DISSERTATION

UNDERSTANDING BLACKLEG IN SEED POTATO OPERATIONS AND THE DEVELOPMENT OF MANAGEMENT STRATEGIES FOR THE CONTROL OF BLACKLEG AND TUBER SOFT ROT, CAUSED BY *Pectobacterium* SPP.

Submitted by

Andrew Joseph Houser

Department of Horticulture and Landscape Architecture

In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Summer 2018

Doctoral Committee:

Advisor: Robert Davidson

Daniel Manter
Mary Stromberger
Stephen Wallner

ABSTRACT

UNDERSTANDING BLACKLEG IN SEED POTATO OPERATIONS AND THE DEVELOPMENT OF MANAGEMENT STRATEGIES FOR THE CONTROL OF BLACKLEG AND TUBER SOFT ROT, CAUSED BY *Pectobacterium* SPP.

Pathogenic bacteria in the genera *Pectobacterium* and *Dickeya* are of major concern in many potato growing regions around the world and are the causal organisms of blackleg and tuber soft rot. Bacteria in these genera are pectolytic, meaning they utilize pectin, of which potatoes are a good source. Crystal Violet Pectate (CVP) media was used in this project to detect the presence of pectolytic bacteria. If this bacteria is present, pits will form on the media. Pitting refers to the presence of a concave indentation in the media resulting from actively growing pectolytic bacteria. This project evaluated different stages of seed potato production to determine possible bacterial reservoirs during a seed cutting operation, optimal timeframe for bacterial screening of potato seed, and the use of different crop rotations to manage these bacteria. In addition, a summary of the findings from this project was used to develop a grower friendly blackleg/tuber soft rot management guide.

The first objective was to evaluate two seed cutting operations and determine whether reservoirs of bacteria were present and could act as possible sources of contamination. Pectolytic bacteria are often spread through the seed cutting process. Cutter surfaces exposed to pectolytic bacteria include cutting knives, belts, rollers, etc. This study evaluated two seed cutting operations for their potential to limit the spread of pectolytic bacteria. Grower 1 used a Milestone seed cutter while grower 2 used a hot cutter with heat sterilized blades. There were no statistical differences between the Milestone and hot seed cutter at reducing the spread of pectolytic bacteria than the Milestone cutter. However, when individual cutter surfaces were compared, there was a higher percentage of pectolytic bacteria on the stationary knives of grower 1 (26.7 % pitting) compared with the hot cutter knives from grower 2 (1.0% pitting).

Results from this study show that the use of heat to sterilize cutting blades reduces the amount of pectolytic bacteria better than stationary knives that were not sanitized.

The second objective was to determine an optimal timing for estimating bacterial levels in a seed crop. Pectolytic bacteria that cause potato blackleg (*Pectobacterium* spp. and *Dickeya* spp.) can often be detected in field-grown, early generation certified seed potato lots, even though the origin seed stocks were tested as free of the bacteria. Because of this, growers have a need for a predictive tool to assist in making good, appropriate decisions about any given seed lot. This project used a crystal violet pectate (CVP) - tuber poke method to screen early generation potato seed to predict possible outbreaks of blackleg. Potato samples from several certified seed operations were tested using this method and compared with summer inspection blackleg readings. Results showed operations that differed in the number of blackleg outbreaks also differed in % pitting when a sample from the same seed crop was tested. At early generations, an increase in % pitting on CVP corresponded to an increase infield blackleg levels. However, in operations with little blackleg present, less pitting was found. The outcome of this project provides potato growers with a potential tool to help predict potential blackleg outbreaks in later generations used for seed production.

The third objective was to evaluate potatoes for blackleg and tuber soft rot levels under different crop rotation systems. One potential management practice that a potato grower can use to reduce disease incidence in their crop is by utilizing different crop rotations. This study evaluated seven different crop rotations in 2013 and 2014, using the potato cultivar Colorado Rose, in the San Luis Valley, CO. Potato seed was inoculated with a slurry of *Pectobacterium atrosepticum* prior to field planting. The potato crop was evaluated to determine the effect each of these rotations had on disease incidence and severity. Blackleg, tuber soft rot and pectolytic bacteria levels were evaluated. Results indicated an increase in pectolytic bacteria population in tubers when Canola, in the plant family Brassicaceae were included in the rotation. When following canola, a decrease in yield and an increase in the number of culls and rotten tubers was also observed in the potato crop. When a potato crop followed the remaining rotational crops, there was no significant changes in yield, grade or disease incidence. Based on this study, the use of

Canola in a crop rotation should not be used if there is a history of blackleg or tuber soft rot within the farming operation.

The final objective of this project was to summarize the findings of this project by developing a grower friendly management guide. This management guide will include results from this project and other contemporary blackleg/tuber soft rot research available that is pertinent to the San Luis Valley potato industry.

AKNOWLEDGEMENTS

To the Triune God, who is the creator and sustainer of all things, without which, there would be no plants or pathogens to study. This project would not be possible except through his guidance and support.

To Dr. Robert Davidson, who has greatly helped me in the design, organization, writing and presentation of this project.

To my wife Lori, who has supported me through this process. Without her love and understanding, this would not have been possible.

I would like to thank the following people for their help, expertise, support and encouragement. They include:

- My committee members: Dr. Robert Davidson, Dr. Dan Manter, Dr. Mary Stromberger, and Dr. Steve Wallner.
- My staff at the Colorado Potato Certification Service: Teresa Almeida, Richard Haslar, Carolyn Keller, Michelle Leckler, Sarah Noller, Jeff Shawcroft, Sarah Shawcroft, and Sharon Yust.
- The staff and seasonal employees at the San Luis Valley Research Center.
- My church family at Valley Church of the Nazarene, for their support, encouragement and prayers.

DEDICATION

To my wife:

Lori Elizabeth,

Your love and support have made this possible.

To my two boys:

Tanner Merritt

&

Josiah Robert,

You guys are a blessing and I learn something new from you every day.

To my mother:

Patricia Ann,

You have been a continuous blessing to me throughout my life.

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LITERATURE REVIEW

Introduction:

Bacteria belonging to the genera *Pectobacterium* and *Dickeya* are causal agents of potato blackleg (stem and foliage disease) and tuber soft rot (tuber disease). Three species within these genera cause the majority of the blackleg and soft rot cases: *Pectobacterium carotovorum* subsp. *carotovorum* (Pcc), *P. atrosepticum* (Pa), and *Dickeya* spp. (Di). All three of these bacteria were formerly grouped in the genus *Erwinia: E. carotovora* subsp. *carotovora* (now Pcc), *E. carotovora* subsp. *atroseptica* (now Pa) and *E. chrysanthemi* (now Di). These bacteria are strait rods with peritrichous flagellae characterized as gram negative, facultative anaerobes that are pectinolytic and non-spore forming. They produce cell wall degrading enzymes that allow for maceration and infiltration of the plant tissue they attack (Czajkowski et al. 2011).

It has been estimated that there are anywhere from $50 to 100 million in losses to potato producers as a result of the tuber soft rots and blackleg, caused by these bacteria (Perombelon and Kelman 1980). The infection of a potato plant by these pectinolytic bacteria can manifest itself in three primary ways. One way is that blackleg symptoms can develop in the stem(s) of the potato plant. Bacteria present in the seed piece, and soil in tropical climates, will migrate up the stem through the vascular tissue. At the base of the stem, water soaked lesions that are light brown to black in color can develop, resembling the appearance of black ink. The vascular tissue is often plugged and is typically discolored as well. The foliage of an affected stem can also have a wilted, chlorotic appearance due to the lack of water and nutrient transport to the affected tissues. Ultimately, this causes an overall sickening of the plant, thereby indirectly reducing tuber yields (Stevenson 2001; DeBoer 2004).

Another way in which the disease can be expressed is tuber soft rot. A seed piece that is infested with bacteria will rot in the field; the bacteria can then spread in the soil and infect daughter tubers at the lenticels or in wounds. Also, tuber lesions can develop at the point where the stolon attaches to the tuber.

The affected tuber tissue is typically cream to tan colored and is soft and granular (Stevenson 2001; DeBoer 2004).

Finally, the less common aerial stem rot can also occur in which lesions can develop in petioles and stems, but not arising from the seed piece. This decay tends to be confined to the above-ground portions of the plant with symptoms similar to that of blackleg. This method of decay does not typically translocate bacteria from the above ground portion of the plant to the tubers in the soil. Yet, aerial stem rot is detrimental to the overall health of the plant, thereby decreasing yield (Stevenson 2001; DeBoer 2004).

These bacteria (Pcc, Pa, and Di) can be spread to healthy tubers during seed cutting, handling, and planting operations. As facultative anaerobes, these bacteria are able to grow rapidly during times of high moisture around the seed piece. They can reproduce either aerobically or anaerobically. High soil temperatures (>20^{o}C) favor seed piece decay. Blackleg in the stems is favored by cool (10-15^{o}C), wet soils at planting, followed by high soil temperatures (>20^{o}C) after plant emergence. These bacteria can be spread to non-infected plants via irrigation water, decaying seed pieces, insects, adverse weather such as hail, and crop debris in soil that contains the bacteria. Wind can also result in wound development in an infected plant, which allows for the bacteria to be spread to an adjacent plant through contact. Bacteria can also move through the stems and stolons of infected plants to the daughter tubers, thereby infecting the daughter tubers. If the daughter tubers are then used to plant the following year's crop, tuber soft rot can develop in storage after harvest or the bacteria can spread to the following year's crop (Stevenson 2001; DeBoer 2004).

Taxonomy and Biology of the Soft Rot Bacteria:

Taxonomy of the Soft Rot Bacteria:

The genera *Pectobacterium* and *Dickeya* belong to the bacterial family Enterobacteriaceae, which contains many microorganisms that are pathogenic to both plant and animal species (Toth et al. 2011). In the last twenty years, with the development of new molecular techniques and phylogenetic analysis, there has been an increased level of genomic work throughout the scientific community, which has resulted in the reorganizing of taxonomy and nomenclature (genera, species, and subspecies) within this family of bacteria. The naming of the soft rot and blackleg causing bacteria within this family have been greatly affected by this. Prior to the early 2000's, the only genus of bacteria causing soft rot and blackleg in potatoes fell in the genus *Erwinia*, although previous attempts had been made to change the genera and species nomenclature through the years (Gardan et al. 2003; Charkowski 2006; Czajkowski et al. 2011).

In 1901, this organism was first described by Hall which he observed in carrots and gave the name *Bacillus carotovorus*. In 1902 de Hall identified this bacteria in potatoes for the first time and gave it the name *Bacillus atrosepticus* (Graham and Dowson 1960). Then in 1917, this genera of bacteria was classified by Winslow et al. in 1917 as *Erwinia* and was named after Erwin Frink Smith, one of the founders of the study of phytobacteriology (Charkowski 2006). In 1945, Walde proposed the genus name *Pectobacterium* for the bacteria, however this name did not gain popularity and *Erwinia* remained the genus name until recently (Graham and Dowson 1960). In the early 2000's, the genus name of *Erwinia* was replaced and divided into two genera, *Pectobacterium* and *Dickeya* (Gardan et al. 2003). Currently, all the bacteria able to cause potato blackleg and tuber soft rot are in these two genera. (Czajkowski et al. 2011). There have been additional species and subspecies of the soft rotting bacteria identified in recent years which include: *Pectobacterium carotovorum* subsp. *brasiliensis* and *Pectobacterium wasabiae* (Marquez-Villavicencio et al. 2011).

The soft rot causing bacteria, primarily *Erwinia carotovora* and *E. atroseptica*, have been seen as a problem when growing many different horticultural crops and have been studied for over 100 years (Charkowski 2006). In the 1970's, another bacterial species, *Dickeya dianthicola* (formerly *Erwinia chrysanthemi*), was identified as causing blackleg and tuber soft rot in the Netherlands. Since then, this bacterium has been discovered in several other European countries, including England and Wales. *Dickeya solani* is another blackleg and tuber soft rot causing bacterial species that was first reported in Israel in 2005, and has since been reported in several European countries as well as the United Kingdom.

D. solani tends to be more aggressive than the other blackleg and tuber soft rot causing Enterobacteria, thus making it of major concern in potato growing areas (Toth et al. 2011; ADHB-Potato Council 2009).

In 2015, there was an increased interest in *Dickeya* sp. and its role in blackleg and tuber soft rot development in the United States. *Dickeya* spp. was identified in Maine and has caused major disease issues there. *D. dianthicola* has been identified in several states after the find in Maine. The concern is that if *D. dianthicola* appeared in Maine, then *D. solani* could be next. Currently, potato production areas are concerned about the potential spread of this bacteria to other parts of the country. Different state seed certification agencies are watching and testing for this bacteria and there is a national effort to reduce the potential spread of this pathogen between states and different potato growers (A.O. Charkowski, personal communication).

D. dianthicola and *D. solani* both favor warmer climates (temperatures exceeding 25^{o}C) than *Pectobacterium* spp. and disease is typically a result of the bacteria moving from the seed piece to the stem base. Both genera of bacteria have also been shown to survive and remain pathogenic in contaminated irrigation water (ADHB-Potato Council 2009).

The Biology and Pathogenicity of the Soft Rot Bacteria:

The Enterobacteria that fall in the genera *Pectobacterium* and *Dickeya* and cause blackleg and tuber soft rot are gram negative, rod shaped, non-spore forming, facultative anaerobes with peritrichous flagella (Charkowski 2006). The soft rot causing bacteria (*Pectobacterium* spp. and *Dickeya* spp.) are necrotrophic, opportunistic pathogens, meaning they cause disease when the plant's resistance is impaired. The presence of free water, resulting in anaerobiosis, can weaken potato tuber resistance and slow wound healing. These bacteria can cause soft rot (tuber) and blackleg (foliar) in the potato plant. Tuber soft rot symptoms can occur when the tuber is in an anaerobic environment (e.g. excess of free water) or has been wounded. The addition of free water can result in anaerobiosis, allowing the bacteria to infiltrate and macerate the tuber tissue, thereby causing rot. Blackleg symptoms occur when bacteria multiply in the mother seed piece and migrate up through the stem. The bacteria will plug the xylem tissue which blocks the transport of water and nutrients to the stem. This results in the wilting and eventual death of the stem (Perombelon 2002; Davidsson 2013).

Pathogenesis of Tuber Soft Rot:

Soft rot causing bacteria, when present, do not always cause disease on a potato tuber. The bacterial cells will often remain dormant or quiescent when in direct contact with the cell wall, even when cell numbers are 10^6 cells/gram of peel tissue. Tuber maceration and disease typically do not occur until bacterial cell numbers are 10^7 cells/gram of peel tissue or higher. This dormancy might be the result of a lack of nutrients, which will reduce bacterial multiplication and proliferation, as well as a lack of free water. While dormant, these bacteria can survive for several months in the tubers lenticels, which is long enough to bridge the gap between growing seasons in a potato cellar. The introduction of free water is typically what will trigger the change from dormant to active in the bacteria (Nielsen 1978; Perombelon 2002).

Anaerobiosis is triggered by the presence of a water film on the tuber surface, which will create an anoxic environment. The presence of a water film will result in two events. First, it prevents oxygen from being able to diffuse across the cell wall. As the tuber cells respire, oxygen is used by the cells. The presence of a water film on the outside of the tuber prevents the diffusion of oxygen from the soil atmosphere. This results in anaerobiosis, which impairs oxygen dependent host resistance systems including the production of phytoalexins, phenolics, and free radicals. The lack of oxygen also inhibits cell wall lignification and suberization, which play an important role in protecting the tuber from pectic enzyme degradation. The second effect of free water on pathogenesis is the swelling of cortical cells and the breaking down of the phelloderm layer in lenticels. Lenticels will open in the presence of free water. This allows the bacteria to penetrate deeper into the tuber, even when a wound is not present. Some plant cells can also lyse due to increased hydrostatic pressure, thereby providing the bacteria with additional nutrients for growth (Perombelon 2002; Czajkowski et al. 2011).

Under aerobic conditions, soft rot bacteria can also cause the rotting of tubers. This will typically happen through the wounding to the tuber. Under this scenario, the tuber’s wound healing and resistance mechanisms are not impaired, as with anaerobic conditions. Bacterial numbers need to be high enough to overcome the tuber's defenses in order for disease development to occur. Basically, the bacteria outcompete the tubers ability to heal. Tuber soft rot can also occur when storage or soil conditions are at relatively high temperatures (>35ºC). This may be a result of a rapid respiration by the tuber cells. The diffusion of oxygen across the cell wall cannot keep up with the cell respiration, resulting in an anaerobiosis (Perombelon 2002).

Tuber soft rot is more likely to occur under anaerobic, rather than aerobic conditions. However, soft rot can occur under both situations, primarily as a result of anaerobiosis. Another reason anaerobic conditions result in a higher incidence of disease is because pectate lyase is produced in higher quantities by soft rot bacteria under these conditions as compared to aerobic conditions. Pectate lyase is one of the more important enzymes used by the bacteria in the process of tuber maceration and gives the bacteria a competitive advantage over the potato tuber (Perombelon 2002).

Pathogenesis of Blackleg:

Unlike soft rot, which affects the tuber mostly under anaerobic conditions, blackleg affects the stem under aerobic conditions. Blackleg develops when conditions are favorable for the multiplication of bacteria during the rotting of the mother seed tuber. Large numbers of bacteria then migrate up the stem causing blackleg. Blackleg disease expression in a potato plant requires either a mother seed piece (e.g., a plant arising from tissue culture; disease-free plants in the greenhouse cannot acquire blackleg from a mother seed piece, etc.) or soil that is heavily contaminated if the seed is free of bacteria (Perombelon 2002; Czajkowski et al. 2011).

Environmental conditions that favor the development of tuber soft rot also favor the development of blackleg. A high soil water level is the major environmental factor affecting the development of blackleg, since this creates an anaerobic environment, thereby resulting in a potentially higher rate of multiplication for the bacteria. Potato cultivars respond differently when conditions are favorable for tuber soft rot and/or blackleg development. A cultivar that is resistant to soft rot might be susceptible to blackleg, and visa-versa. However, the plants' level of resistance to blackleg seems to be more dependent on the ability of the stem to resist blackleg development rather than the mother tuber's resistance to rotting (Perombelon 2002; Charkowski 2015).

Bacteria from the rotting mother tuber are transported passively to the stem xylem vessels. The presence of a lignified barrier at the junction of the stem and tuber may increase the level of blackleg resistance by the plant. It is a physical barrier based on the stem's morphology. Excess lignin in the cell walls have the ability to resist degradation of the cell walls by pectic enzymes released by the bacteria. Cultivars that are resistant to blackleg development develop lignified cell walls sooner than more susceptible cultivars (Perombelon 2002).

Blackleg can develop at different times throughout the season. When blackleg develops early in the season, soon after plant emergence, this is the result of the bacteria originating from the rotten seed piece and overwhelming the plant. Symptoms include stunting, wilting and chlorosis which is caused by a restriction of water flow in the xylem. This is made worse by dry, above ground conditions. Later in the season, this is not always the case as the stems become more independent of the mother tuber. There can often times be high numbers of bacteria in the stem, but no blackleg is observed. Under cool conditions it is not uncommon to see only one stem expressing blackleg symptoms and two or three healthy looking stems coming off of the same mother tuber. When weather conditions are favorable (high soil water, high humidity, and warm temperature), the remaining stems can also succumb to blackleg. Under high relative humidity, the amount of blackleg will increase if the bacteria is present in the stems. This condition results in low transpiration, due to a reduction in water potential. This reduction of water movement, due to greater solute concentrations, can eventually cause the plant cells to leak nutrients into the xylem. The release of these nutrients provide food for the bacteria which are already present in the xylem. This results in the multiplication of the bacteria, thereby plugging the xylem, causing blackleg (Perombelon 2002; Czajkowski et al. 2011).

Under dry atmospheric conditions, the bacteria will travel through the plant but often won't cause blackleg due to the high level of transpiration. Under these conditions the bacteria do not multiply as readily and fail to plug the xylem vessels. Some discoloration in the tops of the stems can result, indicating that there has been some restriction of water flow in the stem, but not enough to kill the stem. Also, bacterial numbers within the stem tend to fluctuate through the growing season, depending on the environment. The microclimate within the plant and in the field plays a major role in blackleg development. The initial seed contamination level also plays a major role in blackleg development. A higher initial number of bacteria in or on the tuber results in a higher chance that the mother tuber will rot. This can lead to a higher rate of bacterial multiplication and plugging of the xylem vessels. The two factors of environment and seed contamination are the leading issues potato farmers have to contend with when managing blackleg in their fields (Perombelon 2002; Charkowski 2015).

Factors Involved in Pathogenesis:

The soft rot causing bacteria use a wide range of plant cell wall degrading enzymes (PCWDEs) as well as other mechanisms to degrade cell wall components. The families of enzymes involved include pectinases, cellulases, hemicellulases, proteases, and xylanases. Each of these have different properties as well as different functions in causing disease. The pectinases are thought to be the most important of the PCWDEs for pathogenesis. These are primarily responsible for the maceration of tissue and ultimately cell death. The pectinases cause the degradation of pectin in the middle lamella between cells. The pectinases used by the bacteria during infection and growth are pectate lyase, pectin lyase, pectin methyl esterase, and polygalacturonase. The different pectinases are expressed extracellularly and used by the bacteria at different times throughout the infection process. Environmental factors such as pH and temperature can influence the expression and activity of different pectinases. Bacteria in the genus *Dickeya* tend to be more virulent at higher temperatures (>30^0C). This may be explained by the use of pectinases by *Dickeya* that are more active at higher temperatures. This may be part of the reason why bacteria in this genus are more active and cause more disease than *Pectobacterium* spp. under higher temperatures (Perombelon 2002; Czajkowski et al. 2011; Charkowski 2015; Davidsson 2013).

The use of quorum sensing is another strategy the soft rot bacteria use to aide in pathogenesis. Quorum sensing is a cell density dependent regulatory system that allows the bacteria to regulate production of certain chemicals when cell numbers are high enough. This is a way the bacteria can prevent the plant cells resistance mechanisms from becoming active until there are enough bacterial cells present to overcome the plants' resistance mechanisms. An example of quorum sensing is the regulation of harpin protein synthesis, which has been identified as being involved in pathogenesis, in Pcc. There is still much to be learned about quorum sensing and how it is involved in disease development caused by soft rot and blackleg causing bacteria (Perombelon 2002; von Bodman et al. 2003; Davidsson 2013).

Several other factors, such as motility, adhesion, and the presence of lipopolysaccharides (LPS) in the bacteria cell walls also play a key role in pathogenesis. Flagella allow the bacteria to swim to the lenticels of a tuber in the presence of free water. Motility does not play a major role in initial infection of the host, but has a major role in getting to the host. Adhesion is also necessary for the bacteria to cause disease through the use of fimbriae or pili. This is a common feature among most enteric pathogenic bacteria. Another factor which is common to most gram negative bacteria are LPS. LPS have been shown to provide the bacteria some protection against antimicrobial compounds produced by the tuber (Perombelon 2002).

Harpin and Avr proteins are also involved in pathogenesis and can cause rapid cell death in the host tissue. Type III secretion systems are used by the bacteria to transport these proteins across the cell membrane. These proteins may be involved in causing the leakage of cellular fluids, which in turn feed the bacteria and allow them to multiply. Although these proteins and the genes encoding them have been identified in plant pathogenesis, much remains to be learned as to the role they play in disease development (Perombelon 2002).

Conditions favorable for disease development:

Infection of Potato Material:

The avenues through which soft rot bacteria are introduced into a potato farming operation are of major concern, and in many cases unknown. Several potential avenues for the introduction of these bacteria have been studied and have been determined in previous work (Meneley and Stanghellini 1976; Lapwood and Harris 1980; Deboer 1989; Ali et al. 2012). Although much work has been done in this area, the initial introduction of these bacteria into field grown progeny of clean nuclear stock originating from greenhouse production has not yet fully been identified (R.D. Davidson, personal communication).

Infected Seed:

Although all avenues of introduction for *Pectobacterium* into a farming operation have not yet been identified, many have been. One of these, and probably the most important, is the use of infected seed in farming operations (Perombelon 2000). Several studies have shown that the primary origin and spread of Pa and Pcc is from seed tubers infected with the bacteria (Toth et al. 2003; Ali et al. 2012). The infected tubers act as an inoculum source for the progeny tubers. The pathogenic bacteria are spread in the soil from the infected mother tubers to the progeny tubers (Lapwood and Harris 1980). The longer a seed lot is grown in the field, the greater the potential for increasing disease. Thus, the amount of pathogenic bacteria and viruses present in and on the seed piece could be higher in the later generation seed (Rowe 1993).

Surface Irrigation Water:

Another possible avenue for the introduction of blackleg causing bacteria into a potato field is through contaminated irrigation water (McCarterzorner et al. 1984; Harrison et al. 1987; Cappaert et al. 1988; Perombelon 1992). McCarter-Zorner et al. (1984) conducted a survey of water sources in Southern Scotland and Colorado. In this study, Pcc was generally present in surface water from drains, ditches, streams, rivers, lakes and reservoirs. Pa was found much less frequently in these same locations. Well water was not identified as a source of the bacteria in southern Scotland and Colorado. In Colorado, a high percentage of water samples (about 66%) taken from the Rio Grande River contained Pcc (McCarterzorner et al. 1984). Cappeaert et al. conducted a survey during 1985 and 1986, and reported that nearly 100% of the surface water samples collected contained Pcc, whereas less than one percent of the samples contained Pa in each of the two years. When well water was sampled, no Pcc or Pa was detected (Cappaert et al. 1988). However, Maddox and Harrison sampled well water from the San Luis Valley, CO and found Pcc as well as Pa (Maddox and Harrison 1988). Harrison found that Pcc and Pa are widespread in both fresh and salt water sources throughout North America. However, no *Dickeya* spp.

were found in this survey (Harrison et al. 1987). In the San Luis Valley, CO the Rio Grande River, Saguache creek, and San Luis Creek all contained *Pectobacterium* spp. (Harrison et al. 1987; Maddox and Harrison 1988).

Pcc has also been collected in rain water. It has been proposed that this may be the result of bacteria being washed out of the atmosphere. If the bacteria is in aerosol form, a rain or irrigation event can wash the bacteria out of the atmosphere, resulting in the contamination of the falling water. This is one possible explanation of why a supposedly bacteria free potato crop can become contaminated with Pcc (McCarterzorner et al. 1984)

Dickeya spp. tends to have a lower survivability in surface water than the *Pectobacterium* spp. *Dickeya* spp. have been found to survive in surface water in Scotland, the Netherlands and Australia. Although *Dickeya* spp. are not commonly found in surface water in North America, they have been found in irrigation ponds containing recycled water in Florida. The survivability of *Dickeya* spp. in surface water is dependent on the buffering effect of the water as well as the presence of nutrients in the water (Toth et al. 2011).

The presence of *Pectobacterium* spp., and to a lesser degree *Dickeya* spp., can result in an increase in the amount of contamination in an irrigated potato field. Both Pcc and Pa can cause aerial stem rot, and surface irrigation water is a possible source for this bacteria (Cappaert et al. 1988; Toth et al. 2011). In a study conducted by Franc et al. (1987), clean potato plants and seed pieces were irrigated with water containing Pcc and Pa. The resulting plants and seed pieces were infected with both Pcc and Pa after irrigation (Franc and Harrison 1987). This demonstrates that irrigating a potato crop with contaminated surface or well water can inoculate the crop with blackleg causing bacteria.

Soil:

The majority of literature available on this topic indicates that the pectolytic bacteria involved in blackleg and tuber soft rot will not survive and remain viable in the soil in the absence of infected plant material (Czajkowski et al. 2011). That said, there is also a body of work indicating that *Pectobacterium* spp. can overwinter in the soil under certain conditions (Meneley and Stanghellini 1976; Deboer 1989).

Meneley and Stanghellini conducted a study where they evaluated field soils for the presence of viable pectolytic bacteria at the University of Arizona Experiment Station. They took soil samples from three different field soils which had been grown to potatoes the previous year. These samples were collected after the winter and at the same time of potato planting the following year. This was to ensure that the bacteria present in the soil when potatoes were typically planted would also be present in the soil sample. The soil samples were put in a media used for the enrichment of pectolytic bacteria and then allowed to incubate at room temperature in an anaerobic environment for 48 hours. Bacteria isolated from this enrichment media went through a series of tests to confirm that they were soft rot bacteria including: gram staining, glucose fermentation, pectate hydrolysis, etc. The bacteria that appeared to be *Pectobacterium* spp. after these tests were then used to inoculate potato plants (cultivar Norgold Russet) to test for pathogenicity. All three of the soil samples tested contained soft rot bacteria *Pectobacterium* spp. that were pathogenic. This study indicates that the soft rot bacteria has the ability to overwinter in the soil in the warmer desert climate of Arizona (Meneley and Stanghellini 1976).

Also, research was conducted in Wisconsin using a similar enrichment technique as used by Meneley and Stanghellini (1976) in addition to tuber baiting and a fluorescent antibody stain procedure. They were able to isolate Pcc and Pa from soil that potatoes had grown in the previous year by using these different techniques. They were also able to isolate these bacteria from tubers and potato plant material that had overwintered in the same fields. It was determined that the populations did decrease over the winter, since the use of direct plating techniques onto CVP did not result in pitting in the spring, but it did result in CVP pitting in the summer when the potato plants were growing. The authors suggested that the soil was not a major source of soft rot bacteria inoculum, however this could be a primary source of *Pectobacterium* spp. inoculum for blackleg and tuber soft rot when tubers that are free from *Pectobacterium* spp. are planted in infested soil (Deboer et al. 1979).

This, however, has not been the case in studies conducted in Scotland on this topic. In Scotland, both Pcc and Pa survived in non-sterile soil for only 3-6 weeks when soil temperatures were above 20^{o}C (Perombelon and Kelman 1980). There has also been research conducted that indicates that the soft rot causing *Pectobacterium* sp. rapidly decrease in soils over time. However, the rapid decline in population does not necessarily indicate that all the bacteria are absent from the soil by the following spring (Deboer 1989).

Something that may be complicating the issue and making it more difficult to get a consensus on the ability of these soft rot bacteria to survive in the soil is the fact that they can easily overwinter on susceptible plant debris and in the rhizosphere of host plants. There are several weed species that are hosts and could be acting as inoculum reservoirs (McCarterzorner et al. 1985; Ali et al. 2012). Also, during potato harvest operations, infected tubers might be left in the field which could give the bacteria a place to inhabit through the winter season in temperate climates (Perombelon and Kelman 1980; Deboer 1989). The overwintering of soft rot bacteria in susceptible plant material could also be the source of *Pectobacterium* spp. in soil samples if the bacteria move from the decaying plant material to the soil at the time the soil samples were collected. It has also been reported that the different species of soft rot causing bacteria have different rates of survivability in the soil. This may explain some of the discrepancies that have been observed when researching the survivability of soft rot bacteria in soil (Deboer 1989).

Wind, Insects:

Pa has also been shown to move and infect injured potato plants via aerosols in the wind and insect transmission (Graham and Harrison 1975; Kloepper et al. 1981). It has been shown that Pa can be picked up from a potato stem that is expressing blackleg symptoms by water aerosols. This has major implications in the spread of Pa from a diseased plant to a healthy plant and could be a potential initial source of the bacteria in potato crops that are planted with seed that is free from Pa bacteria (Graham and Harrison 1975). This is especially true for areas that depend heavily on overhead irrigation, such as the San Luis Valley.

Hail events can also effect a potato crop and can potentially produce aerosols generated by the rain that accompanies the hail as the potato stems are damaged. Bacteria that are picked up by the aerosols can then be transported to adjacent plants (up to four meters or more), which are more likely to be damaged by the hail (Graham and Harrison 1975). The bacteria are much more likely to cause blackleg in a plant if the plant is freshly damaged. Damage in potato plants can also be a concern if potato cull piles, which can harbor bacteria carrying insects, are in close proximity to the plants.

In a study conducted by Kloepper et al (1981), adult fruit flies were shown to transmit blackleg causing bacteria to injured potato plants. The insects picked up the bacteria in a cull pile which was located within 200 meters of the injured plant. The authors also determined that transmission occurred more readily during the warmer parts of the day than in the cooler parts of the day. Potato cull piles, which can harbor pathogenic bacteria and are attractants for bacteria harboring insects, can serve as reservoirs of inoculum which can result in the introduction and spread of the bacteria. Growers are constantly reminded to remove these cull piles from potato fields, in order to reduce blackleg and tuber soft rot levels. Both aerosols from irrigation or rainfall and insects carrying the bacteria from cull piles can serve as sources of Pa inoculum and may provide early generation seed potatoes, which are free from the bacteria when planted, with this inoculum in the field.

Nutrient Imbalances:

Imbalances in nutrient levels in a potato plant can also provide conditions favorable for blackleg and tuber soft rot development (Kumar et al. 1991; Bain et al.1996; Czajkowski et al. 2011). Kumar et al. determined that as the amount of nitrogen applied in season increased, the percentage of rotting tubers in storage also increased. They concluded that the increase of nitrogen also led to a decrease in phenolics in the tuber, thereby reducing the tubers' resistance to rotting (Kumar et al. 1991).

In a study conducted by Bain et al., the addition of gypsum ($CaSO_4$) was shown to decrease the levels of blackleg and tuber soft rot in a field environment. Seed tubers in this study were inoculated with *E. carotovora* subsp. *atroseptica* (Pa) and the soil was amended with gypsum at different rates prior to planting. Calcium levels in the stems and tubers were elevated in the field plots where gypsum amendments were applied. Calcium can improve the structure and stability of plant cell walls which provide an improved resistance to pathogens that depend on the macerating of plant tissue, like Pa. The authors attributed the reduction in blackleg and tuber soft rot to an increase of Calcium levels in the potato plant and daughter tubers, rather than to a change in pH resulting from the addition of CaSO4 (Czajkowski et al. 2011).

Nitrogen and calcium are two plant nutrients that have been found to have an effect on blackleg and tuber soft rot incidence. The proper management of these can improve the potato plants resistance to diseases such as blackleg and tuber soft rot. Nutrition management needs to be part of an overall IPM plan for this disease complex (Czajkowski et al. 2011).

Management of Blackleg and Tuber Soft Rot:

Introduction:

The management of this disease complex tends to be fairly limited, with only a few relatively effective management strategies available to the grower. Due to the current lack of resistant potato cultivars and the impracticality of using chemotherapy and thermotherapy, the use of healthy seed is the most practical way in which to manage this disease (Perombelon 2000). Management of these pathogens and disease development primarily consist of avoidance strategies. Strategies used to minimize disease development include: starting with seed that originates from disease-free, tissue culture sources, planting well suberized seed pieces, minimizing the potential of wounding at harvest, maintaining conditions for good wound healing and good ventilation during storage, using well water rather than surface water to irrigate the crop, and field rotation with a non-host crop (McCarterzorner et al. 1984; Stevenson 2001; Czajkowski et al. 2011).

Another potential management strategy includes the use of potato cultivars that are resistant to disease development. Potato breeders have developed cultivars that exhibit partial resistance, but no cultivar has yet been developed that is fully resistant to blackleg and tuber soft rot. However, some research has been conducted in which wild *Solanum* species have been discovered that exhibit disease resistance. The potential exists for a resistant cultivar to be developed by breeding a commercial potato cultivar with a wild *Solanum* sp., but due to the amount of time required to develop a new potato (it takes

>10 years to develop a new potato cultivar using traditional breeding methods), this solution may be many years away (Czajkowski et al. 2011).

There has also been some success using different chemical treatments to reduce levels of blackleg and tuber soft rot. This has included the application of antibiotics (streptomycin, kasugamycin, etc.) to seed prior to planting or the application of products to the field soil or seed piece in order to make the soil environment unfavorable (altering pH, etc.) to the pathogenic bacteria. Immersion of seed in citric, acetic, ascorbic, formaldehyde and malonic acids have been found to reduce the amount of rotting caused by Pcc. Also, the application of organic and inorganic salts has been shown to inhibit the growth of Pcc and Pa. However, many of these treatments have had only limited success at managing this pathogen (Czajkowski et al. 2011).

One of the primary issues with using chemicals to manage pathogenic bacteria is the placement of chemicals close enough to the bacteria in order to affect them. In the case of bacteria that cause blackleg and tuber soft rot, the bacteria tend to be located in well protected regions, such as the tuber's lenticels, suberized wounds, or even the vascular system of the potato plant. Alternative approaches to using chemical agents or the development of resistant cultivars for the management of this disease may be the use of biological control agents (Czajkowski et al. 2011). Management strategies such as injury reduction of the crop, chemical control agents, biological control agents, crop rotation and cover crops, use of certified seed, as well as some novel management strategies will be discussed in more detail.

Lab Testing for Pa, Pcc, and Di:

There are several means by which to identify the bacterial pathogens that cause blackleg and tuber soft rot (Pa, Pcc, and Di). Visual field and tuber evaluations are typically the initial methods used in the identification of these soft rot bacteria. Visual identification of disease symptoms is a good starting point, however once symptoms are observed it is often too late to salvage the crop. Methods need to be used to identify potential bacterial levels prior to symptom expression in order to protect the potato producer from high risk seed lots (Fraaije et al. 1997).

In order for Pa to cause blackleg in a potato crop, the number of detected Pa cfu's (colony forming units) needs to be $10^2 – 10^4$ per tuber in storage (Aleck and Harrison 1978; Perombelon 2000). The bacteria tend to be located in well protected regions, such as the tuber's lenticels (Czajkowski et al. 2011). When determining the presence of these bacteria on a potato tuber, testing the lenticels and stem end of the tuber is a good strategy (R.D. Davidson, personal communication).

Several lab techniques have been developed to determine approximate bacterial levels from a potato tuber. The use of dilution plating using Crystal Violet Pectate (CVP) media (Cupples and Kelman, 1973) has been used to determine approximate numbers of cfu's per tuber. CVP is a selective media that will develop pits when pectolytic bacteria (e.g. Pa, Pcc, and Di) grow and multiply. These bacteria feed on the pectin in the media, thereby causing pits. The use of CVP can be time consuming and microorganisms other than Pa, Pcc, and Di can also cause pits to develop, if these microbes are pectolytic in nature. So, incubating the plates anaerobically is used to help reduce these other bacteria. Serological techniques, such as immunofluorescence cell/colony staining and ELISA (enzyme-linked immunosorbent assay) can be used as well. With these methods, an enrichment step is required in order to increase the numbers of bacteria present for detection. A problem with these techniques is that there can be false positive and negative reactions due to cross reactions with other saprophytes (Fraaije et al. 1997; Perombelon 2000).

Another technique, which is much more specific, is use of a PCR (Polymerase Chain Reaction) test. This method amplifies a specific segment of DNA from the organism in question using molecular primers. The target section of DNA is specific only to the organism or organisms being tested for. So only a specific section or region of DNA is targeted and amplified from a specific genus or species (Fraaije et al. 1997; Perombelon 2000).

Over the last two decades, there have been several different molecular primer pairs developed for the detection of Pa, Pcc, and Di (Deboer and Ward 1995; Toth et al.1999; Degefu et al. 2009). Using a PCR test is much more specific than using selective media or serological tests, but it is also more costly. The cost of conducting routine PCR tests is continually decreasing, but serological tests or using selective media are still much cheaper, especially when testing at a commercial level (Fraaije et al. 1997; Perombelon 2000).

The use of molecular techniques can determine whether or not the pathogen in question is present, however these techniques cannot determine the viability of the bacteria. Just because the DNA of a particular pathogen is present does not mean it can cause disease. In order to determine the relative level of blackleg and tuber soft rot causing bacteria in any given potato crop, each of these methods (dilution plating on selective media, serological, and molecular/PCR testing) need to be used. The level at which each technique is used is dependent upon the required accuracy and the estimated cost to the grower/researcher.

Injury Reduction (Cut Seed vs. Single Drop Seed, Handling, etc.):

According to Perombelon, tuber contamination by this group of pathogenic bacteria is unavoidable (Perombelon 2000). This, however, does not mean that symptom expression is inevitable. The bacteria may be present on the tuber, but the disease may not develop if conditions are not favorable for disease development. One way a potato farmer can avoid disease expression is to limit the amount of tuber injury (Czajkowski et al. 2011).

In a potato storage, the contents of a rotten tuber can potentially spread to non-symptomatic tubers. Every time potatoes are moved, (e.g., sorting operations, moving of seed for sale, cutting of seed, etc.) the potential is there for spreading the bacteria from a rotten tuber. If the rotten tuber is caused by and contains a pectolytic bacteria, such as Pa or Di, the bacteria can potentially be spread to the healthy tubers in the seed lot. In this case, the bacteria are not spread directly through wounding, but through the movement of the rotten tubers coming in contact with healthy tubers. One way to avoid this potential spread of bacteria is to use single drop seed (this is seed that is of the appropriate size and doesn't need to be cut to be planted) (Czajkowski et al. 2013).

Chemical Control Agents:

One way to minimize the potential spread of the bacteria from symptomatic tubers to healthy tubers is through the use of chemicals and disinfectants. There are a wide range of different compounds that have been evaluated for use as an effective disinfectant. There are limitations for many of the chemical compounds evaluated which include implications for human health, environmental considerations, plant phytotoxicity, and effectiveness as a disinfectant. For example, sodium hypochlorite (aka bleach) has been shown to be effective as a disinfectant seed treatment for managing *D. solani*, however this treatment can lead to issues with tuber phytotoxicity (Czajkowski et al. 2013).

Antibiotics have been shown to be an effective means by which to manage the spread and development of blackleg and tuber soft rot. Treating potato seed pieces with streptomycin, kasugamycin or virginiamycin have been shown to reduce blackleg incidence and tuber decay in storage (Czajkowski et al. 2011). Several antibiotics were evaluated for the suppression of tuber soft rot and found to be effective at reducing the disease symptoms (Wyatt and Lund 1981). Unfortunately, the use of many of these antibiotics are not generally allowed as a seed treatment because of the risk of resistance development in livestock and humans (Czajkowski et al. 2011).

Some compounds, such as benzoic acid (trademarked MennoClean), have been shown to be an effective disinfectant against *D. solani* in vitro and in greenhouse evaluations (Czajkowski et al. 2013). It has also been shown that certain organic and inorganic salts such as aluminum chloride, sodium metabisulfite, aluminum lactate and sodium benzoate have preventative and curative properties for managing soft rot caused by Pa and Pcc (Mills et al. 2006; Yaganza et al. 2014).

Unfortunately, using these compounds for managing pectolytic bacteria levels on and in a given seed piece is somewhat difficult under real world situations. As mentioned earlier, on a seed piece these bacteria tend to inhabit places that are well protected. This makes it much more difficult for the applied compound to come in direct contact with the bacteria. There are potentially alternative ways of applying the disinfectant compounds to the seed piece which may be more effective at coming in contact with the bacteria (e.g. aerosols, application of disinfectants after harvest making sure the tubers were properly dried before going into storage, drying of the tubers after harvest, etc.). However, these practices need more field testing before recommendations on their use can be made (Czajkowski et al. 2011).

Biological Control Agents:

Reasons for Use of Biological Controls:

Biological control is the use of beneficial organisms that employ strategies such as competition with the pathogen (i.e. nutrients, filling a particular niche, etc.), triggering of the plant's defense mechanisms, production of antibiotics or toxins, or improvement of a predator's environment, etc. There are a wide range of ways in which a particular biocontrol agent can disrupt the viability of a particular pathogen. In the case of Pcc and Pa, several potential biocontrol agents have been evaluated in numerous studies (Gross 1988; Cronin et al. 1997; Kastelein et al. 1999; Cladera-Olivera et al. 2006; Czajkowski et al. 2011). Some specific biocontrol agents that will be discussed here are *Bacillus spp.*, *Pseudomonas spp.*, lactic acid bacteria, and the use of bacteriophages as potential management options

Bacillus spp.*:*

A gram positive bacteria, *Bacillus subtilis* BS 107, has been shown to have broad antibiotic activity towards different plant pathogenic fungi and bacteria. A laboratory study conducted in 1998 indicated that this strain of *B. subtilis* had activity against Pcc and Pa. It was evaluated using laboratory plating techniques and cut potato tissue infected with Pcc and Pa. *B. subtilis* was found to produce an antibiotic which reduced Pcc and Pa growth in this study (Sharga and Lyon 1998).

Cladera-Olivera et al. (2006) conducted a study which evaluated the effectiveness of bacteriocin-like substances (BLS) on the management of Pcc. Bacteriocins are antimicrobial peptides that have a bactericidal mode of action. For this study, *Bacillus licheniformis* P40 cells (which produce BLS) were used. This bacteria had been previously isolated from the teleost fish (*Leporinus* sp.), which lives in the Brazilian Amazon basin. The *Bacillus licheniformis* P40 was cultured and BLS was extracted from the cultured bacteria. The BLS was then evaluated for effectiveness against Pcc in inoculated tubers. Results indicated that the diameter of lesions on tubers caused by Pcc were reduced by 30% to 75% (Cladera-Olivera et al. 2006).

Reiter et al. found that *Bacillus megaterium* and *B. macroides* are endophytic in certain potato cultivars, and when infected with Pa, these *Bacillus* sp. were shown to have a negative effect on Pa.

However, these bacteria are present in the infected tubers, but not at levels high enough to fully eliminate disease symptoms. The development of management strategies to increase the numbers of these beneficial endophytic bacteria is a promising avenue of future research in this area (Reiter et al. 2002).

Pseudomonas spp.:

Several species and strains of *Pseudomonas* bacteria have been shown to reduce levels of blackleg and tuber soft rot causing bacteria. Both fluorescent and non-fluorescent *Pseudomonas* have been evaluated. Most of the studies conducted in this area have been in-vitro (Cronin et al. 1997; Czajkowski et al. 2011; Gross 1988; Kastelein et al. 1999).

One study conducted by Kastelein et al. (1999) indicated that the application of several species of florescent pseudomonads have the potential to reduce tuber soft rot caused by Pa, when applied on freshly wounded tubers. A new strategy (green crop lifting) on killing the potato plant when tubers are ready to harvest was evaluated for possible timing of application of several biocontrol agents against Pa. During harvest, the potential for wounding of tubers can be high, which creates an entrance for Pa to infect the tuber. Green crop lifting, in which plants are killed, tubers are removed from the ground and placed on a new soil bed and are then covered by new soil, presents a good strategy for applying a biocontrol agent. It was discovered that when several different species/strains of *Pseudomonas (P. fluorescens & P. putida)* were applied to freshly wounded tubers, the levels of tuber soft rot were reduced by 60% to 85% in storage, depending on the species/strain or mix of strains applied.

In another study, different strains of *Pseudomonas fluorescens*F113 were evaluated for activity against Pa. *P. fluorescens* F113 produces 2,4-diacetylphloroglucinol (DAPG), which is an antibiotic and has been shown to inhibit the growth of Pa. The strains F113Rif and F113G22 were found to have the most activity against Pa on several different media types and in tuber pieces in the soil (Cronin et al. 1997). Gross (1988) evaluated two strains of *P. fluorescens* (W4F68 & W4F393) and one strain of *P. putida* (W4P63) which had been reported as having activity against Pcc in the rhizosphere. The results from this study showed that no antagonistic effects were present between these strains of *Pseudomonas* toward Pcc. It was also learned that two applications (initial concentration was 10^8-10^9cfu/ml - one application as an initial seed treatment and a second as a slurry application approximately eight weeks after planting) of pseudomonads maintained high levels of *Pseudomonas* spp. in the rhizosphere as opposed to a single application (10^2-10^3cfu/gram of root for single application vs. 10^3-10^4cfu/gram of root by end of season). The inactivity of these antagonistic bacteria was possibly due to multiple species of pectolytic bacteria being present in the rhizosphere, thereby reducing the effectiveness of the *Pseudomonas* sp. due to lower numbers of beneficial bacteria compared with the pectolytic bacteria.

An increase in *Pseudomonas* spp. population numbers in the rhizosphere may be a worthwhile strategy for reducing blackleg and tuber soft rot occurrence. However, a limited number of successful field trials evaluating *Pseudomonas* spp. with a potato crop have reduced disease levels. By increasing the survivability of *Pseudomonas* spp. in the soil, the levels of antagonistic antibiotics to Pcc and Pa would be higher. Also, having *Pseudomonas* spp. colonize the particular niches where *Pectobacterium* spp. would typically colonize would greatly increase the benefit of adding this biocontrol to a potato crop. More field trials need to be conducted, possibly with different application methods or field management strategy combinations, in order to maximize the survivability of *Pseudomonas* spp. and decrease Pcc and Pa populations.

Lactic Acid Bacteria:

Another type of bacteria that has shown antagonism toward Pcc are lactic acid bacteria. These are commonly found on fresh produce and in milk products. These bacteria have many different modes of action, including the production of hydrogen peroxide, organic acids and siderophores. They have the ability to inhibit more than one phytopathogen, such as *Botrytis cinerea*, and have a wide range of growth temperatures (8 – 45^{o}C). Some species that have shown antagonistic properties include *Lactobacillus plantarum*, *L. acidophilus*, *L. buchneri*, *Leuconostoc* spp. and *Weissel lacibaria* (Czajkowski et al. 2011).

In another study, four different strains of lactic acid bacteria were shown to be effective against Pcc in vitro. Scatter plots were used to indicate the level of antagonism in the different bacterial strains. Five rankings were assigned to the bacterial strains that were evaluated based on level of antagonism to Pcc: non-significant, low, medium, moderate, and high inhibition. Strains FF441 and MC6 expressed high levels of inhibition, strain PM141 was moderate, and PM249 was medium. Each of the four strains produced hydrogen peroxide. Little work has been done which looks at the effect of lactic acid bacteria when applied to field situations. Also the appropriate timing and formulations of a possible application to a field crop has not yet been determined (Trias et al. 2009). However, the use of lactic acid bacteria as a biocontrol agent for the management of black leg and tuber soft rot has promise.

Crop Rotations and Cover Crops:

It has been known for many years that the use of rotational crops can result in increased crop health and reduced disease levels (Howard 1996; Peters et al. 2003; Larkin and Honeycutt 2006). Rotating crops can maintain a good soil structure and can increase soil organic matter, which reduces soil erosion. Continuous cropping with a susceptible host can increase levels of plant pathogens in the soil which can reduce crop yield and quality (Peters et al. 2003). Crop rotations can reduce soil borne pathogens in three primary ways: disrupting the host-pathogen cycle, altering soil characteristics and soil microbial communities, and directly inhibiting pathogens through the crops production of toxins or by increasing the numbers of antagonistic microorganisms. A two year rotation has a better effect of reducing disease levels than no rotation. In addition, a three year rotation tends to reduce disease better than a two year rotation. The crop planted just prior tends to have the most influence on the following crop, however previous crops also have an impact on disease levels, but to a lesser degree (Larkin et al. 2006). The most important impact a crop has on the soil is the effect on the microbial communities. However, little is known about the specifics of the interactions of a given crop on the microbes present in the soil. Soils planted to a crop tend to have an increased amount of microbial biomass as compared with fallow soil (Larkin and Honeycutt 2006).

Larkin et al. (2007) evaluated the use of three year crop rotations with potato as the third crop in the rotation. These were compared with a continuous potato cropping system. Every rotation evaluated resulted in higher soil microbial populations and an increased microbial community diversity. When canola, barley or sweet corn were planted in the year prior to potato, a decrease in the amount of black scurf and stem canker caused by *Rhizoctonia solani*, a fungal pathogen of potato, was observed when compared with a continuous potato cropping system. However, when soybean or clover preceded potato in the rotation, less disease suppression was observed. The inclusion of barley or canola in the rotation also resulted in an increase in overall bacterial biomass in the soil (Larkin and Honeycutt 2006). Another study found that different crop rotations including barley/timothy grass & timothy grass, barley/under seeded with rye, or mustard & rapeseed/sudan grass & rye decreased the amount of disease caused by *R. solani* and *Streptomyces scabies* (a bacterial pathogen that causes common scab). In this same study, applications of compost to the soil also increased the levels of common scab when compared with the crop rotations and the standard potato rotation (Larkin et al. 2007).

Another study evaluated the use of a three year crop rotation with different crop species and tillage practices, conventional vs minimum tillage. The combination of using a three year rotation and minimum tillage reduced dry rot (*Fusarium spp*.), silver scurf (*Helminthosporium solani*) and black scurf (*R. solani*) in potatoes. Soil from the three year rotations were inoculated with *P. erythroseptica* (the causal agent of pink rot disease) and planted to potatoes in a greenhouse. Pink rot levels were reduced when compared with soil from two year rotations. It was concluded that soil agroecosystems can be managed through different rotation and tillage practices to improve the levels of disease suppression (Peters et al. 2003).

In addition to the suppression of disease causing microbes, different cropping plants can also produce chemicals with allelopathic properties which can be released into the rhizosphere. Depending on the environmental conditions, allelochemicals may be released into the soil at high enough quantities to suppress weed seedling growth (Masiunas et al. 1995; Urbano et al. 2006; Walsh et al. 2014). Crop species such as rice, mustards, sorghum, winter rye, sugar beets, lupins, maize, oats, peas, barley, cucumber, pepper, hairy vetch, and sunflower have been shown to have allelopathic properties (Urbano et al. 2006).

The use of a cover crop has also been shown to be beneficial by controlling weeds. Cover crops typically leave plant residue on the soil surface that the following crop is planted into. Cover crops can limit weed growth through the production of this residue, which provides a physical barrier to the weeds and also through allelopathy (Masiunas et al. 1995; Bezuidenhout et al. 2012). Using rye as a cover crop has been shown to reduce weed populations in a field planted to tomato transplants (Masiunas et al. 1995). Stooling rye and annual ryegrass have also been found to suppress yellow nutsedge (*Cyprus esculentus*) in Maize (Bezuidenhout et al. 2012).

Not all crop rotations or cover crops, however, are beneficial to crop growth. Rotations including buckwheat planted in the year preceding potatoes has been shown to increase the levels of black scurf on the tubers (Peters et al. 2003). The growth in a crop of maize was suppressed following a cover crop of rye (Bezuidenhout et al. 2012). Plants in the family Brassicacea have been shown to produce glucosinolate compounds. This group of compounds can go through enzymatic degradation and produce isothyocyanates and nitriles, which are allelopathic. These compounds can reduce weed seed germination, but can also limit the seed germination of flax and radish (Walsh et al. 2014).

Members of this family are also incapable of forming functioning mycorrhizae because of the production of isothyocyanates, which are antifungal. Mycorrhizal fungi form symbiotic relationships with the roots they colonize. These fungi can take up phosphate from the soil and can transfer much of the phosphate to the roots, which can be used by the plant. If these fungi are absent, the plant cannot benefit from this symbiotic relationship. When maize is followed by *Brassica napus* L. (canola), mycorrhizal colonization of roots is reduced when compared with soy, which reduces yields (Koide and Peoples 2012).

Although there has been a wide range of research evaluating the use of different crop rotations and cover crops and their effect on weeds and plant pathogens, there has been little research evaluating pathogenic pectolytic bacteria in commercial potato production through the use of these practices. Since the use of different species in a crop rotation have been shown to reduce potato diseases such as black scurf, silver scurf, dry rot and pink rot, the potential is there to reduce the incidence of blackleg and tuber soft rot as well. Since the bacteria that cause blackleg and tuber soft rot (Pa, Pcc, & Di) are primarily seed borne, the effects of different rotational crops on the soil could have a major impact on the soil surrounding the potato seed piece, which could have an impact on the seed borne bacteria coming in contact with the soil. So, it would make sense that the use of different crop rotations or cover crops could have an impact on the severity of blackleg and tuber soft rot in a potato crop.

Certified Seed:

The use of certified seed potatoes is considered the foundation to any potato disease management program. Potatoes are typically vegetatively propagated, that is, not derived from true seed. While true seed is used in many countries for production, seed tubers are most often used as the planting material (Davidson 2014). There are several viral, fungal and bacterial diseases that are transmitted in potatoes, but are not transmitted in true seed. The use of vegetatively propagated seed can result in additional problems that other seed produced crops do not have. Vegetatively propagated potatoes can acquire various diseases that can be passed on to subsequent potato crops (Rowe 1993).

Prior to the 20th century, it was observed that potato plants would become less productive or would "run out" of productiveness after being propagated for several years in the field. It was also observed that potatoes produced from true seed would retain their productiveness, however the daughter tubers would not be true to type. By the year 1900, Dutch and German agriculturalists found that certain plants had symptoms (such as leaf curling, crinkling, blotching, etc.) that could be transferred to asymptomatic plants. These symptoms were also associated with lower yields and when plants with these symptoms were removed, the overall health of the crop was improved. This "run out" disease was primarily the result viruses infecting the potatoes, mainly Potato Virus Y (PVY) (Davidson 2014). Scientists from the United States traveled to Europe and saw that the tubers from diseased plants could carry these diseases. These scientists helped the U.S. government set up the National Plant Quarantine Act of 1912 which limited the introduction of new potato diseases into the United States. *Synchytrium endobioticum* (a fungus causing Potato wart) and *Spongospora subterranea* (a protozoan causing powdery scab) were the first two quarantined organisms for potatoes coming into the United States. This led the way for the creation of potato seed certification agencies across the United States and Canada. By

1922, seed certification agencies were in 22 different states across the U.S. Currently, there are approximately 270,000 acres of certified seed potatoes grown throughout the United States and Canada (Rowe 1993).

Not all potatoes grown in North America qualify as certified seed. Potato farmers who choose to raise certified seed have to follow certain guidelines that are enforced by the certified seed agency in their state or province. Each states' certification agency has its own set of guidelines and requirements the seed grower must meet in order to qualify the potato crop as certified. These guidelines can vary among the different states, with certain guidelines that are common among all the states. During each growing season, two or three field inspections are performed by the certification agency to ensure that a seed lot meets the requirements to be classified as certified seed. Field inspectors look for any disease issues, varietal purity, and at the overall health of a given potato lot. Multiple inspections are conducted since diseases can express symptoms at different times during the growing season depending on the potato cultivar being grown and local environmental factors. A winter grow-out is also required by many certification agencies to identify possible disease spread between the last summer field inspection and harvest. Some of the disease issues evaluated by the field inspectors include several potato viruses (including potato virus Y and potato leaf roll virus), bacterial pathogens (including bacterial ring rot and blackleg) and fungal pathogens (including late blight caused by *Phytophthora infestans*). Seed is typically certified through a combination of visual inspections, serological and molecular lab testing depending on the potato cultivar and disease being evaluated (Rowe 1993; Czajkowski et al. 2011; Davidson 2014).

The foundation of most certified seed programs is the use of tissue culture as a disease free starting material. Tissue culture plantlets are tested for a myriad of disease causing pathogens (bacterial, fungal, and viral) affecting potato. Tissue culture plantlets are grown in media and once they reach the proper maturity, are typically transferred and planted in a greenhouse or protected environment. Once the crop is ready for harvest, daughter tubers are collected from the mature tissue culture plantlets and are planted as a potato crop with minimal disease issues for the first field year. The utilization of tissue culture is necessary to start a potato crop at a zero disease level (Davidson 2014)

Certified seed requirements vary between different regions (states, countries, etc.) throughout the world. This is due to different disease pressures as well as political regulations present in various parts of the world. However, all potato inspection services worldwide require the use of disease-free tissue culture plantlets or true seed in the initiation of potato production as well as a limitation on the number of field generations allowed after the initial propagation of the plants. The number of field generations can vary from region to region, with typically only six to eight field generations allowed (Czajkowski et al. 2011).

The management of blackleg in potato production systems has depended on the use of certified seed potatoes during the last half century. The use of certified seed has been instrumental in the limitation of blackleg disease levels in potato production areas throughout the world. However, there are still outbreaks of blackleg and latent infections that are typically not detected with the use of standard certification practices. The use of certified seed is a critical component to managing the spread of black leg and tuber soft rot, but the use of this seed may not completely eliminate the occurrence of this disease from a potato producing operation (Czajkowski et al. 2011).

New Tools for Managing Blackleg and Tuber Soft Rot:

In addition to the use of the aforementioned strategies for managing blackleg and tuber soft rot in a potato crop (e.g. injury reduction, different crop rotations, chemical treatments, biological control agents, certified seed potatoes, etc.), there are other management strategies that are being looked into as control agents for Pcc and Pa as well.

In a study conducted by Mills et al. (2006), different salt compounds were evaluated for their effectiveness against Pcc and Pa. Copper sulfate pentahydrate applied to the seed showed the greatest level of disease control throughout the study, while several other salt compounds also resulted in significant inhibition of Pcc and Pa. More testing in the use of salts as a control strategy needs to be conducted. Application timing, dosages, proper delivery method, and possible mixtures of beneficial organisms need to be tested and evaluated under field conditions before recommendations using these compounds can be made.

A new area of research in the management of Pcc is the use of bacteriophages. Bacteriophages have been discovered that are specific to Pcc. The infection of bacterial cells by bacteriophages results in the lysis of those cells. A study evaluating bacteriophages was conducted in which a fertilizer solution containing bacteriophages specific to Pcc was used on calla lilies. Calla tuber rot is a disease of calla lilies that is caused by the infection of the calla tuber by Pcc. The application of bacteriophages reduced calla tuber rot symptoms by as much as 50%. However, this was shown to be a partial control of calla tuber rot since several bacterial cells escaped being infected by the bacteriophages (Ravensdale et al. 2007). This work was not evaluated with a potato host in mind, but the potential for applying this to a potato situation is high. This avenue of research could have a significant impact in the management of potato tuber rot and should be looked into.

Another potential management strategy for the eradication of *Pectobacterium* spp. is the use of Gliding Arc Discharge technology. A glide arc reactor was developed which uses a nonthermal quenched plasma system with a four-electrode plasma generator. *Pectobacterium* spp. cells that had been grown on a petri dish were killed using this apparatus (Moreau et al. 2007). The application of a modified glide arc reactor in an agricultural setting was not discussed in this paper, but a variation could potentially be used in sanitation of surfaces that have the possibility of being infected by soft rot causing bacteria (i.e. seed cutting surfaces, harvesting equipment, etc.).

These different control strategies have the potential to manage this disease complex. The current research evaluating these have laid a good foundation for blackleg and tuber soft rot causing bacteria management under lab and greenhouse conditions. The future in this area of research is to conduct trials in field situations because the groundwork has been established.

Overview of Project:

Blackleg and tuber soft rot, caused by the bacteria *Pectobacterium carotovorum* subsp. *carotovorum, P. atrosepticum*, and *Dickeya* spp., are diseases of major concern for potato growers throughout the world. This is a difficult disease to manage since pesticides have little effect in controlling the disease and no potato cultivars are fully resistant to it. Currently, the primary method used for management is in handling the potato seed and daughter tubers in a way that limits tuber injury and maintaining conditions in storage that are conducive to good wound healing and unfavorable to tuber soft rot development. This sounds simple enough, but with the large volume of potatoes that a typical potato farmer produces in a given year, this task becomes much more difficult. The areas of management that will be focused on in this project include the preparation of seed for planting, field generation and potato cultivar used, and the rotational crops planted in the years prior to planting potatoes.

Preparing potato seed for planting is a major undertaking which includes the timing of temperature adjustment in storage in relation to the cutting and planting of seed. Potato seed pieces need to have broken dormancy and begin sprouting prior to planting in order to have good emergence and ultimately a good crop. This can be greatly affected through the management of temperature in storage as well as when the seed is cut, if cutting is required. Waiting too long before breaking dormancy will result in poor crop stands and seed pieces rotting in the soil (Rowe 1993; Davidson 2014). Breaking dormancy too early can result in sprouts that are too long that can affect accuracy of planting and can result in the spread of bacterial or viral pathogens, if the sprouts need to be removed (Rowe 1993; Fageria et al. 2015). Cutting of the seed tubers needs to happen in such a way where the freshly cut surface can properly heal prior to planting and also in a way that reduces the spread of bacteria (Rowe 1993; Davidson 2014). The first chapter of this project will evaluate the seed cutting operations of two different certified seed operations, focusing on blackleg and the potential for pectolytic bacteria spread in each operation.

The second chapter will cover the effect field generation and cultivar has on pectolytic bacteria numbers in the seed. Field generation and potato cultivar used can have a major impact on blackleg and tuber soft rot levels in field grown potatoes. The higher the field generation, the more blackleg tends to be present in the seed lot (Rowe 1993). This project will evaluate different seed operations, four field generations, and several different potato cultivars for pectolytic bacteria numbers in the seed. Each seed operation evaluated will have either low or high overall blackleg levels based on seed certification field inspection results.

The third chapter will cover the use of different plant species used in rotation with potato as a means to manage the levels of blackleg and the populations of pectolytic bacteria present on the seed produced. The use of different rotational crops can impact pathogenic bacteria levels in a potato production systems (Deboer 1989). Different monocultures and polyculture species mixes will be evaluated against a standard potato barley rotation. This portion of the project is part of an EPA specialty crops grant.

The final chapter of this project will summarize the findings of this research and other work that pertains to the practical management of blackleg and tuber soft rot. This will be presented in a format that will be digestible by potato producers. This management guideline should aide local potato producers and the potato industry in the management of blackleg and tuber soft rot.

LITERATURE CITED

ADHB-Potato Council, 2007. *Dickeya* spp (*Erwinia chrysanthemi*): What is it…and what can you do? https://potatoes.ahdb.org.uk/publications/dickeya-what-it-and-what-can-you-do? Accessed 15 February 2018.

Aleck, J.R. and M.D. Harrison. 1978. Influence of inoculum density and environment on development of potato. American Potato Journal 55(9): 479-494.

Ali, H.F., M. Ahmad, et al. 2012. Inoculum sources, disease incidence and severity of bacterial blackleg and soft rot of potato. Pakistan Journal of Botany 44(2): 825-830.

Bain, R.A., P. Millard, et al. 1996. The resistance of potato plants to *Erwinia carotovora* subsp *atroseptica* in relation to their calcium and magnesium content. Potato Research 39(2): 185-193.

Bezuidenhout, S.R., C.F. Reinhardt, et al. 2012. Cover crops of oats, stooling rye and three annual ryegrass cultivars influence maize and *Cyperus esculentus* growth. Weed Research 52(2): 153-160.

Cappaert, M.R., M.L. Powelson, et al. 1988. Irrigation water as a source of inoculum of soft rot *Erwinias* for Aerial Stem Rot of Potatoes. Phytopathology 78(12): 1668-1672.

Charkowski, A.O. 2006. Plant-Associated Bacteria: The Soft Rot *Erwinia*. Dordrecht, Netherlands: Springer.

Charkowski, A.O. 2015. Biology and control of *Pectobacterium* in potato. American Journal of Potato Research 92(2): 223-229.

Cladera-Olivera, F., G.R. Caron, et al. 2006. Bacteriocin-like substance inhibits potato soft rot caused by *Erwinia carotovora*. Canadian Journal of Microbiology 52(6): 533-539.

Cronin, D., T. MoenneLoccoz, et al. 1997. Ecological interaction of a biocontrol *Pseudomonas fluorescens* strain producing 2,4-diacetylphloroglucinol with the soft rot potato pathogen *Erwinia carotovora* subsp. *atroseptica*. Fems Microbiology Ecology 23(2): 95-106.

Czajkowski, R., W.J. de Boer, et al. 2013. Chemical disinfectants can reduce potato blackleg caused by '*Dickeya solani*'. European Journal of Plant Pathology 136(2): 419-432.

Czajkowski, R., M.C.M. Perombelon, et al. 2011. Control of blackleg and tuber soft rot of potato caused by *Pectobacterium* and *Dickeya* species: a review. Plant Pathology 60(6): 999-1013.

Davidson, R.D. and K. Xie, 2014. Seed potato production. p. 115-132. In: R. Navarre and M.J. Pavek (eds.). The Potato: Botany, production and uses. CABI, Boston, MA.

Davidsson, P.R., T. Kariola, O. Niemi, and E.T. Palva. 2013. Pathogenicity of and plant immunity to soft rot *Pectobacteria*. Frontiers in Plant Science 4:1-13.

Deboer, S.H. 1989. Bacteria and Potatoes in Crop Rotation Systems. p. 131-152. In: J. Vos, C.D. Vanloon, G.J. Bollen (eds.). Effects of crop rotation on potato production in the temperate zones. Kluwer Academic Publishers, Wageningen, Netherlands.

DeBoer, S.H., I. Rubio. 2004. Blackleg of potato. http://apsnet.org/edcenter/intropp/lessons/prokaryotes/Pages/Blacklegpotato.aspx. Accessed 10 January 2018.

Deboer, S.H., E. Allan, et al. 1979. Survival of *Erwinia carotovora* in Wisconsin soils. American Potato Journal 56(5): 243-252.

Deboer, S.H. and L.J. Ward. 1995. PCR detection of *Erwinia carotovora* subsp *atroseptica* associated with potato tissue. Phytopathology 85(8): 854-858.

Degefu, Y., E. Virtanen, et al. 2009. Pre-PCR processes in the molecular detection of blackleg and soft rot *Erwinia* in seed potatoes. Journal of Phytopathology 157(6): 370-378.

Fageria, M., X.Z. Nie, et al. 2015. Mechanical transmission of potato virus Y (PVY) through seed cutting and plant wounding. American Journal of Potato Research 92(1): 143-147.

Fraaije, B.A., M. Appels, et al. 1997. Detection of soft rot *Erwinia* spp. on seed potatoes: Conductimetry in comparison with dilution plating, PCR and serological assays. European Journal of Plant Pathology 103(2): 183-193.

Franc, G.D. and M.D. Harrison. 1987. The role of contaminated irrigation water in the recontamination of *Erwinia*-free seed potatoes. American Potato Journal 64(8): 438-438.

Gardan, L., C. Gouy, et al. 2003. Elevation of three subspecies of *Pectobacterium carotovorum* to species level: *Pectobacterium atrosepticum* sp nov., *Pectobacterium betavasculorum* sp nov. and *Pectobacterium wasabiae* sp nov. International Journal of Systematic and Evolutionary Microbiology 53: 381-391.

Graham, D.C. and W.J. Dowson, 1960. The coliform bacteria associated with potato blackleg and other soft rots I: Their pathogenicity in relation to temperature. Annals of Applied Biology 48(1): 51-57.

Graham, D.C. and M.D. Harrison. 1975. Potential spread of *Erwinia* spp in aerosols. Phytopathology 65(6): 739-741.

Gross, D.C. 1988. Maximizing rhizosphere populations of fluorescent *Pseudomonads* on potatoes and their effects on *Erwinia carotovora*. American Potato Journal 65(12): 697-710.

Harrison, M.D., G.D. Franc, et al. 1987. Presence of *Erwinia-carotovora* in surface-water in North-America. Journal of Applied Bacteriology 62(6): 565-570.

Howard, R.J. 1996. Cultural control of plant diseases: A historical perspective. Canadian Journal of Plant Pathology-Revue Canadienne De Phytopathologie 18(2): 145-150.

Kastelein, P., E.G. Schepel, et al. 1999. Preliminary selection of antagonists of *Erwinia carotovora* subsp *atroseptica* (Van Hall) dye for application during green crop lifting of seed potato tubers. Potato Research 42(2): 161-171.

Kloepper, J.W., J.W. Brewer, et al. 1981. Insect transmission of *Erwinia-carotovora* var *carotovora* and *Erwinia-carotovora* var *atroseptica* to potato plants in the field. American Potato Journal 58(4): 165-175.

Koide, R.T. and M.S. Peoples. 2012. On the nature of temporary yield loss in maize following canola. Plant and Soil 360(1-2): 259-269.

Kumar, A., V.S. Pundhir, et al. 1991. The Role of Phenols in Potato-Tuber Resistance Against Soft Rot by *Erwinia-carotovora* SSP-*carotovora*. Potato Research 34(1): 9-16.

Lapwood, D. H. and R. I. Harris. 1980. The spread of *Erwinia carotovora* var *atroseptica* (blackleg) and var *carotovora* (tuber soft rot) from degenerating seed to progeny tubers in soil. Potato Research 23(4): 385-393.

Larkin, R.P., T.S. Griffin, et al. 2007. Effects of different soil and crop management strategies on soil microbial communities and soilborne diseases of potato. Phytopathology 97(7): S61-S62.

Larkin, R.P. and C.W. Honeycutt. 2006. Effects of different 3-year cropping systems on soil microbial communities and rhizoctonia diseases of potato. Phytopathology 96(1): 68-79.

Maddox, D.A. and M.D. Harrison. 1988. Presence and population-dynamics of *Erwinia-carotovora* in irrigation water in south central Colorado. Journal of Applied Bacteriology 64(2): 169-182.

Marquez-Villavicencio, M.D., R.L. Groves, et al. 2011. Soft rot disease severity is affected by potato physiology and *Pectobacterium* taxa. Plant Disease 95(3): 232-241.

Masiunas, J.B., L.A. Weston, et al. 1995. The impact of rye cover crops on weed populations in a tomato cropping system. Weed Science 43(2): 318-323.

McCarterzorner, N.J., G.D. Franc, et al. 1984. Soft rot *Erwinia* bacteria in surface and underground waters in southern Scotland and in Colorado, United-States. Journal of Applied Bacteriology 57(1): 95-105.

McCarterzorner, N.J., M.D. Harrison, et al. 1985. Soft rot *Erwinia* bacteria in the rhizoshpere of weeds and crop plants in Colorado, United-States and Scotland. Journal of Applied Bacteriology 59(4): 357-368.

Meneley, J.C. and M.E. Stanghellini. 1976. Isolation of soft-rot *Erwinia* spp from agricultural soils using an enrichment technique. Phytopathology 66(3): 367-370.

Mills, A.A.S., H.W. Platt, et al. 2006. Sensitivity of *Erwinia* spp. to salt compounds in vitro and their effect on the development of soft rot in potato tubers in storage. Postharvest Biology and Technology 41(2): 208-214.

Moreau, M., M.G.J. Feuilloley, et al. 2007. Gliding arc discharge in the potato pathogen *Erwinia carotovora* subsp *atroseptica*: Mechanism of lethal action and effect on membrane-associated molecules. Applied and Environmental Microbiology 73(18): 5904-5910.

Nielsen, L.W. 1978. *Erwinia* species in the lenticels of certified seed potatoes. American Potato Journal 55(12): 671-676.

Perombelon, M.C.M. 1992. Potato blackleg: Epidemiology, host pathogen interaction and control. Netherlands Journal of Plant Pathology 98: 135-146.

Perombelon, M.C.M. 2000. Blackleg risk potential of seed potatoes determined by quantification of tuber contamination by the causal agent and Erwinia carotovora subsp. atroseptica: a critical review. EPPO Bulletin 30(3/4): 413.

Perombelon, M.C.M. 2002. Potato diseases caused by soft rot *Erwinias*: an overview of pathogenesis. Plant Pathology 51(1): 1-12.

Perombelon, M.C.M. and A. Kelman. 1980. Ecology of the soft rot *Erwinias*. Annual Review of Phytopathology 18: 361-387.

Peters, R.D., A.V. Sturz, et al. 2003. Developing disease-suppressive soils through crop rotation and tillage management practices. Soil and Tillage Research 72(2): 181-192.

Ravensdale, M., T.J. Blom, et al. 2007. Bacteriophages and the control of *Erwinia carotovora* subsp *carotovora*. Canadian Journal of Plant Pathology-Revue Canadienne De Phytopathologie 29(2): 121-130.

Reiter, B., U. Pfeifer, et al. 2002. Response of endophytic bacterial communities in potato plants to infection with *Erwinia carotovora* subsp *atroseptica*. Applied and Environmental Microbiology 68(5): 2261-2268.

Rowe, R.C. 1993. Potato health management. APS Press, St Paul, Minn.

Sharga, B.M. and G.D. Lyon. 1998. *Bacillus subtilis* BS 107 as an antagonist of potato blackleg and soft rot bacteria. Canadian Journal of Microbiology 44(8): 777-783.

Stevenson, W.R., 2001. Compendium of Potato Diseases, 2nd edition. The American Phytopathological Society, St. Paul, Minn.

Toth, I.K., L.J. Hyman, et al. 1999. A one step PCR-based method for the detection of economically important soft rot *Erwinia* species on micropropagated potato plants. Journal of Applied Microbiology 87(1): 158-166.

Toth, I.K., L. Sullivan, et al. 2003. Relationship between potato seed tuber contamination by *Erwinia carotovora* ssp *atroseptica*, blackleg disease development and progeny tuber contamination. Plant Pathology 52(2): 119-126.

Toth, I.K., J.M. van der Wolf, et al. 2011. *Dickeya* species: an emerging problem for potato production in Europe. Plant Pathology 60(3): 385-399.

Trias, R., L. Baneras, et al. 2009. Lactic acid bacteria from fresh fruit and vegetables as biocontrol agents of phytopathogenic bacteria and fungi. International Microbiology 11(4): 231-236.

Urbano, B., F. Gonzalez-Andres, et al. 2006. Allelopathic potential of cover crops to control weeds in barley. Allelopathy Journal 17(1): 53-64.

von Bodman, S.B., W.D. Bauer, et al. 2003. Quorum sensing in plant-pathogenic bacteria. Annual Review of Phytopathology 41: 455-482.

Walsh, K.D., D. Sanderson, et al. 2014. Allelopathic effects of camelina (*Camelina sativa*) and canola (*Brassica napus*) on wild oat, flax and radish. Allelopathy Journal 33(1): 83-95.

Wyatt, G.M. and B.M. Lund. 1981. The effect of antibacterial products on bacterial soft rot of potatoes. Potato Research 24(3): 315-329.

Yaganza, E.S., R.J. Tweddell, et al. 2014. Postharvest application of organic and inorganic salts to control potato (*Solanum tuberosum* L.) storage soft rot: Plant tissue-salt physicochemical interactions. Journal of Agricultural and Food Chemistry 62(38): 9223-9231.

CHAPTER 1: EVALUATION OF TWO POTATO SEED CUTTING SYSTEMS FOR PREVENTING THE SPREAD OF PECTOLYTIC BACTERIA.

Summary:

Pathogenic bacteria in the genera *Pectobacterium* and, more recently, *Dickeya* are of major concern in many potato growing regions around the world. These bacteria are pectolytic and often spread through the seed cutting process. Cutter surfaces exposed to pectolytic bacteria include cutting knives, belts, rollers, etc. This study evaluated two seed cutting operations for their potential to limit the spread of pectolytic bacteria. Grower 1 used a Milestone seed cutter while grower 2 used a hot cutter with heat sterilized blades. Results indicate that there was no bacterial spread either with the hot cutter or the Milestone cutter. There was a four-fold increase in pectolytic bacteria on the belts, rollers and knives of the Milestone cutter when compared with the hot cutter. Results from this study show that the use of a hot cutter reduces the spread of pectolytic bacteria better than a conventional cutting operation.

Introduction:

It has been estimated that there are anywhere from $50 to 100 million in losses to potato producers as a result of tuber soft rot and blackleg, caused by soft rotting bacteria in the genera *Pectobacterium* and *Dickeya* (Perombelon and Kelman 1980). The avenues through which soft rot bacteria are introduced into a potato farming operation are of major concern, and in many cases unknown. Several potential avenues for the introduction of these bacteria have been studied and have been determined in previous work (Ali et al. 2012; Deboer 1989; Lapwood and Harris 1980; Meneley and Stanghellini 1976). Most research agrees that the primary origin and spread of *Pectobacterium atrosepticum* and *P. carotovorum* within a seed crop is from seed tubers infected with the bacteria (Ali et al. 2012; Toth et al. 2003). Infected tubers can act as an inoculum source for the progeny tubers. These pathogenic bacteria can spread in the soil from the infected tubers to the progeny tubers as well (Lapwood and Harris 1980).

In potato farming operations in North America the cutting of seed is a common practice in preparation for planting a potato crop. Cutting the seed results in an increase in the number of seed pieces available to plant within very specific size ranges and eye numbers. Since potatoes are vegetatively propagated, a potato tuber has the ability to give rise to a potato plant when conditions are favorable for plant growth, so long as an eye is present and the tuber has broken dormancy (Johnson, 2008). Cutting seed potatoes is used in large part to manage the number of stems and, subsequently overall yield in a potato plant (Arsenault and Christie 2004, Allen 1979; Vokal et al. 1998, Kleinhenz and Bennett 1992; Nolte et al. 2003; Pavek 2014; Davidson 2014).

The cutting of seed typically involves the use of machinery that is specifically designed to cut seed potatoes. Much of the seed cutting in modern potato farming operations happens on a large scale and includes many opportunities for the seed pieces to come into contact with the various belts, rollers, knives and storage containers, and potentially pick up pectolytic bacteria if present on these parts (Rowe 1993). Although the ability to cut seed in potato production can result in cost savings and an increase in efficiency for the farmer, the cutting of seed also has the potential for increases in the spread of potato pathogens (fungi, bacteria, viruses, etc.) among the seed tubers and seed pieces. *Clavibacter michigenensis* subsp. *sepedonicus*, the causal agent of ring rot, and the soft rot causing bacteria (*Pectobacterium* spp. and *Dickeya spp.*) are the bacterial pests of the most concern which might be spread through seed cutting operations (Charkowski 2015; Rowe 1993).

The goal of this project was to identify areas in the seed cutting process that could serve as reservoirs of pectolytic bacteria, primarily from the *Pectobacterium* spp. The cutting practices of two seed potato grower operations were evaluated during the spring of 2014 and 2015. These two operations were chosen because of their differences in summer blackleg levels and the way in which each cuts seed potatoes. The initial load of pectolytic bacteria present in the seed lot was not critical in this study. What was important was whether or not cutting surfaces were acting as bacterial reservoirs and if there was an increase in bacterial levels occurring as the seed was moving through the seed cutter. If differences were not observed between the cutting surfaces, then it could be concluded that reservoirs of bacteria were not present and did not result in the spreading of bacteria as a result of the cutting process.

Materials and Methods:

Different locations on the seed cutter used by each of the two growers were tested to determine pectolytic bacterial levels during the spring cutting seasons of 2014 and 2015. Grower 1 had a history of blackleg (Table 1.1) in summer potato crops over the last seven years while grower 2 had a history of very little blackleg (Table 1.1). Two cultivars, Rio Grande Russet (RIO32-1) and Canela Russet (CAN31-1) were screened at operation #1 while two crops of Canela Russet (CAN31-2 and CAN21-2) were screened at operation #2 (Table 1.2). Each seed lot had potentially different levels of pectolytic bacteria from one another. The screenings were conducted in real time and during actual seed cutting of certified seed lots in order to get a real world pectolytic bacteria evaluation. During every cutter screening, a seed lot was in the process of being cut. At each location during the testing, the entire cutting operation was shut down for approximately 5 minutes prior to collecting samples, which provided an optimum time frame for detecting the presence of bacteria on the seed cutter. Surfaces were screened in this manner in order to decrease the amount of sampling variability that might have resulted if surfaces were screened at different timings relative to when the seed was cut.

Grower 1 used an automated Milestone Potato Seed Cutter (Model 84-D/72-D) and disinfected the circular cutting blades and rollers with Hyamine, a quaternary ammonia compound @ a 1% dilution, (a.i. N-Alkyl dimethyl benzyl ammonium chloride) with a 10 minute soak time between the cutting of each seed lot (Figure 1.1). The majority of seed cutting is automated with the Milestone cutter, except for a few large potatoes that pass through the cutter without being cut. These larger potatoes were cut by hand using stationary knives which were located on the seed cutter, just before the seed was treated with the fungicide Maxim. These stationary knives were not routinely sanitized.

Grower 2 used a Potato Seed Hot Cutter manufactured by Vemco Electrical Contractor Inc., located in the San Luis Valley, CO (Figure 1.2). This company custom builds these seed cutters (vemcoelec.com) based on the premise of the "Forney Hot Cutter" developed in the 1980's (Davidson, personal communication). The cutter uses electricity to continuously run a current through steel blades, raising the temperature of each blade to between 204 and 260^{O}C (400 - 500^{O}F). The actual cutting on this machine was done manually by farm labor physically cutting the potatoes on these blades. A conveyor belt carried the potatoes to the blades and away from the blades once the seed was cut. For both grower 1 and 2, the belts used to carry the seed were not routinely disinfected.

Specific seed cutter surfaces were evaluated for each grower operation as described in Figures 1.1 and 1.2. There were five seed cutter surfaces evaluated for grower 1 and four for grower 2. The surfaces chosen for evaluation differed between the two seed cutters primarily due to the seed cutting system and the seed cutting practices of each grower. The locations on the seed cutter that were screened for reservoirs of bacteria seemed like logical places along the seed cutter to evaluate. These locations were chosen because of the high probability of contact with the uncut and cut seed potatoes.

An autoclaved sterile Q-tip was used to swab one cm^2 area on each surface that was evaluated. Five one cm^2 sections/rep were swabbed at each location. An autoclaved piece of printer paper with a one cm^2 opening was placed on each section prior to swabbing. Only the one cm^2 opening, which allowed exposure to the cutter surface being screened, was swabbed. After swabbing, the Q-tip was immediately placed in an autoclaved test tube filled with one ml of sterile water and was sealed. The test tubes with Q-tips were then brought back to the lab and the water was plated, on fresh Nutrient Agar (NA) media. The plate was then swabbed with the Q-tip so that the water covered the entire surface of the NA plate. The NA plates were placed in an anaerobic chamber for 4 days. A BD GasPak™ EZ Gas Generating Container System was used to generate anaerobic conditions. The amount of time between the swabbing and the transfer of sample water to NA plates was approximately two to three hours. The Q-tip from each tube was used to spread the water over the entire NA plate prior to placing in an anaerobic chamber. Plating onto different media in combination with an anaerobic environment to determine the presence of pectolytic bacteria in seed tubers is a fairly reliable method for detection (Cuppels & Kelman 1974; Deboer and Kelman 1975; Delfosse et al. 1994; Haynes et al. 1997; Nielsen 1978). Several researchers have also used NA to grow bacteria, similar to what was done in this study prior to isolating colonies to a selective media (Ali et al. 2012; Harju and Kankila 1993; Nielsen 1978).

Ten colony forming units (cfu's) were randomly chosen from each NA plate after the 4 day incubation in the anaerobic chamber and were used to inoculate Crystal Violet Pectate (CVP) plates using autoclaved sterile toothpicks. A modified CVP medium was used, similar to that used by Perombelon and Burnett (1991). After inoculation of the CVP, the plates were allowed to incubate in an anaerobic chamber for 4 days prior to evaluation for the presence of pitting. Data were expressed as percentage of cfu’s that caused pitting on CVP.

Since each of the seed cutters and seed lots evaluated were actively being cut by the cooperating growers, tubers with known soft rot were not used as controls due to the possibility of infecting the grower’s seed with the bacteria. Since the majority of field grown asymptomatic potatoes contain some pectolytic bacteria on the tuber surface (Czajkowski et al. 2011), it was determined that adding additional bacteria as a control to the seed cutting operation was not necessary to meet the goals of this project. The goal of this project was to identify areas in the seed cutting process that could serve as reservoirs of pectolytic bacteria, primarily from the *Pectobacterium* spp. and whether or not there should be a concern by growers under a real-world scenarios. The cutter surface just prior to the cutting blades was considered the starting point for each of the grower’s seed cutting operations. If bacterial levels increased or were significantly higher after this point, it would indicate that bacteria was spreading via the cutting of seed.

However, positive and negative controls were used to validate the CVP methodology used to determine pectolytic activity in the lab. For the negative controls, a sterile Q-tip was placed in one ml of sterile water. For the positive controls, a sterile Q-tip was used to collect bacteria from a plate of cultured *Pectobacterium atrosepticum*, which was obtained from the Colorado Potato Certification Service Disease Testing Lab. The Q-tip for the positive control was then placed in one ml of sterile water. Both controls were allowed to incubate for 2-3 hours before plating on NA. The positive and negative controls were then treated in a similar manner to the samples collected from the seed cutters. In the spring 2014 evaluations, there were no positive or negative lab controls evaluated for grower 2's seed cutter screening. For grower 1, two replications of positive and negative controls were used to validate that the tests were working in the lab (positive control had a mean of 75.0% pitting and the negative control had a mean of 0.0% pitting). In the spring of 2015, five replications for the positive and negative controls in the lab were used for both growers (grower 1 had a mean positive control of 86.0% pitting and the negative control had 0.0% pitting; for grower 2 the positive control was 100.0 %, and the negative control was 0.0%).

A nested design was used for this study. Factors include grower/seed cutter (n = 2), cutting surfaces (n = 5 for grower 1 and n = 4 for grower 2), and years are the replications (n = 2). For grower 1, a total of 50 samples were collected: 2 years (the years served as the replicates) x 5 cutting surfaces (operation #1) x 5 analytical replicates per cutting surface. For grower 2, a total of 40 samples were collected: 2 replicates/years x 5 cutting surfaces x 5 analytical replicates per cutting surface. Each operation was analyzed independently of each other. Analysis of variance tests were conducted in SAS (version 9.4, Cary, NC).

A LSD mean separation was used to analyze the data within operations and within years. Means followed by the same letters were not significantly different when p values >=0.10. For analyzing data across years, paired t-tests were used and cutting surfaces were significant when p values <= 0.10. When comparing cutting surfaces between the two operations, paired t-tests were used to determine significance. Data was significant when the p value <= 0.05. SAS 9.4 (PROC GLM) was used to conduct each of the analyses.

Results and Discussion:

Grower 1 Results and Discussion:

For grower 1's seed cutter, there were no statistical differences between cutter surfaces during the cutting of the CAN31-1 and Rio32-1 seed crops in the spring of 2014 and 2015 (Table 1.3).

The level of pitting was not significantly higher in samples collected from the belt after the liquid seed treatment (surface 5) than the rollers, circular knives, stationary knives and the belt under the stationary knives (surfaces 1, 2, 3 and 4). The stationary knives as well as the belt following the treatment of cut seed had higher mean % pitting than the other surfaces. However, there were no statistical differences between any of the surfaces.

One major difference between the two years for grower 1's seed cutting operation was that in 2015 the belt carrying seed away from the liquid seed treater was located outside, which exposed the belt surface to sunlight, lower outside air humidity and air movement from wind. Each of these factors resulted in a drying off of the conveyor belts as well as the cut, treated seed. This created a less conducive environment for bacterial survival and propagation in 2015 as opposed to 2014 (Nolte et al, 1987; Perombelon & Kelman, 1980). Most likely this was the reason the percent of pitting on surface 5 was tended to be higher than surfaces 2, 3 and 4 in 2014, but not in 2015. The use of a liquid seed treater which is not known to inhibit bacterial growth, may have helped the inoculum survive in an enclosed environment which resulted in a level of pitting on surface 5 that was higher than surfaces 2, 3, and 4 in 2014, even though there were no statistical differences.

There were no statistical differences between the circular and stationary knives, however the circular knives had lower pectolytic bacteria numbers than the stationary knives (10.0% vs 27.5%, Table 1.3). The stationary knives are not routinely disinfected, whereas the circular knives are disinfected periodically. This may account for this apparent difference.

Grower 2 Results and Discussion:

When evaluating the seed cutter surfaces for grower 2, there were no significant differences for percent pitting between surfaces across the two years or within each year. This indicates that the cutting of seed did not spread pectolytic bacteria when using the seed cutter used by grower 2. Also, no potential infection reservoirs of pectolytic bacteria were identified at any of the seed cutter surfaces evaluated (Table 1.3).

The seed cutter used by grower 2 in the spring of 2014 and 2015 was located inside the storage facility and the seed treater was located outside. The seed treatment used was a dry formulation and the carrier was fir bark. The use of a dry seed treatment as opposed to a liquid seed treatment, may have had an impact on the amount of bacteria present on the cut seed. This was not directly observed as part of this project, but low blackleg readings during summer field inspections are an indication of this situation. A lower amount of free water coming into contact with the freshly cut or damaged seed is more conductive to suberization (Nolte et al. 1987) and less conducive to multiplication and bacterial growth in *Pectobacterium* spp. (Perombelon 2002). Since the liquid and dry formulations of seed treater do not contain bactericides, but instead contain fungicides, the efficacy of these seed treatments directly against bacteria is negligible. Indirectly, they may reduce sites for secondary infections of the bacteria such as those created by *Fusarium* or other fungal infections (Nolte et al. 2003). However, there were no fungal problems observed in any of the seed on either grower's farm in the years of this project. The presence of free water in the liquid seed treatment can potentially increase the amount of bacteria available for moving onto the seed by spreading any bacteria across the seed piece surface and additionally creating a low oxygen environment for slower suberization and more bacterial multiplication.

Grower 1 versus Grower 2 Results and Discussion:

When comparing the two seed cutting operations, there were four cutting surfaces that were compared against each other. Only four surface comparisons were made because only four comparisons were deemed valid. The surfaces that seemed appropriate for both cutters included the rollers and belts prior to the cutting of seed, two knife locations and the belts under the knives (Figures 1.1 & 1.2).

For comparison #1, there were no significant differences between the two operations and the surfaces the seed came into contact with just prior to being cut (rollers for grower 1 and the belt for grower 2). This indicates that the amount of bacteria present just before seed being cut was statistically equivalent for both operations (Figure 1.3).

When looking at comparison #2 and comparison #4 there were no significant differences (Figure 1.3). The mean percent pitting was lower for grower 2 in both comparisons. However, this was not significant. The relatively high CV values (Table 1.3) and the absence of significant differences between the two growers indicates that the level of variability was high for each of these data set comparisons.

For comparison #3, there were significant differences in the data (Figure 1.3). The percent pitting was significantly higher in the stationary knives from grower 1 than grower 2's hot cutter knives. This can be explained since the stationary knives from grower 1 were not routinely sanitized with a disinfectant, whereas the hot cutter knives from grower 2 are continually heat sterilized.

In addition to the cutting surface comparisons, the overall amount of pitting found over both years and all seed crops evaluated was higher for grower 1 than for grower 2. This is not surprising when comparing the number of symptomatic blackleg plants found on all seed lots during routine summer field inspections conducted by seed certification officials (Table 1.1).

Conclusions:

The goal of this study was to determine possible avenues where pectolytic bacteria might be spreading into potato seed during the seed cutting process and to assess whether or not an adequate level of sanitation was achieved. Two growers with different seed cutting practices and different relative blackleg disease levels in their respective seed operations were evaluated. Grower 1 had issues with blackleg on his potato operation for many years, whereas grower 2 had very little blackleg. Evaluating the different seed cutters used by these operations was helpful in understanding why there were differences in blackleg disease expression as seen in summer field inspections. It is understood that it is difficult to make a direct comparison between bacterial populations on equipment and blackleg in the field. However, by determining bacterial levels which can be spread onto the seed, it is a reasonable assumption that under the appropriate environmental conditions and normal production practices, an association can be made between these levels and blackleg. At least this information provides a good starting point in determining blackleg in the field. This was demonstrated when comparing the two grower operations.

Several significant observations were made as the evaluations took place. First, the use of a liquid seed treatment without proper drying after seed cutting and treatment increased bacterial levels on the conveyor belts after the cutting and treatment of the seed. When the seed and belts were allowed to dry the levels of bacteria did not increase. This is the trend that was observed, even though the data was not statistically significant. This has also been shown by work done by Bdliya et al.(2007). Although a direct comparison between using a wet vs. dry seed treatment was not made on grower 1's operation, a comparison was made between using a wet seed treater where the seed was not properly dried and using the wet seed treater where the seed was properly dried after treatment. When proper drying of the seed did not occur, the belt after the seed had been cut and treated had higher pectolytic bacterial levels than the belt just prior to treatment or either of the cutting blades. However, when the seed and belts were exposed to sunlight and an outdoor environment just after treatment, there were no differences between the belt post seed treatment and any of the cutting surfaces evaluated.

The cost of using a liquid seed treatment or a dry seed treatment is similar. Since grower1 has a history of high blackleg levels in summer field inspections (Table 1.1), a potential fix to his blackleg problem could be to either change the seed cutting practices by using a dry seed treatment or to set up fans, etc. to help dry the seed and the belts after the seed is cut and treated. Changing the location of the seed treater and belts to an outside location could also be used to solve the excess moisture problem. This could potentially expose the seed and the belts to solar radiation and lower relative humidity since the San Luis Valley is located in a desert with less than 10 inches of annual rainfall, mild breezes, and a typical relative humidity level in the 30% range (Colorado Agricultural Meteorological Network 2016). However, springtime in the San Luis Valley can be unpredictable with freezing temperatures and variable weather. This can often prevent the use of equipment and the cutting and treating of seed outside. In the case of unfavorable weather, the use of a dry seed treater instead of a liquid treater or the use of fans to help dry the freshly cut seed could potentially reduce the excess moisture found on the cutting surfaces. This would be a relatively inexpensive solution to the problem of having too much free moisture on the belts.

Another discovery was that by using the hot cutter, there was a reduction in pectolytic bacteria numbers found within the system when comparing the stationary blades of grower and the hot cutter knives of grower 1 (Figure 1.3). The killing of potential pathogens, such as bacteria, through the use of routinely sterilized blades (sterilized either through the use of heat or an effective disinfectant) was shown to be an effective method of sanitation in this study (Figure 1.3). Unlike the drying of cut seed, a change in the method a grower uses to cut seed could be fairly cost prohibitive. However, if the blackleg problem on any given operation was severe enough, the additional cost of a new seed cutter and additional labor might be appropriate.

The amount of pathogenic bacteria present in and on seed tends to be higher in later generation seed stocks. This is one of the reasons for a limited generation seed potato production system (Rowe, 1993). One way to potentially manage the spread of bacterial pathogens, such as pectolytic bacteria like *Pectobacterium* spp., is to always cut the early generation seed first and to limit the number of higher generation seed lots that pass through the seed cutter.

The generation of seed can play a role in the pathogen levels in a seed lot, however, the susceptibility or resistance of a cultivar to a given pathogen can play a much larger role in the presence of a pathogen and its disease severity on the crop. In this study, no differences were observed between the seed crops of the two different cultivars evaluated; Rio Grande Russet and Canela Russet.

Unfortunately, there was not a direct comparison made evaluating the pectolytic bacteria levels in these two cultivars when evaluating the seed cutters since this was outside the scope of this project. While seed piece surfaces were not screened for bacteria, actual inspection readings were taken in the field (Tables 1.1 & 1.2) to evaluate levels of blackleg. This was handled in this manner since pectolytic bacterial levels on the seed piece do not always translate into disease in the field depending on wound healing, environmental conditions, temperature, etc. (Perombelon 2002; Toth et al. 2003).

This study demonstrated that when using a more large scale Milestone cutter as in grower 1's operation, overall pectolytic bacterial numbers were higher when compared with the cutting operation utilized by grower 2. Based on summer field inspections conducted by the Colorado Potato Certification Service, the historic levels of blackleg on grower 1's operation have been much higher than that of grower 2 (Table 1.1). The management of the pectolytic bacteria in grower 1's operation was not adequate to prevent blackleg outbreaks. Since high levels of pectolytic bacteria were not observed for grower 2, we can conclude that the bacterial management practices of grower #2 were adequate to keep bacteria from spreading through the cutting process. From this project is has been determined that the cutting of seed is a potential source of bacterial inoculum that could be contributing to blackleg outbreaks at grower 1's operation. It is recommended that grower 1 employ some of the strategies employed by grower 2 to manage bacterial spread in their seed cutting operation (e.g. using a hot cutter and using a dry seed treater, sanitizing all cutting blades, etc.). Other potential management strategies might also include the use of fans to help dry the seed treated with a liquid seed treater, routine sanitation of the conveyor belts and stationary knives, and the cutting of seed in an outdoor environment.

Tables:

Table 1.1. Total Number of Potato Plants and Seed Lots Expressing Blackleg Symptoms in Summer Field Seasons for Grower 1 & 2, based on Colorado Seed Certification Records (2012 - 2015).							
Grower	Year	# of seed lots evaluated	# plants visually evaluated in summer field inspections	# plants with blackleg	# lots with blackleg	% plants with blackleg	% lots with blackleg
#1	2012	31	69,300	1487	10	2.15	32.26
	2013	26	47,800	49	6	0.10	23.08
	2014	24	45,200	513	11	1.13	45.83
	2015	24	53,800	500	15	0.93	62.50
	Average:	26.3	54,025	637.3	10.5	1.08	40.92
#2	2012	184	110,300	1	1	0.0009	0.54
	2013	170	85,900	6	6	0.0070	3.53
	2014	170	70,700	0	0	0.0000	0.00
	2015	124	110,100	3	3	0.0027	2.42
	Average:	162.0	94,250	2.5	2.5	0.0027	1.62

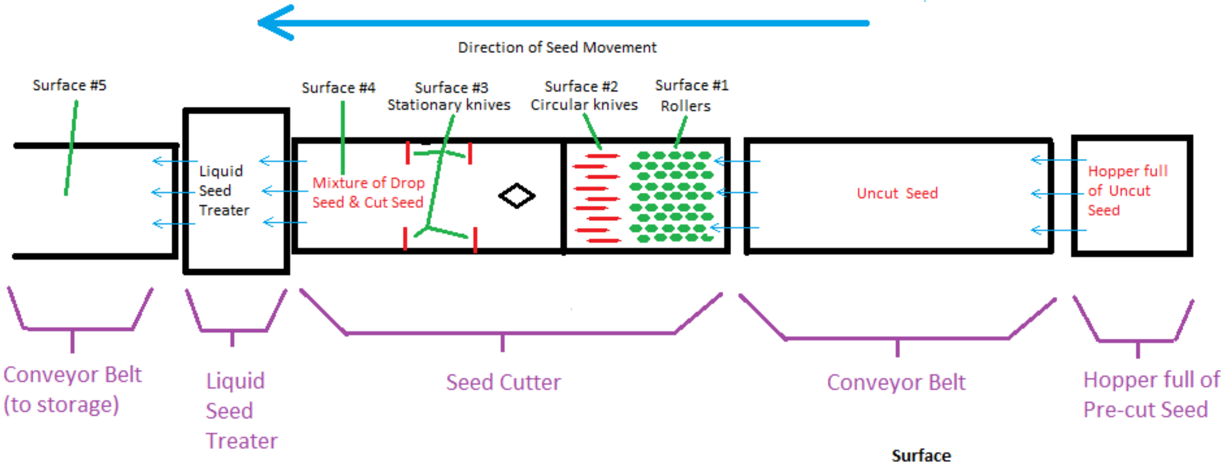
Table 1.2. List of Seed Potato Lots Evaluated in the Spring of 2014 and 2015.					
Date Evaluated	Grower	Variety	Generation	% Blackleg the Previous Summer	% Blackleg the Following Summer
April 11, 2014	1	Canela Russet	3	0.00	0.10
April 6, 2015	1	Rio Grande Russet	3	0.00	0.32
April 22, 2014	2	Canela Russet	3	0.00	*
April 1, 2015	2	Canela Russet	2	0.00	*
* These seed lots were not inspected because they were rejected for mosaic at the winter growout.					

Table 1.3. Evaluation of samples collected from different surfaces on a Milestone Potato Seed Cutter (Grower 1) and Hot Potato Seed Cutter (Grower 2). Percent pits visually observed on CVP media, spring 2014 and spring 2015.

	% pits observed on CVP					% pits observed on CVP		
Cutter Surface (Grower 1)	2014		2015	Mean	Cutter Surface (Grower 2)	2014	2015	Mean
1. Rollers prior to cutters	20.0	ab	14.0	17.0	1. Conveyer Belt (before tubers were cut)	6.0	6.0	6.0
2. Circular knives	10.0	b	10.0	10.0	2. Hot cutter knives (1-2 sec.)	2.0	0.0	1.0
3. Stationary knives	16.7	b	34.0	27.5	3. Belt directly under the hot cutter knives	10.0	2.0	6.0
4. Belt under stationary knives	7.5	b	8.0	7.8	4. Belt (after hot knives, before duster)	10.0	8.0	9.0
5. Belt after the liquid seed treatment	32.5	a	16.0	23.3	5. Hot cutter knives (5 sec)	-	0.0	-
					6. Hot cutter knives (20 sec)	-	0.0	-
LSD (P=0.10)	14.55		NS	NS		NS	NS	NS
CV	67.82		164.63	145.38		150.40	273.00	167.48
P-value	0.0723		0.3840	0.3874		0.5913	0.2067	0.2910

Means followed by the same letter are not significant at P value = 0.05 (n=5).

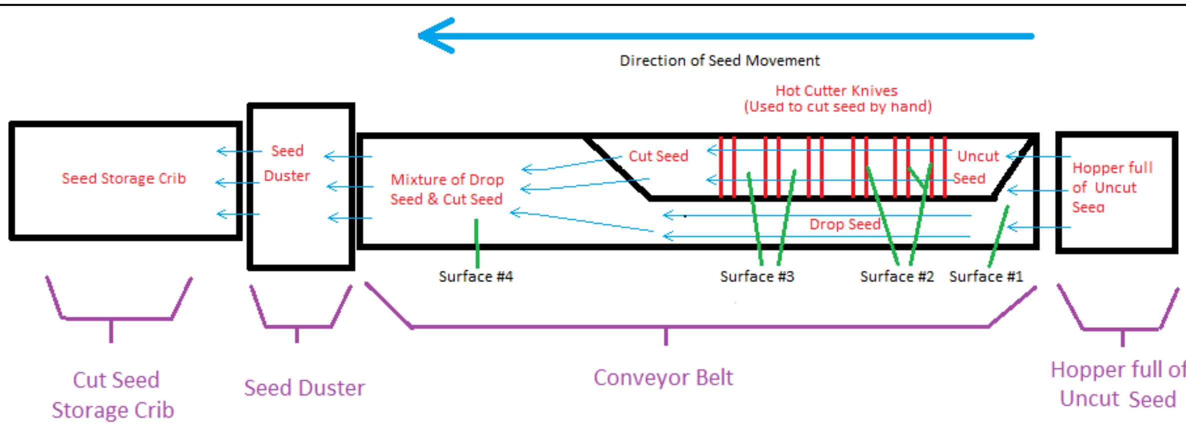
Figures:



Surface

1. Rollers prior to cutters
2. Circular knives
3. Stationary knives
4. Belt under stationary knives
5. Belt after the liquid seed treatment

Figure 1.1. Diagram of the Potato Seed Cutter for Grower 1.



Surface

1. Conveyer Belt before tubers are cut
2. Hot cutter knives
3. Belt directly under the hot cutter knives
4. Belt after the knives, but before the seed duster

Figure 1.2. Diagram of Hot Seed Cutter for Grower 2.

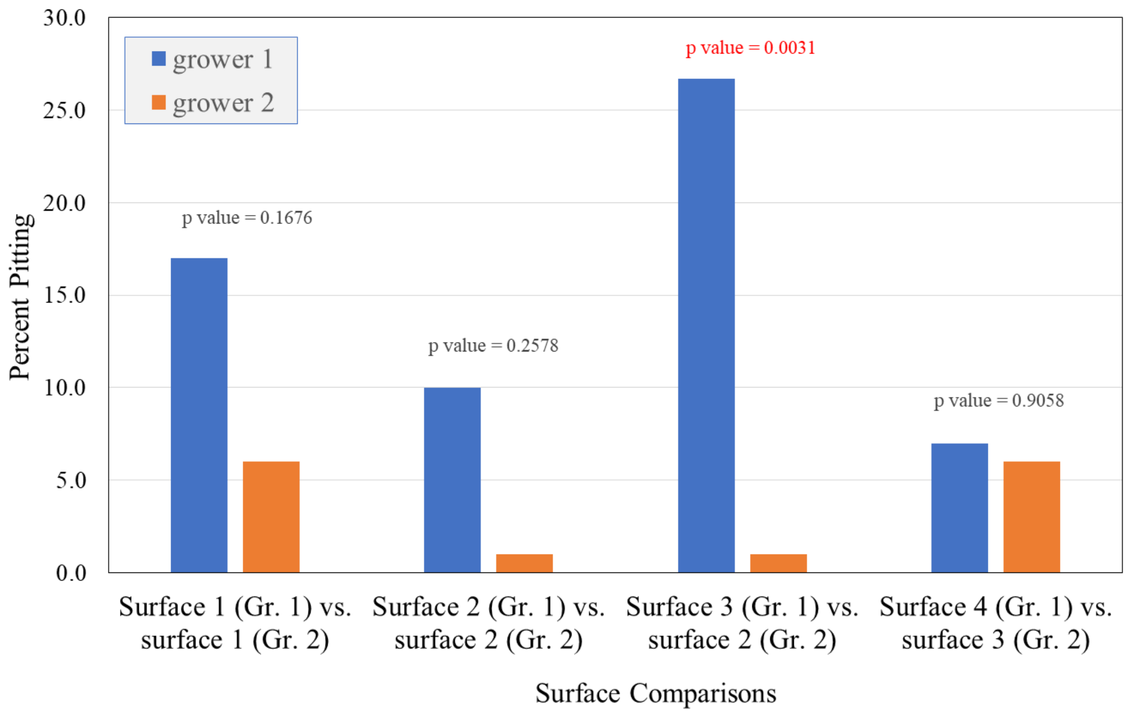


Figure 1.3. Evaluation of samples collected from surfaces on a Milestone Potato Seed Cutter (Grower 1) and Hot Potato Seed Cutter (Grower 2). Percent pits visually observed on CVP media. Mean of 2014 and 2015 screening data for each grower. Seed cutter surfaces significantly different at P=0.05.

LITERATURE CITED

Ali, H.F., M. Ahmad, et al. 2012. Inoculum sources, disease incidence and severity of bacterial blackleg and soft rot of potato. Pakistan Journal of Botany, 44(2), 825-830.

Allen, E.J. 1979. Effects of cutting seed tubers on number of stems and tubers and tuber yields of several potato varieties. Journal of Agricultural Science, 93(AUG), 121-128.

Arsenault, W.J., and B.R. Christie. 2004. Effect of whole seed tuber size and pre-plant storage conditions on yield and tuber size distribution of Russet Burbank. American Journal of Potato Research, 81(6), 371-376.

Bdliya, B.S. and H.U. Haruna. 2007. Efficacy of solar heat in the control of bacterial soft rot of potato tubers caused by *Erwinia carotovora* ssp. *carotovora*. Journal of Plant Protection Research, 47(1), 11-18.

Charkowski, A.O. 2015. Biology and control of *Pectobacterium* in potato. American Journal of Potato Research, 92(2), 223-229.

Colorado Agricultural Meteorological Network. 2016. http://ccc.atmos.colostate.edu/cgi-bin/stationsum form.pl. Accessed on 30 October 2016.

Cuppels, D., & A. Kelman. 1974. Evaluation of selective media for isolation of soft rot bacteria from soil and plant tissue. Phytopathology, 64(4), 468-475.

Czajkowski, R., Perombelon, M.C.M., van Veen, J.A., and van der Wolf, J.M. 2011. Control of blackleg and tuber soft rot of potato caused by *Pectobacterium* and *Dickeya* species: a review. Plant Pathology 60, 999-1013.

Davidson, R.D. and K. Xie, 2014. Seed potato production. p. 115-132. In: R. Navarre and M.J. Pavek (eds.). The Potato: Botany, production and uses. CABI, Boston, MA.

Deboer, S.H. 1989. Bacteria and Potatoes in Crop Rotation Systems. p. 131-152. In: J. Vos, C.D. Vanloon, G.J. Bollen (eds.). Effects of crop rotation on potato production in the temperate zones. Kluwer Academic Publishers, Wageningen, Netherlands.

Deboer, S.H. and A. Kelman. 1975. Evaluation of procedures for detection of pectolytic *Erwinia* spp on potato tubers. American Potato Journal, 52(4), 117-123.

Delfosse, P., F. Aerts, P. Blanquet, and H. Maraite. 1994. Latent infections of pectolytic *Erwinia* spp on seed potato tubers. p. 785-790. In: M. Lemattre, S. Freigoun, K. Rudolph and J.G. Swings (eds.). Plant Pathogenic Bacteria. INRA, Paris, France.

Harju, P., & J. Kankila. 1993. *Erwinia carotovora* contamination of Finnish seed potatoes and the prevalence of bacterial subspecies and serogroups. Agricultural Science in Finland, 2(4), 345-352.

Haynes, K.G., M.J.E. Potts, and R.W. Goth. 1997. Evaluation of the reliability of determining soft rot resistance in potatoes by the tuber slice method. American Potato Journal, 74(4), 265-275.

Kleinhenz, M.D., & M.A. Bennett. 1992. Growth and yield of potato (*Solanum tuberosum* L) cultivars Atlantic and Monona as influenced by seed type and size. American Potato Journal, 69(2), 117-130.

Lapwood, D.H., & R.I. Harris. 1980. The spread of *Erwinia carotovora* var *atroseptica* (blackleg) and var *carotovora* (tuber soft rot) from degenerating seed to progeny tubers in soil. Potato Research, 23(4), 385-393.

Meneley, J.C., & M.E. Stanghellini. 1976. Isolation of soft-rot *Erwinia* spp from agricultural soils using an enrichment technique. Phytopathology, 66(3), 367-370.

Pavek, M. 2014. Seed potato production. p. 115-132. In: R. Navarre and M.J. Pavek (eds.). The potato: Botany, production and uses. CABI, Boston, MA.

Nielsen, L.W. 1978. *Erwinia* species in the lenticels of certified seed potatoes. American Potato Journal, 55(12), 671-676.

Nolte, P., M. Bertram, M. Bateman, and C.S. McIntosh. 2003. Comparative effects of cut and treated seed tubers vs untreated whole seed tubers on seed decay, *Rhizoctonia* stem canker, growth, and yield of Russet Burbank potatoes. American Journal of Potato Research, 80(1), 1-8.

Nolte, P., G.A. Secor, and N.C. Gudmestad. 1987. Wound-healing, decay and chemical treatment of cut potato-tuber tissue. American Potato Journal, 64(1), 1-9.

Perombelon, M.C.M. 2002. Potato diseases caused by soft rot *Erwinias*: an overview of pathogenesis. Plant Pathology, 51(1), 1-12.

Perombelon, M.C.M. and E.M. Burnett. 1991. Two modified crystal violet pectate (CVP) media for the detection, isolation and enumeration of soft rot *Erwinias*. Potato Research, 34(1), 79-85.

Perombelon, M.C.M., & A. Kelman. 1980. Ecology of the soft rot *Erwinias*. Annual Review of Phytopathology, 18, 361-387.

Rowe, R.C. 1993. Potato Health Management. APS Press, St Paul, Minn.

Toth, I.K., L. Sullivan, et al. 2003. Relationship between potato seed tuber contamination by *Erwinia carotovora* ssp *atroseptica*, blackleg disease development and progeny tuber contamination. Plant Pathology, 52(2), 119-126.

Vokal, B., J. Cepl, J. Brecka, and I. Honzik. 1998. Reaction of selected potato varieties to cut planting material. Rostlinna Vyroba, 44(3), 141-147.

CHAPTER 2: THE EFFECT OF FIELD GENERATION, FARMING OPERATION, AND POTATO CULTIVAR ON TUBER LEVELS OF *Pectobacterium* SPP. AND BLACKLEG IN CERTIFIED SEED POTATOES

Summary:

Bacteria that cause potato blackleg (*Pectobacterium* spp. and *Dickeya* spp.) can often be detected in field-grown, early generation certified seed potato lots, even though the origin seed stocks were tested as free of the bacteria. Because of this, growers have a need for a predictive tool to assist in making good, appropriate decisions about any given seed lot. This project used a crystal violet pectate (CVP) - tuber poke method to screen early generation potato seed to predict possible outbreaks of blackleg. Potato samples from several certified seed operations were tested using this method and compared with summer inspection blackleg readings. Results showed operations that differed in the number of blackleg outbreaks also differed in % pitting when a sample from the same seed crop was tested. At early generations, an increase in % pitting on CVP corresponded to an increase in-field blackleg levels. However, in operations with little blackleg present, less pitting was found. The outcome of this project provides potato growers with a potential tool to help predict potential blackleg outbreaks in later generations used for seed production.

Introduction:

Potato blackleg and tuber soft rot, caused by a group of Enterobacteria in the genera *Pectobacterium* and *Dickeya*, is a disease complex that can cause great losses and is the most serious problem in fresh potato shipments (Nolte 2003). Many factors can contribute to the development of blackleg in the foliage of a potato plant. Most research agrees that the primary origin and spread of this bacteria within a potato crop is from seed potatoes infected with the bacteria (Ali et al. 2012; Czajkowski et al. 2011; Toth et al. 2003). However, understanding the spread of these bacterial pathogens and the resulting blackleg development in a potato crop is somewhat complicated and in many cases is unknown

(Skelsey et al. 2016). Blackleg is typically the result of several factors which can include inoculum level in the seed, field generation, cultivar susceptibility, environmental factors, and farming practices. Often, one or more of these factors must be present for blackleg to develop in a potato plant (Charkowski 2015; Perombelon 1992; Perombelon and Kelman 1980).

The use of certified seed potatoes is considered the primary foundation to any potato blackleg management program. While true seed is used in many countries for potato production, vegetatively propagated seed potatoes are most often used as the planting material in the United States (Davidson and Xie 2014). There are several viral, fungal and bacterial diseases, including *Pectobacterium* spp. and *Dickeya* spp., that are transmitted in seed potatoes used for the next crop, but are not transmitted in the first year when true seed is utilized (Rowe 1993).

Research suggests that the longer a seed crop is grown in the field, the greater the potential for increasing disease levels in that crop; thus, the amount of pathogenic bacteria and viruses present in and on the seed piece could be higher in later generation seed. Later generation seed has been planted and replanted for multiple year in field soils (4-5 years), whereas early generation seed had only been planted in the field 0 – 3 years (Rowe 1993). Several potential avenues for the introduction of these bacteria into the seed crop have been studied in previous work (Ali et al. 2012; Deboer 1989; Lapwood and Harris 1980; Meneley and Stanghellini 1976). Of the potential avenues identified, infected potatoes appear to be the primary inoculum source for the progeny tubers. Pathogenic bacteria can either spread directly from the mother seed piece to the progeny potatoes via stolons or from the infected mother seed through the soil to the progeny tubers (Lapwood and Harris, 1980).

Although the plants of some potato cultivars are resistant to foliar expression of *Pectobacterium* spp., there has not been a commercially produced potato cultivar that is fully resistant to tuber soft rot. However, potato cultivars express different levels of susceptibility to blackleg caused by *Pectobacterium* spp.(Charkowski 2015; Chung et al. 2013; Wright et al. 1991). Tubers with high levels of calcium and a high dry matter content have been found to be more resistant to soft rot development than potatoes with lower levels of these factors (Lyon 1989; Tzeng et al. 1990). There are also wild Solanum species with soft rot resistance that exist and breeders are currently looking at these wild types to develop soft rot resistant potato cultivars (Charkowski 2015; Chung et al. 2011).

This project had three main objectives. The first objective was to determine whether pectolytic bacterial numbers found in or on earlier generation seed could be used to determine future blackleg outbreaks. This information, the screening of early generation material, could potentially be used to predict blackleg outbreaks in later generations in a potato crop. The second objective was to evaluate farming operations in their ability to control buildup of pectolytic bacteria and field blackleg and determine which production factors might be critical in controlling the pathogen and disease. The final objective was to determine whether a potato cultivar's susceptibility to blackleg could be predictive of how the cultivar might fare in the field given farming operational differences. The overall purpose of each of these objectives was to develop a screening system to give potato growers a usable tool which would allow prediction of potential blackleg outbreaks in their seed production systems.

Materials and Methods:

Screening Tubers for the Presence of Pectolytic Bacteria:

There are multiple methods to determine the presence and relative levels of pectolytic bacteria in seed potatoes; some are more accurate than others at detecting the presence of this group of bacteria (Koppel 1993). Also, several genera and species of bacteria are pectolytic and can cause tuber soft rot (Lyon 1989). In order to detect the genus *Pectobacterium*, a screening methodology must be fairly specific to these bacteria. Also, several thousand seed potatoes were screened over the course of this project, so the screening method utilized had to produce results rapidly and inexpensively. Because of this, the use of a selective media, Crystal Violet Pectate (CVP), originally developed by Cupples and Kelman, 1974, and later modified by Perombelen and Burnett, 1991, was utilized. A tuber poke technique developed for use in screening nuclear or greenhouse material for seed certification in Colorado was the preferred mechanical technique used to screen the sampled tubers (see addendum).

Crystal Violet Pectate (CVP) Methodology:

A sterile, autoclaved toothpick was used to puncture either the stem end or lenticel of each potato tuber tested. Two toothpicks were used per tuber with each end of the toothpick used for one sample. The stem end at the point of potato stolon attachment to the daughter potato and three lenticels from each seed potato were evaluated. The stem end was not peeled prior to tuber poking, so there was the potential for external (on the surface) bacterial contamination. However, the close proximity of the toothpick poke to the stolon attachment point helped ensure that any bacteria found were most likely a result of transport through the stolon to that area of the tuber; indicating a systemic type infection. After the tuber was punctured with the end of the toothpick, the point was inserted, then twisted into a fresh CVP plate. Twisting of the toothpick was used to ensure that any bacteria on the point were left in the CVP. After inoculation, each CVP plate was placed in an anaerobe jar and was incubated at room temperature for approximately five days. A BD GasPak™ EZ Gas Generating Container System was used to generate anaerobic conditions. The CVP plates were incubated in the anaerobic chamber to reduce the amount of growth of non-pectolytic, aerobic fungi and bacteria. For example, bacteria in the genus *Clostridium* are pectolytic, but do not grow readily under anaerobic conditions (Bain and Perombelon 1988). After the five-day incubation was over, each CVP plate was visually evaluated for pitting (presence of a concave indentation in the media resulting from actively growing pectolytic bacteria) at the toothpick insertion points. A pit in the CVP at the toothpick insertion site indicated that active pectolytic bacteria were present in either the stem end or lenticel from the seed potato. Four seed potatoes were represented on each CVP plate, with enough space in between each toothpick insertion to reduce the possibility of cross contamination between stem end and lenticel insertion points (about 1 cm distance between insertion points).

Objectives 1 and 2:

Early Versus Late Generation Seed Comparisons and Farming Operation Comparisons:

In this study, two different seed crops of Canela Russet potatoes were evaluated over the course of two years, from each of two seed growers. Grower 1 has greater incidences of blackleg outbreaks than grower 2. Each grower harvested their Canela Russet seed crops and put them in storage at the end of the 2013 and 2014 growing seasons. A random sample of 40 tubers from each seed crop was collected in the fall and in the spring for both years of the evaluation (starting fall 2013 and ending spring 2015). For grower 1, the Generation 1 (G1) samples were pulled from storage cribs and the Generation 2 (G2) and Generation 3 (G3) samples were pulled from the postharvest test backup samples. A post-harvest backup sample was used to ensure that a sample could be collected from each seed crop in case the crop was sold or rejected for other reasons. For grower 2, the G1's were collected from a storage crib and the G2 & G3's were collected from the pile (Table 2.1). The stem end and three lenticels from each tuber were tested for the presence of pectolytic bacteria. The poked CVP plates were visually assessed for the formation of pits in the fall 2013, the fall and spring of 2014, and the spring of 2015. The overall % pitting was calculated for each sample.

Objective 2:

Farming Operation Comparison:

Two G4 seed crops of the potato cultivar Satina were evaluated for detection of pectolytic bacteria. The two Satina crops were grown on two different grower operations (grower 2 and grower 3). These two seed crops were chosen based on the % blackleg observed during the certification field inspection in each crop in the summer of 2013, which was the fourth field year (Table 2.2). A sample of 40 seed potatoes was collected and tested for each seed lot at two sampling times, fall, 2013 and spring, 2014 (a total of 80 seed potatoes per crop). Samples were collected from each grower within two weeks prior to plating. The stem end of each seed potato was poked and plated on CVP (Grower 2 - samples plated on November 21, 2013 and May 1, 2014 and read on November 27, 2013 and May 6, 2014 respectively; Grower 3 - samples plated on December 12, 2013 and April 16, 2014 and read on December 17, 2013 and April 21, 2014, respectively). The overall % pitting was calculated for each sample.

Objective 3:

Evaluation of Susceptible Versus Somewhat Resistant Cultivars:

In this part of the project, seed crop samples were evaluated in the fall and spring after the first year in the field and in the fall of the second field year. There were a total of four initial seed crops evaluated, two with a cultivar susceptible to blackleg development, Satina and two with cultivars that were somewhat resistant to blackleg, LaRatte and Canela Russet (Table 2.3). The seed lots came from three different seed potato operations to determine whether individual seed grower operations made a difference in being able to determine cultivar susceptibility level. Again, a tuber poking system was used to evaluate pectolytic bacteria levels in each of the four seed crops. Forty seed potatoes were collected each time samples were obtained from a seed grower (2013 fall, 2014 spring, and 2014 fall). The procedure followed was the same as described earlier.

Analysis:

A completely random experimental design was used for each study in this project, since seed potatoes were selected randomly from each seed crop evaluated. Factors included seed potatoes (n = 40 per seed lot), stem end and lenticel sites from each potato grower operation, cultivar, and year. The number of replicates was 40 for each study (40 seed potatoes tested per sample).The total number of samples for each study was as follows: Evaluation of Canela Russet crops between two farming operation (40 reps x 2 seed crops x 2 sampling points x 4 sample dates = a total of 640 samples), Evaluation of the potato cultivar Satina between two farming operations (40 reps x 2 seed crops x 2 sample dates = a total of 160 samples), and evaluation of susceptible vs. resistant cultivar evaluation (40 reps x 2 potato evaluation points x 4 seed crops x 3 sample dates = a total of 960 samples).

An LSD mean separation was used to analyze the data for each study. For the farming operation comparisons (Canela Russet and Satina cultivars), data was compared between the grower operations, the seed lot generation and the cultivar grown. For the susceptible vs. resistant cultivar screening evaluation, stem end and lenticel data were compared within each sampling time for each sample date. Analysis was completed using paired t-tests and seed sample differences were significant when p values <= 0.05. SAS 9.4 (PROC GLM) was used to conduct each of the analyses.

Results and Discussion:

Objectives 1 & 2:

When comparing the stem end data for the G1 seed crop, there were no significant differences between the Canela Russet seed lots for growers 1 and 2 during the fall or spring after the first year in the field. Samples from grower 2 had an increase in the amount of pitting on CVP in the spring of 2014, however, bacterial numbers decreased in the fall of 2014 and spring of 2015. For grower 1, the amount of pitting continued to increase from the fall of 2013 through the fall of 2014, but decreased in the spring of 2015. In the fall of 2014 the difference between the two growers was significant, with grower 2 having lower bacteria numbers than grower 1 (p value = 0.034) (Figure 2.1). This may be a result of management differences for each grower between the time samples were collected in the spring of 2014 and the fall of 2014. Also, there was a small amount of blackleg (0.1%) observed in grower 1's field year two Canela Russet seed lot in the summer of 2014, but no blackleg was observed in grower 2's field year two seed lot during the same period (Table 2.4). It is also of interest that there was no pitting on CVP observed from the samples collected from either grower in the spring of 2015. This may be an indication that each of the growers managed their seed in such a way as to reduce pectolytic bacteria numbers on the tubers or the environmental conditions at harvest and in storage were conducive to the tubers warding off the bacteria through defense mechanisms, or a combination of both. It is of note that during storage, bacterial numbers found on the surface of the tubers or in the lenticels do typically decrease, so very low levels of bacteria do not necessarily translate into higher levels in the field the following year, assuming the bacteria are not systemic in the tubers. Proper cutting and planting management during the early season can go a long way in reducing blackleg symptoms in the plant. However, when blackleg is found in the field during any inspection, it can be assumed that the lot has a higher "potential" for blackleg expression under the right conditions.

When looking at the lenticel data, grower 1 had a significantly higher amount of pitting than grower 2 in the samples collected in the fall of 2013 and in the spring and fall of 2014 (Figure 2.2). When comparing stem end data with the lenticel data, there was a much higher percentage of pitting in the lenticels for grower 1, which was not the case with grower 2. As with the stem end data, the percentage of pitting was relatively low in the field year 2 samples in the spring of 2015. Overall, grower 1 had a higher percentage of pitting in the lenticels than grower 2, again indicating that there was a higher "potential" for blackleg in grower 1's seed lot.

The year one seed, grown in the summer of 2013, originated from nuclear greenhouse stock grown in 2012 for both growers. Samples from these nuclear stocks had been lab tested for the presence of pectolytic bacteria in 2012 (2012 Colorado Certified Seed Directory). These lab tests came back negative for the presence of pectolytic bacteria on the tubers sampled, which allowed the seed to be certified as a generation one crop for each grower. Therefore, the pitting observed in the samples collected for each grower after field year one was the result of pectolytic bacteria that had been acquired by the tubers sometime after the harvest of the nuclear crop in 2012. This could indicate that a combination of farming practices and environmental conditions at each of the two grower operations were responsible for the pectolytic bacteria present on these seed potatoes. Since grower 1 had overall higher percentages of pitting on CVP than grower 2, we can conclude that the local environment and management strategies used by grower 1 caused a greater increase in overall numbers of pectolytic bacteria present on seed tubers.

When evaluating the stem end and lenticels of Canela Russet seed samples for field year 2 and field year 3, there were no differences between the two growers at any sampling time. In the summer of 2014, 0.80% blackleg was observed in the grower 1generation 3 field, whereas no blackleg was observed in the seed crop of grower 2 (Table 2.5). In 2015, in the generation 4 crop, 0.60% blackleg was observed in the seed on grower 1's field, but none in grower 2's field. While pectolytic bacteria were present in the

Canela Russet seed lot for both grower operations, it is apparent that differences in management practices resulted in blackleg expression for grower 1 and no disease expression for grower 2. These results also indicate that as seed moves through the field generations, the expression of blackleg is more likely to show up in successive years. That is, low levels of blackleg early in the cycle as seen in the first field years do not always translate into field symptoms, but as the crop moves through successive field generations, there is a greater likelihood of seeing field symptoms.

Throughout this project, several early (generation 1 and generation 2) and late (generation 3 and generation 4) seed lots were evaluated using the CVP methodology. In many of these evaluations, the earlier generation seed samples had an equal or higher overall % pitting than samples from later generations (data not presented). In a study conducted by Perombelon et.al. (1980), it was also found that an increase in pectolytic bacteria numbers did not always carry over to the higher generations. Perhaps screening earlier generation seed stock for presence of pectolytic bacteria provides better information for predicting future blackleg outbreaks than screening later generations.

Objective 2:

When evaluating seed samples of the cultivar Satina for seed growers2 and 3, there were no significant differences between lenticels (data not presented). The % pitting for the potato seed stem ends was significantly lower in grower 2 than in grower 3 (Figure 2.3). Also, in the fall of 2013 the stem ends had a significantly higher amount of pitting than the lenticels for grower 3 (22.50% vs. 5.83%, p value = 0.0019). In the summer of 2013, a high amount of blackleg was observed in the seed crop from grower3 (Table 2.2). The sample evaluated was pulled from this crop. Since the bacteria can travel from a symptomatic plant to the daughter tuber via the stolon, it would explain why the tuber stem ends showed a higher percentage of pitting on the plates in grower 3's crop. A higher % pitting would indicate that there was a relatively high level of pectolytic bacteria in the seed lot that was carried over from the summer field season. This difference in % pitting between the two lots was not detected when the sample was evaluated in the spring of 2014, indicating that the fall might be a better time to evaluate a seed sample for pectolytic bacterial activity. Another explanation might be two-fold. Grower 3's tubers could have seen a decrease in bacterial populations during the storage season, a common occurrence as tubers move into the holding phase of storage if tuber healing is good and conditions are less favorable for bacterial growth. During this time bacterial numbers can decrease as a result (Pringle and Robinson 1996; Vanvuurde and Devries 1994). Grower 2's tubers could have seen an increase in bacterial populations since the samples were stored near other lots with high levels of bacteria present. Soft rot bacteria can move in storage when conditions are right and lots with high bacteria levels are being sorted nearby (Graham and Harrison 1975; Perombelon 1992). The results from this portion of the project indicated that the evaluation of a seed lot using CVP can be used to determine relative blackleg levels after the field blackleg levels have been determined. Can this test be used to consistently determine outbreak potential for later generations?

Objective 3:

Four seed crops of potato cultivars with different levels of susceptibility to blackleg were evaluated from three different farming operations. The first field year (generation 1) was used for each cultivar/seed crop to determine pectolytic bacteria differences. Each of the seed crops evaluated were the generation 1 and subsequent generation 2 seed lots or first and second years in the field. There are a couple of reasons why only the earlier generations were evaluated. As mentioned earlier, an increase in the number of years in the field does not always translate into an increase in bacteria numbers in the seed, even though blackleg levels tend to increase as years in the field increase in susceptible cultivars. Also, due to the effect disease has on potato seed production and certification disease tolerances, earlier generation material is much more consistent when evaluating because of possible rejections which would prevent the seed from being saved and planted back for later generations. Using later generation seed crops would have resulted in a greater probability of a seed crop rejection by seed certification officials, due to a failure to meet required tolerances for diseases such as PVY, blackleg, etc. This would have prevented an evaluation of the seed crop the following year.

When analyzing the Canela Russet and LaRatte seed crops, cultivars which tend to be more resistant to blackleg, there was no statistical difference in % pitting observed between the stem ends or lenticels (data not presented). For the samples from the two Satina crops, there was a significant difference between the amount of pitting for the stem end and lenticels (Figures 2.4 and 2.5). In both cases, the % pitting was higher in the stem ends than in the lenticels in the fall of 2014 (40% vs. 22.5% for grower 4, 22.5% vs. 5.8% for grower3). It did not matter whether the seed lot was grown within a seed operation that historically had a problem with blackleg (grower 4) or a grower with a minimal blackleg problem (grower 3). This might indicate that in cultivars with higher susceptibility to pectolytic bacterial population increases and blackleg symptom expression, more systemic infections of *Pectobacterium* spp. and thus higher levels of bacteria in the stolon attachment area of the tuber may be observed.

Based on summer blackleg levels from certification field inspections, there appears to be little or no blackleg present in each of these crops over three years (Table 2.3). Again, given that pectolytic bacteria were present in and on the tubers which were planted back each year and the cultivar was susceptible, the lack of blackleg could be the result of very good farming practices or excellent environmental conditions which were not conducive to blackleg expression. Within a seed crop, many plants can be asymptomatic and won't express blackleg symptoms at numbers high enough to detect in a routine field inspection until later generations (generation 3 or higher). The generation 3 Satina lot from grower 4 would have been a good check for this since grower 4 has had a history of blackleg based on certification records (Colorado Certified Seed Directory – 2005 to 2015). However, it was not planted back by the grower in the summer of 2015, so the follow-up evaluation was not possible.

Conclusions:

The aim of this project was to determine a way to predict potential outbreaks of blackleg in a potato crop. Several objectives have been presented in this research which were conducted using certified seed from several certified seed operations. The first objective, determining whether pectolytic bacteria numbers from earlier generation seed could be used to determine future blackleg outbreaks was shown to be true. This corresponds well to the findings of Lund and Kelman, 1977. Using a tuber poke/mist chamber setup, they were able to screen seed lots for soft rot presence. A soft rot potential index was developed to predict soft rot levels in seed lots. Their findings showed that higher soft rot potentials in given seed lots often led to more rot and blackleg in the field.

When evaluating grower's 1 and 2 seed lots, differences in pectolytic bacterial populations were higher after the generation 1 potato crop in grower 1 than in grower 2. This corresponded to certification inspection data during the summer of 2014 where blackleg was observed in grower 1's seed crop but not in the seed crop of grower 2. Pectolytic bacteria are present in a plant expressing blackleg and can travel through the stolon to the stem end of the daughter tubers, which could explain this finding (De Boer 2002). This is further confirmed since blackleg was observed during inspections in the summer of 2014 in grower 1's lot and very low levels of pectolytic bacteria were found in the stem ends of the seed potato sample in the fall of 2014 for grower 2.

When looking at percent pitting from the lenticels of the seed potatoes, the numbers for grower 1 were consistently higher than for grower 2. This was quite evident when evaluating the generation 1 seed crop in the fall of 2013 and spring 2014. However, when evaluating the samples from the generation 2 and generation 3 seed, this was not observed. The historical differences in blackleg levels for these grower operations correspond relatively well to the % pitting in the seed samples following the generation 1 crop (grower 2 tends to have lower levels of blackleg within the operation than grower1). However, this correlation does not continue in the second and third field generations. This discrepancy between numbers of bacteria in the seed and field blackleg levels across several generations has been observed in previous studies (Perombelon et al. 1980). This discrepancy was observed in grower 1's samples, where there was an increased level of blackleg as the number of years in the field also increased, yet the levels of pectolytic bacteria decreased as the number of years in the field increased. Reasons for this discrepancy could be differences in the soil microbiome surrounding the daughter tubers for the later generation crops. There may be changes in the physiology of the later generation daughter tubers that either provide for a different environment, thereby changing the bacterial populations of the pathogen or their competitors. The might decrease the level of pitting observed on the CVP plates and could reduce the accuracy of the

CVP test. A later generation daughter tuber also might be more susceptible to blackleg development and require fewer pathogenic bacteria in order for blackleg to develop in the crop. There needs to be further evaluations of this phenomenon before we can say this is the case in all situations.

The second objective was to evaluate farming operations in their ability to control buildup of pectolytic bacteria and field blackleg and determine which factors might be critical in controlling the pathogen and disease. When comparing the two operations used in Objective 1, there were several differences between these two operations. Differences included the manner in which seed was cut prior to planting. Grower 2 used a hot cutter type system for cutting all of his seed while grower 1used a more traditional cutting system with disinfectants sprayed periodically. Grower 2 had a greenhouse and grew most of their nuclear stock in-house, whereas grower 1 purchased all nuclear stock from other sources. The seed operations are located roughly 20 miles apart with grower 2's fields located more on the western edge of the San Luis Valley which is quite isolated from other operations and grower 1is located more in the heart of the potato growing area in the SLV, etc. Grower 2 used a storage system for storing early generation material isolated from higher generation material where grower 1 usually stored the early generation material separately, but within a larger storage that contained later generation seed stocks. The differences between grower 2 and grower 1 need to be further investigated to determine what these growers are doing differently in their production practices to cause such differences in pectolytic bacteria numbers as well as blackleg levels, but on the surface it would appear that there are two main differences which might account for the disparity in bacterial numbers: First is the cutting system utilized, and second is the storage system.

When evaluating the stem ends of seed potatoes from a Satina lot, field generation 4, the % pitting data confirmed the high level of blackleg from the previous summer's crop. Other research has indicated that pectolytic bacteria numbers in higher generation seed lots do not always correlate with higher blackleg levels in the field (Perombelon et al., 1980). This has been confirmed in this study when evaluating the more resistant cultivars Canela Russet and LaRatte. However, a later generation correlation between blackleg and pectolytic bacteria numbers in a highly susceptible cultivar like Satina, as was observed in this study, should not be ignored, even when there are few blackleg symptoms seen in the field. A follow up study would be to pull and test samples on the generation 3 seed lot preceding a high blackleg year and determine whether the outbreak could be predicted as well. Further evaluation of this phenomenon needs to be continued, which is outside of the scope of this project.

The final objective was to determine whether a potato cultivar's susceptibility to blackleg could be predictive of how the cultivar might fare in the field given farming operational differences. The results from the stem end evaluations of the more resistant Canela Russet and LaRatte, and susceptible, Satina cultivars showed that early generation testing could be used to determine cultivar susceptibility to blackleg. For this project, data were collected from the stem end of the seed potato and from the lenticels for different reasons. Pectolytic bacteria found in the stem end of a potato are an indication that the bacteria entered the stem end through the stolon and have systemically infected the tuber. There is a high probability that most bacteria found in the stem end of a potato come into contact with the stem end through the stolon attachment. As a daughter tuber develops, water, photosynthates, starch, etc. are transported to the tuber via the stolon. Along with nutrients and photosynthates that allow the daughter tuber to continue to bulk and grow, microbes such as bacteria can also travel along the stolon to the daughter tuber. In the case of blackleg, a potato plant expressing foliar symptoms of this disease can transport the bacterial pathogens to the daughter tubers through the stolon. The daughter tubers acquisition of pathogenic bacteria from the mother plant would most likely be seen in the stem end of the potato. By testing the stem ends of the daughter potatoes we can get a feel for the amount of bacteria that were transmitted to the potatoes from the mother plant via the stolon (De Boer 2002).

On the other hand, bacteria present in the lenticels do not necessarily contact the daughter potato via the stolon. Bacteria detected in the lenticels are typically coming from other avenues, not necessarily from a diseased mother plant (De Boer 2002). The pectolytic bacteria found in the lenticels could be coming from any of the following sources: a blackleg symptomatic mother plant or infected tubers, contaminated rain or irrigation events, insect spread, etc.(Czajkowski et al. 2011; De Boer 2002; Graham and Harrison 1975; Harrison et al. 1987; Kloepper et al. 1981). It is of note that bacteria found in the lenticels may or may not give rise to blackleg. If the bacteria in the lenticels do not ever break into the tuber from the lenticel region or are not in lenticels near the germinating sprout, they often have no impact on the potential for blackleg in the crop. However, while this may be true, it is still a good idea to test the lenticels to determine which microorganisms are present on the tuber and to acknowledge that bacteria in the lenticels can potentially give rise to blackleg if bacteria are somehow moved into the tuber (Nielsen 1978; Perombelon 1992).

The overall purpose of each of these objectives was to develop a rapid, inexpensive screening system to give potato growers a usable tool which would allow prediction of potential blackleg outbreaks in their seed production systems. This research indicates that the screening of early generation seed stocks can give a legitimate indication of the "potential" for blackleg in the field the following season. Also, screening the stem ends of seed potatoes for pectolytic bacteria might be a good tool to determine whether a potato cultivar is susceptible to blackleg. Based on this research, there were differences observed in a susceptible cultivar versus two that were less susceptible. The methodology used in this paper evaluates early generation seed using a CVP testing procedure which allows for a prediction of blackleg susceptibility without having to test the later generation stock. The testing of later generation seed could produce conflicting bacteria numbers based on the data from this study and from the Perombelon study (1980). The method used in this paper could potentially be used on crops from a seed operation that may or may not have a history of blackleg. When there is no significant difference in % pitting between stem ends and lenticels, it indicates that there is not an excess of pectolytic bacteria at the stem end of the seed potato.

If the grower has a problem with blackleg, but is unsure if the cultivar they are planting is susceptible to blackleg, this test could provide useful information to help make decisions about whether they should or should not flush out a seed lot that might develop blackleg before it reaches later generations. In addition, potato breeders could work with farmers using this blackleg prediction model to determine the susceptibility of different cultivars to blackleg. This would provide a real-world scenario which might improve on the development of blackleg resistant cultivars.

Tables:

Table 2.1. Canela Russet Samples that were Collected from Grower 1 & 2 During the 2013/14 and 2014/15 Potato Storage Seasons.								
Season/Year sample was collected	Grower	Lot #	Field Year	Date Plated on CVP	Date Read	# of Tubers Screened	cut (1) or uncut (2)	Storage
Fall 2013	1	CAN11	FY1	17-Jan	22-Jan	40	2	crib
Fall 2013	1	CAN21	FY2	24-Dec	29-Dec	40	2	b sample
Spg 2014	1	CAN11	FY1	7-May	14-May	40	1	crib
Spg 2014	1	CAN21	FY2	7-May	14-May	40	1	b sample
Fall 2014	1	CAN21	FY2	11-Dec	20-Dec	40	2	b sample
Fall 2014	1	CAN31	FY3	12-Dec	20-Dec	40	2	b sample
Spg 2015	1	CAN21	FY2	8-Apr	14-Apr	40	1	b sample
Spg 2015	1	CAN31	FY3	9-Apr	14-Apr	40	1	b sample
Fall 2013	2	CAN21	FY2	10-Dec	14-Dec	40	2	pile
Fall 2013	2	CAN11	FY1	10-Dec	14-Dec	40	2	crib
Spg 2014	2	CAN11	FY1	16-Apr	21-Apr	40	1	crib
Spg 2014	2	CAN21	FY2	24-Apr	29-Apr	40	1	pile
Fall 2014	2	CAN11	FY1	15-Dec	26-Dec	40	2	crib
Fall 2014	2	CAN21	FY2	15-Dec	26-Dec	40	2	pile
Fall 2014	2	CAN31	FY3	15-Dec	26-Dec	40	2	pile
Spg 2015	2	CAN21	FY2	19-May	26-May	40	1	pile
Spg 2015	2	CAN31	FY3	19-May	26-May	40	1	pile

Table 2.2. Satina Lot Comparison for Grower 2 and Grower 3 (Field Year Four).						
Grower	Cultivar	Year	Field Year	Field Generation	Acres in Lot	% Blackleg[a]
2	Satina	2013	FY4	G4	22.00	0.00
2	Satina	2014	FY5	G5	NA[b]	NA[b]
3	Satina	2013	FY4	G4	47.60	6.00
3	Satina	2014	FY5	G5	NA[b]	NA[b]

[a]Each lot is inspected by trained seed potato inspectors. Percent blackleg is based on the visual evaluation of 1% of the number of acres in each seed lot (or a minimum of 1,000 plants for seed lots with less than 10 acres). The total number of plants expressing blackleg sympoms are used to calculate the percentatge of blackleg: (# blackleg plants/# plants evaluated)*100.

[b]NA = Information is Not Available because seed lot was not planted by the grower in 2014.

Table 2.3. Potato Cultivar Comparison for Growers 2, 3, and 4 (Field Year One, Two and Three).						
Grower	Cultivar	Year	Field Year	Field Generation	Acres in Lot	% Blackleg[a]
2	Canela Russet	2013	FY1	G1	1.03	0.00
2	Canela Russet	2014	FY2	G2	5.00	0.00
2	Canela Russet	2015	FY3	G3	27.50	0.00
3	Satina[b]	2013	FY1	G1	0.40	0.00
3	Satina[b]	2014	FY2	G2	1.80	0.00
3	Satina[b]	2015	FY3	G3	13.30	0.00
4	Satina[b]	2013	FY1	G1	0.25	0.00
4	Satina[b]	2014	FY2	G2	3.00	0.00
4	Satina[b]	2015	FY3	G3	NA[c]	NA[c]
4	La Ratte	2013	FY1	G1	1.20	0.00
4	La Ratte	2014	FY2	G2	4.00	0.00
4	La Ratte	2015	FY3	G3	20.00	0.05

[a]Each lot is inspected by trained seed potato inspectors. Percent blackleg is based on the visual evaluation of 1% of the number of acres in each seed lot (or a minimum of 1,000 plants for seed lots with less than 10 acres). The total number of plants expressing blackleg sympoms are used to calculate the percentatge of Blackleg: (# blackleg plants/# plants evaluated)*100.

[b]In 2013, the G4 Satina lot for grower 4 was 6.0% blackleg and the G4 Satina lot for grower 1 was 10% blackleg. This indicates that blackleg levels in the cultivar Satina tend to be high in later generations.

[c]NA = Information is Not Available because seed lot was not planted by the grower in 2014.

Table 2.4. Canela Russet Lot Comparison for Grower 1 and Grower 2 (Field Year One, Two, and Three).					
Grower	Year	Field Year	Field Generation	Acres in Lot	% Blackleg (Summer Inspection)[a]
1	2013	FY1	G1	0.20	0.00
1	2014	FY2	G2	1.50	0.10
1	2015	FY3	G3	9.25	0.00
2	2013	FY1	G1	1.03	0.00
2	2014	FY2	G2	5.00	0.00
2	2015	FY3	G3	27.50	0.00

[a]Each lot is inspected by trained seed potato inspectors. Percent blackleg is based on the visual evaluation of 1% of the number of acres in each seed lot (or a minimum of 1,000 plants for seed lots with less than 10 acres). The total number of plants expressing blackleg sympoms are used to calculate the percentatge of blackleg: (# blackleg plants/# plants evaluated)*100.

Table 2.5. Canela Russet Lot Comparison for Grower 1 and Grower 2 (Field Year Two, Three, and Four).

Grower	Year	Field Year	Field Generation	Acres in Lot	% Blackleg (Summer Inspection)[a]
1	2013	FY2	G2	1.50	0.00
1	2014	FY3	G3	12.00	0.80
1	2015	FY4	G4	57.50	0.60
2	2013	FY2	G2	0.96	0.00
2	2014	FY3	G3	6.70	0.00
2	2015	FY4	G4	42.00	0.00

[a]Each lot is inspected by trained seed potato inspectors. Percent blackleg is based on the visual evaluation of 1% of the number of acres in each seed lot (or a minimum of 1,000 plants for seed lots with less than 10 acres). The total number of plants expressing blackleg sympoms are used to calculate the percentatge of blackleg: (# blackleg plants/# plants evaluated)*100.

Figures:

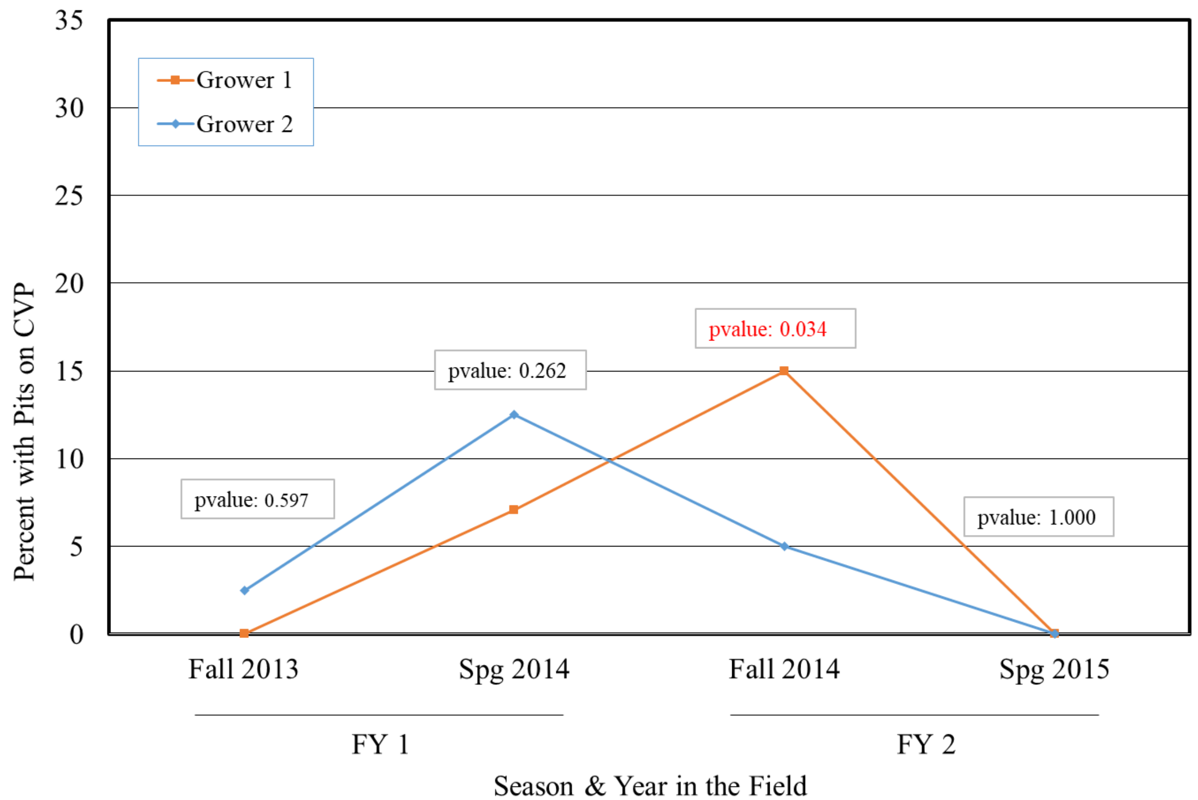


Figure 2.1. Grower 1 vs Grower 2 Pitting Evaluation of Seed Tuber Stem Ends in Canela Russet for Field Years 1 and 2.

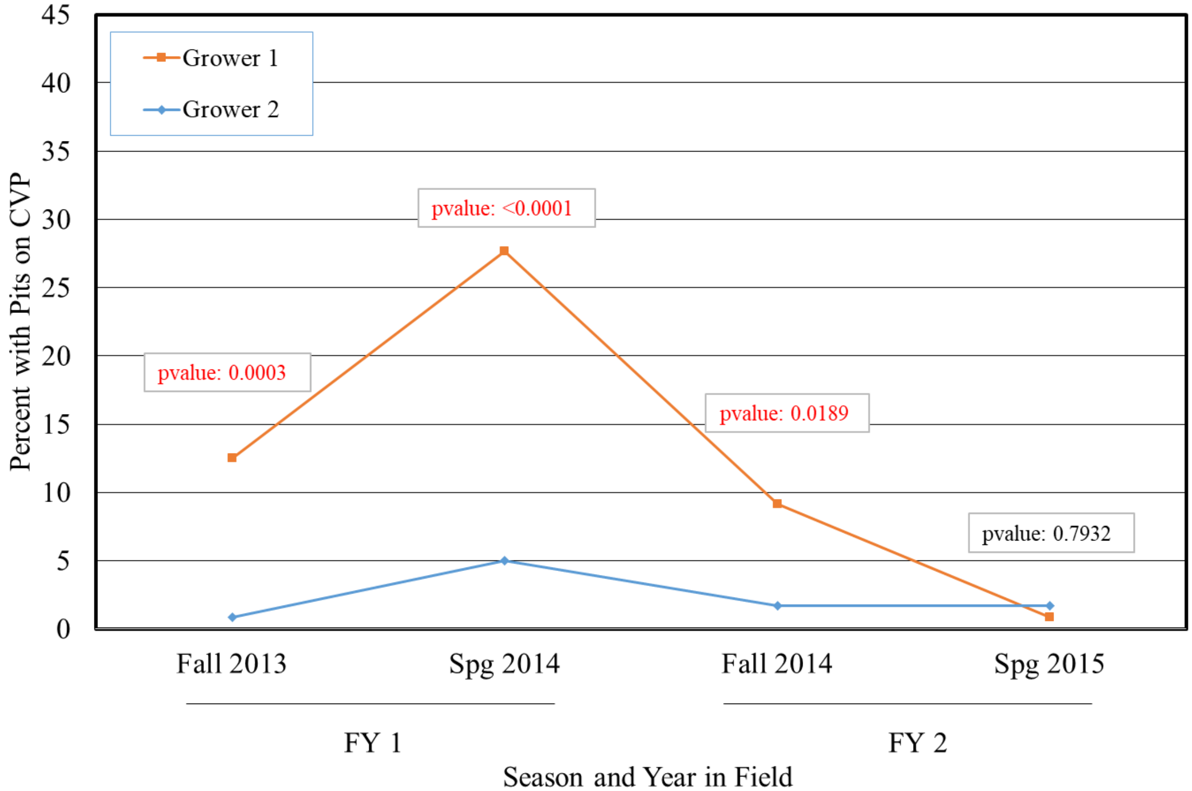


Figure 2.2. Grower 1 vs Grower 2 Pitting Evaluation of Seed Tuber Lenticels in Canela Russet for Field Years 1 and 2.

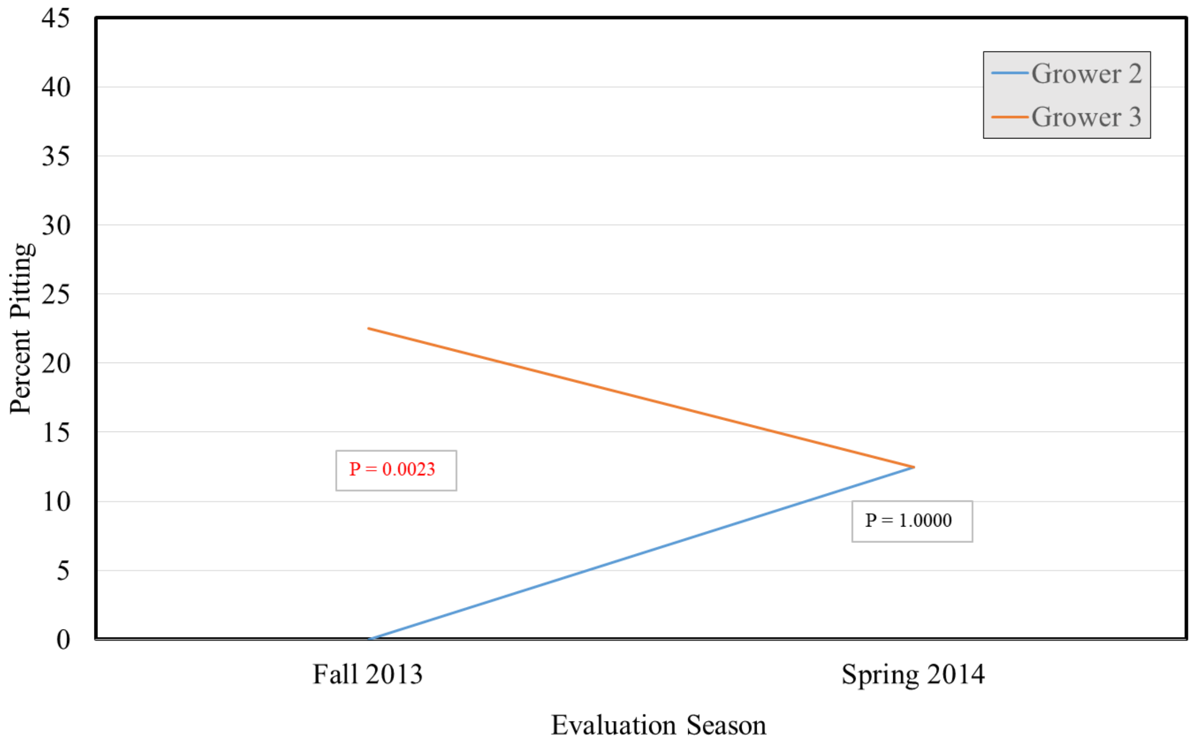


Figure 2.3. Evaluation of the Potato Cultivar Satina (Field Generation Four) Under Two Different Seed Operations to Determine the Percentage of Pitting caused by Pectolytic Bacteria - Stem Ends, 2013 Crop.

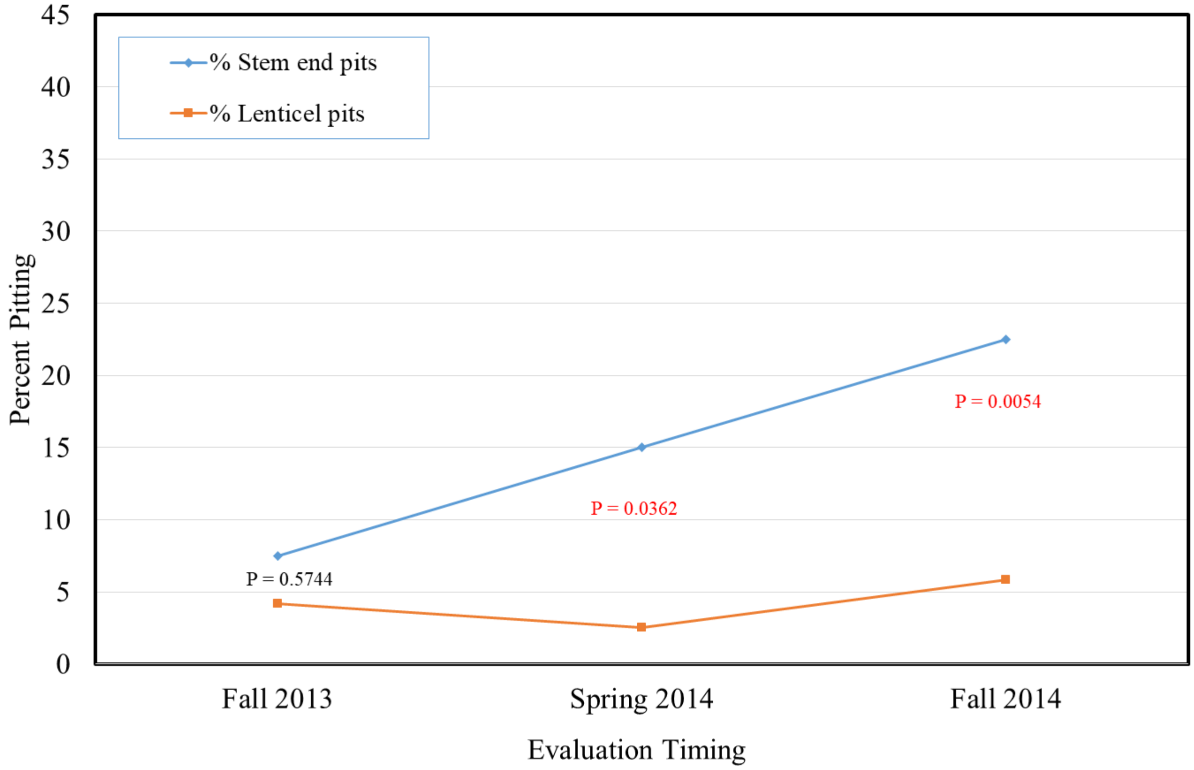


Figure 2.4. Grower 4 Satina Seed Crop - Pitting Evaluation of Stem End verses Lenticels (Starting with Field Year 1 Seed).

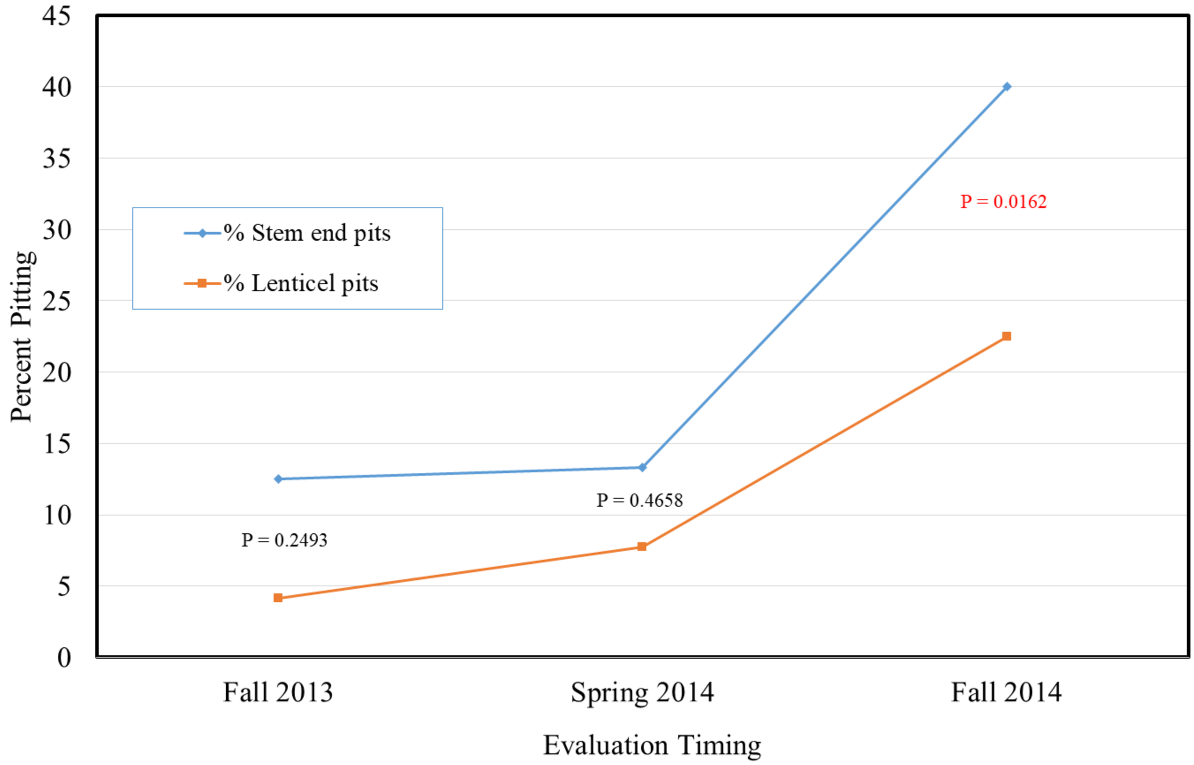


Figure 2.5. Grower 3 Satina Seed Crop - Pitting Evaluation of Stem End verses Lenticels (Starting with Field Year 1 Seed).

LITERATURE CITED

Ali, H.F., M. Ahmad, M. Junaid, A. Bibi, A. Ali, M. Sharif, B. Ali, K. Nawab, and A. Sadozai. 2012. Inoculum sources, disease incidence and severity of bacterial blackleg and soft rot of potato. Pakistan Journal of Botany 44: 825-830.

Bain, R.A. and M.C.M. Perombelon. 1988. Methods of testing potato cultivars for resistance to soft rot of tubers caused by *Erwinia-carotovora* subsp *atroseptica*. Plant Pathology 37: 431-437.

Charkowski, A.O. 2015. Biology and control of *Pectobacterium* in potato. American Journal of Potato Research 92: 223-229.

Chung, Y.S., N.J. Goeser, X.K. Cai, and S. Jansky. 2013. The effect of long term storage on bacterial soft rot resistance in potato. American Journal of Potato Research 90: 351-356.

Chung, Y. S., K. Holmquist, D.M. Spooner, and S.H. Jansky. 2011. A test of taxonomic and biogeographic predictivity: Resistance to soft rot in wild relatives of cultivated potato. Phytopathology 101: 205-212.

Cuppels, D. and A. Kelman. 1974. Evaluation of selective media for isolation of soft rot bacteria from soil and plant tissue. Phytopathology 64: 468-475.

Czajkowski, R., M.C.M. Perombelon, J.A. van Veen, and J.M. van der Wolf. 2011. Control of blackleg and tuber soft rot of potato caused by *Pectobacterium* and *Dickeya* species: a review. Plant Pathology 60: 999-1013.

Davidson, R.D. and K. Xie. 2014. Seed Potato Production. p. 115-132. In: R. Navarre and M.J. Pavek (eds.). The potato botany, production and uses. CABI, Boston, MA.

De Boer, S.H. 2002. Relative incidence of *Erwinia carotovora* subsp *atroseptica* in stolon end and peridermal tissue of potato tubers in Canada. Plant Disease 86: 960-964.

Deboer, S.H. 1989. Bacteria and Potatoes in Crop Rotation Systems. p. 131-152. In: J. Vos et al. (eds.). Effects of Crop Rotation on Potato Production in the Temperate Zones. Kluwer Academic Publishers, Wageningen, Netherlands.

Graham, D.C., and Harrison, M.D. 1975. Potential Spread of *Erwinia* spp in Aerosols. Phytopathology 65: 739-741.

Harrison, M.D., G.D. Franc, D.A. Maddox, J.E. Michaud, and N.J. McCarterzorner. 1987. Presence of *Erwinia-carotovora* in surface-water in North America. Journal of Applied Bacteriology 62: 565-570.

Kloepper, J.W., J.W. Brewer, and M.D. Harrison. 1981. Insect transmission of *Erwinia-carotovora* var *carotovora* and *Erwinia-carotovora* var *atroseptica* to potato plants in the field. American Potato Journal 58: 165-175.

Koppel, M. 1993. Methods of assessing potato-tubers for resistance to bacterial Soft-Rot. Potato Research 36: 183-188.

Lapwood, D.H. and R.I. Harris. 1980. The spread of *Erwinia carotovora* var *atroseptica* (blackleg) and var carotovora (tuber soft rot) from degenerating seed to progeny tubers in soil. Potato Research 23: 385-393.

Lund, B.M. and A. Kelman. 1977. Determination of potential for development of bacterial soft rot of potatoes. American Potato Journal 54: 211-225.

Lyon, G.D. 1989. The biochemical basis of resistance of potatoes to soft rot *Erwinia* spp: A review. Plant Pathology 38: 313-339.

Meneley, J.C. and M.E. Stanghellini. 1976. Isolation of soft-rot *Erwinia* spp from agricultural soils using an enrichment technique. Phytopathology 66: 367-370.

Nielsen, L.W. 1978. *Erwinia* species in the lenticels of certified seed potatoes. American Potato Journal 55: 671-676.

Nolte, P., J.S. Miller, B.D. Geary, and D.L. Corsini. 2003.Disease Management. p. 163. In: C. Jeffery, Stark, and S.L. Love (eds.). Potato Production Systems. University of Idaho Agricultural Communications, Moscow, ID.

Perombelon, M.C.M. 1992. Potato blackleg: Epidemiology, host pathogen interaction and control. Netherlands Journal of Plant Pathology 98: 135-146.

Perombelon, M.C.M., and E.M. Burnett. 1991. Two modified crystal violet pectate (CVP) media for the detection, isolation and enumeration of soft rot Erwinias. Potato Research 34: 79-85.

Perombelon, M.C.M. and A. Kelman. 1980. Ecology of the soft rot Erwinias. Annual Review of Phytopathology 18: 361-387.

Perombelon, M.C.M., R. Lowe, C.E. Quinn, and I.A. Sells. 1980. Contamination of pathogen-free seed potato stocks by *Erwinia-carotovora* during multiplication: Results of a 6-year montoring study. Potato Research 23: 413-425.

Pringle, R.T. and K. Robinson. 1996. Storage of seed potatoes in pallet boxes: The role of tuber surface moisture on the population of *Erwinia* bacteria. Potato Research 39: 205-222.

Rowe, R.C. 1993. Potato health management. APS Press, St Paul, Minn.

Skelsey, P., J.G. Elphinstone, G.S. Saddler, S.J. Wale, and I.K. Toth. 2016. Spatial analysis of blackleg-affected seed potato crops in Scotland. Plant Pathology 65: 570-576.

Toth, I.K., L. Sullivan, J.L. Brierley, A.O. Avrova, L.J. Hyman, M. Holeva, L. Broadfoot, M.C.M. Perombelon, and J. McNicol. 2003. Relationship between potato seed tuber contamination by *Erwinia carotovora* ssp *atroseptica*, blackleg disease development and progeny tuber contamination. Plant Pathology 52: 119-126.

Tzeng, K.C., R.G. McGuire, and A. Kelman. 1990. Resistance of tubers from different potato cultivars to soft rot caused by *Erwinia-carotovora* subsp *atroseptica*. American Potato Journal 67: 287-305.

Vanvuurde, J. W. L., and Devries, P. M. (1994). Population-Dynamics of *Erwinia-carotovora* subsp *atroseptica* on the Surface of Intact and Wounded Seed Potatoes During Storage. *Journal of Applied Bacteriology* 76, 568-575.

Wright, P. J., Crowhurst, R. N., Anderson, J. A. D., and Dale, J. R. (1991). Evaluation fo Potato Cultivars and Breeding Lines for Susceptibility to Tuber Soft Rot Induced by *Erwinia-carotovora* subsp *atroseptica*. *New Zealand Journal of Crop and Horticultural Science* 19, 187-190.

CHAPTER 3: THE EVALUATION OF DIFFERENT CROP ROTATIONS TO MANAGE BLACKLEG, TUBER SOFT ROT, AND PECTOLYTIC BACTERIAL POPULATIONS ON POTATO TUBERS

Summary:

Pathogenic bacteria in the genera *Pectobacterium* and *Dickeya* are of major concern in many potato growing regions around the world and are the causal organisms of blackleg and tuber soft rot. Bacteria in these genera are pectolytic, meaning they utilize pectin, of which potatoes are a good source. One management practice that a potato grower can use to reduce disease incidence in their crop is by utilizing different crop rotations. This study evaluated seven different crop rotations in 2013 and 2014, using the potato cultivar Colorado Rose, in the San Luis Valley, CO. Potato seed was inoculated with a slurry of *Pectobacterium atrosepticum* prior to field planting. The potato crop was evaluated to determine the effect each of these rotations had on disease incidence and severity. Blackleg, tuber soft rot and pectolytic bacteria levels were evaluated. Results indicated an increase in pectolytic bacteria population in tubers when plant species in the family Brassicaceae (canola and camolina) were included in the rotation. When following canola, a decrease in yield and an increase in the number of culls and rotten tubers was also observed in the potato crop. Based on this study, the use of brassica species in a crop rotation should not be used if there is a history of blackleg or tuber soft rot within the farming operation.

Introduction:

For many years it has been known that the use of rotational crops can result in increased crop health and reduced disease levels (Howard 1996; Larkin and Honeycutt 2006; Peters et al. 2003). Rotating crops can maintain a good soil structure and can increase soil organic matter, which reduces soil erosion. Rotating crops on a given piece of ground increases microbial diversity. Microorganisms such a fungi can improve soil structure through the development of hyphae, which can better hold the soil together. Some crop species can deplete the soil of organic matter, whereas others can increase the organic matter content in the soil. Continuous cropping with a susceptible host can increase levels of plant pathogens in the soil which reduces crop yield and quality. The use of crop rotations can reduce soil borne pathogens in three primary ways: disrupting the host-pathogen cycle, altering soil characteristics and soil microbial communities, directly inhibiting pathogens through the crop's production of toxins or by increasing the numbers of antagonistic microorganisms (Peters et al. 2003).

The length of time or number of seasons between crops has an impact on the effectiveness of crop rotations. In a study by Larkin and Honeycutt (2006), a two year rotation was shown to reduce disease levels more effectively than no rotation. Also, a three-year rotation tended to reduce potential disease incidence better than a two year rotation. The crop planted just prior tends to have the most influence on the following crop, however previous crops also have an impact on disease levels, but to a lesser degree. The most important impact a crop has on the soil is its effect on the microbial communities. However, little is known about the specifics of the interactions of a given crop on the microbes present in the soil. Soils planted to a crop tend to have an increased amount of microbial biomass as compared with fallow soil, due to root exudates providing nutrients and food to the microbes in the soil (Larkin and Honeycutt 2006).

Larkin et al. evaluated the use of three year crop rotations with potato as the third crop in the rotation. These were compared with a continuous potato cropping system. Every rotation evaluated resulted in higher soil microbial biomass and an increased microbial community diversity. When canola, barley or sweet corn were planted in the year prior to potato, a decrease in the amount of black scurf and stem canker caused by *Rhizoctonia solani*, a fungal pathogen of potato, was observed when compared with a continuous potato cropping system. However, when soybean or clover preceded potato in the rotation, less disease suppression was observed. The inclusion of barley or canola in the rotation also resulted in an increase in overall bacterial biomass in the soil, which could improve the breakdown of excess plant material, thereby increasing the levels of usable micronutrients available for the subsequent crop (Larkin and Honeycutt 2006).   Another study by Larkin et al. found that different crop rotations including barley/timothy grass and timothy grass, barley/under-seeded with rye, or mustard &

rapeseed/sudangrass and rye decreased the amount of disease caused by *R. solani* and *Streptomyces scabies* (a bacterial pathogen that causes common scab in potatoes). In this same study, applications of compost to the soil also increased the levels of common scab when compared with the crop rotations and the standard potato rotation (Larkin et al. 2007).

Another study evaluated the use of a three-year crop rotation with different crop species and tillage practices (conventional vs minimum tillage). The combination of using a three-year rotation and minimum tillage reduced the dry rot (*Fusarium spp*.), silver scurf (*Helminthosporium solani*) and black scurf (*R. solani*) in potatoes. Soil from the three-year rotations were inoculated with *P. erythroseptica* (the causal agent of pink rot disease) and planted to potatoes in a greenhouse. Pink rot levels were reduced when compared with soil from two year rotations. It was concluded that soil agroecosystems can be managed through different rotation and tillage practices to improve the levels of disease suppression (Peters et al. 2003).

In addition to the suppression of disease causing microbes, different cropping plants can also produce chemicals with allelopathic properties which can be released into the rhizosphere. Depending on the environmental conditions, allelochemicals may be released into the soil at high enough quantities to suppress weed seedling growth (Masiunas et al. 1995; Urbano et al. 2006; Walsh et al. 2014). Crop species such as rice, mustards, sorghum, winter rye, sugar beets, lupins, maize, oats, peas, barley, cucumber, pepper, hairy vetch, and sunflower have been shown to have allelopathic properties (Urbano et al. 2006).

The use of a cover crop has also been shown to be beneficial in controlling weeds. Cover crops typically leave plant residue on the soil surface available when the following crop is planted. Cover crops can limit weed growth through the production of this residue, which provides a physical barrier to the weeds and also through allelopathy (Bezuidenhout et al. 2012; Masiunas et al. 1995). Using rye as a cover crop has been shown to reduce weed populations fields planted to tomato transplants (Masiunas et al. 1995). Stooling rye and annual ryegrass have also been found to suppress yellow nutsedge (*Cyprus esculentus*) in maize (Bezuidenhout et al. 2012).

Not all crop rotations or cover crops, however, are beneficial to crop growth (Peters et al., 2003). Rotations including buckwheat planted in the year preceding potatoes have been shown to increase the levels of black scurf on the tubers (Peters et al. 2003). The growth in a crop of maize was suppressed following a cover crop of rye (Bezuidenhout et al. 2012). Plants in the family Brassicacea produce glucosinolate compounds, which can go through enzymatic degradation and produce isothyocyanates and nitriles. These compounds are allelopathic and can reduce weed seed germination, but can also limit the seed germination of flax and radish (Walsh et al. 2014).

Members of this family are also incapable of forming functioning mycorrhizae because of the production of isothyocyanates, which are also antifungal. Mycorrhizal fungi form symbiotic relationships with the roots they colonize. These fungi can take up phosphate from the soil and can transfer much of the phosphate to the roots, which can be used by the plant. If these fungi are absent, the plant cannot benefit from this symbiotic relationship. When maize is followed by *Brassica napus* L. (canola), mycorrhizal colonization of roots is reduced when compared with soy, which reduces yields (Koide and Peoples 2012).

Although there has been a wide range of research evaluating the use of different crop rotations and cover crops and their effect on weeds and plant pathogens, there has been little research evaluating pathogenic pectolytic bacteria in commercial potato production using these practices. Since the use of different species in a crop rotation have been shown to reduce potato diseases such as black scurf, silver scurf, dry rot and pink rot, the potential is there to reduce the incidence of blackleg and tuber soft rot as well. Since the bacteria that cause blackleg and tuber soft rot (*Pectobacterium atrosepticum, P. carotovorum,* and *Dickeya* sp.) are primarily seed born, the effects of different rotational crops on the soil can have a major impact on the soil surrounding the potato seed piece, which could have an impact on the seed borne bacteria coming in contact with the soil. The use of different crop rotations or cover crops could have an impact on the severity of blackleg and tuber soft rot in a potato crop. The majority of literature available on this topic indicates that the pectolytic bacteria involved in blackleg and tuber soft rot will not survive and remain viable in the soil in the absence of infected plant material (Czajkowski et al. 2011).

It has been estimated that there are anywhere from $50 to 100 million in losses to potato producers as a result of tuber soft rot and blackleg, caused by these bacteria (Perombelon and Kelman 1980).The purpose of this study was to determine the effect of different crop rotations on potato yield and quality, blackleg and tuber soft rot incidence and severity, and pectolytic bacterial numbers in potato seed. An estimation of pectolytic bacterial numbers was calculated in order to determine the potential for disease development in the subsequent potato crop the following year.

Materials and Methods:

Rotational Crops:

A total of seven different crop rotations were evaluated for overall yield, levels of blackleg, tuber soft rot and pectolytic bacterial population numbers on seed potatoes in storage. Two-year and three-year crop rotations were evaluated. In the San Luis Valley, the two-year rotation of potato/barley is the standard rotation, which was used as the control in this study. Crop rotations were started in the 2011 and 2012 growing seasons, in close proximity to each other within the same field, which ensured uniform management within a given year and allowed for data collection from the potato crop for each rotation in the 2013 and 2014 growing seasons. The species of agricultural crops evaluated in this study were chosen because of their ability to grow well in the high alpine environment of the San Luis Valley of Colorado (Table 3.1).

Seed Inoculation with P. atrosepticum:

In both years, one row of seed tubers inoculated with *Pectobacterium atrosepticum* was planted in a row adjacent to a row of healthy potatoes, which served as an untreated control. There was an additional row of untreated potatoes on each side of the plot. A culture of *P. atrosepticum* was used for the inoculations. In order to ensure that the bacteria used was pathogenic, six colonies were collected using sterile toothpicks which were then used to inoculate a freshly cut potato slice with the bacteria.

Freshly cut potato slices from the cultivar Sangre S10 were used. The freshly cut and inoculated tuber slice was then placed in a petri dish and covered with a moistened paper towel and was allowed to incubate for 72 hours at room temperature. After incubation, three of the six colonies had resulted in a rot pocket on the tuber slice. Bacteria were isolated from the three rot pockets and plated on 15 nutrient agar plates and placed in an anaerobe jar for 36 hours (A BD GasPak™ EZ Gas Generating Container System was used to generate anaerobic conditions), which ensured a high level of pathogenicity in the bacteria.

To make the bacterial slurry for inoculation, four of the NA plates were used and distilled water was used to wash the bacteria from the plates. Serial dilutions were used to achieve a bacterial concentration of 10^5 cfu/ml. A general purpose UV/Vis spectrophotometer (Beckman Coulter DU®720) was used to determine an absorbance of 1.2, which was then used to determine the concentration of bacteria. A standard curve developed by Aleck and Harrison (1978) showing the absorbance readings from several different dilutions of *Erwina carotovora* was utilized to obtain the proper concentration. Seed from the potato cultivar Colorado Rose, which is susceptible to tuber soft rot, was freshly cut and inoculated with the bacterial slurry. To inoculate the potato seed pieces, the bacterial slurry was sprayed on the seed pieces (May 23, 2013 and May 19, 2014) using a roller table and a stationary sprayer (SURFLO electric pump). The fresh cut seed was allowed to suberize in paper sacks for 24 hours prior to planting in the field.

Data Collection:

Field plots were 6.2 meters long and consisted of two rows nested between another row on each side of the plot (4 rows total), with 86 cm spacing between rows and 30 cm between seed pieces within rows. One middle row was planted to seed that was inoculated with the bacterial slurry and another middle row was not inoculated, to be used as a control. Data was collected from the uninoculated and inoculated potato rows. Blackleg incidence was determined through the collection of several visual readings in August of each year (2013 – August 16, 21 and September 17; 2014 – July 23, August 5, 20, 28 and September 3), which is when blackleg tends to express in the San Luis Valley. At harvest, 10 potato plants were harvested from the middle of each plot and all tubers were evaluated for yield, grade, and the presence of tuber soft rot (2013 – September 18 and 19; 2014 – September 23and 24). Also, one tuber sample was collected from each rotational treatment (20 tubers per treatment per replication).

The stem end and three lenticels were evaluated on each of the 20 tubers sampled for the presence of *Pectolytic* bacteria. This was accomplished by poking the stolon end and three random lenticels on the tuber surface on each tuber. Autoclaved, sterile toothpicks were used for this. The end of the toothpick that punctured the tuber was then plated on crystal violet pectate (CVP) media. Next, the CVP plate was put in an anaerobic environment for 5-7 days. Finally, the plates were visually assessed for the formation of pits (2013 – October 21 through November 19; 2014 – November 9 through December 7).

Plot Management and Experimental Design:

The study was irrigated using a solid set sprinkler system. The plots were irrigated with 48.8 cm (2013) and 40.4 cm (2014) of water, respectively. For both years, a preplant fertilizer with the formulation of 80N-60P-0K-25S-2.5Z was applied and 60 units of N was applied through the sprinkler after tuber set. The following pesticides were applied: Matrix @ 105.2 g/ha, Eptam @ 5.23 L/ha (2013) and Chateau @ 70.2 g/ha, Dual Magnum @ 1.75 L/ha, Sencor @ 370.5 g/ha (2014). The plots were planted on May 23, 2013 and May 20, 2014.

A randomized complete block design was used for this study. Data was collected on two rows (one inoculated and one uninoculated) from the middle 3.1 meters of each plot in 2013 and 2014. Factors included crop rotation (n=7), inoculation (n=2), tubers tested (n=20) and year (n = 2). The number of replicates per crop rotation was 4 per year, for a total of 2,240 samples: 4 replicates x 7 crop rotations x 2 inoculations x 20 tubers x 2 years. A LSD mean separation was used to analyze the data across crop rotations and years. Means followed by the same letters were not significantly different when p values >=0.05. For comparing inoculated and uninoculated samples, paired t-tests were used and crop rotations were significant when p values <= 0.05. SAS 9.4 (PROC GLM) was used to conduct each of the analysis of variance tests (version 9.4, Cary, NC).

Results and Discussion:

Yield and Disease Incidence

When evaluating the uninoculated potato crop, there were no significant differences between treatments for size and quality profiles, total hundred weight (CWT), number of rotten tubers, or percent tubers with rot. This indicated that when bacterial levels were relatively low in potato seed, different crop rotations did not tend to have a major impact on blackleg or tuber soft rot. This provided evidence that the use of these rotations will not result in higher levels of blackleg and tuber soft rot when compared with the standard potato/barley rotation (data not included).

When evaluating the yields of the inoculated potatoes, a significant difference was observed in the percent culls (Figure 3.1). The rotation including canola (Trt. 3) had the highest percent culls (8.6%) when compared with the other rotations. The potato/barley, potato/barley/sudan and potato-rye/cocktail/sudan rotations (Trts 1,4,7) had the lowest percent culls of any of the treatments (2.6, 3.0, & 3.6% respectively).

Culls are typically characterized by the presence of misshaped tubers, growth cracks, and other external defects. When canola and camolina were grown the year prior to potatoes, there was a significantly higher number of culls than in the standard potato/barley rotation in the potatoes inoculated with *P. atrosepticum*. These crop species are known to produce allelochemicals which can negatively affect the following crop's growth. Also, canola and camolina do not form a symbiotic relationship with arbuscular mycorrhizae which can also negatively affect the subsequent potato crop (Koide and Peoples 2012; Walsh et al. 2014). Mycorrhizae provide potatoes with essential micronutrients which are, in part, responsible for a higher percentage of US Number 1's (this refers to the highest grade of potatoes based on the federal potato standards) in a potato crop (Duffy and Cassells 2000). The absence of mycorrhizae can then be an attributing factor in the production of more cull potatoes in a potato crop. Since this difference was observed in the potatoes harvested from the seed potatoes that were inoculated with *P. atrosepticum* and not in the uninoculated potatoes, it can be deduced that inoculating the seed with a pathogenic bacteria affected the harvestable crop negatively when the potatoes were followed by a crop of canola or camolina. This may be a consequence of the potato plant having to invest more resources in warding off an attack by the pathogenic bacteria, thereby putting fewer resources towards the production of tubers which could result in the production of more atypical tubers. Conversely, we can conclude that when potatoes with high levels of pectolytic bacteria are planted in a field having a rotational crop other than canola or camolina, the potato crop is better suited to defend itself against a bacterial pathogen, such as *P. atrosepticum*. The potato plant can allocate fewer resources towards defense mechanisms and more toward tuber production. When a rotational crop that promotes mycorrhizal growth is planted the year proceeding potatoes, the potato plants have a better chance of warding off disease caused by pathogenic bacteria.

When comparing potato yields (cwt a^{-1}) from the uninoculated and inoculated rows, the total yields were significantly lower (270 cwt a^{-1}) in the inoculated rows than in the uninoculated row (370 cwt a^{-1}) when the potato crop followed canola (Table 3.2). Also, when comparing the total number rotten tubers, the inoculated rows were significantly higher than the uninoculated rows in the potato crop following canola (Table 3.3). This indicates that planting potatoes which are more susceptible to developing tuber soft following canola increases the likelihood of having higher levels of tuber soft rot at harvest.

As mentioned previously, canola does not form a relationship with mycorrhizae. This can negatively affect the soil microbiome for potatoes, since potatoes rely on a relationship with mycorrhizae for obtaining essential nutrients. Without this relationship, the tubers are more likely to develop various problems, such as tuber soft rot, which was observed in this study. However, in this study, there were no measurements taken, other than overall potato yield and grade, that indicated what the mycorrhizal levels were.

The crop species rye and sudan can provide benefit as well as potential harm to a potato crop. Rye and sudan also produce allelochemicals, which can have an adverse effect on different plant species (Masiunas et al. 1995). The production of these allelochemicals may have an adverse effect on potato plant defense systems, which would explain the higher levels of soft rot. However, the effect on potato plant defense systems needs to be validated through additional field and lab trials before conclusions can be made.

Pectolytic Bacteria Numbers (Stem End and Lenticels)

When comparing the different rotations planted with inoculated potato seed, the pitting data is somewhat conflicting (Figures 3.2 & 3.3). When looking at the stem end (Figure 2), there is no significant difference between most rotations, except between the potato/barley/cocktail (12.5% pitting) and potato-rye/cocktail/Sudan (5.6% pitting) rotations. Since the seven species cocktail is included in both rotations it is difficult to make a conclusion as to why this difference is present between the two rotations.

When evaluating the lenticel pitting data (Figure 3.3), the potato/barley (3.5% pitting), potato-rye/sudan (5.7% pitting) and potato/barley/camolina (4.2% pitting) rotations expressed the lowest percentage of pitting when compared with the other rotations. The rotations that included canola and the seven-species cocktail mix had the highest percentage of pitting. The cocktail does not contain a member in the Brassicacea family (Table 3.1), but produced percent pitting levels that were as high as the rotation with canola. The cocktail mix contains sudan, however the Potato-Rye/Sudan rotation had relatively low percent pitting levels. The inclusion of sudan or canola does not appear to be an indicator for what the level of pitting will be in the subsequent potato crop. Further testing needs to be done to validate these results.

Pectolytic Bacteria Numbers (Inoculated Seed vs Uninoculated Seed):

When tuber samples were evaluated for each of the different crop rotations, only the rotation that included canola showed a significant difference between the uninoculated and inoculated treatments at a p=0.05 level (Table 3.4). There was a significant difference between the uninoculated and inoculated treatments when evaluating both the stem ends as well as lenticels. Potato/barley/canola was the only rotation showing this difference when compared with the other rotations.

A possible explanation of this might be that since canola does not form a symbiotic relationship with mycorrhizae, some of the micro and macro nutrients essential to the potato plants' health and defense mechanisms were absent (Dabrowska et al. 2014; McGonigle et al. 2011; Mustafa et al. 2016; Valetti et al. 2016). This could explain why the potatoes from this rotation had more pectolytic bacteria in the stem end and lenticels of the harvested tubers than the other rotations. Higher levels of these bacteria in the stem ends as well as the lenticels of the tubers indicate that the chance of disease developing in the next cropping generation is higher. When seed from the potatoes in this rotation are planted the following year, the chance of blackleg and tuber soft rot developing are higher due to higher bacteria levels in the seed. Also, there was an increased amount of soft rot observed in this rotation when the seed was inoculated with *P. atrosepticum* (Table 3.3). Since canola was planted in the cropping year just prior to potatoes, additional experimentation needs to be done determining how the rotation order can affect the levels of bacteria and disease.

There was only a significant difference when the seed was inoculated with the bacteria in the canola rotation. This indicates that high bacteria numbers only appear to be an issue when pectolytic bacteria are at high levels in the system. This will not always be the case for every potato farmer. Only those farmers that have historically had problems with blackleg and tuber soft rot would be at risk when using canola as a rotational crop. Another factor would be the potato cultivar used by the grower as well, since different cultivars have varying levels of resistance and susceptibility to blackleg and soft rot (Czajkowski et al. 2011).

Canola could potentially still be in the cropping rotation and not result in an increase in tuber soft rot or % culls if it was not planted the year prior to potatoes. For example, if Barley were planted prior to potatoes, the negative effects of the canola may be reduced since barley, not canola, would have more impact on the potatoes due to its' being planted the previous cropping year. The use of different potato cultivars as well as a different rotation order are things that need to be evaluated to have a clearer picture of how canola effects the development of blackleg and tuber soft rot.

Conclusions:

The use of different rotational crop species has been used by farmers for centuries for the improvement of crop yield and quality. In recent years, there has been an increased interest in the use of different rotational crops. This is in part due to the negative reputation synthetic pesticides and fertilizers have concerning the environment and human health. There is much debate as to exactly what effect these products have. Nevertheless, the negative perception of these products is real. When using rotational crops, the impact different crop species have on the primary cash crop is of great importance. If there is too much of a negative effect by the crop used as a rotation (e.g. reduction in yield, increase in disease, etc.), a different crop species should be used in the rotation. This study evaluated seven different crop rotation systems with potatoes being the primary cash crop. A special emphasis was put on the effect these rotations had on the management of blackleg and tuber soft rot in a potato crop.

Most of the rotations used did not result in any significant difference when compared with the standard barley/potato rotation. There were no differences in yield or grade between the different rotations evaluated. This indicates that the crop species including barley, sudan, rye, canola, camolina and a seven-species mix can be used in a rotation with potatoes. The differences came when the potato seed was inoculated with the bacteria *P. atrosepticum*, the causal agent of blackleg and tuber soft rot. When these bacteria were present in high numbers in the seed, the crop rotations which included the brassicas, canola and camolina, resulted in higher percent culls, higher pectolytic bacterial numbers and higher levels of tuber soft rot. These differences were not observed when seed not inoculated with *P. atrosepticum* was used.

The use of brassica species in a field rotation with potatoes should be used with caution in farming operations that have a history of blackleg and tuber soft rot. Results from this study indicate that the use of crop species such as canola have a negative effect on potato crop health when high levels of pectolytic bacteria are present, but don't negatively impact the crop when low levels of pectolytic bacteria are present. Crop rotations that did not include the brassicas resulted in no differences in percent culls or tuber soft rot levels. The crop used in the rotation did not result in a significant change in potato quality for most of the rotations evaluated. Potato growers, as with all farmers, need to assess the pros and cons of each rotational crop they use to make sure they are not putting their farming operations at unnecessary risk for disease development and potential crop failure.

Tables:

Table 3.1. List of Crop Rotations Evaluated to Determine Effectivess at Reducing Blackleg and Tuber Soft Rot Incidence in Potato. SLV Research Center, Center CO (2013 & 2014).

Treatment #	Year 1	Year 2	Year 3	Year 4
1	Potato	Barley	Potato	Barley
2	Potato-Rye[a]	Sudan GM[b]	Potato-Rye[a]	Sudan GM[b]
3	Potato	Barley	Canola	Potato
4	Potato	Barley	Sudan GM[b]	Potato
5	Potato	Barley	Camolina	Potato
6	Potato	Barley	7 Species Cocktail GM[bc]	Potato
7	Potato-Rye[a]	7 Species Cocktail GM[bc]	Sudan GM[b]	Potato

[a]Potatoes were planted in the Spring and Rye was planted after potato harvest in the fall.

[b]GM = Green Manure; These crops were chopped and incorporated into the soil late in the growing season.

[c]The 7 species cocktail consisted of Arvika Field Pea (28%), Sorghum Sudan (22%), Buckwheat (14%), Oats (14%), Common Vetch (13%), Tiller Radish (6%), and Turnip (3%).

Table 3.2. Evaluation of potato yield as affected by challenge inoculation with *Pectobacterium atrosepticum*, mean of 2013 & 2014 data.

Trt #	Treatment	Total CWT a^{-1} (UI)[a]	Total CWT a^{-1} (I)[b]	Total CWT a^{-1} (Pairwise comparison between UI & I)[c]
1	Potato/Barley	364.2	347.3	0.7024
2	Potato-Rye/Sudan GM	352.4	283.7	0.1239
4	Potato/Barley/Canola	370.1	269.8	0.0254
5	Potato/Barley/Sudan GM	363.8	362.1	0.9696
6	Potato/Barley/Camolina	358.3	304.5	0.2268
10	Potato/Barley/Cocktail GM	303.5	354.9	0.2794
11	Potato-Rye/Cocktail GM/Sudan GM	366.3	310.0	0.2056
LSD		NS	NS	
CV		23.44	30.17	-
P value		0.72	0.36	-

[a]Potato seed was not inoculated (UI) with *Pectobacterium atrosepticum* when planted. Total yield is expressed as hundred weight per acre (CWT a^{-1}), 1-3.1 meter row per treatement per replication was harvested, mean of four replications and both years.

[b]Potato seed was uninoculated (UI) with *Pectobacterium atrosepticum* when planted. Total yield is expressed as hundred weight per acre (CWT a^{-1}), 1-3.1 meter row per treatement per replication was harvested, mean of four replications and both years.

[c]The pairwise comparisons between UI and I was made across years for the total CWT a^{-1} at harvest. P-values greater than 0.05 indicate that the I and UI potato rows are are not significantly different from each other (mean of 2013 & 2014 data, four replications per year).

Table 3.3. Evaluation of potato seed challenge inoculated with *Pectobacterium atrosepticum* to determine effect on soft rot incidence, mean of 2013 & 2014 data.

Trt #	Treatment	Number Rotten Tubers (UI)[a]	Number Rotten Tubers (I)[b]	Pairwise comparison between UI & I[c]
1	Potato/Barley	0.3	0.3	1.0000
2	Potato-Rye/Sudan GM	0.1	0.8	0.0605
3	Potato/Barley/Canola	0.0	0.9	0.0092
4	Potato/Barley/Sudan GM	0.3	0.3	1.0000
5	Potato/Barley/Camolina	0.3	0.4	0.7048
6	Potato/Barley/Cocktail GM	0.1	0.8	0.1115
7	Potato-Rye/Cocktail GM/Sudan GM	0.3	0.5	0.4492
LSD		NS	NS	-
CV		253.05	151.73	-
P value		0.90	0.57	-

[a]Potato seed was not inoculated (UI) with *P. atrosepticum* when planted. Total number of tubers expressing symptoms of soft rot at harvest, mean of four replications and both years.

[b]Potato seed was inoculated (I) with *P. atrosepticum* when planted. Total number of tubers expressing symptoms of soft rot at harvest, mean of four replications and both years.

[c]The pairwise comparison between UI and I was made across years for the total number of rotten tubers at harvest. P-values greater than 0.05 indicate that the I and UI potato rows are not significantly different from each other (mean of 2013 & 2014 data, four replications per year).

Table 3.4. Evaluation of potatoes both challenge inoculated and uninoculated with *Pectobacterium atrosepticum* to determine effect on stem end and lenticel pitting on CVP, mean of 2013 and 2014 data.			
		% Pitting Data[a]	
Trt #	Treatment	Stem end	Lenticel
1	Potato/Barley	0.6946	0.6463
2	Potato-Rye/Sudan GM	0.4062	0.0770
3	Potato/Barley/Canola	0.0497[b]	0.002[c]
4	Potato/Barley/Sudan GM	0.4323	0.8185
5	Potato/Barley/Camolina	0.0774	0.0665
6	Potato/Barley/Cocktail GM	0.3354	0.1255
7	Potato-Rye/Cocktail GM/Sudan GM	0.2751	0.3205

[a]The pairwise comparisons between samples collected from field plots planted with Inoculated seed (I) and seed that was uninoculated (UI) were made across years for the % pitting data. P-values greater than 0.10 indicate that the I and UI potato rows are are not significantly different from each other (mean of data from 2013 and 2014 and four replications per year).

[b]Percent pitting was higher in the tuber stem ends from the samples pulled from the seed inoculated with *P. atrosepticum* (11.3%) compared with the seed that was not inoculated (5.0%).

[c]Percent pitting was higher in the tuber lenticels from the samples pulled from the seed inoculated with *P. atrosepticum* (10.4%) compared with the seed that was not inoculated (4.8%).

Figures:

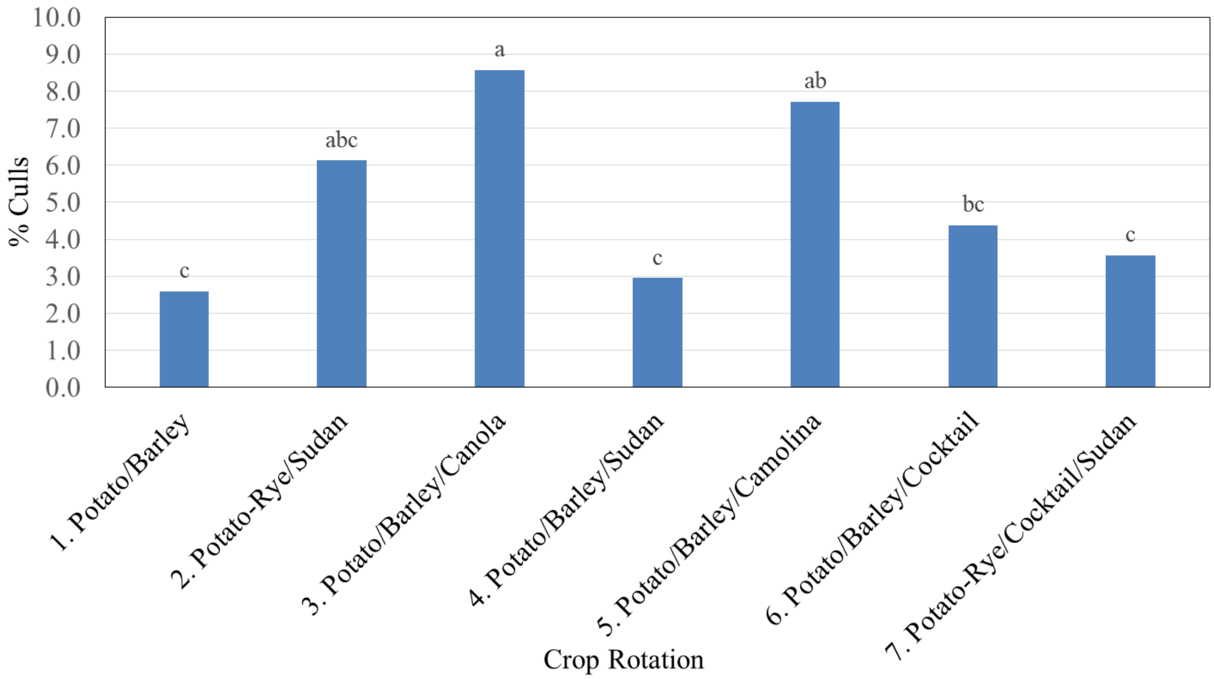


Figure 3.1. Evaluation of Rotational Crops for Potato Quality at Harvest. The seed was inoculated with *Pectobacterium atrosepticum* at planting. Culls are defined as any tuber not meeting grade standards (e.g. misshaped, green coloration, etc.). Percent culls are based on tuber weight at harvest. Data collected on one 10 foot row per treatment per replication, mean of 2013 and 2014 data. Means followed by the same letters are not significantly different at P=0.05 (LSD).

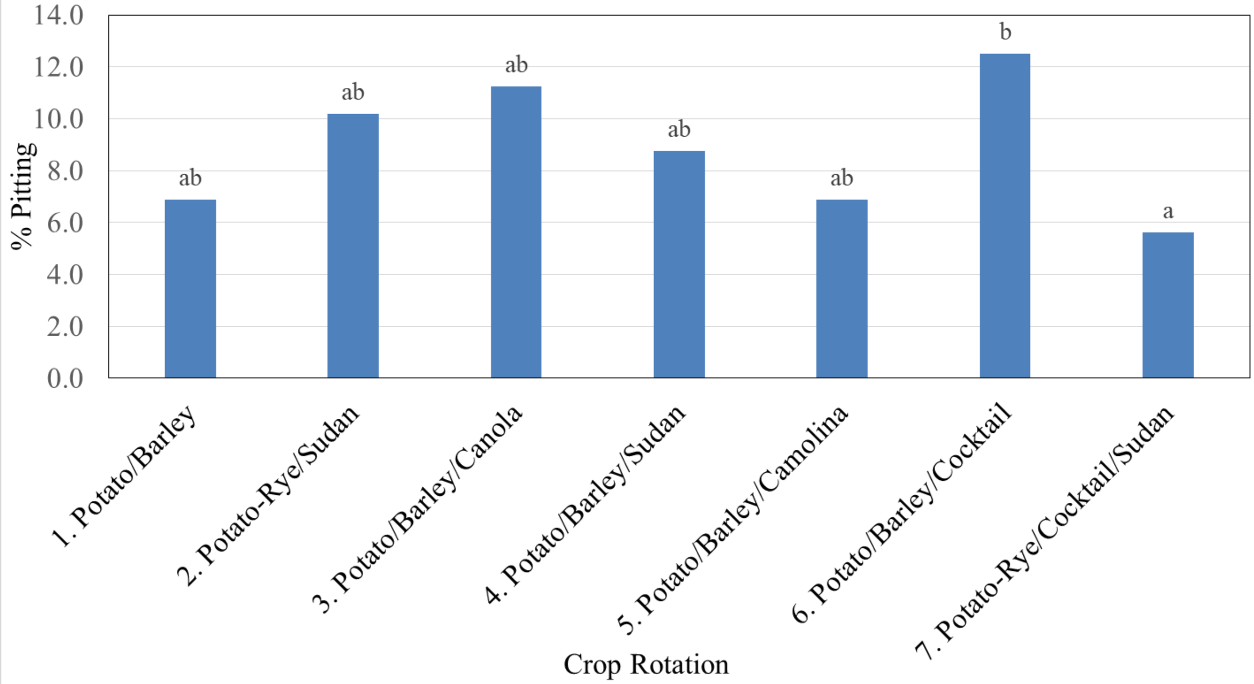


Figure 3.2. Evaluation of Tuber Samples for Pectolytic Bacteria using pitting reaction to Crystal Violet Pectate (CVP) Media: Stem End Data. Percent pitting is defined as the number of pits develping on CVP media after being inoculated with tuber stem end material by a sterile toothpick. A sample of 20 tubers per crop rotation per replication was used to determine pitting percentage, mean of 2013 and 2014 data. Means followed by the same letters are not significantly different at P=0.05 (LSD).

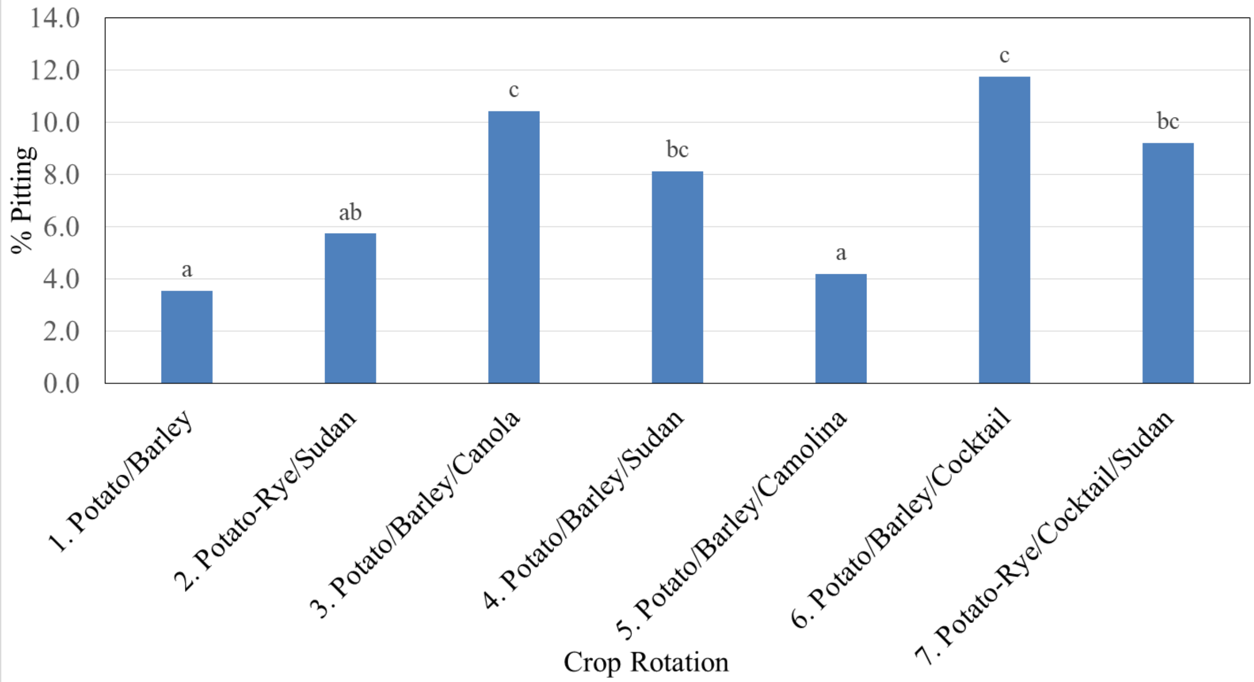


Figure 3.3. Evaluation of Tuber Samples for Pectolytic Bacteria using pitting reaction to CVP Media: Lenticel Data. Percent pitting is defined as the number of pits develping on CVP media after being inoculated with tuber stem end material by a sterile toothpick. A sample of 20 tubers per crop rotation per replication was used to determine pitting percentage. Three randomly selected lenticels per tuber were used to detemine percent pitting, mean of 2013 and 2014 data. Means followed by the same letters are not significantly different at P=0.05 (LSD).

LITERATURE CITED

Aleck, J.R. and M.D. Harrison. 1978. Influence of inoculum density and environment on development of potato blackleg. American Potato Journal 55: 479-494.

Bezuidenhout, S.R., C.F. Reinhardt, and M.I. Whitwell. 2012. Cover crops of oats, stooling rye and three annual ryegrass cultivars influence maize and *Cyperus esculentus* growth. Weed Research 52: 153-160.

Czajkowski, R., M.C.M. Perombelon, J.A. van Veen, and J.M. van der Wolf. 2011. Control of blackleg and tuber soft rot of potato caused by *Pectobacterium* and *Dickeya* species: A review. Plant Pathology 60: 999-1013.

Dabrowska, G., C. Baum, A. Trejgell, and K. Hrynkiewicz. 2014. Impact of arbuscular mycorrhizal fungi on the growth and expression of gene encoding stress protein - metallothionein BnMT2 in the non-host crop *Brassica napus* L. Journal of Plant Nutrition and Soil Science 177: 459-467.

Duffy, E.M., and A.C. Cassells. 2000. The effect of inoculation of potato (*Solanum tuberosum* L.) microplants with arbuscular mycorrhizal fungi on tuber yield and tuber size distribution. Applied Soil Ecology 15: 137-144.

Howard, R.J. 1996. Cultural control of plant diseases: A historical perspective. Canadian Journal of Plant Pathology-Revue Canadienne De Phytopathologie 18: 145-150.

Koide, R.T., and M.S. Peoples. 2012. On the nature of temporary yield loss in maize following canola. Plant and Soil 360: 259-269.

Larkin, R.P., T.S. Griffin, O. Olanya, G.C. Starr, and C. Honeycutt. 2007. Effects of different soil and crop management strategies on soil microbial communities and soilborne diseases of potato. Phytopathology 97: S61-S62.

Larkin, R.P., and C.W. Honeycutt. 2006. Effects of different 3-year cropping systems on soil microbial communities and *Rhizoctonia* diseases of potato. Phytopathology 96: 68-79.

Masiunas, J.B., L.A. Weston, and S.C. Weller. 1995. The impact of rye cover crops on weed populations in a tomato cropping system. Weed Science 43: 318-323.

McGonigle, T.P., M. Hutton, A. Greenley, and R. Karamanos. 2011. Role of mycorrhiza in a wheat-flax versus canola-flax rotation: A case study. Communications in Soil Science and Plant Analysis 42: 2134-2142.

Mustafa, G., B. Randoux, B. Tisserant, J. Fontaine, M. Magnin-Robert, A.L.H. Sahraoui, and P. Reignault. 2016. Phosphorus supply, arbuscular mycorrhizal fungal species, and plant genotype impact on the protective efficacy of mycorrhizal inoculation against wheat powdery mildew. Mycorrhiza 26: 685-697.

Perombelon, M.C.M., and A. Kelman. 1980. Ecology of the soft rot *Erwinias*. Annual Review of Phytopathology 18: 361-387.

Peters, R.D., A.V. Sturz, M.R. Carter, and J.B. Sanderson. 2003. Developing disease-suppressive soils through crop rotation and tillage management practices. Soil and Tillage Research 72: 181-192.

Urbano, B., F. Gonzalez-Andres, and A. Ballesteros. 2006. Allelopathic potential of cover crops to control weeds in barley. Allelopathy Journal 17: 53-64.

Valetti, L., L. Iriarte, and A. Fabra. 2016. Effect of previous cropping of rapeseed (*Brassica napus* L.) on soybean (*Glycine max*) root mycorrhization, nodulation, and plant growth. European Journal of Soil Biology 76: 103-106.

Walsh, K.D., D. Sanderson, L.M. Hall, S. Mugo, and M.J. Hills. 2014. Allelopathic effects of camelina (*Camelina sativa*) and canola (*Brassica napus*) on wild oat, flax and radish. Allelopathy Journal 33: 83-95.

CHAPTER 4: MANAGEMENT GUIDELINES FOR BLACKLEG AND TUBER SOFT ROT ON A POTATO FARMING OPERATION

Introduction:

Bacteria belonging to the genera *Pectobacterium* and *Dickeya* cause blackleg (stem and foliar disease) and tuber soft rot (tuber disease) in potatoes. These bacteria (formerly grouped in the genus *Erwinia*) are facultative anaerobes, favoring an environment with a lack of oxygen but can be functional with relatively good growth in an environment with oxygen (Czajkowski et al. 2011). It has been estimated that there are between $50 to 100 million in losses to potato producers as a result of tuber soft rot and blackleg (Perombelon et al. 1980).

The infection of a potato plant by these bacteria can cause disease in three primary ways. Blackleg disease can develop in the stem(s) of the potato plant. Bacteria present in the seed piece, as well as in soil if grown in tropical climates, will migrate up the stem through the vascular tissue into both the below and above ground portions in the diseased stem tissues.

Blackleg symptoms include water soaked lesions that are light brown to black in color in the potato stem, resembling the appearance of black ink (Figure 4.1). The foliage of an affected stem can also have a wilted appearance due to the lack of water and nutrient transport within the stem tissues. Ultimately, this causes an overall sickening of the plant, thereby indirectly reducing tuber yields (DeBoer 2004; Stevenson 2001). Another way in which the disease can be expressed is tuber soft rot. A seed piece that is infested with bacteria often rots in the field; decaying tubers release large numbers of bacteria into the root zone which can then spread in the soil and contaminate daughter tubers at the lenticels or in wounds from cultivating, harvesting, etc. Also, tuber lesions can develop at the point where the stolon attaches to the tuber. The affected tuber tissue is typically cream to tan colored and is soft and granular (Figure 4.2). There is also a well-defined margin between the healthy and rotten potato tissue (DeBoer 2004; DeBoer 2008; Stevenson 2001).

Finally, the less common aerial stem rot can occur when lesions develop in petioles and stems. In this case, bacteria do not typically originate from the seed piece. Instead, the bacteria originate from other infected plants, infested irrigation water, insects, etc. and land on healthy plants which may have wounds from any number of sources. This decay tends to be confined to the above-ground portions of the plant with symptoms similar to that of blackleg. This method of decay normally does not translocate bacteria from the above ground portion of the plant to the tubers in the soil. Aerial stem rot is detrimental to the overall health of the plant and can decrease yield (DeBoer 2004; Stevenson 2001) .

In 2015, there was an increased interest in *Dickeya* spp. and their role in blackleg and tuber soft rot development in the United States. *Dickeya* spp. was identified in Maine and has caused major blackleg outbreaks there. *D. dianthicola* has been identified in several states after the find in Maine. The concern is that if *D. dianthicola* appeared in Maine, could *D. solani* be next? *D. solani* is a much more virulent species and causes greater damage than *D. dianthicola*. Currently, potato production areas are concerned about the potential spread of *Dickeya* spp. to other parts of the country. State seed certification agencies are watching for this species and testing for its presence when needed. There is a national effort to reduce the potential spread of this pathogen between states and different potato growers (Charkowski 2016).

D. dianthicola and *D. solani* both favor warmer climates than *Pectobacterium* spp. and disease is typically a result of the bacteria moving from the seed piece to the stem base. Both genera of bacteria have also been shown to survive and remain pathogenic in contaminated irrigation water (ADHB-Potato Council 2007).

In the San Luis Valley of Colorado, *Dickeya* spp. has been identified in winter test backup seed samples for certification. It is still unclear at this time whether the climate in the San Luis Valley is favorable for blackleg development from this bacteria. More investigations will be conducted by seed certification officials and potato pathologists from Colorado State University in the upcoming years to determine the risk *Dickeya* poses to the potato producers in the San Luis Valley (Colorado Potato Certification Service 2017 Records; A. Charkowski, personal communication).

Both *Pectobacterium* spp. and *Dickeya* spp. can be spread to healthy tubers during seed cutting, handling, and planting operations and are able to rapidly multiply during times of high moisture around the seed piece. This is especially critical when tubers with a pulp temperature lower than the soil are planted. This encourages moisture to form around the seed piece helping to reduce O_2 near the tuber surface and increases the likelihood that soft rotting bacteria can freely multiply. Unsuberized, fresh cut seed planted into these conditions can be even more problematic. Also, the use of effective seed treaters like fungicides which help control problems like *Fusarium* and pink rot are essential if soft rot decay is potentially a problem. *Pectobacterium* spp. are excellent secondary invaders through wounds made by these other pathogens and soft rot may be much worse in a field when tubers are also infected with these other diseases (Stark and Love 2003). Thus, high soil temperatures (>68 ºF) coupled with wet soils tend to favor seed piece decay. Blackleg can appear in the stems when tubers encounter cool (50-59 ºF), wet soils at planting, followed by high soil temperatures (>68 ºF) after plant emergence.

These bacteria can be spread to non-infected plants through irrigation water, decaying seed pieces, insects, adverse weather such as hail, and crop debris in soil that contains the bacteria. Wind can also result in wound development in an infected plant, which allows for the bacteria to be spread to an adjacent plant through contact. Bacteria movement can also occur through the stems and stolons of infected plants to the daughter tubers, thereby infecting the daughter tubers. Infection of these daughter tubers often remains latent through the storage season. If the daughter tubers are then used to plant the following year's crop the bacteria can be carried over to the following year's crop. Field blackleg can develop and tuber soft rot can develop in storage after harvest continuing the cycle through field generations (DeBoer, 2004; Stevenson, 2001). It is of note that whenever there are high bacterial densities present, such as around a rotting tuber, a high level of contamination to other healthy plant parts is more likely (Czajkowski et al, 2011).

There are several techniques a potato grower can use to manage and reduce the amount of blackleg and soft rot in a potato lot. The best known management strategies are presented in this article. Among the very best approaches a grower can use is to carefully monitor seed lots and flush out those lots which have higher levels of blackleg or soft rot based upon inspection results, testing, or other available evidence such as how the lot maintained during the storage season. When purchasing seed, always buy the best certified seed available with the lowest known blackleg levels. If a certification program does not have this information available, growers should insist on obtaining this information either through direct testing of the seed lot or working closely with others to monitor the seed during its growth. Over the years, those growers who purchase or plant seed with the lowest blackleg readings usually have few if any blackleg or soft rot problems during the season and into storage.

Another strategy is the use of proper vine desiccation. If green vines are not desiccated, but chopped while green and blackleg is present in the field, a very small percentage of blackleg (less than 1%) can translate into a very high percent of infected plants (over 30%) with bacteria traveling to the daughter tubers within a two week period (Davidson, unpublished data). However, if green vines are chopped and an effective, fast acting desiccant like sulfuric acid is utilized within 48 hours of chopping, bacterial transmission is stopped. Adjusting all equipment to minimize plant and/or tuber wounds, proper disinfection of all equipment contacting any part of the potato during the growing season or at harvest, and minimizing healthy tuber contact with infected or rotting tubers during piling are also essential strategies. When harvesting and piling potatoes, a 0.5% level of rotted tubers in the field can result in a 5% or more infection in the crop the following year just from rotted tubers rolling around during the harvest and piling operation and coating healthy tubers with bacterial slime (R.D. Davidson, unpublished data). However, if one looks at the start of the growing season, potentially one of the most critical of all factors in controlling spread of *Pectobacterium* spp. is during the seed cutting phase of production.

Seed Cutting Techniques:

In potato farming operations in North America the cutting of seed is a common practice in preparation for planting a potato crop. Cutting seed results in an increase in the number of seed pieces available to plant within very specific size ranges and eye numbers. Since potatoes are vegetatively propagated, a potato tuber has the ability to give rise to a potato plant when conditions are favorable for plant growth, so long as an eye is present and the tuber has broken dormancy. The cutting of seed typically involves the use of machinery that is specifically designed to cut seed potatoes. Much of the seed cutting in modern potato farming operations happens on a large scale and includes many opportunities for the seed pieces to come into contact with the various belts, rollers, knives and storage containers, and potentially pick up blackleg and soft rot causing bacteria if present on these parts (Rowe 1993). While the ability to cut seed in potato production can result in cost savings and an increase in efficiency for the farmer, the potential to spread potato pathogens (fungi, bacteria, and viruses) among the seed tubers and seed pieces also increases. *Clavibacter michigenensis* subsp. *sepedonicus*, the causal agent of ring rot, and the soft rot causing bacteria (*Pectobacterium* spp. and *Dickeya spp.*) are the bacterial pests of most concern which might be spread through seed cutting operations (Charkowski 2015; Rowe 1993).

A research project conducted in the San Luis Valley during the spring of 2014 and 2015 identified areas in the seed cutting process that could promote the spread of blackleg and soft rot causing bacteria. The cutting practices of two seed potato growers were evaluated. Grower 1 had a history of blackleg and grower 2 had a history of very little blackleg. Grower 1 used an automated Milestone Potato Seed Cutter (Model 84-D/72-D) and disinfected the circular cutting blades and rollers with Hyamine, a quaternary ammonia compound prepared as a 1% dilution, (a.i. N-Alkyl dimethyl benzyl ammonium chloride) with a 10 minute soak time between the cutting of each seed lot (Figure 4.3). The majority of seed cutting is automated with the Milestone cutter, except for a few large potatoes that pass through the cutter, which are cut by hand using stationary blades. These stationary knives were not routinely sanitized.

Grower 2 used a Potato Seed Hot Cutter manufactured by Vemco Electrical Contractor Inc., located in the San Luis Valley, CO (Figure 4.4). This company custom builds these seed cutters (vemcoelec.com) based on the premise of the "Forney Hot Cutter" developed in the 1980's (Davidson personal communication). The cutter uses electricity to continuously run a current through steel blades, raising the temperature of each blade to between 204 and 260ºC (400 – 500ºF). The cutting on this machine was done by farm labor physically cutting the potatoes on these blades. A conveyor belt carried the potatoes to and from the blades once the seed was cut. For both growers, the belts that carried the seed were not routinely disinfected.

Different locations on the seed cutter used by each operation were tested to determine bacterial levels. The surfaces evaluated on each seed cutter were the knives (both stationary and circular) and the belts (both before and after the fungicide seed treatment was applied to the cut seed).

A sterilized moistened Q-tip was used to collect the bacterial sample from the surface of either the knives or belts for each seed cutter surface evaluated. The Q-tips were then brought back to the lab and were swabbed on fresh Nutrient Agar (NA) media and incubated in an environment lacking oxygen for four days. Ten colony forming units (cfu's) were randomly chosen from each NA plate after the 4 day incubation and were used to inoculate Crystal Violet Pectate (CVP) plates using autoclaved sterile toothpicks. A modified CVP medium was used, similar to that used by Perombelon and Burnett (1991). After inoculation of the CVP, the plates were allowed to incubate in an anaerobic chamber for 4 days prior to evaluation for the presence of pitting. Any active colonies of soft rot/blackleg causing bacteria resulted in pitting on the CVP plates (Figure 4.5). Evaluating the different seed cutters used by these operations was helpful in understanding why there were differences in blackleg disease expression as seen in summer field inspections. Several significant observations were made as the evaluations took place. First, the use of a liquid seed treatment tended to increase bacterial levels on the conveyor belts after the cutting and treatment of the seed, although this was not statistically significant. When the seed and belts were allowed to dry or when a seed treatment with a dry formulation was used, the levels of bacteria were reduced, which was also shown by work done by Bdliya et.al. (2007). The cost of using a liquid seed treatment or a dry seed treatment is similar. Since grower 1 has a history of high blackleg levels in summer field inspections, a potential fix to his blackleg problem could be to either change the seed cutting practices by using a dry seed treatment or to set up fans to help dry the seed and the belts after the seed is cut and treated. Changing the location of the seed treater and belts to an outside location could also be used to solve the excess moisture problem. This could potentially expose the seed and the belts to solar radiation and lower relative humidity since the San Luis Valley is located in a desert with less than 10 inches of annual rainfall, mild breezes, and a typical relative humidity level in the 30% range (2016). However springtime in the San Luis Valley can be unpredictable with freezing temperatures and variable weather. This can often prevent the use of equipment and the cutting and treating of seed outside. In the case of unfavorable weather, the use of a dry seed treatment instead of a liquid treatment or the use of fans to help dry the freshly cut seed could greatly reduce the excess moisture found on the cutting surfaces. This would be a relatively inexpensive solution to the problem of having too much free moisture on the belts.

Another discovery was that by using the hot cutter and proper sanitation, there was a reduction in pectolytic bacteria numbers found within the system (Figure 4.6). The killing of potential pathogens, such as bacteria, through the use of a heat sterilized blade was shown to be an effective method of sanitation in this study. Unlike the drying of cut seed, a change in the method a grower uses to cut seed could be fairly cost prohibitive. However, if the blackleg problem on any given operation was severe enough, the additional cost of a new seed cutter and additional labor might be worth it.

One final note, there have been numerous resources spent on using physical control methods to help manage soft rot and blackleg. Among these are the use of antibiotic seed treaters after cutting (normally Streptomycin based products), biologicals, hot water dips, formaldehyde, etc. Some of these approaches have been more successful than others. Growers should verify which of these approaches may be feasible and how they may work on their own operations.

Differences in Management Practices:

Avenues of introducing *Pectobacterium* into a farming operation have not been fully elucidated, but there has been some progress. One avenue for introducing bacteria into the system, and probably the most important, is the use of infected seed in farming operations (Perombelon 2000). Several studies have shown that the primary origin and spread of *P. atrosepticum* and *P. carotovorum* is from seed tubers infected with the bacteria (Ali et al. 2012; Toth et al. 2003). The infected tubers act as an inoculum source for the progeny tubers. Large numbers of pathogenic bacteria are released into the soil rhizosphere as the mother tubers rot and are spread to the progeny tubers during the season (Lapwood and Harris 1980). The longer a seed lot is grown in the field, the greater the potential for increasing disease. Thus, the amount of pathogenic bacteria and viruses present in and on the seed piece could be higher in later generation seed (Rowe, 1993).

A research project evaluating two seed potato growers in the San Luis Valley, CO evaluated two Generation 4 (G4) seed crops of the potato cultivar Satina. Each of these crops were tested for relative pectolytic bacterial levels in the seed. The two Satina crops were grown on two different grower operations (grower 2 and grower 3). These two seed crops were chosen based on the % blackleg observed during the certification field inspection in each crop in the summer of 2013, which was the fourth field year (Table 4.1). A sample of 40 seed potatoes was collected and tested for each seed lot at two sampling times. The stem end of each seed potato was poked and plated on CVP and read in the fall of 2013 and spring of 2014.

The % pitting for the potato seed stem ends was significantly lower in grower 2 than in grower 3 (Figure 4.7). Also, in the fall of 2013, the stem ends had a significantly higher amount of pitting than the lenticels for grower 3 (22.50% vs. 5.83%, p value = 0.0019). In the summer of 2013, a high amount of blackleg was observed in the seed crop from grower 3 (Table 4.1). Since bacteria can travel from a symptomatic plant to the daughter tuber via the stolon, it would explain why the tuber stem ends showed a higher percentage of pitting on the CVP plates in grower 3's crop. A higher % pitting would indicate that there was a relatively high level of pectolytic bacteria in the seed lot that was carried over from the summer field season. This difference in % pitting between the two lots was not detected when the sample was evaluated in the spring of 2014, indicating that the fall might be a better time to evaluate a seed sample for pectolytic bacterial activity. Another explanation might be two-fold. Grower 3's tubers could have seen a decrease in bacterial populations during the storage season, a common occurrence as tubers move into the holding phase of storage if conditions are less favorable for bacterial growth. As a result bacterial numbers can decrease during this time (Pringle and Robinson 1996; Vanvuurde and Devries

1994). Additionally, more rapid cooling of the storage to the holding temperature with good airflow to reduce low O_2 conditions can be beneficial to keeping bacterial numbers at bay (Bohl and Johnson, 2011). Grower 2's tubers could have seen an increase in bacterial populations since the samples were stored with other seed lots with high levels of bacteria present. Soft rot bacteria can move in storage when conditions are right and seed lots with high bacterial levels are being sorted nearby (Graham and Harrison, 1975; Perombelon, 1992). Results from this study indicate that differences in bacterial numbers in seed between growers can be detected and can correspond to blackleg readings in the field. The % pitting data confirmed the high level of blackleg from the previous summer's Satina crop. However, this is not always the case (Perombelon et al. 1980). More work needs to be conducted before a reliable test can be used to predict blackleg outbreaks, but this research does confirm that differences exist between growers. If differences exist, then it can be deduced that alternative management strategies can be used to manage bacteria levels, which can then result in a reduction of blackleg.

Crop Rotation:

Rotating crops can maintain a good soil structure and can increase soil organic matter, which reduces soil erosion. Continuous cropping with a susceptible host can increase levels of plant pathogens in the soil which reduces crop yield and quality. The use of crop rotations can reduce soil borne pathogens in three primary ways: 1) disrupting the host-pathogen cycle, 2) altering soil characteristics and soil microbial communities and3) directly inhibiting pathogens through the crop's production of toxins or by increasing the numbers of antagonistic microorganisms (Peters et al. 2003). The length of time or number of seasons between crops has an impact on the effectiveness of crop rotations. For example, a two-year rotation reduces disease levels more effectively than no rotation. Also, a three-year rotation tends to reduce potential disease incidence better than a two-year rotation. The crop planted just prior tends to have the most influence on the following crop, however, the previous crops also have an impact on disease levels, but to a lesser degree (Larkin and Honeycutt 2006).

A study was conducted in the San Luis Valley, CO with the purpose of determining the effect of different crop rotations on potato yield and quality, blackleg and tuber soft rot incidence and severity in potato seed. Two-year and three-year crop rotations were evaluated, making for a total of seven different rotations. Crop rotations were started in the 2011 and 2012 growing seasons and data was collected in the 2013 and 2014 growing seasons. The species of agricultural crops evaluated in this study were chosen because of their ability to grow well in the high alpine environment of the San Luis Valley of Colorado (Table 4.2).

In both years, one row of seed tubers inoculated with pathogenic *Pectobacterium atrosepticum* was planted in a row adjacent to a row of healthy potatoes, which served as an untreated control. Seed from the potato cultivar Colorado Rose, which is susceptible to tuber soft rot, was freshly cut and inoculated with the bacterial slurry. Data was collected from the uninoculated and inoculated potato rows. Blackleg incidence was determined through the collection of several visual readings in August of each year which is when blackleg tends to express in the San Luis Valley. At harvest, ten potato plants were harvested from the middle of each plot and all tubers were evaluated for yield, grade, and the presence of tuber soft rot. The study was irrigated using a solid set sprinkler system. A randomized complete block design was used for this study. Data was collected on two rows (one inoculated and one uninoculated) from the middle 10 feet of each plot in 2013 and 2014. An LSD mean separation and paired t-tests were used to analyze the data across crop rotations and years.

When evaluating the uninoculated potato crop, there were no significant differences between crop rotations for size and quality profiles, total yield, or number of rotten tubers. This indicated that when bacterial levels were relatively low in potato seed, different crop rotations did not tend to have a major impact on blackleg or tuber soft rot. This provided evidence that the use of these rotations will not result in higher levels of blackleg and tuber soft rot when compared with the standard potato/barley rotation (data not presented). However, in other studies (Larkin and Honeycutt 2006, Walsh et al. 2014) rotations have been shown to have a significant impact on blackleg levels.

When evaluating the yields of the inoculated potatoes, a significant difference was observed in the percent culls (Figure 4.8). The rotation including canola (Trt. 3) had the highest percent culls (8.6%) when compared with the other rotations. The potato/barley, potato/barley/sudan and potato-rye/cocktail/sudan rotations (Trts 1,4,7) had the lowest percent culls of any of the rotations evaluated.

Culls are typically characterized by the presence of misshapen tubers, growth cracks, and other external defects. When canola and camolina were grown the year prior to potatoes, there was a significantly higher number of culls than in the standard potato/barley rotation in the potatoes inoculated with *P. atrosepticum*. These crop species are known to produce allelochemicals which can negatively affect the following crop's growth. Also, canola and camolina do not form a symbiotic relationship with arbuscular mycorrhizae which can negatively affect the subsequent potato crop (Koide and Peoples 2012; Walsh et al. 2014). Mycorrhizae provide potatoes with essential micronutrients which are, in part, responsible for a higher percentage of US number 1's in a potato crop (Duffy and Cassells 2000). The absence of mycorrhizae can be an attributing factor in the production of more cull potatoes in a potato crop. Since this difference was observed in the potatoes inoculated with *P. atrosepticum* and not in the uninoculated potatoes, it can be deduced that inoculating the seed with a pathogenic bacteria affected the harvestable crop negatively when the potatoes were followed by a crop of canola or camolina. Conversely, we can conclude that when potatoes with high levels of pectolytic bacteria are planted in a field following a rotational crop other than canola or camolina, the potato crop is better suited to defend itself against a bacterial pathogen, such as *P. atrosepticum*. When a rotational crop that promotes mycorrhizal growth is planted the year proceeding potatoes, the potato plants have a better chance of warding off disease caused by pathogenic bacteria.

When comparing the number of rotten tubers, the inoculated rows had significantly higher numbers than the uninoculated rows following canola (Table 4.3). This indicates that planting potatoes which are more susceptible to developing tuber soft following canola increases the likelihood of having higher levels of tuber soft rot at harvest. When *P. atrosepticum* is present in high numbers in potato seed, the crop rotations which include the brassicas, canola and camolina, result in higher percent culls and higher levels of tuber soft rot. However, crop rotations that did not include the brassicas did not result in difference in percent culls or tuber soft rot levels. The crop used in the rotation did not result in a significant change in potato quality for most of the rotations evaluated.

Surface Irrigation Water:

Another possible avenue for the introduction of blackleg causing bacteria into a potato field is through contaminated irrigation water (Cappaert et al. 1988; Harrison et al. 1987; McCarterzorner et al. 1984; Perombelon 1992). McCarter-Zorner et al. conducted a survey of water sources in Southern Scotland and Colorado. It was found that *P. carotovorum* was generally present in surface water from drains, ditches, streams, rivers, lakes and reservoirs. Well water was not identified as a source of the bacteria in southern Scotland and Colorado. In Colorado, a high percentage of water samples taken from the Rio Grande River contained *P. carotovorum* (McCarterzorner et al. 1984). However, Maddox and Harrison sampled well water from the San Luis Valley, CO and found *P. carotovorum* as well as *P. atrosepticum* (Maddox and Harrison 1988). No *Dickeya* spp. was found in this survey (Harrison et al. 1987). In the San Luis Valley, CO the Rio Grande River, Saguache Creek, and San Luis Creek all contained *Pectobacterium* spp. (Harrison et al. 1987; Maddox and Harrison 1988).

P. carotovorum has also been collected in rain water. It has been proposed that this may be the result of bacteria being washed out of the atmosphere. If the bacteria is in aerosol form, a rain or irrigation event can wash the bacteria out of the atmosphere, resulting in the contamination of the falling water. This is one possible explanation of why a supposedly bacteria-free potato crop can become contaminated with *P. carotovorum* (McCarterzorner et al. 1984).

Dickeya spp. tends to have a lower survivability in surface water than the *Pectobacterium* spp. Although *Dickeya* spp. are not commonly found in surface water in North America, they have been found in irrigation ponds containing recycled water in Florida. The survivability of *Dickeya* spp. in surface water is dependent on the buffering effect of the water as well as the presence of nutrients in the water

(Toth et al. 2011). These reports show that irrigating a potato crop with contaminated surface water can inoculate the crop with blackleg causing bacteria.

Soil Contamination:

The majority of literature available on this topic indicates that the pectolytic bacteria involved in blackleg and tuber soft rot will not survive and remain viable in the soil in the absence of infected plant material (Czajkowski et al. 2011). However, there is also a body of work indicating that *Pectobacterium* spp. can overwinter in the soil under certain conditions (DeBoer 1989; Meneley and Stanghellini 1976). Something that may be complicating the issue and making it more difficult to get a consensus on the ability of these soft rot bacteria to survive in the soil is the fact that they can easily overwinter on susceptible plant debris and in the rhizosphere of host plants. There are also several weed species that are hosts and could be acting as inoculum reservoirs (Ali et al. 2012; McCarterzorner et al. 1985). Also, during potato harvest operations, infected tubers left in the field could give the bacteria a habitat for the winter season in temperate climates (DeBoer 1989; Perombelon and Kelman 1980). It has also been reported that the different species of soft rot causing bacteria have different rates of survivability in the soil. This may explain some of the discrepancies that have been observed when researching the survivability of soft rot bacteria in soil (DeBoer 1989).

Wind and Insects as a Source of Contamination:

P. atrosepticum has also been shown to move and infect injured potato plants via aerosols in the wind and insect transmission (Graham and Harrison 1975; Kloepper et al. 1981). It has been shown that *P. atrosepticum* can be picked up from a potato stem that is expressing blackleg symptoms by water aerosols. This has major implications in its spread from a diseased plant to a healthy plant and could be a potential initial source of the bacteria in potato crops that are planted with seed that is free from the bacteria (Graham and Harrison 1975). This is especially true for areas that depend heavily on overhead irrigation, such as the San Luis Valley.

Hail events can also affect a potato crop and can potentially produce aerosols generated by the rain that accompanies the hail as the potato stems are damaged. Bacteria that are picked up by the aerosols can then be transported up to four meters or more to adjacent plants, which are more likely to be damaged by the hail (Graham and Harrison 1975). The bacteria is much more likely to cause blackleg in a plant if the plant is freshly damaged. Damage in potato plants can also be a concern if potato cull piles, which can harbor bacteria carrying insects, are in close proximity to the plants.

In a study conducted by Kloepper et al. (1981), adult fruit flies were shown to transmit blackleg causing bacteria to injured potato plants. The insects picked up the bacteria in a cull pile which was located within 217 yards of the injured plant. The authors also determined that transmission occurred more readily during the warmer parts of the day than in the cooler parts of the day. Potato cull piles, which can harbor pathogenic bacteria and are attractants for bacteria harboring insects, can serve as reservoirs of inoculum which can result in the introduction and spread of the bacteria. Growers should remove these cull piles from potato fields in order to reduce blackleg and tuber soft rot levels. Aerosols from overhead irrigation, rainfall and insects carrying the bacteria from cull piles may serve as sources of inoculum for early generation seed potatoes.

Nutrient Imbalances:

Imbalances in nutrient levels in a potato plant can also provide conditions favorable for blackleg and tuber soft rot development (Bain et al. 1996; Czajkowski et al. 2011; Kumar et al. 1991). Kumar et al. (1991) determined that as the amount of nitrogen applied in season increased, the percentage of rotting tubers in storage also increased. They concluded that the increase of nitrogen also led to a decrease in phenolics in the tuber, thereby reducing the tubers' resistance to rotting.

In a study conducted by Bain et al. (1996), the addition of gypsum ($CaSO_4$) was shown to decrease the levels of blackleg and tuber soft rot in a field environment. Seed tubers in this study were inoculated with *P. atrosepticum* and the soil was amended with gypsum at different rates prior to planting. Calcium levels in the stems and tubers were elevated in the field plots where gypsum amendments were applied. Calcium can improve the structure and stability of plant cell walls which provide an improved resistance to pathogens that depend on the macerating of plant tissue, like *P. atrosepticum* (Czajkowski et al. 2011).

Nitrogen and calcium are two plant nutrients that have been found to have an effect on blackleg and tuber soft rot incidence. Also, their levels found in the plants at the time of maturation can play a major role in good skin set. The proper management of these can improve the potato plants resistance to diseases such as blackleg and tuber soft rot. Nutrition management needs to be part of an overall integrated pest management plan for this disease complex (Czajkowski et al. 2011).

Conclusions:

Blackleg and tuber soft rot are diseases of major concern in the potato industry throughout the world. This article describes some of the more practical methods a potato farmer can use to manage the bacteria (*Pectobacterium* spp. and *Dickeya* spp.) that cause these diseases. Since there are no completely resistant cultivars currently available, it is important to use an integrated approach to management. The following is a prioritized list of management practices a farmer can use in his or her battle against blackleg and tuber soft rot.

Utilizing all of these techniques to manage these bacteria may not be practical for every farming operation. However, the use of several of these strategies will help aid in the reduction of blackleg and tuber soft rot. It was the aim of this article to give the potato farmer tools he or she can use to combat this disease complex on their farming operation. It is the hope of the authors of this article that the farmer will use the management techniques discussed here in combination with new technologies as they become available. Blackleg and tuber soft rot, as is the case with most diseases, will change and adapt to different environments and physical pressures over time, and so should the farmer as we continue to combat this disease complex.

Prioritized List of Blackleg and Tuber Soft Rot Management Strategies:

1. Plant certified seed with known, low levels of blackleg.
2. When practical, plant potato cultivars that are resistant to blackleg and soft rot.
3. Plant lower generation seed whenever economically feasible (Generation 3 or 4 if possible).
4. Avoid contamination by soft rotting bacteria at all stages of production. Especially important is to minimize injury to the plants during the field season and to potatoes during harvest. Also, avoid desprouting seed prior to planting when practical, as this can spread the bacteria if present (AHDB-Potato Council 2007).
5. Plant single drop seed instead of cut seed pieces whenever possible. Make sure seed is properly warmed (50^0F with 90% relative humidity) and suberized, or if using fresh cut seed, make sure soil temperatures are appropriate to reduce the chance for free moisture and low O_2 conditions around the seed piece. Always try to use a good seed treatment to avoid problems with diseases like *Fusarium*, pink rot or others which can allow entry of the soft rotting bacteria as secondary invaders when present in the seed being planted.
6. If cutting seed is a necessity, try to disinfect the blades during and after cutting with an effective disinfectant. When using liquid seed treaters, keep excess moisture to a minimum on the cut surface and avoid any presence of free water to allow for proper healing.
7. Using steam as well as disinfectants to sterilize equipment will help to reduce spread of soft rot causing bacteria.
8. Do not plant potatoes back to back in subsequent years. Plant rotational crops in between potato crops. A three-year rotation is better than a two-year rotation to avoid any potential bacteria residing in decaying plant material.
9. When planning what crop rotation to use, avoid some crop species in the Brassicaceae family (e.g. Canola, Camolina, etc.) if there is a history of blackleg or tuber soft rot on the farming operation.

10. Avoid the use of surface irrigation water if possible, as this can harbor blackleg causing bacteria. This is especially critical if the field has suffered a wound causing event such as hail or wind.
11. As irrigation is used, avoid overwatering of the potato crop as this can encourage bacterial growth and movement in the soil due to the availability of free water. This is especially critical later in the season when excess moisture can swell the lenticels on the daughter tubers. Swollen lenticels can rupture, allowing the bacteria easy access into the interior of the tuber. This problem is often seen when early run table stock potatoes out of storage are washed and have rotted, collapsed regions around the lenticels. The best way to handle this problem is to allow the potatoes further time to heal in storage and make sure air flow is adequate to the pile. This usually clears up the problem within two to three weeks.
12. Do not keep cull piles in close proximity to the potato crop. Bacteria present in the culled potatoes can spread to the potato crop through wind movement, insect spread, etc.
13. Monitor nutrient levels in the petioles and fertilize to maintain good nutrient balance in the crop. Avoid excess nitrogen and low calcium later in the season to improve skin set and tuber maturity.
14. If blackleg is present in the field make sure to use a good vine desiccant to avoid plant to plant contamination and work to achieve good skin set. Especially critical at harvest is to keep all equipment properly adjusted to minimize wounds; keep it properly sanitized; and keep chains and pilers full to minimize tuber rolling. Pick out all rotted tubers during harvest and keep debris to a minimum going into the storage to avoid poor air flow within the pile. Free moisture is one of the few critical components of developing soft rot in storage. Frozen tubers, tubers decaying as a result of pink rot, late blight or *Fusarium*, or moving wet potatoes into storage all can provide copious amounts of free moisture.
15. Consider a faster cool down timing to the holding temperature in storage to reduce potential infection while making sure proper wound healing has occurred. If rotted tubers are still present, consider higher air flow to dry the rotted areas and keep relative humidity and oxygen content as high as possible. As always, monitor the pile during the season frequently to avoid hot spots or

regions where low O_2 may occur. If hot spots occur, regulate the air going to the pile to try to reduce the heat in the pile or move the affected portions of the pile to keep additional problems to a minimum.

Tables:

Table 4.1. Seed Lot Comparison of Two Certified Seed Growers, San Luis Valley, Colorado. (Potato Cultivar Satina, 2013 & 2014)						
Grower	Cultivar	Year	Field Year	Field Generation	Acres in Lot	% Blackleg[a]
2	Satina	2013	FY4	G4	22.00	0.00
2	Satina	2014	FY5	G5	NA[b]	NA[b]
3	Satina	2013	FY4	G4	47.60	6.00
3	Satina	2014	FY5	G5	NA[b]	NA[b]

[a]Each lot is inspected by trained seed potato inspectors. Percent blackleg is based on the visual evaluation of 1% of the number of acres in each seed lot (or a minimum of 1,000 plants for seed lots with less than 10 acres). The total number of plants expressing blackleg sympoms are used to calculate the percentatge of blackleg: (# blackleg plants/# plants evaluated)*100.

[b]NA = Information is Not Available because seed lot was not planted by the grower in 2014.

Table 4.2. List of Crop Rotations Evaluated to Determine Effectivess at Reducing Blackleg and Tuber Soft Rot Incidence in Potato. SLV Research Center, Center CO (2013 & 2014).				
Treatment #	Year 1	Year 2	Year 3	Year 4
1	Potato	Barley	Potato	Barley
2	Potato-Rye[a]	Sudan GM[b]	Potato-Rye[a]	Sudan GM[b]
3	Potato	Barley	Canola	Potato
4	Potato	Barley	Sudan GM[b]	Potato
5	Potato	Barley	Camolina	Potato
6	Potato	Barley	7 Species Cocktail GM[bc]	Potato
7	Potato-Rye[a]	7 Species Cocktail GM[bc]	Sudan GM[b]	Potato

[a]Potatoes were planted in the Spring and Rye was planted after potato harvest in the fall.

[b]GM = Green Manure; These crops were chopped and incorporated into the soil late in the growing season.

[c]The 7 species cocktail consisted of Arvika Field Pea (28%), Sorghum Sudan (22%), Buckwheat (14%), Oats (14%), Common Vetch (13%), Tiller Radish (6%), and Turnip (3%).

Table 4.3. Evaluation of potato seed challenge inoculated with *Pectobacterium atrosepticum* to determine effect on soft rot incidence, mean of 2013 & 2014 data.				
Trt #	Treatment	Number Rotten Tubers (UI)[a]	Number Rotten Tubers (I)[b]	Pairwise comparison between UI & I[c]
1	Potato/Barley	0.3	0.3	1.0000
2	Potato-Rye/Sudan GM	0.1	0.8	0.0605
3	Potato/Barley/Canola	0.0	0.9	0.0092
4	Potato/Barley/Sudan GM	0.3	0.3	1.0000
5	Potato/Barley/Camolina	0.3	0.4	0.7048
6	Potato/Barley/Cocktail GM	0.1	0.8	0.1115
7	Potato-Rye/Cocktail GM/Sudan GM	0.3	0.5	0.4492
LSD		NS	NS	-
CV		253.05	151.73	-
P value		0.90	0.57	-

[a]Potato seed was not inoculated (UI) with *P. atrosepticum* when planted. Total number of tubers expressing symptoms of soft rot at harvest, mean of four replications and both years.

[b]Potato seed was inoculated (I) with *P. atrosepticum* when planted. Total number of tubers expressing symptoms of soft rot at harvest, mean of four replications and both years.

[c]The pairwise comparison between UI and I was made across years for the total number of rotten tubers at harvest. P-values greater than 0.05 indicate that the I and UI potato rows are not significantly different from each other (mean of 2013 & 2014 data, four replications per year).

Figures:

Figure 4.1. A potato plant showing typical blackleg.

Figure 4.2. A potato exhibiting soft rot caused by *Pectobacterium* spp. Notice the well-defined margin between the rotten and healthy tissues.

Figure 4.3. A milestone seed cutter in the process of cutting potato seed, variety Canela Russet.

Figure 4.4. A “Forney Hot Cutter” used to manually cut potato seed.

Figure 4.5. Crystal Violet Pectate (CVP) media with pits caused by soft rot

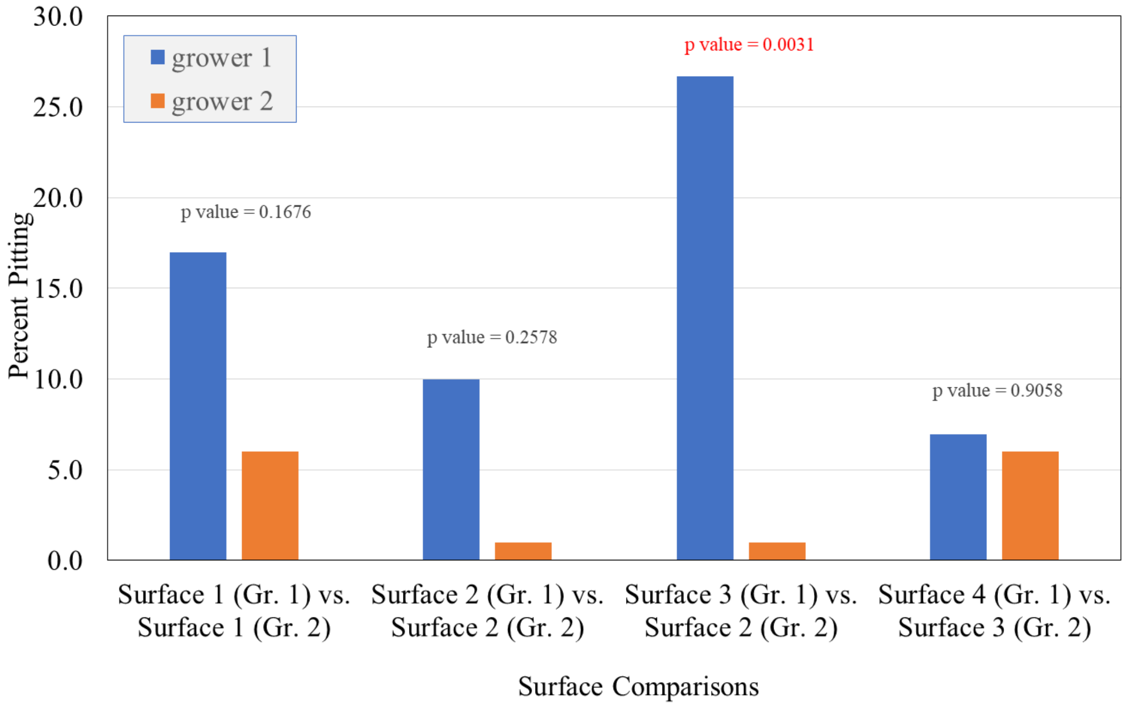


Figure 4.6. Evaluation of samples collected from different surfaces on a Milestone Potato Seed Cutter (Grower 1) and Hot Potato Seed Cutter (Grower 2). Percent pits visually observed on CVP media. Mean of 2014 and 2015 data for each grower. Seed cutter surfaces significantly different at P=0.05.

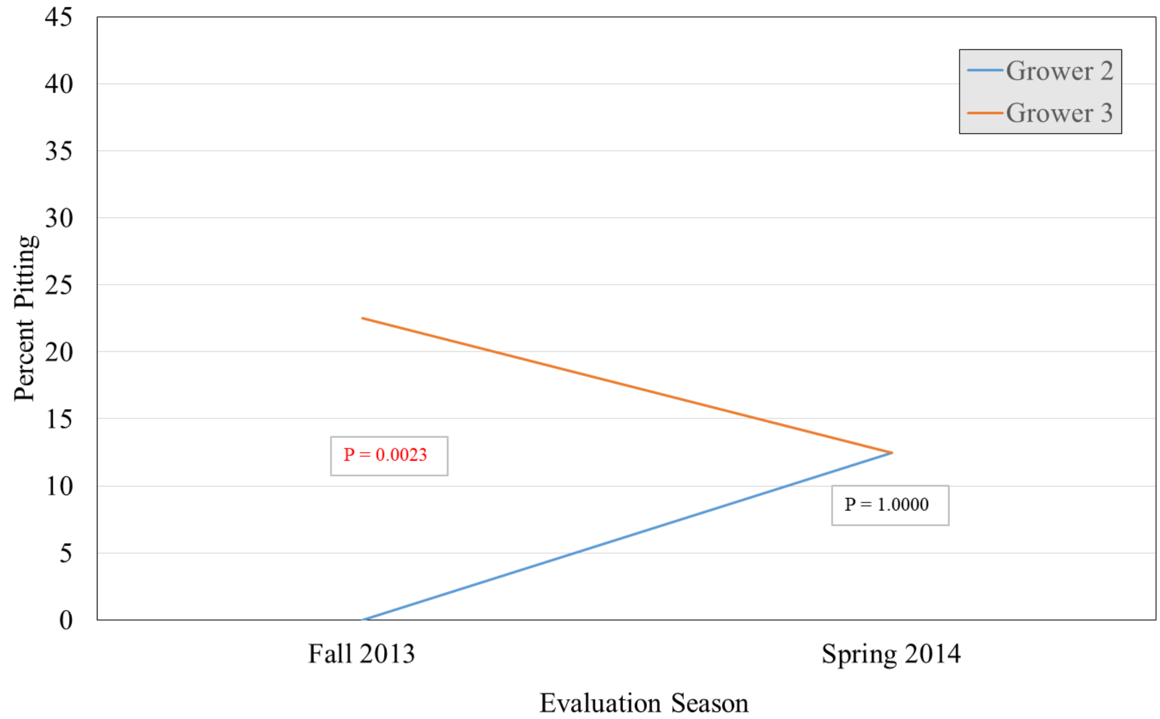


Figure 4.7. Evaluation of the Potato Cultivar Satina (Field Generation Four) Under Two Different Seed Operations to Determine the Percentage of Pitting caused by Pectolytic Bacteria - Stem Ends, 2013 Crop.

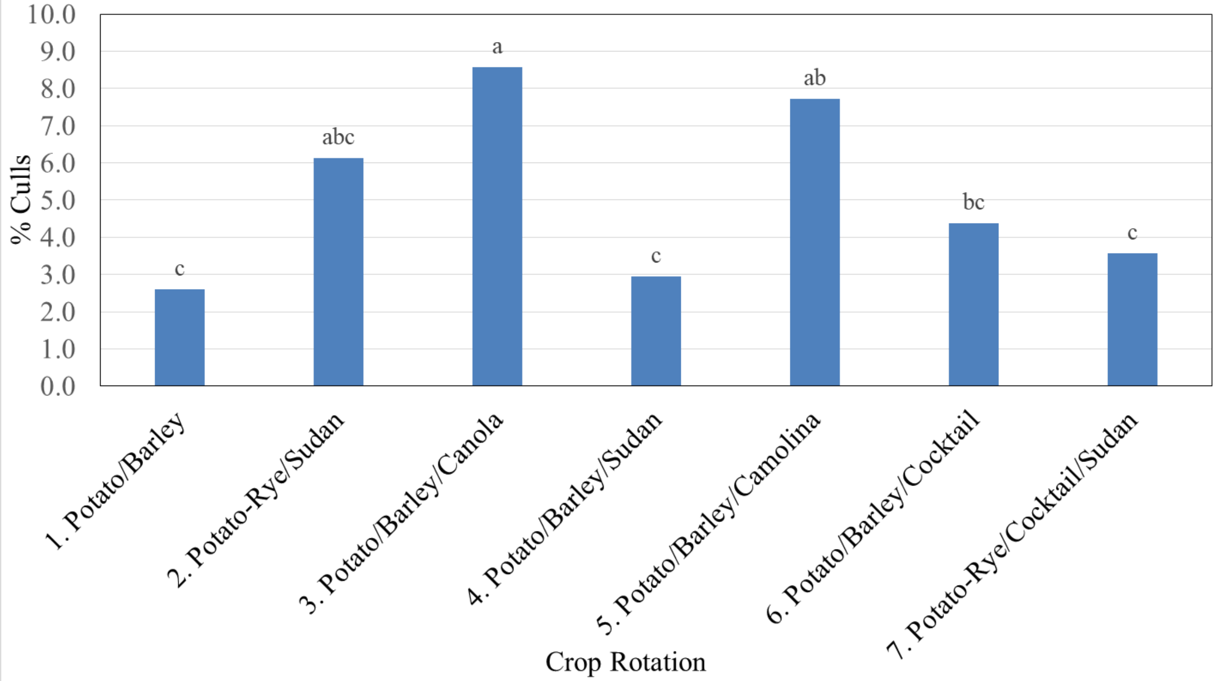


Figure 4.8. Evaluation of Rotational Crops for Potato Quality at Harvest. The seed was inoculated with *Pectobacterium atrosepticum* at planting. Culls are defined as any tuber not meeting grade standards (e.g. misshaped, green coloration, etc.). Percent culls are based on tuber weight at harvest. Data collected on one 10 foot row per treatment per replication, mean of 2013 and 2014 data. Means followed by the same letters are not significantly different at P=0.05 (LSD).

LITERATURE CITED

ADHB-Potato Council, 2007. *Dickeya* spp (*Erwinia chrysanthemi*): What is it…and what can you do? https://potatoes.ahdb.org.uk/publications/dickeya-what-it-and-what-can-you-do? Accessed 15 February 2018.

Ali, H.F., M. Ahmad, M. Junaid, A. Bibi, A. Ali, M. Sharif, B. Ali, K. Nawab, and A. Sadozai. 2012. Inoculum sources, disease incidence and severity of bacterial blackleg and soft rot of potato. Pakistan Journal of Botany 44: 825-830.

Bain, R.A., P. Millard, and M.C.M. Perombelon. 1996. The resistance of potato plants to *Erwinia carotovora* subsp *atroseptica* in relation to their calcium and magnesium content. Potato Research 39: 185-193.

Bohl, W. and D. Johnson (eds.). 2011. Commercial Potato Production in North America, APJ Supplement 57. p. 67-72. Potato Association of America, Orono, ME

Bdliya, B.S. and H.U. Haruna. 2007. Efficacy of solar heat in the control of bacterial soft rot of potato tubers caused by *Erwinia carotovora* ssp. *carotovora*. Journal of Plant Protection Research, 47(1), 11-18.

Cappaert, M.R., M.L. Powelson, G.D. Franc, and M.D. Harrison. 1988. Irrigation water as a source of inoculum of soft rot Erwinias for aerial stem rot of potatoes. Phytopathology 78: 1668-1672.

Charkowski, A.O. 2015. Biology and control of *Pectobacterium* in potato. American Journal of Potato Research 92: 223-229.

Colorado Agricultural Meteorological Network. 2016. http://ccc.atmos.colostate.edu/cgi-bin/stationsum form.pl. Accessed on 30 October 2016.

Colorado Potato Certification Service records, 2017. Colorado State University, Center, Colorado.

Czajkowski, R., M.C.M. Perombelon, J.A. van Veen, and J.M. van der Wolf. 2011. Control of blackleg and tuber soft rot of potato caused by *Pectobacterium* and *Dickeya* species: A review. Plant Pathology 60: 999-1013.

Deboer, S.H. 1989. Bacteria and Potatoes in Crop Rotation Systems. p. 131-152. In: J. Vos, C.D. Vanloon, G.J. Bollen (eds.). Effects of crop rotation on potato production in the temperate zones. Kluwer Academic Publishers, Wageningen, Netherlands.

DeBoer, S.H., I. Rubio. 2004. Blackleg of potato. http://apsnet.org/edcenter/intropp/lessons/ prokaryotes/Pages/Blacklegpotato.aspx. Accessed 10 January 2018.

DeBoer, S.H. 2008. Managing Soft Rot and Ring Rot. p. 171-174. In: D.A. Johnson (ed.). Potato Health Management, 2nd edition. The American Phytopathological Society, St. Paul, MN.

Duffy, E.M. and A.C. Cassells. 2000. The effect of inoculation of potato (*Solanum tuberosum* L.) microplants with arbuscular mycorrhizal fungi on tuber yield and tuber size distribution. Applied Soil Ecology 15: 137-144.

Graham, D.C. and M.D. Harrison. 1975. Potential spread of *Erwinia* spp in aerosols. Phytopathology 65: 739-741.

Harrison, M.D., G.D. Franc, D.A. Maddox, J.E. Michaud, and N.J. McCarterzorner. 1987. Presence of *Erwinia carotovora* in surface water in North America. Journal of Applied Bacteriology 62: 565-570.

Kloepper, J.W., J.W. Brewer, and M.D. Harrison. 1981. Insect transmission of *Erwinia carotovora* var *carotovora* and *Erwinia carotovora* var *atroseptica* to potato plants in the field. American Potato Journal 58: 165-175.

Koide, R.T. and M.S. Peoples. 2012. On the nature of temporary yield loss in maize following canola. Plant and Soil 360: 259-269.

Kumar, A., V.S. Pundhir, and K.C. Gupta. 1991. The role of phenols in potato-tuber resistance against soft rot by *Erwinia-carotovora* ssp-*carotovora*. Potato Research 34: 9-16.

Lapwood, D.H. and R.I. Harris. 1980. The spread of *Erwinia carotovora* var *atroseptica* (blackleg) and var carotovora (tuber soft rot) from degenerating seed to progeny tubers in soil. Potato Research 23: 385-393.

Larkin, R.P. and C.W. Honeycutt. 2006. Effects of different 3-year cropping systems on soil microbial communities and rhizoctonia diseases of potato. Phytopathology 96: 68-79.

Maddox, D.A. and M.D. Harrison. 1988. Presence and population dynamics of *Erwinia carotovora* in irrigation water in south central Colorado. Journal of Applied Bacteriology 64: 169-182.

McCarterzorner, N.J., G.D. Franc, M.D. Harrison, J.E. Michaud, C.E. Quinn, I.A. Sells, and D.C. Graham. 1984. Soft rot *Erwinia* bacteria in surface and underground waters in southern Scotland and in Colorado, United-States. Journal of Applied Bacteriology 57: 95-105.

McCarterzorner, N.J., M.D. Harrison, G.D. Franc, C.E. Quinn, I.A. Sells, and D.C. Graham. 1985. Soft rot *Erwinia* bacteria in the rhizosphere of weeds and crop plants in Colorado, United-States and Scotland. Journal of Applied Bacteriology 59: 357-368.

Meneley, J.C. and M.E. Stanghellini. 1976. Isolation of soft rot *Erwinia* spp from agricultural soils using an enrichment technique. Phytopathology 66: 367-370.

Perombelon, M.C.M. 1992. Potato blackleg: Epidemiology, host pathogen interaction and control. Netherlands Journal of Plant Pathology 98: 135-146.

Perombelon, M.C.M. 2000. Blackleg risk potential of seed potatoes determined by quantification of tuber contamination by the causal agent and *Erwinia carotovora* subsp. *atroseptica*: A critical review. EPPO Bulletin 30: 413.

Perombelon, M.C.M., and E.M. Burnett. 1991. Two modified crystal violet pectate (CVP) media for the detection, isolation and enumeration of soft rot *Erwinias*. Potato Research 34: 79-85.

Perombelon, M.C.M. and A. Kelman. 1980. Ecology of the soft rot *Erwinias*. Annual Review of Phytopathology 18: 361-387.

Perombelon, M.C.M., R. Lowe, C.E. Quinn, and I.A. Sells. 1980. Contamination of pathogen-free seed potato stocks by *Erwinia carotovora* during multiplication: Results of a 6-Year monitoring study. Potato Research 23: 413-425.

Peters, R.D., A.V. Sturz, M.R. Carter, and J.B. Sanderson. 2003. Developing disease-suppressive soils through crop rotation and tillage management practices. Soil and Tillage Research 72: 181-192.

Pringle, R.T. and K. Robinson. 1996. Storage of seed potatoes in pallet boxes: The role of tuber surface moisture on the population of *Erwinia* bacteria. Potato Research 39: 205-222.

Rowe, R.C. 1993. Potato health management. American Phytopathogical Association, APS Press, St Paul, MN.

Stark, J. and S. Love (eds.). 2003. Potato production systems. p. 161-163. U. of Idaho Extension, U. of Idaho Communicaions, Moscow, Idaho.

Stevenson, W.R., R. Loria, G. Franc, and P. Weingarter (eds.). 2001. Compendium of Potato Diseases, 2nd edition. The American Phytopathological Society, APS Press, St. Paul, MN.

Toth, I.K., L. Sullivan, J.L. Brierley, A.O. Avrova, L.J. Hyman, M. Holeva, M., L. Broadfoot, M.C.M. Perombelon, and J. McNicol. 2003. Relationship between potato seed tuber contamination by *Erwinia carotovora* ssp *atroseptica*, blackleg disease development and progeny tuber contamination. Plant Pathology 52: 119-126.

Toth, I.K., J.M. van der Wolf, G. Saddler, E. Lojkowska, V. Helias, M. Pirhonen, L. Tsror, and J.G. Elphinstone. 2011. *Dickeya* species: an emerging problem for potato production in Europe. Plant Pathology 60: 385-399.

Vanvuurde, J.W.L. and P.M. Devries. 1994. Population dynamics of *Erwinia carotovora* subsp *atroseptica* on the surface of intact and wounded seed potatoes during storage. Journal of Applied Bacteriology 76: 568-575.

Walsh, K.D., D. Sanderson, L.M. Hall, S. Mugo, and M.J. Hills. 2014. Allelopathic effects of camelina (*Camelina sativa*) and canola (*Brassica napus*) on wild oat, flax and radish. Allelopathy Journal 33: 83-95.

ADDENDUM TO CHAPTER 2

Introduction:

The main objective of this study was to evaluate which method of determining pectolytic activity was most accurate and cost effective. A wrapped tuber method and a modified Crystal Violet Pectate (CVP) method were compared to determine this. The modified CVP medium used was similar to that used by Perombelon and Burnett (1991).

Materials and Methods:

There are multiple methods that can be used to determine the presence and relative levels of pectolytic bacteria in seed potatoes. Some are more accurate than others at detecting the presence of this group of bacteria (Koppel 1993). Also, several genera and species of bacteria are pectolytic and can cause tuber soft rot (Lyon 1989). In order to detect the genus *Pectobacterium* and *Dickeya*, a screening methodology needs to be used that is fairly specific to these bacteria. Also, several thousand seed potatoes will be screened over the course of this PhD project, so the screening method used needs to produce results rapidly and be inexpensively. Two different screening methodologies were used and evaluated to determine which test was better to use over the course of this project.

Three seed lots were evaluated from two different certified seed grower operations (Table 5.1). The seed lot from grower 1 was the potato cultivar Canela Russet, field generation 3 (G3). The other two seed lots were from grower 2 and were both the potato cultivar LaRatte - one was a field generation one (G1) and the other was a field generation two (G2). A random sample of 40 seed potatoes were collected from each of the three seed lots in the spring of 2014. The stem end (the point of potato stolon attachment to the daughter potato) and three lenticels from each seed potato were evaluated. A sterile, autoclaved toothpick was used to puncture each location on the potatoes. The autoclave used was a Market Forge Machine, the temperature reached 121ºC at a pressure of 17 psi to achieve sterilization. Two toothpicks were used per potato, both ends of each toothpick were used to collect potato material from the stem end and three lenticels. After the stem end or lenticel was punctured with the toothpick point, the point was inserted, then twisted into a fresh CVP plate. A modified CVP medium was used, similar to that used by Perombelon and Burnett (1991) Twisting was used to ensure that any bacteria on the point was left in the CVP. The seed potato was then wrapped in a paper towel that was moistened with sterile water and further wrapped in aluminum foil. The Canela Russet sample was punctured and wrapped on May 23, 2014(G3) and the LaRatte samples on May 13th (G1) and May 14th (G2), respectively.

Tuber Wrapping Methodology:

Each wrapped seed potato was incubated at room temperature for three days prior to evaluation. After three days, each tuber was unwrapped and the locations where the sterile toothpicks punctured either the lenticel or stem end were visually assessed for the presence of a rot pocket at the puncture site. The seed potatoes from the Canela Russet lot were evaluated on May 26, 2014 (G3), while the LaRatte potatoes were evaluated on May 16th (G1) and 17th (G2), respectively.

Crystal Violet Pectate Methodology:

After the sterile toothpick that had punctured either the stem end or lenticel was inserted into the CVP plate, each plate was placed in an anaerobe jar and was incubated at room temperature for approximately five days. A BD GasPak^{TM} EZ Gas Generating Container System was used to generate anaerobic conditions. Pectolytic bacteria digest pectin which causes pits to form on CVP media when incubated at room temperature for 4 to 7 days. The inoculated CVP plates were incubated in the anaerobic chamber to reduce the amount of growth of non-pectolytic, aerobic fungi and bacteria. For example, bacteria in the genus *Clostridium* are pectolytic, but do not grow readily under anaerobic conditions (Bain and Perombelon 1988). After the five day incubation was over, each CVP plate was visually evaluated for pitting at the toothpick insertion points. A pit in the CVP at the toothpick insertion site indicated that active pectolytic bacteria was present in either the stem end or lenticel from the seed potato. Four seed potatoes were represented on each CVP plate, with enough space in between each toothpick insertion to reduce the possibility of cross contamination between stem end and lenticel insertion points (about 1 cm distance between insertion points). The Canela Russet G3 CVP plates were read on May 29, 2014 and LaRatte CVP plates were read on May 18 (G1) and May 19 (G2), respectively.

Analysis:

A completely random experimental design was used for this evaluation since seed potatoes were selected randomly from each seed crop evaluated. A sample of 40 seed potatoes were collected and evaluated individually for each seed crop tested. Factors included seed potatoes (n = 40 per seed lot), stem end and lenticel sites from each potato, grower operation, cultivar, and year. The number of replicates was 40 for each study (40 seed potatoes tested per sample). The total number of samples was as follows: 40 reps x 3 seed lots x 2 treatments x 2 potato evaluation points = a total of 480 samples. An LSD mean separation was used to analyze the data for each study. Each pairing was analyzed independently of the other pairings (e.g. LAR21 CVP was compared only with LAR21 wrapped when looking at stem end data). Means followed by the same letters were not significantly different when p values >=0.05. A Least Squares Means Analysis using Proc GLM was used to perform the analysis (SAS version 9.4, Proc GLM, Cary, NC).

Results and Discussion:

When comparing the two methods (tuber wrapping vs CVP) for determining pectolytic bacteria activity in seed potatoes, the wrapping method had statistically higher levels of bacteria, represented by rot pockets, for each evaluation except for the Canela Russet stem ends (Figure 5.1). Also, no blackleg was found in the any of the three seed crops either in 2013 or 2014 (Table 5.1). Since there was no blackleg present in the plants the year before or the year after the seed potato evaluation was conducted, the CVP method appears to provide bacteria numbers that match better with the summer blackleg levels.

Samples for this trial were collected from three seed crops off two potato grower operations (LaRatte G1 and LaRatte G2 from grower 1, and Canela Russet from grower 2). Grower 1 has historically had high levels of blackleg and grower 2 has had relatively low levels of blackleg in recent years (Colorado Potato Certification Service Annual Seed Directories – 2005 to 2015). These blackleg levels may explain why the Canela Russet lot had low levels of pitting in CVP and a low number of rot pockets in the wrapped tubers when looking at stem ends. The reason stem ends were tested was because *Pectobacterium* sp. can travel through the vascular system in an infected potato plant and infect the daughter tubers at the attachment point, which is the stem end (De Boer 2002). If no blackleg is present in the summer crop, it could be deduced that zero or very low levels of bacteria were present at the stem end.

Conclusions:

Of the two detection methods evaluated, the method using CVP media in combination with an anaerobic environment was the most accurate and cost effective for this investigation. The use of CVP as a detection method has been shown by other research to be a fairly accurate and cost effective method for determining the presence of soft rot causing bacteria in seed potatoes (Cuppels and Kelman 1974; Helias et al. 2012; Hyman et al. 2001; Perombelon and Burnett 1991). The wrapping method overall resulted in a much higher amount of rot pocket formation than pitting detected using the CVP method. This may be the result of several factors. One might be that the tuber is not a selective media, like the CVP plate. This would allow other organisms to cause rot on the tubers that typically would not result in a pit when on CVP. Also, any pectolytic microbes present on the surface of the tuber, in close proximity to where the tuber was wounded with the toothpick, may add to the development of rot pockets.

Two methods for determining pectolytic bacteria levels were compared and evaluated to determine which method would provide more accurate results when looking at summer blackleg readings. It was found that using the CVP plating method, similar to that used by Perembelon and Burnett (1991), was the most reliable of the two methods evaluated and will be used for the remainder of the tests in this PhD project.

Table:

Table 5.1. Summer Visual Blackleg Readings for the CVP vs Seed Wrapping Evaluation.					
Grower	Cultivar	Year	Field Year	Acres in Lot	% Blackleg[a]
1	La Ratte	2013	FY1	1.2	0
1	La Ratte	2014	FY2	4.0	0
1	La Ratte	2013	FY2	3.0	0
1	La Ratte	2014	FY3	10.0	0
2	Canela Russet	2013	FY3	3.8	0
2	Canela Russet	2014	FY4	NA[b]	NA[b]

[a]Each lot is inspected by trained seed potato inspectors. Percent blackleg is based on the visual evaluation of 1% of the number of acres in each seed lot (or minimum of 1,000 plants for seed lots with less than 10 acres). The total number of plants expressing blackleg symptoms are used to calculate the percentage of blackleg: e.g. (# blackleg plants/total # of plants evaluated)*100.

[b]The FY3 Canela Russet lot was not planted back in 2014, so no data is available.

Figure:

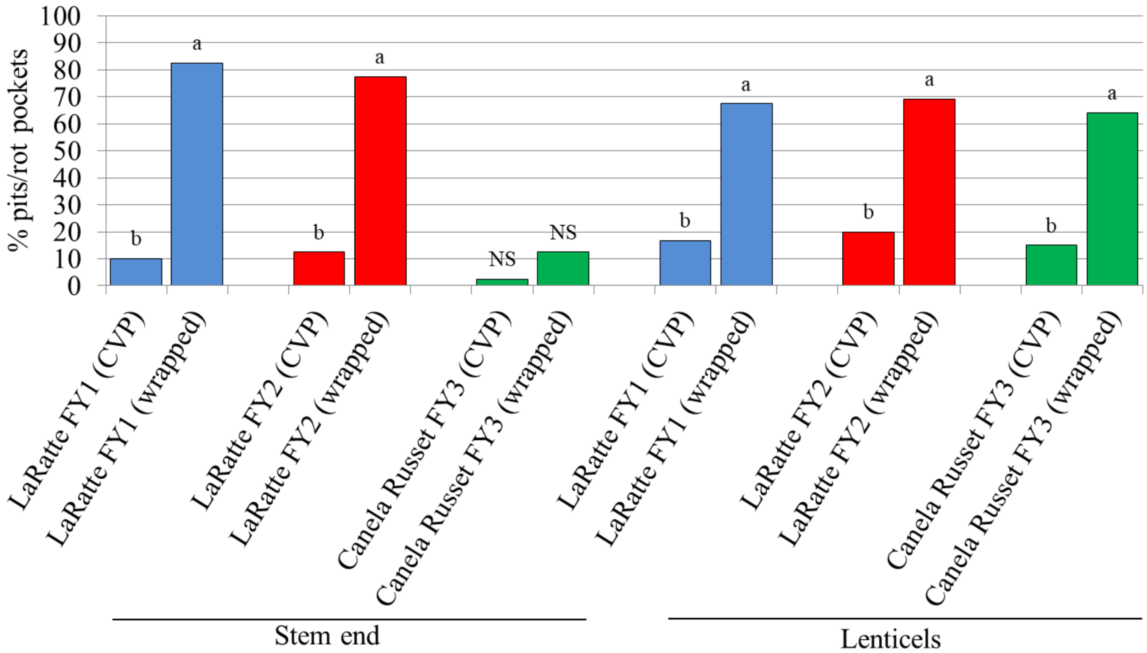


Figure 5.1. Evaluation of Two Methods for Detecting Pectolytic Bacterial Levels in Seed Potatoes. Samples were collected in the spring of 2014. Generation in the field is represented as follows: Field year one = FY1, two = FY2, three = FY3. Both LaRatte seed lots were grown by grower 1 and the Canela Russet lot by grower 2. Data represents a mean of 40 seed pieces per seed lot. Each pairing was analyzed independently of the other pairings (e.g. LaRatte FY1 CVP was compared only with LaRatte FY1 wrapped for the stem end, LSD separation, p=0.05).

LITERATURE CITED

Bain, R.A., and M.C.M. Perombelon. 1988. Methods of testing potato cultivars for resistance to soft rot of tubers caused by *Erwinia-carotovora* subsp *atroseptica*. Plant Pathology 37: 431-437.

Cuppels, D. and A. Kelman. 1974. Evaluation of selective media for isolation of soft rot bacteria from soil and plant tissue. Phytopathology 64: 468-475.

De Boer, S.H. 2002. Relative incidence of *Erwinia carotovora* subsp *atroseptica* in stolon end and peridermal tissue of potato tubers in Canada. Plant Disease 86: 960-964.

Helias, V., P. Hamon, E. Huchet, J.V.D. Wolf, and D. Andrivon. 2012. Two new effective semiselective crystal violet pectate media for isolation of *Pectobacterium* and *Dickeya*. Plant Pathology 61: 339-345.

Hyman, L.J., L. Sullivan, I.K. Toth, and M.C.M. Perombelon. 2001. Modified crystal violet pectate medium (CVP) based on a new polypectate source (Slendid) for the detection and isolation of soft rot E*rwinias*. Potato Research 44: 265-270.

Koppel, M. 1993. Methods of assessing potato-tubers for resistance to bacterial soft-rot. Potato Research 36: 183-188.

Lyon, G.D. 1989. The biochemical basis of resistance of potatoes to soft rot *Erwinia* spp.: A review. Plant Pathology 38: 313-339.

Perombelon, M.C.M. and E.M. Burnett. 1991. Two modified crystal violet pectate (CVP) media for the detection, isolation and enumeration of soft rot *Erwinias*. Potato Research 34: 79-85.