6 only.
[b] Severity index data are for Scab Severity Group 6 only.
[c] Weighted Severity index data are for Scab Severity Groups 1 through 6; each severity index 1 through 6 was multiplied by 1, 2, 3, 4, 5 and 6, respectively then divided by a constant (21) to express the severity data as an index from 1–100.
[d] ns= no significant differnces among treatment means.

Soil oxygen (%) patterns were similar among all tillage treatments throughout most of the year in 2012 or 2013, with a few exceptions (Fig. 5.1). There were no significant differences in soil oxygen (%) among tillage treatments in 2012 or 2013 ($p > 0.05$). In 2012, average soil oxygen content of four soil oxygen probes from hilling to harvest were 19.4, 19.5, and 19.7% for chisel plow, moldboard plow, and minimal disturbance treatments, respectively. In 2013, average soil oxygen content of four soil oxygen probes from hilling to harvest were 19.6, 19.5, and 19.4% for chisel plow, moldboard plow, and minimal disturbance treatments, respectively. Average soil oxygen content was within the expected range, which is usually between 19 and 20% (Sullivan and Krieger 2001). In 2012, soil oxygen levels remained constant until around Aug, when soil oxygen (%) decreased in the chisel plow treatment compared to the other tillage methods. In 2013, the minimal disturbance had lower soil oxygen (%) early in the season, compared to the other tillage treatments, then in late Jul soil oxygen increased and remained consistent with other treatments.
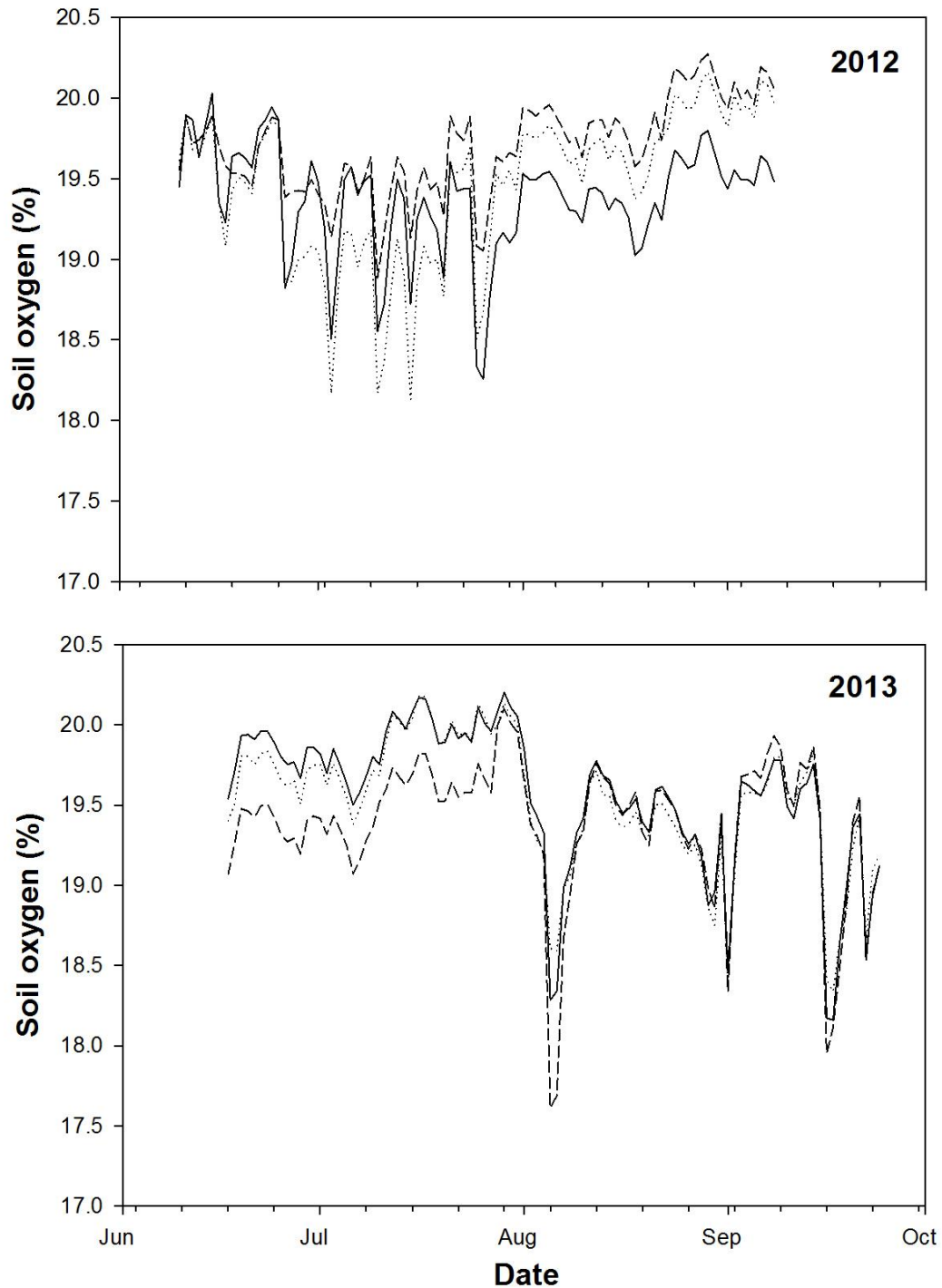
Figure 5.3 Summary of the 2012 and 2013 soil oxygen (%) content at the Clarksville Research Center, Michigan State University, Clarksville, MI. Chisel plow (solid line), moldboard plow (dotted line), minimal disturbance (dashed line) represent the average soil oxygen (%) collected from four soil oxygen sensors for each day starting 2-h after hilling, when the oxygen sensors were positioned in potato hill.

At MRC, there were significant differences between tillage treatments and potato cultivar on the overall scab severity index [($p < 0.05$); Table 5.5]. PCS overall incidence was 96% or greater for all treatments with overall severity values ranging from 10.2 to 13.4. Moldboard plowing significantly reduced PCS overall severity compared to chisel and minimal disturbance treatments. 'Dark Red Norland' and 'Snowden' had a significantly lower PCS overall severity indices compared to 'Russet Norkotah'. Upon analyzing the lesion classes individually, PCS incidence and severity of PCS within the lesion type class group 6 (SG − 6) were significantly lower for 'Dark Red Norland' compared to other cultivars (Table 5.5). All three cultivars had significant differences in total yield, with 'Dark Red Norland' the highest (35.1 t/ha), followed by 'Snowden' (24.1 t/ha), and 'Russet Norkotah' (16.4 t/ha). There were significant interactions between cultivar and tillage treatments in relation to total yield and the overall PCS severity index ($p < 0.05$) with 'Dark Red Norland' * Moldboard having statistically higher total yield at 42.4 t/ha compared to other treatments (Table 5.5).

At CRC, there were no significant differences between tillage treatments and cultivar on scab incidence or severity and no significant effect on total yield ($p > 0.05$; Table 5.6). There were no significant differences among treatments in the individual lesion class SG − 6. Furthermore, there was no significant interaction between cultivar and tillage practices. PCS was severe in this trial and the overall incidence was 96% or greater for all treatments with overall severity values ranging from 10.6 to 12.3. Total yield ranged from 15.7 to 20.0 t/ha (Table 5.6).

Table 5.5 Effects of tillage type (moldboard plow, chisel plow, and minimal disturbance) and potato cultivar ('Snowden', 'Russet Norkotah', and 'Dark Red Norland') on the incidence and severity of potato common scab and total potato yield at the Montcalm Research Center, Michigan State University, Entrican, MI in 2013.

Treatment	Incidence SG6[a] (%)		Scab Index SG6[b] (0-100)		Scab Index Overall[c]		Total Yield (t/ha)	
Chisel…………….....…	60.8	ns[d]	47.7	ns	12.1	a[e]	23.1	ns
Moldboard………….…	50.2		34.8		10.6	b	28.9	
Minimal disturbance.......	61.1		47.6		12.0	a	23.5	
Snowden……….....……	73.2	a	51.5	a	10.8	b	24.1	b
Russet Norkotah….........	59.3	a	50.2	a	12.4	a	16.4	c
Dark Red Norland……...	39.5	b	28.4	b	11.5	ab	35.1	a
Moldboard * Red………	37.0	c	24.6	d	10.9	bc	42.37	a
Chisel * Red…………....	45.5	bc	35.1	bcd	12.4	ab	31.83	b
Minimal dist. * Red…...	36.0	c	25.7	cd	11.1	bc	31.2	b
Moldboard * Snowden...	72.0	ab	49.6	a-d	10.2	c	27.9	b
Chisel * Snowden……...	78.0	a	54.2	ab	10.6	bc	22.3	bc
Minimal dist. * Snowden	69.5	ab	50.7	abc	11.5	abc	21.9	bc
Minimal dist. * Russet…	77.5	a	66.4	a	13.4	a	17.6	c
Moldboard * Russet……	41.5	c	30.3	bcd	10.6	bc	16.5	c
Chisel * Russet………….	59.0	abc	54.0	ab	13.3	a	15.0	c

[a] Severity of common scab was measured as an index calculated by counting the number of tubers (n = 50) falling in class 0:0= 0%; 1:1 to 1:6; 2:1 to 2:6; 3.1 to 3:6; 4.1 to 4:6; 5.1 to 5:6; and 6.1 to 6:6 where the first number is the type of lesion (0= no lesions; 1= superficial discrete; 2= coalescing superficial; 3= raised discrete; 4= raised coalescing; 5= pitted discrete; 6= pitted coalescing surface area of tuber covered with tuber lesions (surface and pitted) and the second number is surface area affected (1= 1 lesion to 2%; 2= 2.1-5%; 3= 5.1-10%; 4= 10.1-25%; 5= 25.1%-50%; 6, > 50% surface area). These incidence data are for Scab Severity Group 6 only.
[b] Severity index data are for Scab Severity Group 6 only.

Table 5.5 (cont'd)

[c] Weighted Severity index data are for Scab Severity Groups 1 through 6; each severity index 1 through 6 was multiplied by 1, 2, 3, 4, 5 and 6, respectively then divided by a constant (21) to express the severity data as an index from 1–100.

[d] ns= no significant differnces among treatment means.

[e] Values followed by the same letter are not significantly different at $p = 0.05$ (Honest Significant Difference; Tukey Multiple Comparison) and analyses for main effects of cultivation type, cultivar and interactions between the variables were discrete.

Table 5.6 Effects of tillage type (moldboard plow, chisel plow, and minimal disturbance) and cultivar ('Snowden', 'Russet Norkotah', and 'Dark Red Norland') on the incidence and severity of potato common scab and total potato yield at the Clarksville Research Center, Michigan State University, Clarksville, MI in 2013.

Treatment	Common scab incidence and severity index			
	Incidence SG6[a] (%)	Scab Index SG6[b] (0-100)	Scab Index Overall[c]	Total Yield (t/ha)
Chisel…………….……	42.3 ns[d]	30.4 ns	12.0 ns	19.7 ns
Moldboard……….……	33.7	21.5	11.7	17.9
Minimal disturbance.....	36.4	44.4	10.8	16.6
Snowden…………..……	54.6 a[e]	47.1	11.4	15.7
Russet Norkotah…........	33.5 ab	26.2	12.3	18.2
Dark Red Norland….....	24.3 b	23.0	10.6	20.0

[a] Severity of common scab was measured as an index calculated by counting the number of tubers (n = 50) falling in class 0:0= 0%; 1:1 to 1:6; 2:1 to 2:6; 3.1 to 3:6; 4.1 to 4:6; 5.1 to 5:6; and 6.1 to 6:6 where the first number is the type of lesion (0= no lesions; 1= superficial discrete; 2= coalescing superficial; 3= raised discrete; 4= raised coalescing; 5= pitted discrete; 6= pitted coalescing surface area of tuber covered with tuber lesions (surface and pitted) and the second number is surface area affected (1= 1 lesion to 2%; 2= 2.1-5%; 3= 5.1-10%; 4= 10.1-25%; 5= 25.1%-50%; 6, > 50% surface area). These incidence data are for Scab Severity Group 6 only.

[b] Severity index data are for Scab Severity Group 6 only.

[c] Weighted Severity index data are for Scab Severity Groups 1 through 6; each severity index 1 through 6 was multiplied by 1, 2, 3, 4, 5 and 6, respectively then divided by a constant (21) to express the severity data as an index from 1–100.

[d] ns= no significant differnces among treatment means.

[e] Values followed by the same letter are not significantly different at $p = 0.05$ (Honest Significant Difference; Tukey Multiple Comparison) for effect of cultivar only.

There were no significant differences between ammonium sulfate (AS) treatments on overall scab incidence or severity compared to the control ($p > 0.05$; Table 5.7). PCS was severe in this trial with an overall incidence of 100%. There were no significant differences in PCS incidence or severity identified in lesion type group 6 [SG – 6, (Table 5.7)]. Total yield ranged from 23.4 to 24.6 t/ha and was not statistically different among treatments. ES had inconsistent results based on pH responses, but did reduce the pH on all treatments consisting of 280 kg AS/ha and the treatment consisting of 140 kg AS/ha had variable effects on pH. The average pH of the site was 6.4 to 6.8 before AS application and was 6.2 to 6.8 after, with no significant effect on soil pH in this study ($p > 0.05$).

Table 5.7 Effects of ammonium sulfate (AS) at 0, 140, and 280 kg AS/ha on the incidence and severity of potato common scab and total yield at the Clarksville Horticultural Research Station, Michigan State University, Clarksville, MI in 2012.

	Common scab incidence and severity index			
Treatment	Incidence SG6[a] (%)	Scab Index SG6[b] (0-100)	Scab Index Overall[c]	Total Yield (t/ha)
AS 280 kg/ha……..........	56.5 ns[d]	44.3 ns	12.6 ns	24.2 ns
AS 140 kg/ha…...…….	49.5	37.5	12.2	24.6
Untreated Check….......	47.0	35.9	12.5	23.4

[a] Severity of common scab was measured as an index calculated by counting the number of tubers (n = 50) falling in class 0:0= 0%; 1:1 to 1:6; 2:1 to 2:6; 3.1 to 3:6; 4.1 to 4:6; 5.1 to 5:6; and 6.1 to 6:6 where the first number is the type of lesion (0= no lesions; 1= superficial discrete; 2= coalescing superficial; 3= raised discrete; 4= raised coalescing; 5= pitted discrete; 6= pitted coalescing surface area of tuber covered with tuber lesions (surface and pitted) and the second number is surface area affected (1= 1 lesion to 2%; 2= 2.1-5%; 3= 5.1-10%; 4= 10.1-25%; 5= 25.1%-50%; 6, > 50% surface area). These incidence data are for Scab Severity Group 6 only.
[b] Severity index data are for Scab Severity Group 6 only.
[c] Weighted Severity index data are for Scab Severity Groups 1 through 6; each severity index 1 through 6 was multiplied by 1, 2, 3, 4, 5 and 6, respectively then divided by a constant (21) to express the severity data as an index from 1–100.
[d] ns= no significant differnces among treatment means.

Severe PCS developed on the majority of tubers from all treatments in the chloropicrin and oxamyl trial and overall incidence was close to 95% on average, discounting treatment effects. There was 70% incidence within the lesion classification group 6 (SG − 6) alone. Treatments with mean incidence of scab in the SG − 6 class ranged from 66.5 to 76.5, and no treatments were significantly different from the untreated control (Table 5.8). Treatments within the SG − 6 severity classification ranged from 49.6 to 59.7% and no treatment had significantly lower indices in comparison to the untreated control. The mean severity of scab in the weighted overall scab index rating ranged from 18.9 to 21.2 and no treatments had significantly lower indices in comparison to the untreated control, although chloropicrin (104.4 kg/ha) significantly reduced PCS in comparison to the oxamyl program. Total yield ranged from 37.3 to 50.0 t/ha. No soil treatment increased total yield in comparison to the untreated control, although chloropicrin (104.4g kg/ha) significantly increased total yield in comparison to the oxamyl program (Table 5.8). No symptoms of phytotoxicity were observed in this trial.

Common scab was severe in the crop protection efficacy trial with 98% or greater overall incidence for all treatments. Treatments with mean scab incidence in the SG − 6 class ranged from 55.0 to 69.5%, and no treatments were significantly different from the untreated control (Table 5.9). Treatments with mean severity of scab in the SG − 6 class ranged from 41.5 to 54.1 and no treatment had significantly lower indices in comparison to the untreated control (Table 5.9). Overall weighted scab index rating ranged from 15.5 to 18.1 and no treatments had significantly lower indices in comparison to the untreated control. *Streptomyces lydicus* had significantly higher SG − 6 and overall weighted scab indices compared to the untreated control.

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Total yield ranged from 17.7 (untreated control) to 24.2 t/ha and treatments were not significantly different (Table 5.9). No phytotoxicity symptoms were observed in this trial.

Table 5.8 Efficacy of crop protection programs on the incidence and severity of potato common scab and total potato yield at the Montcalm Research Center, Michigan State University, Entrican, MI in 2011.

Treatment	Common scab incidence and severity index			
	Incidence SG6[a] (%)	Scab Index SG6[b] (0-100)	Scab Index Overall[c]	Total Yield (t/ha)
Oxamyl 4.9 L/ha (B[d]);				
Oxamyl 2.5 L/ha (C)………..……...	72.0 ns[e]	58.5 ns	21.2 a[f]	37.3 b
Chloropicrin 78.5 kg/ha (A)………..	71.5	58.5	21.0 ab	39.3 ab
Chloropicrin104.0 kg/ha (A)…….....	66.5	49.6	18.9 b	46.0 a
Chloropicrin 152.0 kg/ha (A)………	70.0	52.8	19.1 ab	43.5 ab
Chloropicrin 184.0 kg/ha (A)………	76.5	59.7	20.4 ab	41.1 ab
Untreated Check……………....……	71.5	54.8	19.2 ab	42.0 ab

[a] Severity of common scab was measured as an index calculated by counting the number of tubers (n = 50) falling in class 0:0= 0%; 1:1 to 1:6; 2:1 to 2:6; 3.1 to 3:6; 4.1 to 4:6; 5.1 to 5:6; and 6.1 to 6:6 where the first number is the type of lesion (0= no lesions; 1= superficial discrete; 2= coalescing superficial; 3= raised discrete; 4= raised coalescing; 5= pitted discrete; 6= pitted coalescing surface area of tuber covered with tuber lesions (surface and pitted) and the second number is surface area affected (1= 1 lesion to 2%; 2= 2.1-5%; 3= 5.1-10%; 4= 10.1-25%; 5= 25.1%-50%; 6, > 50% surface area). These incidence data are for Scab Severity Group 6 only.

[b] Severity index data are for Scab Severity Group 6 only.

[c] Weighted Severity index data are for Scab Severity Groups 1 through 6; each severity index 1 through 6 was multiplied by 1, 2, 3, 4, 5 and 6, respectively then divided by a constant (21) to express the severity data as an index from 1–100.

[d] Application timings, A= Soil treatments were applied using a tractor-mounted soil injection system calibrated to deliver 78.5, 109.8, 152.4, and 183.8 kg/ha chloropicrin into prepared seedbeds four-row plots (22.9-cm by 21.3-m at 86.4-cm row spacing) on 13 Oct, 2011.; B= Oxamyl was applied as an in-furrow at planting treatment at 4.9 L/ha on 8 May, 2012; C= Oxamyl was applied at hilling (2.5 L/ha) on 12 Jun, 2012.

[e] ns= no significant differnces among treatment means.

[f] Values followed by the same letter are not significantly different at $p = 0.05$ (Honest Significant Difference; Tukey Multiple Comparison).

Table 5.9 Efficacy of crop protection programs on the incidence and severity of potato common scab and total potato yield at the Montcalm Research Center, Michigan State University, Entrican, MI in 2012.

Treatment	Common scab incidence and severity index			
	Incidence SG6[a] (%)	Scab Index SG6[b] (0-100)	Scab Index Overall[c]	Total Yield (t/ha)
B. subtilis 4.7 L/ha (A[d])……...….	63.5 abc[e]	45.8 a-d	16.1 abc	22.9 a
B. subtilis 9.4 L/ha (A)……...….	55.0 c	42.3 cd	15.5 c	24.2 a
PCNB 11.7 L/ha (A)……….....….	58.0 bc	41.5 d	15.6 bc	22.0 ab
B. subtilis 4.7 L/ha +				
PCNB 5.9 L/ha (A)………….…..	58.0 bc	43.6 bcd	16.1 abc	22.8 a
S. lydicus 1.7 kg/ha (A)………...	69.5 a	54.1 a	18.1 a	23.8 a
Untreated Check…………….....	60.0 abc	43.1 bcd	15.7 bc	17.7 ab

[a] Severity of common scab was measured as an index calculated by counting the number of tubers (n = 50) falling in class 0:0= 0%; 1:1 to 1:6; 2:1 to 2:6; 3.1 to 3:6; 4.1 to 4:6; 5.1 to 5:6; and 6.1 to 6:6 where the first number is the type of lesion (0= no lesions; 1= superficial discrete; 2= coalescing superficial; 3= raised discrete; 4= raised coalescing; 5= pitted discrete; 6= pitted coalescing surface area of tuber covered with tuber lesions (surface and pitted) and the second number is surface area affected (1= 1 lesion to 2%; 2= 2.1-5%; 3= 5.1-10%; 4= 10.1-25%; 5= 25.1%-50%; 6, > 50% surface area). These incidence data are for Scab Severity Group 6 only.

[b] Severity index data are for Scab Severity Group 6 only.

[c] Weighted Severity index data are for Scab Severity Groups 1 through 6; each severity index 1 through 6 was multiplied by 1, 2, 3, 4, 5 and 6, respectively then divided by a constant (21) to express the severity data as an index from 1–100.

[d] Application timings, A= Soil treatments were applied in furrow at planting into prepared seedbeds on 13 Oct, 2011.

[e] Values followed by the same letter are not significantly different at $p = 0.05$ (Honest Significant Difference; Tukey Multiple Comparison).

5.4 DISCUSSION

The locations of the field trials (MRC and CRC) were ideal because a) these sites have soil types typical of the majority of the soils where potatoes are grown in MI; and b) have a history of PCS. The trials were conducted using PCS susceptible cultivars (Lindholm et al., 2007) grown in Michigan for chip and tablestock production (Douches et al., 2012; 2013). Environmental conditions were conducive for PCS in 2012 and 2013 with overall incidence 96% or greater in all trials across all cultivars and experimental treatments. The exact environmental conditions that are conducive to PCS in potatoes are elusive (Epp, 2013); however, in Michigan there tends to be an annual confluence of such conditions (Loria et al., 2001; Wharton et al., 2007b; Hao et al., 2009; Rosenzweig et al., 2012; Wanner et al., 2014). In recent years national cultivar trials have been established and Michigan often has the highest incidence and severity (Wanner, 2009).

The application of elemental sulfur (ES) was implemented in the fall because it takes several months for it to oxidize to sulfate ions. The sulfate ions in the soil solution can then be readily utilized by plants or affect soil pH. In the ES experimental field trials, there were no significant effects on overall PCS incidence or severity (Tables 5.1; 5.3; 5.4). The application of ES did reduce soil pH in most experimental field trials (Tables 5.1; 5.3; 5.4), although the reduction was minimal overall. One possible explanation is that the conversion of elemental sulfur to sulfate ions (sulfur oxidation) did not take place until the following summer, after soil sampling in the spring. Microbial organisms in the soil that release hydrogen ions and reduce soil pH are greatly responsible for the oxidation process (Lawrence and Germida, 1988). Microbial activity is greatly reduced in the spring and fall and almost absent in the winter. Oxidation of

elemental sulfur reaction is a slow process and changes pH gradually over time, possibly contributing to the minimal effects in these studies.

Potato common scab causing species are known to persist at pH between 5.2 and 7.0 (Loria, 2001; Powelson and Rowe, 2008) and soils at MRC and CRC were within this range. Severity of PCS usually increases with increasing soil pH with an optimal range for infection at 5.0-8.0 (Lambert and Manzer, 1991). Lacey and Wilson (2001) reported that pH was one of strongest indicators of PCS infection for *S. scabies* and the threshold for infection was 5.0-5.2, with no scab symptoms observed in soils with pH 4.9 or less. The purpose of this study was to see what effect ES would have on incidence and severity of PCS, not to reduce the soil pH out of the optimal range. Excessive, possibly phytotoxic, ES would be needed to reduce soil pH to levels below the optimal range of scab infection at these locations, which would be very expensive and not a practical means for commercial potato growers to manage the disease. In addition, pH below 5.0 may interfere with nutrient uptake. Furthermore, some *Streptomyces* spp. are known to cause PCS at a pH as low as 4.5 (Lindholm et al., 1997) and these have been reported in Michigan (Hao et al., 2009). Thus, lowering the soil pH might not be an effective PCS management strategy.

Ammonium sulfate (AS) is one of the cheapest and easiest forms of sulfur to obtain in North America (Pavlista, 2005). Sulfate present in soil solution can be immediately taken up by plants and can alter soil pH (Pavlista, 2005). In the AS experimental trial, there were no significant differences between AS treatments on overall scab incidence or severity compared to the control. The application of AS did reduce soil pH in most experimental field plots, however the reduction was minimal and not sufficient to impact common scab. PCS was severe in the ammonium sulfate trial with an incidence of 100% regardless of treatment. The results of the

field trial were similar to that of Mizuno et al., (2000) who found a single application of ammonium sulfate was ineffective at controlling PCS in some soil types. Furthermore, emergence of the potato crop was variable in this field trial, thus tuber initiation occurred at different times. As an effective management strategy, AS must be incorporated into the hill at tuber initiation (Hammerschmidt et al., 1986). In this study, proper timing for the incorporation of AS at tuber initiation was attempted but unsuccessful due to the uneven plant stand.

Historically, sulfur has been used to reduce PCS severity with success in some locations (Dees and Wanner, 2012). Applications of sulfur to reduce common scab, without altering soil pH has been reported as early as the 1920's (Martin, 1923). The mechanism is not well known or understood, however the conversion of sulfur to volatile forms may act as a biocide (Pavlista, 2005). Another possibility is that the addition of sulfur could stimulate beneficial soil microbial communities that are antagonistic to plant pathogenic *Streptomyces* spp. (Kinkel et al., 2011; Meng et al., 2012), although analysis of the soil microbial community was not done in the current study studies.

Soil tillage practices can be used as a cultural management strategy to reduce potato diseases (Gudmestad et al., 2007). The impact of tillage on plant disease development has been highly variable, depending on the specific regional crop-pathogen-environment interactions (Sumner et al., 1981). In these experimental field trials, the impact of tillage practices were variable in relation to PCS incidence and severity. In a few trials, the moldboard plow treatment significantly reduced overall scab severity indices compared to the chisel plow and minimal-no till treatments (Table 5.2 and 5.5), although it was not reproduced at the alternate location or in subsequent years. A possible explanation is that the moldboard plow provided more soil inversion, thus burying PCS inoculum; however this was not measured. Moldboard plowing has

been shown to have positive effects in the management of some soil-borne potato diseases including potato early die, relative to conventional tillage (Gudmestad et al., 2007). However, chisel compared to moldboard plowing can reduce the incidence and severity of some potato diseases including Rhizoctonia stem and stolon canker, caused by *R. solani* (Leach et al., 1993). Furthermore, the tillage techniques had no significant effects on soil oxygen content. Soil oxygen content in these field trials averaged 19 to 20%, which is within the normal range for agriculture fields (Sullivan and Krieger, 2001), regardless of the tillage technique implemented.

Host resistance is thought to be the most important and practical method for controlling PCS (Loria et al., 2001; Haynes et al., 2007; 2010). However there are no cultivars completely resistant to PCS and tubers can become diseased especially when conditions are conducive and inoculum is plentiful (Loria, 2001; Wanner et al., 2014). The mechanism of cultivar tolerance to PCS is not well understood, which complicates effective and successful breeding. Despite this several cultivars have been developed that are very tolerant (Douches et al., 2012; 2013)

In the experimental trial using different potato cultivars at MRC, 'Dark Red Norland' and 'Snowden' had significantly lower PCS overall severity indices compared to 'Russet Norkotah'. 'Dark Red Norland' is considered a moderately susceptible cultivar compared to the other two highly susceptible cultivars (Lambert et al., 2006), but was not the case in the PCS cultivar trial in the current study. Reasons for 'Snowden' having significantly less PCS compared to 'Russet Norkotah' are unknown. 'Russet Norkotah' is one of the most susceptible cultivars to PCS based on a trial with 17 cultivars (Lambert et al., 2006). Douches et al., (2012; 2013) reported similar responses in potato cultivar performance in Michigan with 'Dark Red Norland' performing better than 'Russet Norkotah' in relation to PCS; however in those field experiments, Douches et al.,

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(2012; 2013), reported that 'Dark Red Norland' and 'Russet Norkotah' had lower PCS indices compared to 'Snowden'.

According to the field study at MRC, 'Dark Red Norland' interacted positively with moldboard tillage to reduce the incidence and severity of PCS and this combination could possibly be utilized as a management strategy for potatoes intended for the fresh market in fields with a history of PCS. At CRC, there were no significant differences between tillage treatments and cultivar on scab incidence or severity and no significant effect on total yield. Reasons for the variability in cultivar performance is unknown, but it appears cultivar performance can be site specific.

Chemical control and antimicrobial compounds can be used as a management strategy for PCS control, but have shown variable success (Dees and Wanner, 2012; Wanner et al., 2014). Common scab was severe in the crop protection program trial, with 98% or greater overall incidence (Table 5.8 and 5.9). Overall severity indices were significantly higher for the treatments containing *S. lydicus* compared to the untreated control. Treatments with pentachloronitrobenzene (PCNB) were not effective at controlling PCS in this field study. These results were different than Davis (1976), who reported a reduction of PCS with the application of PCNB; however PCNB was effective only when soil moisture levels were high in that study. In a more recent study, PCNB did not statistically reduce PCS compared to the untreated control (Jordan et al., 2011), but soil moisture was not measured in that trial.

The oxamyl/chloropicrin trial had a high incidence and severity of PCS. No treatments had significantly lower overall incidence or indices in comparison to the untreated control. Jordan et al. (2011) showed variable effects in PCS control with the application of chloropicrin, but total yield has been consistently reported to increase yield compared to non-fumigated

controls. In the current study, the chloropicrin treatments had no effect in total potato yield and were not statistically different from the untreated check.

Maintaining soil moisture levels near field capacity during the two-six weeks during tuber initiation can inhibit PCS infection (Lapwood, et al. 1973; Loria, 2001). Maintaining soil moisture is essential for control of PCS regardless of the management strategy (Davis et al., 1976). Soil moisture levels were near field capacity in 2012 and 2013 at MRC and in 2013 CRC, although this management strategy didn't appear to be effective in controlling PCS, similar to the report by Lapwood et al. (1973). Furthermore, a few studies have shown that irrigation can result in higher PCS levels (Larkin et al., 2011). Therefore, any single management strategy, such as maintaining soil moisture levels near field capacity, may not adequately minimize the occurrence and severity of PCS.

5.6 CONCLUSION

Potato common scab is an important disease that affects potato tuber quality. Effective PCS control remains elusive, partly due to the lack of knowledge in the biology of pathogenic *Streptomyces* spp. An increased understanding of the mechanisms of pathogenicity and virulence is needed to identify effective PCS management strategies. On a quality scale, many tubers harvested in these trials would be hard to market. No treatment in any trial reduced common scab incidence or severity to a level that would be acceptable to commercial potato growers or processors. The high levels of PCS would result in possible rejection or at a minimum a price reduction, due to poor quality. Overall, no treatment was effective in controlling PCS, possibly because environmental conditions were conducive for PCS during these field seasons, thus contributing to high disease pressure at both locations.

Results were inconsistent and varied depending on locations, similar to other studies. The pH was marginally lowered by the addition of ES in this study at all locations, and not reduced to a level that is required to reduce the incidence or severity of PCS. These field trials indicated that management of PCS requires an integrated approach that combines the use of host resistance, cultural control methods, and possibly chemical control methods. Further research is needed to identify factors contributing to potato common scab and identifying management strategies for adequate control.

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