# AN ABSTRACT OF THE THESIS OF

Jessie Brazil for the degree of Master of Science in Botany and Plant Pathology presented on April 14, 2020.

Title: Diversity and Impact of Soft Rot Pathogens of Potato in the Columbia Basin.

Abstract approved: _____

Kenneth Frost

Bacterial soft rot of potato (*Solanum tuberosum*), caused by *Pectobacterium* and *Dickeya* species, is among the most common and destructive potato diseases in the United States. These pathogens cause a variety of vascular wilts, and in potato cause a disease complex that includes tuber soft rot, blackleg, aerial stem rot, and lenticel rot. The Columbia Basin of Northeastern Oregon and Southwestern Washington is a valuable potato producing region. Two projects were conducted at the Hermiston Agricultural Research and Extension Center in Hermiston, Oregon, to address knowledge gaps surrounding soft rot of potato.

The distribution and identities of the multiple bacterial pathogens that cause soft rot of potato has not been recently characterized in the Columbia Basin. The first project was conducted to characterize the genetic diversity of soft rot pathogens of potato in the Columbia Basin. In 2018 and 2019, 25 and 120 diseased plant samples, respectively, that exhibited symptoms of soft rot and originated from the Columbia Basin were received and analyzed using diagnostic PCR assays to identify the isolates. Fifty-four soft rot pathogens were detected in 51 of the samples, that included *P. carotovorum*

(61.1%), *P. atrosepticum* (20.4%)*, Dickeya* species (5.6%), *P. parmentieri* (9.3%), and *P. brasiliense* (3.7%). Twenty-eight bacterial isolates were obtained in culture, although no *Dickeya* spp. were recovered. The identity of these isolates was confirmed through a phylogenetic assessment of *dnaX*, *pelY*, and 16s rRNA sequences. We found that in 2018 and 2019, the soft rot pathogens of potato that were present in the Columbia Basin were *P. carotovorum*, *P. atrosepticum*, *P. brasiliense*, and *P. parmentieri*. We concluded that although *Dickeya* spp. may be present, *Pectobacterium* spp. were the dominant pathogens associated with soft rot of potato in the Columbia Basin in 2018 and 2019.

Potato yield loss associated with seed-borne infections of soft rot pathogens has not been recently estimated. The second project was conducted to determine the yield losses associated with seed-borne soft rot infections to determine how potato yields vary as a function of inoculum prevalence in seed potato in Eastern Oregon. In 2018, potato seed of ‘Lamoka’ and ‘Russet Burbank’ cultivars were inoculated with *Pectobacterium carotovorum* and *Dickeya chrysanthemi* and in 2019, potato seed of ‘Lamoka’ and ‘Russet Burbank’ cultivars were inoculated with *Pectobacterium atrosepticum* and *Pectobacterium parmentieri*. In each year, inoculated and non-inoculated seed potato was mixed to create planting stock with 0%, 5%, 10%, 20%, and 30% incidence of soft rot. The resulting 20 treatments (2 cultivars x 2 strains x 5 doses of inoculum) were planted in the field and managed using grower practices typical for the region. Emergence, plant health, and blackleg incidence was monitored throughout the growing season. Potatoes from each plot were harvested, graded, and total yield for each plot was calculated by weight. We observed that an increase of bacterial inoculum

in the potato seed lead to lower emergence rates for the ‘Lamoka’ cultivar treatments in 2018. Lower rates of emergence lead to lower yields. We did not observe effects of the treatments in either year on plant health. Based on the results of this study, in Eastern Oregon, a 0- 30% incidence of soft rot bacteria in ‘Russet Burbank’ cultivar potato seed does not contribute to lowered emergence, plant health, or yield. A 5 - 30% incidence of soft rot bacteria in ‘Lamoka’ seed may impact plant emergence and yield.

# Diversity and Impact of Soft Rot Pathogens of Potato in the Columbia Basin

by
Jessie Brazil

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented April 14, 2020
Commencement June 2020

Master of Science thesis of Jessie Brazil presented on April 14, 2020

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Jessie Brazil, Author

# ACKNOWLEDGEMENTS

I would like to sincerely thank Dr. Kenneth Frost for the help, guidance, and support he has given me through each stage of the process over the past three years. I thank Dr. Ken Johnson, Dr. Christopher Mundt, and Dr. Seri Robinson for taking the time to serve on my graduate committee. I would also like to thank the members of the HAREC Plant Pathology Laboratory – Dr. Hannah Rivedal, Victoria Skillman, and Cassandra Funke, for their help, advice, and comradery during my stay in Hermiston. Finally, I would like to thank my family and friends for their love and support.

# CONTRIBUTION OF AUTHORS

Dr. Kenneth Frost secured funding for this research, designed the projects, provided guidance, and reviewed the chapters. Dr. Hannah Rivedal offered advice for lab work conducted in Chapter 2, assisted with the phylogenetic analysis in Chapter 2, and reviewed the chapters. Victoria Skillman assisted with field preparation, data collection, and statistical analysis in Chapter 3.

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# Chapter 1

# General Introduction

Jessie Brazil

Potatoes (*Solanum tuberosum)* are among the world's most important food crops, both in terms of area cultivated and total weight produced, and are consumed by over a billion people worldwide on a daily basis (Douches et al. 1996; Camire et al. 2009; Birch et al. 2012). In 2017, the total global potato production was estimated at 388,191,000 tonnes, with 19,302,600 hectares harvested (FAOSTAT 2019). Potatoes are high in several micronutrients, such as carbohydrates, starch, proteins, vitamin C, and fiber, and are good sources of vitamins B1, B3 and B6, and minerals such as potassium, phosphorus and magnesium, and also contain folate, pantothenic acid, and riboflavin (FAO 2008; Camire et al. 2009; White et al. 2009; Birch et al. 2012; Obidiegwu et al. 2015).

Several important diseases that affect potato production and yield worldwide are caused by pectinolytic soft rot bacteria, including tuber soft rot, blackleg, aerial stem rot, and lenticel rot (Nykyri et al. 2012; Mansfield et al. 2012; Stevenson et al. 2001). Soft rot bacterial species that cause disease of potato are from two genera, *Pectobacterium* and *Dickeya*, and are collectively known as the soft rot Pectobacteriaceae (SRP; formerly Enterobacteriaceae) (Pérombelon and Kelman 1980; Pérombelon 2002; Adeolu et al. 2016). SRP are gram negative, rod-shaped, non-spore forming, facultative anaerobes with peritrichous flagella (Charkowski 2006) and are characterized by their ability to produce several pectinolytic enzymes, which degrade primary plant cell walls and the middle lamella resulting in host tissue maceration (Garibaldi and Bateman 1971; Abbott and Boraston 2008; Pérombelon 2002; Barras et al. 1994). The maceration of host tissues represents the primary symptom of the diseases caused by SRP, including the wet, soft rot of tubers and the blackened, necrotic stem tissue of blackleg (Garibaldi and

Bateman 1971). Broadly, SRP are capable of causing vascular wilts or soft rots on a wide range of host plants. Approximately 50% of angiosperm orders can be affected including many economically important species of horticultural and ornamental plants (Ma et al. 2007; Samson et al. 2005; Charkowski 2018). SRP are the most common and widely studied causal agents of soft rot and blackleg of potato (Charkowski 2018). Species of SRP that have been reported to cause soft rot and blackleg on potato include *Pectobacterium carotovorum, P. atrosepticum, P. brasiliense, P. odoriferum, P. parmentieri, P. polaris, Dickeya dianthicola, D. dadantii, D. chrysanthemi,* and *D. solani* (Czajkowski et al. 2011; Portier et al. 2019; Dees et al. 2017; Khayi et al. 2016; Gardan et al. 2003).

The taxonomy of the SRP has undergone numerous changes over the past hundred years, and historically has not been consistent in literature due to these continuous changes (Charkowski 2006). Prior to 2005, the SRP were classified in the genus *Erwinia*. Potato blackleg and soft rot were first described in Europe between 1878 and 1901, and the SRP responsible for the symptoms were given various names (Hellmers 1959). The genus *Erwinia* was established in 1917 by Winslow et al., and included the plant pathogenic Enterobacteria, and was named after the phytobacteriologist Erwin Frink Smith, although some literature referred to the SRP as *Bacillus carotovorus* (Charkowski 2006; Lacey 1926). Throughout the 20th century, various SRP were classified as either *Erwinia chrysanthemi* or *Erwinia carotovora* (Charkowski 2006; Burkholder et al. 1953). In 1945, Waldee proposed moving the pectinolytic *Erwinia* to a novel genus, *Pectobacterium*, but there was not widespread or consistent use of the new genus name until the 1990s, when Hauben et al. (1998) and

Kwon et al. (1997) used 16S rRNA gene sequences to reexamine the *Erwinia* phylogeny and delineated several pectinolytic subspecies of *Erwinia* to *Pectobacterium.* In 2005, Samson et al. proposed further taxonomic reclassification of all strains of *Pectobacterium chrysanthemi* to be transferred to the novel genus *Dickeya*, named after the American phytopathologist Robert Dickey, based on genetic differences within the genus *Pectobacterium* (Samson et al. 2005; Motyka et al. 2017; Czajkowski et al. 2015). Since 2005, further taxonomic revisions have taken place due to new techniques to examine the genetic relatedness, and will continue to take place (Motyka et al. 2017).

Regardless of taxonomic classification, the SRP are capable of causing crop loss at every stage of potato production, from seed generation, at planting, during the growing season, at harvest, during transport, and in storage (Charkowski 2018). Symptoms of potato soft rot and blackleg caused by different SRP are phenotypically indistinguishable (Toth et al. 2011). In the field, early season symptoms of soft rot of potato can include poor emergence, stunted growth, and plant death (Czajkowski et al. 2011; Pérombelon and Kelman 1980). SRP can travel from infected tubers up the vascular system of the plant, resulting in inky, blackened, necrotic tissue at the base of the stem, which is known as blackleg, although tuber soft rot does not always result in the development of blackleg (Powelson and Franc 2011; Pérombelon and Kelman 1980). As the vascular system of a stem with blackleg becomes blocked with bacteria in the growing plant, foliar symptoms such as wilting and chlorosis may occur, and stems with blackleg may also become hollowed out, and the plant may collapse from lack of stem structure. Aerial stem rot is the result of bacterial infection of the stem through a wound in the plant canopy and subsequent colonization of the vascular system leading

to blackened necrotic stems in the upper canopy (Johnson et al. 2011; Huang 1986). Blackleg can be distinguished from aerial stem rot because blackleg results from SRP infection of the seed piece which moves up the stem, and aerial stem rot results from SRP infection in the canopy and moves down the stem (Pérombelon and Kelman 1987).Tuber soft rot can develop later in the growing season, when the bacteria is spread to healthy progeny tubers from infected mother tubers or from infected neighboring plants (Pérombelon and Kelman 1980). Tubers infected with the soft rot bacteria appear rotted, wet, and slimy, and infection from secondary organisms can produce a foul smell (Hugouvieux-Cotte-Pattat et al. 2014; Czajkowski et al. 2011). Finally, a disease known as lenticel rot occurs when SRP infect and cause rotting of tuber lenticels resulting in a pockmark appearance of the tuber surface (Adams 1974).

SRP can also asymptomatically colonize host tissue. Due to this ability, the presence of SRP is often not detected, which is significant in the potato industry because potatoes are vegetatively propagated. The primary inoculum source resulting in SRP infections in a potato field is thought to occur due to planting infected asymptomatic seed pieces (Pérombelon 1974; Rossman et al. 2018). Additionally, SRP-infected seed potatoes can be distributed long distances to different growing regions for planting, where environmental conditions may be favorable for the bacteria to cause disease and spread to healthy progeny tubers (Czajkowski et al. 2010). Other potential sources of SRP inoculum within a field include soil and water, on contaminated tools and equipment, and alternative plant hosts (Czajkowski et al. 2011). Finally, insects may also spread the bacteria from infected plants to healthy plants in the field (Rossmann et al. 2018), but, to our knowledge, only one study has been conducted to

examine the relative importance of insects for the spread of SRP in the potato cropping system.

Currently there are no registered chemical products that are curative after SRP have infected a potato, and disease management has relied heavily on cultural practices despite decades of research on soft rot bacteria (Czajkowski et al. 2011). One of the most important factors driving soft rot and blackleg disease development is the amount of initial bacterial inoculum contaminating the seed tubers at planting (Pérombelon 1992; Aleck and Harrison 1978). Thus, planting clean seed is the most important aspect of soft rot disease management (Pérombelon and Kelman 1987; Czajkowski et al. 2011). However, the occurrence of disease, soft rot and blackleg incidence and severity, is highly variable and dependent on environmental conditions. The ability for the bacteria to cause disease can be affected by soil moisture and water level (Pérombelon et al. 1989), soil and air temperature (Pérombelon 1992), and soil and plant nutrition (McGovern et al. 1985; Bain et al. 1996; Graham and Harper 1966). Potato cultivar can also influence disease incidence and severity, as there are varying degrees of resistance among cultivars and cultivar resistance can vary among growing seasons (Pérombelon and Salmond 1995).

During the growing season, plants that show symptoms of soft rot must be removed entirely to avoid spreading the pathogen to healthy plants and remove soil inoculum (Czajkowski et al. 2011). Good equipment sanitation practices will also help in avoiding the spread of the pathogen throughout the field. Copper compounds may be used to prevent the pathogen from spreading but may be toxic to plants. During harvest and storage, it is important to avoid wounding tubers, which may provide entry for SRP.

Remove tuber that have symptoms of soft rot to avoid contamination. Storage should be cool and dry and have good aeration.

SRP have caused substantial yield loss in potato crops in Europe and the Middle East in the early 2000s. In 2009, Tsror et al. estimated that loses due to *Dickeya* species in Israel resulted in 20-25% yield reductions when disease incidence was over 15% (Motyka et al. 2017; Tsror et al. 2009). Prior to 2015, Dickeya species were not known to cause disease of potato in the U.S. However, in 2015, potato producers in the Eastern United States experienced a blackleg outbreak, attributed to *Dickeya dianthicola*, that resulted in substantial yield losses. Much of the recent research on the diversity and pathogenicity of SRP causing disease of potato has been conducted in Europe and the identity of SRP causing disease in different U.S. potato production regions is not well characterized, especially after the most recent taxonomic revision of SRP in 2005.

Potatoes (*Solanum tuberosum*) are a valuable crop in the United States, worth $3.77 billion in 2017 (USDA-NASS 2018).The Columbia Basin in the Pacific Northwest has an ideal climate for potato production. This region includes parts of Washington, Oregon, and Idaho, and produces more than half of the United States' potatoes (USDA-NASS 2016). Most of the potatoes produced in this area are for the processing market (Dung et al. 2015).The Columbia Basin is known for its high yields and productivity due to its long growing season, well-draining sandy soils, warm days and cool nights, good light intensity, and the presence of abundant irrigation water. Much of the research studying the SRP has been conducted in Europe, where the climate and cultural practices differ from the Columbia Basin. There were two main objectives for this thesis:

1) to characterize the diversity of soft rot causing bacteria in the Columbia Basin to understand which bacterial soft rot species were present and responsible for causing the disease and 2) to determine how varying amounts of initial SRP incidence in potato seed affect plant emergence, plant health, and yield in the Columbia Basin.

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# Chapter 2

## Diversity of *Pectobacterium* and *Dickeya* species responsible for causing blackleg and soft rot of potato in the Columbia Basin

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**Abstract**

Bacterial soft rot of potato (*Solanum tuberosum*) caused by *Pectobacterium* and *Dickeya* species results in considerable yield loss throughout the United States. These pathogens cause a variety of vascular wilts, and in potato cause a disease complex that includes tuber soft rot, blackleg, aerial stem rot, and lenticel rot. The distribution and identities of the multiple bacterial pathogens that cause soft rot of potato has not been recently characterized in the Columbia Basin of Eastern Oregon and Washington, which is an important and productive potato growing region. This study was conducted to characterize the genetic diversity of soft rot pathogens of potato in the Columbia Basin. In 2018 and 2019, 25 and 120 diseased plant samples, respectively, that exhibited symptoms of soft rot and originated from the Columbia Basin were received and analyzed using diagnostic PCR assays to identify the isolates. Fifty-four soft rot pathogens were detected in 51 of the samples that included *P. carotovorum* (61.1%), *P. atrosepticum* (20.4%)*, Dickeya* species (5.6%), *P. parmentieri* (9.3%), and *P. brasiliense* (3.7%). Twenty-eight bacterial isolates were obtained in culture, although no *Dickeya* spp. were recovered. We confirmed the identity of these isolates through a phylogenetic assessment of *dnaX*, *pelY*, and 16s rRNA sequences. We found that in 2018 and 2019, the soft rot pathogens of potato that were present in the Columbia Basin were *P. carotovorum*, *P. atrosepticum*, *P. brasiliense*, and *P. parmentieri*. We concluded that although *Dickeya* spp. may be present, *Pectobacterium* spp. were the dominant pathogens associated with soft rot of potato in the Columbia Basin in 2018 and 2019.

## Introduction

Bacterial soft rot and blackleg of potato (*Solanum tuberosum*) are potentially devastating diseases in every potato growing region of the United States and around the world (Nykyri et al. 2012; Mansfield et al. 2012). *Pectobacterium* and *Dickeya* species, known as the soft rot *Pectobacteriaceae* (SRP; formerly *Enterobacteriaceae*) (Adeolu et al. 2016) are the most common and widely studied causal agents of soft rot of potato (Charkowski 2018). SRP are capable of causing vascular wilts or soft rots on a wide range of host plants, including many species of horticultural and ornamental importance (Samson et al. 2005; Ma et al. 2007; Charkowski 2018). Species of SRP that have been reported to cause soft rot diseases of potato include *Pectobacterium carotovorum*, *P. atrosepticum*, *P. brasiliense*, *P. odoriferum*, *P. parmentieri*, *P. polaris*, *Dickeya dianthicola*, *D. dadantii*, *D. chrysanthemi*, and *D. solani* (Czajkowski et al. 2011; Portier et al. 2019; Dees et al. 2017; Khayi et al. 2016; Gardan et al. 2003).

The SRP were previously classified in the genus *Erwinia*, but underwent taxonomic reclassification based on genetic differences within the genus; pectinolytic *Erwinia* were transferred to both *Pectobacterium* and the novel genus *Dickeya* (Samson et al. 2005; Czajkowski et al. 2015). In 2014 and subsequent years, northeastern and midwestern states, such as Maine, New York, Michigan, and Wisconsin, experienced an outbreak of soft rot and blackleg disease, which resulted in potato fields having poor emergence and, in the worst cases, complete crop loss (Charkowski 2018). Since then, SRP have gained much interest in the United States, especially because the current distribution of the different bacterial species affecting potato remains largely unknown. In addition, the diversity of these pathogens has not been recently characterized in all

potato producing regions of the U.S., especially after the taxonomic reorganization and reclassification of the *Erwinia* genus and development of more refined ways to examine the genetic differences among SRP.

SRP produce pectinolytic enzymes that degrade pectin in the middle lamella and primary plant cell walls, which results in soft, wet, rotted disease symptoms (Garibaldi and Bateman 1971; Abbott and Boraston 2008). SRP cause multiple diseases of potato, including tuber soft rot, blackleg, aerial stem rot, and lenticel rot (Stevenson et al. 2001). Tuber soft rot is the result of SRP colonizing the tuber and macerating the host tissue, causing a wet, soft, foul-smelling rot. Tuber soft rot can be a problematic disease at every stage of potato production, including planting, harvest, transport, and storage. Potato blackleg is the result of bacteria from infected tubers moving up the stem resulting in necrosis of the vascular system and causing the base of the stem to become black and inky (Powelson and Franc 2011). However, tuber soft rot does not always result in the development of blackleg (Pérombelon and Kelman 1980; Czajkowski et al. 2011). Aerial stem rot is the result of a bacterial infection of the stem through a wound in the plant canopy and subsequent colonization of the vascular system leading to blackened necrotic stems in the upper canopy (Johnson et al. 2011). Lenticel rot is the result of the bacteria infecting the lenticels of the tuber and creating pockmark rot on the surface of the tubers (Adams 1974; Inglis et al. 2011).

Infection from SRP are highly variable in the environment. The ability for the bacteria to cause disease can be affected by soil moisture and water level (Pérombelon et al. 1989), soil and air temperature (Pérombelon 1992), soil and plant nutrition (McGovern et al. 1985; Bain et al. 1996; Graham and Harper 1966), potato cultivar

resistance, (Pérombelon and Salmond 1995), and the amount of initial bacterial inoculum contaminating the seed tubers at planting (Pérombelon 1992; Aleck and Harrison 1978). Management strategies for SRP depend on the exclusion of the pathogen, primarily through limited generation seed production, seed certification programs, and the sanitation of tools and equipment (Czajkowski et al. 2011). SRP are most commonly spread long distances through infected asymptomatic seed pieces (Rossmann et al. 2018; Pérombelon 1974). It is believed that the bacteria can survive in soil and water, on contaminated tools and equipment, and on alternative hosts, which may be additional sources of infection within the field (Czajkowski et al. 2011). Insects may also spread the bacteria from infected plants to healthy plants in the field (Rossmann et al. 2018). Once plants become infected with SRP, there are currently no curative measures available to salvage the plants (Czajkowski et al. 2011).

Potato is a major staple crop in the United States. In 2016, 1 million acres of potatoes were grown in thirty states that corresponded to $3.74 billion dollars in farm gate value and 57% of that potato production occurred in the northwestern states of Oregon, Washington, and Idaho (USDA-NASS 2017). The Columbia Basin of Eastern Oregon and Washington is an extremely productive and valuable potato growing region due to its long growing season, well-draining sandy soils, warm days and cool nights, high light intensity, and the presence of abundant irrigation water (Lamb Weston n.d.). However, in this important potato producing region, the diversity of the bacterial soft rot pathogens causing diseases has not been recently characterized. The aim of this study was to isolate bacteria associated with bacterial soft rot diseases of potato and confirm

the identity of the isolates using diagnostic PCR assays and through a phylogenetic assessment of *dnaX*, *pelY*, and 16S rRNA gene regions.

## Materials and Methods

### Collection of diseased potato plants and tubers

In 2018 and 2019, samples of diseased potato plants were obtained multiple ways. 1) Potato plants and tubers with symptoms of soft rot, aerial stem rot, lenticel rot, or blackleg submitted by farmers and crop consultants to the Hermiston Agricultural Research and Extension Center (HAREC) Disease Diagnostic Clinic in Hermiston, Oregon were used for this study. 2) Clinic samples of other crops used in rotation with potato with soft rot symptoms, such as tomato and hemp, were also collected. 3) Symptomatic potato plants and tubers were collected from grower fields in the Hermiston, OR area and from field experiments conducted at HAREC, including the commercial seed lot trial.

Plant samples submitted to the HAREC Disease Diagnostic Clinic were processed upon arrival. Plant samples collected from grower fields and HAREC were bagged, transported to the laboratory and processed immediately, or stored at 4ºC and processed within 48 hours. Processing included washing the infected area and visually examining the cleaned potato tissue. Pieces of stem or tuber tissue were excised at the margin between healthy and symptomatic tissue and used for bacterial isolation and DNA extraction and PCR detection of SRP, which were conducted in parallel.

### Bacterial isolation

Plant tissue was surface sterilized with a 10% Clorox (Clorox Company, Oakland, CA) solution for 30 seconds and rinsed three times with sterile distilled water for 30 seconds. The plant tissue was placed in a 1.5 ml tube containing 500 µl of sterile distilled water and macerated with a sterile pestle. The macerated tissue was incubated at room temperature for five to ten minutes before being spread on Crystal Violet Pectate agar (CVP) (Hélias et al. 2012) with a sterile loop. CVP agar plates were incubated at 28°C for 24 hours. Bacterial colonies forming pits in the CVP medium were streaked onto fresh CVP medium until a single colony could be obtained.

To prepare each isolate for storage, a single colony was taken off the agar with a sterile toothpick and dropped into a sterile culture tube containing 5 ml of Luria-Bertani (LB) broth (Difco, Franklin Lakes, NJ). Culture tubes were incubated in a shaking incubator for 24 hours at 120 rpm at 28°C. If bacterial growth was observed in the culture tubes after 24 hours and the presence of an SRP was confirmed by PCR (described below), 500 µl of the LB broth culture was added to a cryogenic culture tube with 500 µl of 40% glycerol solution. The tube was vortexed to ensure the bacteria were suspended in the glycerol solution and stored in a -80°C freezer.

**DNA extraction and PCR detection of SRP**

To quickly confirm the presence and identity of SRP from each plant sample or bacterial isolate, DNA was extracted using the Dellaporta nucleic acid extraction protocol (Dellaporta et al. 1983). A multiplex polymerase chain reaction (PCR) was performed using the extracted DNA as a template. The multiplex PCR included primer pairs EXPccF/EXPccR, Y45/46, and DF/DR that specifically detect *P. carotovorum* or *P. parmentieri*, *P. atrosepticum*, and *Dickeya* species, respectively (Humphris et al. 2015;

Potrykus et al. 2014). PCR was performed in a Bio-Rad T100 Thermal Cycler (Bio-Rad, Hercules, CA) with the following program: denaturation (94 °C for 4 minutes), 30 cycles of denaturation (94°C for 45 seconds), annealing (62°C for 1:30 minutes), and extension (72 °C for 1:30 minutes, and then final extension (72°C for 3 minutes) (Potrykus et al 2014). Agarose gel electrophoresis was used to determine the sizes of the amplified products with a Bio-Rad PowerPac Basic (Bio-Rad, Hercules, CA) gel box in a 2% agarose gel made with 1x Tris-acetate-EDTA buffer stained with Biotium GelRed (Biotium, Hayward, CA), and the amplicons were visualized under UV light. When there was production of a 550 bp amplicon consistent with the detection of *P. carotovorum*/*P. parmentieri*, the PCR products were Sanger sequenced using EXPccF/EXPccR primers. Cleaned PCR product was submitted to the Oregon State University (OSU) Center for Genome Research and Biocomputing (CGRB). Results were compared to the National Center for Biotechnology Information (NCBI) BLAST database to differentiate between these two species.

**Sequence typing**

For each of the stored isolates, three gene regions were sequenced, the 16S rRNA region, *pelY* pectate lyase gene, and the *dnaX* partial gene region. A 1400 bp segment of the universal bacterial 16S rRNA region was amplified from the bacterial DNA samples using 27F/1495R primers (Lane 1991). A 434-bp segment of the *pelY* gene region was amplified from the bacterial DNA samples using Y1/Y2 primers (Darrasse et al. 1994). A 535-bp segment of the *dnaX* partial gene region was amplified from the bacterial DNA samples using dnaXF/dnaXR primers (Slawiak et al. 2009). PCR product sizes were confirmed using agarose gel electrophoresis. Amplicons were

cleaned using the Promega Wizard PCR Product Clean Up Kit (Promega, Madison, WI) and total DNA in the final volume was adjusted to 25 ng as required by the sequencing center. The *dnaX*, 16S, and *pelY* samples were sequenced using standard Sanger sequencing at the OSU CGRB in Corvallis, OR.

**Phylogenetic analysis**

Phylogenetic trees of the axenic bacterial isolate collection were constructed from amplified regions of 16S, *dnaX*, and *pelY* genes. Reference sequences were obtained from the NCBI database. Reference sequences included *P. carotovorum*, *P. atrosepticum*, *P. brasiliense*, *P. parmentieri, D. solani*, and *D. dianthicola* (Table 2.1). Sequence analyses were conducted in Geneious (Biomatters Ltd. Auckland, NZ). Forward and reverse sequences were aligned in Geneious to create a consensus sequence for each gene region of each isolate. Multiple sequence alignments (MSA) of partial 16S, *dnaX*, and *pelY* gene regions were constructed using MAFFT Version 7.450 with the G-INS-I algorithm (Katoh and Standley, 2013), and were manually corrected in Geneious. A maximum likelihood tree reconstruction was performed using RAxML (Randomized Axelerated Maximum Likelihood) Version 8 (Stamatakis, 2014) under the GTR-CAT approximation. Following tree construction, SRP species were mapped onto the tree to determine if there was clustering of isolates and if the SRP isolates clustered with reference isolates. This data was overlaid onto the final trees using the ggtree package (Yu et al. 2017) available in R (R core team, 2017).

**Results**

**Diseased potato plants and tubers**

In the 2018 and 2019 growing seasons, 25 and 120 diseased plant samples, respectively, were received or sampled and evaluated in this study. The majority of the diseased plant samples were submitted by local growers (120), and an additional 25 diseased potato plant samples were collected from grower fields or HAREC field experiments (Table 2.2). Potato samples were received from farm operations located in the Columbia Basin of Oregon and Washington and from Idaho. Several other crops (i.e. hemp and tomato) with symptoms consistent with SRP infection were also examined as part of this study. A total of 145 samples were diseased and exhibited symptoms of tuber soft rot, blackleg, and aerial stem rot symptoms (Table 2.2) including slimy tubers, chlorotic wilted leaves, and hollowed out necrotic stems.

**PCR testing of plant samples and bacterial isolates**

Out of the 145 total plant samples tested, 54 SRP were detected in 51 of the samples using multiplex PCR (Table 2.2). There were multiple SRP species present in three samples. The *P. carotovorum/P. parmentieri* complex was most commonly detected (74.1%), followed by *P. atrosepticum* (20.4%), and *Dickeya* species (5.6%) (Table 2.2). Multiple SRP were detected from three samples including one sample infected with *P. carotovorum/P. parmentieri* and *P. atrosepticum* (2.0%), one with *P. atrosepticum and Dickeya* spp. (2.0%), and one with *P. carotovorum/P. parmentieri* and *Dickeya* spp. (2.0%).

Detections in the *P. carotovorum/P. parmentieri* category were later refined to differentiate between both species based on sequence data. Several of the samples were determined to be *P. parmentieri* or *P. brasiliense.* After adjustments based on sequencing data, the proportion of detections was determined to be: 61.1% *P.*

*carotovorum*, 20.4% *P. atrosepticum,* 5.6% *Dickeya* species, 9.3% *P. parmentieri*, and 3.7% *P. brasiliense* (Table 2.2).

Twenty-eight bacterial isolates were obtained from the 51 diseased plant samples, but no *Dickeya* spp. were recovered in culture. Based on PCR, the isolate collection was comprised of 75% *P. carotovorum,* 7.1% *P. parmentieri*, 10.7% *P. atrosepticum,* 7.1% *P. brasiliense*, and 0% *Dickeya* species (Table 2.2).

**Phylogenetic analysis of bacterial isolates**

We sequenced and aligned a 1400-bp segment of the universal bacterial 16S rRNA region, a 434-bp segment of the *pelY* gene region, and a 535-bp segment of the *dnaX* partial gene region from each of the 28 bacterial isolates to create three phylogenetic trees showing the relationship among the isolates and reference species from the NCBI database.

The maximum likelihood tree reconstruction of the partial 16S rRNA gene sequences contained 22 isolates (6 isolates were removed due to poor sequencing quality) and 18 reference sequences, but did not yield distinct clades (Figure 2.1). In part, this may be due to the fact that only part of the 16S rRNA gene region was sequenced and did not contain enough information to resolve species groupings.

The maximum likelihood tree reconstruction of the partial *dnaX* gene sequences contained 28 isolates and 11 reference sequences (Figure 2.2), and the maximum likelihood tree reconstruction of the partial *pelY* gene sequences contained 28 isolates and four reference sequences (Figure 2.3). Both *DnaX* and *pelY* tree reconstructions resolved all reference strains, resulting in four distinct clades representing *Pectobacterium atrosepticum*, *Pectobacterium brasiliense*, *Pectobacterium*

*carotovorum*, and *Pectobacterium parmentieri,* each with greater than 70% bootstrap support (Figures 2.2 and 2.3).

In the *dnaX* tree, *Pectobacterium* species clustered separately from the rooted *Dickeya* species (100% bootstrap support; Figure 2.2). The *P. parmentieri* and *P. atrosepticum* clades separated from the *P. carotovorum* and *P. brasiliense* isolates (90% bootstrap support; Figure 2.2). Both the *P. parmentieri* and *P. atrosepticum* clades had 100% bootstrap support (Figure 2.2). The *P. brasiliense* isolates clustered within the *P. carotovorum* clade and had greater than 98% bootstrap support (Figure 2.2). The *P. carotovorum* isolates separated into three clusters, one set with three isolates (97% bootstrap support), a set with 11 isolates that was closely related to *P. brasiliense* (>70% bootstrap support), and a final set with six isolates and the two reference species (Figure 2.2). This final set was highly supported as separate from the two previously described *P. carotovorum* clades (100% bootstrap support), but had less than 70% overall bootstrap support (Figure 2.2).

In the *pelY* tree, the *Pectobacterium atrosepticum* clade clustered separately from the *P. parmentieri*, *P. brasiliense*, and *P. carotovorum* isolates (100% bootstrap support; Figure 2.3). The *P. parmentieri* clade separated from the *P. carotovorum* and *P. brasiliense* isolates (100% bootstrap support; Figure 2.3). Both the *P. parmentieri* clade (100% bootstrap support) and the *P. brasiliense* clade (99% bootstrap support) were highly supported (Figure 2.3). The *P. brasiliense* isolates clustered apart the *P. carotovorum* clade with greater than 77% bootstrap support (Figure 2.3). The *P. carotovorum* clade had 81% bootstrap support (Figure 2.3).

The grouping of the isolates collected in this study was consistent among the two gene regions, providing support for the taxonomic identification of the bacterial isolates. The taxonomic assignments of the 28 isolates based on the phylogenetic relationships and the multiplex PCR analysis were also consistent (Table 2.3).

**Geographic origin of SRP isolates**

Based on PCR detection and sequence identification of the bacterial isolates sampled in this study, *Pectobacterium carotovorum* was the most prevalent species of SRP detected in the Columbia Basin. *Pectobacterium carotovorum* was detected in 65% (26/40) of the diseased potatoes originating in Oregon, followed by *P. atrosepticum*, *P. parmentieri*, *P. brasiliense*, and mixed detections of *P. atrosepticum* and *P. carotovorum* or *Dickeya* spp. detected in 15%, 10%, 2.5% and 7.5% of the disease potato samples, respectively (Table 2.4). From potatoes originating in Washington, there were two detections of *P. carotovorum* (22.22%), three detections of *P. atrosepticum* (33.33%), one detection each of *Dickeya* spp., *P. parmentieri*, and *P. brasiliense* (11.11% each), and one mixed detection of *P. atrosepticum* and *P. carotovorum* (11.11%; Table 2.4). *P. carotovorum* was detected from two potato samples from Idaho (100%; Table 2.4).

**Disease symptoms associated with SRP**

Tuber soft rot was the most commonly observed disease symptom observed and evaluated in this study, followed by blackleg and aerial stem rot (Table 2.5). There were 21 detections of *P. carotovorum* associated with tuber soft rot (60%), five detections of *P. atrosepticum* (14.29%), one detection of *Dickeya* spp. (2.86%), four detections of *P. parmentieri* (11.43%), one detection of *P. brasiliense* (2.86%), and three mixed

detections of *P. atrosepticum* and *P. carotovorum* or *Dickeya* spp. (8.57%) (Table 2.5). There were nine detections of blackleg associated with *P. carotovorum* (60%), three detections of *P. atrosepticum* (20%), and one detection each of *P. parmentieri* (6.67%), *P. brasiliense* (6.67%), and a mixed infection of *P. carotovorum* and *P. atrosepticum* (6.67%). Aerial stem rot was not commonly observed and *P. atrosepticum* was detected in the one plant with aerial stem rot that was evaluated (Table 2.5).

**Seasonal detection of SRP**

Late in the growing season (July through September) was when the highest number of submissions with SRP detections (30 in total between both years) were received to be evaluated in this study (Table 2.6). During this time of the growing season, there were 18 detections of *Pectobacterium carotovorum* (60%), five detections of *P. parmentieri* (16.67%), four detections of *P. atrosepticum* (13.33%) two detections of *P. brasiliense* (6.67), and one detection of a mixed sample of *P. atrosepticum* and *P. carotovorum* (3.33%). During the early season (April through June) there were 19 samples submitted with SRP detections. These included 10 detections of *P. carotovorum* (52.63%), five detections of *P. atrosepticum* (26.32%), three mixed detections of *P. atrosepticum* and *P. carotovorum* or *Dickeya* spp. (15.79%), and one detection of a *Dickeya* spp. (5.26%). During the storage season (October through March), there were two samples submitted with detections of *P. carotovorum* (Table 2.6).

**Potato cultivars with soft rot disease**

Most SRP was detected from ‘Russet Norkotah,’ ‘Russet Burbank,’ and ‘Ivory Russet’ cultivars (Table 2.7; Supplementary Table 2.1). There were 20 ‘Russet

Norkotah' samples that were associated with *P. atrosepticum* (5%), *P. carotovorum* (80%), *P. brasiliense* (5%), and with mixed infections of *P. atrosepticum* and *Dickeya* spp. (10%) (Table 2.7). There were six 'Russet Burbank' submissions where *P. carotovorum* (83.33%) and *P. atrosepticum* (16.67%) were detected. There were six 'Ivory Russet' submissions where *P. atrosepticum* (33.33%), *P. carotovorum* (33.33%), *P. parmentieri* (16.67%), and a mixed infection of *P. atrosepticum* and *P. carotovorum* (16.67%) were detected (Table 2.7).

**Discussion**

In 2018 and 2019, the soft rot pathogens of potato that were present in the Columbia Basin of Eastern Oregon and Washington were *P. atrosepticum*, *P. brasiliense*, *P. carotovorum*, and *P. parmentieri*. *Pectobacterium carotovorum* and *P. atrosepticum* were the dominant species in the surveyed samples. This is different from the findings of Ma et al. 2018, who reported that *Dickeya* spp. comprised nearly half of the bacterial species responsible for causing blackleg in New York state. In part, the difference could be due to disease sampling strategy. For example, Ma et al. 2018 sampled primarily potatoes with blackleg, whereas we sampled all potato soft rot diseases. However, even when we look exclusively at potatoes expressing blackleg from the Columbia Basin, the composition of bacterial species differed from those found in New York.

In the Columbia Basin, the predominant species associated with blackleg were *P. carotovorum* and *P. atrosepticum*. Additionally, blackleg was not prevalent in the Columbia Basin, possibly due to the dry conditions during the growing season

(Pérombelon et al. 1989). *Dickeya* spp. were detected in only 5.6% of the potatoes infected with SRP and were mostly detected in association with *Pectobacterium* species. This result is similar to the finding of Ali et al. (2012), who found that from 2007-2009 *Erwinia carotovora* subsp*. atroseptica* (*Pectobacterium atrosepticum*) and *Erwinia carotovora* subsp*. carotovora* (*Pectobacterium carotovorum*) were the main causal organisms of blackleg and soft rot in Pakistan. Our findings may be similar to this study because the arid climate of the Columbia Basin may be more similar to Pakistan than to New York state and the U.S East coast. Additionally, *Dickeya* spp. may be rare in the Pacific Northwest because potato seed certification programs and increased efforts to prevent the spread of *Dickeya* spp. after their U.S. arrival was detected in 2015 may have been effective in limiting spread into the Columbia Basin.

Although *Dickeya* spp. were detected by PCR in a small proportion of diseased plant samples, we were unable to recover *Dickeya* spp. in culture. *Dickeya* spp. are known to grow more slowly than *Pectobacterium* spp. on the CVP medium used to culture SRP from diseased plants and their presence on CVP can be difficult to detect (A. Charkowski and G. Secor, personal communication, August 8, 2017). Additionally, *Dickeya* spp. may lose the pectinolytic ability to create pits in CVP agar, so after the initial transfer of pectinolytic colonies to a new CVP plate *Dickeya* spp. colonies might not have been detected due to the lack of pitting. Others have used CVP to successfully culture *Dickeya* spp., and CVP medium remains the most widely used medium for culturing all SRP.

It is generally thought that optimal temperatures for *Pectobacterium* spp. to cause disease are lower compared to *Dickeya* spp. (Toth et al. 2011), and temperature

can influence which SRP species is present and primarily responsible for causing disease (Smadja et al. 2004). Additionally, temperature is known to influence the production of pathogenicity factors of SRP. Thus, one hypothesis is that plants infected with more than one SRP species are more likely to develop soft rot disease, and cause more severe disease, because collectively, multiple SRP have a broader optimal temperature range over which they can cause disease than a single SRP. From this current study, we do not have any data that would support or negate this hypothesis. However, in several cases, multiple SRP were detected from a single symptomatic sample (i.e., about 8% of the samples,) documenting that infections comprised of multiple SRP do readily occur in potato fields.

Three different genes/gene regions (16S, *dnaX*, *pelY*) were analyzed to examine the phylogenetic relationships of the 28 bacterial isolates that were recovered in culture from diseased plant samples in this survey. The phylogenetic trees for the *dnaX* and *pelY* gene regions produced four distinct clades that represented *P. atrosepticum*, *P. brasiliense*, *P. carotovorum*, and *P. parmentieri*, but the 16S phylogenetic tree did not yield distinct clades. The fact that the full ~1530 bp length 16S rRNA was not sequenced may be why the phylogenetic analysis resulted in poor clustering. However, previous phylogenetic analyses of *Pectobacterium* species based only on 16S rRNA gene sequences have also been unable to resolve all species (or former *P. carotovorum* subspecies) in their studies (Nabhan et al. 2012). Both the *dnaX* and *pelY* phylogenetic trees resulted in a similar grouping of isolates into distinct clades and the identities of the SRP isolates derived from the phylogenetic analyses were consistent with the results of the multiplex PCR. This finding suggests the species assignments from the

multiplex PCR results were accurate for differentiating between *P. carotovorum* and *P. atrosepticum*, although further testing is required to distinguish between *P. carotovorum, P. parmentieri,* and *P. brasiliense* when *P. carotovorum* is detected is initially detected. The primers used in the multiplex PCR (Humphris et al. 2015; Potrykus et al. 2014) are the standard used by many agencies, such as United States Department of Agriculture's Animal and Plant Health Inspection Service (USDA APHIS), to detect *Pectobacterium* and *Dickeya* species and continue to be reliable.

Prior to this study, *Pectobacterium parmentieri* and *Pectobacterium brasiliense* had not been reported in Oregon, and *Pectobacterium brasiliense* had not yet been reported in Washington (Dung et al. reported *Pectobacterium wasabiae* (now *Pectobacterium parmentieri)* causing aerial stem rot in 2012). It is possible that these bacterial species have been present in the Columbia Basin, but were not previously detected due to the lack of molecular tools available to easily and accurately characterize them. This is apparent based a 2012 article by De Boer et al. that includes a table referencing a strain of *Pectobacterium wasabiae* isolated from Oregon in 1970-1985. Also, due to the recent taxonomic reclassifications of the SRP, these previously unreported pathogens might have been identified as *P. carotovorum* or *P. atrosepticum*, which are ubiquitous in all potato producing regions in the US. Although the composition of SRP causing disease in potato may be changing in the Columbia Basin production region of the U.S., much of the management tactics used to suppress these diseases will remain the same. In the future, it will be important to better understand the threats posed by the current SRP or risk for establishment of new SRP under climate change scenarios (Skelsey et al. 2018). Continued surveillance and identification of SRP in the

Columbia Basin will remain and important component to mitigating crop losses due to soft rot diseases.

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## Tables

**Table 2.1**. Reference isolates obtained from NCBI used in phylogenetic analysis of soft rot isolates collected from the Columbia Basin.

| Species | Accession number[a] | Substrate[b] | Location[c] | Reference[d] | Gene Region[e] | Base pair range[f] | Target[g] |
|---|---|---|---|---|---|---|---|
| *Pectobacterium parmentieri* | CP026983.1 | Potato | Poland | Zoledowska,S. 2018 | CG | | 16S |
| *Pectobacterium parmentieri* | CP026980.1 | Potato | Poland | Zoledowska,S. 2018 | CG | | 16S |
| *Pectobacterium parmentieri* | CP026977.1 | Potato | Poland | Zoledowska,S. 2018 | CG | | 16S |
| *Pectobacterium carotovorum* | AF373187.1 | NA | Canada | Fessehaie et al. 2014 | 16S | | |
| *Pectobacterium carotovorum* | AF373185.1 | NA | Canada | Fessehaie et al. 2014 | 16S | | |
| *Pectobacterium carotovorum* subsp. *carotovorum* | CP003776.1 | NA | Korea | Park et al. 2014 | CG | | 16S |
| *Pectobacterium carotovorum* subsp. *brasiliense* | CP020350.1 | Cucumber | China | Li et al. 2014 | CG | | 16S |
| *Pectobacterium carotovorum* subsp. *brasiliense* | CP024780.1 | Cucumber | China | Huang et al. 2017 | CG | | 16S |
| *Pectobacterium carotovorum* subsp. *brasiliense* | CP009769.1 | Chinese cabbage | China | Sui et al. 2017 | CG | | 16S |
| *Pectobcaterium atrocepticum* | AF373183.1 | NA | NA | Fessehaie et al. 2014 | 16S | | |
| *Pectobcaterium atrocepticum* | AF373181.1 | NA | Germany | Fessehaie et al. 2014 | 16S | | |
| *Pectobcaterium atrocepticum* | CP009125.1 | NA | Belarus | Nikolaichik et al. 2016 | CG | | 16S |
| *Dickeya solani* | CP024710.1 | Potato | Germany | Golanowska et al. 2018 | CG | | 16S |
| *Dickeya solani* | CP015137.1 | Potato | Netherlands | Khayi et al. 2016 | CG | | 16S |
| *Dickeya solani* | CP017453.1 | Potato | Finland | Khayi et al. 2017 | CG | | 16S |
| *Dickeya dianthicola* | CP031560.1 | Potato | ME, USA | Ma et al. 2017 | CG | | 16S |
| *Dickeya dianthicola* | CP017638.1 | Potato | NA | Khayi et al. 2017 | CG | | 16S |
| *Dickeya dianthicola* | LC042601.1 | Carnation | Japan | Febryani et al. 2015 | 16S | | |

**Table 2.1.** (Continued).

| Species | Accession number | Substrate | Location | Reference | Gene Region | Base pair range | Target |
|---|---|---|---|---|---|---|---|
| *Pectobacterium carotovorum* subsp. *carotovorum* | MK516950.1 | Potato | USA | Portier et al. 2019 | dnaX | | |
| *Pectobacterium carotovorum* subsp. *carotovorum* | MK516909.1 | Potato | Denmark | Portier et al. 2019 | dnaX | | |
| *Dickeya solani* | CP024711.1 | Potato | Poland | Golanowska et al. 2018 | CG | | dnaX |
| *Dickeya solani* | CP024710.1 | Potato | Germany | Golanowska et al. 2018 | CG | | dnaX |
| *Dickeya solani* | CP017453.1 | Potato | Finland | Khayi et al. 2017 | CG | | dnaX |
| *Dickeya dianthicola* | CP017638.1 | Potato | NA | Khayi et al. 2017 | CG | | dnaX |
| *Pectobacterium carotovorum* subsp. *brasiliense* | MK516959.1 | Water | France | Portier et al. 2019 | dnaX | | |
| *Pectobcaterium atrocepticum* | MF954606.1 | Potato | NY, USA | Ma et al. 2017 | dnaX | | |
| *Pectobacterium parmentieri* | MF954603.1 | Potato | NY, USA | Ma et al. 2017 | dnaX | | |
| *Pectobacterium parmentieri* | MF954604.1 | Potato | NY, USA | Ma et al. 2017 | dnaX | | |
| *pectobacterium parmentieri* | MF954605.1 | Potato | NY, USA | Ma et al. 2017 | dnaX | | |
| *Pectobacterium parmentieri* | CP026979.1 | NA | Poland | Zoledowska,S. 2018 | CG | 2559236-2559648 | PelY |
| *Pectobcaterium atrocepticum* | CP007744.1 | NA | China | Zhu et al. 2014 | CG | 2347553-2347964 | PelY |
| *Pectobacterium carotovorum* | CP021894.1 | Potato | Finland | Niemi et al. 2017 | CG | 2650971-2651383 | PelY |
| *Pectobacterium carotovorum* subsp. *brasiliense* | CP009769.1 | Chinese cabbage | China | Sui et al. 2014 | CG | 2497850-2498274 | PelY |

[a] Accession numbers from the National Center for Biotechnology Information.
[b] Substrate or host plant from which the isolate was collected from. NA = information not available.
[c] Country the isolate was collected from. NA = information not available.
[d] Author(s) who submitted the sequences and year of submission.
[e] Gene region used in alignment. Where references were complete genomes or listed as “chromosome,” base pair and target gene regions were included. CG = complete genome.
[f] Base pair ranges used in alignment for references from complete genomes.
[g] Gene region used in alignment for references from complete genomes.

**Table 2.2.** List of positive bacterial soft rot isolates collected from the Columbia Basin from 2018- 2019.

| Isolate | Date Collected | Location[a] | Host[b] | Symptom[c] | Cultivar[d] | PCR Result[e] | Culture[f] |
|---|---|---|---|---|---|---|---|
| JB1 | 6/26/18 | OR | Potato | Blackleg | Russet Burbank | Pcc | No |
| JB4 | 6/26/18 | OR | Potato | Soft rot | Russet Burbank | Pcc | No |
| JB15 | 8/17/18 | OR | Potato | Soft rot | Ranger Russet | Pp | No |
| JB17 | 9/5/18 | OR | Potato | Soft rot | Ranger Russet | Pp | Yes |
| JB29 | 4/29/19 | WA | Potato | Soft rot | Clearwater | Pba | No |
| JB30 | 4/29/19 | WA | Potato | Soft rot | Clearwater | Pba | No |
| JB31 | 4/29/19 | WA | Potato | Soft rot | Clearwater | Dickeya | No |
| JB33 | 4/30/19 | OR | Potato | Soft rot | NA | Pba | No |
| JB35 | 4/30/19 | OR | Potato | Soft rot | NA | Pba | No |
| JB38 | 5/13/19 | OR | Potato | Soft rot | Norkotah | Pcc | Yes |
| JB39 | 5/13/19 | OR | Potato | Soft rot | Norkotah | Pcc | Yes |
| JB45 | 5/13/19 | OR | Potato | Soft rot | Norkotah | Pcc | No |
| JB46 | 5/13/19 | OR | Potato | Soft rot | Norkotah | Pcc | Yes |
| JB47 | 5/13/19 | OR | Potato | Soft rot | Norkotah | Pba Dickeya | No |
| JB53 | 5/13/19 | OR | Potato | Soft rot | Norkotah | Pcc | Yes |
| JB54 | 5/13/19 | OR | Potato | Soft rot | Norkotah | Pcc Dickeya | No |
| JB55 | 5/13/19 | OR | Potato | Soft rot | Norkotah | Pcc | Yes |
| JB56 | 5/13/19 | OR | Potato | Soft rot | Norkotah | Pcc | Yes |
| JB59 | 5/13/19 | OR | Potato | Soft rot | Norkotah | Pcc | No |
| JB61 | 5/13/19 | OR | Potato | Soft rot | Norkotah | Pba | No |
| JB63 | 5/13/19 | OR | Potato | Soft rot | Norkotah | Pcc | Yes |
| JB87 | 7/11/19 | OR | Potato | Soft rot | Ivory Russet | Pba | Yes |
| JB88 | 7/12/19 | OR | Potato | Soft rot | NA | Pcc | Yes |
| JB90 | 7/12/19 | OR | Potato | Soft rot | NA | Pcc | Yes |

**Table 2.2.** (Continued).

| Isolate | Date Collected | Location | Host | Symptom | Cultivar | PCR Result | Culture |
|---|---|---|---|---|---|---|---|
| JB94 | 7/15/19 | ID | Potato | Blackleg | Russet Burbank | Pcc | Yes |
| JB95 | 7/15/19 | ID | Potato | Blackleg | Russet Burbank | Pcc | Yes |
| JB96 | 7/15/19 | OR | Potato | Blackleg | Russet Burbank | Pcc | Yes |
| JB99 | 7/16/19 | OR | Potato | Blackleg | Ivory Russet | Pcc | Yes |
| JB100 | 7/16/19 | OR | Potato | Blackleg | Ivory Russet | Pcc | Yes |
| JB103 | 7/18/19 | OR | Hemp | Blackleg | NA | Pcc | Yes |
| JB105 | 7/26/19 | WA | Potato | Blackleg | Ivory Russet | Pcc Pba[g] | Yes |
| JB106 | 7/26/19 | WA | Potato | Blackleg | Ivory Russet | Pp | Yes |
| JB107 | 7/26/19 | WA | Potato | Blackleg | Ivory Russet | Pba | Yes |
| JB113 | 8/15/19 | OR | Potato | Aerial stem rot | Russet Burbank | Pba | Yes |
| JB121 | 9/8/19 | OR | Potato | Blackleg | NA | Pcc | No |
| JB122 | 9/8/19 | OR | Potato | Blackleg | NA | Pcc | Yes |
| JB123 | 9/8/19 | OR | Potato | Blackleg | NA | Pba | No |
| JB124 | 9/8/19 | OR | Potato | Blackleg | NA | Pb | Yes |
| JB126 | 9/10/19 | WA | Potato | Soft rot | Norkotah | Pcc | No |
| JB127 | 9/10/19 | WA | Potato | Soft rot | Norkotah | Pb | Yes |
| JB133 | 9/10/19 | WA | Potato | Soft rot | Norkotah | Pcc | Yes |
| JB134 | 9/10/19 | OR | Potato | Soft rot | Atlantic | Pcc | No |
| JB135 | 9/10/19 | OR | Potato | Soft rot | Atlantic | Pcc | No |
| JB138 | 9/13/19 | OR | Potato | Soft rot | Russet Norkotah | Pcc | No |
| JB139 | 9/13/19 | OR | Potato | Soft rot | Russet Norkotah | Pcc | No |
| JB140 | 9/13/19 | OR | Potato | Soft rot | Russet Norkotah | Pcc | No |
| JB141 | 9/13/19 | OR | Potato | Soft rot | Russet Norkotah | Pcc | No |
| JB142 | 9/13/19 | OR | Potato | Soft rot | Russet Norkotah | Pcc | No |
| JB143 | 9/19/19 | OR | Potato | Soft rot | Umatilla | Pcc | Yes |

**Table 2.2.** (Continued).

| Isolate | Date Collected | Location | Host | Symptom | Cultivar | PCR Result | Culture |
|---|---|---|---|---|---|---|---|
| JB144 | 9/19/19 | OR | Potato | Soft rot | Umatilla | Pcc | Yes |
| JB145 | 9/19/19 | OR | Potato | Soft rot | Umatilla | Pcc | Yes |

[a] State where the plant sample was grown or originated from.
[b] Plant species of sample.
[c] Soft rot symptom observed on plant sample.
[d] Cultivar or variety of plant sample. NA = information not available.
[e] Multiplex PCR result or sequence BLAST result for sequenced isolates. Pcc = *Pectobacterium carotovorum*, Pba = *Pectobacterium atrocepticum*, Pp = *Pectobacterium parmentieri*, Pb = *Pectobacterium brasiliense*, and Dickeya = *Dickeya spp.* In some instances multiple pathogens were detected in the same plant sample.
[f] Indicates whether a culture of the detected isolate was stored in glycerol in -80°C freezer.
[g] In Isolate JB105, Pcc and Pba were detected in plant sample, but only Pcc was recovered in culture for isolate collection.

**Table 2.3.** PCR results of soft rot isolates collected from the Columbia Basin from 2018- 2019 compared to *pelY, dnaX,* and 16S DNA sequencing results.

| Isolate | PCR Result[a] | *pelY* Result | *dnaX* Result | 16S Result[b] |
|---|---|---|---|---|
| JB17d | Pp | Pp | Pp | Pp |
| JB38B | Pcc, Dsp, Pba | Pcc | Pcc | Pcc |
| JB39A | Pcc | Pcc | Pcc | Pcc |
| JB46x4 | Pcc | Pcc | Pcc | Pcc |
| JB53B | Pcc | Pcc | Pcc | Pcc |
| JB55x3 | Pcc | Pcc | Pcc | Pcc |
| JB56A | Pcc | Pcc | Pcc | Pcc |
| JB63B | Pcc | Pcc | Pcc | Pcc |
| JB87A | Pba | Pba | Pba | Pba |
| JB88B | Pp | Pp | Pp | Pp |
| JB90A | Pp | Pp | Pp | Pp |
| JB94B | Pcc | Pcc | Pcc | Pcc |
| JB95A | Pcc | Pcc | Pcc | NA |
| JB96A | Pcc | Pcc | Pcc | Pcc |
| JB99 | Pcc | Pcc | Pcc | NA |
| JB100A | Pcc | Pcc | Pcc | Pcc |
| JB103D | Pcc | Pcc | Pcc | NA |
| JB105D | Pba Pcc | Pcc | Pcc | NA |
| JB106A | Pp | Pp | Pp | NA |
| JB107B | Pba | Pba | Pba | Pba |
| JB113A | Pba | Pcc | Pcc | Pcc |
| JB121A | Pcc | Pcc | Pcc | Pcc |
| JB124A | Pb | Pb | Pb | Pb |
| JB127A | Pb | Pb | Pb | Pb |
| JB133A | Pcc | Pcc | Pcc | Pcc |
| JB143A | Pcc | Pcc | Pcc | Pcc |
| JB144A | Pcc | Pcc | Pcc | NA |
| JB145A | Pcc | Pcc | Pcc | Pcc |

[a] Pcc = *Pectobacterium carotovorum*, Pba = *Pectobacterium atrocepticum*, Pp = *Pectobacterium parmentieri*, Pb = *Pectobacterium brasiliense*, and Dsp = *Dickeya spp.* In some instances multiple pathogens were detected in the same plant sample.

[b] NA = not available due to low sequencing quality.

**Table 2.4.** Number and percent of SRP species detected in symptomatic potato plants originating from Idaho, Oregon, and Washington from 2018- 2019.

| | Location (State) [a] | | |
|---|---|---|---|
| Pathogen[b] | Oregon | Washington | Idaho |
| Pba | 6 (15.0) | 3 (33.3) | 0 (0.0) |
| Pcc | 26 (65.0) | 2 (22.2) | 2 (100.0) |
| Dsp | 0 (0.0) | 1 (11.1) | 0 (0.0) |
| Pp | 4 (10.0) | 1 (11.1) | 0 (0.0) |
| Pb | 1 (2.5) | 1 (11.1) | 0 (0.0) |
| Pba + Pcc(Dsp) | 3 (7.5) | 1 (11.1) | 0 (0.0) |
| Total overall | 40 | 9 | 2 |

[a] State where the plant sample was grown or originated from.
[b] Pathogen detected in sample. Pcc = *Pectobacterium carotovorum*, Pba = *Pectobacterium atrocepticum*, Pp = *Pectobacterium parmentieri*, Pb = *Pectobacterium brasiliense*, Dsp = *Dickeya* species, and Pba + Pcc(Dsp) indicates a mixed infection of *P. atrosepticum* and *P. carotovorum* or *Dickeya* species.

**Table 2.5.** Number and percent of SRP species detected in symptomatic potato plants exhibiting blackleg, tuber soft rot, and aerial stem rot disease symptoms collected from the Columbia Basin from 2018- 2019.

| | Disease (Symptom) [a] | | |
|---|---|---|---|
| Pathogen[b] | Blackleg | Tuber Rot | Arial Stem Rot |
| Pba | 3 (20.0) | 5 (14.29) | 1 (100.0) |
| Pcc | 9 (60.0) | 21(60.0) | 0 (0.0) |
| Dsp | 0 (0.0) | 1 (2.86) | 0 (0.0) |
| Pp | 1 (6.67) | 4 (11.43) | 0 (0.0) |
| Pb | 1(6.67) | 1 (2.86) | 0 (0.0) |
| Pba + Pcc(Dsp) | 1 (6.67) | 3 (8.57) | 0 (0.0) |
| Total overall | 15 | 35 | 1 |

[a] Disease symptom observed on the plant sample.
[b] Pathogen detected in sample. Pcc = *Pectobacterium carotovorum*, Pba = *Pectobacterium atrocepticum*, Pp = *Pectobacterium parmentieri*, Pb = *Pectobacterium brasiliense*, Dsp = *Dickeya* species, and Pba + Pcc(Dsp) indicates a mixed infection of *P. atrosepticum* and *P. carotovorum* or *Dickeya* species.

**Table 2.6.** Number and percent of SRP species detected in symptomatic potato plants collected in the early season (April – June), late season (July - September), and during the storage season (October – March) from the Columbia basin from 2018- 2019.

| | Season [a] | | |
|---|---|---|---|
| Pathogen[b] | Early (April - June) | Late (July - Sept) | Storage (Oct - March) |
| Pba | 5 (26.32) | 4 (13.33) | 0 (0.0) |
| Pcc | 10 (52.63) | 18 (60.0) | 2 (100.0) |
| Dsp | 1 (5.26) | 0 (0.0) | 0 (0.0) |
| Pp | 0 (0.0) | 5 (16.67 | 0 (0.0) |
| Pb | 0 (0.0) | 2 (6.67) | 0 (0.0) |
| Pba + Pcc(Dsp) | 3 (15.79) | 1 (3.33) | 0 (0.0) |
| Total overall | 19 | 30 | 2 |

[a] Season when the plant sample was submitted or collected.

[b] Pathogen detected in sample. Pcc = *Pectobacterium carotovorum*, Pba = *Pectobacterium atrocepticum*, Pp = *Pectobacterium parmentieri*, Pb = *Pectobacterium brasiliense*, Dsp = *Dickeya* species, and Pba + Pcc(Dsp) indicates a mixed infection of *P. atrosepticum* and *P. carotovorum* or *Dickeya* species.

**Table 2.7.** Number and percent of SRP species detected in symptomatic potato plants from different cultivars collected from the Columbia Basin in 2018- 2019.

| | Cultivar [a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Pathogen[b] | Russet Burbank | Ranger Russet | Clearwater | Russet Norkotah | Ivory russet | Atlantic | Umatilla | NA |
| Pba | 1 (16.67) | 0 (0.0) | 2 (66.67) | 1 (5.0) | 2 (33.33) | 0 (0.0) | 0 (0.0) | 3 (33.33) |
| Pcc | 5 (83.33) | 0 (0.0) | 0 (0.0) | 16 (80.0) | 2 (33.33) | 2 (100.0) | 3 (100.0) | 5 (55.56) |
| Dsp | 0 (0.0) | 0 (0.0) | 1 (33.33) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Pp | 0 (0.0) | 2 (100.0) | 0 (0.0) | 0 (0.0) | 1 (16.67) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Pb | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (5.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (11.11) |
| Pba + Pcc(Dsp) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 2 (10.0) | 1 (16.67) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Total overall | 6 | 2 | 3 | 20 | 6 | 2 | 3 | 9 |

[a] Cultivar of the plant sample.

[b] Pathogen detected in sample. Pcc = *Pectobacterium carotovorum*, Pba = *Pectobacterium atrocepticum*, Pp = *Pectobacterium parmentieri*, Pb = *Pectobacterium brasiliense*, Dsp = *Dickeya* species, and Pba + Pcc(Dsp) indicates a mixed infection of *P. atrosepticum* and *P. carotovorum* or *Dickeya* species..

**Supplementary Table 2.1.** List of plant samples collected from the Columbia Basin from 2018- 2019 tested for soft rot bacteria.

| Isolate | Date Collected | Location[a] | Host[b] | Symptom[c] | Cultivar[d] | PCR Result[e] | Culture[f] |
|---|---|---|---|---|---|---|---|
| JB1 | 6/26/18 | OR | Potato | Blackleg | Russet Burbank | Pcc | No |
| JB2 | 6/26/18 | OR | Potato | Blackleg | Russet Burbank | - | No |
| JB3 | 6/26/18 | OR | Potato | Blackleg | Russet Burbank | - | No |
| JB4 | 6/26/18 | OR | Potato | Soft rot | Russet Burbank | Pcc | No |
| JB5 | 6/26/18 | OR | Potato | Blackleg | Lamoka | - | No |
| JB6 | 7/13/18 | OR | Potato | Blackleg | Lamoka | - | No |
| JB7 | 8/13/18 | WA | Potato | Soft rot | NA | - | No |
| JB8 | 8/13/18 | WA | Potato | Soft rot | NA | - | No |
| JB9 | 8/13/18 | WA | Potato | Soft rot | NA | - | No |
| JB10 | 8/13/18 | WA | Potato | Soft rot | NA | - | No |
| JB11 | 8/13/18 | WA | Potato | Soft rot | NA | - | No |
| JB12 | 8/13/18 | WA | Potato | Soft rot | NA | - | No |
| JB13 | 8/13/18 | WA | Potato | Soft rot | NA | - | No |
| JB14 | 8/13/18 | WA | Potato | Soft rot | NA | - | No |
| JB15 | 8/17/18 | OR | Potato | Soft rot | Ranger Russet | Pp | No |
| JB16 | 8/17/18 | OR | Potato | Soft rot | Ranger Russet | - | No |
| JB17 | 9/5/18 | OR | Potato | Soft rot | Ranger Russet | Pp | **Yes** |
| JB18 | 9/5/18 | OR | Potato | Soft rot | Ranger Russet | - | No |
| JB19 | 9/5/18 | OR | Potato | Soft rot | Ranger Russet | - | No |
| JB20 | 9/5/18 | OR | Potato | Soft rot | Ranger Russet | - | No |
| JB21 | 9/5/18 | OR | Potato | Soft rot | Ranger Russet | - | No |
| JB22 | 9/5/18 | OR | Potato | Soft rot | Ranger Russet | - | No |
| JB23 | 9/5/18 | OR | Potato | Soft rot | Ranger Russet | - | No |
| JB24 | 9/5/18 | OR | Potato | Soft rot | Ranger Russet | - | No |

**Supplementary Table 2.1.** (Continued).

| Isolate | Date Collected | Location | Host | Symptom | Cultivar | PCR Result | Culture |
|---|---|---|---|---|---|---|---|
| JB25 | 9/5/18 | OR | Potato | Soft rot | Ranger Russet | - | No |
| JB26 | 4/17/19 | WA | Potato | Soft rot | Shepody | - | No |
| JB27 | 4/17/19 | WA | Potato | Soft rot | Shepody | - | No |
| JB28 | 4/29/19 | WA | Potato | Soft rot | Clearwater | - | No |
| JB29 | 4/29/19 | WA | Potato | Soft rot | Clearwater | Pba | No |
| JB30 | 4/29/19 | WA | Potato | Soft rot | Clearwater | Pba | No |
| JB31 | 4/29/19 | WA | Potato | Soft rot | Clearwater | Dickeya | No |
| JB32 | 4/30/19 | OR | Potato | Soft rot | NA | - | No |
| JB33 | 4/30/19 | OR | Potato | Soft rot | NA | Pba | No |
| JB34 | 4/30/19 | OR | Potato | Soft rot | NA | - | No |
| JB35 | 4/30/19 | OR | Potato | Soft rot | NA | Pba | No |
| JB36 | 4/30/19 | OR | Potato | Soft rot | NA | - | No |
| JB37 | 4/30/19 | OR | Potato | Soft rot | NA | - | No |
| JB38 | 5/13/19 | OR | Potato | Soft rot | Norkotah | Pcc | **Yes** |
| JB39 | 5/13/19 | OR | Potato | Soft rot | Norkotah | Pcc | **Yes** |
| JB40 | 5/13/19 | OR | Potato | Soft rot | Norkotah | - | No |
| JB41 | 5/13/19 | OR | Potato | Soft rot | Norkotah | - | No |
| JB42 | 5/13/19 | OR | Potato | Soft rot | Norkotah | - | No |
| JB43 | 5/13/19 | OR | Potato | Soft rot | Norkotah | - | No |
| JB44 | 5/13/19 | OR | Potato | Soft rot | Norkotah | - | No |
| JB45 | 5/13/19 | OR | Potato | Soft rot | Norkotah | Pcc | No |
| JB46 | 5/13/19 | OR | Potato | Soft rot | Norkotah | Pcc | **Yes** |
| JB47 | 5/13/19 | OR | Potato | Soft rot | Norkotah | Pba Dickeya | No |
| JB48 | 5/13/19 | OR | Potato | Soft rot | Norkotah | - | No |
| JB49 | 5/13/19 | OR | Potato | Soft rot | Norkotah | - | No |

**Supplementary Table 2.1.** (Continued).

| Isolate | Date Collected | Location | Host | Symptom | Cultivar | PCR Result | Culture |
|---|---|---|---|---|---|---|---|
| JB50 | 5/13/19 | OR | Potato | Soft rot | Norkotah | - | No |
| JB51 | 5/13/19 | OR | Potato | Soft rot | Norkotah | - | No |
| JB52 | 5/13/19 | OR | Potato | Soft rot | Norkotah | - | No |
| JB53 | 5/13/19 | OR | Potato | Soft rot | Norkotah | Pcc | **Yes** |
| JB54 | 5/13/19 | OR | Potato | Soft rot | Norkotah | Pcc Dickeya | No |
| JB55 | 5/13/19 | OR | Potato | Soft rot | Norkotah | Pcc | **Yes** |
| JB56 | 5/13/19 | OR | Potato | Soft rot | Norkotah | Pcc | **Yes** |
| JB57 | 5/13/19 | OR | Potato | Soft rot | Norkotah | - | No |
| JB58 | 5/13/19 | OR | Potato | Soft rot | Norkotah | - | No |
| JB59 | 5/13/19 | OR | Potato | Soft rot | Norkotah | Pcc | No |
| JB60 | 5/13/19 | OR | Potato | Soft rot | Norkotah | - | No |
| JB61 | 5/13/19 | OR | Potato | Soft rot | Norkotah | Pba | No |
| JB62 | 5/13/19 | OR | Potato | Soft rot | Norkotah | - | No |
| JB63 | 5/13/19 | OR | Potato | Soft rot | Norkotah | Pcc | **Yes** |
| JB64 | 5/13/19 | OR | Potato | Soft rot | Norkotah | - | No |
| JB65 | 5/13/19 | OR | Potato | Soft rot | Norkotah | - | No |
| JB66 | 5/13/19 | OR | Potato | Soft rot | Norkotah | - | No |
| JB67 | 5/13/19 | OR | Potato | Soft rot | Norkotah | - | No |
| JB68 | 5/13/19 | OR | Potato | Soft rot | Norkotah | - | No |
| JB69 | 5/17/19 | OR | Potato | Soft rot | Ivory Russet | - | No |
| JB70 | 5/16/19 | OR | Potato | Soft rot | Russet Burbank | - | No |
| JB71 | 5/16/19 | OR | Potato | Soft rot | Russet Burbank | - | No |
| JB72 | 5/22/19 | ID | Potato | Blackleg | Shepody | - | No |
| JB73 | 5/22/19 | OR | Potato | Blackleg | All Bue | - | No |
| JB74 | 5/22/19 | OR | Potato | Blackleg | Atlantic | - | No |

**Supplementary Table 2.1.** (Continued).

| Isolate | Date Collected | Location | Host | Symptom | Cultivar | PCR Result | Culture |
|---|---|---|---|---|---|---|---|
| JB75 | 5/22/19 | OR | Potato | Blackleg | Atlantic | - | No |
| JB76 | 6/5/19 | OR | Potato | Blackleg | Shepody | - | No |
| JB77 | 6/5/19 | OR | Potato | Blackleg | Shepody | - | No |
| JB78 | 6/5/19 | OR | Potato | Blackleg | Clearwater | - | No |
| JB79 | 6/5/19 | OR | Potato | Blackleg | Clearwater | - | No |
| JB80 | 6/5/19 | AB | Potato | Blackleg | Russet | - | No |
| JB81 | 6/5/19 | AB | Potato | Blackleg | Russet | - | No |
| JB82 | 6/25/19 | OR | Potato | Soft rot | NA | - | No |
| JB83 | 6/25/19 | OR | Potato | Soft rot | NA | - | No |
| JB84 | 6/25/19 | OR | Potato | Soft rot | NA | - | No |
| JB85 | 6/25/19 | OR | Potato | Soft rot | NA | - | No |
| JB86 | 7/8/19 | WA | Potato | Soft rot | Ranger Russet | - | No |
| JB87 | 7/11/19 | OR | Potato | Soft rot | Ivory Russet | Pba | **Yes** |
| JB88 | 7/12/19 | OR | Potato | Soft rot | NA | Pcc | **Yes** |
| JB89 | 7/12/19 | OR | Potato | Soft rot | NA | - | No |
| JB90 | 7/12/19 | OR | Potato | Soft rot | NA | Pcc | **Yes** |
| JB91 | 7/15/19 | OR | Hemp | Blackleg | NA | - | No |
| JB92 | 7/15/19 | OR | Hemp | Blackleg | NA | - | No |
| JB93 | 7/15/19 | OR | Hemp | Blackleg | NA | - | No |
| JB94 | 7/15/19 | ID | Potato | Blackleg | Russet Burbank | Pcc | **Yes** |
| JB95 | 7/15/19 | ID | Potato | Blackleg | Russet Burbank | Pcc | **Yes** |
| JB96 | 7/15/19 | OR | Potato | Blackleg | Russet Burbank | Pcc | **Yes** |
| JB97 | 7/16/19 | OR | Potato | Blackleg | Ivory Russet | - | No |
| JB98 | 7/16/19 | OR | Potato | Blackleg | Ivory Russet | - | No |
| JB99 | 7/16/19 | OR | Potato | Blackleg | Ivory Russet | Pcc | **Yes** |

**Supplementary Table 2.1.** (Continued).

| Isolate | Date Collected | Location | Host | Symptom | Cultivar | PCR Result | Culture |
|---|---|---|---|---|---|---|---|
| JB100 | 7/16/19 | OR | Potato | Blackleg | Ivory Russet | Pcc | **Yes** |
| JB101 | 7/17/19 | OR | Tomato | Soft rot | NA | - | No |
| JB102 | 7/18/19 | OR | Potato | Soft rot | Clearwater | - | No |
| JB103 | 7/18/19 | OR | Hemp | Blackleg | NA | Pcc | **Yes** |
| JB104 | 7/19/19 | OR | Hemp | Blackleg | NA | - | No |
| JB105 | 7/26/19 | WA | Potato | Blackleg | Ivory Russet | Pba Pcc | **Yes** |
| JB106 | 7/26/19 | WA | Potato | Blackleg | Ivory Russet | Pp | **Yes** |
| JB107 | 7/26/19 | WA | Potato | Blackleg | Ivory Russet | Pba | **Yes** |
| JB108 | 8/2/19 | OR | Potato | Soft rot | Russet Burbank | - | No |
| JB109 | 8/2/19 | OR | Potato | Soft rot | Russet Burbank | - | No |
| JB110 | 8/14/19 | OR | Hemp | Blackleg | NA | - | No |
| JB111 | 8/14/19 | OR | Hemp | Blackleg | NA | - | No |
| JB112 | 8/14/19 | OR | Potato | Soft rot | NA | - | No |
| JB113 | 8/15/19 | OR | Potato | Aerial stem rot | Russet Burbank | Pba | **Yes** |
| JB114 | 8/22/19 | OR | Potato | Blackleg | Russet Burbank | - | No |
| JB115 | 8/22/19 | OR | Potato | Blackleg | Russet Burbank | - | No |
| JB116 | 8/22/19 | OR | Potato | Blackleg | Russet Burbank | - | No |
| JB117 | 8/22/19 | OR | Potato | Blackleg | Russet Burbank | - | No |
| JB118 | 8/22/19 | OR | Potato | Blackleg | Russet Burbank | - | No |
| JB119 | 8/22/19 | OR | Potato | Blackleg | NA | - | No |
| JB120 | 8/28/19 | OR | Potato | Soft rot | Umatilla | - | No |
| JB121 | 9/8/19 | OR | Potato | Blackleg | NA | Pcc | No |
| JB122 | 9/8/19 | OR | Potato | Blackleg | NA | Pcc | **Yes** |
| JB123 | 9/8/19 | OR | Potato | Blackleg | NA | Pba | No |
| JB124 | 9/8/19 | OR | Potato | Blackleg | NA | Pb | **Yes** |

**Supplementary Table 2.1.** (Continued).

| Isolate | Date Collected | Location | Host | Symptom | Cultivar | PCR Result | Culture |
|---|---|---|---|---|---|---|---|
| JB125 | 9/10/19 | WA | Potato | Soft rot | Norkotah | - | No |
| JB126 | 9/10/19 | WA | Potato | Soft rot | Norkotah | Pcc | No |
| JB127 | 9/10/19 | WA | Potato | Soft rot | Norkotah | Pb | **Yes** |
| JB128 | 9/10/19 | WA | Potato | Soft rot | Norkotah | - | No |
| JB129 | 9/10/19 | WA | Potato | Soft rot | Norkotah | - | No |
| JB130 | 9/10/19 | WA | Potato | Soft rot | Norkotah | - | No |
| JB131 | 9/10/19 | WA | Potato | Soft rot | Norkotah | - | No |
| JB132 | 9/10/19 | WA | Potato | Soft rot | Norkotah | - | No |
| JB133 | 9/10/19 | WA | Potato | Soft rot | Norkotah | Pcc | **Yes** |
| JB134 | 9/10/19 | OR | Potato | Soft rot | Atlantic | Pcc | No |
| JB135 | 9/10/19 | OR | Potato | Soft rot | Atlantic | Pcc | No |
| JB136 | 9/13/19 | OR | Potato | Soft rot | Russet Norkotah | - | No |
| JB137 | 9/13/19 | OR | Potato | Soft rot | Russet Norkotah | - | No |
| JB138 | 9/13/19 | OR | Potato | Soft rot | Russet Norkotah | Pcc | No |
| JB139 | 9/13/19 | OR | Potato | Soft rot | Russet Norkotah | Pcc | No |
| JB140 | 9/13/19 | OR | Potato | Soft rot | Russet Norkotah | Pcc | No |
| JB141 | 9/13/19 | OR | Potato | Soft rot | Russet Norkotah | Pcc | No |
| JB142 | 9/13/19 | OR | Potato | Soft rot | Russet Norkotah | Pcc | No |
| JB143 | 9/19/19 | OR | Potato | Soft rot | Umatilla | Pcc | **Yes** |
| JB144 | 9/19/19 | OR | Potato | Soft rot | Umatilla | Pcc | **Yes** |
| JB145 | 9/19/19 | OR | Potato | Soft rot | Umatilla | Pcc | **Yes** |

[a] State where the plant sample was grown or originated from.
[b] Plant species of sample.
[c] Soft rot symptom observed on plant sample.
[d] Cultivar or variety of plant sample. NA = information not available

[e] Multiplex PCR result or sequence BLAST result for sequenced isolates. Pcc = *Pectobacterium carotovorum*, Pba = *Pectobacterium atrocepticum*, Pp = *Pectobacterium parmentieri*, Pb = *Pectobacterium brasiliense*, and Dickeya = *Dickeya spp.* In some instances multiple pathogens were detected in the same plant sample. - = negative PCR result.
[f] Indicates whether a culture of the detected isolate was stored in glycerol in -80°C freezer.

## Figures

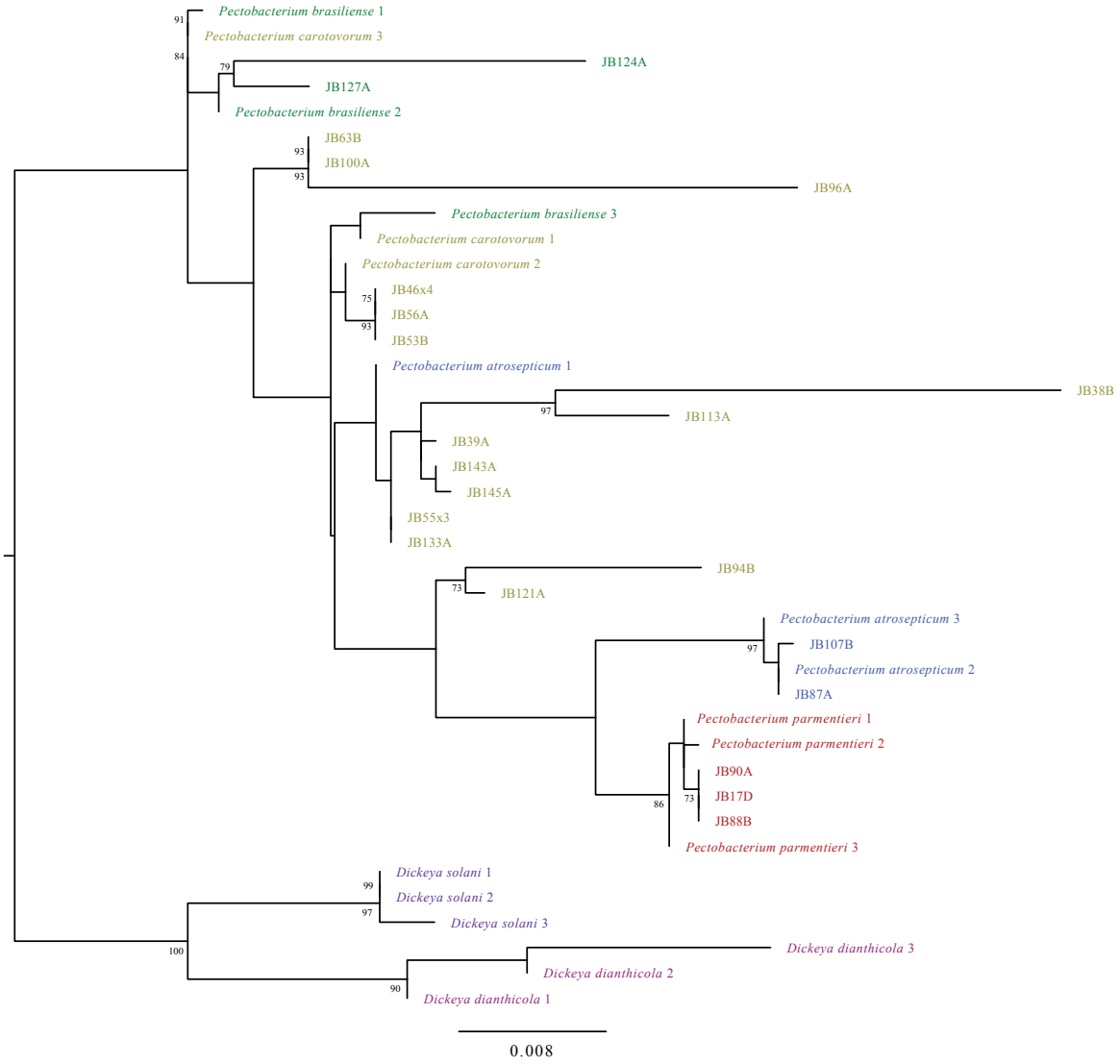


**Figure 2.1.** Phylogenetic tree, derived by RAxML analysis of the partial 16S gene region of 22 bacterial strains isolated from the Columbia Basin representing *Pectobacterium atrosepticum, P. brasiliense, P. carotovorum,* and *P. parmentieri* rooted to *Dickeya spp.* Species designation of isolates highlighted by color. Reference isolates have full Latin binomials. Numbers at branch points indicate bootstrap support values greater than 70%.

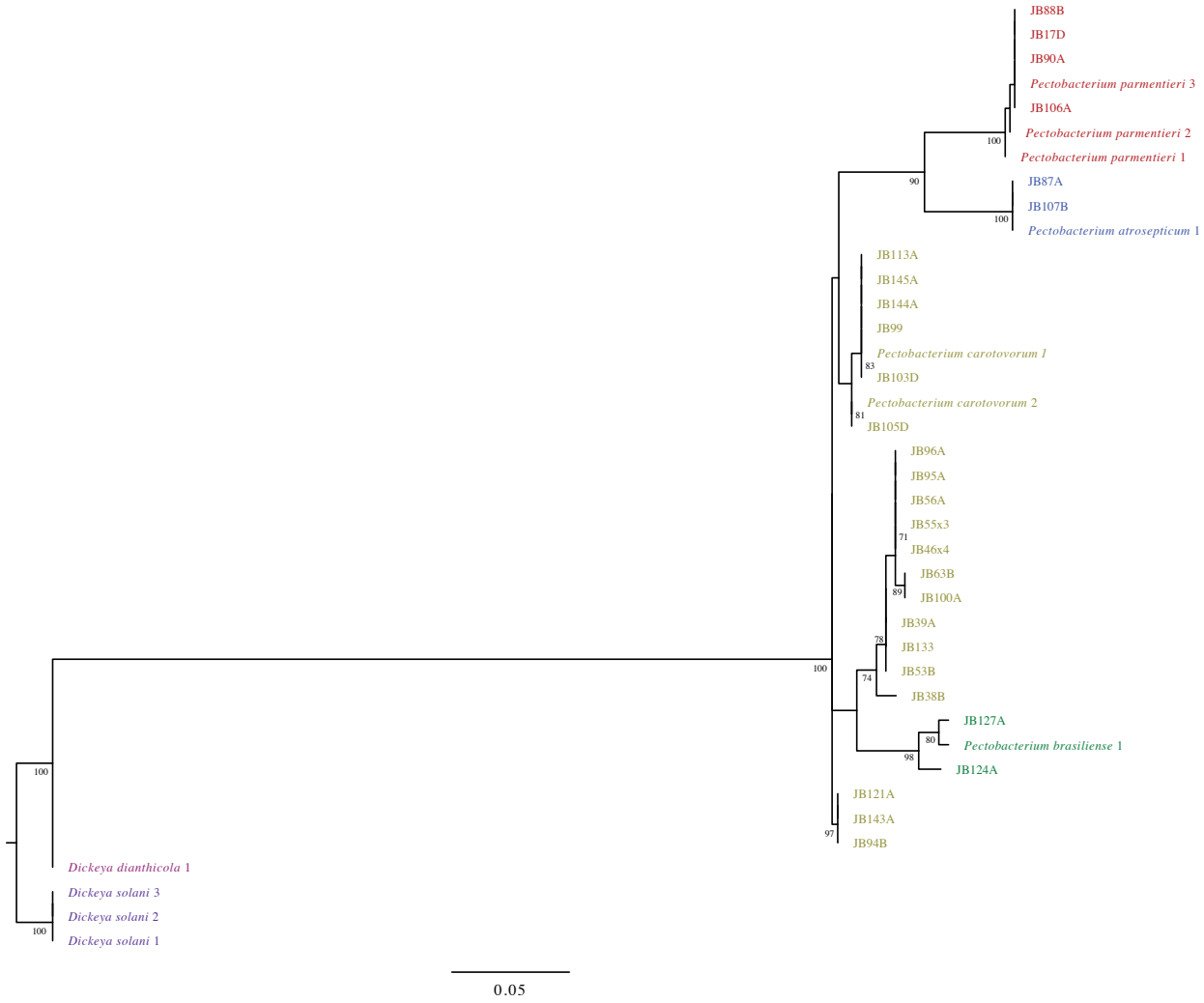


**Figure 2.2.** Phylogenetic tree, derived by RAxML analysis of the partial *dnaX* gene region of 28 bacterial strains isolated from the Columbia Basin representing *Pectobacterium atrosepticum, P. brasiliense, P. carotovorum,* and *P. parmentieri* rooted to *Dickeya spp.* Species designation of isolates highlighted by color. Reference isolates have full Latin binomials. Numbers at branch points indicate bootstrap support values greater than 70%.

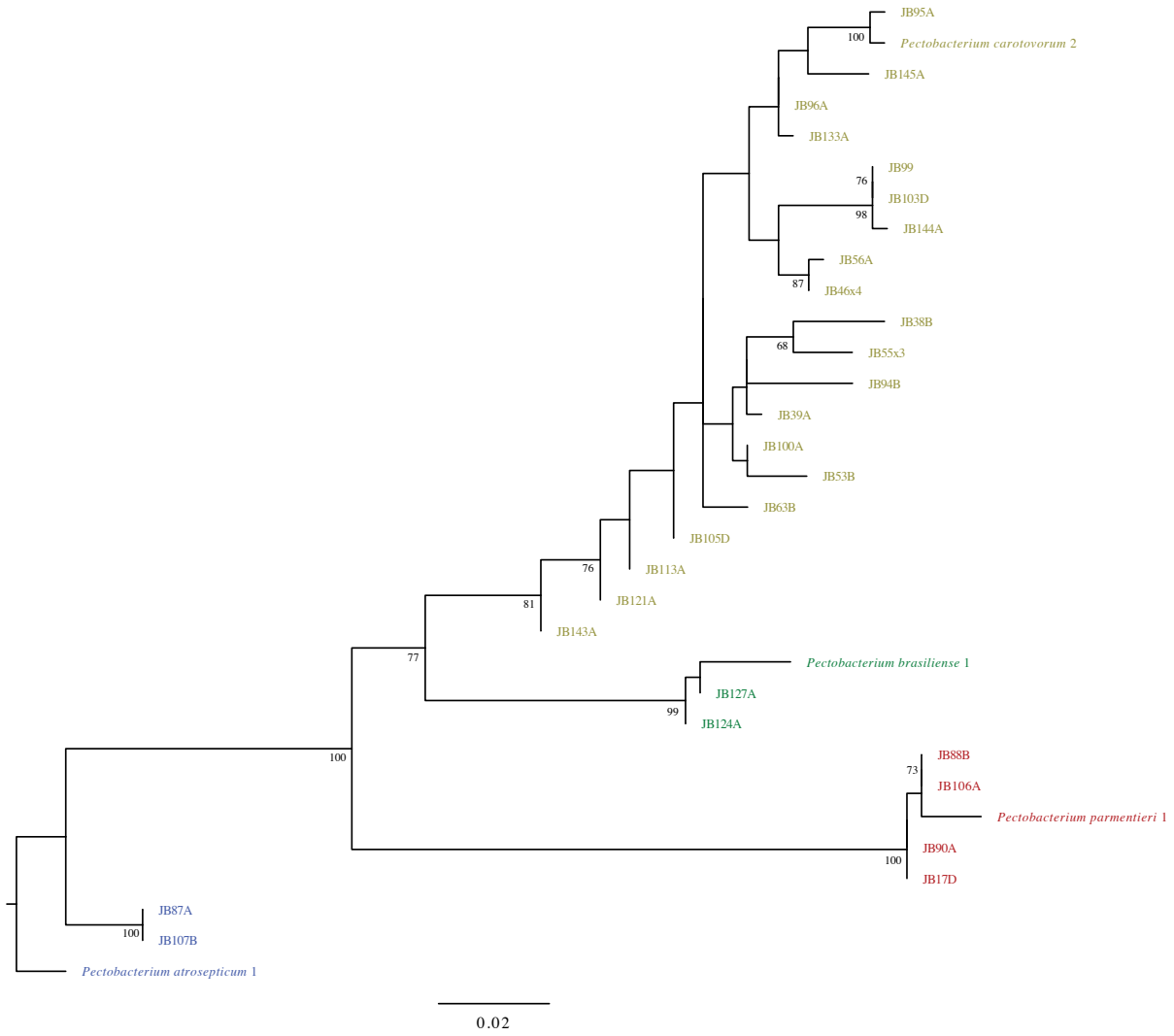


**Figure 2.3.** Phylogenetic tree, derived by RAxML analysis of the partial *pelY* gene region of 28 bacterial strains isolated from the Columbia Basin representing *Pectobacterium atrosepticum, P. brasiliense, P. carotovorum,* and *P. parmentieri.* Species designation of isolates highlighted by color. Reference isolates have full Latin binomials. Numbers at branch points indicate bootstrap support values greater than 70%.

# Chapter 3

## Yield as a function of soft rot bacteria (*Pectobacterium* spp. and *Dickeya* spp.) prevalence in seed potato in Eastern Oregon

Jessie Brazil, Hannah Rivedal, Victoria Skillman, Kenneth Frost

**Abstract**

Bacterial soft rot of potato (*Solanum tuberosum*), caused by *Pectobacterium* and *Dickeya* species, is a concerning disease complex that is capable of severely limiting crop yield. Potato yield loss associated with seed-borne infections of these pathogens has not been recently estimated. The objective of this study was to determine the yield losses associated with seed-borne bacterial soft rot infections to determine how potato yields vary as a function of inoculum prevalence in seed potato in Eastern Oregon. In 2018, potato seed of 'Lamoka' and 'Russet Burbank' cultivars was inoculated with *Pectobacterium carotovorum* and *Dickeya chrysanthemi,* and in 2019, potato seed of the same cultivars was inoculated with *Pectobacterium atrosepticum* and *Pectobacterium parmentieri*. In each year, inoculated and non-inoculated seed potato was mixed to create planting stock with 0%, 5%, 10%, 20%, and 30% incidence of soft rot. The resulting 20 treatments (2 cultivars x 2 strains x 5 levels of initial of inoculum) were planted in the field and managed using production practices typical for the region. Emergence, plant health, and blackleg incidence was monitored throughout the growing season. Potatoes from each plot were harvested, graded, and total yield for each plot was calculated by weight. We observed that an increase of bacterial inoculum in the potato seed led to lower emergence rates for the 'Lamoka' cultivar treatments in 2018. At 33 days after planting, non-inoculated 'Lamoka' seed had the highest emergence (38.13%) and 30% initial *Dickeya chrysanthemi* incidence in 'Lamoka' seed had the lowest emergence (15.63%). Emergence of the 'Russet Burbank' cultivar was not influenced by the initial percent of inoculated seed planted and had higher mean final emergence than the 'Lamoka' cultivar treatments. The lower rates of emergence led to

lower yields in the ‘Lamoka’ seed. In 2018, the total yield of the ‘Lamoka’ non-inoculated seed had the highest yields at 27.52 tons per acre and 30.13 tons per acre for the *Pectobacterium carotovorum* and *Dickeya chrysanthemi* treatments, respectively. In 2018, the ‘Lamoka’ seed with 5%, 10%, and 20% initial *Pectobacterium carotovorum* incidence and 20% and 30% initial *Dickeya chrysanthemi* incidence had the lowest total yields. We did not observe effects of the treatments in either year on Horsfall- Barratt plant disease score ratings or AUDPC. In this study, the differences in cultivar resistance and susceptibility were apparent. In the Columbia Basin, ‘Russet Burbank’ is known as a more resistant cultivar to soft rot pathogens, and ‘Lamoka’ is regarded as more susceptible. The findings of this study suggest careful evaluation of cultivar resistance for planting when the presence of SRP in seed is known. Based on the results of this study, 0- 30% incidence of soft rot bacteria in ‘Russet Burbank’ cultivar potato seed does not contribute to lowered emergence, plant health, or yield. A 5- 30% incidence of soft rot bacteria in ‘Lamoka’ seed may impact plant emergence and yield.

**Introduction**

Potatoes (*Solanum tuberosum*) are a valuable crop in the United States, worth $3.77 billion in 2017 (USDA-NASS 2018). Bacterial soft rot pathogens of potato are capable of limiting potato crop production and value due to the diseases they cause (Toth et al. 2011). Soft rot is caused by pectinolytic bacteria from two different genera, *Pectobacterium* and *Dickeya*, and are collectively known as the soft rot Pectobacteriaceae (SRP; formerly Enterobacteriaceae) (Pérombelon and Kelman 1980; Pérombelon 2002; Adeolu et al. 2016). SRP are gram negative, rod-shaped, non-spore

forming, facultative anaerobes with peritrichous flagella (Charkowski 2006). SRP are capable of causing disease on a wide range of ornamentally and horticulturally important plants, such as potato, corn, carrot, and tomato (Hauben et al. 1998; Martinez-Cisneros et al. 2014; Waleron et al. 2014; Caruso et al. 2016). In potato, SRP are capable of limiting yield value at every stage of production, during seed production and transport, at planting, throughout the growing season, at harvest, and in storage.

On potato, SRP are able to cause multiple diseases, including tuber soft rot, blackleg, aerial stem rot, and lenticel rot (Stevenson et al. 2001). Multiple species of SRP have been reported to cause soft rot disease on potato, including *Pectobacterium carotovorum*, *P. atrosepticum*, *P. brasiliense*, *P. odoriferum*, *P. parmentieri*, *P. polaris*, *Dickeya dianthicola*, *D. dadantii*, *D. chrysanthemi*, and *D. solani* (Czajkowski et al. 2011; Portier et al. 2019; Dees et al. 2017; Khayi et al. 2016). Historically, the different diseases were thought to be associated with specific bacterial species. For example, *P. carotovorum* was associated primarily with tuber soft rot and *P. atrosepticum* was primarily associated with blackleg. However, more recently, multiple bacterial species have been isolated and determined to be the cause of multiple soft rot diseases; the association between a SRP species and disease is not as straightforward as once thought (Pérombelon 2002; Czajkowski et al. 2011; Toth et al. 2011). Symptoms of soft rot of potato include poor emergence, plant stunting, chlorosis, wet, rotten tubers, necrotic stem tissue, and plant death (Pérombelon and Kelman 1980).

There are numerous ways potatoes can become infected by SRP, but the main source of soft rot infections in a field is from planting asymptomatically infected seed pieces (Pérombelon 1974; Rossman et al. 2018). Soft rot bacteria often

asymptomatically colonize host tissue (Pérombelon and Kelman, 1980) causing latent infections that are not detected during seed potato production. Asymptomatically infected potatoes grown for seed can then be distributed long distances to different growing regions for planting, where environmental conditions may be favorable for the bacteria to initiate disease and lead to yield loss (Czajkowski et al. 2010). This can be a significant challenge for the potato industry since spread of the pathogen to new locations could occur well before disease symptoms are expressed and disease severity can vary greatly depending on the final planting location.

After planting, the incidence and severity of soft rot and blackleg that develops from infected seed can be highly variable and is dependent on environmental conditions; the same potato seed lot planted in different locations can have different soft rot disease outcomes (Czajkowski et al. 2011). The ability for the bacteria to cause disease can be affected by soil moisture and water level (Pérombelon et al. 1989), soil and air temperature (Pérombelon 1992), and soil and plant nutrition (McGovern et al. 1985; Bain et al. 1996; Graham and Harper 1966). Potato cultivar can also influence disease incidence and severity, as there are varying degrees of resistance among cultivars, and that resistance can also vary across growing seasons (Pérombelon and Salmond 1995). In addition, there may be multiple pathogens, alone or in combinations, causing disease which could result in variation in the disease outcome (van der Wolf et al. 2016). For these reasons, the risk of losses due to soft rot pathogens present in seed potato has been challenging to assess and quantify.

Limited generation seed production and seed certification programs have been successful for reducing the spread of SRP in seed potato, but the level of control is

inconsistent year-to-year because pathogen virulence and symptom expression vary by environmental conditions and bacterial species (Czajkowski et al. 2011). Unlike other bacterial pathogens where there is zero tolerance for detection in the seed, SRP contamination of seed potatoes is sometimes documented but only blackleg incidence is noted on the North American Certified Seed Potato Health Certificate. Although variable state-to-state, seed lots with documented presence of SRP (i.e. blackleg) at low prevalence (e.g., < 3.0% in Oregon,) are still within tolerances for certification in the U.S. In the Columbia Basin of Oregon and Washington, the prevalence of soft rot bacteria in seed tubers that will result in yield loss has not been recently established with contemporary bacterial species present in the potato cropping system. The purpose of this research was to asses yield loss associated with seed-borne bacterial soft rot infections to determine how potato yields vary as a function of SRP prevalence in seed potato.

## Materials and methods

### Inoculum preparation

In 2018, cultures of *Pectobacterium carotovorum* (P.c.) and *Dickeya chrysanthemi* (D.c.) from the American Type Culture Collection (ATCC) were used for inoculation (ATCC, Manassas, VA). In 2019, inoculum was prepared from cultures of *Pectobacterium atrosepticum* (P.a.) and *Pectobacterium parmentieri* (P.p.) (Chapter 2; Isolate JB17) isolated from diseased potatoes from the Columbia Basin.

In both years, axenic cultures of each bacterium were grown on nutrient agar for 48 hours at 28°C, collected from plates, and diluted with ¼ strength Ringer's solution to

an optical density at 600nm of 0.1 OD (c.$10^8$ cells $mL^{-}1$). Bacterial suspensions were used to inoculate seed potatoes and, in 2019, 2 ul/l Tween was added to the bacterial suspension prior to inoculation by vacuum infiltration (van der Wolf et al. 2017).

**Tuber inoculation and seed tuber mixing**

In 2018, seed for potato varieties ‘Russet Burbank’ and ‘Lamoka’ were sourced from a local grower and cut prior to inoculation. Potatoes of both varieties were placed in a vacuum desiccator, completely submersed in a bacterial suspension, and placed under a vacuum of 0.7 bars for 15 minutes. The vacuum was released and the potatoes remained in the bacterial suspension for an additional 10 minutes. The inoculated potatoes were mixed with non-inoculated potatoes to establish planting stocks differing in prevalence of inoculated tubers. Treatments were established for each cultivar with prevalence of 0%, 5%, 10%, 20%, and 30% inoculated tubers based on fresh weight. In 2018 there was a total of 20 treatments (2 cultivars x 2 strains x 5 levels of initial inoculum; Table 3.1).

After reviewing the 2018 experimental protocols with collaborators in other states, some changes were made to the inoculation procedure for 2019. In 2019, ‘Russet Burbank’ seed was sourced from a local grower and ‘Lamoka’ seed was procured by collaborators in MI to be used as a standard variety and seed source for all locations (i.e. MI, ND, and OR) conducting similar studies. Whole potatoes for both cultivars were inoculated prior to cutting. Potatoes of both varieties were placed in a vacuum desiccator, completely submersed in a bacterial suspension, and placed under a vacuum of 0.7 bars for 15 minutes. The vacuum was released and the potatoes remained in the bacterial suspension for an additional 10 minutes. In 2019, inoculated

potatoes were stored in a cold room for one week and were then cut and mixed with non-inoculated potatoes to establish planting stocks made up of 0%, 5%, 10%, 20%, and 30% inoculated tubers to create 20 treatments (Table 3.2). In 2019, prevalence was established based on the number of potato seed pieces required to plant each plot.

**Field preparation and experiment establishment**

The field experiment was conducted at the Hermiston Agricultural Research and Extension Center (HAREC) south of Hermiston, Oregon during the 2018 and 2019 growing seasons. The field used had fine sandy loam soil with approximately 65% sand, 25% silt, 10% clay, and less than 1% organic matter. In both years, the previous crop before experiment establishment was winter wheat and fall tillage practices included ripping to 0.46 meters (18”), discing twice, and roller-harrowing. Prior to potato planting in each year, wheat was killed with an herbicide application and the field was disced and roller-harrowed.

On May 2, 2018, furrows were opened with a commercial potato planter with the closing discs removed. An in-furrow application of fertilizer, Admire (8.7 fl oz./acre), Ridomil (0.42 fl. oz./1000 row feet), and Quadris (0.8 fl. oz./1000 row feet) occurred when furrows were open. This experiment included four replicates arranged in a randomized complete block design. Seed pieces for each of the 20 treatments were hand-planted into the open furrows, spaced 12 inches apart within rows. Rows were 34 inches apart and each plot was four rows (i.e., 11.3 ft) wide and 20 ft long, a total of 0.0052 acres. Immediately after planting, furrows were closed and hills were formed using a Lilliston rolling cultivator (Bigham Brothers, Lubbock, TX).

In 2019, the experiment was planted on May 24. Seed pieces of ‘Russet Burbank’ were planted with a commercial drop planter. An in-furrow application of fertilizer, Admire (8.7 fl. oz. /acre), Ridomil (0.42 fl. oz./1000 row feet), and Quadris (0.8 fl. oz./1000 row feet). Treatments that included the cultivar ‘Lamoka’ were planted on May 31 by hand because ‘Lamoka’ seed was not available when the other variety was planted. Again, the experiment included four replicates arranged in a randomized complete block design. Seed pieces for each of the 20 treatments were hand-planted into the open furrows, spaced 0.30 meters (12”) apart within rows. Rows were 0.86 meters (34”) apart and each plot was four rows (i.e., 3.44 meters (11.3’)) wide and 6.10 meters (20’) long, a total of 0.0052 acres.

After planting in both 2018 and 2019, potatoes were maintained by the HAREC field staff according to the typical commercial practices of the Columbia Basin. Prior to harvest, potatoes were vine-killed with Reglone on 24 September and 10 September of 2018 and 2019, respectively. Harvest occurred on 11 October and 24 September in 2018 and 2019.

**Data collection**

Throughout the growing season, measurements were taken to monitor plant health. Plant emergence was assessed at the beginning of the growing season, starting at 22 days after planting (DAP) for the 2018 trial and 17 DAP for the 2019 trial. Emergence was assessed approximately biweekly thereafter until maximum plant emergence occurred at 47 DAP for the 2018 trial and 34 DAP for the 2019 trial (Table 3.3). Additional, disease assessments occurred on August 21, 31, September 13 and 21 in 2018 and on August 1, 8, 15, 22, 29, September 5 and 10 in 2019. Disease was

assessed visually per plot using a modified Horsfall-Barratt scale. Plant disease symptoms were scored per plot from 0 to 11, with 0 being symptomless (or 0% affected) and 11 being completely dead (or 100% affected), and scores between 0 and 11 were estimated based on the percentage of plants with symptoms in each plot (Horsfall and Barratt, 1945). The plant health scores were converted to the corresponding percent mid-point value. Area under the disease progress curve (AUDPC) was calculated for each plot with the following formula: $\Sigma_i^{n-1}$ $[(Y_i + Y_{i+1})/2](t_{i+1} - t_i)$, where $Y_i$ = the percent mid-point value at the $i$th observation, $t_i$ = time (days after planting) at the $i$th observation, and $n$ = number of observations (Madden et al. 2007). Plants were also visually assessed for the presence of blackleg symptoms when emergence and disease were assessed and the number of plants exhibiting blackleg symptoms in each plot was recorded. Potatoes were harvested from each plot and stored for approximately two weeks before being graded by weighing potatoes grouped in the following categories: Culls, <3 oz., 4 to 6 oz., 6 to 10 oz., and >10 oz. Specific gravity, an estimate of the dry matter content of tubers, was estimated for a subset of potatoes harvested from each plot as an indication of tuber quality (Edgar 1951).

**Statistical analysis**

Statistical analysis was conducted using R Studio (R Core Team 2018). Analysis of variance with a Fisher's Protected Least Squared Difference (LSD) mean separation test was used to detect treatment differences (McDonald 2014; Shaffer 1995; Steel et al. 1997). Due to the difference in experimental factors occurring in 2018 and 2019, analyses were not conducted to compare years. Analyses were conducted within year

data to determine if the treatments had any effect on final plant emergence, plant health, or yield.

## Results

### 2018

*Effect of treatments on emergence*

In the 2018 trial, there was no significant effect of the initial SRP incidences in seed on emergence of the potatoes for the ‘Russet Burbank’ cultivar (Table 3.4). Emergence of the ‘Lamoka’ cultivar differed as a function of the initial SPR incidences in seed at 29 days after planting (P= 0.033) and 33 days after planting (P= 0.017; Table 3.4). At 29 days after planting, non-inoculated ‘Lamoka’ seed had the highest emergence, 35.00% and 34.38% for the P.c. and D.c. treatments, respectively, and was different from ‘Lamoka’ seed with 30% initial D.c. incidence, which had the lowest emergence (15.63%; Table 3.4). At 33 DAP, non-inoculated ‘Lamoka’ seed had the highest emergence (38.13%) and 30% initial D.c. incidence in ‘Lamoka’ seed had the lowest emergence (15.63%; Table 3.4). Overall, emergence of the ‘Russet Burbank’ cultivar was not influenced by the initial percent of inoculated seed planted and had higher mean final emergence (88.19% at 47 DAP) than the ‘Lamoka’ cultivar treatments (32.25% mean emergence at 47 DAP) (Figure 3.1).

*Effect of treatments on plant health*

There was no effect of the initial SRP incidence in seed on plant health on each individual score date or as an average of all scores for the season for both cultivars (Table 3.5). There was also no effect of the initial SRP incidences in seed on AUDPC

for the ‘Lamoka’ cultivar (P= 0.578) and the ‘Russet Burbank’ cultivar (P= 0.813; Table 3.5). Only two plants of the ‘Lamoka’ cultivar developed blackleg symptoms.

*Effect of treatments on yield and grade*

The effect of the initial SRP incidences in seed on total yield differed between the bacterial species used for inoculum and between cultivar (Table 3.6). Overall, the total yield and yield within the various size categories of potato for the ‘Russet Burbank’ cultivar were not influenced by the initial percent of inoculated seed planted (Table 3.6). For the ‘Lamoka’ cultivar, there were differences in yield for the >12 oz. size category (P= 0.033) and the total yield (P= 0.015) and total yield minus culls (P= 0.025; Table 3.6). For the >12 oz. size category and the ‘Lamoka’ cultivar, non-inoculated seed for the D.c. treatment had the highest yield at 19.69 tons per acre and 5%, 10%, and 20% initial incidences of P.c. and 30% initial incidence of D.c. had the lowest yields (Table 3.6). The ‘Lamoka’ non-inoculated seed had the highest total yields, 27.52 tons per acre and 30.13 tons per acre for the P.c. and D.c. treatments, respectively. ‘Lamoka’ seed with 5%, 10%, and 20% initial P.c. incidence and 20% and 30% initial D.c. incidence had the lowest yields (Table 3.6). The total yield of the ‘Lamoka’ seed inoculated with *D. chrysanthemi* differed as a function of the initial SPR incidences in seed, with the 30% initial incidence dose having the lowest average total yield (16.99 tons per acre) and the non-inoculated seed having the highest average total yield (30.13 tons per acre; Table 3.6). This trend was also apparent when comparing mean total weight after removing the culled potatoes from the datasets (Table 3.6). When removing culls, the non-inoculated seed had the highest mean total yield (29.05 tons per acre) and the 30% initial incidence dose had the lowest mean total yield (16.99 tons per acre; Table 3.6).

For the ‘Lamoka’ cultivar total yield minus the cull weight, the non-inoculated seed had the highest yields (26.51 tons per acre for the P.c. treatment and 29.05 tons per acre for the D.c. treatment; Table 3.6). The ‘Russet Burbank’ cultivar had higher total yields than the ‘Lamoka’ cultivar (Figure 3.2). The ‘Russet Burbank’ cultivar had both higher total yields and emergence than the ‘Lamoka’ cultivar (Figure 3.3).

**2019**

*Effect of treatments on emergence*

In the 2019 trial, there was no significant effect of the initial SPR incidences in seed on emergence of the potatoes for the ‘Lamoka’ cultivar (Table 3.7). There were differences in the ‘Russet Burbank’ cultivar at 17 days after planting (P= 0.029; Table 3.7). For the ‘Russet Burbank’ cultivar at 17 days after planting, non-inoculated seed for the P.p. treatment and 20% initial P.a. incidence had the highest percent emergence (both had 92.50% emergence), and 20% initial P.p. incidence had the lowest percent emergence (76.88%; Table 3.7). The ‘Russet Burbank’ cultivar had higher final mean emergence (93.06% at 34 DAP) than the ‘Lamoka’ cultivar (63.25% at 34 DAP) (Figure 3.4).

*Effect of treatments on plant health*

There was no effect of the initial SRP incidences in seed on plant health on each individual score date or as an average of all scores for the season for any of the cultivars (Table 3.8). There was also no effect of the initial SRP incidences on AUDPC for the ‘Lamoka’ cultivar (P= 0.772) or the ‘Russet Burbank’ cultivar (P= 0.472). No plants of any cultivar were observed with blackleg symptoms.

*Effect of treatments on yield and grade*

For both the ‘Lamoka’ and ‘Russet Burbank’ cultivars, there was no effect of the initial SRP incidence in seed on total yield or yield within the various size categories of potatoes (Table 3.9). The ‘Russet Burbank’ cultivar had higher final mean total yields than the ‘Lamoka’ cultivar (Figure 3.5). The ‘Russet Burbank’ cultivar had higher final mean total yields and higher final emergence than the ‘Lamoka’ cultivar (Figure 3.6).

**Discussion**

Seedborne soft rot infections are a major factor limiting potato yield. It has long been established that increasing the amount of initial inoculum contaminating potato seed will increase seed piece decay and blackleg infections (Aleck and Harrison, 1978). In both years of this study there was no effect of the treatments on plant health scores or AUDPC. In 2018 and 2019, blackleg was not commonly observed in the Columbia Basin of Northeastern Oregon and Southeastern Washington (Chapter 2). This could be due to the dry growing conditions of the region (Pérombelon et al. 1989). Temperature and humidity are known to affect both the soft rot pathogens present and the ability for the pathogens to cause disease (Pérombelon and Kelman 1980; Smadja et al. 2004). Blackleg symptoms are more common under wet conditions, whereas in dry conditions, infected plants tend to become stunted and chlorotic (Pérombelon and Kelman 1980; Czajkowski et al. 2011). The arid Columbia Basin contrasts with other potato growing regions in the United States, such as the more humid climate of New York, where Ma et al. 2018 found that blackleg was the most common symptom observed.

In this study, differences due to the role of cultivar resistance was apparent. We observed that an increase of bacterial inoculum in the potato seed led to lower

emergence rates in 2018 for the ‘Lamoka’ cultivar early in the growing season at 29 and 33 days after planting. Lower rates of emergence in ‘Lamoka’ seed lead to a decrease in the resulting yield. This was not observed with the ‘ Russet Burbank’ cultivar. In addition, in both 2018 and 2019, the ‘Lamoka’ cultivar emergence was much lower compared to the ‘Russet Burbank’ cultivar. The ‘Lamoka’ cultivar treatments’ lowered emergence also corresponded with yields being lower than ‘Russet Burbank’ in both years. This finding might suggest careful evaluation of cultivar resistance for planting when the presence of SRP in seed is known. In Eastern Oregon, ‘Russet Burbank’ is a widely grown cultivar that is believed to have some resistance to soft rot pathogens (Thangavel et al. 2014). ‘Lamoka’ is a chipping cultivar that is susceptible to blackleg and soft rot and not widely grown in the Pacific Northwest. Nevertheless, growers should consider soft rot resistance in their cultivar selection, try to select resistant cultivars for planting, and purchase seed with a low initial amount of bacterial inoculum in potato seed to increase emergence rates and enhance yields (Bo et al. 2019).

There were differences among the pathogens used for inoculation. This was expected because there are known differences in virulence and pathogenicity among the different soft rot species. At 33 DAP in 2018, the 30% initial *Dickeya chrysanthemi* incidence treatment had the lowest emergence. This, however, was unexpected because *Pectobacterium* species were thought to rot tubers faster than *Dickeya* species (Charkowski 2018). Thus it was expected that the 30% initial *Pectobacterium carotovorum* incidence treatment would have the lowest emergence due to rotting of the seed. This finding also differs with van der Wolf et al. 2016, who found in a similar experiment conducted in the Netherlands that among seed vacuum infiltrated with

various *Pectobacterium* and *Dickeya* species, species of *Pectobacterium* had higher numbers of plants exhibiting symptoms than *Dickeya* species early in the season. In 2019, when *Dickeya* species were not used, the ‘Russet Burbank’ cultivar at 17 days after planting, the 20% initial *Pectobacterium atrosepticum* incidence had the highest percent emergence (as well as non-inoculated), and the 20% initial *Pectobacterium parmentieri* incidence had the lowest percent emergence. This finding is also different from that of van der Wolf et al. 2016, who found that seed inoculated with *Pectobacterium atrosepticum* had a higher percentage of diseased plants in the field than *Pectobacterium wasabiae* (now *Pectobacterium parmentieri*). It is important to examine the pathogenicity of the different bacterial species in different growing regions because it appears their ability to cause disease varies as a function of their geographic location.

The results of this study indicate that 0- 30% incidence of soft rot bacteria in ‘Russet Burbank’ cultivar potato seed does not contribute to lowered emergence, plant health, or yield. A 5- 30% incidence of soft rot bacteria in ‘Lamoka’ seed may impact plant emergence and yield. These results can only be extrapolated to the growing conditions of Eastern Oregon, and further research will be required to confirm these thresholds. Further research on higher dosages of soft rot bacteria, such as 35% and 40%, would provide further insight into the thresholds of ‘Russet Burbank’ cultivar seed. In addition, future studies in different growing regions could evaluate the inoculum thresholds of other cultivars that are widely grown in those regions, because soft rot and blackleg disease varies widely between regions and climates. Knowledge of inoculum

thresholds for additional cultivars would be very beneficial to growers when making cultivar selections and purchasing seed.

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## Tables

**Table 3.1.** List of treatments, potato cultivars, bacterial species and inoculation dose percentages used in the 2018 trial in Hermiston, OR.

| Treatment | Cultivar | Bacterial Species | Dose |
|---|---|---|---|
| 1 | Lamoka | *P. carotovorum* | 0% |
| 2 | Lamoka | *P. carotovorum* | 5% |
| 3 | Lamoka | *P. carotovorum* | 10% |
| 4 | Lamoka | *P. carotovorum* | 20% |
| 5 | Lamoka | *P. carotovorum* | 30% |
| 6 | Lamoka | *D. chrysanthemi* | 0% |
| 7 | Lamoka | *D. chrysanthemi* | 5% |
| 8 | Lamoka | *D. chrysanthemi* | 10% |
| 9 | Lamoka | *D. chrysanthemi* | 20% |
| 10 | Lamoka | *D. chrysanthemi* | 30% |
| 11 | Russet Burbank | *P. carotovorum* | 0% |
| 12 | Russet Burbank | *P. carotovorum* | 5% |
| 13 | Russet Burbank | *P. carotovorum* | 10% |
| 14 | Russet Burbank | *P. carotovorum* | 20% |
| 15 | Russet Burbank | *P. carotovorum* | 30% |
| 16 | Russet Burbank | *D. chrysanthemi* | 0% |
| 17 | Russet Burbank | *D. chrysanthemi* | 5% |
| 18 | Russet Burbank | *D. chrysanthemi* | 10% |
| 19 | Russet Burbank | *D. chrysanthemi* | 20% |
| 20 | Russet Burbank | *D. chrysanthemi* | 30% |

**Table 3.2.** List of treatments, potato cultivars, bacterial species and inoculation dose percentages used in the 2019 trial in Hermiston, OR.

| Treatment | Cultivar | Bacterial Species | Dose |
|---|---|---|---|
| 1 | Lamoka | *P. parmentieri* | 0% |
| 2 | Lamoka | *P. parmentieri* | 5% |
| 3 | Lamoka | *P. parmentieri* | 10% |
| 4 | Lamoka | *P. parmentieri* | 20% |
| 5 | Lamoka | *P. parmentieri* | 30% |
| 6 | Lamoka | *P. atrosepticum* | 0% |
| 7 | Lamoka | *P. atrosepticum* | 5% |
| 8 | Lamoka | *P. atrosepticum* | 10% |
| 9 | Lamoka | *P. atrosepticum* | 20% |
| 10 | Lamoka | *P. atrosepticum* | 30% |
| 11 | Russet Burbank | *P. parmentieri* | 0% |
| 12 | Russet Burbank | *P. parmentieri* | 5% |
| 13 | Russet Burbank | *P. parmentieri* | 10% |
| 14 | Russet Burbank | *P. parmentieri* | 20% |
| 15 | Russet Burbank | *P. parmentieri* | 30% |
| 16 | Russet Burbank | *P. atrosepticum* | 0% |
| 17 | Russet Burbank | *P. atrosepticum* | 5% |
| 18 | Russet Burbank | *P. atrosepticum* | 10% |
| 19 | Russet Burbank | *P. atrosepticum* | 20% |
| 20 | Russet Burbank | *P. atrosepticum* | 30% |

**Table 3.3.** Mean percent potato plant emergence at different number of days after planting (DAP) in the 2018 trial conducted in Hermiston, OR.[a]

| | | | Lamoka | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Organism[b]** | **Dose[c]** | **22 DAP** | **29 DAP** | **33 DAP** | **36 DAP** | **40 DAP** | **42 DAP** | **43 DAP** | **47 DAP** |
| **1** | | **0** | 16.25 a | 35.00 a | 38.13 a | 38.13 a | 39.38 a | 40.63 ab | 40.00 ab | 41.25 ab |
| **2** | | **5** | 6.88 a | 20.00 bc | 24.38 bcd | 29.38 ab | 28.75 ab | 32.50 abc | 31.88 abc | 33.13 abc |
| **3** | **P.c** | **10** | 7.50 a | 20.00 bc | 23.75 cd | 27.50 ab | 27.50 ab | 30.63 abc | 30.63 abc | 31.25 abc |
| **4** | | **20** | 8.13 a | 24.38 abc | 28.75 abc | 30.00 ab | 30.00 ab | 30.00 bc | 29.38 bc | 29.38 bc |
| **5** | | **30** | 9.38 a | 21.25 bc | 25.00 bcd | 24.38 b | 25.63 b | 27.50 c | 25.63 c | 25.63 c |
| **6** | | **0** | 13.13 a | 34.38 a | 36.25 ab | 38.75 a | 39.38 a | 43.13 a | 43.75 a | 43.13 a |
| **7** | | **5** | 11.88 a | 25.63 abc | 33.13 abc | 30.00 ab | 30.63 ab | 33.75 abc | 33.13 abc | 34.38 abc |
| **8** | **D.c** | **10** | 13.75 a | 31.25 ab | 32.50 abc | 32.50 ab | 31.25 ab | 34.38 abc | 33.13 abc | 33.75 abc |
| **9** | | **20** | 7.50 a | 21.25 bc | 23.13 cd | 20.00 b | 21.88 b | 23.13 c | 23.75 c | 25.00 c |
| **10** | | **30** | 8.13 a | 15.63 c | 15.63 d | 21.25 b | 22.50 b | 25.00 c | 25.63 c | 25.63 c |
| | | LSD | NA | 12.18 | 11.98 | NA | NA | NA | NA | NA |
| | | F (P-value) | 0.71 | 0.03 | 0.02 | 0.11 | 0.14 | 0.07 | 0.13 | 0.06 |
| | | | **Russet Burbank** | | | | | | | |
| **Treatment** | **Organism** | **Dose** | **22 DAP** | **29 DAP** | **33 DAP** | **36 DAP** | **40 DAP** | **42 DAP** | **43 DAP** | **47 DAP** |
| **11** | | **0** | 38.13 b | 75.63 a | 77.50 c | 85.00 b | 87.50 a | 88.75 a | 86.25 ab | 86.25 a |
| **12** | | **5** | 54.38 ab | 80.63 a | 83.13 abc | 91.25 ab | 92.50 a | 90.63 a | 85.00 b | 84.38 a |
| **13** | **P.c** | **10** | 41.88 ab | 83.13 a | 90.63 a | 95.00 a | 93.75 a | 91.25 a | 87.50 ab | 91.25 a |
| **14** | | **20** | 50.00 ab | 76.25 a | 80.63 bc | 83.75 b | 91.25 a | 90.00 a | 83.75 b | 86.25 a |
| **15** | | **30** | 48.75 ab | 88.13 a | 88.13 ab | 92.50 ab | 93.75 a | 94.38 a | 93.75 a | 88.75 a |
| **16** | | **0** | 40.63 b | 88.13 a | 90.63 a | 90.63 ab | 90.63 a | 94.38 a | 90.00 ab | 85.00 a |
| **17** | | **5** | 40.63 b | 79.38 a | 83.75 abc | 86.88 ab | 91.25 a | 94.38 a | 91.25 ab | 91.88 a |
| **18** | **D.c** | **10** | 58.75 a | 86.25 a | 88.75 ab | 91.25 ab | 93.75 a | 96.88 a | 85.63 b | 91.25 a |
| **19** | | **20** | 50.63 ab | 87.50 a | 83.75 abc | 92.50 ab | 93.75 a | 95.00 a | 89.38 ab | 88.13 a |
| **20** | | **30** | 53.13 ab | 80.63 a | 90.00 ab | 92.50 ab | 91.88 a | 92.50 a | 89.38 ab | 88.75 a |
| | | LSD | NA | NA | NA | NA | NA | NA | NA | NA |
| | | F (P-value) | 0.29 | 0.42 | 0.12 | 0.27 | 0.90 | 0.60 | 0.27 | 0.56 |

[a] Tables separated by cultivar. Data were analyzed in R statistical environment and treatment means were separated using ANOVA with a Fisher's Protected Least Squared Difference (LSD) mean separation test (P=0.05). Different letters indicate differences among treatments. Mean separation not performed if F-test was not significant at P = 0.05.

[b] SRP species used for treatment inoculation. P.c = *Pectobacterium carotovorum*. D.c = *Dickeya chrysanthemi*.
[c] Dose level of inculcation. 0 = non inoculated, 5 = 5% inoculated seed, 10 = 10% inoculated seed, 20 = 20% inoculated seed, 30 = 30% inoculated seed.

**Table 3.4.** Mean Horsfall-Barratt potato plant disease score percent midpoints, averages, and area under severity progress curve (AUDPC) at various number of days after planting (DAP) in the 2018 trial conducted in Hermiston, OR.[a]

| | | | Lamoka | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Organism[b]** | **Dose[c]** | **111 DAP** | **121 DAP** | **134 DAP** | **145 DAP** | **Average** | **AUDPC** |
| **1** | | **0** | 17.38 ab | 16.63 b | 88.63 a | 95.63 a | 54.56 ab | 1617.50 ab |
| **2** | | **5** | 21.25 ab | 27.13 b | 81.50 a | 93.75 a | 55.91 ab | 1661.25 ab |
| **3** | **P.c** | **10** | 20.88 ab | 39.00 ab | 75.25 a | 95.13 a | 57.56 ab | 1722.50 ab |
| **4** | | **20** | 12.63 b | 28.00 ab | 76.25 a | 94.13 a | 52.75 ab | 1576.25 ab |
| **5** | | **30** | 18.50 ab | 34.25 ab | 81.50 a | 96.00 a | 57.56 ab | 1730.00 ab |
| **6** | | **0** | 16.13 ab | 18.50 b | 75.25 a | 91.88 a | 50.44 b | 1477.50 b |
| **7** | | **5** | 16.13 ab | 28.00 ab | 74.38 a | 95.63 a | 53.53 ab | 1582.50 ab |
| **8** | **D.c** | **10** | 18.50 ab | 56.25 a | 83.88 a | 98.00 a | 64.16 a | 1983.75 a |
| **9** | | **20** | 30.38 a | 34.25 ab | 74.25 a | 97.88 a | 59.19 ab | 1726.25 ab |
| **10** | | **30** | 13.75 ab | 43.75 ab | 79.13 a | 98.75 a | 58.84 ab | 1791.25 ab |
| | | LSD | NA | NA | NA | NA | NA | NA |
| | | F (P-value) | 0.73 | 0.22 | 0.76 | 0.73 | 0.66 | 0.58 |
| | | | **Russet Burbank** | | | | | |
| **Treatment** | **Organism** | **Dose** | **111 DAP** | **121 DAP** | **134 DAP** | **145 DAP** | **Average** | **AUDPC** |
| **11** | | **0** | 8.00 a | 11.88 a | 63.38 a | 82.88 a | 41.53 a | 1206.88 a |
| **12** | | **5** | 12.63 a | 21.75 a | 56.13 a | 84.75 a | 43.81 a | 1265.63 a |
| **13** | **P.c** | **10** | 15.00 a | 37.88 a | 64.75 a | 81.00 a | 49.66 a | 1506.25 a |
| **14** | | **20** | 12.63 a | 11.50 a | 45.25 a | 78.50 a | 36.97 a | 1023.13 a |
| **15** | | **30** | 20.13 a | 20.13 a | 47.50 a | 87.63 a | 43.84 a | 1215.00 a |
| **16** | | **0** | 12.63 a | 13.75 a | 67.25 a | 90.00 a | 45.91 a | 1323.13 a |
| **17** | | **5** | 26.00 a | 42.88 a | 80.38 a | 92.75 a | 60.50 a | 1826.25 a |
| **18** | **D.c** | **10** | 22.13 a | 29.50 a | 60.88 a | 96.13 a | 52.16 a | 1495.00 a |
| **19** | | **20** | 22.13 a | 21.00 a | 61.88 a | 94.25 a | 49.81 a | 1410.63 a |
| **20** | | **30** | 9.13 a | 19.00 a | 59.50 a | 82.88 a | 42.63 a | 1245.00 a |
| | | LSD | NA | NA | NA | NA | NA | NA |
| | | F (P-value) | 0.74 | 0.54 | 0.91 | 0.67 | 0.80 | 0.81 |

[a] Data were analyzed in R statistical environment and treatment means were separated using ANOVA with a Fisher's Protected Least Squared Difference (LSD) mean separation test (P=0.05). Different letters indicate differences among treatments. Mean separation not performed if F-test was not significant at P = 0.05.
[b] SRP species used for treatment inoculation. P.c = *Pectobacterium carotovorum*. D.c = *Dickeya chrysanthemi*.
[c] Dose level of inculcation. 0 = non inoculated, 5 = 5% inoculated seed, 10 = 10% inoculated seed, 20 = 20% inoculated seed, 30 = 30% inoculated seed.

**Table 3.5.** Mean potato yield (tons per acre) by size grade, total yield, and total yield minus culls in the 2018 trial conducted in Hermiston, OR.[a]

| | | | Lamoka | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Organism[b]** | **Dose[c]** | **Culls** | **< 4 oz** | **4-8 oz** | **8-12 oz** | **> 12 oz** | **Total** | **Total - culls** |
| **1** | | **0** | 1.01 a | 1.14 a | 2.32 ab | 5.87 ab | 17.18 ab | 27.52 a | 26.51 a |
| **2** | | **5** | 1.25 a | 0.84 abc | 1.59 ab | 3.29 c | 11.21 c | 18.18 b | 16.93 b |
| **3** | **P.c** | **10** | 0.36 a | 0.88 abc | 1.26 b | 3.69 bc | 10.84 c | 17.03 b | 16.67 b |
| **4** | | **20** | 0.82 a | 0.80 abc | 1.53 ab | 4.28 abc | 11.44 c | 18.88 b | 18.06 b |
| **5** | | **30** | 1.04 a | 0.82 abc | 1.18 b | 6.38 a | 14.52 abc | 23.93 ab | 22.89 ab |
| **6** | | **0** | 1.08 a | 1.04 ab | 2.30 ab | 6.02 ab | 19.69 a | 30.13 a | 29.05 a |
| **7** | | **5** | 1.43 a | 1.09 ab | 1.80 ab | 3.86 bc | 15.65 abc | 23.83 ab | 22.40 ab |
| **8** | **D.c** | **10** | 1.67 a | 1.18 a | 2.61 a | 6.18 ab | 12.78 bc | 24.42 ab | 22.75 ab |
| **9** | | **20** | 0.52 a | 0.53 c | 1.18 b | 3.24 c | 12.38 bc | 17.86 b | 17.34 b |
| **10** | | **30** | 0.79 a | 0.66 bc | 1.19 b | 4.12 abc | 10.23 c | 16.99 b | 16.19 b |
| | | LSD | NA | NA | NA | NA | 5.71 | 8.00 | 8.09 |
| | | F (P-value) | 0.69 | 0.09 | 0.17 | 0.06 | 0.03 | 0.02 | 0.03 |
| | | | **Russet Burbank** | | | | | | |
| **Treatment** | **Organism** | **Dose** | **Culls** | **< 4 oz** | **4-8 oz** | **8-12 oz** | **> 12 oz** | **Total** | **Total - culls** |
| **11** | | **0** | 3.99 abc | 2.07 ab | 4.62 a | 9.54 ab | 22.95 a | 43.16 a | 39.18 ab |
| **12** | | **5** | 4.51 ab | 2.39 ab | 4.10 a | 7.36 b | 16.24 a | 34.61 a | 30.09 b |
| **13** | **P.c** | **10** | 5.13 a | 2.40 ab | 4.74 a | 9.88 a | 17.83 a | 39.98 a | 34.85 ab |
| **14** | | **20** | 3.26 bc | 2.44 ab | 4.72 a | 9.53 ab | 23.17 a | 43.12 a | 39.86 a |
| **15** | | **30** | 3.78 abc | 2.36 ab | 5.55 a | 10.30 a | 21.22 a | 43.21 a | 39.43 ab |
| **16** | | **0** | 3.69 abc | 1.87 b | 3.70 a | 9.36 ab | 20.64 a | 39.26 a | 35.57 ab |
| **17** | | **5** | 3.56 abc | 2.51 ab | 4.78 a | 9.69 ab | 16.94 a | 37.48 a | 33.92 ab |
| **18** | **D.c** | **10** | 2.76 c | 2.38 ab | 5.28 a | 9.33 ab | 20.71 a | 40.47 a | 37.70 ab |
| **19** | | **20** | 4.29 abc | 1.93 ab | 4.15 a | 10.97 a | 18.53 a | 39.87 a | 35.58 ab |
| **20** | | **30** | 4.67 ab | 2.54 a | 3.65 a | 11.04 a | 21.12 a | 43.02 a | 38.36 ab |
| | | LSD | NA | NA | NA | NA | NA | NA | NA |
| | | F (P-value) | 0.25 | 0.40 | 0.62 | 0.18 | 0.60 | 0.64 | 0.58 |

[a] Data were analyzed in R statistical environment and treatment means were separated using ANOVA with a Fisher's Protected Least Squared Difference (LSD) mean separation test (P=0.05). Different letters indicate differences among treatments. Mean separation not performed if F-test was not significant at P = 0.05.
[b] SRP species used for treatment inoculation. P.c = *Pectobacterium carotovorum*. D.c = *Dickeya chrysanthemi*.
[c] Dose level of inculcation. 0 = non inoculated, 5 = 5% inoculated seed, 10 = 10% inoculated seed, 20 = 20% inoculated seed, 30 = 30% inoculated seed.

**Table 3.6.** Mean percent potato plant emergence at different days after planting (DAP) in the 2019 trial conducted in Hermiston, OR.[a]

| | | | Lamoka | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Organism[b]** | **Dose[c]** | **17 DAP** | **20 DAP** | **24 DAP** | **27 DAP** | **31 DAP** | **34 DAP** |
| **1** | | **0** | 30.00 bc | 49.38 a | 55.00 a | 55.63 a | 56.25 a | 59.38 a |
| **2** | | **5** | 40.00 abc | 57.50 a | 63.75 a | 67.50 a | 67.50 a | 67.50 a |
| **3** | **P.p** | **10** | 44.38 ab | 58.75 a | 63.13 a | 62.50 a | 63.75 a | 66.25 a |
| **4** | | **20** | 35.00 abc | 49.38 a | 53.75 a | 56.25 a | 60.00 a | 54.38 a |
| **5** | | **30** | 28.75 c | 51.25 a | 56.25 a | 56.25 a | 57.50 a | 59.38 a |
| **6** | | **0** | 49.38 a | 61.25 a | 66.25 a | 66.25 a | 66.25 a | 68.13 a |
| **7** | | **5** | 38.75 abc | 51.25 a | 60.00 a | 63.75 a | 61.88 a | 60.63 a |
| **8** | **P.a** | **10** | 33.75 bc | 56.25 a | 58.13 a | 60.00 a | 60.00 a | 66.25 a |
| **9** | | **20** | 38.75 abc | 56.25 a | 58.75 a | 60.63 a | 60.63 a | 63.75 a |
| **10** | | **30** | 32.50 bc | 53.13 a | 61.25 a | 61.25 a | 62.50 a | 66.88 a |
| | | LSD | NA | NA | NA | NA | NA | NA |
| | | F (P-value) | 0.15 | 0.73 | 0.77 | 0.69 | 0.89 | 0.53 |
| | | | **Russet Burbank** | | | | | |
| **Treatment** | **Organism** | **Dose** | **17 DAP** | **20 DAP** | **24 DAP** | **27 DAP** | **31 DAP** | **34 DAP** |
| **11** | | **0** | 92.50 a | 94.38 ab | 97.50 ab | 96.88 abc | 95.63 a | 93.75 a |
| **12** | | **5** | 90.00 ab | 93.13 ab | 94.38 ab | 94.38 bc | 96.25 a | 91.25 a |
| **13** | **P.p** | **10** | 83.75 abc | 95.00 ab | 95.63 ab | 96.25 abc | 95.63 a | 91.25 a |
| **14** | | **20** | 76.88 c | 93.75 ab | 97.50 ab | 100.00 a | 96.25 a | 93.13 a |
| **15** | | **30** | 85.00 abc | 92.50 b | 96.25 ab | 96.25 abc | 96.88 a | 91.88 a |
| **16** | | **0** | 86.25 ab | 91.88 b | 93.13 b | 92.50 c | 91.88 a | 94.38 a |
| **17** | | **5** | 91.88 ab | 98.13 a | 98.75 a | 98.75 ab | 95.00 a | 89.38 a |
| **18** | **P.a** | **10** | 83.13 bc | 96.25 ab | 95.63 ab | 96.88 abc | 96.25 a | 96.25 a |
| **19** | | **20** | 92.50 a | 93.13 ab | 96.88 ab | 96.25 abc | 93.75 a | 91.88 a |
| **20** | | **30** | 84.38 abc | 92.50 b | 95.63 ab | 96.88 abc | 97.50 a | 97.50 a |
| | | LSD | 9.21 | NA | NA | NA | NA | NA |
| | | F (P-value ) | 0.03 | 0.41 | 0.65 | 0.31 | 0.97 | 0.79 |

[a] Data were analyzed in R statistical environment and treatment means were separated using ANOVA with a Fisher's Protected Least Squared Difference (LSD) mean separation test (P=0.05). Different letters indicate differences among treatments.
Mean separation not performed if F-test was not significant at P = 0.05.
[b] SRP species used for treatment inoculation. P.c = *Pectobacterium carotovorum*. D.c = *Dickeya chrysanthemi*.
[c] Dose level of inculcation. 0 = non inoculated, 5 = 5% inoculated seed, 10 = 10% inoculated seed, 20 = 20% inoculated seed, 30 = 30% inoculated seed.

**Table 3.7.** Mean Horsfall-Barratt disease score percent midpoints, averages, and area under severity progress curve (AUDPC) at various days after planting (DAP) in the 2019 trial conducted in Hermiston, OR.[a]

| | | | Lamoka | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Organism[b]** | **Dose[c]** | **70 DAP** | **77 DAP** | **84 DAP** | **91 DAP** | **98 DAP** | **Average** | **AUDPC** |
| **1** | | **0** | 4.50 a | 7.88 a | 6.75 ab | 9.00 a | 11.25 a | 7.88 a | 220.50 a |
| **2** | | **5** | 4.88 a | 6.75 a | 7.88 ab | 9.00 a | 11.25 a | 7.95 a | 221.81 a |
| **3** | **P.p** | **10** | 4.88 a | 5.63 a | 6.00 ab | 9.00 a | 13.50 a | 7.80 a | 208.69 a |
| **4** | | **20** | 3.75 a | 5.63 a | 4.88 b | 6.75 a | 9.00 a | 6.00 a | 165.38 a |
| **5** | | **30** | 5.63 a | 5.63 a | 6.75 ab | 10.13 a | 15.00 a | 8.63 a | 229.69 a |
| **6** | | **0** | 4.50 a | 7.88 a | 6.75 ab | 11.25 a | 16.13 a | 9.30 a | 253.31 a |
| **7** | | **5** | 3.75 a | 6.75 a | 9.00 a | 9.00 a | 11.25 a | 7.95 a | 225.75 a |
| **8** | **P.a** | **10** | 4.50 a | 9.00 a | 6.75 ab | 7.88 a | 12.38 a | 8.10 a | 224.44 a |
| **9** | | **20** | 4.88 a | 7.50 a | 6.75 ab | 11.25 a | 13.50 a | 8.78 a | 242.81 a |
| **10** | | **30** | 4.50 a | 4.88 a | 5.63 ab | 6.75 a | 9.00 a | 6.15 a | 168.00 a |
| | | LSD | NA | NA | NA | NA | NA | NA | NA |
| | | F (P-value) | 0.95 | 0.90 | 0.52 | 0.60 | 0.95 | 0.83 | 0.77 |
| | | | **Russet Burbank** | | | | | | |
| **Treatment** | **Organism** | **Dose** | **70 DAP** | **77 DAP** | **84 DAP** | **91 DAP** | **98 DAP** | **Average** | **AUDPC** |
| **11** | | **0** | 0.38 a | 0.75 a | 2.25 a | 3.00 b | 5.63 a | 2.40 b | 63.00 b |
| **12** | | **5** | 0.38 a | 0.75 a | 1.50 a | 5.63 ab | 4.50 a | 2.55 b | 72.19 b |
| **13** | **P.p** | **10** | 0.38 a | 1.13 a | 1.50 a | 3.00 b | 5.63 a | 2.33 b | 60.38 b |
| **14** | | **20** | 0.38 a | 0.75 a | 1.50 a | 4.50 ab | 6.75 a | 2.78 ab | 72.19 b |
| **15** | | **30** | 1.13 a | 1.88 a | 1.50 a | 3.75 b | 5.63 a | 2.78 ab | 73.50 ab |
| **16** | | **0** | 0.38 a | 0.75 a | 1.50 a | 3.75 b | 6.75 a | 2.63 b | 66.94 b |
| **17** | | **5** | 1.88 a | 1.88 a | 2.25 a | 7.88 a | 13.88 a | 5.55 a | 139.13 a |
| **18** | **P.a** | **10** | 0.75 a | 1.13 a | 1.50 a | 4.88 ab | 5.63 a | 2.78 ab | 74.81 ab |
| **19** | | **20** | 0.75 a | 0.38 a | 1.50 a | 4.13 b | 12.75 a | 3.90 ab | 89.25 ab |
| **20** | | **30** | 1.13 a | 1.50 a | 1.50 a | 5.63 ab | 6.75 a | 3.30 ab | 87.94 ab |
| | | LSD | NA | NA | NA | NA | NA | NA | NA |
| | | F (P-value) | 0.70 | 0.50 | 0.57 | 0.25 | 0.59 | 0.49 | 0.47 |

[a] Data were analyzed in R statistical environment and treatment means were separated using ANOVA with a Fisher's Protected Least Squared Difference (LSD) mean separation test (P=0.05). Different letters indicate differences among treatments. Mean separation not performed if F-test was not significant at P = 0.05.
[b] SRP species used for treatment inoculation. P.c = *Pectobacterium carotovorum*. D.c = *Dickeya chrysanthemi*.
[c] Dose level of inculcation. 0 = non inoculated, 5 = 5% inoculated seed, 10 = 10% inoculated seed, 20 = 20% inoculated seed, 30 = 30% inoculated seed.

**Table 3.8.** Mean potato yield (tons per acre) by size grade, total yield, and total yield minus culls in the 2019 trial conducted in Hermiston, OR.[a]

| | | | Lamoka | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Organism[b]** | **Dose[c]** | **Culls** | **< 4 oz** | **4-6 oz** | **6-10 oz** | **> 10 oz** | **Total** | **Total - culls** |
| **1** | | **0** | 0.99 a | 1.85 a | 2.13 b | 3.71 b | 5.22 ab | 13.90 a | 12.91 a |
| **2** | | **5** | 0.23 ab | 2.18 a | 4.14 a | 5.49 ab | 5.36 ab | 17.39 a | 17.16 a |
| **3** | **P.p** | **10** | 0.25 ab | 1.96 a | 4.12 a | 5.13 ab | 3.25 b | 14.71 a | 14.46 a |
| **4** | | **20** | 0.23 ab | 1.96 a | 3.13 ab | 5.63 ab | 4.27 ab | 15.22 a | 14.99 a |
| **5** | | **30** | 0.66 ab | 1.79 a | 3.50 ab | 5.44 ab | 6.54 a | 17.93 a | 17.27 a |
| **6** | | **0** | 0.16 b | 2.09 a | 3.54 ab | 6.90 a | 5.02 ab | 17.71 a | 17.55 a |
| **7** | | **5** | 0.44 ab | 2.20 a | 3.58 ab | 4.45 ab | 4.99 ab | 15.65 a | 15.21 a |
| **8** | **P.a** | **10** | 0.36 ab | 2.20 a | 3.38 ab | 4.60 ab | 4.17 ab | 14.71 a | 14.35 a |
| **9** | | **20** | 0.49 ab | 2.40 a | 3.95 a | 4.58 ab | 5.70 ab | 17.13 a | 16.64 a |
| **10** | | **30** | 0.40 ab | 2.17 a | 3.54 ab | 5.69 ab | 5.09 ab | 16.90 a | 16.50 a |
| | | LSD | NA | NA | NA | NA | NA | NA | NA |
| | | F (P-value) | 0.58 | 0.99 | 0.45 | 0.44 | 0.55 | 0.82 | 0.77 |
| | | | **Russet Burbank** | | | | | | |
| **Treatment** | **Organism** | **Dose** | **Culls** | **< 4 oz** | **4-6 oz** | **6-10 oz** | **> 10 oz** | **Total** | **Total - culls** |
| **11** | | **0** | 3.67 b | 4.05 a | 6.25 a | 9.90 a | 4.33 a | 28.20 a | 24.54 a |
| **12** | | **5** | 4.94 ab | 3.55 ab | 5.41 a | 8.82 ab | 5.45 a | 28.18 a | 23.24 a |
| **13** | **P.p** | **10** | 5.54 a | 3.41 ab | 5.30 a | 6.91 ab | 5.33 a | 26.49 a | 20.95 a |
| **14** | | **20** | 4.07 ab | 3.56 ab | 5.75 a | 8.00 ab | 6.86 a | 28.23 a | 24.16 a |
| **15** | | **30** | 4.43 ab | 3.69 ab | 5.59 a | 7.29 ab | 5.36 a | 26.36 a | 21.94 a |
| **16** | | **0** | 4.07 ab | 3.36 ab | 5.13 a | 6.74 b | 6.36 a | 25.67 a | 21.59 a |
| **17** | | **5** | 4.90 ab | 3.39 ab | 6.23 a | 8.56 ab | 6.57 a | 29.65 a | 24.74 a |
| **18** | **P.a** | **10** | 5.02 ab | 2.98 b | 5.87 a | 8.74 ab | 4.63 a | 27.24 a | 22.22 a |
| **19** | | **20** | 4.57 ab | 4.22 a | 5.77 a | 8.02 ab | 6.28 a | 28.87 a | 24.29 a |
| **20** | | **30** | 4.21 ab | 3.36 ab | 5.84 a | 8.90 ab | 5.92 a | 28.23 a | 24.02 a |
| | | LSD | NA | NA | NA | NA | NA | NA | NA |
| | | F (P-value) | 0.60 | 0.34 | 0.95 | 0.57 | 0.70 | 0.94 | 0.88 |

[a] Data were analyzed in R statistical environment and treatment means were separated using ANOVA with a Fisher's Protected Least Squared Difference (LSD) mean separation test (P=0.05). Different letters indicate differences among treatments. Mean separation not performed if F-test was not significant at P = 0.05.

[b] SRP species used for treatment inoculation. P.c = *Pectobacterium carotovorum*. D.c = *Dickeya chrysanthemi*.
[c] Dose level of inculcation. 0 = non inoculated, 5 = 5% inoculated seed, 10 = 10% inoculated seed, 20 = 20% inoculated seed, 30 = 30% inoculated seed.

## Figures

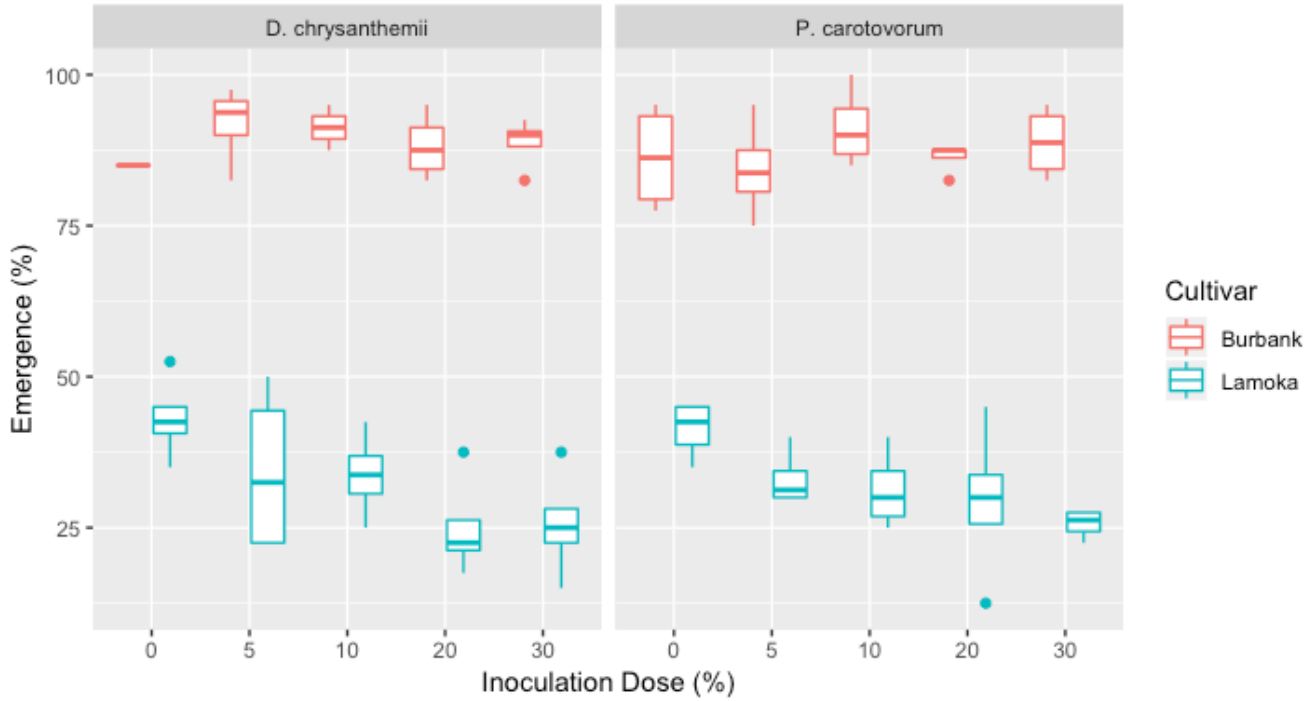


**Figure 3.1.** Average final plant emergence (%) versus the prevalence of inoculated seed tubers planted in 2018. Treatments included two cultivars of potato, 'Russet Burbank' and 'Lamoka,' inoculated with either *Dickeya chrysanthemi* or *Pectobacterium carotovorum* to establish five different levels of inoculated seed tubers (i.e. 0%, 5%, 10%, 20%, or 30%) for planting.

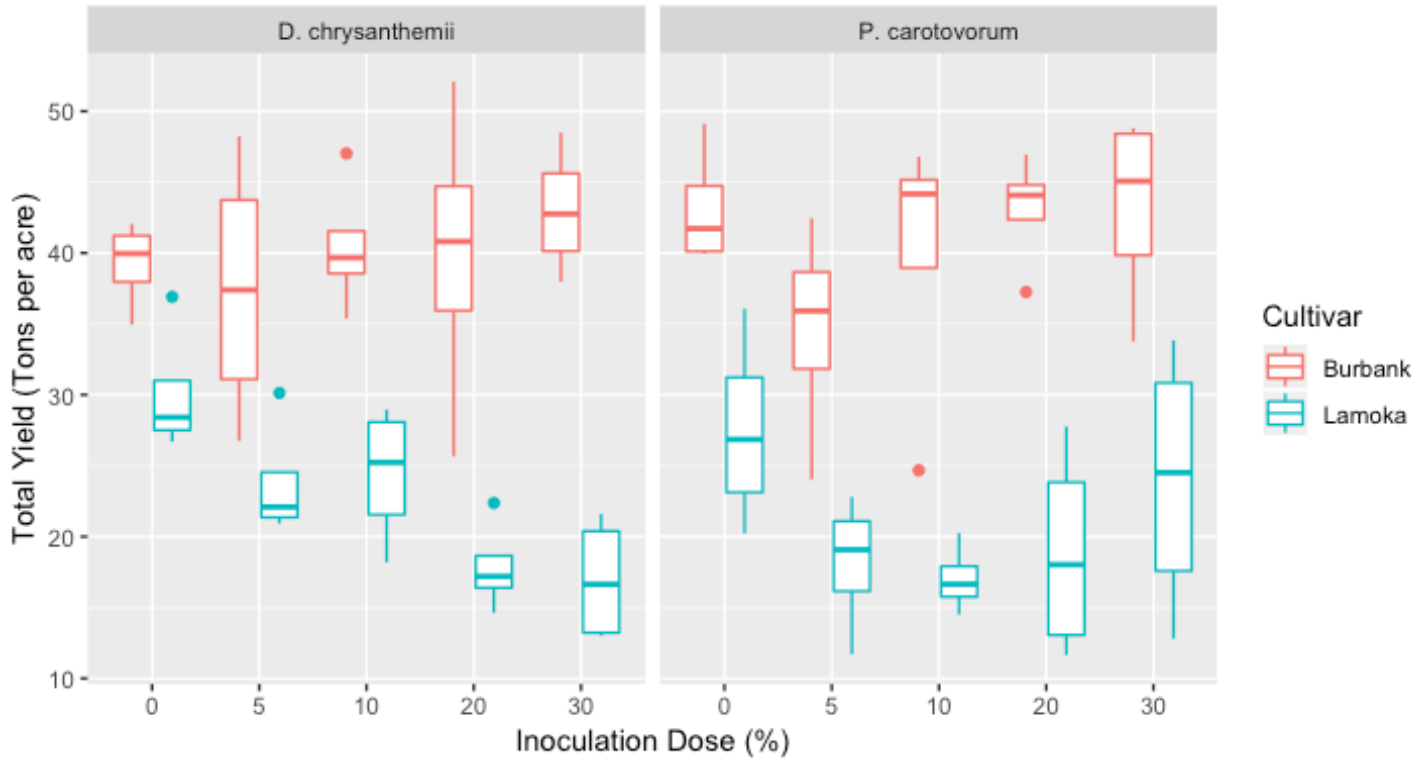


**Figure 3.2.** Average total potato yield (tons per acre) versus the prevalence of inoculated seed tubers planted in 2018. Treatments included two cultivars of potato, 'Lamoka' and 'Russet Burbank,' inoculated with either *Dickeya chrysanthemi* or *Pectobacterium carotovorum* to establish five different levels of inoculated seed tubers (i.e. 0%, 5%, 10%, 20%, or 30% inoculated) for planting.

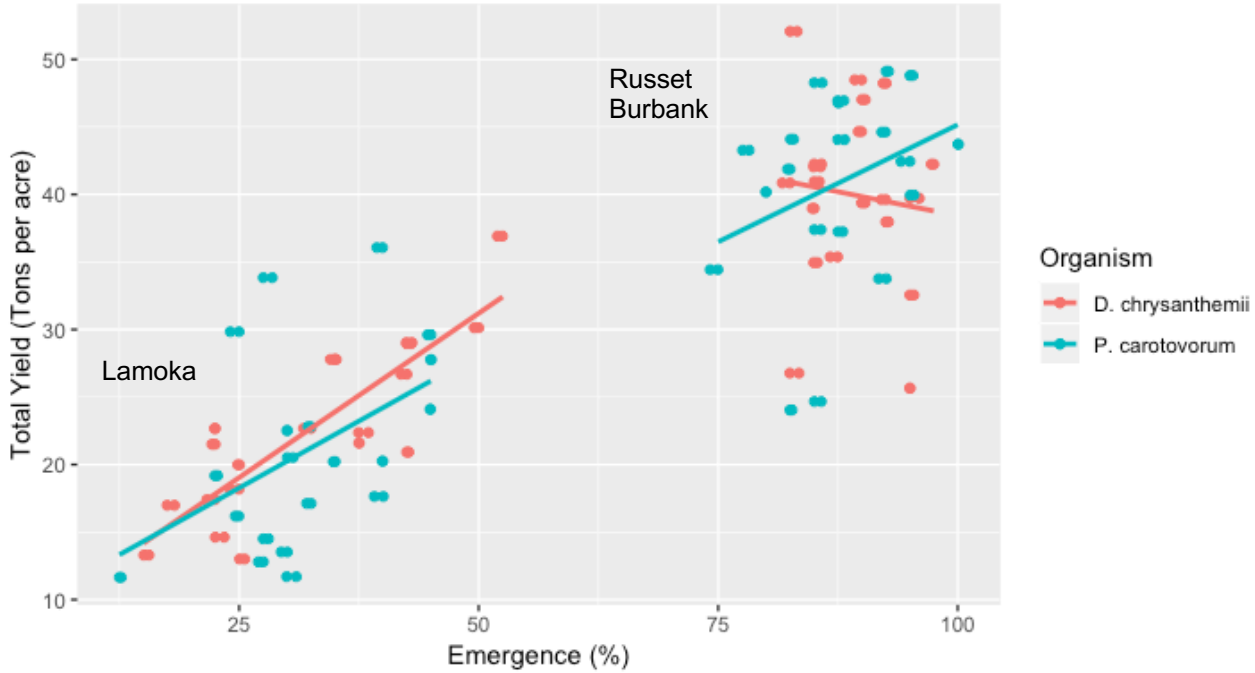


**Figure 3.3**. 2018 trial average total potato yield (tons per acre) as a function of the average percent emergence of potato plants from two different cultivars, 'Lamoka' and 'Russet Burbank,' that were inoculated with five different levels (0%, 5%, 10%, 20%, or 30%) of *Dickeya chrysanthemi* or *Pectobacterium carotovorum*.

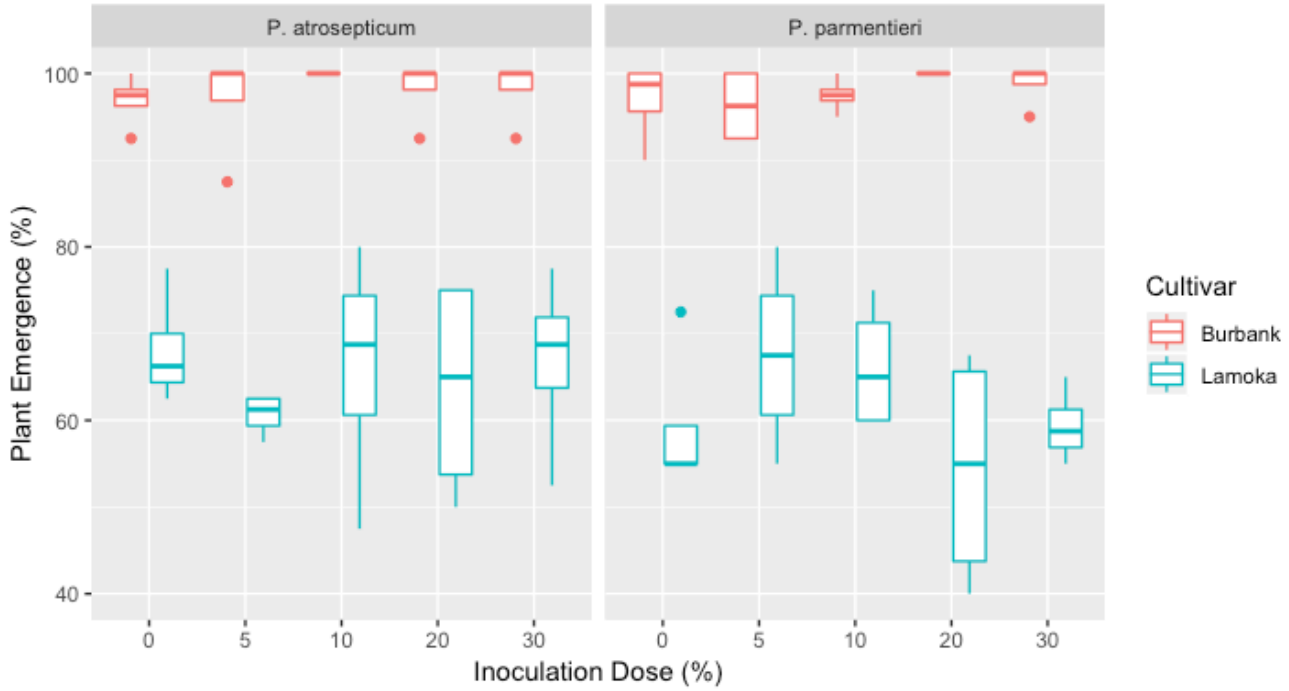


**Figure 3.4**. Average final plant emergence (%) versus the prevalence of inoculated seed tubers planted in 2019. Treatments included two cultivars of potato, 'Russet Burbank' and 'Lamoka,' inoculated with either *Pectobacterium atrosepticum* or *Pectobacterium parmentieri* to establish five different levels of inoculated seed tubers (i.e. 0%, 5%, 10%, 20%, or 30%) for planting.

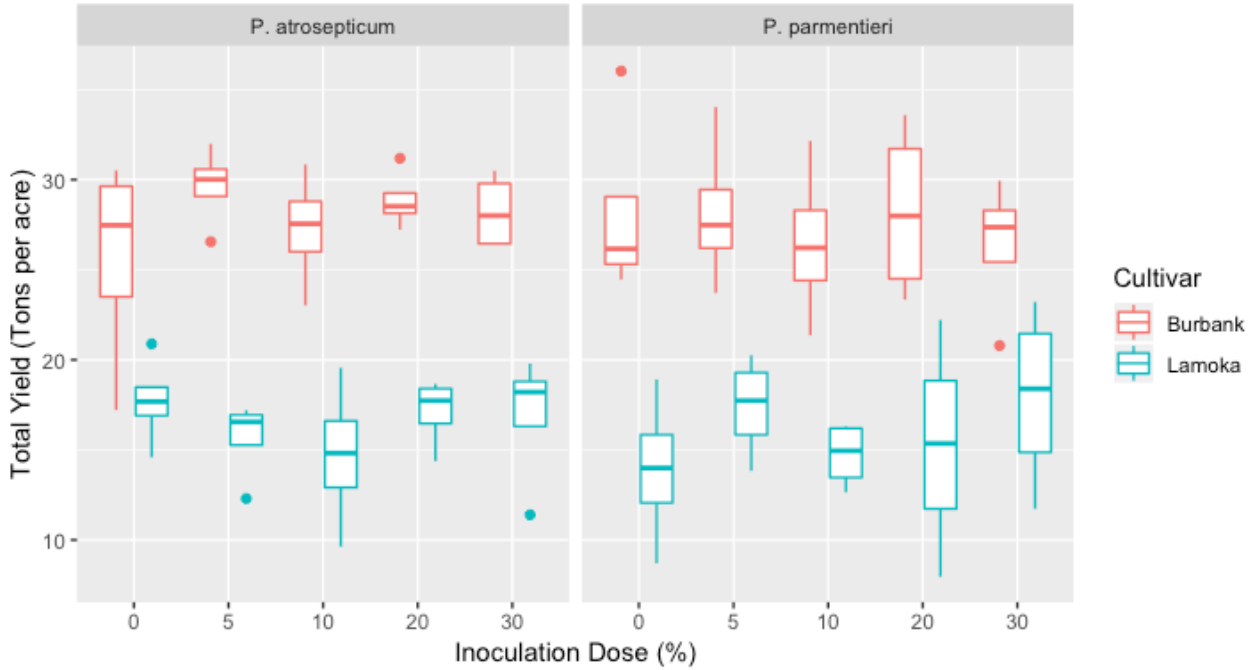


**Figure 3.5.** Average total potato yield (tons per acre) versus the prevalence of inoculated seed tubers planted in 2019. Treatments included two cultivars of potato, 'Lamoka' and 'Russet Burbank,' inoculated with either *Pectobacterium atrosepticum* or *Pectobacterium parmentieri* to establish five different levels of inoculated seed tubers (i.e. 0%, 5%, 10%, 20%, or 30% inoculated) for planting.

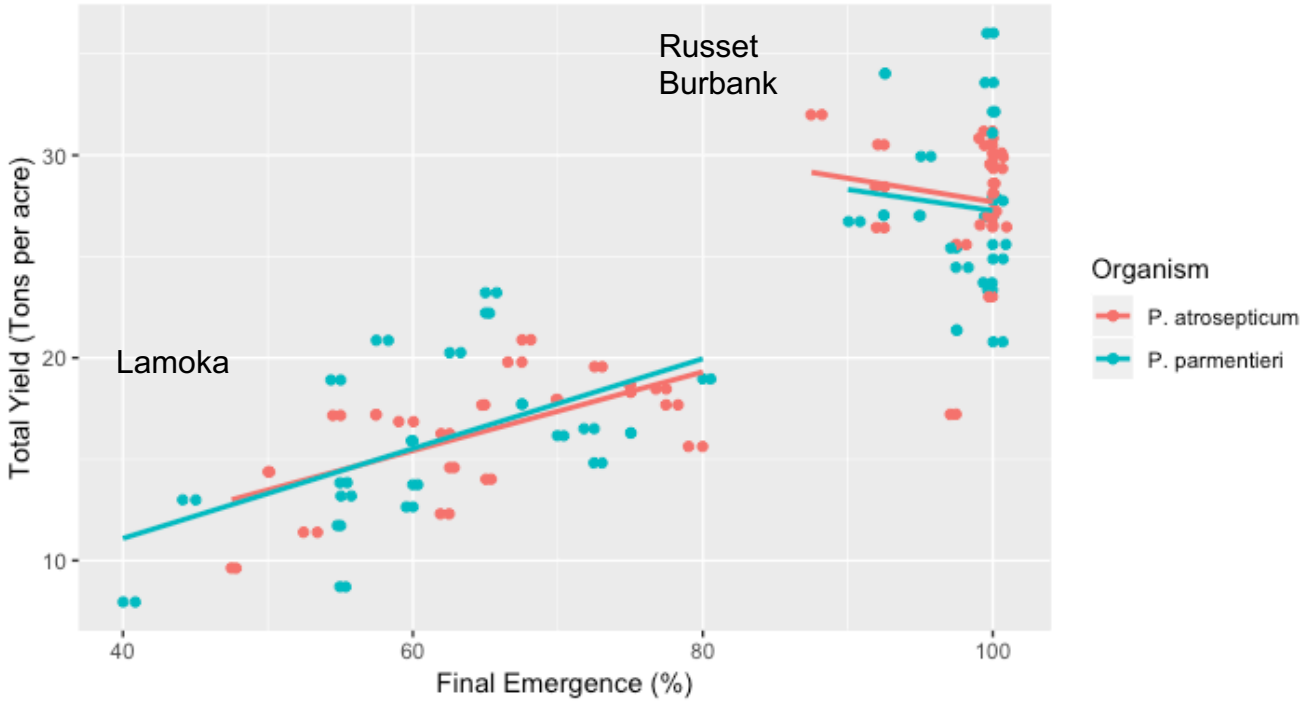


**Figure 3.6.** 2019 trial average total potato yield (tons per acre) as a function of the average percent emergence of potato plants from two different cultivars, 'Lamoka' and 'Russet Burbank,' that were inoculated with five levels (0%, 5%, 10%, 20%, or 30%) of *Pectobacterium atrosepticum* or *Pectobacterium parmentieri*.

# Chapter 4

# General Conclusion

Jessie Brazil

The distribution and identities of soft rot pathogens of potato had not been recently characterized in the Columbia Basin. The purpose of research in Chapter 2 was to isolate bacteria associated with soft rot of potato samples from the Columbia Basin and confirm their identity using diagnostic PCR and a phylogenetic assessment of *dnaX*, *pelY*, and 16S gene regions. We tested 145 plant samples and detected 54 soft rot pathogens in 51 samples with the following breakdown: 61.1% *P. carotovorum*, 20.4% *P. atrosepticum,* 5.6% *Dickeya* species, 9.3% *P. parmentieri*, and 3.7% *P. brasiliense*. We obtained 28 bacterial isolates in pure culture compromised of 75% *P. carotovorum,* 7.1% *P. parmentieri*, 10.7% *P. atrosepticum,* 7.1% *P. brasiliense*, and 0% *Dickeya* species. We were unable to successfully culture *Dickeya* spp. We created 3 phylogenetic trees that further validated the taxonomic assignments of the pathogens. We concluded that *P. carotovorum* was the dominant soft rot species causing disease in the Columbia Basin, followed by *P. atrosepticum* and *P. parmentieri. Dickeya* species and P. brasiliense were less common. P. parmentieri and *P. brasiliense* had not been previously reported in Oregon, and *P. brasiliense* had not been reported in Washington. This survey provided an estimate of soft rot pathogens present in the Columba Basin in 2018 and 2019.

Due to the asymptomatic nature of soft rot pathogens in potato seed, to avoid further introduction of new soft rot pathogens to the Columbia Basin, it is important for growers to use clean certified seed and good sanitation. In this disease system, it is important to screen for the presence of soft rot bacteria using laboratory diagnostic techniques to ensure clean seed. Based on the diversity of the soft rot pathogens identified in the Columbia Basin through this research, measures taken to reduce the

prevalence of *Dickeya* species in seed potato may have been effective at reducing this pathogen's entry into the Columbia Basin, as *Dickeya* species have become the primary cause of blackleg in other potato producing regions in the U.S., such as New York state (Ma et al. 2018).

Future research to gain insight of the diversity of the soft rot causing pathogens in the Columbia Basin and to monitor the species present is required because of the ever changing taxonomy of the pathogens and the development of more refined ways to identify pathogens. Future work should involve collecting isolates each growing seasoning and obtaining more cultures of the isolated pathogens. Specifically, it would be important to obtain *Dickeya* species in culture and provide an accurate estimate of the *Dickeya* species present in the Columbia Basin. In addition, pathogenicity tests of the isolates should be conducted to determine which species of soft rot bacteria are most aggressive in the unique growing region of the Columbia Basin and which cultivars may carry some resistance or tolerance to soft rot diseases. This information would be important to growers in the region who face disease pressure from these pathogens but do not have an accurate estimate of the risk posed by the various bacterial species or the relative susceptibility of the cultivars they grow.

Yield loss as a function of soft rot pathogen prevalence in seed tubers had not been recently estimated. The purpose of the research in Chapter 3 was to quantify yield losses as a function of soft rot pathogen prevalence in seed potato to help determine the potential value associated with maintenance of clean seed potato through seed certification programs. This could help determine the costs growers might be willing to pay to ensure the seed they sell or purchase does not have a high prevalence of

bacterial soft rot pathogens. This was done by conducting a two year field trial in Hermiston, Oregon. In 2018, potato seed of ‘Lamoka’ and ‘Russet Burbank’ cultivars were inoculated with *Pectobacterium carotovorum* and *Dickeya chrysanthemi* and in 2019, potato seed of ‘Lamoka’ and ‘Russet Burbank’ cultivars were inoculated with *Pectobacterium atrosepticum* and *Pectobacterium parmentieri*. In each year, inoculated and non-inoculated seed potato was mixed to create planting stock with 0%, 5%, 10%, 20%, and 30% incidence of soft rot. The resulting 20 treatments (2 cultivars x 2 strains x 5 doses of inoculum) were planted in the field and measurements to assess plant emergence, plant health, and yield were taken during the growing season. In both years of this study, there was no effect of the treatments on plant health scores or AUDPC. We found that in 2018, an increase of bacterial inoculum led to lower emergence for the ‘Lamoka’ cultivar treatments, and the ‘Lamoka’ cultivar treatments yield was also lower than the ‘Russet Burbank’ treatments.

Although this research is not sufficient to modify current practices, based on the results of this study, some threshold guidelines may improve yield in Eastern Oregon. The results of this study indicate that 0- 30% incidence of soft rot bacteria in ‘Russet Burbank’ cultivar potato seed does not contribute to lowered emergence, plant health, or yield. However, we found that a 5- 30% incidence of soft rot bacteria in ‘Lamoka’ seed may impact plant emergence and yield. Thus, cultivar selection may be critical to minimize yield loss due to soft rot diseases. Best practices for managing soft rot of potato and ensuring the highest yields possible is for growers to purchase and plant seed that is certified to be SRP-free when possible and to use proper sanitation techniques on their farm to avoid spreading the pathogens.

Future research performed to determine seed thresholds of soft rot bacteria should use higher bacterial prevalence, such as 35% or 40%, to better estimate of the losses caused by soft rot bacteria. Future research efforts to evaluate cultivar performance following inoculation with different SRP could help growers to select cultivars that would reduce their risk for incurring yield losses. Inoculation of different cultivars with different SRP species, alone or as SRP species mixtures, would further our understanding of how each cultivar interacts with a SRP species, as cultivar susceptibility is a major factor affecting yield losses. These pathogenicity studies could, in part, help to elucidate the genetic factors responsible for virulence in the pathogen or resistance/susceptibility in potato. It would also be important to conduct future studies in other growing regions to understand how different climates and different soft rot pathogens affect plant health, emergence, and yield.

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# Appendix

# First Report of Soft Rot of Potato (*Solanum tuberosum*) Caused by *Pectobacterium parmentieri* in Oregon

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Published in *Plant Disease*

Brazil, J.A., Rivedal, H.M., Frost, K.E. 2020. First Report of Soft Rot of Potato (Solanum tuberosum) Caused by Pectobacterium parmentieri in Oregon. *Plant Disease.* https://doi.org/10.1094/PDIS-09-19-1857-PDN

Bacterial soft rot of potato is an important disease caused by *Pectobacterium* and *Dickeya* species that can lead to severe yield loss (Toth et al. 2011). In August 2018, Ranger Russet potato tubers with soft rot symptoms were collected from a field at the Hermiston Agricultural Research and Extension Center. Symptomatic tubers occurred at approximately 5% incidence in the field. The tuber tissue was soft, wet, rotted, and cream to tan in color. Symptomatic tuber tissue was excised and surface sterilized with a 10% NaOCl solution for 30 s and rinsed three times with sterile distilled water for 30 s. The tuber tissue was macerated in 100 µl of sterile distilled water with a sterilized pestle and streaked on crystal violet pectate (CVP) medium (Hélias et al. 2012). Single colonies that formed pits in the agar after two days were streaked on CVP medium and a single colony that formed pits was transferred to nutrient agar medium and stored. The isolate was Gram-negative, failed to produce fluorescent pigment on King's B medium, and displayed facultative anaerobic respiration on Hugh-Leifson medium (Holt et al. 1994). DNA was extracted from the isolate using the Dellaporta extraction protocol (Dellaporta et al. 1983). A multiplex polymerase chain reaction (PCR) was performed that uses primer pairs to detect *Pectobacterium carotovorum* subsp. *carotovorum /P.*

*parmentieri* (ExpccF/ExpccR), *P. atrosepticum* (Y45/Y46), and *Dickeya* species (DF/DR) (Potrykus et al. 2014). The PCR resulted in the production of a 550 bp amplicon that is consistent with detection of *P. carotovorum* subsp. *carotovorum /P. parmentieri.* PCR was also performed using primer pairs 27F/1495R targeting the 16S rRNA region, resulting in an amplicon of 200-400 bp (Neilan et al. 1997), and recAF/recAR targeting the *recA* gene, resulting in an amplicon of 730 bp (Waleron et al. 2002). The PCR products were sequenced at the Oregon State University Center for Genome Research and Biocomputing using Sanger sequencing and BLAST was used to compare the nucleotide sequences to sequences in the NCBI database. The ExpccF/ExpccR amplified region (GenBank accession no. MN366344) shared 99.6% identity (525/527 bp) with *P. parmentieri* strain SCC3193 (GenBank accession no. CP003415); the 16S rRNA region (GenBank accession no. MN366318) shared 100% identity (969 bp) with *P. parmentieri* strain IFB5408 (GenBank accession no. CP026977); and the *recA* region (GenBank accession no. MN366345) shared 100% identity (677 bp) with *P. parmentieri* strain IFB5485, GBBC 1786 (GenBank accession no. CP026981) from Belgium (Zoledowska et al. 2018). To determine the pathogenicity of the isolate, six potato tubers were sterilized with a 30% NaOCl solution for 30 minutes. Three tubers were stab inoculated with the isolate and three tubers were stabbed without the isolate to serve as controls. The tubers were kept in a moist chamber at 28°C, and within three days soft rot symptoms similar to the initial infected tuber's symptoms occurred on the inoculated tubers. Bacteria were reisolated and extracted from the tubers as above. PCR using the primer pair ExpccF/ExpccR was performed, and the sequenced results (GenBank accession no. MN339552) showed

99.6% similarity (522/524 bp) to the strain used for inoculation (GenBank accession no. MN366344), fulfilling Koch's postulates. To our knowledge, this is the first report of soft rot of potato caused by *P. parmentieri* in Oregon, adding to our understanding of the diversity of soft rot bacteria that cause disease in an important potato growing region.

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