MANAGEMENT OF SOIL AND SEED BORNE DISEASES OF POTATO (*SOLANUM TUBEROSUM*)

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ABSTRACT

MANAGEMENT OF SOIL AND SEED BORNE DISEASES OF POTATO (*SOLANUM TUBEROSUM*)

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There are numerous important potato diseases that are annual production concerns for growers in Michigan. This study included three projects. The first project evaluated commercial cultivars and advanced breeding lines (ABL) with foliar resistance to late blight via tuber tests, and integrated disease management with foliar resistant varieties/ABLs in combination with fungicide seed treatments against four of genotypes of *P. infestans*. Commercial cultivars and ABLs showed differential responses to genotypes of *P. infestans* based on both experiments. Overall, ABLs and commercial cultivars in combination with seed treatments performed better based on plant stand compared to inoculated non-treated controls. A second project evaluated conventional and quantitative PCR primers for detection of *Pectobacterium* and *Dickeya* spp. to monitor the presence and spread of these bacteria in the field and in seed-lots for certification. In two consecutive years samples from the field had both *Dickeya* and *Pectobacterium* spp. Whereas, tubers from seed-lots from Michigan seed production did not test positive for *Dickeya* spp. and overall relatively low levels of *Pectobacterium* spp. within acceptable tolerance. Three conventional PCR primers were reliable and were able to differentiate *Pectobacterium* down to species. Finally, a third project characterized *Streptomyces* spp. recovered from potato and turnip phenotypically based on virulence, pathogenicity, and morphologically, and determined the diversity and phylogenetic relationships among species. This study identified morphological, molecular and phylogenetically differences among *Streptomyces* spp. isolated from turnip and potato.

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IMPORTANCE OF THE POTATO

The potato (*Solanum tuberosum* L) is native to the Andes Mountain of South America, and has been cultivated for approximately 10,000 years (Salaman, 1970). Potatoes are the number one non-cereal food crop, and the third most important food crop after wheat (*Triticum aestivum* L.), and rice (*Oryza sativa* L.). Potatoes are grown in more than 100 countries, in temperate, subtropical and tropical regions. The top five producers of potato are China, India, the Russian Federation, Ukraine and the US producing 99.1, 43.7, 31.1, 21.8, and 19.9 million t, respectively (FAO, 2017). Potatoes are the leading vegetable in the US. Michigan ranks number 7 among other states in production of potatoes and produces approximately 0.81 million t annually.

The potato is in the *Solanaceae* family, which includes more than 90 genera and about 3000 species. Potato divides into 4 cultivated species *Solanum tuberosum*, *S. ajanhuiri*, *S. curtilobum*, and *S. juzepczurii,* and the most commonly grown species is *Solanum tuberosum* (Hawkes, 1990). The genome of the potato is complex due to chromosome numbers that can vary from diploid, triploid, tetraploid, pentaploid to hexaploid (Spooner and Bamberg, 1994).

SOIL AND SEED BORNE DISEASES

Potato is plagued by more than 40 pests and diseases caused by fungi, viruses, viroids, phytoplasma, oomycetes, insects and nematodes. Pathogens that cause disease on potatoes can be air, soil and seed borne and can cause damage on all parts of the plant (Fiers et al., 2011). Potatoes also are affected by abiotic disorders such as insufficient plant nutrients, excess moisture, low oxygen levels, drought and other stresses.

Commercial potatoes are usually grown from cut potato tuber pieces (Johnson and Powelson, 2008). This practice results in wounds that enable pathogens to enter the host during the cutting

and handling process. There are certain bacteria such as *Pectobacterium* and *Dickeya* that can cause seed decay. These bacteria produce pectolytic enzymes that break down cell wall structures and the middle lamella. Once the potato plant gets infected, plants tissue starts to decay and discoloration initiates from the base of the stem and makes its way up to the plant. Usually both of these bacteria can overwinter in tubers and initial inoculum is an infected tuber. If the tuber is infected, then it may decay during the storage or if the tuber is asymptomatic, it may decay in the soil after planting.

There are also foliar, and soilborne pathogens that infect potato at different growth stages. *Phytophthora infestans* causes late blight of potato and infections can start during seed handling and ultimately infect whole plant. This pathogen has a polycyclic disease cycle and can be a problem throughout the whole growing season. This disease primarily is managed by varietal resistance and fungicide application.

Another soilborne bacterial disease is common scab, caused by *Streptomyces* spp. These bacteria are saprophytes and live in the soil, but several cause diseases on potatoes and other crops. This disease is ranked as the fourth most important potato disease worldwide (Hao et al., 2009; Loria et al., 1997).

LATE BLIGHT OF POTATOES

INTRODUCTION

"Phytophthora infestans has been the subject of so many investigations and controversies that it fills one of the most romantic chapters in the history of biological research" – (Berg, 1926). This statement by Berg is still true today after more than 170 years. *Phytophthora infestans* is still one of the most destructive disease of potatoes, tomatoes and other members of the Solanaceous family

worldwide. Late blight is a threat in all potato production areas, but it is particularly important in regions with both rain-fed and irrigated agriculture, and moderate temperatures.

Late blight is an annual production concern in Michigan. Primary means of managing this disease rely on multiple foliar fungicide applications, and cultural practices. However, there are other management tools available, such as planting resistant cultivars, removing primary inoculum source (ex. volunteers) and seed treatments prior to planting.

Nomenclature. *Phytophthora infestans* was first named as *Botrytis infestans* by Montagne. Later, it was renamed to *Phytophthora infestans* by Anton de Bary. The current scientific and preferred name is *Phytophthora infestans* (Mont.) de Bary. Other common name is Phytophthora blight, and international common names are blight of potato, and blight of tomato (CABI, 2017).

Distribution. Late blight is common in many countries and in all areas where potatoes and tomatoes are grown. One of the reasons why it is widely distributed around the world is due to infected tubers. Infected tubers can be shipped and sold in places where late blight is not wide spread. If growers purchase and plant infected seeds, they may introduce late blight into areas where it has not been previously found or where it is uncommon.

Economic importance. Late blight is an extremely destructive pathogen when the weather conditions are favorable, and it can destroy an entire field within days (Fry et al., 1993). The disease can impact potato production in many ways such as reducing yield, lowering tuber quality, diminishing storability, and increasing cost for production due to the high number of fungicide applications (Nowicki et al., 2011). If left unmanaged, it can result complete crop failure.

Hosts. It is known that *P. infestans* infectious more than 80 species, but only 25% of these were able to produce lesions in the laboratory environment (Erwin and Ribeiro, 1996a). However, in

agriculture the two economically important main hosts are potato and tomato. These two crops can be grown together can promote late blight spread and infection from one host to another.

Symptoms. *Phytophthora infestans* infects leaves, stems, and tubers. Early symptoms on leaves and stems are small, dark and black lesions. In the presence of moisture and water, the lesions expand and become water-soaked. Lesions develop chlorotic borders, then quickly expand and become necrotic. Usually the first lesions appear on the lower leaves due to the microclimate under the canopy. However, if weather conditions are favorable, lesions might develop on the upper leaves as well. On the leaf, a lesion starts to develop near the petiole or at the edge of the leaf where water accumulates and/or dew stays longer. Lesions are not restricted by veins. White mycelia can develop on the abaxial side of the leaf. This mycelial growth is useful to differentiate late blight from other foliar diseases of potatoes. On stems, lesions can develop by either direct infection, or from direct extension from infected leaves. Lesions may start developing at the point of attachment to the stem, where water accumulates. Infected stems become weakened and may collapse, leading to plant death (Henfling, 1987). On tubers, late blight lesion is irregularly shaped and has brown to purplish areas on the skin. Under the skin there is a tan to brown discoloration of the tissue. Infected tuber tissue is usually dry and granular (Kirk et al., 2004).

CAUSAL ORGANISM (*PHYTOPHTHORA INFESTANS*)

Taxonomic description. *Phytophthora infestans* is an oomycete. It is in the order Peronosporales and the family Peronosporaceae. Until the latter part of the 20th century, based on morphologically and physiologically features, *Phytophthora* species was classified as a true fungi (Erwin and Ribeiro, 1996b). However, molecular techniques and biochemical analysis suggested that oomycetes do not share taxonomic affinity with fungi (Cooke and Lees, 2004).

Biology. The name *Phytophthora* came from the Greek *phyto* meaning plant and *phthora*-destroyer. It can reproduce both sexually and asexually. The asexual organism or mycelium requires a living host such as potato tubers, or other crop debris on solanaceous families to overwinter. On the other hand, sexual oospores can survive in the soil and in the absence of a host for many years (Drenth et al., 1995).

Asexual cycle. Asexual reproduction is common in Michigan and in the United States (Goodwin et al., 1998), and results in production of sporangia on both primary and secondary inoculum. Primary inoculum is sporangia, which can infect directly or indirectly. Direct infection occurs in humid and warm weather (<18° C), when sporangia directly infect leaves and/or tubers via germ tube and forms appressorium (Hardham, 2001). Indirect infection occurs if there is free water and the temperature is below 18° C. These conditions are favorable for motile zoospores to be released from the sporangia. Both sporangia and zoospores can infect the foliage and tubers. The zoospores encyst, form a germ tube, special structure apressorium and infection peg that directly penetrates the host tissue via an epidermal cell or through stomata. After infection mycelium can grow intra and intercellular. Another specialized feeding structure, haustoria, can form, but this happens rarely. Once infection takes place, this tissue become colonized and results in more mycelium that produce more sporangia and the infection process continues and spreads quickly, since both zoospores and sporangia spread via free water or wind. Tubers are infected when sporangia or zoospores are washed down into the soil. Mycelia can infect tubers via lenticels, eyes, or through any mechanical wound (Darsow, 2004). Asymptomatic infected tubers may be harvested, stored and can serve as a primary inoculum next season that can start an epidemic in the following year.

Sexual cycle. *Phytophthora infestans* is a heterothallic organism, thus it requires opposite mating types to come into contact to produce oogonium and antheridia. Upon fertilization, the oogonium

produces oospores. Oospores are known to survive harsh conditions compared to mycelium and sporangia (Drenth et al., 1995). The oospores are formed better at low temperature conditions (15-20). The oospores are found in the potato stem tissue, but not in the leaf tissue. This could be because stem tissue can survive blight attack longer then leaf tissue (Mosa et al., 1991). Tubers get infected via oospores that drop off from the stems and decompose to the soil. There is very little is known about oospore production in the soil and how they infect potato tubers (Andrivon, 1995).

P. infestans genotypes in the US. Currently there are six genotypes (US-7, US-8, US-11, US-22, US-23 and US-24) of *P. infestans* occurring in the USA (https://usablight.org/node/52). The US-1 genotype was the first global and aggressive genotype. However, new genotypes appeared and displaced US-1 (Hu et al., 2012). In early 2000, the US-8 genotype become predominant and aggressive in both foliage and tubers. However, in 2009-2010, the US-22 genotype appeared and was responsible for late blight epidemics in Eastern and Midwestern U.S. (Danies et al., 2013). The new US-22 genotype was found in Michigan and displaced US-8 genotype (Rojas et al., 2014). However, US-22 genotype did not last long in Michigan and in other states of the US and a new US-23 genotype appeared and displaced US-22. Since 2012 US-23 genotype has been the most predominant on potatoes and tomatoes across the country (https://usablight.org/node/52).

DISEASE CYCLE AND EPIDEMIOLOGY

The primary inoculum of *P. infestans* is mycelium that infect tubers. In the US pathogen mainly overwinters as mycelium. *Phytophthora infestans* can survive from year to year in the infected tubers in storage, in cull piles in the field, or in infected tubers that are left behind after harvest and do not freeze over the winter. In the spring, mycelium grows from infected tubers and produces sporangia. The polycyclic part of this disease causes production of sporangia and zoospores that

can be dispersed by wind or water and infect new parts of the same plants or new plant throughout the growing season. Sporangia and zoospores from infected plants can be washed down to the soil and infect newly formed tubers (Kirk et al., 2004).

Humidity, warm temperature, and moisture are essential for successful late blight development. Humidity above 90% is favorable for sporangia formation. Sporangia usually form on the abaxial side of the leaf or on infected stems. Optimum temperatures for sporulation are between 18-22° C. However, sporulation can occur in a wide range of temperatures (3-26° C). Zoospores require cooler temperatures (below 18° C) and moisture/water to swim. Under warmer temperatures (21-26° C), direct germination occurs via germ tube. Each sporangium contains 6 to 8 zoospores. Each zoospore can initiate an infection. Favorable conditions for late blight include cool nights, warm and humid days, high relative humidity, frequent rainfall and moderate temperatures (10-25° C). This type of weather can create ideal conditions for late blight epidemics, and can destroy whole fields of potato within days (Schumann and D'Arcy, 2000).

MANAGEMENT OF LATE BLIGHT

Currently, primary means of managing late blight include foliar fungicide application during the growing season, and cultural practices. However, to effectively manage late blight, there is a need to implement an integrated management approach. Using a combination of management tools such as planting resistant varieties, cultural practices, chemical sprays in conjunction with weather forecast predictions is very important.

Cultivar resistance. The most commonly grown potato cultivars are not resistant to late blight. However, there are newly bred varieties that have durable late blight resistance and example of these varieties are Sarpo Mira and Cooperation 88. These varieties have not acquired a large market share yet because growers, consumers and processors prefer other established varieties.

Cultural practices. Cultural practices are important and the primary management approach for late blight. After harvesting potatoes, volunteer potatoes and cull piles need to be removed from the fields. As mentioned above, the pathogen overwinters in tubers, and crop debris. Removing infected tubers in and near the field is crucial and helps to eliminate primary inoculum sources for next season. Cull piles should be kept as small as possible and should be covered with plastic to increase temperatures to break down potato tissue. Volunteer potatoes need to be killed with herbicides. Planting certified seeds free from pathogen is important. Tubers that are suspected of being infected have to be removed. Select fields with good water infiltration and drainage. It is important to avoid irrigation late night/evening and early morning and apply irrigation during the daylight hours to allow leaves to dry.

Chemical control. Protectant fungicides such as chlorothalonil, mancozeb, azoxystrobin and others should be used as a preventive manner, in conjunction with forecasting tools. If susceptible potato varieties planted or late blight pressure is high fungicide applications may be needed weekly. Fungicide applications before symptoms is expressed is more effective.

Seed treatments. Seed treatments are part of the chemical control and is often simpler and less expensive control measure compare to the foliar treatment. Moreover, with seed treatment plant growth may be more vigorous and may produce higher yields if the seed pieces are free from pathogens. Seed treatments are done after seed cutting, prior handling operations, thus can be a preventive approach to decrease the disease transmission. It is important that seed treatments have to be done quickly after cutting the seeds (Powelson et al., 2002).

SUMMARY

Potato late blight caused by *P. infestans* is an important disease of potatoes worldwide. It can lead to a loss of an entire field within days if no control managements take place (Fry et al., 1993). Late

blight is an annual concern for Michigan. This polycyclic disease can be controlled by multiple fungicide applications during the growing season. However, fungicide applications are expensive and other tools of management need to be practice. In years conducive to late blight, 10-15 foliar fungicide applications can be made per season. The mail goal of this research was designed to combine planting resistant varieties in conjunction with seed treatments prior planting to decrease the late blight incidence.

BLACK LEG, SOFT ROT AND AERIAL STEM ROT OF POTATOES

INTRODUCTION

Black leg, aerial stem rot and soft rot caused by *Dickeya* and *Pectobacterium* species are economically important pathogens of vegetables, fruits and ornamental plants (Table 1) (Perombelon, 2002; Golanowska and Łojkowska, 2016).

Table 1**.** Characterization of *Dickeya* and *Pectobacterium* species and their host range

Species name	Host	References
Dickeya dianthicola	*Dianthus spp.* *Cichorium intybus* *Cynara scolymus* *Dahlia variabilis* *Kalanchoe blossfeldiana* *Lycopersicon esculentum* ***Solanum tuberosum***	Samson et al. (2005)
Dickeya solani	***Solanum tuberosum***	Van der Wolf et al. (2014b)
Dickeya zeae	*Zea mays* *Ananas comosus* *Brachiaria ruziziensis* *Chrysanthemum* spp. *Musa* spp. *Nicotiana tabacum* *Oryza sativa* ***Solanum tuberosum***	Samson et al. (2005)
Dickeya dadantii	*Dianthus* spp. ***Solanum tuberosum*** *Ananas comosus* *Pelargonium* *Euphorbia* *Zea mays* *Philodendron* *Saintpaulia* *Musa* spp.	Samson et al. (2005)
Dickeya chrysenthemi bv. *chrysenthemi*	*Chrysanthemum* spp. ***Solanum tuberosum*** *Lycopersicon esculentum* *Cichorium intybus* *Helianthus annuus*	Samson et al. (2005)
Dickeya chrysenthemi bv. *parthenii*	*Parthenium* *Cynara scolymus* *Philodendron*	Samson et al. (2005)

Table 1. (cont'd)

Dickeya paradisiaca	*Musa* spp. *Zea mays* ***Solanum tuberosum***	Samson et al. (2005)
Dickeya dieffenbachiae	*Dieffenbachia,* *Lycopersicon esculentum* *Musa* spp.	Samson et al. (2005)
Dickeya fangzhongdai	*Pyrus pyrifolia*	Tian et al. (2016)
Dickeya aquatica	Water	Parkinson et al. (2014)
Pectobacterium carotovorum subsp. carotovorum	***Solanum tuberosum*** *Beta vulgaris*	Hauben et al. (1998)
Pectobacterium carotovorum subsp. brasiliense	***Solanum tuberosum***	Hauben et al. (1998)
Pectobacterium carotovorum subsp. odoriferum	***Solanum tuberosum*** *Cichorium intybus*	Hauben et al. (1998)
Pectobacterium permienteri (wasabiae)	*Armoracia rusticana* ***Solanum tuberosum***	
Pectobacterium atrosepticum	***Solanum tuberosum*** *Lycopersicon esculentum* *Cichorium intybus*	Gardan et al. (2003)
Pectobacterium betavascularum	*Beta vulgaris*	Gardan et al. (2003)

The first report of this disease was in potato in Europe over 40 years ago (Toth et al., 2011). Losses from the pathogen in most of the European countries had remained low until the outbreak in early 2000 (Toth et al., 2011). This outbreak was associated with the emergence of a new bacterial species (Toth et al., 2011). Since then, extensive research has been conducted in Europe on *Pectobacterium* and *Dickeya* spp. (Czajkowski et al., 2011; Toth et al., 2011). In the US, *Pectobacterium* is endemic in potato production. However, recently an outbreak of *Dickeya* was reported in the US during the 2015 growing season. *Dickeya dianthicola* was found in several states and caused poor emergence in potato fields (Jiang et al., 2016; Rosenzweig et al., 2016b).

The bacteria can survive and move via infected seed or plant material. Locally the bacteria can spread via soil, water, air, infected plant remains, alternative hosts or agricultural equipment. Generally, this pathogen is considered a seed-borne disease. However, it can infect plants via natural openings such as stomata, hydathodes, or wounds (Motyka et al., 2017). The symptoms of *Dickeya* and *Pectobacterium* are similar and difficult to distinguish. Furthermore, sometimes infection can be asymptomatic. Due to these difficulties, lab-based diagnostic tools are required to accurately identify infection. There are different approaches to manage these diseases, however the first step for effective management of the disease is an accurate and reliable diagnosis. Cultural management practices can be used at different stages of the growing season. Most important is planting disease free seeds, avoiding contamination during seed preparation, harvest and storage.

Distribution. *Dickeya* and *Pectobacterium* are widely distributed bacteria. They are known to be found in many geographical areas due to a wide variety of hosts, and their ability to survive in various climatic conditions. Many *Dickeya* and *Pectobacterium* spp. can be found in both tropical and subtropical areas.

Economic importance. *Dickeya* and *Pectobacterium* spp. are among the top ten economically important bacterial plant pathogens (Mansfield et al., 2012). However, it is challenging to determine the total economic impact in many different geographical locations due to many susceptible hosts and variability of aggressiveness of these pathogens. For example, losses in potato crop are mainly in the seed certification sector. This is because seed tubers that are infected with bacteria will get rejected and/or downgraded during seed certification process. For example, in the Netherlands, where much of the production of potatoes is for certified seed, it has been estimated that direct losses from these bacteria is around 30%. Conversely in Poland, where 90% of potato production is for table and processing, average losses have been estimated to be between 5-30% (Toth et al., 2011; Motyka et al., 2017).

Hosts. Both *Pectobacterium* and *Dickeya* have a wide host range. There are a number of important crop and ornamental species that these bacteria can infect, among them are potato, corn, banana, chrysanthemum, sunflower, and ornamental species (Perombelon, 2002; Toth et al., 2011; Samson et al., 2005). Diseases caused by *Dickeya* was primarily restricted in more tropical and subtropical climates. However, recently due to the climatic conditions this has changed and outbreaks of *Dickeya* have been reported in non-tropical areas (Toth et al., 2011; Rosenzweig et al., 2016b; Jiang et al., 2016). The host range of specific *Pectobacterium* genera depend on species type. *Pectobacterium carotovorum* has the widest host range, but is most prevalent in potato (Perombelon, 2002). Moreover, *P. atrosepticum* appears to be host specific and is pathogenic on potatoes. Another economically important *Pectobacterium* species is *P. wasabiae*, was originally isolated from the diseased rhizomes of roots of Japanese horseradish (*Wasabia japonica*) (Masao Goto and Matsumoto, 1987). Subsequently also *P. wasabiae* has been isolated from potato plant

and tubers in various countries and recently has been re classified *P. parmentieri* (Khayi et al., 2016).

Soft rot symptoms of *Dickeya* and *Pectobacterium* spp. on potato tubers under humid and wet conditions appear as black, slimy, water-soaked rot lesions. Usually the bacteria enter the tuber via lenticels, the stolon end or wounds. The decay in the tuber can rapidly spread and macerate the tissue resulting in soft rot. The tuber, after exposure to air, become brown or black. Soft rot of potato is odorless, however secondary infection by other pathogens can result in the development of a foul smell. The bacteria can easily spread in storage due to liquid from rotten tubers and can cause rotting pockets in stored tuber lots. Infected seed tubers can result poor emergence or can cause foliar symptoms. Soft rot disease on potato can cause losses in the field and post-harvest in storage. Foliar symptoms include curled leaves and wilted plants. Both *Pectobacterium* and *Dickeya* spp. can block the vascular system and plants may express chlorotic symptoms and also may resemble water deficiency. Soft rot can also infect stems and roots which eventually can kill the whole plant (Czajkowski et al., 2011; Motyka et al., 2017; Rosenzweig et al., 2016a).

Blackleg symptoms usually appear on the lower stem of the potato plant. This can be caused by either *Dickeya* or *Pectobacterium* spp. The bacteria can move up from the infected tuber to the stem. Symptoms on the stems are similar to tuber symptoms under humid and (Rudd Jones, 1950) wet conditions, mainly black, dark lesions. The lesion forms at the base of the stem and can quickly girdle the plant. Infected stems become soft, water-soaked and can infect the entire plant and lead to the death. The foliar symptoms of infected plants include: stunting; chlorosis; and curling at the margin as disease progresses. Under dry conditions, foliar symptoms may include stunting, wilting, chlorosis, and shriveling of leaves and stems.

Dickeya and *Pectobacterium* spp. also cause aerial stem rot. The early symptoms of aerial stem rot are irregular brown to black lesions on the plant. Lesions can rapidly grow, especially under wet and humid conditions, and infect the entire stem and result in soft and decaying rot. Decaying tissue is similar to soft rot of tubers and blackleg and are slimy, water-soaked and black. Symptoms under dry conditions are also similar to blackleg where stems can desiccate and shrivel.

CAUSAL ORGANISM (*DICKEYA* AND *PECTOBACTERIUM* SPECIES)

Taxonomic description and history. *Dickeya* and *Pectobacterium* belong to the kingdom Bacteria, phylum Proteobacteria, class Gammaproteobacteria, order *Enterobacterales* and family *Pectobacteriaceae* (Adeolu et al., 2016). The taxonomical classification of these bacteria has been revised over the years due to improvement of phylogenetic and systematic analyses (Davidsson et al., 2013; Motyka et al., 2017). This recent taxonomic change was conducted by Adeolu at al., 2016. The authors created a new order, *Enterobacterales* and new family *Pectobacteriaceae.* This new taxonomic study moved *Pectobacterium* and *Dickeya* from *Enterobacteriaceae* family into the new *Pectobacteriaceae* family.

The first identification of these bacteria was at the beginning of the 20th century. L.R. Jones described *Bacillus carotovorus* as the first soft rot pathogen on vegetables. Until, 1998 *Dickeya* and *Pectobacterium* were in the *Erwinia* genera but were separated into two species *Erwinia carotovora* and *Erwinia chrysenthemi* (Hauben et al., 1998). However, in 1998 they were changed to genus *Pectobacterium* as *Pectobacterium carotovorum* and *Pectobacterium chrysenthemi* (Hauben et al., 1998). The genus *Pectobacterium* currently includes six species and four subspecies: *Pectobacterium aroidearum*, *Pectobacterium cacticida*, *Pectobacterium betavasculorum*, *Pectobacterium atrosepticum*, *Pectobacterium parmentieri* (aka *wasabiae*) and *Pectobacterium carotovorum*; *Pectobacterium carotovorum* subsp. *actinidiae*, *Pectobacterium*

carotovorum subsp. *brasiliense*, *Pectobacterium carotovorum* subsp. *carotovorum*, and *Pectobacterium carotovorum* subsp. *odoriferum* (Gardan et al., 2003; Nabhan et al., 2013). Samson et al (2005) first described the genus *Dickeya* in 2005 and divided it into seven species; *Dickeya dadantii*, *Dickeya dieffenbachia*, *Dickeya fangzhongdai, Dickeya chrysenthemi*, *Dickeya paradisiaca*, *Dickeya zeae*, and *Dickeya dianthicola* (Samson et al., 2005; Tian et al., 2016). Recently, *Dickeya dieffenbachiae* was reclassified as *Dickeya dadantii* sub-species *dieffenbachiae* (Reverchon et al., 2016). Recent studies added two more species to the list, *Dickeya aquatica* isolated from freshwater rivers, and *Dickeya solani*.

Biology. *Dickeya* and *Pectobacterium* are small gram-negative rod shape motile bacteria with peritrichous flagella. They are facultative anaerobes and can survive in both aerobic and anaerobic conditions. The anaerobic life style of these bacteria is important because they can weaken the host plant's resistance system like production of phytoalexins, disruption of plant cell membranes and free radicals (Motyka et al., 2017). These bacteria do not produce spores and are not able to survive in harsh conditions or in the soil without a host. *Dickeya* and *Pectobacterium* spp. are necrotrophs, however they also can be endophytes. The asymptomatic stage of the disease is common. This can result from host resistance or unfavorable conditions such as drought, acidic stress, nutrient deficiency, and other stresses. Low levels of oxygen and high humidity are favorable conditions for infection these bacteria.

Plant cell wall degrading enzymes. One of the most important features of *Dickeya* and *Pectobacterium* species is the production of plant cell wall degrading enzymes (PCWDE) (Davidsson et al., 2013). There are number of PCWDE such as polygalacturonase, pectate lyase, cellulase, xylanase and protease. The name of the disease soft rot comes from the ability of the bacteria to produce pectinases that destroy pectin in the plant cell wall. The main enzymes are

pectate and pectin lyases that cleave α-1,4 glycosidic linkages by the β-elimination. Additionally, bacteria secrete cellulases, xylaneses and proteases which also play a role in the breakdown of the cell wall components.

DISEASE CYCLE AND EPIDEMIOLOGY

Dickeya and *Pectobacterium* usually overwinter in the potato tuber, which is a primary source of inoculum. The bacteria can also overwinter in plant debris. In the spring and early summer, bacteria from infected tubers can spread into young stems and roots. The bacteria moves intracellularly, dissolve the plant cell walls and ultimately softens the mother tuber. Daughter tubers may become infected with *Dickeya* or *Pectobacterium* as they develop later in the season. There are many modes of transmission of these bacteria such as rain, irrigation, insects, wounds during handling and planting. Rain and irrigation may spread bacteria from infected plants to non-infected ones. Insects can vector bacteria by causing wounds on the plants thus, allowing bacteria to gain access. Insects can also transmit bacteria from infected plants to non-infected plants. Wounds during harvest or the handling process, provides bacteria a point of entry into the host. Asymptomatic tubers in storage can become the primary inoculum for the next season. *Dickeya* and *Pectobacterium* spp. can survive in alternate hosts and plant debris. *Dickeya* has also been found in surface water which indicates that it can live in the irrigation or other environmental sources (Reverchon and Nasser, 2013).

MANAGEMENT OF BLACK LEG, SOFT ROT AND AERIAL STEM ROT

Primary mean of management of black leg, soft rot and aerial stem rot is to avoid or prevent the disease. Once the disease has been initiated, it becomes difficult to manage. Control measures include avoiding infected seed, sanitation of equipment during seed cutting and planting, harvesting and grading. In places where the bacteria not have been reported, biosecurity measures

need to be taken. Infected seeds are the main source of introduction to new areas. *Dickeya* and *Pectobacterium* free seed need to be planted and seed stock need to be monitored for the pathogens. If *Dickeya* and/or *Pectobacterium* occurs in the field, there are a number of measures that can be taken to reduce the spread of inoculum. These measures include scouting for symptomatic plants and eradicating them; avoiding mechanical harvesting; cleaning and disinfecting equipment; harvesting in dry weather; minimizing damage and bruising during harvest and grading; eradicating alternate hosts and another sources (e.g. other crops, weed spp., insects or contaminated water). *Dickeya* species can live in surface water, and irrigation may spread bacteria. Thus, limiting irrigation from sources where bacteria has been found is important.

DIAGNOSTIC TOOLS

Usually it is difficult to differentiate between *Dickeya* and *Pectobacterium* species. Diagnostic tools are essential and effective in detecting the presence and differentiating these bacteria. Detection can also help avoid planting or exporting *Dickeya* or *Pectobacterium* infected seed. A commonly used method to a diagnose these bacteria is isolation on selective crystal violet pectate (CVP) single and/or double layer medium (Cuppels and Kelman, 1974). The CVP media usually is used to select all pectinolytic bacteria and is not reliable in differentiating in *Dickeya* and *Pectobacterium*. There are other selective media and other characteristics such as colony color, pigmentation, and shape that can be used to detect *Dickeya* and *Pectobacterium* spp. Serological and biochemical analyses can also be used, but lack sensitivity and specificity. Currently the most effective method for detection and differentiation of these bacteria are DNA based methods, such as conventional PCR, qPCR and sequencing. There are a number of primers that have been developed from specific regions in both *Dickeya* and *Pectobacterium* in the genomes. The most widely used primer for *Dickeya* species is primer set ADE1/ADE2 which is based on pectate lyase

gene. For *Pectobacterium* there are several species-specific primers such as Expcc for *P. carotovorum* and Y45/46 for *P. atrosepticum*. Combined methods such as multiplex PCR, qPCR and sequencing tools are also available for detection.

SUMMARY

Dickeya and *Pectobacterium* spp. are the causal agents of the black leg, aerial stem and soft rot on potatoes, vegetables and ornamental crops. These pectinolytic bacteria are responsible for significant economic losses around the world, particularly in potato production. Current management practices include prevention, such as early detection and identification, monitoring disease spread, planting disease free seed, and sanitation during seed handling. The disease was first reported in Europe but had not been an issue in the US until 2015. There are multiple methods to differentiate and detect these bacteria, currently the most effective techniques are DNA based methods such as PCR, qPCR and sequencing. A number of specific primers based on both *Dickeya* and *Pectobacterium* genomes have been developed and have been used in Europe and the US. The main goal of this research is to evaluate specificity of currently available PCR and real time PCR primers to develop quick and proper identification methods.

COMMON SCAB OF POTATO AND TURNIP

INTRODUCTION

Common scab is a bacterial disease that is caused by several species of the genus *Streptomyces*. Common scab is one of the most important disease on potato worldwide and it has been identified as the fourth major potato disease in the US and is of particular concern in Michigan potato production (Hao et al., 2009; Loria et al., 1997). *Streptomyces* species also infect: root crops such as turnip, radish, beet, carrot, and sweet potato (Labeda and Lyons, 1992; Goyer and Beaulieu, 1997; Lapwood et al., 1976). Recently turnip growers in Michigan have reported scab on turnip.

The common scab disease results in a poor-quality crop, symptoms such as such as corky, pitted and deep pitted lesions on the tuber or roots and thus can decrease the marketability of the crop and can result in significant economic loses. Some *Streptomyces* species can cause yield loss, for example *S. ipomoeae* on sweet potatoes. However, most of the species have little or no effect on total yield loss (Dees and Wanner, 2012; Takeuchi T et al., 1996). Consistent management of this disease is elusive.

Distribution. *Streptomyces* species are distributed globally. Due to their wide hosts they have been found in many geographic areas. *S. scabies* is the most common species that causes scab in potato production worldwide. This includes: Europe; South Africa; Australia; New Zealand, Israel; United States; Canada; Japan and India (Miyajima et al., 1998; Loria et al., 1997).

Economic Importance. Severe damage can reduce the quality of harvested tubers. Scab infected tubers have poor market quality because of the appearance of common scab and also lower starch content. Tubers that are heavily infected have reduced germination rate and not suitable for planting.

Hosts. The host range for *Streptomyces* species includes most higher plants. The evidence of broad host range was demonstrated by Hooker (Hooker, 1949). Potato is one of the most studied host. However, there are other economically important crops including: turnip (*Brassica rapa* L.); beet (*Beta vulgaris* L.); carrot (*Daucus carota* L.); radish (*Raphanus sativus* L.); peanut (*Arachis hypogaea* L.); rutabaga (*Brassica napobrassica* L.); sweet potato (*Ipomoea batatas)* and parsnip (*Pastinaca sativa* L.).

Symptoms. Typically scab symptoms are limited to tuber; however, bacteria have been reported to infect the stolon (Bäng, 1979). Early symptoms on tuber are browning, and hypertrophy. Symptoms develop into circular, irregular lesions (5-10 mm in diameter) and often coalesce.

Ultimately at harvest lesions may become shallow, raised or deep-pitted corky, depending on environmental conditions, and strain virulence (Stevenson et al., 2001; Loria et al., 1997). Multiple lesion types can occur on a single tuber or root. The type of lesion is believed to be associated with host (e.g. potato cultivar), the aggressiveness of the *Streptomyces* species, time of infection and environmental conditions (Loria et al., 1997).

CAUSAL ORGANISM (*STREPTOMYCES* SPECIES)

Taxonomic description and history. *Streptomyces* belongs to the kingdom bacteria, phylum Actinobacteria, class Actinobacteria, order Actinomycetales and family Streptomycetaceae. The bacteria was first isolated and described in 1890 in North America by Thaxter (Thaxter, 1891) . Thaxter named the bacteria *Oospora scabies* and characterized this organism as a melanin producer, with grey spores borne on spiral chains (Thaxter, 1891). Since the first identification the species has been renamed several times. In 1948 it was changed to *Streptomyces scabies*, than in 1997 it was changed to *Streptomyces scabiei* to follow grammatical convention (Lambert and Loria, 1989). Both names have been found in the literature, however more commonly it is known as *S. scabies*.

Biology. *Streptomyces* are gram-positive, filamentous, spore-bearing bacteria that are soil inhabitants. However, *Streptomyces* have also been isolated from marine organisms and from animals. *Streptomyces* species resemble fungi due to their filamentous morphology. The bacteria produce thin hyphae with few or no cross walls. The spores of the *Streptomyces* species be different based on species. For example *S. scabies* spores are gray, smooth, contains melanin, whereas *S. cylindrical* spores usually produce in mature spiral chains of 20 or more (Lambert and Loria, 1989). Spores usually release from the top of the hyphae. The *Streptomyces* can survive in the soil for decades.

DISEASE CYCLE AND EPIDEMIOLOGY

The *Streptomyces* are saprophytic organism and they can overwinter in the soil or on the surface of the tuber. The bacteria spread from one place to another by splash irrigation or rain water, wind, on infected tubers, farm equipment, insects and nematodes. The bacteria consists of slender, branched mycelium sometimes with few or no cross walls. The mycelium is composed from thin branched sporogenous hyphae. Each sporogenous hyphae breaks into small cylindrical spores (Lambert and Loria, 1989). The spores germinate and enter the host tissue via wounds, lenticels, and stomata (Locci, 1994; Agrios, 2005). Bacteria grows between and through the cells. A corky layer forms around the lesion and layers pushes the infected area outward. The periderm of the potato breaks down and a scab lesion form. Once the first layer is penetrated a new lesion form. If tubers get infected they can survive as an inoculum for next season (Agrios, 2005).

PATHOGENICITY FACTORS

The pathogenicity of *Streptomyces* spp. depends on a phytotoxin thaxtomin (Loria et al., 2008). There are eleven genes in the thaxtomin family that have been identified, characterized and associated with pathogenicity in *Streptomyces* spp. (Bignell et al., 2014). The most commonly produced by *Streptomyces* spp. is a thaxtomin A, however there are other phytotoxins that shown to be produced but in a lower amount (Loria et al., 2008; Bignell et al., 2014). Thaxtomin is a cyclic dipeptide and was first discovered by Russell King and his colleagues in New Brunswick (King et al., 1989; Lawrence et al., 1990). The primary mode of action of thaxtomin A is to inhibit cellulose biosynthesis in the plant tissue, but it also can cause cell hypertrophy (cell expansion), stunting of the root and shoot, tissue necrosis, programmed cell death, and can alter Ca^{+}, H^{+} and ion influx (Loria et al., 2008; Loria et al., 1997; Loria et al., 2006). The gene cluster that contains all necessary genes of the biosynthesis of thaxtomin is in a large chromosome region that functions

as a pathogenicity island (PAI) (Kers et al., 2004; Loria et al., 2006). The PAI can be horizontally transferred from pathogenic *Streptomyces* spp. to non-pathogenic spp. which then create a new pathogenic strain (Loria et al., 2006). Thaxtomin biosynthesis genes are clustered in the first section of the PAI and it is called "toxicogenic region" (Lerat et al., 2009). The toxicogenic region include genes such as *txtA*, *txtB*, *txtC*, *txtR* and *NOS* (Lerat et al., 2009). The *txtA* and *txtB* genes are responsible for production non-ribosomal peptide synthase; *txtC* gene is responsible for a cytochrome P450 monoxygenase; and the NOS gene responsible for a nitric oxide synthase and a cellobiose-binding regulatory protein (Lerat et al., 2009). The second part of the PAI called is "colonization region". This segment of the PAI contains more genes and they are responsible in virulence and not associated with pathogenicity (Lerat et al., 2009). For example, of the colonization region genes are Nec1 and *TomA* genes. Nec1 gene is required for colonization by the pathogen and play a role in the suppression of plant defense genes (Joshi et al., 2007). *TomA* gene is a homologue of tomatinase gene that can detoxify phytoanticipins that play role in the defense response against pathogens (Kers et al., 2005).

MANAGEMENT OF COMMON SCAB OF POTATO AND TURNIP

Potato common scab is difficult to manage due to the poor understanding of the disease occurrence, severity, bacteria and host mechanism (Dees and Wanner, 2012). The disease is more severe during the warm and dry conditions with soil temperature above 22°C. Warm conditions are favorable for growth of the pathogen and also for production of thaxtomin (Stevenson et al., 2001). The important factors that impact scab severity and its management include; genetic background of pathogen; diversity of virulence; the endemic nature of bacteria in the soil; ability of live outside the host; lack of information on the interaction between host and bacteria; environmental factors such as pH, soil temperature, soil moisture and interaction of other soil organisms can contribute

occurrence and severity of common scab (Dees and Wanner, 2012; Johnson and Powelson, 2008). Thus, it is a complex and difficult task to manage common scab, therefore an integrated approach such as cultural, chemical, biological and resistant varieties is necessary (Braun et al., 2017b).

Cultivar resistance. Host plant resistance is considered to be an ideal choice for managing soilborne diseases including common scab (Fiers et al., 2011; Braun et al., 2017a). US potato breeding programs have prioritized breeding for a common scab resistance in recent decades. There are a number of cultivars that have been reported to be resistant to common scab in Europe and in the US. Some examples of resistant cultivars in the US: Alta Crown; Freedom Russet; GemStar Russet; Kalkaska; Liberator; Marcy; McBride; Megachip; Millennium Russet; Owyhee Russet; Premier Russet; Summit Russet; Teton Russet, and Western Russet (Braun et al., 2017a).

Chemical control. For chemical control soil fumigants are available. There are limited soil fumigants on the market that can be used. Some soil fumigants have been removed from the market due to environmental concerns. The primary soil fumigants in the US are pentachloronitrobenzene (PCNB) and chloropicrin. The soil fumigants have shown some efficacy against common scab however, it can be cost prohibitive. Soil fumigants are still under investigation and continued to be tested in the US and in Canada.

Cultural practices. Crop rotations, green manures, organic soil amendments, and cover crops are some of the cultural practices to manage common scab. There are numerous publications in the literature that have tested cropping diversity, long and short period rotations, green manures, fall cover crops and number of organic soil amendments (Larkin et al., 2011; Larkin and Honeycutt, 2006; Peters et al., 2003, 2004). The result of these studies has been mixed. Some crops such as canola, rapeseed, winter rye with on a 3-year rotation have resulted in suppression of common scab. However, timothy and barley with 3 years of rotation did not suppress common scab.

Additionally, green manures have shown mixed results. In fields following corn or alfalfa from previous years showed suppression of *Streptomyces.* However, green manures can play role in the pathogen inhibitory mode. Number of different soil amendments have been tested that decrease severity of common scab and other soilborne diseases. Most of the soil amendments that have tested used industrial or agricultural waste products.

Biological control. Commercially available biological controls are available and can be an alternative method to other management strategies. Researchers have studied a number of non-pathogenic bacteria and fungi as potential biological control agents against common scab and other diseases (Meng et al., 2011; Kinkel et al., 2012; Meng et al., 2013). The non-pathogenic *Streptomyces*, *Pseudomonas*, *Bacillus* species have been investigated and showed mixed results. Typically, biological agents work well in the controlled environment and not in the field. A number of biological agents have been shown antagonistic activity against *Streptomyces* species. Some findings suggest that using biological control agents with combination of traditional methods such as acidification can be effective under field conditions. More work needs to be done to efficiently recommend effective biological control methods.

SUMMARY

The main goal of this research is to characterize genotypically and phenotypically S*treptomyces* species that are recovered from potato and turnip in Michigan. The disease mainly affects tuber and root quality. Tuber and root crops that affected may be rejected further processing or fresh market. Currently there is no effective strategies available to manage common scab. The development of phenotypic and genotypic information of current population of *Streptomyces* species in Michigan may inform and improve management strategies and help growers to better protect their crop.

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CHAPTER 2: INTEGRATION OF RESISTANT CULTIVARS IN COMBINATION WITH SEED TREATMENTS TO MANAGE SEED-BORNE LATE BLIGHT IN POTATO

ABSTRACT

Late blight of potato (*Solanum tuberosum* L.), caused by *Phytophthora infestans* (Mont.) de Bary is still a threat to a potato and tomato production worldwide. It affects foliage, tuber yield and storability in potato. Five commercial cultivars, three susceptible to *P. infestans* (Atlantic, Snowden and Russet Norkotah), two with foliar resistance to *P. infestans* (Missaukee and Jacqueline Lee), and nine advanced breeding lines (ABL) with foliar resistance to *P. infestans* from the Michigan State University potato breeding and genetics program were used in the tuber susceptibility test and seed treatment studies. Tubers for susceptibility test were inoculated with US-23 genotype of P. *infestans*. To assess tuber tissue infection, digital scanner and image analysis software was used. The severity of tuber blight development represented by tissue necrosis and relative average reflection intensity was calculated. The 2015 result showed that severity of tuber blight between cultivars and ABL were significant. However, in 2018 all cultivars and ABL's were not statistically different from each other, except cultivars Snowden and Jacqueline Lee. For seed treatment trials tubers were cut and inoculated with a mixture of US-8, US-22, US-23 and US-24 genotypes of *P. infestans* and treated with either one or combination of fungicides Nubark Mancozeb, Reason or Emesto Silver. The experiment included two controls (non-inoculated non-treated and inoculated non-treated) and inoculated and treated with fungicides. Cut tuber pieces were planted and number of emerged plants was recorded over a period. Final plant stand (%) and relative area under the emergence progress curve was calculated.

Overall, fungicide treatments and the non-inoculated and non-treated control had greater higher number of plants and rate of emergence in comparison to inoculated and non-treated

control. In the field trials among the commercial cultivars the higher number of plants and better rate of the emergence over time were observed in Snowden, Jacqueline Lee and Missaukee compared to Atlantic and Russet Norkotah. In the greenhouse experiments with all four tested *P. infestans* genotypes, ABLs showed similar results, except for the ABL MSQ131-A and MSS428-2 had lower number of emerged plants or no plants and the rate of emergence was lower compared to other ABLs evaluated.

INTRODUCTION

Late blight is caused by the oomycete *Phytophthora infestans* (Mont de Bary). In the 170 years since its identification, late blight remains a major threat to potato and tomato production worldwide (Guenthner et al., 2001; Fry and Goodwin, 1997). In the last two decades, the population of *P. infestans* has been dynamic and new strains with different virulence were discovered (Rojas et al., 2014).

Phytopthtora infestans is a heterothallic organism requiring two mating types, A1 and A2. In the US, the pathogen is commonly reproduced by asexual spores known as sporangia (Hu et al., 2012). Before 1980, the A1 mating strain was the only lineage in the US. However, this has changed and genotypes with both mating types (A1 and A2) were reported (Ristaino, 2002).

Early in 2000, the US-1 clonal (A1 mating type) lineage of *P. infestans*, which was the most prevalent worldwide, was displaced by newer genotypes such as US-8 (A2), US-7 (A2), US-11 (A1) and US-14 (A2) (Saville et al., 2016; Hu et al., 2012; Ristaino, 2002). Devastating new genotypes such as US-22 (A1), US-23 (A2) and US-24 (A2) occurred in 2009 that displaced many of the genotypes found previously (Peters et al., 2014; Alkher et al., 2015; Danies et al., 2013) . The genotype US-22 was predominant in 2010/11 in much of north central and eastern part of the United States. However, since 2012, US-23 genotype has been predominant in Michigan, Midwest and across the United States. The newer genotypes of *P. infestans* have reported to be different in phenotypic and epidemiological characteristics, mating types and sensitivity to metalxyl (Hu et al., 2012; Danies et al., 2013). For example, US-8 and US-22 genotypes were aggressive on both foliage and tubers and both are A2 mating types, whereas, US-23 and US-24 are A1 mating types (Rojas et al., 2014; Mambetova et al., 2018; Hu et al., 2012).

The current management strategies of late blight disease are heavily rely on foliar fungicide applications. The ideal management would be using resistant varieties, however newer cultivars released since 2000 have foliar resistance/tolerance, but not in tubers (Douches et al., 2010; Douches et al., 2001; Meier et al., 2015). Tuber infection can lead to tuber rot in storage and can increase loss for seed and consumption (Kirk et al., 2009; Kirk et al., 1999). Moreover, infected tubers can be a source of inoculum for the next growing season. In severe cases, US-8 aggressive genotype of *P. infestans* can either kill the developing sprouts outright, resulting in non-emergence, or late emergence with uneven plant stand (Kirk et al., 2001). Thus, aggressive genotypes on tubers can lead to more severe economic losses.

The changes in the genetic diversity of the population of *P. infestans* are not predictable. However, the sudden appearance and predominance of the US-22, US-23 and US-24 genotypes, highlighted the need to develop disease management and breeding in preparation for further genetic variability in the pathogen population. Knowledge of competitive interactions among pathogen genotypes, and the role of resistant cultivars and advanced breeding lines (ABL) will help us to develop a better understanding of the factors which govern strain selection and stability. Potato breeding programs have developed foliar late blight resistance ABLs, however the ABLs were never tested for tuber susceptibility. Therefore, the objectives of this study were i) evaluate commercial cultivars and foliar resistant ABLs against *P. infestans* US-23 genotype via tuber susceptibility test ii) `and evaluate the potential of different genotypes of *P. infestans* to impact establishment in final plant stand and plant emergence in commercial cultivars and foliar late blight resistant ABLs combined with seed treatments.

MATERIAL AND METHODS

Potato variety and ABLs selection. Five commercial cultivars and nine ABLs were used for evaluation. The ABLs and commercial varieties were from the Michigan State University (MSU) potato breeding and genetics program and from Michigan seed growers. Two cultivars, Missaukee and Jacqueline Lee, had foliage resistance to late blight, and the other three cultivars Atlantic, Snowden and Russet Norkotah were susceptible to late blight. The ABLs were tested previously at MSU potato breeding and genetics program and have shown foliage resistance to late blight (personnel communication with Dr. Douches) (Table 2).

Phytophthora infestans isolates. *Phytophthora infestans* genotype US-23 was used for susceptibility tests in the growth chamber and for field trials, since it has been the predominant genotype in Michigan since 2012 (Table 3). For greenhouse seed treatments study four (US-8, US-22, US-23 and US-24) genotypes of *P. infestans* were used (Table 3). Cultures of *P. infestans* were grown on pea agar in the dark at 18^{o} C for 14 days. For field and greenhouse seed treatment trials, inoculum was produced by harvesting sporangia by flooding the surface of the petri dish with sterile distilled water and gently scraping of the *P.infestans* culture with a sterile plastic rod to remove the mycelia and sporangia. The suspension was strained through four layers of cheesecloth to remove mycelia and the concentration was adjusted to 2 x 10^{6} sporangia ml^{-1} using a hemacytometer. The suspension then was incubated in the refrigerator at 4^{o} C for 4 h prior inoculation to release zoospores. For tuber susceptibility test, colonized plugs were used for inoculation.

Tuber susceptibility test. Tuber susceptibility test was conducted in the growth chamber during the spring of 2015 and 2018 (Table 2). Tubers were washed, and surface sterilized prior to

Table 2**.** List of commercial cultivars and advanced breeding lines tested in the growth chamber, greenhouse and field trials in 2015, 2016, 2017 and 2018

Cultivar/Advanced breeding line (ABL)		Foliar resistance to late blight	Field trials		Greenhouse trials		Tuber susceptibility tests	
			2015	2016	2016	2017	2015	2018
Atlantic	Cultivar	No[a]	X[b]	X	X	X	X	-[c]
Jacqueline Lee	Cultivar	Yes[d]	X	X	X	X	X	X
Missaukee	Cultivar	Yes	X	X	X	X	X	X
Russet Norkotah	Cultivar	No	X	X	X	X	X	-
Snowden	Cultivar	No	X	X	X	X	X	X
MSL211-3	ABL	Yes	X	X	X	X	X	X
MSQ131-A	ABL	Yes	X	X	X	X	X	X
MSS576-5SPL	ABL	Yes	X	X	X	-	X	-
MSS487-2	ABL	Yes	X	X	X	X	X	X
MSS428-2	ABL	Yes	X	-	X	X	X	-
MSS176-1	ABL	Yes	X	X	X	X	X	-
MSS206-2	ABL	Yes	X	X	X	X	X	X
MST145-2	ABL	Yes	-	-	-	-	X	X
MSV235-2PY	ABL	Yes	X	X	X	X	X	-

[a] Cultivars/Advanced breeding lines with a foliar late blight resistance
[b] Cultivars/Advanced breeding lines tested
[c] Cultivars/Advanced breeding lines did not test
[d] Cultivars/Advanced breeding lines with no foliar late blight resistance

Table 3**.** Characteristics of *Phytophthora infestans* isolates used in this study including mating types, metalaxyl sensitiviy, primary host and presence in Michigan

Genotypes	Mating type	Met[a]	Host[b]	Reported in MI as of 2017
US-8	A2	R	P	Yes
US-22	A2	I	P/T	Yes
US-23	A1	S	P/T	Yes
US-24	A1	S	P	No

[a] Met= metalaxyl sensitivity (S=sensitive; I=intermediate; R=resistant)
[b] Primarily host of the specific genotype; P=potato; T= tomato

inoculation. A 5-mm diameter sterilized cork-borer was used to remove a plug near the apical end of tuber. A sterilized needle was used to insert colonized agar with *P. infestans* inside the tuber and for control non-colonized agar plug was used. The potato plug was returned to close the wound and petroleum jelly was placed on top of the plug to seal it. Five inoculated and five non-inoculated potato tubers for each cultivar/line/replicate were placed in 2.3 kg mesh bags. After inoculation, tubers were placed in sterilized plastic crates in a complete randomized block design (RCBD) with four replicates for a total of 40 tubers. The crates were covered with plastic bags and wet towels were placed inside the bags. The crates were stored in a controlled environmental growth chamber (Conviron, model MTR 30, Winnipeg, Manitoba, CA) for 30 days at 10^{o} C. After 30 days, disease incidence was measured using a digital scanner and image analysis software (Niemira et al., 1999; Rojas et al., 2014).

Inoculation and seed treatment. Seed treatment studies were conducted in the greenhouse and in the field. Commercial cultivars and ABL tubers were cut, into two or three sections longitudinally (depending on the size of tuber) ensuring the presence of viable sprouts on each seed-piece. The cut seed pieces were immersed in a mixture of 2 x 10^6 sporangia ml^{-1} mycelium and zoospores of *P. infestans* US-23 and/or US-8, US-22 and US-24 genotypes for 30 min then dried for 1 h prior to treating with fungicides. Treatments applied to seed pieces were (i) non-inoculated, (ii) inoculated with *P. infestans*, and (iii) inoculated with *P. infestans* and treated with either Emesto Silver (active ingredients: penflufen, prothioconozole, Bayer CropScience, NC, USA) at label rate (0.31 fl oz per 100 lbs. of seed pieces), Nubark Mancozeb (active ingredients: zinc ion and manganese ethylene bisdithiocarbamate, Wilbur-Ellis Company, WA, USA) at label rate (1 lb per 100 lbs. whole or cut seed pieces), and Reason 500 (active ingredient: fenamidone, Bayer

CropScience, NC, USA) at label rate (0.15 fl oz per 100 lbs. of seed pieces). Treated seed pieces were planted the following day.

Field experiments. Inoculated and treated seed pieces were planted at the Michigan State University Clarksville Research Center, Clarksville, MI on 28 May of 2015 and on 31 May of 2016 into two-row by 10-ft plots (10-in between plants to give a target population of 10 plants at 34-in row spacing) replicated four times in a RCBD. Plots were irrigated as needed with sprinklers and were hilled immediately before sprays began. Weeds were controlled by hilling and with Dual 8E (2 pt on 5 Jun 2015 and 10 Jun 2016), Poast (1.5 pt on 17 Jul 2015 and 21 Jul 2016). Insects were controlled with Admire 2F (20 fl oz at planting), Sevin 80S (1.25 lb on 17 and 31 Jul 2015 and 21 and 31 Jul 2016), Thiodan 3EC (2.33 pt on14 Aug 2015 and 18 Aug 2016) and Pounce 3.2EC (8 oz on 17 Jul 2015 and 21 Jul 2016). The number of emerged plants was recorded over a 42-day period after planting both years. Final plant stand (%) and the relative area under the emergence progress curve (RAUEPC) was calculated. Meteorological variables were measured with a Campbell weather station located at the farm from 1 May to 31 Oct in 2015 and 2016 respectively.

Greenhouse experiments. Inoculated and treated seed pieces were planted in the greenhouse at Michigan State University, East Lansing, MI on 12 Jan of 2016 and on 1 Feb of 2017 in 4-inch pots (3.8 L) containing potting compost mix (Suremix perlite, Michigan Growers Products, Inc. Galesburg, MI, USA). Plants in the pot were placed in a RCBD with four replications and were maintained at 16 h photoperiod. Pots were irrigated at 3-5-day intervals and were fertilized weekly (20-20-20, Proturf and Peters Professional®, Brantford, ON, Canada). The number of emerged plants were recorded over a 21-day in 2016 and 19-day in 2017 period after planting. Final plant stand (%) and the RAUEPC was calculated.

DATA COLLECTION AND ANALYSES

Tuber susceptibility test. The severity of tuber blight development represented by necrotic tissue and measured by relative average reflective intensity (RARI) was determined. The RARI was calculated based on the following formula: RARI (%) = (1-mean average reflective intensity (ARI) treatment/mean ARI control) x100; RARI has a minimum value of zero (no darkening) and maximum value of 100 (tuber surface completely darkened). Relative average intensity reflection data was analyzed using ANOVA and means were compared using Tukey's HSD test (alpha=0.05).

Field and greenhouse experiments. The RAUEPC was calculated by dividing the area under the emergence progress curve (AUEPC) by the maximum AUEPC (100 X duration of emergence period) from planting to full emergence. Data was analyzed using ANOVA and means were compared using Tukey's HSD test (alpha=0.05). There were significant differences in the potato seed tuber emergence and final plant stand for plants inoculated with the four different genotypes of *P.infestans*, and between years, therefore data were analyzed separately for each *P. infestans* genotypes. Statistical analysis of field experiments also showed significant differences between years, therefore data from two years were analyzed separately.

RESULTS

Tuber susceptibility. In 2015, Russet Norkotah had the highest RARI value of 25.98 and was statistically higher compared to other cultivars and six ABLs (Table 4). The rest of the cultivars were not significantly different from each other (Table 4). MSS576-5SPL had significantly higher RARI value of 22.45 compared to five ABLs (Table 4). In 2018, Snowden and Jacqueline Lee had significantly lower RARI values of 1.73 and 1.40 compared to other cultivars and ABLs except

for MST145-2 with RARI value of 7.64%. The rest of the cultivars and ABLs were not significantly different from each other (Table 4).

Table 4**.** Effect of tuber tissue late blight as mean Relative Area Reflection Intensity (RARI%) in commercial potato cultivars and advanced breeding lines (ABL) after inoculation with US-23 genotypes of *Phytophthora infestans*

Variety/ABL	**Mean RARI (%)[a]**			
	2015	Significance	2018	Significance
Russet Norkotah	25.98	a[b]	n/a	n/a
MSS576-5SPL	22.45	ab	n/a	n/a
MST145-2	16.95	abc	7.64	ab
MSL211-3	16.70	abc	13.12	a
Missaukee	15.29	bcd	12.62	a
MSS487-2	14.01	bcd	10.34	a
MSQ131-A	11.97	cd	11.31	a
Snowden	11.95	cd	1.73	b
Atlantic	11.68	cd	n/a	n/a
Jacqueline Lee	11.03	cd	1.40	b
MSV235-2PY	10.03	cd	n/a	n/a
MSS428-2	9.02	cd	n/a	n/a
MSS176-1	6.94	d	15.20	a
MSS206-2	5.35	d	10.67	a

[a] Normalized tuber tissue darkening score expressed as RARI (%) = (1-mean ARI treatment/mean ARI control) x 100; % RARI has a minimum value of zero (no darkening) and maximum value of 100 (tuber surface completely darkened)

[b] Values followed by the same letter are not significantly different at $p = 0.05$ (Least Square Means Tukey HSD)

Field experiments. Atlantic had significantly lower final plant stand, 26.0% and 32.0% in 2015 and 2016, respectively except Russet Norkotah 39.0% and 46.5% in 2015 and 2016, respectively and Snowden 49.5% in 2016 (Table 5). Similarly, Atlantic had lower RAUEPC values 15.0% and 15.3% in 2015 and 2016, respectively and was significantly lower compared to two other cultivars (Table 5). In 2015, ABLs were not significantly different from each other, except MSV235-2PY had significantly higher final plant stand 76.0% compared to MSS487-2 53.0% (Table 5). Similarly, in 2016 ABLs were not significantly different from each other except MSV235-2PY 83.5% was significantly higher in final plant stand from MSQ131-A (58.0%) (Table 5). MSV235-2PY had the highest RAUEPC values 42.6%, and 39.2% in 2015 and 2016, respectively, and it was significantly higher from two ABLs MSQ131-A 27.9% and MSS487-2 25.0% in 2015 and four ABLs MSQ131-A 19.5%; MSS206-2 22.5%; MSS487-2 25.4% and MSS576-5SPL 29.4% in 2016. In 2015, no significant differences among treatments in final plant stand, except non-treated inoculated control. In 2016, non-inoculated non-treated control had significantly higher final plant stand compared to the rest of the treatments, except Reason+Nubark Mancozeb treatment. In 2015, no significant difference among fungicide treatments in RAUEPC (Table 5). The non-treated inoculated control had significantly lower final plant stand and RAUEPC in 2016 and 2017 respectively, compared to other treatments (Table 5).

Table 5**.** Field trials (2015 and 2016) on effect of seed treatments for management of seed-borne *Phytophthora infestans* US-23 genotype in different potato cultivars and advanced breeding lines (ABL), plant stand (%), relative area under emergence progress curve values (RAUEPC 0-100)

Variety/ABL	**Final Plant Stand (%)**				**RAUEPC (%)**			
	2015		2016		2015		2016	
Atlantic	26.0	e[a]	32.0	d	15.0	e	15.3	f
Jacqueline lee	49.0	cd	75.0	ab	22.9	def	24.2	cdef
Missaukee	73.0	ab	73.5	ab	38.6	ab	29.2	bcd
Russet Norkotah	39.0	de	46.5	cd	19.2	ef	15.3	f
Snowden	65.5	abc	49.5	cd	38.5	ab	26.5	bcde
MSL211-3	70.0	abc	76.0	ab	35.7	abcd	34.2	ab
MSQ131-A	56.0	abcd	58.0	bc	27.9	bcde	19.5	ef
MSS176-1	70.5	abc	77.5	ab	38.8	ab	33.3	abc
MSS206-2	74.0	ab	65.5	abc	40.6	ab	22.5	def
MSS428-2	64.5	abc	n/a	n/a	36.4	abc	n/a	n/a
MSS487-2	53.0	bcd	73.5	ab	25.0	cdef	25.4	bcde
MSS576-5SPL	75.5	ab	74.5	ab	37.9	ab	29.4	bcd
MSV235-2PY	76.0	a	83.5	a	42.6	a	39.2	a
Treatments								
Non-inoculated non-treated control	78.8	a	79.4	a	42.5	a	31.4	a
Nubark Mancozeb	68.1	ab	62.7	b	36.8	ab	25.9	b
Emesto Silver+Nubark Mancozeb	65.8	b	65.4	b	34.6	b	25.7	b
Reason+Nubark Mancozeb	67.9	ab	79.7	a	35.3	b	31.4	a
Inoculated control	24.0	c	39.9	c	12.0	c	16.4	c

[a] Values followed by the same letter are not significantly different at $p = 0.05$ (Least Square Means Tukey HSD)

US-8. Jacqueline Lee had the lowest final plant stand 18,5% and 18,5% in 2016 and 2017, respectively and was significantly lower compared two other cultivars Atlantic 32.0%, and Snowden 38.0% in 2015 and one cultivar Snowden 40.5% in 2016 (Table 6). Similarly, Jacqueline Lee had the lowest RAUEPC values of 4.8% and 6.4% in 2016 and 2017, respectively and was significantly lower compared to the cultivars Atlantic, Russet Norkotah and Snowden with RAUEPC values of 12.2%, 12.2% 14.5%, respectively in 2016 and cultivar Snowden with RAUEPC value of 15.2% in 2017. In 2016, no significant differences among ABLs in final plant stand except, MSV235-2PY and MSL211-3 with final plant stands of 36.5% and 34.6%, respectively that were significantly higher compared to MSQ131-A with final plant stand of 21.0% (Table 6). Similarly, in 2017 there were no significant differences among ABLs in final plant stand except, MSS428-2 had significantly lower plant stand (0.5%). In 2016, MSV235-2PY had significantly higher RAUEPC (16.8%) compared to the rest of the ABLs, except MSL211-3 with 13.7% emergence (Table 6). In 2017, MSS428-2 had a significantly lower RAUEPC value (0.2%) compared to the rest of the ABLs. Also, MSS576-5SPL had significantly lower RAUEPC value (7.2%) from ABLs MSQ131-A, MSS487-2 and MSV235-2PY with RAUEPC values of 12.5%, 12.5% and 13.1%, respectively (Table 6). In 2016, cultivars and ABLs treated with Reason+Nubark Mancozeb had significantly higher final plant stand compared to other treatments, except for the non-inoculated and non-treated control (Table 6). In 2017, all three fungicide treatments had a significantly higher RAUEPC compared to controls. There was no significant differences among fungicide treatments RAUEPC in 2016 and 2017. The non-treated inoculated control had significantly lower final plant stand and RAUEPC values of 1.7%, 7.9%, 0.7 and 2.5% in 2016 and 2017, respectively (Table 6).

Table 6**.** Greenhouse trials (2016 and 2017) on effect of seed treatments for management of seed-borne *Phytophthora infestans* US-8 genotype in different potato cultivars and advanced breeding lines (ABL), plant stand (%), relative area under emergence progress curve values (RAUEPC 0-100)

Variety/ABL	**Final Plant Stand (%)**				**RAUEPC (%)**			
	2016		2017		2016		2017	
Atlantic	32.0	abc[a]	31.0	ab	12.2	abcde	10.4	abc
Jacqueline lee	18.5	d	18.5	b	4.8	fg	6.4	c
Missaukee	26.3	abcd	20.2	b	8.9	bcdef	7.1	c
Russet Norkotah	29.0	abcd	31.5	ab	12.2	abcd	8.1	bc
Snowden	38.0	a	40.5	a	14.5	ab	15.2	a
MSL211-3	34.6	ab	30.3	ab	13.7	abc	9.9	bc
MSQ131-A	21.0	cd	34.0	ab	2.4	g	12.5	ab
MSS176-1	30.0	abcd	37.5	a	7.2	defg	11.2	abc
MSS206-2	25.0	bcd	31.5	ab	5.3	fg	10.3	abc
MSS428-2	24.0	bcd	0.5	c	7.4	degf	0.2	d
MSS487-2	26.0	abcd	33.5	ab	8.2	cdefg	12.5	ab
MSS576-5SPL	24.0	bcd	31.0	ab	6.3	efg	7.2	c
MSV235-2PY	36.5	ab	33.0	ab	16.8	a	13.1	ab
Treatments								
Non-inoculated non-treated control	38.1	ab	23.5	b	12.1	a	7.8	b
Nubark Mancozeb	31.9	bc	32.9	a	11.6	a	10.8	a
Emesto Silver+Nubark Mancozeb	29.0	c	38.7	a	9.7	a	13.4	a
Reason+Nubark Mancozeb	39.6	a	40.6	a	11.9	a	13.3	a
Inoculated control	1.7	d	7.9	c	0.7	b	2.5	c

[a] Values followed by the same letter are not significantly different at $p = 0.05$ (Least Square Means Tukey HSD).

US-22. There were no significant differences in plant stand among cultivars in 2016 when inoculated with US-22 genotype. There were no significant differences in plant stand among cultivars in 2017, except for Snowden which had significantly higher plant stand compared to Jacqueline Lee, with percent emergence of 28.0% and 15.5%, respectively (Table 7). Snowden had the highest RAUEPC values of 13.7% and 10.9% in 2016 and 2017, respectively and was significantly higher from Jacqueline Lee with RAUEPC values of 4.4% and 4.8% in 2016 and 2017, respectively, and Missaukee with RAUEPC value of 5.1% in 2017 (Table 7). In 2016, MS176-1 had significantly higher final plant stand (35.5%) from ABL MSQ131-A (22.0%). In 2017, there were no significant differences among ABLs, except for MSS428-2 which had significantly lower final plant stand (0%). In 2016, MSV235-2PY had significantly higher a RAUEPC value (12.8%) compared to ABLs MSQ131-A and MSS576-5SPL with a RAUEPC values of 2.8% and 5.3%, respectively. In 2017, MSV235-2PY (10.1%) and MSS487-2 (8.4%) had significantly higher a RAUEPC values compared to ABLs MSS428-2 (0%) and MSS576-5SPL (3.7%) (Table 7). Cultivars and ABLs treated with Reason+Nubark Mancozeb had the highest final plant stand values of 44.6% and 40.0% in 2016 and 2017, respectively and was significantly higher from Emesto Silver+Nubark Mancozeb (31.7%) and non-treated inoculated control (2.9%) in 2016 and all treatments in 2017 (Table 7). In 2016, there were no significant differences among treatments in RAUEPC except inoculated control had significantly lower RAUEPC value of 0.8% (Table 7). In 2017, cultivars and ABLs treated with Reason+Nubark Mancozeb had significantly higher RAUEPC (13.2%) compared to other treatments. Nubark Mancozeb (9.1%) and Emesto Silver+Nubark Mancozeb (10.5%) treatments were not significantly different from each other; however, they were significantly higher compared to non-inoculated non-treated (0.3%) and non-treated inoculated (0.9%) (Table 7).

Table 7**.** Greenhouse trials (2016 and 2017) on effect of seed treatments for management of seed-borne *Phytophthora infestans* US-22 genotype in different potato cultivars and advanced breeding lines (ABL), plant stand (%), relative area under emergence progress curve values (RAUEPC 0-100)

Variety/ABL	**Final Plant Stand (%)**				**RAUEPC (%)**			
	2016		2017		2016		2017	
Atlantic	35.0	ab[a]	24.0	ab	10.3	abcd	9.3	abc
Jacqueline lee	29.0	abc	15.5	b	4.4	de	4.8	cd
Missaukee	32.5	abc	16.5	ab	9.7	abcd	5.1	cd
Russet Norkotah	31.5	abc	22.0	ab	11.0	abc	7.1	abcd
Snowden	40.0	a	28.0	a	13.7	a	10.9	a
MSL211-3	35.5	ab	20.5	ab	12.0	ab	5.9	bcd
MSQ131-A	22.0	c	22.5	ab	2.8	e	8.1	abcd
MSS176-1	35.5	ab	24.0	ab	7.9	adcde	7.1	abcd
MSS206-2	31.5	abc	23.5	ab	7.0	bcde	8.1	abcd
MSS428-2	28.5	bc	0	c	7.4	degf	0	e
MSS487-2	31.5	abc	23.5	ab	7.6	bcde	8.4	abc
MSS576-5SPL	27.5	bc	17.0	ab	5.3	cde	3.7	de
MSV235-2PY	34.0	ab	22.0	ab	12.8	ab	10.1	ab
Treatments								
Non-inoculated non-treated control	40.0	a	1.0	c	11.3	a	0.3	c
Nubark Mancozeb	40.0	a	27.3	b	11.3	a	9.1	b
Emesto Silver+Nubark Mancozeb	31.7	b	29.4	b	8.6	a	10.5	b
Reason+Nubark Mancozeb	44.6	a	40.0	a	11.0	a	13.2	a
Inoculated control	2.9	c	1.9	c	0.8	b	0.9	c

[a] Values followed by the same letter are not significantly different at $p = 0.05$ (Least Square Means Tukey HSD)

US-23. There were no significant differences among cultivars in the final plant stand in 2016 and 2017 (Table 8). Snowden had significantly higher RAUEPC values of 19.9%; 15.3% in 2016 and 2017, respectively compared to cultivars Jacqueline Lee and Russet Norkotah with plant stand values of 8.5% and 12.0% respectively in 2016. In 2017, cultivars Jacqueline Lee (7.6%), Missaukee (8.6%) and Russet Norkotah (7.5%) had significantly lower RAUEPC compared to Snowden (15.3%) and Atlantic (10.7%) (Table 8). There were no significant differences among ABLs in final plant stand in 2016, except for MSV235-2PY which had a significantly higher plant stand compared to MSQ131-A3, with stand plant values of 47.0% and 30.5% respectively. In 2017, there were no significant differences among ABLs, except for MSS428-2 which had significantly lower final plant stand value of 2.5% (Table 8). In 2016, MSV235-2PY had significantly higher RAUEPC with value of 21.6% compared to other six ABLs. In 2017, there were no significant differences among ABLs in RAUEPC, except for MSS428-2 which had a significantly lower RAUEPC value 0.8%. In 2016, there were no significant differences among treatments, except for the non-treated inoculated control which had significantly lower final plant stand value of 26.7% (Table 8). In 2017, cultivars and ABLs treated with Nubark Mancozeb had significantly higher final plant stand value of 40.2% compared to Emesto Silver+Nubark Mancozeb and Reason+Nubark Mnacozeb with plant stand of 25.0% and 33.1% respectively (Table 8). Similarly, cultivars and ABLs treated with Nubark Mancozeb had the highest RAUEPC values of 15.7% and 12.5% in 2016 and 2017, respectively and were significantly higher from Reason+Nubark Mancozeb and inoculated control in 2016 and Emesto Silver+NM in 2017 (Table 8).

Table 8**.** Greenhouse trials (2016 and 2017) on effect of seed treatments for management of seed-borne *Phytophthora infestans* US-23 genotype in different potato cultivars and advanced breeding lines (ABL), plant stand (%), relative area under emergence progress curve values (RAUEPC 0-100)

Variety/ABL	**Final Plant Stand (%)**				**RAUEPC (%)**			
	2016		2017		2016		2017	
Atlantic	41.5	ab[a]	33.5	a	16.0	bc	10.7	ab
Jacqueline lee	35.5	bc	25.5	a	8.5	ef	7.6	b
Missaukee	41.0	abc	37.5	a	15.3	bc	8.6	b
Russet Norkotah	38.5	abc	28.5	a	12.0	cdef	7.5	b
Snowden	46.0	ab	41.0	a	19.9	ab	15.3	a
MSL211-3	43.5	ab	37.5	a	13.7	abc	11.2	ab
MSQ131-A	30.5	c	38.0	a	6.7	f	13.1	ab
MSS176-1	39.5	abc	37.0	a	14.2	cd	12.0	ab
MSS206-2	43.0	ab	35.5	a	11.4	cdef	11.5	ab
MSS428-2	36.5	abc	2.5	b	11.4	cdef	0.8	c
MSS487-2	40.5	abc	39.0	a	12.6	cde	13.4	ab
MSS576-5SPL	37.5	abc	37.0	a	9.8	def	9.5	ab
MSV235-2PY	47.0	a	35.5	a	21.6	a	13.7	ab
Treatments								
Non-inoculated non-treated control	41.0	a	29.8	bc	14.7	ab	9.5	ab
Nubark Mancozeb	45.2	a	40.2	a	15.7	a	12.5	a
Emesto Silver+Nubark Mancozeb	45.6	a	25.0	c	15.3	ab	8.1	b
Reason+Nubark Mancozeb	41.7	a	33.1	abc	12.9	b	10.3	ab
Inoculated control	26.7	b	36.5	ab	9.1	c	11.5	a

[a] Values followed by the same letter are not significantly different at $p = 0.05$ (Least Square Means Tukey HSD)

US-24. In 2016, there were no significant differences among cultivars in the final plant stand (Table 9). In 2017, Snowden had significantly higher final plant stand of 45.0% compared to Jaqueline Lee and Missaukee with final plant of 23.0% and 38.0% respectively (Table 9). Similarly, Snowden had significantly higher RAUEPC values 20.9% and 19.3% in 2016 and 2017, respectively compared to Jacqueline Lee with RAUEPC values of 8.7% and 7.7% in 2016 and 2017, respectively, and Russet Norkotah with RAUEPC value of 13.0% in 2016 (Table 9). In 2016, MSV235-2PY had significantly higher final plant stand of 44.0% compared to MSQ131-A and MSS428-2 with final plant stand of 20.0% and 28.5% (Table 9). In 2017 there were no significant differences among ABLs in final pant stand, except for MSS428-2 which had significantly lower final plant stand of 0%. In 2016 MSV235-2PY had significantly higher RAUEPC value of 25.5% compared to all other ABLs. In 2017 there were no significant differences among ABLs, except for MSS428-2 which had significantly lower RAUEPC value of 0% (Table 9). There were no significant differences among fungicide treatments in final plant stand and in RAUEPC in 2016 and 2017, respectively (Table 9). In 2016, non-inoculated and non-treated control had significantly higher final plant stand of 38.7% and RAUEPC values of 15.2% compared to non-treated inoculated control with final plant stand of 9.4% and RAUEPC values of 3.9%. In 2017, there were no significant differences among non-inoculated non-treated and non-inoculated and treated controls in final plant stand and in RAUEPC (Table 9).

Table 9**.** Greenhouse trials (2016 and 2017) on effect of seed treatments for management of seed-borne *Phytophthora infestans* US-24 genotype in different potato cultivars and advanced breeding lines (ABL), plant stand (%), relative area under emergence progress curve values (RAUEPC 0-100)

Variety/ABL	**Final Plant Stand (%)**				**RAUEPC (%)**			
	2016		2017		2016		2017	
Atlantic	38.0	ab[a]	39.0	a	16.2	bc	15.3	ab
Jacqueline lee	36.0	ab	23.0	b	8.7	ef	7.7	c
Missaukee	36.0	ab	38.0	b	15.5	bcd	11.6	abc
Russet Norkotah	38.5	ab	43.5	a	13.0	cde	15.0	ab
Snowden	44.0	a	45.0	a	20.9	ab	19.3	a
MSL211-3	36.5	ab	43.5	a	15.3	bcd	14.1	abc
MSQ131-A	20.0	c	43.0	a	4.9	f	16.8	ab
MSS176-1	38.0	ab	45.5	a	13.9	cde	15.6	ab
MSS206-2	39.0	ab	n/a	n/a	13.9	cde	14.1	ab
MSS428-2	28.5	bc	0.0	c	10.5	cdef	0.0	d
MSS487-2	36.0	ab	40.5	a	12.0	cde	16.2	ab
MSS576-5SPL	34.5	ab	44.0	a	9.1	def	13.6	abc
MSV235-2PY	44.0	a	39.5	a	25.5	a	19.7	a
Treatments								
Non-inoculated non-treated control	38.7	b	29.8	b	15.2	a	10.4	c
Nubark Mancozeb (NM)	44.8	a	40.9	a	16.8	a	15.9	a
Emesto Silver+NM	42.9	ab	40.4	a	17.2	a	15.4	ab
Reason+NM[b]	44.6	a	40.8	a	16.0	a	15.0	ab
Inoculated control	9.4	c	29.8	b	3.9	b	12.3	bc

[a] Values followed by the same letter are not significantly different at $p = 0.05$ (Least Square Means Tukey HSD)

DISCUSSION

In tuber susceptibility experiments, the US-23 genotype was used because of its predominance in Michigan since 2012. Control treatments in both years did not show any late blight symptoms. In tuber susceptibility experiments performed in 2015 the *P. infestans*, US-23 genotype was more aggressive and tuber necrosis was higher compared to experiments performed in 2018. In 2015, cultivars and ABLs that had less tuber necrosis were Snowden, Atlantic, Jacqueline Lee, MSV235-2PY, MSS428-2 and MSS176-1. Kirk et al., (2009) also reported that Atlantic and Jacqueline Lee showed less disease incidence and more tuber resistance in the field against *P. infestans* US-1, US-8, US-4 and US-11 genotypes (Kirk et al., 2009). Reduced tuber necrosis symptoms on tubers in 2018 experiments could be due to temporal factors, because cultivars and ABLs were evaluated at a later time than in 2015. In 2015, tuber susceptibility experiments were performed in January and in 2018 the experiments were performed at the end of April. A number of studies have reported mixed results, where in one study stability of resistance of potato tissue remained the same with age in susceptible potato cultivars, but decreased with age of tested tubers in resistant cultivars (Bhatia and Young, 1985). Conversely, another study found that tuber age increases resistance in some cultivars but decreases in others (Lebecka et al., 2006). Thus, suggesting that in our study testing time may affected tuber response.

Field experiments combined foliar late blight resistant ABLs with seed treatments in an effort to better manage both foliar and tuber late blight on potato. In 2015 and 2016, there were no foliar symptoms of late blight observed. Late blight symptoms on the stem of plants were detected in greenhouse experiments after seed inoculation. Kirk et al., (2009) reported similar results and did not express foliar late blight symptoms in the field. However, foliar late blight symptoms were observed in the controlled environment study with the US-8 genotype. Another study by Wharton

et al., (2012) reported similar findings, with 100% tuber infection rate, no foliar late blight symptoms observed in the field, except one plant in the inoculated non-treated control. Among the commercial cultivars, the higher number of plants and better rate of the emergence over time were observed in Snowden, Jacqueline Lee and Missaukee compared to Atlantic and Russet Norkotah. The performance of ABLs were similar in terms of the rate of emergence over the same time period and in total number of plants. The fungicide treatments Nubark Mancozeb alone or in combination of either Emesto Silver, or Reason showed similar effectiveness in total number of plants and in rate of emergence over time compared to the non-inoculated non-treated controls. Whereas, the non-treated inoculated control had significantly a lower number of emerged plants, therefore suggesting that tubers did not emerge due to rotting. Several other studies reported similar results and suggest a negative correlation between inoculated tubers and rate of emergence (Wharton et al., 2012; Kirk et al., 2009; Rojas et al., 2014).

In greenhouse experiments, no foliar late blight symptoms were observed, however symptoms did occur in tubers and lower parts of the stem (Figure 1). The four (US-8, US-22, US-

Figure 1**.** Symptoms of late blight on lower parts of the stem (pointing with errors) in the greenhouse experiment using US-23 genotype of *Phytophthora infestans*

Figure 2**.** Symptom of soft rot secondary infection in the greenhouse experiment using US-23 genotype of *Phytophthora infestans*

23 and US-24) genotypes of *P. infestans* resulted in different responses in cultivars and ABLs. Overall, Nubark Mancozeb alone or in combination of either Reason or Emesto Silver did not show any significant differences among one another. However, all three fungicide treatments and non-inoculated non-treated controls had higher plant stand and the rate of emergence of the plants over time were higher compared to inoculated non-treated control. Additionally, in experiments with *P. infestans* US-8 and US-22 genotypes, the inoculated non-treated control had a significantly lower number of emerged plants compared to the non-inoculated and non-treated and three fungicide treatments Nubark Mancozeb, Reason+Nubark Mancozeb and Emesto Silver+Nubark Mancozeb. Other studies have reported similar results where US-8 and US-22 were more aggressive compared to other *P. infestans* genotypes (Rojas et al., 2014; Kirk et al., 2009). In experiments with US-23 and US-24, mixed results were observed. In some years, inoculated and non-treated treatments were not different from non-inoculated and non-treated in terms of the number of emerged plants. Suggesting that the US-23 and US-24 genotypes may not be as aggressive as US-8 and US-22. The secondary bacterial infection with soft rot were recorded in non-inoculated and non-treated control and this could be another explanation for lower number of emerged plants (Figure 2). The number of plants and rate of emergence over time in all commercial cultivars and ABLs using US-23 and US-24 *P. infestans* genotypes in both years were similar, except ABL MSS428-2. In 2017, ABL MSS428-2 in the experiment with US-23 and US-24 showed poor or no emergence. In the experiment with the US-8 genotype cultivars had a similar number of emerged plants and similar rate of emergence over time, except Jacqueline Lee. Jacqueline Lee had low number of plants in both years. In a study by James et al., (2003) Jacqueline Lee was determined susceptible to US-8 genotype in tuber assays. Conversely, a study by Rojas et al., (2014) reported that Jacqueline Lee was the least susceptible among other tested cultivars

against a number of *P. infestans* genotypes including US-8 and US-22. Advanced breeding lines also performed similarly in terms of the total of emerged plants and rate of emergence over time, except, MSQ131-A and MSS428-2. Similarly, to 2017 experiments using US-23 and US-24 genotypes, MSS428-2 had significantly reduced emergence compared to other ABLs. In experiments with the US-22 genotype cultivars had similar number of emerged plants and showed similar rate of emergence over time. Advanced breeding lines also performed similarly in terms of the total emerged plants and rate of emergence, except, MSQ131-A and MSS428-2 which had low or no emerged plants. Overall, in experiments with all four tested *P. infestans* genotypes, the ABL MSQ131-A had lower number of emerged plants or no plants in the case of MSS428-2 and the rate of emergence was lower compared to other ABLs evaluated.

Seed treatments with fungicides are commonly used in commercial potato production to control seed-borne diseases (Wharton et al., 2012). Planting healthy and non-infected potato seeds is crucial, however, according to this study inoculated and treated seed tubers had similar numbers of emerged plants compared to non-inoculated and non-treated control. Since no foliar late blight was found in both years, we were not able to differentiate between susceptible and resistant cultivars and ABLs. In conclusion, this study shows that combination of foliar and seed treatments can protect infected seed tubers. The integrated management with foliar, tuber and seed treatment is a good approach. Evaluating ABL with newly reported genotypes of *P. infestans* is important.

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LITERATURE CITED

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CHAPTER 3: EVALUATION OF CONVENTIONAL AND REAL-RIME POLYMERASE CHAIN REACTION METHODS TO DETECT *DICKEYA* AND *PECTOBACTERIUM* SPECIES.

ABSTRACT

Blackleg, aerial stem rot and tuber soft rot are caused by either *Dickeya* spp. or *Pectobacterium* spp. Blackleg caused by *Pectobacterium* spp. is common, but *Dickeya* spp. has not been an issue until an outbreak in 2015. The outbreak resulted in high incidence of this disease in the US and in Michigan. Symptoms of *Dickeya* and *Pectobacterium* spp. are often indistinguishable. The bacteria can move via asymptomatic infected seed tubers. Accurate diagnostics are essential to detect these bacteria, particularly for seed-certification. The objectives of this research included: i) to evaluate the fidelity of available PCR primers for *Dickeya* and *Pectobacterium* spp. and ii) determine the spread and presence soft rot causing bacteria in Michigan potato production by surveying infected plant tissue from the fields and dormant tubers from seed production lots. Eleven total PCR and qPCR primers were tested for detect and differentiate *Dickeya* and *Pectobacterium* spp. using infected plant tissue and dormant tubers. A total of 150 symptomatic field samples were received between 2016 and 2017 years. Among them, 98 samples were positive for *Dickeya* spp. and 45 samples were positive for *P. carotovorum*/*parmentieri* and *atrosepticum* and the remaining samples were negative for all assays. In addition, in the fall of 2016 and 2017 4800 dormant tubers were tested originating from Michigan. In both years no seed-lot tested positive for *Dickeya*, only *Pectobacterium* spp. Among 11 tested PCR and qPCR primers, two primers did not amplify either *Pectobacterium* or *Dickeya* spp., and five primers showed inconsistent results. Two primers need to be validated further. In conclusion, there is one specific primer pair that can consistently and accurately detect *Dickeya* spp., and two specific primer pairs for *Pectobacterium* spp. but there are no primers that can detect mixed samples effectively.

INTRODUCTION

Potatoes (*Solanum tuberosum* L.), are the most important non-grain food crop in the world. However, potatoes are susceptible to numerous soil and seed-borne and foliar diseases. Worldwide there are about 40 seed and soil-borne diseases that affect tubers of potato, which is the economically important part of the crop (Fiers et al., 2011). Blackleg, aerial stem rot and soft rot caused by either *Dickeya* or *Pectobacterium* species are among bacterial seed transmitted diseases that result in significant losses in potato production.

Early in the 2000s, a blackleg outbreak in Europe occurred, caused by an emerging species of pectolytic bacteria. *Dickeya solani* became a major cause of soft rot disease in Europe that resulted in downgrading in seed potato lots (Toth et al., 2011). A similar *Dickeya* outbreak occurred in the US in 2015 (Charkowski, 2018; Ma et al., 2018). The first reports of *Dickeya dianthicola* was confirmed in several states (Jiang et al., 2016; Rosenzweig et al., 2016b). By the end of 2015, the bacteria had been reported in 10 states, including multiple seed potato producing states. *Dickeya* and *Pectobacterium* spp. can be present in asymptomatic seed tubers and can become a primary source of inoculum for infection and spread. This was likely the cause of black leg epidemic in the US in 2015. The infected seed tubers shipped to multiple locations and spread the disease across potato production areas.

All of the known *Dickeya* and *Pectobacterium* spp. are able to cause soft rot symptoms on tubers, however not all of the species can cause black leg or aerial stem rot (Charkowski, 2018). Typically, soft rot and black leg infected potato tubers are diagnosed visually, and it is an insufficient method to distinguish *Dickeya* and *Pectobacterium* since both symptoms caused by these two bacterial pathogens are closely identical. Also, both *Dickeya* and *Pectobacterium.* can be present in the same plant sample, making it harder to distinguish from one another. Scientists

studied and developed molecular assays to detect and distinguish among species (Toth et al., 2011; Van Vaerenbergh et al., 2012). There are a number of PCR primers that have been developed from specific regions of the genomes in both *Dickeya* and *Pectobacterium*. However, the pathogen has reemerged in the US and there is limited information about the specificity of available primers. Therefore, the objective of this research included: i) to evaluate the fidelity of available PCR primers for *Dickeya* and *Pectobacterium* spp. and ii) determine the spread and presence of soft rot causing bacteria in Michigan potato production by surveying infected plant tissue from the fields and dormant tubers from seed production lots.

MATERIALS AND METHODS

Symptomatic samples. Symptomatic tuber and stem samples from various growers from Michigan were received during spring and summer of 2016 and 2017 years. A total of 150 symptomatic samples were received over two years. Samples were washed with tap water to remove excess soil. Tissue samples from the margin of symptomatic and healthy part of the tuber and stems were cut using a sterilized scalpel and placed into 2 ml tubes. Samples were crushed in buffer or H_2O to allow the bacteria to stream out of the samples and 100 µl aliquot of the suspension was plated onto selective media crystal violet pectate (CVP) (1.02 g $CaCl_2 \cdot H_2O$, 1.0 g Tryptone, 5.0 g Tri-sodium citrate, 2.0 g $NaNO_3$, 1.5 mL 0.1% crystal violet, 4.0 g agar, 2.8 mL 5 M NaOH, and 18.0 g pectin) and 500 µl was used for DNA extraction. The bacterial suspension plated on CVP were grown for 48 h at 27° C. Single colonies were obtained for pure culture and isolates were stored long term in 20% glycerol solution in -80° C. Total genomic DNA were extracted from plant tissue and pure bacterial cultures using the DNeasy Power Soil Kit (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions with slight modifications.

Asymptomatic samples. Asymptomatic dormant tuber samples from various seed potato lots were received in the fall of 2016 and 2017. A total of twelve seed lots were received in both years. Each seed lot sub-samples contained 400 tubers. Seed lots were further sub divided into groups of 20. Tuber stem ends from groups of 20 potatoes were removed with 5-mm diameter sterilized cork-borer and placed into 50 ml Erlenmyer flask with 25 ml of quarter-strength sterile Ringer's buffer (2.25 g NaCl, 0.105 g KCl, 0.12 g $CaCl_2 \cdot 6H_2O$ and 0.05 g of sodium bicarbonate). Flasks were placed onto a rotary shaker (Lab-line Orbit, Melrose Park, IL) at 100 rpm for 2 hours to allow bacteria to stream out of samples. After 2 hours, 5 ml of suspension was removed from the flask and placed into a 15 ml sterile centrifuge tubes with 5 ml of pectate solution (0.64 g $MgSO_4 \cdot 7H_2O$, 2.16 g $(NH_4)2SO_4$, 2.16 g K_2HPO4, 1.5 g pectin). The tops of tubes were loosely tightened, and tubes were placed into anaerobic bags with indicators (BD Bio-Bag™ Type C, Sparks, MD) in an incubator at 37-38° C for 48 hours. After incubation, samples were centrifuged at 14,000 rpm for 5 min and pelleted. The supernatant was removed from the tube and the pellet was resuspended in distilled H_2O. Subsequently, the resuspended sample was used for DNA extraction, and a 100 µl aliquot was plated onto CVP, and a sub-sample was stored long-term in 20% glycerol solution at -80° C. The DNA extractions were performed as described above.

Conventional and quantitative polymerase chain reaction. Conventional PCR and quantitative PCR primers were used for differentiation and detection (Table 10). Conventional PCR reactions consisted of: 9.5 µl of Go Taq® Master Mix 2X (Promega, Madison, WI), 1 µl of 10 µM of reverse and forward primer, 12.5 µl of distilled H_2O and 2 µl of genomic DNA, in a 25 µl reaction volume. Each PCR assay included positive control (known *Dickeya* or *Pectobacterium* spp.), negative control (oomycete) and water control. Amplifications were performed on a thermocycler (MyCycler, Bio-Rad, Hercules, CA) using appropriate cycling parameters (Table 10). PCR produc

Table 10**.** List of *Dickeya* or *Pectobacterium* species, assay types, conventional and real time polymerase chain reaction species specific primers, PCR cycling conditions, product sizes and references that were used in this study

Species/subspecies	Assay type	Name, Sequence, Primers (5'- 3')	PCR cycling conditions	PCR product size (bp)	References
Dickeya spp.	Conventional	**PelADE1** GAT CAG AAA GCC CGC AGC CAG AT **PelADE2** CTG TGG CCG ATC AGG ATG GTT TTG TCG TGC	95 ° C for 5m; 30 cycles; 94 ° C for 60s; 62 ° C for 90s, 72 ° C for 120s; 72 ° C for 5m	420	(Nassar et al., 1996)
	Conventional	**DF** AGA GTC AAA AGC GTC TTG **DR** TTT CAC CCA CCG TCA GTC	105.5° C; 95 ° C for 4m; 30 cycles: 94 ° C for 45s; 62 ° C for 90s, 72 ° C for 90s; 72 ° C for 5m	133	(Laurila et al., 2010)
	Conventional/ Real-time	**EchF1** CCC TGT TAC CGC CGT GAA ***EchF2*** CTG ACA AGT GAT GTC CCC TTC GTC TAG AGG	2 min at 50° C, 10 min at 95° C, followed by 40 cycles and 15 sec for 95° C and 1 min for 60° C		(van der Wolf et al., 2013)
	Conventional/ Real-time	***D. chrysenthemiF*** CGA TTT CCC GGC AAG TGT ***D. chrysenthemiR*** TGG CAA AAG GGC TGA ATTG	2 min at 50° C, 10 min at 95° C, followed by 40 cycles and 15 sec for 95° C and 1 min for 60° C		(van der Wolf et al., 2013)
	Conventional/ Real-time	***D. solaniF*** ACA CTA CAG CGC GCA TAA AC CAC CAA AGT GGA AGA TAC CC ***D. solaniR*** CCA GGC CGT GCT CGA AAT CC CTT CGT CGA TCA GGT AAA CC	2 min at 50° C, 10 min at 95° C, followed by 40 cycles and 15 sec for 95° C and 1 min for 60° C		(van der Wolf et al., 2013)
	Conventional/ Real-time	***D. dianthicolaF*** GCC GTA TCC ATC ATG CTT ACC ***D. dianthicolaR*** AAC GGG CGA TAG TCG TCT TG	2 min at 50° C, 10 min at 95° C, followed by 40 cycles and 15 sec for 95° C and 1 min for 60° C		(van der Wolf et al., 2013)
	Conventional/ Real-time	***fliC-1*** TAT CAA CAG CGC CAA AGA CAA CGC ***fliC-2*** ACG GCT CAT GTT GGA TAC TTC GTT	95 ° C for 1m; 35 cycles: 95 ° C for 20s; 55 ° C for 30s, 72 ° C for 45s; 72 ° C for 5m	650	(Van Vaerenbergh et al., 2012)

Table 10. (cont'd)

	Conventional/ Real-time	***dnaXF*** TAT CAG GTY CTT GCC CGT AAG TGG ***dnaXR*** TCG ACA TCC ARC GCY TTG AGA TG	95 ° C for 1m; 35 cycles: 95 ° C for 20s; 55 ° C for 30s, 72 ° C for 45s; 72 ° C for 5m	535	(van der Wolf et al., 2014)
	Conventional/ Real-time	***gyrBF*** TAA GTT YGA CGA YAA CTC STAYAA RGT ***gyrBR*** CCC CTT CCA CCA GGT ASA GTT C	2 min at 50° C, 10 min at 95° C, followed by 40 cycles and 15 sec for 95° C and 1 min for 60° C	974	(Marrero et al., 2013)
Pectobacterium carotovorum/ parmentieri	Conventional	**ExpccF** GAA CTT CGC ACC GCC GAC CTT CTA **ExpccR** GCC GTA ATT GCC TAC CTG CTT AAG	95 ° C for 4m; 30 cycles: 94 ° C for 45s; 62 ° C for 90s, 72 ° C for 90s; 72 ° C for 5m	420/550	(Kang et al., 2003)
Pectobacterium atrosepticum	Conventional	**Y45** TCA CCG GAC GCC GAA CTG TGG CGT **Y46** TCG CCA ACG TTC AGC AGA ACA AGT	95 ° C for 4m; 30 cycles: 94 ° C for 45s; 62 ° C for 90s, 72 ° C for 90s; 72 ° C for 5m	439	(Frechon et al., 1998)

were separated on 1% (w/v) agarose gel in 0.5×Tris-borate-EDTA (5.4g Tris-base, 2.75g Boric Acid, 2 ml 0.5M EDTA, 1000ml H_2O) stained with GelRed Nucleic Acid Stain (Phenix Research Products, Chandler, NC) by electrophoresis and visualized by UV exposure using the Gel Doc 2000 apparatus (Bio-Rad, Hercules, CA). The size of amplicon of interest was determined using 100 bp DNA ladder (NE Biolab, Ipswich, MA).

The qPCR reactions consisted of: 10 µl of iTaq ^{TM} Universal Probes Supermix (Bio-Rad, Hercules, CA), 1 µl of 10 µM of forward and reverse primer, 2 µl of genomic DNA and 7 µl of H_2O, in a 20 µl reaction volume. Reactions were carried in StepOnePlus^{TM}Real-Time PCR System (Applied Biosystems, Foster, CA) using the following cycling parameters: 2 min at 50^{o} C, 10 min at 95^{o} C, followed by 40 cycles and 15 sec for 95^{o} C and 1 min for 60^{o} C (Table 10). Each sample was replicated three times. After the PCR was completed, amplification plots of the standard curve, tested samples and controls were collected from the software.

RESULTS

Primer specificity. Out of nine primers for *Dickeya* spp. two primers (*gyrB* and *D. chrysenthemi*) did not amplify genomic DNA of controls, and two primer pairs (*dnaX* and *D. dianthicola*) amplified both *Dickeya* and *Pectobacterium* spp. and were unable to distinguish between them. Five primer pairs amplified only *Dickeya* spp. when it was not mixed with *Pectobacterium* spp. These included: *D. solani*; *Ech*; *Flic*; *PelADE* and *Dr/Df*. However, all five primers amplified both *Dickeya* and *Pectobacterium* spp. in the mixed samples and could not distinguish among the bacterial genera. The *Dr/Df* primer showed inconsistent results with *Dickeya* spp. samples. Overall, *PelADE* primer was the most consistent in conventional PCR and when confirmed with traditional cultural based approaches. The primer pair *ExpccF/R* amplified both *P. carotovorum* and *P. parmentieri*, however the PCR amplicon size is different between species, for *P.*

carotovorum and for *P. parmentieri* (used to known as *wasabiae*) the expected sizes are 420 bp and 550 bp, respectively. The primer *Y45/46* amplified *P. atrosepticum* consistently. Sub-samples from symptomatic plant tissue were also tested via qPCR using *Flic* and *Ech* primers. Both *Flic* and *Ech* primers detected pure *Dickeya dianthicola* DNA of the positive control. Both primers did not detect pure *Pectobacterium* DNA sample. However, both primers amplified samples that were mixed with *Dickeya* and *Pectobacterium* spp. and with different ratio present in the sample with negative and water control being undetermined (Table 11).

Table 11. Quantitative PCR (qPCR) and cycle threshold (ct) value mean in 5 *Dickeya* positive control with different DNA concentrations, three cv. Lamoka samples, three ratios of *Pectobacterium carotovorum* sub species *carotovorum* (*Pcc*) and *Dickeya dianthicola* (*Dd*), negative (oomycete) and water samples

Samples ID	**Description (DNA concentration)**	**Cycle threshold mean**
2802[a]	Positive control (55)[b]	15.27078
2802	Positive control (5.5)[b]	19.41421
2802	Positive control (0.55)[b]	23.53942
2802	Positive control (0.055)[b]	27.96969
2802	Positive control (0.0055)[b]	33.05048
1907[c]	*Dd* positive sample	29.54498
Lamoka 1[d]	*Pcc* positive sample	39.85369
Lamoka 3[d]	*Pcc* positive sample	37.85369
Lamoka 10[d]	*Pcc* positive sample	38.85369
Pcc[e]	*Pcc* positive sample	38.85369
*Pcc*75:*Dd*25[f]	75:25 ratio sample	18.11978
*Pcc*50:*Dd*50[f]	50:50 ratio sample	16.78828
*Pcc*25:*Dd*75[f]	25:75 ratio sample	16.17356
Negative	Oomycete negative control	Undetermined
Water	Distilled autoclaved water	Undetermined

[a]2802= *Dickeya* positive control
[b] positive control with different number of DNA concentration for standard curve
[d] Samples from commercial fields
[e] *Pcc*= positive control
[f] *Pcc* and *Dd* different ratios

Symptomatic samples. A total of 150 symptomatic tuber and stem samples were collected from potato production fields in 2016 and 2017 (Table 12). All of the samples were used for genomic DNA extraction, conventional PCR and qPCR and traditional cultural based approaches. Among all samples, 98 tested positive for *Dickeya* spp. using the conventional primer pairs *PelADE*, 25 tested positive for *Pectobacterium* spp. (*P. carotovorum/P. parmentieri*) using primer pairs *ExpccF/R* and 20 of them were tested positive for *P. atrosepticum* using primer pairs *Y45/Y46*. Thirteen samples tested positive for *Dickeya* and *P. carotovorum/parmentieri* species. Ten samples tested positive for *Dickeya* spp. and *P. atrosepticum*. Nine samples tested positive for *P. carotovorum/parmentieri* and *P. atrosepticum* (Table 12). Symptomatic samples that plated onto CVP all formed pitted cavities in the media and were confirmed as pectolytic.

Asymptomatic samples. In 2016 400 subsamples of dormant tubers were received from 4 seed-lots (1600 tubers total; Table 13). Samples from seed-lots were combined and a total of 80 sub-samples were used for DNA extraction and PCR. Among 80 samples 19 of them tested positive for *P. carotovorum/P. parmentieri* using primer pairs *ExpccF/R* and five of them tested positive for *P. atrosepticum*, and none of the tested positive for *Dickeya* spp. (Table 13). Similarly, in 2017 400 subsamples of dormant tubers were received from 8 seed-lots (3200 tubers total; Table 14). Samples were combined and total of 160 sub-samples were used for DNA extraction and PCR. Among 160 samples 6 of them tested positive for *P. carotovorum/P. parmentieri* using primer pairs *ExpccF/R* and none of the samples were tested positive for *Dickeya* and *P. atrosepticum* (Table 14).

Table 12**.** Results of conventional polymerase chain reaction (PCR) of three (*PelADE1/2*, *ExpccF/R*, and *Y45/46*) set of primers from field samples from summer and fall of 2016 and 2017

	Conventional PCR assays		
Variety	***PelADE1/2***	***ExpccF/R***	***Y45/46***
Norwis	+[a]	+	-[b]
Norwis	+	+	-
Norwis	+	+	-
Lamoka	+	+	-
Lamoka	+	+	-
Lamoka	+	+	-
N/A[c]	+	+	+
N/A	+	+	+
N/A	+	+	+
N/A	+	+	+
N/A	+	+	+
N/A	+	+	+
N/A	+	-	-
N/A	+	-	-
N/A	+	-	-
N/A	+	-	-
N/A	+	-	-
N/A	+	-	-
N/A	+	-	-
N/A	+	-	-
N/A	+	-	-
N/A	+	-	-
N/A	+	-	-
N/A	+	-	-
N/A	+	-	-
N/A	+	-	-
N/A	-	-	-
N/A	-	-	+
N/A	-	-	-
N/A	-	-	-
N/A	-	-	-
N/A	-	-	-
N/A	-	-	-
N/A	-	-	-
N/A	-	-	-
N/A	-	-	-
FL2137	-	+	+
FL2137	-	+	-

Table 12. (cont'd)

FL2137	-	+	-
FL2137	-	+	-
FL2137	-	+	-
FL2137	-	+	-
FL2137	-	+	-
Reba	+	-	-
Reba	+	-	-
Reba	+	-	-
Reba	+	-	-
Reba	+	-	-
Reba	+	-	-
Lamoka	+	-	+
Lamoka	+	-	+
Lamoka	+	-	+
Lamoka	+	-	+
Lamoka	+	-	-
Lamoka	+	-	-
Lamoka	+	-	-
Lamoka	+	-	-
Lamoka	+	-	-
Lamoka	+	-	-
Lamoka	+	-	-
Lamoka	+	-	-
Lamoka	+	-	-
SJ176	+	-	-
SJ176	+	-	-
SJ176	+	-	-
SJ176	+	-	-
SJ176	+	-	-
SJ176	+	-	-
SJ176	+	-	-
SJ176	+	-	-
SJ176	+	-	-
SJ176	+	-	-
SJ176	+	-	-
SJ176	+	-	-
FL1867	+	-	-
FL1867	+	-	-
FL1867	+	-	-
FL1867	+	-	-
FL1867	+	-	-
FL1867	+	+	-
FL1867	+	-	-

Table 12. (cont'd)

FL1867	+	-	-
FL1867	+	-	-
FL1867	+	-	-
FL1867	+	-	-
FL1867	+	-	-
Silverton Russet	+	-	-
Silverton Russet	+	-	-
Silverton Russet	+	-	-
Silverton Russet	+	-	-
Silverton Russet	+	-	-
Silverton Russet	+	-	-
Silverton Russet	+	-	-
Silverton Russet	+	-	-
Silverton Russet	+	-	-
Silverton Russet	+	-	-
Silverton Russet	+	-	-
Silverton Russet	+	-	-
Caribou	+	-	-
Caribou	+	-	-
Caribou	+	-	-
Caribou	+	-	-
Caribou	+	-	-
Caribou	+	-	-
Caribou	+	-	-
Caribou	+	-	-
Caribou	+	-	-
Caribou	+	-	-
Caribou	+	-	-
Caribou	+	-	-
FL2312	+	-	-
FL2312	+	-	-
FL2312	+	-	-
FL2312	+	-	-
FL2312	+	-	-
FL2312	+	-	-
FL2312	-	-	-
FL2312	-	-	-
FL2312	-	-	-
FL2312	-	-	-
FL2312	-	-	-
FL2312	-	-	-
FL2312	-	-	-
N/A	-	+	+

Table 12. (cont'd)

N/A	-	+	+
N/A	-	-	+
N/A	-	-	+
N/A	-	+	-
N/A	-	+	-
N/A	-	+	-
N/A	-	+	-
N/A	-	-	-
N/A	-	-	-
N/A	-	-	-
N/A	-	-	-
N/A	-	-	+
N/A	-	-	+
N/A	-	-	+
N/A	-	-	+
N/A	-	-	-
N/A	-	-	-
N/A	-	-	-
N/A	-	-	-
N/A	-	-	-
N/A	-	-	-
N/A	-	-	-
N/A	-	-	-

[a] + Presence of the gene
[b] - Absence of the gene
[c] N/A-Not available

Table 13**.** Results of conventional polymerase chain reaction (PCR) of three (*PelADE1/2, ExpccF/R*, and *Y45/Y46*) set of primers from fall 2016 seed-lots samples

	Conventional PCR assays		
Variety	***PelADE1/2***	***ExpccF/R***	***Y45/46***
Lamoka 1	-[a]	+[b]	-
Lamoka 2	-	+	-
Lamoka 3	-	+	-
Lamoka 4	-	+	-
Lamoka 5	-	+	-
Lamoka 6	-	+	-
Lamoka 7	-	+	-
Lamoka 8	-	-	-
Lamoka 9	-	-	-
Lamoka 10	-	-	-
Lamoka 11	-	-	-
Lamoka 12	-	-	-
Lamoka 13	-	+	-
Lamoka 14	-	-	-
Lamoka 15	-	-	-
Lamoka 16	-	+	-
Lamoka 17	-	-	-
Lamoka 18	-	-	-
Lamoka 19	-	-	-
Lamoka 20	-	-	-
Snowden 1-1	-	+	-
Snowden 1-2	-	-	-
Snowden 1-3	-	+	-
Snowden 1-4	-	-	-
Snowden 1-5	-	-	-
Snowden 1-6	-	-	-
Snowden 1-7	-	+	-
Snowden 1-8	-	+	-
Snowden 1-9	-	+	-
Snowden 1-10	-	-	-
Snowden 1-11	-	-	-
Snowden 1-12	-	-	-
Snowden 1-13	-	-	-
Snowden 1-14	-	-	-
Snowden 1-15	-	-	-
Snowden 1-16	-	+	-
Snowden 1-17	-	+	-
Snowden 1-18	-	+	-
Snowden 1-19	-	+	-

Table 13. (cont'd)

Snowden 1-20	-	+	-
Snowden 2-1	-	-	-
Snowden 2-2	-	-	-
Snowden 2-3	-	-	-
Snowden 2-4	-	-	-
Snowden 2-5	-	-	-
Snowden 2-6	-	-	-
Snowden 2-7	-	-	-
Snowden 2-8	-	-	+
Snowden 2-9	-	-	-
Snowden 2-10	-	-	-
Snowden 2-11	-	-	-
Snowden 2-12	-	-	-
Snowden 2-13	-	-	+
Snowden 2-14	-	-	+
Snowden 2-15	-	-	-
Snowden 2-16	-	-	+
Snowden 2-17	-	-	+
Snowden 2-18	-	-	-
Snowden 2-19	-	-	+
Snowden 2-20	-	-	-
Snowden 3-1	-	-	-
Snowden 3-2	-	-	-
Snowden 3-3	-	-	-
Snowden 3-4	-	-	-
Snowden 3-5	-	-	-
Snowden 3-6	-	-	-
Snowden 3-7	-	-	-
Snowden 3-8	-	-	-
Snowden 3-9	-	-	-
Snowden 3-10	-	-	-
Snowden 3-11	-	-	-
Snowden 3-12	-	-	-
Snowden 3-13	-	+	-
Snowden 3-14	-	-	-
Snowden 3-15	-	-	-
Snowden 3-16	-	-	-
Snowden 3-17	-	-	-
Snowden 3-18	-	-	-
Snowden 3-19	-	-	-
Snowden 3-20	-	-	-

[a] - Absence of the gene
[b] + Presence of the gene

Table 14**.** Results of conventional polymerase chain reaction (PCR) of three (*PelADE1/2*, *ExpccF/R*, and *Y45/46*) set of primers from fall 2017 seed-lots samples

		Conventional PCR assays		
Variety	**Field Year**	***PelADE1/2***	***ExpccF/R***	***Y45/46***
Nadina 1	FY5	-[a]	-	-
Nadina 2	FY5	-	-	-
Nadina 3	FY5	-	-	-
Nadina 4	FY5	-	-	-
Nadina 5	FY5	-	-	-
Nadina 6	FY5	-	-	-
Nadina 7	FY5	-	-	-
Nadina 8	FY5	-	-	-
Nadina 9	FY5	-	-	-
Nadina 10	FY5	-	-	-
Nadina 11	FY5	-	-	-
Nadina 12	FY5	-	-	-
Nadina 13	FY5	-	-	-
Nadina 14	FY5	-	-	-
Nadina 15	FY5	-	-	-
Nadina 16	FY5	-	-	-
Nadina 17	FY5	-	-	-
Nadina 18	FY5	-	-	-
Nadina 19	FY5	-	-	-
Nadina 20	FY5	-	-	-
Atlantic 1	FY4	-	-	-
Atlantic 2	FY4	-	-	-
Atlantic 3	FY4	-	-	-
Atlantic 4	FY4	-	-	-
Atlantic 5	FY4	-	-	-
Atlantic 6	FY4	-	-	-
Atlantic 7	FY4	-	-	-
Atlantic 8	FY4	-	-	-
Atlantic 9	FY4	-	-	-
Atlantic 10	FY4	-	-	-
Atlantic 11	FY4	-	-	-
Atlantic 12	FY4	-	-	-
Atlantic 13	FY4	-	-	-
Atlantic 14	FY4	-	-	-
Atlantic 15	FY4	-	-	-
Atlantic 16	FY4	-	-	-
Atlantic 17	FY4	-	-	-
Atlantic 18	FY4	-	-	-
Atlantic 19	FY4	-	-	-

Table 14. (cont'd)

Atlantic 20	FY4	-	-	-
Russet Burbank 1	FY4	-	-	-
Russet Burbank 2	FY4	-	-	-
Russet Burbank 3	FY4	-	-	-
Russet Burbank 4	FY4	-	-	-
Russet Burbank 5	FY4	-	-	-
Russet Burbank 6	FY4	-	-	-
Russet Burbank 7	FY4	-	-	-
Russet Burbank 8	FY4	-	-	-
Russet Burbank 9	FY4	-	-	-
Russet Burbank 10	FY4	-	-	-
Russet Burbank 11	FY4	-	-	-
Russet Burbank 12	FY4	-	-	-
Russet Burbank 13	FY4	-	-	-
Russet Burbank 14	FY4	-	-	-
Russet Burbank 15	FY4	-	-	-
Russet Burbank 16	FY4	-	-	-
Russet Burbank 17	FY4	-	-	-
Russet Burbank 18	FY4	-	-	-
Russet Burbank 19	FY4	-	-	-
Russet Burbank 20	FY4	-	-	-
Lamoka 1	FY4	-	-	-
Lamoka 2	FY4	-	-	-
Lamoka 3	FY4	-	-	-
Lamoka 4	FY4	-	-	-
Lamoka 5	FY4	-	-	-
Lamoka 6	FY4	-	-	-
Lamoka 7	FY4	-	-	-
Lamoka 8	FY4	-	-	-
Lamoka 9	FY4	-	-	-
Lamoka 10	FY4	-	-	-
Lamoka 11	FY4	-	-	-
Lamoka 12	FY4	-	-	-
Lamoka 13	FY4	-	-	-
Lamoka 14	FY4	-	-	-
Lamoka 15	FY4	-	-	-
Lamoka 16	FY4	-	-	-
Lamoka 17	FY4	-	-	-
Lamoka 18	FY4	-	-	-
Lamoka 19	FY4	-	-	-
Lamoka 20	FY4	-	-	-
Manistee 1	FY2	-	-	-
Manistee 2	FY2	-	-	-

Table 14. (cont'd)

Manistee 3	FY2	-	-	-
Manistee 4	FY2	-	-	-
Manistee 5	FY2	-	-	-
Manistee 6	FY2	-	-	-
Manistee 7	FY2	-	-	-
Manistee 8	FY2	-	-	-
Manistee 9	FY2	-	-	-
Manistee 10	FY2	-	-	-
Manistee 11	FY2	-	-	-
Manistee 12	FY2	-	-	-
Manistee 13	FY2	-	-	-
Manistee 14	FY2	-	-	-
Manistee 15	FY2	-	-	-
Manistee 16	FY2	-	-	-
Manistee 17	FY2	-	-	-
Manistee 18	FY2	-	-	-
Manistee 19	FY2	-	-	-
Manistee 20	FY2	-	-	-
Manistee 1	FY3	-	-	-
Manistee 2	FY3	-	-	-
Manistee 3	FY3	-	-	-
Manistee 4	FY3	-	-	-
Manistee 5	FY3	-	-	-
Manistee 6	FY3	-	-	-
Manistee 7	FY3	-	-	-
Manistee 8	FY3	-	-	-
Manistee 9	FY3	-	-	-
Manistee 10	FY3	-	-	-
Manistee 11	FY3	-	-	-
Manistee 12	FY3	-	-	-
Manistee 13	FY3	-	-	-
Manistee 14	FY3	-	-	-
Manistee 15	FY3	-	-	-
Manistee 16	FY3	-	-	-
Manistee 17	FY3	-	-	-
Manistee 18	FY3	-	-	-
Manistee 19	FY3	-	-	-
Manistee 20	FY3	-	-	-
Snowden 1	FY3	-	-	-
Snowden 2	FY3	-	-	-
Snowden 3	FY3	-	-	-
Snowden 4	FY3	-	-	-
Snowden 5	FY3	-	-	-

Table 14. (cont'd)

Snowden 6	FY3	-	+[b]	-
Snowden 7	FY3	-	+	-
Snowden 8	FY3	-	+	-
Snowden 9	FY3	-	-	-
Snowden 10	FY3	-	-	-
Snowden 11	FY3	-	-	-
Snowden 12	FY3	-	+	-
Snowden 13	FY3	-	-	-
Snowden 14	FY3	-	-	-
Snowden 15	FY3	-	+	-
Snowden 16	FY3	-	-	-
Snowden 17	FY3	-	-	-
Snowden 18	FY3	-	-	-
Snowden 19	FY3	-	-	-
Snowden 20	FY3	-	+	-
Snowden 1	FY4	-	-	-
Snowden 2	FY4	-	-	-
Snowden 3	FY4	-	-	-
Snowden 4	FY4	-	-	-
Snowden 5	FY4	-	-	-
Snowden 6	FY4	-	-	-
Snowden 7	FY4	-	-	-
Snowden 8	FY4	-	-	-
Snowden 9	FY4	-	-	-
Snowden 10	FY4	-	-	-
Snowden 11	FY4	-	-	-
Snowden 12	FY4	-	-	-
Snowden 13	FY4	-	-	-
Snowden 14	FY4	-	-	-
Snowden 15	FY4	-	-	-
Snowden 16	FY4	-	-	-
Snowden 17	FY4	-	-	-
Snowden 18	FY4	-	-	-
Snowden 19	FY4	-	-	-
Snowden 20	FY4	-	-	-

[a] - Absence of the gene
[b] + Presence of the gene

DISCUSSION

The 2015 outbreak of potato black leg disease in the US became a concern and most critical disease problem for potato growers. The disease was detected and confirmed in all major potato producing states by 2018 (Charkowski, 2018). Since 2015, there have been two research papers and 7 first reports on blackleg and aerial stem rot disease (Charkowski, 2018; Jiang et al., 2016; McNally et al., 2017b; Rosenzweig et al., 2016b; Ma et al., 2018; Zeng et al., 2018; Patel et al., 2018; Ge et al., 2017; McNally et al., 2017a). In response to occurrence of black leg, aerial stem rot and soft rot diseases, this study evaluated the specificity of available PCR and qPCR primers and identified reliable primers in identification of *Dickeya* and *Pectobacterium* spp. additionally, determined the spread and presence of soft rot causing bacteria in Michigan potato production by surveying infected plant tissue from the fields and dormant tubers from seed production lots.

The primer set *Df/Dr* according to Laurila et al., (2010) amplified a 133 bp fragment from *D. dianthicola* strain and did not recognize the *Pectobacterium* strains. However, our results with the same primer set were inconsistent. Nassar et al., (1996) tested conserved regions about 420 bp long of the *PelADE* cluster specific to *E. chrysenthemi* (now known as *Dickeya chrysenthemi*) and amplified 78 strains of *E. chrysenthemi* and found the primer pairs to be specific to *E. chrysenthemi* and suggested the primer set could be used for diagnostic and identification. This study found *PelADE1/2* primer set was consistent, and reliable enough to detect *Dickeya* spp., but could not differentiate among *Dickeya* species. The primer set *ExpccF/R* amplified a single band of expected size (550 bp) of 29 *P. carotovorum* subspecies *carotovorum* strains and three *P. wasabiae* (now known as *parmentieri*) strains, but not from the other *P. carotovorum* subspecies *atrosepticum*, *betavasculorum* or *odoriferum*, or from other *Erwinia* spp. or bacterial genera (Kang et al., 2003). Similarly, in this study the *ExpccF/R* amplified *P. carotovorum* and *P. parmentieri* species but

none of the other *Pectobacterium* and *Dickeya* species. (Frechon et al., 1998) examined 65 *Erwinia carotovorum* subspecies *atrosepticum* (now known as *P. atrosepticum*) and all strains gave a positive result using the *Y45/46* primer set. In contrast, the 53 *Erwinia carotovorum* subspecies *carotovorum* strains and 44 other plant pathogenic bacteria including other pectolytic *Erwinia* spp. and 21 saprophytic bacteria tested negative (Frechon et al., 1998). Similarly, in our study *Y45/46* primer set consistently amplified *P. atrosepticum*. van der Wolf et al., (2013) reported that *Dickeya* primer sets *D. chrysenthemi* and *D. dianthicola* can be useful for rapid characterization of *Dickeya* spp. in surveys and in research on dissemination, survival, plant colonization and the effect of management strategies. However, in our study *D. dianthicola* primer set did not differentiate between *Dickeya* and *Pectobacterium* spp. The primer set *D. chrysenthemi* did not amplify the *D. dianthicola* positive control suggesting that it could be species specific. The same study concluded that the *D. solani* primer set is not suitable for testing plant propagation material, due to the occurrence of false-positive reactions. Additionally, this study found that the *D. solani* primer set amplified *D. dianthicola*, suggesting it is not species-specific. The *gyrB* primer was modified for detection of *Dickeya* spp. by Marrero et al., (2013). However, in this study the *gyrB* primer set did not amplify *Dickeya* spp. Van Vaerenbergh et al., (2012) reported that *Dickeya Flic* primer was effective for diagnosis of *D. solani* in potato plants and tubers, however in this study the *Flic* primer detected *D. dianthicola* and suggested it is not species specific. Therefore, based on the primer specificity data, all further testing with field and seed lots testing were done using primer sets PelADE1/2 to detect *Dickeya* spp., *ExpccF/R* for *P. carotovorum* and *P. parmentieri*, and *Y45/46* for *P. atrosepticum*. Similarly, recent reports that have detected either or both *Dickeya* and *Pectobacterium* spp. since the 2015 outbreak used the same PCR primers (McNally et al., 2017b; Ma et al., 2018).

The importance of evaluating and finding a reliable diagnostic method is especially important for seed lot testing. Currently, a blackleg disease test is not required for seed certification, however growers are requesting soft rot, blackleg disease test to be done prior to purchasing potato seeds. Thus, accurate, quick and inexpensive diagnostic methods are essential.

In conclusion there are no specific primers that can detect *Dickeya* up to the subspecies level and no primers that can detect both *Dickeya* and *Pectobacterium* spp. in the mixed samples. The most reliable and accurate primer sets for detection of *Dickeya* spp. are *PelADE1/2*, for *P. carotovorum* and *parmentieri ExpccF/R* and for *P. atrosepticum Y45/46*. These primers can be used for detection using symptomatic plant tissue, and asymptomatic dormant tuber materials. However further validation needs to be done to identify species specific primers to detect *Dickeya* spp. and primers that can detect mixed samples with *Dickeya* and *Pectobacterium* spp, since presence of both bacteria in the same plant can occur. Further development of high-fidelity primers is important in detection of introduced or exotic pectolytic bacteria potato production in the future for the management of soft rot diseases.

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LITERATURE CITED

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CHAPTER 4: GENOTYPIC AND PHENOTYPIC CHARACTERIZATION OF PATHOGENIC *STREPTOMYCES* SPECIES ON POTATO AND TURNIP IN MICHIGAN

ABSTRACT

Common scab of potato is caused by *Streptomyces* spp. of soil-inhabiting filamentous bacteria. There are over 500 species of *Streptomyces*, however few of them are plant pathogenic. To date, at least 13 different species of *Streptomyces* can infect and cause disease on potato, turnip and other crops. *Streptomyces scabies* is the most common and widely distributed pathogenic species among the streptomyces. For this study, we characterized plant-pathogenic *Streptomyces* spp. associated with common scab infected potato and turnip tubers harvested in Michigan. A total of 51 putative isolates, 20 from turnip roots and 31 from potato tubers successfully isolated. The isolates were identified by morphological and molecular methods and phylogenetic relationship was determined between isolates. Out of 51 isolated 48 isolates that caused pitted and surface lesions have genes encoding for thaxtomin synthase (*txtA* and *txtAB*), tomatinase (*tomA*) and a necrosis protein (*nec1*). The maximum-parsimony analysis of 3 locus revealed three main similar clustered groups, with some exceptions. Overall, isolates from turnip and potato that are recovered from superficial lesions shared similar molecular characteristics and grouped together in the phylogenetic analysis. Whereas, potato isolates recovered from pitted lesions shared similar molecular and morphological characteristics and grouped together in phylogenetic analysis. Therefore, suggesting that *Streptomyces* spp. that caused superficial lesions could be different from species that cause pitted lesions.

INTRODUCTION

Potato (*Solanum tuberosum*) production in 2016 in the US occupied over 416,826 hectares, with an estimated value of $3.74 billion (USDA-NASS, 2017, accessed August 30, 2018). Common scab (CS) caused by *Streptomyces* spp. of soil-inhabiting filamentous bacteria (Lambert and Loria, 1989; Loria et al., 1997). The disease is an annual concern for potato growers in the US and worldwide (Loria et al., 2008; Wanner and Kirk, 2015). In surveys among potato growers CS typically ranks fourth among limiting factors to potato production in the US, particularly in the North Central region due to the favorable environmental conditions (Hao et al., 2009; Rosenzweig et al., 2012). Additionally, CS can also cause disease on root and tuber crops such as turnip (*Brassica rapa*), radish (*Raphanus raphanistrum* subsp. *sativus*), sweet potato (*Ipomoea batatas*), peanut (*Arachis hypogaea*), rutabaga (*Brassica napobrassica*), and carrots (*Daucus carota* subsp. *sativus*) (Loria et al., 1997; Rosenzweig, 2014). Turnip (*Brassica rapa*) production according to USDA-NASS in 2012 occupied 174 hectares with an estimated value of over a $1 million of revenue. Michigan is a national leader for growing potatoes for chip production and for root crop production including turnips (Anonymous, 2011). Recently, turnip production has been affected by marketability issues caused by an emerging soil-borne disease called common scab.

There are more than 500 *Streptomyces* spp. however only 13 species have been found to cause disease worldwide (Wanner and Kirk, 2015; Santos-Cervantes et al., 2017). The most reported and well recognized species of *Streptomyce*s associating with CS in potato is *S. scabies*. However, other *Streptomyces* spp. have been reported in different parts of the world which were associated with common and netted scab that are included: *S. europaeiscabiei; S. stelliscabiei* (Bukhalid et al., 2002)*; S. bottropensis; S. acidiscabies; S. turgidiscabies; S. reticuliscabiei* (Bouchek-Mechiche et al., 2000; Faucher et al., 1992; Kreuze et al., 1999)*; and S. aureofaciens*

(Kreuze et al., 1999; Park et al., 2003) . The number of pathogenic *Streptomyces* species may evolve due to horizontal gene transfer (HGT) (Loria et al., 2006). Horizontal gene transfer is a mechanism in bacteria that allows gene transfer from pathogenic species to non-pathogenic species, therefore resulting in a new pathogenic species. There is a cluster of genes that are associated with pathogenicity of *Streptomyces* species and located in a large chromosome region called pathogenicity island (PAI) (Loria et al., 2006). The PAI genes have been described and used for identification and taxonomy of *Streptomyces* spp. The first part of the region of the PAI cluster contains genes for synthesis of the pathogenicity determinant thaxtomin (*txtA*, *txtB*, *txtC*, *txtR* and *NOS*) and the second part of the cluster contains genes for pathogenicity factors tomatinase (*tomA*) and a necrosis-inducing protein (*nec1*). These genes also presently have been used for detection of pathogenic isolates using polymerase chain reaction (Hao et al., 2009; Wanner, 2006).

Pathogenic *Streptomyces* spp. have one or more genes that are associated with pathogenicity (*nec1*, *txtAB*, and *tomA*). *TxtAB* is the gene (operon) responsible for the biosynthesis of the plant toxin thaxtomin, which is the main factor for common scab symptom expression in potato. *Nec1* and *tomA* are also associated with pathogenicity and virulence, although their function is a source of debate. Pathogenicity 'islets' can be located in distinct locations or clustered in the same region of the genome, depending on the species. Strain DS3024, an Idaho strain (Wanner, 2007a), and two other strains (Wanner and Haynes, 2009) have been identified that are genetically distinct from other known pathogenic *Streptomyces* spp. and lack *nec1*. Pathogenic *Streptomyces* spp. missing both pathogenicity-associated genes *nec1* and *tomA* have also been found (Wanner and Haynes, 2009).

Symptomology on both tuber and root crops is similar. Symptoms appear as corky, erumpent, raised lesions on the tuber and root surface (Figure 3). According to Wanner, (2006)

Figure 3**.** Symptoms on turnip roots appear as a corky, erumpent, and raised lesions

there has been many discussions on whether different types of lesions and symptoms on potato are caused by different *Streptomyces* spp. Additionally, several studies have reported (Bouchek-Mechiche et al., 2000; Faucher et al., 1993; Goyer et al., 1996) that different lesions (netted vs. pitted vs. superficial) have been produced by different *Streptomyces* spp. Other studies have found that one or more species can occur on the same tuber or even the same lesion. This could explain different types of lesion on the same tuber. In order to understand the importance of pathogenic *Streptomyces* populations, especially since the recent report on turnips and other root crops, it is necessary to have better idea of the diversity in turnip and potato production fields in Michigan. Therefore, the objective of this study were the following: i) collect pathogenic *Streptomyces* species causing scab in Michigan potato and turnip production ii) characterize phenotypically virulence and pathogenicity on potato and turnip using tuber disc and seedling assays, respectively iii) characterize pathogenicity factors using PCR primers and iv) to determine the diversity of *Streptomyces* species associated with scab on potato and turnip in MI and to understand their phylogenetic relationships influenced by phenotype.

MATERIALS AND METHODS

Sample collection and *Streptomyces* isolation. A total of 51 isolates were collected from commercial potato and turnip grower fields in Michigan in 2016 (Table 15). Potato isolates were obtained from symptomatic pitted and superficial lesions and turnip isolates were collected from superficial lesions only. Both turnip roots and potato tubers were washed under running tap water and blotted dry on paper towel. Isolations were carried out following the protocol by Wanner, (2004) with slight modifications. Briefly, a small piece of symptomatic plant tissue from turnip or potato was excised and surface sterilized in 1% NaOCl for 1 min. After several rinses with ddH20 the tissue was put in tube with ddH20 and placed in a water bath (model) at 60° C for 30 min

Table 15. *Streptomyces* spp. isolates collected in Michigan and reference isolates downloaded from the NCBI database

Isolate ID	Species	Host	Type of lesion	Location	Accession number
1 Turnip	*Streptomyces spp.*	Turnip	Superficial	Michigan, USA	-
2 Turnip	*Streptomyces spp.*	Turnip	Superficial	Michigan, USA	-
3 Turnip	*Streptomyces spp.*	Turnip	Superficial	Michigan, USA	-
4 Turnip	*Streptomyces spp.*	Turnip	Superficial	Michigan, USA	-
5 Turnip	*Streptomyces spp.*	Turnip	Superficial	Michigan, USA	-
6 Turnip	*Streptomyces spp.*	Turnip	Superficial	Michigan, USA	-
7 Turnip	*Streptomyces spp.*	Potato	Superficial	Michigan, USA	-
8 Turnip	*Streptomyces spp.*	Potato	Superficial	Michigan, USA	-
10 Turnip	*Streptomyces spp.*	Potato	Pitted	Michigan, USA	-
1 Turnip A	*Streptomyces spp.*	Potato	Pitted	Michigan, USA	-
1 Turnip B	*Streptomyces spp.*	Potato	Pitted	Michigan, USA	-
2 Turnip A	*Streptomyces spp.*	Potato	Pitted	Michigan, USA	-
2 Turnip B	*Streptomyces spp.*	Potato	Pitted	Michigan, USA	-
3 Turnip A	*Streptomyces spp.*	Potato	Pitted	Michigan, USA	-
3 Turnip B	*Streptomyces spp.*	Potato	Superficial	Michigan, USA	-
4 Turnip A	*Streptomyces spp.*	Potato	Pitted	Michigan, USA	-
4 Turnip B	*Streptomyces spp.*	Potato	Pitted	Michigan, USA	-
5 Turnip A	*Streptomyces spp.*	Potato	Superficial	Michigan, USA	-
5 Turnip B	*Streptomyces spp.*	Potato	Superficial	Michigan, USA	-
6 Turnip A	*Streptomyces spp.*	Potato	Superficial	Michigan, USA	-
11 Pit	*Streptomyces spp.*	Potato	Superficial	Michigan, USA	-

Table 15. (cont'd)

7 Pit	*Streptomyces spp.*	Potato	Superficial	Michigan, USA	-
6 Surface C	*Streptomyces spp.*	Potato	Superficial	Michigan, USA	-
6 Surface B	*Streptomyces spp.*	Potato	Superficial	Michigan, USA	-
6 Surface A	*Streptomyces spp.*	Potato	Superficial	Michigan, USA	-
5 Surface D	*Streptomyces spp.*	Potato	Superficial	Michigan, USA	-
5 Surface C	*Streptomyces spp.*	Potato	Superficial	Michigan, USA	-
5 Surface B	*Streptomyces spp.*	Potato	Superficial	Michigan, USA	-
5 Surface A	*Streptomyces spp.*	Potato	Superficial	Michigan, USA	-
4 Pit C	*Streptomyces spp.*	Potato	Pitted	Michigan, USA	-
3 Surface B	*Streptomyces spp.*	Potato	Superficial	Michigan, USA	-
Control 6 Pit D-2	*Streptomyces spp.*	Potato	Pitted	Michigan, USA	-
Control 6 Pit D-1	*Streptomyces spp.*	Potato	Pitted	Michigan, USA	-
Control 6 Pit A-3	*Streptomyces spp.*	Potato	Pitted	Michigan, USA	-
Control 6 Pit A-2	*Streptomyces spp.*	Potato	Pitted	Michigan, USA	-
Control 6 Pit A-1	*Streptomyces spp.*	Potato	Pitted	Michigan, USA	-
Control 6 Pit A	*Streptomyces spp.*	Potato	Pitted	Michigan, USA	-
Control 5 Surface B-1	*Streptomyces spp.*	Potato	Superficial	Michigan, USA	-
Control5 Surface B	*Streptomyces spp.*	Potato	Superficial	Michigan, USA	-
Control2 Surface	*Streptomyces spp.*	Potato	Superficial	Michigan, USA	-
3-3	*Streptomyces scabiei*	Potato	Pitted	Michigan, USA	-
3-2	*Streptomyces scabiei*	Potato	Pitted	Michigan, USA	-
3-1	*Streptomyces scabiei*	Potato	Pitted	Michigan, USA	-
1-9	*Streptomyces scabiei*	Potato	Pitted	Michigan, USA	-

Table 15. (cont'd)

1-8	*Streptomyces scabiei*	Potato	Pitted	Michigan, USA	-
1-7	*Streptomyces scabiei*	Potato	Pitted	Michigan, USA	-
1-6	*Streptomyces scabiei*	Potato	Pitted	Michigan, USA	-
1-4	*Streptomyces scabiei*	Potato	Pitted	Michigan, USA	-
1-3	*Streptomyces scabiei*	Potato	Pitted	Michigan, USA	-
1-2	*Streptomyces scabiei*	Potato	Pitted	Michigan, USA	-
1-1	*Streptomyces scabiei*	Potato	Pitted	Michigan, USA	-
H112	*Mycobacterium tuberculosis*	Human	-	India	CP019613.1
Unknown	*Streptomyces bottropensis*	Potato	Unknown	Japan	D63868.1
Unknown	*Streptomyces neyagawaensis*	Potato	Unknown	Japan	D63869.1
Unknown	*Streptomyces diastatochromogenes*	Potato	Unknown	Japan	D63867.1
Unknown	*Streptomyces acidiscabies*	Potato	Unknown	USA	D63865.1
rrnD	*Streptomyces coelicolor*	Saprophyte	Unknown	Unknown	Y00411.1
ATCC 700248	*Streptomyces turgidiscabies*	Potato	Unknown	Japan	NR_040828.2
IBSBF 2086	*Streptomyces reticuliscabies*	Potato	Unknown	Brazil	KY783747.1
HER21	*Streptomyces stelliscabie*	Potato	Superficial	Michigan, USA	HM018115.1
AB026199	*Streptomyces scabiei*	Potato	Unknown	Japan	AB026199.1
ATCC49173	*Streptomyces scabiei*	Potato	Unknown	Canada	NR_116531.1
267	*Streptomyces scabiei*	Unknown	Unknown	Sweden	Y15500.1

After 30 min, individual bacterial suspensions originating from a single lesion were spread onto water agar (15 g agar and 1 L distilled water) media. Plates were incubated at 30° C for 2-3 days, and individual characteristic bacterial colonies were picked with a sterile inoculation loop and transferred to obtained pure culture.

Pathogenicity tests on tuber disc assay. Snowden cultivar was used due to its susceptibility to common scab and its commercial use throughout the state of Michigan and Midwestern US potato production regions for the tuber disc pathogenicity assay. Tubers were washed and dried with paper towel before use. Whole tubers were sterilized by flaming in 70% ethanol prior to cutting into discs. A tuber disc (20 mm in diameter by 7 mm in height) was bored using a cork-borer (size of the cork borer). The discs were rinsed three times in a sterile ddH20 and placed in Petri plates with moist filter paper. Putative *Streptomyces* spp. inoculum was prepared from cultures grown on oat bran agar media (20 g oat bran; 250µL salt solution (0.1 g FeSO4*7H2O; 0.1 g MnCl2*4H2O; 0.1g ZnSO4*7H2O), 15 g agar and 1L distilled water) for 8 days. A 5 mm cork-borer was used to make a plug from the putative *Streptomyces* spp. culture and for control a plug from oatmeal agar were placed on top of the tuber discs. Each Petri plate contained 5 tuber discs and three replicates total 15 tuber discs. The tuber discs were incubated in the dark at 22 to 24° C for 7 days. Tuber discs were evaluated visually based on the area of necrosis on of the plant tissue.

Pathogenicity tests on turnip seedling assay. Turnip seedling pathogenicity test was done *in vitro*. Seeds of turnip of "Purple Top White Globe" variety were surface sterilized for 3 min in NaOCl, rinsed twice in sterile ddH20 and placed on moist filter paper (Whatman 100mm no.1, Maidstone, England) overnight at 20-25° C under 24 h of photoperiod for germination. Five uniformly germinated seeds were selected and placed onto oat bran agar media. Isolates were streaked on the section of the oat bran agar media and germinated turnip seed were placed above the inoculated

area. Each isolate was replicated three times. As a control turnip seedling were placed onto non-inoculated oat bran agar media plates. Turnip seedlings were evaluated by measuring the length of the seedling after one week and compared to the non-inoculated control.

DNA extraction. Genomic DNA was extracted from all 51 isolates that was grown on oat bran agar media at 25° C for 10 days. The mycelia form each isolate were scraped off from the plate using a sterilized toothpick and subsequent of tissue was used for DNA extraction using the DNeasy Power Soil Kit (Qiagen Inc., Valencia, CA) according to the manufacturer's protocol with a slight modification.

PCR amplification and DNA sequencing. The extracted genomic DNA was used as a template for polymerase chain reaction amplification. The genes included the bacterial 16S rRNA, two housekeeping genes *recA* (recombinase A) and *rpoB* (RNA polymerase, ß-subunit) (Guo et al., 2008) , two genes for synthesis of the pathogenicity determinant thaxtomin *txtA* and *txtAB* and two genes for pathogenicity factors *nec1* (necrosis-inducing protein) and *tomA* (tomatinase) (Wanner, 2006). PCR reactions were performed on a BioRad MyCycler thermocycler (Hercules, CA) using appropriate parameters and cycling conditions for each locus (Table 16). The PCR product visualized on a 1.2 % agarose gel and stained with GelRed (Phenix Research Products, Chandler, NC). The PCR products were purified with the Wizard SV Gel and PCR Clean-up System (Promega Corporation, WI, USA) and submitted to Michigan State University Genomics Core Facility for sequencing. Both forward and reverse strands were sequenced using the PCR primers for each locus (Table 16). Sequences were edited in CLC Main Workbench (Qiagen, Aarhus, Denmark), and were aligned using Muscle 3.8.31 implemented in Mesquite 3.51 with default parameters (Maddison and Maddison, 2011). For identification of resulting gene regions sequenc

Table 16**.** Primer pairs and cycling conditions used in polymerase chain reaction detection of genes in the Streptomyces isolates including 16S rRNA bacterial gene, two housekeeping genes and four genes for the *Streptomyces* pathogenicity island (PAI)

Gene	Primer pair (5'- 3')	Annealing t (°C)	PCR cycling conditions	PCR product size (bp)	References
16S rRNA	CATTCACGGAGAGTTTGATCC ACTTTCGCTTCTTCCCTGCT	55	95 ° C for 3m; 35 cycles: 95 ° C for 20s; 55 ° C for 30s, 72 ° C for 45s; 72 ° C for 5m	1531	
recA	CCGCRCTCGCACAGATTGAACGSCAATTC GCSAGGTCGGGGTTGTCCTTSAGGAAGTTGCG	60	95 ° C for 5m; 30 cycles: 95 ° C for 30s; annealing for 30s, 72 ° C for 90s; 72 ° C for 10m; ending 4 ° C hold	913	(Guo et al., 2008)
rpoB	GAGCGCATGACCACCCAGGACGTCGAGGC CCTCGTAGTTGTGACCCTCCCACGGCATGA	65	95 ° C for 5m; 30 cycles: 95 ° C for 30s; annealing for 30s, 72 ° C for 90s; 72 ° C for 10m; ending 4 ° C hold	994	(Guo et al., 2008)
txtA	CACGTACGCGCAGTTCAATG AGATGATGTAGGCGGGACTC	48	95 ° C for 3m; 40 cycles: 95 ° C for 20s; annealing for 30s, 72 ° C for 2m; ending 4 ° C hold	398	(Wanner, 2006)
txtAB	CCACCAGGACCTGCTCTTC TCGAGTGGACCTCACAGATG	48	95 ° C for 3m; 40 cycles: 95 ° C for 20s; annealing for 30s, 72 ° C for 2m; ending 4 ° C hold	385	(Wanner, 2006)
tomA	GAGGCGTTGGTGGAGTTCTA GAGGCGTTGGTGGAGTTCTA	55	95 ° C for 3m; 40 cycles: 95 ° C for 20s; annealing for 30s, 72 ° C for 2m; ending 4 ° C hold	392	(Wanner, 2006)
Nec1	ATGAGCGCGAACGGAAGCCCCGGA GCAGGTCGTCACGAAGGATCG	60	95 ° C for 3m; 40 cycles: 95 ° C for 20s; annealing for 30s, 72 ° C for 2m; ending 4 ° C hold	700	(Wanner, 2006)

were compared using the BLAST algorithm with sequences of closely related species in the National Center for Biotechnology Information (NCBI) GenBank database.

Sequence alignment and phylogenetic analysis. Sequence and phylogenetic analyses were performed using PAUP version 4.0a163 (Swofford, 2011). Maximony-parsimony (MP) method was utilized to build phylogenetic trees. It was assumed that insertions and deletions (indels) are single evolutionary events, therefore indels greater than two basepairs in length were coded as a single character as the outgroup for all analyses. The following reference sequences of *Streptomyces* spp. were included: *S. stelliscabiei*; *S. scabies*; *S. scabies*; *S. scabies*; *bottropensis*; *S. neyagawaensis*; *S. diastatochromogenes*; *S. acidiscabies*; *S. coelicolor*; *S. turgidiscabies*; *S. reticuliscabiei* and outgroup (*M. tuberculosis*). Reference and outgroup sequences were downloaded from the NCBI database (Table 15). Sequences were aligned with the isolate queries using Muscle 3.8.31 implemented in Mesquite 3.51 with default parameters (Maddison and Maddison, 2011). Maximum-parsimony trees were calculated using a heuristic search with three bisection-reconnection (TBR) branch-swapping and 10 replicates with random sequence addition. For MP trees, internal branch support was assessed by bootstrap analysis of 1000 replicates with 10 random additions per replicate using the FastStep algorithm.

RESULTS

A total of 51 putative *Streptomyces* spp. isolates were obtained, 20 isolates were recovered from turnip roots and 31 isolates were recovered from potato tubers. *Streptomyces* isolates were variable in their appearance (Table 17). Turnip isolates colonies had aerial mycelium of dark grey to white. Substrate mycelium of turnip isolates were mostly whitish grey, dark/grey with a buffed appearance (Table 17). Turnip isolates were also buff with diffusible pigments (Table 17). Aerial mycelium of potato isolates from superficial lesions were dark grey to grey (Table 17). Substrate

mycelium were mostly dark grey or buff (Table 17). Potato isolates from superficial lesions had buff or red pigment (Table 17). Aerial mycelium of colonies isolated from pitted lesions on potato were grey with white, grey or dark grey (Table 17). Substrate mycelium of colonies isolated from pitted potato lesions were whiteish grey, to grey and grey with red (Table 17). Additionally, two strains isolated from pitted lesions had red and pink pigments (Table 17).

Pathogenicity assays. In the tuber disc pathogenicity assay of the 20 turnip isolates only 5 produced a necrotic response (Table 18). Among 11 potato isolates from superficial lesions three isolates produced a necrotic response (Table 18). Seventeen of the potato isolates from pitted lesions produced a necrotic response (Table 18). In the turnip seedling pathogenicity assay no significant differences were observed among all 51 isolates based on seedling length (data not shown).

Molecular characterization. The presence or absence of the *txtA*, *txtAB*, *nec1* and *tomA* genes was assessed for all 51 isolates. Out of 20 turnip isolates, four had the *txtA* gene locus, five had the *txtAB* gene locus, three had the *nec1* locus, and sixteen had the *tomA* gene locus (Table 18). Out of 31 potato isolates seventeen had the *txtA* gene locus, twenty-one had the *txtAB* locus, seventeen had the *nec1* locus, and 27 *tomA* gene locus.

DNA sequencing. PCR amplifications of 16S rRNA and *rpoB* amplified 45 out of 51 isolates and resulted in sequenced regions of 1327 and 913bp, respectively. However, PCR amplifications of *recA* amplified only 37 out of 51 isolates and resulted in sequenced regions of 994bp. Although sequencing results originated from PCR products, no heterozygous sites were observed in DNA sequencing chromatograms. All turnip and superficial potato isolates were compared using the BLAST algorithm with sequences of closely related species and subsequently identified as *Streptomyces* spp. All pitted isolates from potato were compared using the BLAST algorithm with

Table 17. Morphological characteristics of 51 *Streptomyces* isolates on oat bran agar media

Strain	Aerial mycelium	Substrate mycelium	Pigment
1 Turnip	dark grey	buff	buff
2 Turnip	dark grey	grey/buff	buff
3 Turnip	grey	whitish grey	no
4 Turnip	grey	grey	no
5 Turnip	dark grey/white colonies	dark grey	no
6 Turnip	grey	dark grey	no
7 Turnip	grey	whitish grey	no
8 Turnip	grey	grey	no
10 Turnip	dark grey	dark grey	no
1 Turnip A	grey/white colonies	grey/buff	no
1 Turnip B	grey	grey	no
2 Turnip A	grey	whitish grey	no
2 Turnip B	grey	whitish grey	no
3 Turnip A	dark grey/dark colonies	whitish grey	no
3 Turnip B	dark grey/dark and white colonies	whitish grey	no
4 Turnip A	dark grey	grey	no
4 Turnip B	grey	grey	no
5 Turnip A	dark grey	dark grey	no
5 Turnip B	dark grey	dark Grey	no
6 Turnip A	dark grey	whitish grey	no
Control 2 Surface	white with little grey	buff	no
3 Surface B	grey	dark grey	no
5 Surface A	grey	dark grey	no
5 Surface B	grey with white colonies	dark grey	no
Control 5 Surface B	white with little grey	buff	buff
Control 5 Surface B-1	white with little grey	buff	red
5 Surface C	grey	dark grey	no
5 Surface D	grey	dark grey	no
6 Surface A	grey	dark grey	no
6 Surface B	grey	buff	red
6 Surface C	grey/white colonies	dark grey	no
4 Pit C	grey	whitish grey	no

Table 17. (cont'd)

7 Pit	dark grey	dark grey	no
11 Pit	grey/white colonies	grey with red colonies	red
Control 6 Pit D-2	grey/white colonies	whitish grey	no
Control 6 Pit D-1	grey/white colonies	whitish grey	no
Control 6 Pit A-3	grey/white colonies	whitish grey	no
Control 6 Pit A-2	grey/white colonies	whitish grey	no
Control 6 Pit A-1	grey/white colonies	whitish grey	no
Control 6 Pit A	grey/white colonies	whitish grey	no
3-3	grey/white colonies	grey	no
3-2	grey	whitish grey	no
3-1	grey	whitish grey	no
1-9	grey/white colonies	whitish grey	no
1-8	grey/white colonies	whitish grey	no
1-7	grey/white colonies	grey	no
1-6	grey/white colonies	grey	no
1-4	grey/white colonies	grey	no
1-3	grey/white colonies	whitish grey	pink
1-2	grey/white colonies	whitish grey	no
1-1	grey/white colonies	whitish grey	no

Table 18**.** Summary of presence of 16S rRNA, two housekeeping genes, four marker genes for the Streptomyces pathogenicity island (PAI) and response from tuber disc pathogenicity assay in 51 *Streptomyces* isolates

Isolates	**16S rRNA**	***recA***	***rpoB***	***txtA***	***txtAB***	***nec1***	***tomA***	**Pathogenicity**
1 Turnip	+[a]	+	+	-[b]	-	-	-	-[c]
2 Turnip	+	-	+	+	+	-	+	-
3 Turnip	+	+	+	-	-	-	+	-
4 Turnip	+	+	+	-	-	-	+	-
5 Turnip	+	+	+	+	+	+	+	+[d]
6 Turnip	+	+	+	-	-	-	+	-
7 Turnip	+	+	+	-	-	+	+	+
8 Turnip	+	+	+	-	-	-	+	-
10 Turnip	+	+	+	+	+	-	-	-
1 Turnip A	+	+	+	-	-	-	+	-
1 Turnip B	+	+	+	-	-	-	+	-
2 Turnip A	+	+	+	-	-	-	+	-
2 Turnip B	+	+	+	-	-	-	+	-
3 Turnip A	+	+	+	-	-	-	+	+
3 Turnip B	-	+	+	+	+	-	-	+
4 Turnip A	+	+	+	-	-	-	-	-
4 Turnip B	+	+	+	-	-	-	+	-
5 Turnip A	+	+	+	-	-	-	+	-
5 Turnip B	+	+	+	-	-	+	+	+
6 Turnip A	+	+	+	-	+	-	+	n/a[d]
11 Pit	+	+	+	+	+	-	+	+
7 Pit	+	-	-	-	+	-	+	-
6 Surface C	+	-	+	-	-	-	+	-
6 Surface B	+	-	+	-	-	-	+	-
6 Surface A	+	+	+	-	-	-	+	-

Table 18. (cont'd)

5 Surface D	+	-	+	-	-	-	+	-
5 Surface C	+	+	+	-	-	-	+	-
5 Surface B	+	-	+	+	+	-	+	-
5 Surface A	+	-	+	-	-	-	+	+
4 Pit C	+	-	+	-	-	-	+	-
3 Surface B	+	-	+	-	-	-	-	-
Control 6 Pit D-2	+	+	-	+	+	+	+	+
Control 6 Pit D-1	+	+	+	+	+	+	+	+
Control 6 Pit A-3	+	-	+	+	+	+	+	+
Control 6 Pit A-2	+	-	+	+	+	+	+	+
Control 6 Pit A-1	+	+	+	+	-	+	+	+
Control 6 Pit A	+	+	+	-	+	+	+	+
Control 5 Surface B-1	+	+	+	-	+	+	-	+
Control 5 Surface B	+	+	+	-	+	+	-	+
Control 2 Surface	+	+	+	-	-	+	-	+
3-3	+	+	+	+	+	-	+	+
3-2	+	+	+	+	+	+	+	+
3-1	+	+	+	+	+	-	+	+
1-9	+	+	+	+	+	+	+	+
1-8	+	+	+	+	+	+	+	+
1-7	+	-	-	+	+	+	+	+
1-6	+	+	+	-	+	+	+	+
1-4	+	-	-	+	+	+	+	+
1-3	+	-	-	+	+	+	+	+
1-2	+	-	+	+	+	-	+	+
1-1	-	-	+	+	+	+	+	-

[a] - Absence of the gene
[b] + Presence of the gene
[c] - Not pathogenic
[d] + Pathogenic
[e] n/a- not available

sequences of closely related species as well and all were identified *Streptomyces scabiei*, with the except two were identified as *Streptomyces* spp.

Single-locus analyses. Datasets from 16S rRNA and *rpoB* included 57 taxa, and the *recA* gene consisted of 47 total taxa including reference sequences and an outgroup. Analysis of the 16S rRNA gene contained a total of 1194 characters with 884 parsimony-informative positions. The *rpoB* gene dataset contained 1501 characters with 502 parsimony-informative positions. The *recA* gene dataset contained 1277 characters with 503 parsimony-informative positions. The 16S rRNA gene contained the most nucleotide diversity of the genes sequenced followed by *recA* and *rpoB.*

To investigate whether the three single-locus datasets resulted in similar phylogenies, each dataset was analyzed individually using maximum-parsimony. The maximum-parsimony analysis of 3 loci revealed three main similar clustered groups, with some exceptions.

16S ribosomal RNA gene. For the 16SrRNA gene ten turnip isolates were grouped together, another two turnip isolates (1T and 10T) were phylogenetically distinct from other turnips with 100% bootstrap value (Figure 4). All pitted isolates grouped together, except, two isolates (7Pit and 4 PitC) that were grouped with superficial isolates from potato (Figure 4). Pitted isolates that clustered together also clustered with reference *Streptomyces* isolates. Superficial isolates from potato mostly clustered together, except four isolates, where two (6SA and 5SC) clustered together and were distinct from other superficial isolates with 100% bootstrap support, one (6SB) grouped with 2 turnip isolates and a reference non-pathogenic *Streptomyces* isolate and one (5SA) clustered with a turnip isolate and were distinct from all other isolates with 64% boostrap support (Figure 4). *Mycrobacterium tuberculosis* was used as an outgroup and was distinct from all other isolates.

Recombinase (recA) gene. For the recombinase (*recA*) gene phylogeny most of the isolates from superficial lesions from turnip and potato clustered together, with some exceptions. Two turnip

isolates (3TA and 2TB) were distinct from other isolates with 100% bootstrap support, and one turnip isolate (8T) was distinct from other isolates with 100% bootstrap support as well (Figure 5) All pitted isolates clustered together with 97% bootstrap support and were distinct from superficial isolates both from turnip and potato (Figure 5) All isolates were able to be clustered with reference *Streptomyces* isolates. The outgroup *M. tuberculosis* was distinct from all isolates (Figure 5).

Polymerase B subunit gene. For the polymerase B subunit (*rpoB*) gene phylogeny all pitted isolates from potato clustered together with 100% bootstrap support, except two isolates (Figure 6). Turnip and potato isolates from superficial lesion were scattered throughout the phylogenetic tree (Figure 6) Eight turnip isolates clustered together with 96% bootstrap support, 4 other turnip isolates clustered together with 71% bootstrap support, and three other turnip isolates grouped together with one superficial potato isolate with 97% bootstrap support (Figure 6). Eight superficial potato isolates, one turnip and one pitted isolate clustered together with 53% bootstrap support. One turnip isolate (2TA) was distinct from all isolates with 100% bootstrap support. All isolates were able to be clustered with reference *Streptomyces* isolates (Figure 6). The outgroup *M. tuberculosis* was distinct from other isolates (Figure 6).

Figure 4**.** Maximum-parsimony tree resulting from the analysis of the sequence 16S rRNA gene of 45 *Streptomyces* spp. isolates and 12 reference *Streptomyces* species. *Mycrobacterium tuberculosis* was designed as the outgroup. Numbers on the branches represent bootstrap support from 1000 replications (values greater than 50% displayed on the tree)

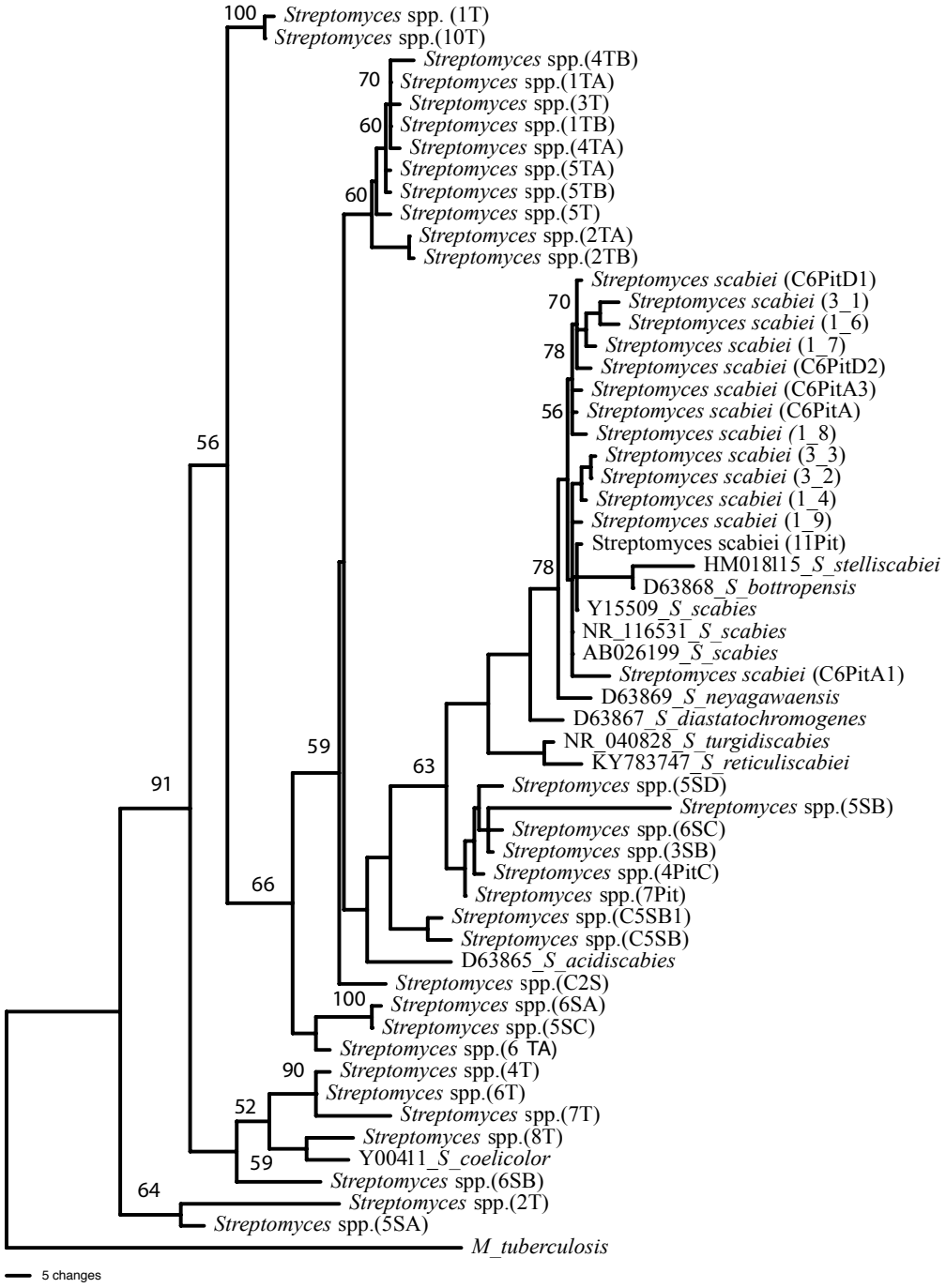


Figure 5**.** Maximum-parsimony tree resulting from the analysis of the sequence Recombinase gene (*recA*) of 35 *Streptomyces* spp. isolates, and 12 reference *Streptomyces* species. *Mycrobacterium tuberculosis* was designed as the outgroup. Numbers on the branches represent bootstrap support from 1000 replications (values greater than 50% displayed on the tree)

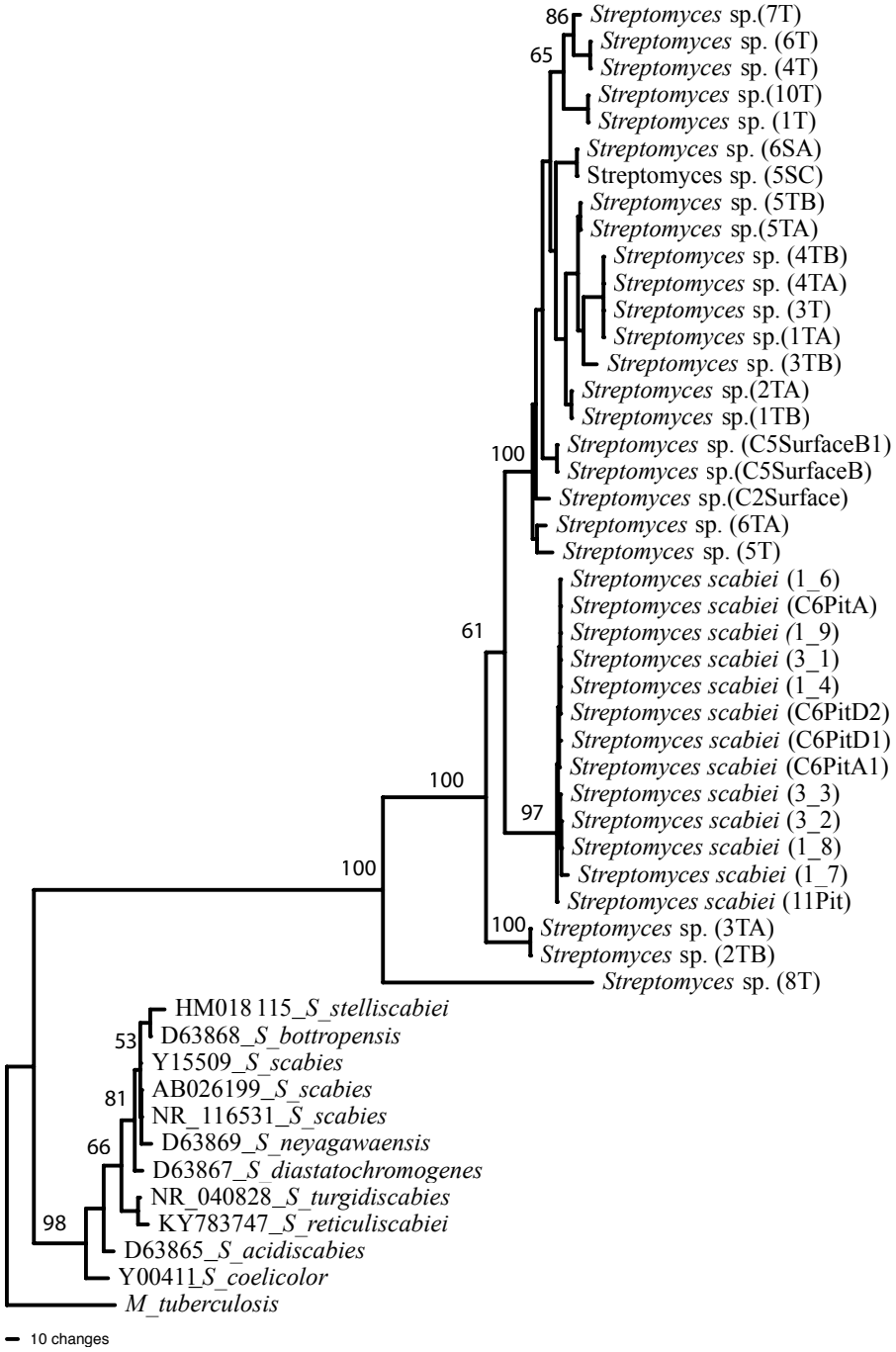
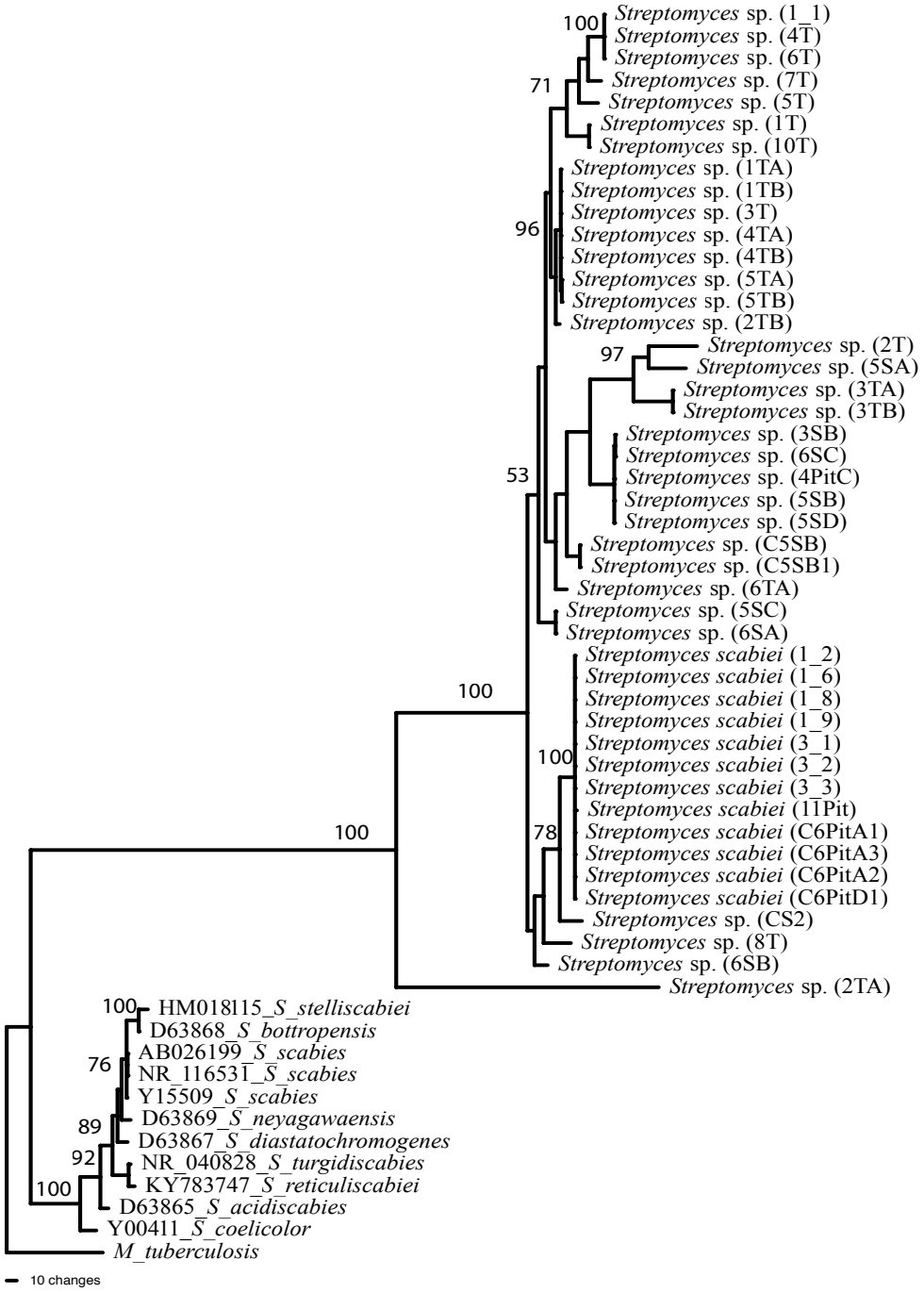


Figure 6**.** Maximum-parsimony tree resulting from the analysis of the sequence Polymerase B subunit gene (*rpoB*) of 45 isolates of *Streptomyces* spp. 12 reference *Streptomyces* species. *Mycrobacterium tuberculosis* was designed as the outgroup. Numbers on the branches represent bootstrap support from 1000 replications (values greater than 50% displayed on the tree)



DISCUSSION

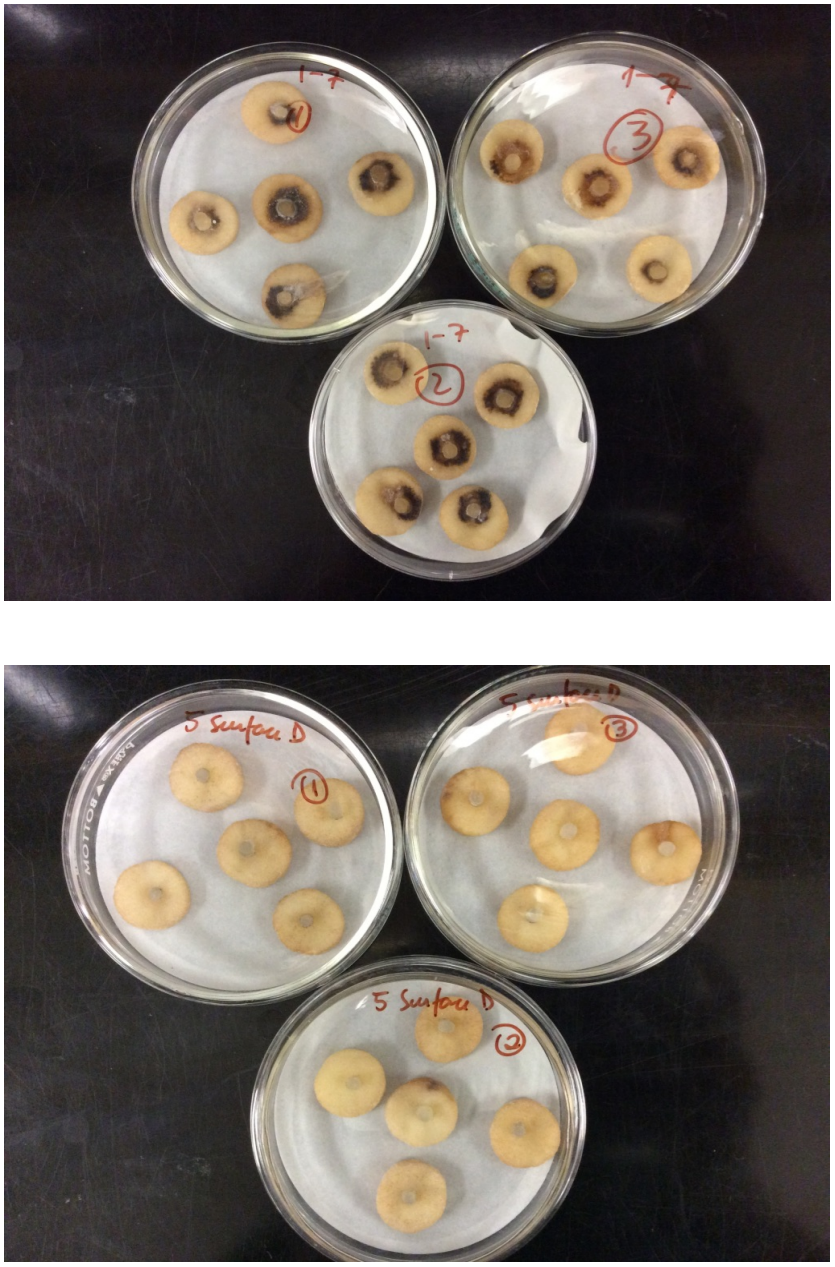
Common scab of potatoes is a widespread disease of potatoes and other root crops (Loria et al., 1997). A number of different *Streptomyces* species are able to cause common scab (Wanner, 2006; Wanner and Kirk, 2015). The most common and widely distributed species of pathogenic *Streptomyces* is *S. scabies*. However, there are CS causing species around the world. In the US and particularly in Michigan based on study by Wanner, (2006) four *Streptomyces* species are pathogenic on potato.

Overall turnip roots did not produce deep pitted lesions, only superficial lesions, therefore all the turnip isolates were collected from superficial lesions. Whereas on potato tubers both deep pitted and superficial lesions were observed therefore isolates were recovered from both lesion types. Previous studies by Lehtonen et al., (2004) isolated at least two different *Streptomyces* spp. from the same field, tuber or from a single lesion. It has been demonstrated that severity of scab symptoms is related to the susceptibility of the host, however the possibility that different species of *Streptomyces* could be responsible for different phenotypic characteristics, particularly for lesion type. In pathogenicity assays on tuber disks most of the turnip and potato isolates from superficial lesions did not result in necrosis (Figure 7). Conversely, most of the potato isolates from pitted lesions produced necrosis on tuber discs (Figure 7). Numerical differences were observed in the turnip seedling pathogenicity assay based on seedling length, but no significant differences were found among isolates compared to the non-inoculated control. All the isolates resulted in numerically lower seedling length compared to non-inoculated control. Notably isolates did suppress root growth, but root measurements were not recorded.

Thaxtomin production is one of the characteristics of pathogenic isolates (Wanner and Kirk, 2015). Thaxtomin targeted PCR revealed that only a few turnip and potato isolates from

superficial lesions possessed the target for t*xtA* and *txtAB* genes. Most isolates from pitted potato lesions contained both *txtA* and *txtAB* genes. Wanner, (2004) reported that all, except one *Streptomyces* isolate from radish had the *txtA* gene locus. Similarly, most of the turnip and potato isolates from superficial lesions lacked the *nec1* gene. The *nec1* gene has been reported to be present in pathogenic *Streptomyces* species (Lapaz et al., 2017; Wanner, 2009). The compound that causes a necrotic response that is produced by *nec1* containing species has not been identified, but it is distinct from thaxtomin (Wanner, 2004). Several studies reported that non-pathogenic

Figure 7**.** Potato tuber discs inoculated with *Streptomyces* isolate that recovered from pitted lesion showing necrosis (top picture). Potato tuber discs inoculated with S*treptomyces* isolate recovered from superficial lesion showing no necrosis (bottom picture)



isolates did not have the *nec1* gene, whereas *nec1* was present in pathogenic isolates (Wanner, 2004; Bukhalid et al., 1998; Kreuze et al., 1999). In our study, turnip and potato isolates from superficial lesions that contained *nec1* gene also resulted in necrosis in tuber disc assays. Most of the isolates that lacked the *nec1* gene did not result in necrosis. Conversely, Wanner, (2004) found that isolates that lack the *nec1* gene produced a spectrum of scab lesion types and severity of pathogenicity related to seedling emergence and survival. The role of the *nec1* gene in pathogenicity and as a virulence factor is not well understood in *Streptomyces* species, however isolates that lack the *nec1* gene in this study were collected from symptomatic roots/tubers. Another PAI related gene the tomatinase homolog-encoding gene *tomA* is present in most of the common *Streptomyces* spp. including: *S. scabiei*; *S. acidiscabies* and *S. tugridiscabies* (Wanner, 2007b). In this study the *tomA* gene was present in most turnip, and potato *Streptomyces* spp. isolates. Similar results were reported by St-Onge et al., (2008) where all pathogenic isolates contained the *tomA* gene.

Potato isolates from pitted lesions in the three phylogenetic topologies clustered similarly. Moreover, all the pitted isolates contained all PAI related genes, except three isolates that were missing *nec1* and two were missing *txtA* genes. Additionally, all pitted isolates clustered with reference pathogenic *Streptomyces* species. Thus, indicating that the isolates are likely closely related to one of the pathogenic species. Similarly, a study by Fyans et al., (2015) found that thaxtomin-A producing isolates all clustered together with reference isolates of *S. scabies*, and *S. europaeiscabiei*. Two pitted isolates that did not cluster with the rest of isolates were missing *txtA* and *nec1* and they did not produce a necrotic response on tuber discs. Additionally, these two isolates clustered in the 16S rRNA phylogeny with potato isolates from superficial lesions, and all had the *tomA* gene. In the 16S rRNA phylogeny turnip isolates that clustered together all had *tomA*,

except two isolates had thaxtomin and *nec1* genes. Another two turnip isolates that were distinct from the other turnip isolates lack both *tomA* and *nec1* genes and did not produce a necrotic response on tuber discs. One turnip isolate that was also distinct from the rest of turnip isolates had all PAI genes but lacked the *nec1* gene. Additionally, in the 16S rRNA phylogeny most potato isolates from superficial lesions clustered together and these isolates contained either two or one of the following genes: *tomA*; *nec1*; and/or *txtAB*. However, all these isolates lack the *txtA* gene. A study by Leiminger et al., (2013) reported that the presence of the *txtAB* gene resulted in the ability to produce thaxtomin A, and is considered as a pathogenicity determinant for pathogenic *Streptomyces*. Similarly, another study by Lapaz et al., (2017) also reported that isolates that lack *txtAB* genes were still able to cause disease. Two isolates recovered from superficial potato lesions that were distinct from the rest of the superficial isolates posed the *tomA* gene and lacked all the other PAI related genes. Isolates that clustered with non-pathogenic *S. coelicolor* also had only the *tomA* gene and lacked the other PAI related genes. The *nec1* and *tomA* genes are part of the PAI, however they are not located in the same chromosomal region as the thaxtomin biosynthesis gene cluster (Dees et al., 2013). Therefore, these two genes could independently transfer to different *Streptomyces* species (Dees et al., 2013). The phylogeny resulting from *recA* and *rpoB* genes revealed similarly clustered groups as the 16S rRNA phylogeny with the following exception, in *rpoB* phylogeny one potato isolate from a pitted lesion clustered with turnip isolates. This isolate was also identified as *Streptomyces* spp, whereas, other the isolates recovered from pitted potato lesions were identified as *S. scabies*. However, this isolate contained all four PAI related genes. Notably this isolate was not included in the other phylogenetic analyses. Another turnip isolate was distinct from the rest of the isolates and only contained the *tomA* gene and lacked the other PAI related genes. In the *recA* phylogeny 2 turnip isolates were distinct from the other isolates and

yet another isolate recovered from turnip was distinct from the rest of the isolates and all three only contained the *tomA* gene and lacked the other PAI related genes.

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