INOCULATION AND SPREAD OF DICKEYA IN POTATOES

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Field experiments were conducted in two different growing environments to evaluate the spread and movement of *Dickeya dadantii*. A procedure to inoculate seed potatoes with *Dickeya dadantii* was developed to use during this study. Spread of *Dickeya dadantii* from inoculated potato seed to healthy potato seed during the handling, cutting and planting procedures was not detected at either location. Spread of *Dickeya dadantii* from inoculated seed to surrounding progeny tubers in the field was documented in both locations. In Florida, 33% of progeny tubers tested positive for *Dickeya* using PCR, and in North Dakota, 13% of the progeny tubers tested positive. Stunting was observed in plants grown from *Dickeya dadantii* inoculated seed tubers in North Dakota, but not in Florida. These results indicate that *Dickeya dadantii* may spread during the seed handling and cutting processes and can spread in the field from infected seed tubers to progeny tubers.

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INTRODUCTION

Bacterial soft rot is a ubiquitous disease of cultivated potato (*Solanum tuberosum L.*), affecting all plant parts, in the field and storage (Czajkowski et al. 2010). Soft rot bacteria were first classified as *Bacillus*, but were moved to the genus *Erwinia* (Charkowski et al. 2014). In 2005, *Erwinia* was reclassified into two different genera: *Pectobacterium* and *Dickeya* spp., to reflect various physiological differences (Samson et al. 2005). Further classification described *Dickeya* as having six species (*D. dianthicola, D. zeae, D. aquaticus, D. chrysanthemi, D. dadantii,* and *D. solani),* and *Pectobacterium* with four species (*P. parmentieri, P. atrosepticum, P. carotovorum,* and *P. brasiliense)* (Samson et al. 2005). Although the primary causes of tuber soft rot are *Pectobacterium* spp., both *Pectobacterium* and *Dickeya* can cause seed decay, blackleg symptoms of the potato plant in the field, and soft rot of the tuber in storage (Czajkowski et al. 2011). *Dickeya* spp. are more aggressive at higher temperatures (approximately 30 °C), more limited to the vascular tissue, and require lower inoculum levels to initiate disease, compared to *Pectobacterium* (Toth et al. 2011).

Today, increasing amounts of blackleg are being caused by several *Dickeya* spp. including *Dickeya dadantii*, *Dickeya dianthicola*, and *Dickeya solani*. Although *Dickeya* spp. were discovered much earlier, they were sporadic in causing disease on most potatoes until the early twenty first century (Raoul des Essarts et al. 2016), when *Dickeya* was the primary cause of blackleg in most European countries and Israel due to international tuber seed trade distribution (Toth et al. 2011). *Dickeya* in the United States and Canada was first identified on potato plants in Florida and Ontario, and became a significant problem in the United States and Canada in 2014 (Jiang et al. 2016). These locations were planted with seed potatoes from Maine (Jiang et al. 2016). The pathogen has been widely distributed throughout the US, by seed potatoes (Gary

Secor, personal communication). In 2015 and 2016, the pathogen became more widespread, causing serious economic losses in chip and table potatoes, and today, is now documented in 22 states and two provinces (Gary Secor, personal communication).

Many aspects of disease caused by *Dickeya* spp. are not understood, including spread and movement in the field and during production. The specific objectives of this study were to:

1) Develop an efficient inoculation method for *Dickeya* spp. in potato tubers
2) Determine the spread of *Dickeya dadantii* during the seed potato handling and cutting process
3) Determine the natural spread of *Dickeya dadantii* in a commercial potato field

CHAPTER 1. LITERATURE REVIEW

Host

The potato (*Solanum tuberosum subsp. tuberosum* L.) is a herbaceous crop native to the Andean highlands of South America (Hooker 1981), including, Chile, Peru, Bolivia, and Ecuador (Raker and Spooner 2002). It is cultivated worldwide and is the fourth most important food crop in the world by total production, after rice (*Oryza sativa*), maize (*Zea mays*), and wheat (*Triticum aestivum*) (Stark and Love 2003). Widely produced genotypes (*Solanum tuberosum subsp. tuberosum*) were introduced into Europe from South America during the late sixteenth century and moved into North America in the early eighteenth century (Stark and Love. 2003).

Initially, only a few potato genotypes were introduced to Europe, limiting the genetic base of the population (Czajkowski et al. 2011). An absence of chemical control for potato pathogens, combined with the limited genetic base in the population, led to limited resistance to potato diseases and resulted in lower yields and reduced tuber quality (Czajkowski et al. 2011). Genetics aside, potatoes provided valuable, and abundant amounts of food for the poor and hungry (Stark and Love. 2003), as they are a good source of carbohydrates, vitamin C, vitamin B_6, fiber, potassium, and magnesium (Zaheer and Akhtar 2016). The lack of disease resistance and chemical control was aggressively exposed in Ireland when Irish growers selected for a single trait, yield, and neglected to select for disease resistance. Lack of genetic diversity and chemical control resulted in a highly susceptible population to the oomycete, *Phytophthora infestans* (Mont) de Bary, eventually leading to the Irish potato famine (Yoshida et al. 2013). During the Irish potato famine, approximately one million people died, and an additional one million were forced to flee the country (Turner 2005) because of the lack of the main staple

potato crop. This potato endemic sparked the need and desire for plant pathology research and provided a starting point for understanding potato pathogens (Agrios 1988).

Pathogen

Annually, an estimated 22% of potatoes are lost due to bacterial, fungal, and viral diseases and pests, equaling around fifty-eight million metric tons of potatoes (Czajkowski et al. 2011). For example, *Ralstonia solanacearum* causing bacterial wilt of potato, can cause yield losses of 33 to 90% (Yanetri Asi Nion and Toyota 2015), while bacterial ring rot, caused by *Clavibacter michiganensis* subsp. *sepedonicus* can cause disease after remaining latent in the vascular system of tubers, leaves and stems (Pietraszko et al. 2018). Late blight, caused by the oomycete *Phytophthora infestans* (Mont) de Bary, is one of the most devastating and costly diseases of potato across the globe (Wang et al. 2016) and potato leaf role virus (PLRV) may reduce yields by 40 to 60% (Wang et al. 2011). Colorado potato beetle is a devastating pest to potato production, having the ability to completely defoliate an entire commercial field if left unchecked (Alyokhin et al. 2014). Another contributor to overall losses are two bacterial diseases, blackleg and soft rot. Potato blackleg and soft rot of potato are caused by bacteria that secrete pectinases, proteases, cellulases and xylanases, although pectinase is known to be the most important for pathogenicity (Collmer and Keen 1986). *Dickeya* spp. (formerly *Erwinia chrysanthemi*) and *Pectobacterium* spp. (formerly *Erwinia atrosepticum* and *carotovorum*), are the cause of blackleg and soft rot (Samson et al. 2005). These bacteria are gram negative, non-sporulating, facultative anaerobes, and are motile with straight rods and peritrichous flagella (Czajkowski et al. 2011). They cause soft rot of fleshy vegetables and fruits such as potatoes, carrots, onions, radishes, and tomato (Ma et al. 2007). Soft rotting bacteria are commonly found whenever plant tissues are rotting, often giving off a foul odor due to the volatilization of

substances being released amid the disintegration of plant tissue by the bacteria (Agrios 1988). Degraded tissues become watery, mushy, and soft, with excretion of bacterial ooze.

Soft rot bacteria overwinter in soil, in debris, on contaminated equipment, or inside insects (Agrios 1988). Soft rotting bacteria enter potato plants and tubers through wounds, or natural openings such as lenticels. Once they gain access to host tissue, soft rot bacteria use the intercellular spaces to multiply rapidly, and can produce large amounts of pectinase (Agrios 1988). Pectinase produced by the bacteria predominantly degrades the middle lamella, separating the cells from one another, causing degradation and leakage, resulting in soft rot decay. During infection, plant cells lose their water and shrivel (Agrios 1988). Symptoms of soft rot in potato tubers are water-soaked lesions and rot. The lesions quickly grow and become sunken, soft, and gelatinous (Agrios 1988). Discoloration may occur, along with wrinkled or blistered tissue. As the lesions grow they develop a cream-colored discoloration. The disintegration process can occur in as little as three to five days (Agrios 1988).

In the field, soft rot bacteria can cause severe damage and a range of symptoms. Symptoms caused by the bacteria commonly start at the top of the plant with necrosis of the upper leaves with subsequent soft rotting of the stems (Toth et al. 2011). The stem commonly turns black in color, watery, and becomes shriveled (Toth et al. 2011). When this occurs, the whole plant will frequently become wilted, stunted, and ultimately die (Agrios 1988).

The minimum temperature for soft rot bacterial growth is 5 °C (Agrios 1988). Optimal growing temperatures range from approximately 25 to 35 °C (du Raan et al. 2015), but previous research has also shown *Dickeya solani* can cause severe rotting symptoms of potato plants at a temperature range of approximately 18 to 25 °C (Czajkowski et al. 2013). The maximum

temperature for growth of soft rotting bacteria is approximately 43 °C (du Raan et al. 2015). Temperatures must reach above approximately 50 °C to kill the bacteria (Agrios 1988).

Disease Cycle

Primary infection of tubers and stems begins with bacteria that reside in the overwintering host (Agrios 1988). Bacteria can overwinter inside tubers that are rotting from the previous season, insect pupae, in most surface water, and for a short time in the soil (Agrios 1988). Bacteria commonly reside in the roots and tubers of the potato plant (Czajkowski et al. 2010). Once inside the roots or tuber, the bacteria will multiply exponentially in intercellular spaces and in collapsed cells. Bacteria will then continue to cause further infection in the original host, or move to tubers or roots of adjacent plants (Czajkowski et al. 2010). In storage, bacterial infection can spread quickly from tuber to tuber via natural openings or wounds received during harvest and handling, leading to economic losses (Agrios 1988). The primary insect vector for soft rot bacteria is the seed-corn maggot (*Delia platura* (Meigen)). Infected larvae may vector the bacteria into the potato seed piece (Agrios 1988). Overwintering bacteria in the soil can infect through natural openings or wounds in the host.

Soft rot bacteria initially feed on liquids excreted by the broken cells on the wound surface (Agrios 1988), and after infection, multiply rapidly, producing pectinolytic and cellulolytic enzymes, resulting in maceration of the host tissues (Agrios 1988).

Management

Management of soft rot is limited. Cultural practices such as prevention and sanitization have proven to be useful (Agrios 1988). Quarantine can prevent pathogen introduction; however, in areas where *Dickeya* has been found, quarantine is ineffective, since the pathogen has already

been introduced. Fields that have a history of soft rot bacteria should be well-drained (Agrios 1988).

Crop rotation with broccoli, watermelon, and other non-hosts can be useful (Ma et al. 2007). Removal of the susceptible host for at least one growing season will eliminate the pathogen, as it does not survive for more than six months (Czajkowski et al. 2010). Proper care and handling during harvest and storage is also important. Avoiding damage during handling will minimize tuber injury, thus limiting entry points for the pathogen (Czajkowski et al. 2011), and damaged tubers should be allowed to properly wound heal to prevent bacterial infection (De Boer and Rubio 2004). If infected tubers are stored, they should be discarded as soon as possible to prevent subsequent spread (Agrios 1988). Storage bins should be dry and clean. Temperatures of 4 °C will inhibit development of new soft rot infection, as the minimal temperature for growth is 5 °C (Agrios 1988).

Post-harvest testing of seed lots for *Dickeya* spp. is also important. Previous research has suggested that *Dickeya* spp. can remain quiescent in the peel of the tuber with bacterial populations ranging from < 101 to 106 cells/g (Perombelon 2002). Polymerase chain reaction (PCR) tests of seed lots can confirm the presence of *Dickeya* spp. in seed tubers. A testing protocol of 400 tubers per seed lot, containing 25 cores per sample with 16 samples per seed lot is suggested (Gary Secor, personal communication). Chemical and biological controls have not provided reliable control of soft rot (Agrios 1988). Recent research has suggested multiple *Dickeya* spp. and *Pectobacterium* spp. can be detected simultaneously (Potrykus et al. 2014) and seed lots testing positive for *Dickeya* should be discarded (Gary Secor, personal communication).

Epidemiology

Today, little is known about the in-field spread of *Dickeya* spp. *Pectobacterium atrosepticum* has been known to spread both in the field due to contaminated soils, and during harvest due to contaminated equipment (Czajkowski et al. 2010). In contaminated soil, bacteria may move in free soil water created by high rainfall or over irrigation, and into open lenticels of potato tubers. It is possible that *Dickeya* spp. can spread and infect the same way. Recent research has shown high amounts of infection in the stem-end of progeny tubers (Czajkowski et al. 2010). It is possible that when the mother tuber rots, the bacteria colonize the stems of the newly growing plant, becoming systemic, and subsequently colonizing stem-ends of progeny tubers (Czajkowski et al. 2010). It is also possible for soil inhabiting soft rotting bacteria to colonize roots of mother plants and adjacent plants, thus further colonizing the plant and progeny tubers (Czajkowski et al. 2010). Furthermore, research has shown *Dickeya* spp. have the capacity to migrate in free water up to 10 meters (Czajkowski et al. 2010). With rotting tubers in the soil and appropriate environmental conditions such as high temperatures and free water, bacteria multiply to high populations and are released and disseminated through the soil when rainfall occurs (Czajkowski et al. 2010). Infected haulms may be another mechanism for spread if rainfall occurs during the period between haulm destruction and harvest, as the systemic bacteria can be passed from destructed haulm to adjacent plants (de la Pasture 2016). Lastly, *Dickeya* spp. have a wide host range, including banana (*Musa acuminate*), rice (*Oryza sativa)*, sugarcane (*Saccharum officinarum*), and broom-corn (*Sorghum bicolor*) (Ma et al. 2007), and thus soil inoculum may originate from other infected hosts including other crops or weeds (Czajkowski et al. 2010).

Recent Research

Dickeya spp. can cause disease on multiple fleshy vegetable crops and ornamentals across the globe (Toth et al. 2011). Initially described as *Erwinia*, molecular diagnostic tools have allowed researchers to differentiate *Erwinia* into two different genera: *Dickeya* and *Pectobacterium* (Samson et al. 2005). Based on molecular characteristics, *Dickeya* is currently divided into six species (*D. dianthicola, D. zeae, D. aquaticus, D. chrysanthemi, D.dadanti*, and *D. solani*), and *Pectobacterium* into four species (*P. parmentieri, P. atrosepticum*, *P. carotovorum*, and *P. brasiliense*) (Suarez et al. 2017).

In the past four years, *Dickeya* spp. have become a prevalent issue in the United States (Gary Secor, personal communication). Soft rot of potato is commonly caused by *Pectobacterium* (Ma et al. 2007), but *Dickeya* spp. have had a significant impact on potato production in the United States (John Nordgaard, personal communication). *Dickeya* was discovered in 2014 in potato fields located in Florida and Ontario, planted with seed potatoes from Maine (Jiang et al. 2016), and later identified as *Dickeya dianthicola* (Steve Johnson, personal communication). This observation has lead researchers to believe *Dickeya* spp., such as *D. dianthicola* and *D. dadantii* are moved with seed potatoes. In 2015 and 2016, the pathogen became widespread, causing serious economic losses in chip and table potatoes, particularly in the eastern US (Steve Johnson, personal communication). Currently, 22 state, and two Canadian provinces in North America, have confirmed the presence of *Dickeya* (Gary Secor, personal communication).

Symptoms caused by *Dickeya* spp. in potato are similar to other blackleg and soft rot causing pathogens, with some differences. Symptoms include stand loss, in season stunting and wilting, vascular necrosis, and hollowing of the pith (Czajkowski et al. 2011). Compared to

Pectobacterium spp., *Dickeya* spp. are more aggressive, cause disease at lower inoculum levels, are systemic in the vascular system, and cause disease at higher temperatures (Toth et al. 2011). *Dickeya* spp. are xylem systemic, causing xylem necrosis and discoloration (Gary Secor, personal communication), while *Pectobacterium* spp. are not systemic (Gary Secor, personal communication). *Dickeya* spp. have a higher optimal growth temperature of approximately 30 °C (du Raan et al. 2015) and thrive in warmer climates than *Pectobacterium* spp. *Dickeya* spp. can also be present in the lenticels of progeny tubers grown from infected mother plants, while *Pectobacterium* spp. remain on the outside of the lenticels (Toth et al. 2011).

CHAPTER 2. DEVELOPMENT OF AN EFFICENT INOCULATION METHOD FOR *DICKEYA DADANTII*

Introduction

Dickeya spp. are known to be seed tuber pathogens (Toth et al. 2011). *Dickeya* contaminated seed lots may result in economic losses due to downgrading and subsequent rejection of the seed lot, caused by an inability to meet certification standards (van der Wolf et al. 2017). Since 2014, *Dickeya* spp., specifically *D. dianthicola* (Jiang et al 2016) and *D. dadantii*, have become widespread on the East Coast of the United States and are causing significant amounts of disease in the field (Gary Secor, personal communication). Symptoms caused by *Dickeya* spp. include necrosis and chlorosis of the stem or leaves, stunting or wilting of the whole plant, and complete death of the plant (Czajkowski et al. 2011).

Dickeya spp. have demonstrated the ability to persist in a quiescent state inside the tuber, even when high numbers of bacteria are present (Perombelon 2002). Dormancy has been observed after tubers are inoculated with high concentrations, up to 10^6 cells/g, of bacteria resulting in symptomless infection (Perombelon 2002). Symptomless infection by *Dickeya* can persist until favorable environmental conditions occur, and bacteria can survive in seed to the next growing season (Motyka et al. 2017). Possible reasons for bacterial dormancy include lack of available free water, low nutrient required for growth, and possible host resistance (Perombelon 2002). The numbers of bacteria vary depending on the storage conditions. If storage conditions are dry, bacteria populations remain low. If tubers remain wet for a long period of time, bacterial populations will increase, although they generally do not reach above concentrations that will cause disease symptoms of tubers if proper storage guidelines are met (Perombelon 2002).

Bacteria remain dormant until favorable conditions for multiplication occur, such as prolonged free water surrounding the tubers, or injury to the tuber (Motyka et al. 2017). *Dickeya* spp. are facultative anaerobes and can grow in low oxygen conditions caused by a film of water, blocking gas exchange by the lenticels (Perombelon 2002). The anaerobic environment diminishes the oxygen dependent host resistance, hinders cell wall lignification, and suberization of the tuber (Perombelon 2002) and favors bacterial growth. Other factors allowing for disease development are prolonged wet spring weather, speed of migration of bacteria through the plants vascular system, temperature, and the amount of inoculum (Toth et al. 2011). Temperature, soil moisture, and amount of inoculum are significant factors in disease development, with symptoms commonly expressed amid high temperatures and favorable soil moisture (Czajkowski et al 2010). *Dickeya* spp. are known to thrive in a variety of optimal growth temperatures (Tsror et al. 2012), with a range of approximately 25 to 35°C, and with a maximum temperature for growth of 43°C (du Raan et al. 2015). Previous research has shown that *Dickeya* spp. will develop disease with smaller amounts of inoculum, when compared with other soft rot causing pathogens such as *Pectobacterium* spp. (Toth et al. 2011). In this study, two inoculation methods were tested for development of in-field *Dickeya* sp. symptoms, by inoculating potato tubers with *Dickeya dadantii* in either the stem-end or lenticels.

Materials and Methods

Tuber Collection and Preparation

Seed potatoes used for the inoculation study were cv. Atlantic produced at Black Gold Farms at Park River, ND. Seed potatoes, were tested prior to inoculation, using standard testing protocols of 400 tubers per seed lot, by PCR using *pelADE* primer pairs (Nassar et al. 1996) at North Dakota State University, to insure seed was free of *Dickeya* spp.

Isolate Preparation and Inoculation

Dickeya dadantii isolate 1309-17b4 collected in 2015 from potatoes in the winter potato certification test plot (courtesy Alan Westra) in Hawaii, were used for the treatment inoculations. Bacteria were grown on nutrient agar (deionized water, 500 mL; nutrient broth, 4.5g; and agar, 7.5g) for approximately two days at 30 °C, and were collected by adding 12-15 mL of autoclaved water into the Petri plate in four increments, plates were scraped with a sterile inoculation loop to remove bacteria, and collected in a sterile flask. Bacterial solutions were adjusted to a concentration of 10^8 cfu/mL for stem-end inoculations, and a concentration of 10^7 cfu/mL for vacuum infiltration of lenticels. Inoculations with water only were used as controls for the experiment. Each tuber was hand washed prior to inoculation to remove excess dirt, and allowed to air dry over night at room temperature.

Stem-end inoculations were conducted by placing 5 µl of bacterial solution at a concentration of 10^8 cfu/mL on the stem-end of the tuber and stabbing the stem-end 10 times with a sterilized sharp needle. Tubers were air dried and stored at 10 °C, until transport to the field.

Tubers were inoculated by vacuum infiltration by placing 6 to 8 tubers into a “Space Saver” vacuum desiccator containing 1L of 10^7 cfu/mL-1 bacterial solution or 1L of water (control). The vacuum desiccator was then connected to a Gast Vacuum pump with a vacuum of 600mmHg for 15 minutes. The tubers were removed from the vacuum, soaked in the bacterial solution for an additional 10 minutes, air dried at room temperature, and stored at 10 °C until transport to the field. The bacterial solution was changed every fourth cycle of 15 minutes with a fresh solution. This process was repeated with a separate desiccator and vacuum pump for bacterial and water inoculations to avoid contamination.

Field Trials

Trials were planted at two locations, Black Gold Farms near Live Oak, Florida (30.30 N, -82.98 W) on 15 Feb 2017, and at the Oakes Irrigation Research Site near Oakes, North Dakota (46.07 N, -98.09 W; elevation 399 m), on 15 May 2017. The soil at Live Oak, FL, is an Alpin fine sand, Thermic, coated Lamellic Quartzipsamments with a soil pH of 6.5 (USDA-NRCS 2017). The soil type at Oakes, ND, is an Embden coarse-loamy, mixed, superactive, frigid Pachic Hapludolls (14% clay, 70% sand, 16% silt), with a soil pH of 6.6 (USDA-NRCS 2017). The trials were planted as a randomized complete block design (RCBD) with five treatments and four replicates; each treatment and replicate consisted of 25 single drop tubers of cv. Atlantic (Figure 1). The treatments were: non-inoculated, vacuum infiltrated with *Dickeya dadantii*, vacuum infiltrated with water, stem-end inoculated with *Dickeya dadantii*, and stem-end inoculated with water. Buffer rows were planted outside of, and between replicates.

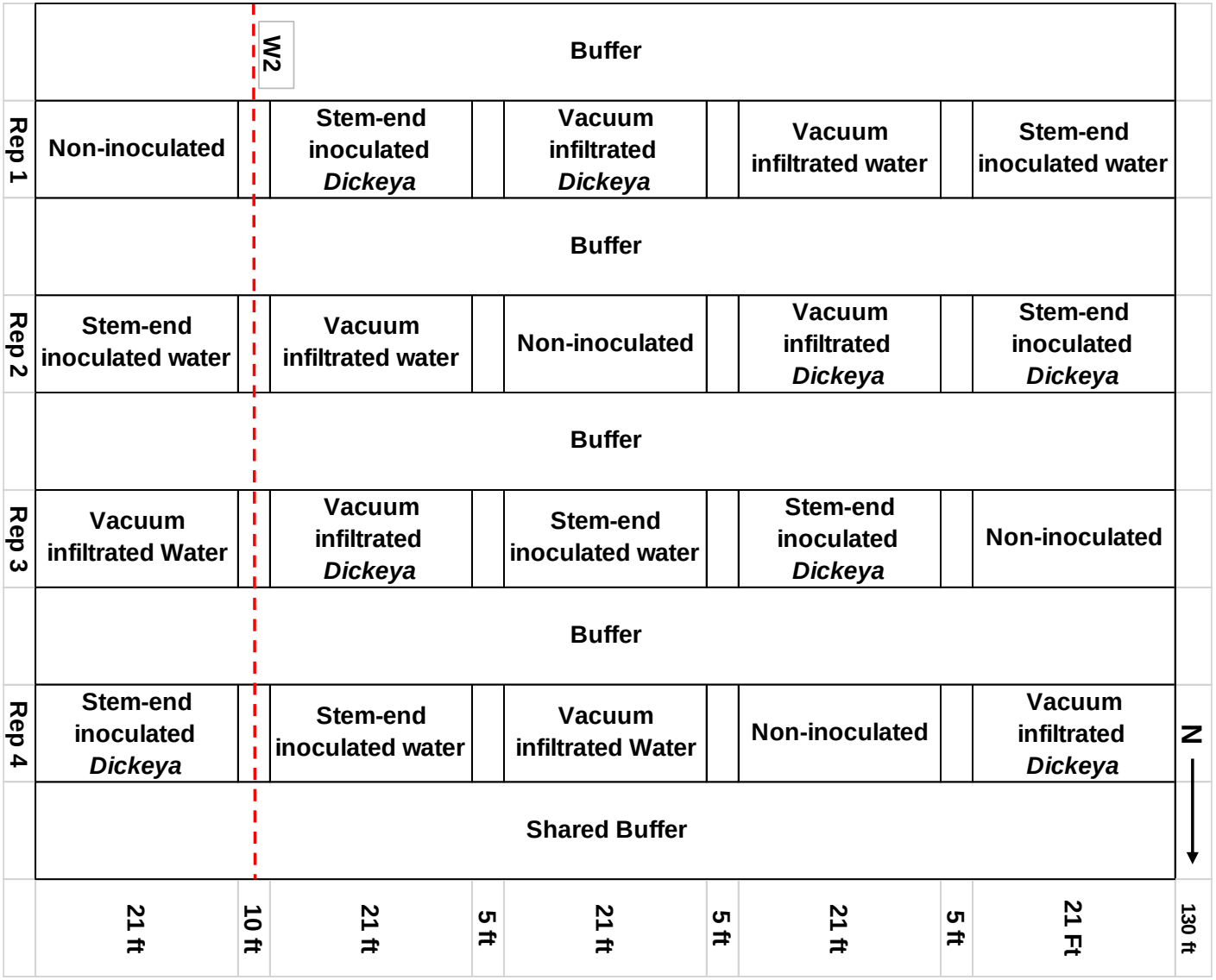


Figure 1. Trial design for the inoculation methods trials used at Live Oak, FL, and Oakes, ND, in 2017.
*Red Dash Line: Irrigator wheel track

At Live Oak, FL, whole inoculated tubers, non-inoculated tubers, and water inoculated tubers were planted on 15 Feb 2017, in a RCBD with four replicates. Seed was planted at a 25 cm with-in row spacing, and 86 cm between rows, at a seed depth of 12 cm. Daily average soil temperatures for 15 Feb 2017, were 20.4º C with an average air temperature of 20.1º C, an average relative humidity of 79%, with no natural rainfall, and no irrigation (Florida Automated Weather Network 2017). Overhead irrigation was applied two days after planting (DAP) and every one to seven days throughout the growing season, with a minimum uniform rate of 0.5 cm and maximum uniform rate of 0.8 cm. Fertilization, weed control, disease control, and irrigation, were typical of grower practice for Florida potato cultivation guidelines (Zotarelli et al. 2017). Climactic conditions in Florida were overall normal for growing potatoes (Florida Automated Weather Network. 2017) throughout the growing season (Table 1).

Table 1. Climatic conditions at Live Oak, FL, through the 2017 growing season.

Year	Month	Days After Planting	Total Irrigation	Total Rainfall	Total Water	Average Soil Temperature	Average Air Temperature
			cm	cm	cm	°C	°C
2017	Feb	1-14	10.7	1.8	12.5	18.3	17.1
2017	Mar	15-45	20.6	2.1	22.7	19.7	17.1
2017	Apr	46-75	19.1	11.6	30.7	25.2	21.7
2017	May	76-97	10.7	9.5	20.2	27.9	24.2
	Average		15.3	6.3	21.5	22.8	20
	Total		61.1	25	86.1	NA	NA
	Max		0.3	11.6	30.7	35.2	34.8
	Min		0	0	12.5	8.1	-1.3

Note- Adapted from the Florida Automated Weather Network, University of Florida, 2017. (Soil temperatures measured at a depth of 10 cm) (Average air temperatures measured at two meters)

Table 2. Climatic conditions at Oakes, ND, through the growing season in 2017.

Year	Month	Days After Planting	Total Irrigation	Total Rainfall	Total Water	Average Soil Temperature	Average Air Temperature
			cm	cm	cm	°C	°C
2017	May	1-17	3.2	3.7	6.9	12.8	13.3
2017	Jun	18-47	6.4	5.4	11.8	20	19.4
2017	Jul	48-78	16.5	2.2	18.7	24.4	22.2
2017	Aug	79-100	7.6	9.8	10.4	21.1	18.3
	Average		8.4	5.3	12.0	19.6	18.3
	Total		33.7	21.1	54.8	NA	NA
	Max		16.5	9.8	18.7	24.4	29.4
	Min		3.2	2.2	6.9	12.8	6.1

Note- Adapted from the North Dakota Agricultural Weather Network, North Dakota State University, 2017 (Turf soil temperatures measured at a depth of 10 cm) (Average air temperatures measured at two meters)

At Oakes, ND, all treatments were planted using the same design, number of replicates, seed spacing, and planting depth as used at Live Oak, FL. Fertilization, weed control, disease control and irrigation, were performed according to North Dakota State University potato production guidelines (Bissonnette et al. 1993). Previous crops grown at Oakes, ND, over the past three years were irrigated soybean (*Glycine max* L.) in 2016, dryland wheat (*Triticum aestivum* L.) in 2015, and irrigated soybean (*Glycine max* L.) in 2014. The previous three year rotation at Oakes, ND, was maize (*Zea mays* L.), soybeans (*Glycine max* L.) and potatoes (*Solanum tuberosum L.*). Throughout the growing season, rainfall occurred naturally, with a total of 21.1cm from 15 May 17 to 31 Aug 2017 (North Dakota Agricultural Weather Network 2017) (Table 2). Supplemental overhead center pivot irrigation provided a total of 33.7 cm of water from 11 May 2017 to harvest (31 Aug 17) (North Dakota Agricultural Weather Network 2017) (Table 2). Irrigation water was applied weekly to bi-weekly, as needed with no less than 1.27 cm and no more than 1.91 cm of water per application.

Data Collection

Stand counts were recorded by counting each emerged potato plant and converted to percentage stand by dividing the number of plants emerged per treatment (X), by the number of seed tubers planted per treatment (25), and multiplied by 100. Stand was counted at 43 and 58 days after panting (DAP) at Live Oak, FL, and at 24 and 30 DAP at Oakes, ND. Visual counts for blackleg disease were made at 43, 58, and 75 DAP at Live Oak, FL, and 39, 45, 51, 59, 72, 77, and 87 DAP at Oakes, ND. Plant height (cm) was measured 45, 51, 60, and 65 DAP at Oakes, ND, but was not done at Live Oak, FL. Three plants were randomly chosen per plot and height measured in cm, from the soil line, to the apex of the plant using a standard meter stick.

Data Analysis

Data were subject to analysis of variance (ANOVA) to determine if there were statistical differences among treatments using PROC ANOVA in SAS 9.4 (Statistical Analysis Software, version 9.4. SAS Institute Inc., 100 SAS Campus Dr., Cary, NC 27513). Significant differences were found (Appendices A and B), therefore data were analyzed and presented by site. Treatment means were separated using a T-test to calculate least significant differences (LSD) ($p=0.05$).

Results and Discussion

Both inoculation methods with *Dickeya dadantii* resulted in significant stand reduction at Live Oak, FL, at 43 DAP, but not at Oakes, ND at 30 DAP, compared to the non-treated check and infiltrate water (Appendix A). Stand reduction was greater by vacuum infiltration than by stab inoculation (Table 3). Neither inoculation method resulted in significant stand reductions at Oakes, ND, when compared to the non-inoculated treatments and water controls (Table 3).

Table 3. Percent stand of non-inoculated seed, *Dickeya dadantii* inoculated seed, and water inoculated seed, at two locations in 2017.

Treatment	Florida	North Dakota
Non-inoculated	93 A[a]	91
Infiltrate *Dickeya*	41 C	88
Infiltrate water	90 A	90
Stab *Dickeya*	55 B	91
Stab water	95 A	89

[a]Numbers followed by the same letter in a column are not significantly different according to an ANOVA procedure using a t-Test (LSD) analysis at P=0.05.

There are several possible explanations for the location differences between the two sites. Live Oak, FL, had higher average soil temperatures (+5.5 ºC) and higher average air temperatures (+3.8 ºC) than Oakes, ND (Tables 1 and 2). Higher temperatures favor *Dickeya* spp. compared with other soft rot bacteria (Toth et al. 2011). Lojkowska et al. 2010 showed *Dickeya* spp. can cause disease at a wide range of temperatures (18 to 28 ºC). During the month of planting at Live Oak, FL, the average soil temperature was 18.3 ºC and the average air temperature was 17.1 °C, while the average soil temperatures at Oakes, ND, were 12.8 °C, with an average air temperature of 13.3 °C (Table 1 and 2). Because of higher temperatures at Live Oak, FL, *Dickeya* in the tuber may have had a more conducive environment for disease development and symptom expression, resulting in a significant percentage stand loss at Live Oak, FL, and not at Oakes, ND.

Previous research has shown that *Dickeya solani* demonstrates variability in virulence under different field conditions (Czajkowski et al. 2013). This could be true for other *Dickeya* spp., such as *Dickeya dadantii* or *Dickeya dianthicola*. In greenhouse experiments, *D. solani* colonized roots of potato more rapidly at higher temperatures (28 ºC), suggesting greater bacterial activity and movement at higher temperatures (Czajkowski et al. 2013). At Live Oak, FL, average soil temperatures of 25.2 °C were observed at time stand counts were determined

(DAP, Table 1), suggesting that environmental conditions at Live Oak, FL, were hot enough for *Dickeya* development and disease expression.

Regardless, both methods are effective inoculation methods that can be used in Florida but not North Dakota, for future field spread and movement trials, due to differences in the environments at each location. It also explains in part, why more *Dickeya* disease is found in Florida compared to North Dakota.

Plant Height Differences Results

Significant differences in plant height among treatments was found at Oakes, ND (Appendix B). *D. dadantii* inoculated treatments resulted in significant reductions in plant height up to 60 DAP. At 45 and 51 DAP, *Dickeya dadantii* inoculated treatments were significantly shorter compared to non-inoculated, stab water and vacuum water treatments (Table 4). At 60 DAP, only *D. dadantii* infected treatments showed statistical differences, compared to water or non-inoculated treatments. At 65 DAP there was no statistical difference between treatments (Table 4). Similar reductions in plant height due to treatments were not observed in the Live Oak, FL trial (data not shown).

Table 4. Effect of *Dickeya dadantii* inoculation treatments on plant height (cm) at 45, 51, 60 and 65 DAP at Oakes, ND, in 2017.

Treatment	45 DAP	51 DAP	60 DAP	65 DAP
Non-inoculated	60.3 A_a	69.0 AB	71.9 A	66.6
Infiltrate *Dickeya*	28.6 D	45.7 D	60.7 B	64.0
Infiltrate water	52.2 B	65.9 B	72.8 A	64.0
Stab *Dickeya*	38.4 C	56.3 C	64.7 B	63.8
Stab water	55.3 AB	72.3 A	72.3 A	66.0

[a]Numbers followed by the same letter in a column are not significantly different according to an ANOVA procedure using a t-Test (LSD) analysis at P=0.05.

Data demonstrated that plant height differences were a result of bacterial infection of the seed piece that reduced plant growth up to 60 DAP. Lack of noticeable plant height differences

at Live Oak, FL, may have been due to frost damage incurred. The trial at Live Oak, FL, was damaged by frost at 30 DAP on 16 Mar 17. Temperatures during the early morning of 16 Mar 17 reached from -3.3ºC to -2.2ºC, with a wind speed of eight to 12.9 kph, for approximately three hours (Florida Automated Weather Network. 2017). During the evening prior to the frost, plots were hilled, and irrigated through the night as a frost damage prevention method. At the time of frost, plants were approximately 10 cm to 20 cm tall. Above ground portions of the plant were damaged, but below ground, seed pieces were not damaged, allowing the plants to recover and resume normal growth (Fig. 2).

Figure 2. Visual symptoms of frost damage at 30 DAP at Live Oak, Florida in 2017. (a) Non-inoculated plants exhibiting symptoms of stunting. (b) Non-inoculated plants exhibiting symptoms of necrosis, chlorosis, and stunting. (c) Magnified image of necrosis and chlorosis of frost damaged plants.

To infect the plant systemically, *Dickeya* spp. commonly colonize roots of growing plants (Czajkowski et al. 2011). The cold temperatures that led to frost may have caused the *Dickeya* to replicate more slowly, or stop replicating all together. When seed tubers rot, the bacterial

population can increase, allowing them to infect the roots of the potato plant and subsequently enter into the vascular system (Czajkowski et al. 2011). Due to the low temperatures and frost damage to the above ground portion of the plant, bacteria may not have reached the population needed to colonize the roots and ultimately the vascular system of the potato plant.

Another possible explanation for the plant height differences observed at Oakes, ND, but not at Live Oak, FL, may be the difference in air and soil temperature up to 30 DAP. At Oakes, ND, during the first month of growth, average air and soil temperatures did not reach above 13.3ºC (Table 2). Optimal growing temperatures for *Dickeya* range from approximately 25 to 35°C (du Raan et al. 2015), but have also been shown to cause disease at a lower range of temperatures including 18 to 28°C (Lojkowska et al. 2010). Due to these below optimal bacterial growing conditions, *D. dadantii* may have remained in a quiescent state until temperatures rose to more conducive levels. Temperatures closer to optimal growing conditions could be observed (average soil and air temperatures above 18 ºC) during the second month of the growing season (Table 2). Once more conducive temperatures were reached, the bacteria may have become more active and replicated, thus infecting the growing plants to cause stunting symptoms. When compared against Oakes, ND, temperatures were much higher at planting at Live Oak, FL. These higher temperatures may have caused either the mother tuber to rot, or immediate blackleg in growing plants instead of delayed stunting symptoms as seen at Oakes, ND. Similar research conducted by van der Wolf et al. (2017) showed that amounts of precipitation did not correlate with symptom expression of *Dickeya* spp. infected plants. Similarly, disease development at both locations did not correlate with amounts of precipitation (both irrigation and natural rainfall) in these trials.

In-Field Observations

Live Oak, FL, trials were evaluated two times for blackleg during the season. At Live Oak, FL, during the first collection at 43 DAP, four samples consisting of one to three rotting stems were tested for *Dickeya* by PCR using *pelADE* primers (Nassar et al., 1996). Two samples tested positive for *D. dadantii*. During the second collection at 58 DAP, 25 blackleg samples were collected. Of the 25 plants, 15 samples tested positive for *D. dadantii*. Two samples that were water inoculated tested negative. All samples collected expressed blackleg symptoms.

At Oakes, ND, observations were made seven times during the growing season. Five more observations were recorded at Oakes, ND, compared to Live Oak, FL, because of the convenience of traveling to trial locations. Sample collections at Oakes, ND, were made at 39, 45, 51, 59, 72, 77, and 87 DAP. At 39 DAP, two samples were collected based on stunting symptoms only. No blackleg or wilting was observed in any inoculated or non-inoculated treatments. Of the two samples collected, one of them tested positive for *Dickeya*. At 45 DAP, two samples were collected, and both tested positive for *Dickeya*. At 51 DAP, 22 samples were randomly collected throughout all inoculated treatments and six samples from all non-inoculated or water inoculated treatments, based on stunting symptoms only. Of the combined 28 samples taken, one sample from a vacuum inoculated treatment tested positive for *Dickeya dadantii*. At 59 DAP, one sample tested negative for *Dickeya.* At 72 DAP, one sample from a stem-end inoculated treatment tested positive for *Dickeya.* At 77 DAP, seven samples with blackleg symptoms tested negative for *Dickeya*. At 87 DAP, no plants were found showing blackleg symptoms. The reason that all replicates were not sampled at each sampling date was because samples were taken based on blackleg symptom expression only. Overall, blackleg plants found at Live Oak, FL, outnumbered the blackleg plants found at Oakes, ND, 17 to 4.

CHAPTER 3. SPREAD OF D*ICKEYA DADANTII* DURING THE SEED HANDLING AND CUTTING PROCESSES

Introduction

Bacteria have many methods by which they can be moved and spread. The soft rotting bacteria *Dickeya* and *Pectobacterium*, can spread from infected tubers to clean tubers, both in the field and in storage (Tsror et al. 2012). Tubers can become contaminated during plant growth, although harvesting and subsequent handling and grading have been considered the most probable time for infection to spread (Tsror et al. 2012). *Pecotobacterium atrosepticum* can spread by mechanical cultivation and during harvest, when a wounded tuber contacts an infected, rotting tuber (Czajkowski et al 2010). Once an infected tuber rots, the soft rotting bacteria can multiply exponentially, thus creating enough inoculum to cause subsequent infections during both harvest and handling (Czajkowski et al 2010). Once a clean tuber contacts an infected tuber, the clean tuber may become infected through natural openings, such as lenticels or cracks on the periderm (Czajkowski et al 2010). Bacteria on the periderm commonly die quickly, but bacteria that reside inside the lenticels and wounds can remain viable until the next growing season (Czajkowski et al 2010). Production of clean seed stocks has been identified as one of the best prevention methods for bacterial spread (Tsror et al. 2012). Potato seed certification requires that seed fields be scouted two to three times through the growing season and the number of plants demonstrating blackleg symptoms be recorded (Czajkowski et al. 2014). Blackleg plants are then rouged and discarded. Seed certification cannot detect latent infections which may persist in the lenticels. In subsequent growing seasons, asymptomatic tubers may have the ability to transmit bacteria to clean tubers through contact during the cutting and handling processes and in-field infections (Czajkowski et al. 2014). This possible mechanism of spread and infection is cause for

concern. The most viable method of long distance transmission for *Dickeya* and *Pectobacterium* is known to be contaminated seed tubers (Tsror et al. 2012). Latent infections of seed lots are common and can be tested by laboratory methods including culturing and PCR (Motyka et al. 2017). It is important to know if bacteria in contaminated tubers can spread to healthy tubers. The objective of this trial was to determine if *Dickeya dadantii* can spread during the handling and cutting of seed potatoes.

Materials and Methods

Treatment Preparation and Trial Design

A trial was planted at Black Gold Farms near Live Oak, Florida (30.30 N, -82.98 W) on 15 Feb 2017, and a trail was planted at the Oakes Irrigation Research Site near Oakes, North Dakota (46.07 N, -98.09 W; elevation 399 m) on 15 May 2017. The soil at Live Oak, FL, is an Alpin fine sand, Thermic, coated Lamellic Quartzipsamments with a soil pH of 6.5 (USDA-NRCS 2017). The soil type at Oakes, ND, is an Embden coarse-loamy, mixed, superactive, frigid Pachic Hapludolls (14% clay, 70% sand, 16% silt), with a soil pH of 6.6 (USDA-NRCS 2017). Trials were planted in a RCBD, with eight treatments and four replicates. Each treatment consisted of 100 single drop tubers, with 25 tubers per treatment in each replicate. Treatments also consisted of either cut or whole tubers that were either rolled with *Dickeya* infected or non-infected tubers and/or treated with firbark and healed for three days to simulate the suberization process or planted immediately to prevent suberization (Table 5). Healthy whole or cut tubers of cv. Atlantic were treated before or after cutting by rolling in a 50 gallon container with ten cut infected seed pieces or non-infected seed pieces and sharp stones to simulate injury during the cutting and handling processes. Firbark (if included in treatment) was added after infected or non-infected treatment seed. All cut treatments were cut three day before planting. Non-infected

tubers of cv. Atlantic previously tested negative by PCR (Nassar et al. 1996) and seed lot cv. "22" with 42% infection by *Dickyea dianthicola* were used in this trial. All cutting was done by hand, at both locations, and all seed was hand planted at 25 cm with-in row spacing and 86 cm between rows, at a depth of 12 cm.

Table 5. Treatments for the seed cutting and inoculation trials at Oakes, ND, and Live Oak, FL.

#	Treatment	Cut seed	Inoculation
1	Non-treated/Unhealed	No	No
2	Non-treated/Unhealed	Yes	No
3	Non-treated/Unhealed	No	Yes
4	Non-treated/Unhealed	Yes	Yes
5	Firbark/ Heal 3 Days	No	No
6	Firbark/ Heal 3 Days	Yes	No
7	Firbark/ Heal 3 Days	No	Yes
8	Firbark/ Heal 3 Days	Yes	Yes

Data Collection

Stand counts were recorded by counting each emerged potato plant, and converted to percentage stand by dividing the number of plants emerged per treatment (X), by the number of seed tubers planted per treatment (25), and multiplied by 100. At Live Oak, FL, stand counts were recorded at 43 DAP. Only one stand count was conducted because of frost that damaged previously emerged tubers (Fig. 2). Three separate visual counts of each treatment for potential soft rot in treated seed pieces and blackleg in plants were made 43, 58, and 75 DAP at Live Oak, FL.

At Oakes, ND, stand counts were recorded 24 and 30 DAP using the same methods as at Live Oak, FL. Visual observations for disease were made 39, 45, 51, 59, 72, 77, and 87 DAP at Oakes, ND, using the same methods as at Live, Oak, FL.

Data Analysis

Data were subject to analysis of variance and an orthogonal comparison to determine if there were statistical differences among treatments using PROC ANOVA in SAS 9.4 (Statistical Analysis Software, version 9.4. SAS Institute Inc., 100 SAS Campus Dr., Cary, NC 27513). Significant differences were found (Appendices C and D), therefore data were analyzed and presented by site. Treatment means were separated using a T-test to calculate LSD (p=0.05).

Results and Discussion

There were no significant differences of mean percentage stand between treatments at Live Oak, FL (Table 6) (Appendix C). Although, at Oakes, ND, there were significant differences in stand percentage across treatments (Table 7) (Appendix C). Results suggest cutting versus whole seed, firbark treatments, and suberization for three days did not have an impact on the spread of *Dickeya* during seed cutting and handling of potatoes at Live Oak, FL.

Table 6. Mean percentage stand for the seed cutting and handling trial at Live Oak, FL, at 43 DAP, in 2017.

#	Treatment	Cut seed	Inoculation	Mean Stand (%)
1	Non-treated/Unhealed	No	No	95
2	Non-treated/Unhealed	Yes	No	85
3	Non-treated/Unhealed	No	Yes	96
4	Non-treated/Unhealed	Yes	Yes	82
5	Firbark- Heal 3 Days	No	No	90
6	Firbark- Heal 3 Days	Yes	No	87
7	Firbark- Heal 3 Days	No	Yes	96
8	Firbark- Heal 3 Days	Yes	Yes	82

Table 7. Mean percentage stand for the seed cutting and handling trial at Oakes, ND, at 30 DAP, in 2017.

#	Treatment	Cut seed	Inoculation	Mean Stand (%)
1	Non-treated/Unhealed	No	No	91 A[a]
2	Non-treated/Unhealed	Yes	No	69 B
3	Non-treated/Unhealed	No	Yes	91 A
4	Non-treated/Unhealed	Yes	Yes	70 B
5	Firbark- Heal 3 Days	No	No	94 A
6	Firbark- Heal 3 Days	Yes	No	79 B
7	Firbark- Heal 3 Days	No	Yes	91 A
8	Firbark- Heal 3 Days	Yes	Yes	73 B

[a]Numbers followed by the same letter in a column are not significantly different according to an ANOVA procedure using a t-Test (LSD) analysis at P=0.05.

However, at Oakes, ND, all cut treatments had a significantly lower percentage mean stand, compared to uncut treatments. Orthogonal comparison demonstrated significant differences when comparing cut and whole seed ($P<0.05$) (Appendix D). As an explanation, average soil and air temperatures from 1 to 17 DAP, were approximately 13 °C (Table 2), which may have caused germination, root and stem growth to be delayed, thus discouraging the rapid establishment of the potato plant and making it more prone to infection from soil pathogens. In addition, cut seed pieces were not treated with fungicide to prevent fungal infection, which may have allowed fungal soil pathogens to infect more easily, thus causing the reduction of stand. It is also possible that *Dickeya dianthicola* spread to the cut seed pieces, subsequently causing stand loss. The wound caused by cutting may have allowed entry, thus causing disease symptoms in the form of stand loss.

Although, when comparing cut and whole seed, at both locations, neither treatment expressed in-field blackleg symptoms. A logical explanation for this was that there was either not enough *Dickeya dianthicola* transferred from infected seed to clean seed during seed cutting and handling to cause symptom expression, or conditions through the growing season were not

conducive. However, at Live Oak, FL, data from other trials (Ch. 2 and 4) suggest conditions were conducive for disease expression. Excessive amounts of precipitation and high temperatures in both the soil and air (Table 1), should have facilitated blackleg expression if the bacteria were present, even with low amounts of inoculum as previous research has shown that *Dickeya* spp. will develop disease with smaller amounts of inoculum, when compared with other soft rot causing pathogens such as *Pectobacterium* spp. (Toth et al. 2011). *Dickeya* spp. also thrive in high temperatures, and high amounts of moisture (Czajkowski et al 2010). At Oakes, ND, environmental conditions were not as conducive for bacterial expression as those observed at Live Oak, FL. Blackleg expression was not observed. After tumbling cut *Dickeya* infected tubers with non-infected tubers, similar results of no disease symptoms caused by *Dickeya* spp., have been observed by Steve Johnson, University of Maine (personal communication) when *Dickeya* infected seed was cut and tumbled with whole and cut non-infected seed, and no stand loss or blackleg was observed. Data suggest that *Dickeya* spp. may spread during the cutting and handling process of potatoes (Ch. 3 Results).

CHAPTER 4. IN-FIELD SPREAD OF D*ICKEYA DADANTII*

Introduction

Blackleg is a ubiquitous disease of potato characterized by symptoms of stem decay and black discoloration starting at the base of the stem (De Boer et al. 2012). Two genera primarily cause blackleg, *Pectobacterium* and *Dickeya* (Czajkowski et al. 2011). Both pathogens can remain latent in lenticels of seed tubers for several generations, thus risking unknown spread (Perombelon 2002). Both soft rot causing pathogens have been known to cause losses in seed potato production due to downgrading or rejection of seed lots due to latent infection (van der Wolf et al. 2017). In the past three years, *Dickeya* spp. have spread in the United States, particularly along the East Coast, causing significant economic loss (Jiang et al. 2016). *Dickeya* spp. are Gram-negative, facultative anaerobes, that produce pectinolytic enzymes to macerate host tissue (Perombelon 2002). Long distance movement of *Dickeya* spp. has been primarily attributed to contaminated seed tubers (Tsror et al. 2012). To date, the most useful management technique to limit spread has been the production of pathogen-free seed (Czajkowski et al. 2009). Polymerase chain reaction analysis of seed lots, using *pelADE* primer pairs (Nassar et al. 1996) and a standard testing protocol of 400 tubers per seed lot, has proven to be most effective in the US (Gary Secor, personal communication).

Compared to *Pectobacterium* spp., *Dickeya* spp. are more aggressive, can spread through the plants vascular system, have higher optimal growth temperatures, approximately 25 to 35 °C (du Raan et al. 2015), but have also been shown to cause severe rotting symptoms at approximately 18 to 25 °C (Czajkowski et al. 2013), and cause disease at lower inoculum levels (Toth et al. 2011). Surviving for no more than six months (Czajkowski et al. 2010), *Dickeya* seems to be less hardy, surviving for a shorter time in the soil compared to *Pectobacterium* (Toth

et al. 2011). As a seed-borne disease, it is likely that *Dickeya* spp. infect via natural openings (lenticels) or tuber wounds (Czajkowski et al. 2010). One important environmental factor for blackleg development is the amount of soil water (Czajkowski et al. 2011). Previous research conducted by Czajkowski et al. (2010) has shown that *Dickeya* can infect both lenticels in field tubers, and roots of potato plants in greenhouse infected soils. Research demonstrated that once roots are infected, *Dickeya* spp. can move systemically through the vascular system and eventually induce typical blackleg symptoms (Czajkowski et al. 2010). Once in the vascular system, bacteria can become latent, and overwinter in the infected host (Czajkowski et al. 2010). Because of recent infections in the Eastern United States, many questions have been asked regarding how *Dickeya* spreads in the field. Czajkowski et al. (2010) also proposed two possible methods of spread to progeny tubers: bacteria may either be moved directly from the mother tuber to progeny tubers via the plants vascular system, through the stolon and into the progeny tuber, or bacteria may infect the roots of plants, move through the vascular system, and subsequently infect progeny tubers. If conditions are conducive, *Dickeya* can move to adjacent plants and cause infection by wounds or natural openings (Czajkowski et al. 2010).

The objective of this study was to evaluate the natural spread and movement of *Dickeya* sp. in the field. Field experiments were conducted in 2017, at Live Oak, Florida, and at Oakes, North Dakota, by planting *Dickeya* sp. infected seed tubers surrounded by clean seed tubers. Expression of blackleg symptoms throughout the growing season, and the spread of *Dickeya* sp. to progeny tubers of surrounding plants, was evaluated.

Materials and Methods

The trial was planted at Black Gold Farms near Live Oak, Florida (30.30 N, -82.98 W) on 15 Feb 2017, and at the Oakes Irrigation Research Site near Oakes, North Dakota (46.07 N, -

98.09 W; elevation 399 m) on 15 May 2017. The soil at Live Oak, FL was an Alpin fine sand, Thermic, coated Lamellic Quartzipsamments with a soil pH of 6.5 (USDA-NRCS 2017). The soil type at Oakes, ND, was an Embden coarse-loamy, mixed, superactive, frigid Pachic Hapludolls (14% clay, 70% sand, 16% silt), with a soil pH of 6.6 (USDA-NRCS 2017).

Culture Isolation and Preparation

All bacterial cultures and aqueous bacterial solutions used for inoculation were prepared using the protocol as described in Chapter 2.

Tuber Inoculation

Forty whole seed tubers of cv. "22" were inoculated by vacuum infiltration with an aqueous solution of a *Dickeya dadantii* at 10^7 cfu/mL^{-1} and were used as the inoculated treatment. The control treatment consisted of forty vacuum infiltrated tubers with distilled, autoclaved water. Vacuum infiltration was the same as described in Chapter 2.

Planting

Spread Trials

Whole seed tubers of a susceptible variety, cv. "22", inoculated with *Dickeya dadantii* and water by vacuum infiltration, were planted in the field, and surrounded by four cv. Reba seed tubers, in a RCBD with two treatments and four replicates. Each treatment consisted of ten whole tubers inoculated with *Dickeya dadantii* or water that were hand planted. Non-inoculated seed (cv. Reba) free of *Dickeya* spp. was planted surrounding the seed in front of, behind, and adjacent to, the *Dickeya dadantii* inoculated seed (Fig. 3). In the figure, blue dots represent non-infected tubers and red a dot represents a *Dickeya dadantii* inoculated tuber. Surrounding seed was previously tested for *Dickeya* spp. using the standard testing protocol of 400 tubers per seed lot by PCR using *pelADE* primer pairs (Nassar et al. 1996), to ensure seed was free of *Dickeya*

spp. Seed was spaced 25 cm apart within each row, rows were 86 cm apart, and the planting depth was 12 cm. Through the growing season, trials at Live Oak, FL, were irrigated each day it did not rain naturally. Approximately 0.8 cm of irrigation water was applied each irrigation to provide adequate water. At, Oakes, ND, trials were irrigated accordingly (approximately 1-3 days) to maintain soil water holding capacity at approximately 80%.

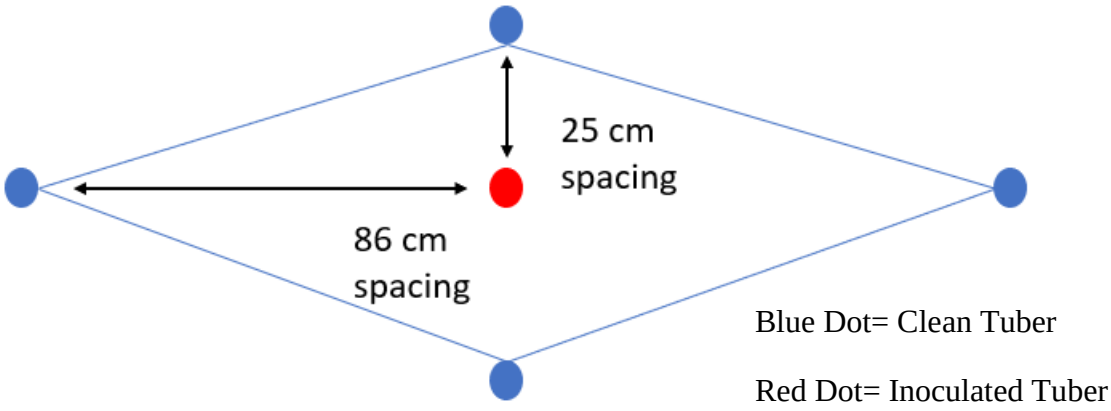


Figure 3. Planting diagram for spread trials at Live Oak, FL, and Oakes, ND, conducted in 2017.

Symptom Assessment

Spread Trials

At Live Oak, FL, treatments were evaluated for *Dickeya dadantii* three times through the growing season. Three separate visual observations for disease symptoms caused by *Dickeya* were conducted at 43, 58, and 75 DAP. Plant stems (20 to 30 cm long) exhibiting blackleg symptoms, were placed into a medium one-quart, sealed, air and water tight plastic freezer bag along with 10 mL of autoclaved water, and macerated so that DNA could be extracted using Power Plant® Pro DNA Isolation Kit (Qiagen.Venlo, The Netherlands) and subsequently tested for *Dickeya* spp. using conventional PCR with *pelADE* primers (Nassar et al. 1996).

At Oakes, ND, treatments were evaluated for *Dickeya dadantii* seven times throughout the growing season. Evaluations were conducted at 39, 45, 51, 59, 72, 77, and 87 DAP. Evaluations and testing procedures were the same as at Live Oak, FL.

Harvest

Spread Trials

Four tubers from each cv. Reba plant surrounding each inoculated cv. "22" seed tuber were hand harvested on 21 May 2017 (97 DAP) at Live Oak, FL, and on 22 Aug 2017 (98 DAP) at Oakes, ND. Samples consisting of four tubers from each surrounding plant were placed into a pre-numbered, water resistant, paper bag and bags were stored in a shaded, cool (approximately 21°C) building and allowed to air dry. Once completely harvested, samples were shipped to the lab at North Dakota State University and stored at 10 °C until testing.

Maceration and Incubation

One stem-end core and one peel sample, from each cv. Reba tuber, were tested for *Dickeya* spp. Each stem-end core sample was removed from each of the four tubers using a #5 cork borer (10 mm) and combined into one sample bag with 5 µl of autoclaved water, labeled, macerated, and incubated at 30 °C for approximately 24 hours. Each peel sample (5x3 cm) from each of the four tubers was removed using a potato peeler (approximately 1 cm deep), combined in a sample bag with 10 µl of autoclaved water, macerated, and incubated at 30 °C for approximately 24 hours.

DNA Extraction

DNA was extracted from all samples and tested for *Dickeya* spp. using the Power Plant® Pro DNA Isolation Kit (Qiagen.Venlo, The Netherlands) following the manufacturer's instructions. Extracted DNA samples were stored at -20 °C until PCR testing.

PCR Protocol

A conventional PCR was conducted for each DNA sample to detect *Dickeya* spp. using *pelADE* primer pair (Nassar et al. 1996), with a Bio-Rad PCR machine. Reagents used per sample were 5 µl of buffer (5x Green GoTaq® Reaction Buffer), 2.5 µl of MgCl2, 2.5 µl of dNTPs, 0.625 µl of forward primer, 0.625 µl of reverse primer, 0.1 µl of GoTaq, 8.65 µl of Sigma water, and 5 µl of DNA. After an initial denaturation step of 94 °C for two minutes, 30 PCR cycles were run: denaturation (94 °C, 45 sec), annealing (62 °C, 45 sec), and extension (72 °C, 1 minute and 30 seconds), followed by 10 minutes at 72 °C, and held at 4 °C for an infinite amount of time.

Progeny Tuber Expression Trial

Two separate trials utilizing progeny tubers collected at harvest from Live Oak, FL or progeny tubers from Oakes, ND, 2017, were planted at two separate fields in 2018, in the same environments at Black Gold Farms near Live Oak, FL (30.30 N, -82.98 W) (Field names: Corbett A and Corbett C) on 14 Feb 18. The soil at Live Oak, FL was a Alpin fine sand, Thermic, coated Lamellic Quartzipsamments with a soil pH of 6.5 (USDA-NRCS 2017). The purpose of these trials was to determine if *Dickeya* infected, second generation progeny tubers would express disease symptoms when planted the next growing season. To do this, tubers that tested positive for *Dickeya* were replanted and evaluated for disease expression. Each trial was planted in a RCBD with two treatments: *Dickeya* infected progeny tubers and non-infected, PCR tested, progeny tubers harvested from surrounding plants of the spread trial in the previous year. The Live Oak, FL, progeny tuber trial contained 100 infected and 100 non-infected samples, with each sample consisting of four tubers per sample for a total of 25 samples per replicate. The Oakes, ND, progeny tuber trial consisted of 40 infected and 40 non-infected samples, with each

sample consisting of four tubers per sample for a total of 10 samples per replicate. Tubers were hand planted with one tuber cv. Red La Soda in-between each sample. After every five samples, three tubers, cv. Red La Soda, were planted as spacers. Seed was spaced 31 cm apart within each row, with 86 cm between rows. Stand counts were recorded at 25, 41, and 77 DAP, by counting the number of emerged plants in each sample and dividing the total number of seed tubers planted in each sample (four), to calculate the total percent emerged plants per sample. Plant height was recorded at 41 and 53 DAP by measuring each above ground plant from the soil line, to the apex of the plant. Blackleg expression was recorded by visually evaluating each above ground plant, in each treatment, to check for disease expression caused by *Dickeya*, at 41, 53, and 77 DAP throughout the growing season.

Data Analysis

Spread Trial 2017 and Progeny Tuber Trial 2018

Data were subject to analysis of variance to determine if there were statistical differences among treatments using PROC ANOVA in SAS 9.4 (Statistical Analysis Software, version 9.4. SAS Institute Inc., 100 SAS Campus Dr., Cary, NC 27513). Significant differences were found (Appendices G, H, I and J), therefore data were analyzed and presented by site. Treatment means were separated using a T-test to calculate LSD (p=0.05).

Results & Discussion

Spread Trials- Field Results 2017

No difference between treatments was found for stand or blackleg during the growing season at Live Oak, FL (data not shown). Nineteen plants with blackleg symptoms were identified and of the 19 plants, 17 were from plants surrounding a *Dickeya dadantii* inoculated seed piece and two stems, approximately 10 to 20 cm in length, were sampled from plants

surrounding a water infiltrated seed piece. Of the 17 plant stems tested from a plant surrounding a *D. dadantii* inoculated seed piece, 11 (65.0%) samples tested positive for *Dickeya* spp. when tested by conventional PCR using *pelADE* primers (Nassar et al. 1996). Of the two plants sampled from a water infiltrated treatment, both were negative for *Dickeya dadantii*.

No differences in stand were found between treatments in the trial grown at Oakes, ND (data not shown). Through the growing season, a total of four plants with blackleg symptoms were sampled. Of the four plant stems sampled, all were from plants surrounding a *Dickeya dadantii* inoculated seed piece. One of four samples tested positive for *Dickeya dadantii* by conventional PCR using *pelADE* primers (Nassar et al. 1996).

Spread Trial-Tuber Testing 2017

One hundred four of 320 samples (33%) of the progeny harvested cv. Reba tuber groups tested positive for *Dickeya* from the Live Oak, FL trial (Appendix E). Stem-end core test results had the highest incidence of *Dickeya dadantii* with 76 positive samples of 320 (24%). Of the 76 positive stem-end core samples, 58 were from tubers harvested from plants adjacent to a *Dickeya dadantii* inoculated seed piece (76%), and 18 were from tubers harvested from plants that surrounded a water inoculated seed piece (24%). Of the peel samples, 41 of 320 (13%) tested positive for *Dickeya dadantii*. Of the positive peel samples, 16 (39%) were sampled from tubers harvested from plants adjacent to a *Dickeya dadantii* inoculated seed piece, and 25 (61%) were from tubers harvested from plants adjacent to a water inoculated seed piece. Positive samples coming from rows containing plants adjacent to a *Dickeya dadantii* inoculated seed piece were significantly greater than samples harvested from plants adjacent to a water inoculated seed piece at Live Oak, FL (Fig. 4) (Appendix G). When comparing peel and stem-end core tests, significant differences among treatments were observed at Live Oak, FL (Fig. 6) (Appendix H).

Thirteen of 320 samples tested positive for *Dickeya dadantii* in both the peel and stem-end core (5%).

At Oakes, ND, PCR analysis of the progeny tubers resulted in a total of 40 positive samples of 320 total plants sampled (13%) (Appendix F). Peel test results showed the highest amount of positive tests for *Dickeya dadantii* with a total of 38 positive samples of the 320 peels that were sampled (12%). Of the positive samples, 22 were from tubers harvested from plants surrounding a *Dickeya dadantii* inoculated seed piece (58%) while 16 were from tubers harvested from plants that surrounded a water inoculated seed piece (42%). Furthermore, of the core samples tested, two of 320 (1%) samples were positive for *Dickeya dadantii*. Of the positive samples, one was sampled from a plant surrounding a *D. dadantii* inoculated seed piece (50%) and one was sampled from a plant surrounding a water inoculated seed piece (50%). Positive samples harvested from rows containing plants adjacent to a *D. dadantii* inoculated seed piece were not significantly different, compared to samples harvested from plants adjacent to a water inoculated seed piece at Oakes, ND (Fig. 5). When comparing peel and stem-end core tests, *Dickeya dadantii* positive peel tests were significantly greater, compared to stem-end core tests at Oakes, ND (Fig. 7) (Appendix H). One of the 320 samples showed positive results in both the core and the peel (0.3%).

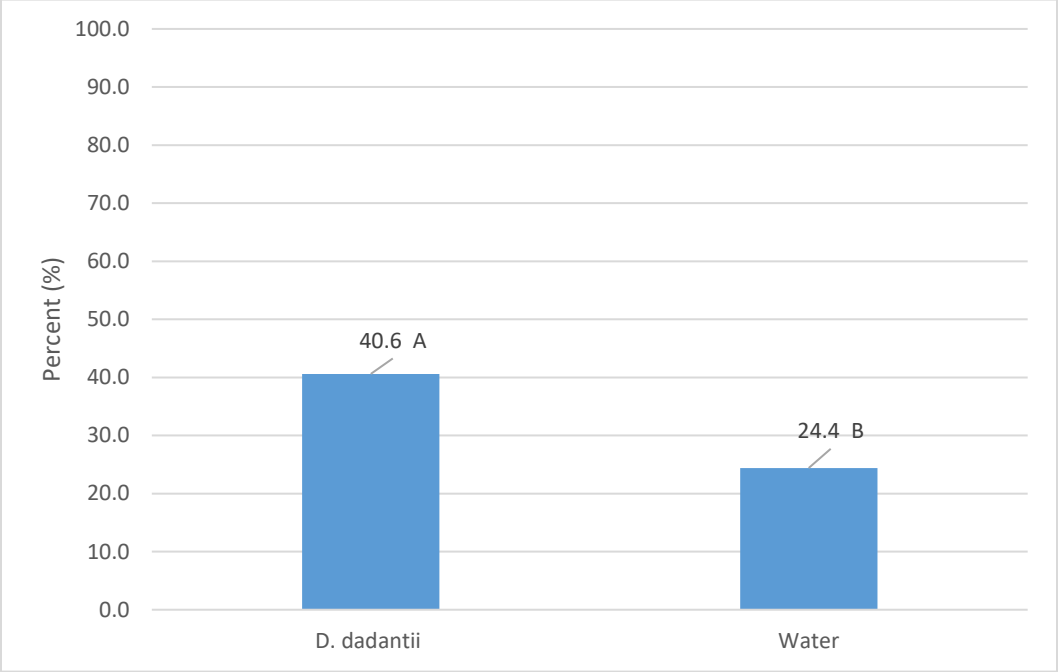


Figure 4. Percentage of progeny tuber sample groups that tested positive for *Dickeya dadantii* from Live Oak, FL, in 2017.

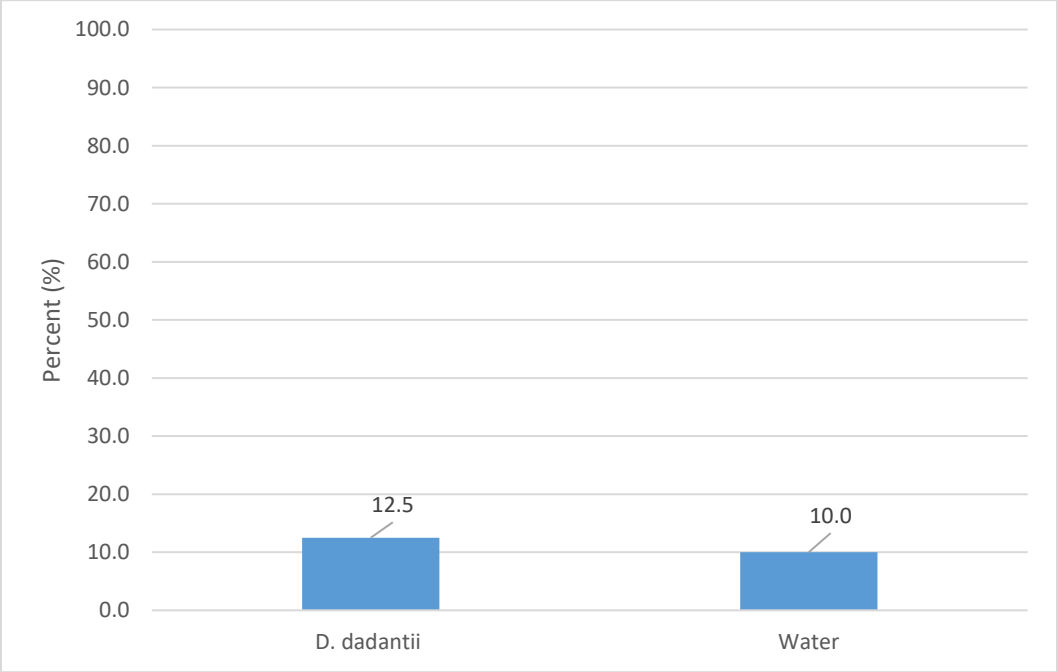


Figure 5. Percentage of progeny tuber sample groups that tested positive for *Dickeya dadantii* from Oakes, ND, in 2017.

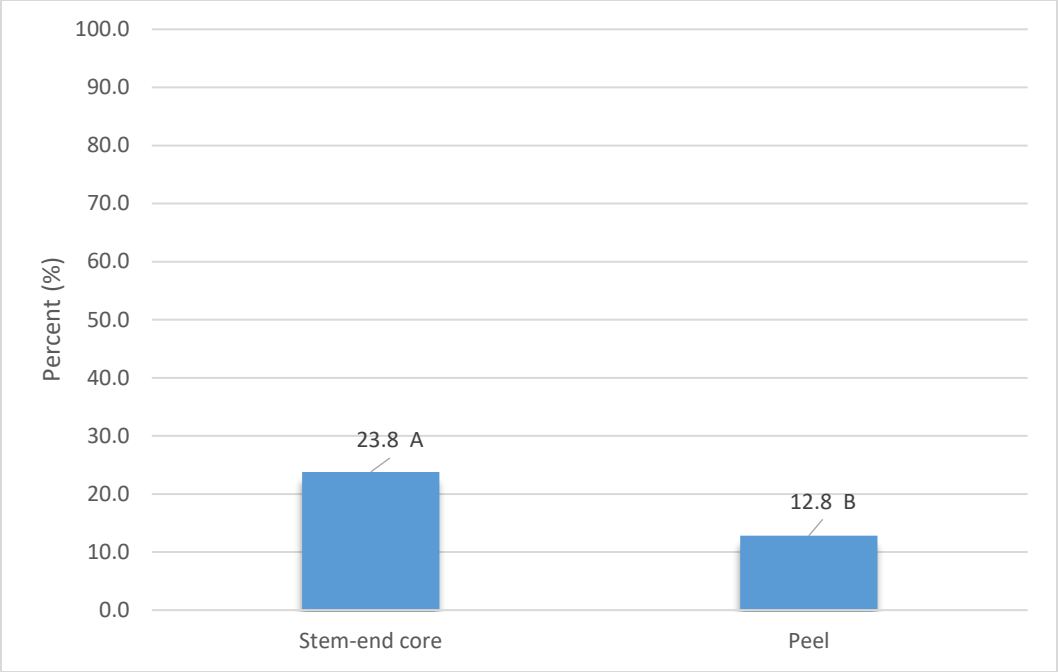


Figure 6. Percentage incidence of progeny tuber sample groups testing positive for *Dickeya dadantii* from Live Oak, FL, 2017.

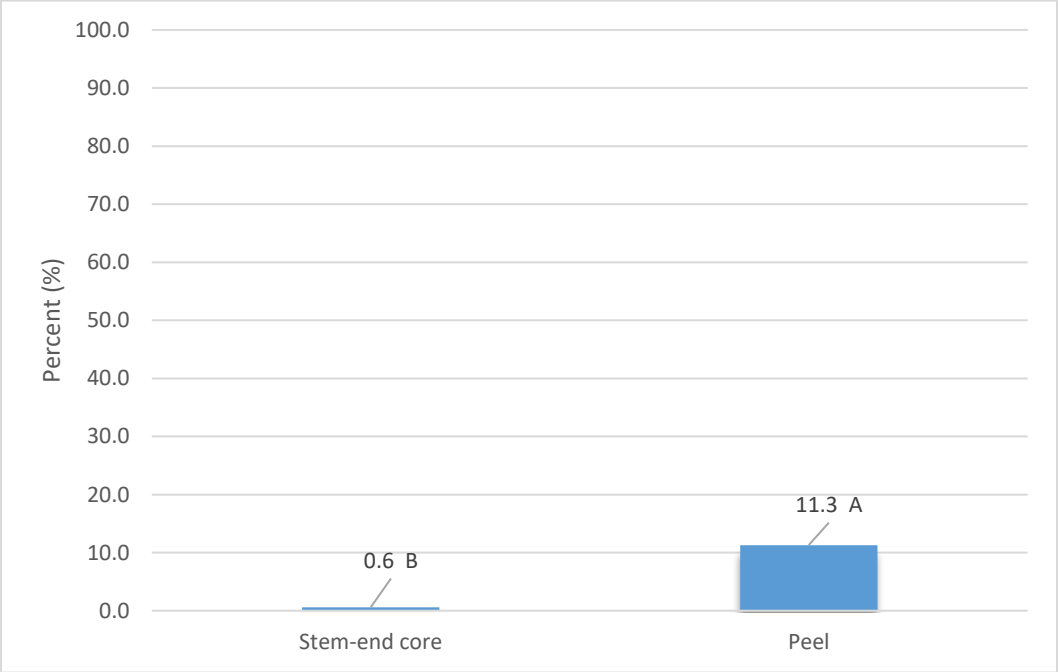


Figure 7. Percentage incidence of progeny tuber sample groups testing positive for *Dickeya dadantii* from Oakes, ND, in 2017.

The total number of infected progeny tubers at Live Oak, FL, suggests the incidence of bacterial spread to progeny tubers and adjacent plants was 33%. Previous research conducted by van der Wolf et al. (2017) found *D. solani* infected plants produced infected progeny tubers at a rate of 27% when inoculated by vacuum infiltration at a density of 10^6 CFU mL^{-1} (van der Wolf et al. 2017), similar to our results of 33%.

Similar results were also found in field experiments conducted in the Netherlands in 2008 and 2009 when tubers were vacuum inoculated with different strains of *Dickeya solani* and *Dickeya dianthicola* (Czajkowski et al 2013). Half of the strains used had progeny tubers that demonstrated an infection rate ranging from 30 to 70%, with the other remaining strains demonstrating an infection rate of approximately 5 to 100% (Czajkowski et al 2013). These results coincide with results found at Live Oak, FL and Oakes, ND, suggesting that aside from vacuum infiltration being an efficient inoculation method, *Dickeya* sp. can spread to progeny tubers if environmental conditions, such as high temperature (above 18 °C) and free water, are present.

There are several possible explanations for bacterial spread and disease expression at Live Oak, FL. Factors such as soil type and texture, soil water-holding capacity, amount of irrigation water applied to the field, and the amount of time in which water was applied. In sandy soil, available water can greatly decrease in a small amount of time if not properly irrigated or adequate rainfall occurs. Due to an inability to hold the water needed for optimum plant growth, overhead irrigation was applied frequently at Live Oak, FL (Table 1), to maintain 80% available soil moisture. Through the growing season, 61 cm of total irrigation water was applied to the trial at Live Oak, FL. This large quantity of water could be a feasible method of transportation for bacterial spread from mother tuber, or mother plant, to progeny tubers and/or surrounding plants.

Irrigation aside, natural rainfall at Live Oak, FL, often occurred in a short time span. For example, at 48-49 DAP, 32 cm of rain fell in a 48-hour timespan (Florida Automated Weather Network 2017). At the time the precipitation fell, average soil temperatures were 24 ºC and average air temperatures were 21 ºC (Florida Automated Weather Network 2017), well above the minimal optimal growing temperature for *Dickeya* spp. of 18 ºC (Lojkowska et al. 2010). This excessive amount of water in a short amount of time may have helped create a more conducive environment for bacterial spread from plant to plant, and/or plant to progeny tuber. Previous literature suggests that if the mother tuber rots, *Dickeya* can be released into the soil and move from rotting mother tubers to other progeny tubers via soil water (Czajkowski et al. 2011). Excessive amounts of water in a short period of time, combined with the sandy soil and optimal temperatures at Live Oak, FL, may have created a conducive environment for the bacteria to spread from mother tuber and plant to the progeny tubers on the same plant or to progeny tubers of surrounding plants. Overall, Live Oak, FL received a total of 86 cm of water through the growing season, while Oakes, ND only received 55 cm of water (Table 1 and 2). Oakes, ND, did not have any large amounts of natural rainfall in a short period of time as occurred at Live Oak, FL. The largest amount of precipitation at Oakes, ND was not observed until 90-91 DAP when 7 cm of natural rainfall accumulated over a 48- hour timespan (Table 2). The amount of water in Live Oak, Fl, may have aided in the spread of soft rot bacteria to progeny tubers and may help explain why a greater amount of spread was observed at Live Oak, FL, than at Oakes, ND.

Interestingly, both peel and core testing resulted in positive samples that came from water inoculated treatments at both Live Oak, FL and Oakes, ND. Although this could have been caused by contamination, research has shown that *Dickeya* can move via free water in soil up to 10 m (Czajkowski et al. 2010). Trials were designed with row spacing of 86 cm (<1 m) and

contained one buffer row between each *Dickeya dadantii* inoculated treatment and water inoculated treatment with a maximum distance of plants grown from clean seed to a *Dickeya dadantii* inoculated treatment of approximately ≤ 3.5 m, well within the spreading distance of *Dickeya*.

In *Dickeya dadantii* and water inoculated treatments, *D. Dadantii* positive samples were found in peel and core tissues tested from Live Oak, FL, and Oakes, ND, thus revealing differences in bacterial location within the plant. If *Dickeya* is detected in the stem-end core, logically the bacteria entered progeny tubers through the roots and into the vascular system. This is due to the inability of the bacteria to enter the vascular system via lenticels, as there is no entrance into the vascular system through the periderm of the tubers without wounding. On the contrary, if *Dickeya* is found in the peel, it is reasonable to assume bacteria entered through the lenticels on the periderm of the tuber.

Compared to Live Oak, FL, Oakes, ND, has several environmental and climatic differences, which may explain the smaller amount of infected progeny tubers observed. First, the soil and air temperatures at Oakes, ND, at planting were approximately 4 to 5 ºC less than at Live Oak, FL, which may have inhibited bacteria from rotting inoculated seed pieces, and thus reducing the amount of potential inoculum (Table 2). Although, as the season progressed, soil and air temperatures at both locations rose and remained at similar levels, aside from the last month of the growing season (76-100 DAP). At this time, soil and air temperatures at Oakes, ND, were approximately 5 to 6 °C less than those recorded at Live Oak, FL. This decrease in temperature may have reduced bacterial activity, and perhaps the spread to progeny tubers. Overall, Live Oak, FL, had a higher average soil temperature (3 ºC) and a higher average air temperature (2 ºC), when compared to Oakes, ND (Table 1 and 2).

Another difference between each trial location was the amount of precipitation (irrigation and natural rainfall). Live Oak, FL, recorded more precipitation than Oakes, ND, by approximately 31.3 cm, primarily due to irrigation (+27.4 cm). Although Oakes, ND, trails were irrigated, the amount of required irrigation was less due to greater soil water holding capacity and organic matter content. It is possible *Dickeya* bacteria did not have the continuous amounts of free water needed to cause significant amounts of blackleg and infect progeny tubers at Oakes, ND. One could speculate the saturation of soil water in the Oakes, ND, soil profile may have had a conducive effect on the bacteria to progeny tuber movement, although, data suggests continuously draining free water, combined with constant new additions of precipitation help the bacteria to move from plant to plant and plant to tuber.

Overall, data suggests spread can and will occur from infected mother seed pieces to progeny tubers of surrounding plants at both Live Oak, FL, and Oakes, ND, when environmental conditions are conducive.

Progeny Tuber Expression Trial 2018

Percent stand counts resulted in significant differences among treatments at Live Oak, FL, but not Oakes, ND. *Dickeya* infected treatments demonstrated percent stand of 93.8, while non-infected treatments resulted in percentage stand of 98.8 (Table 8) (Appendix I). Plant height was also significantly different among treatments in Live Oak, FL, but not at Oakes, ND. *Dickeya* infected treatments demonstrated plant heights of 23.9, while non-infected treatments resulted in percentage stand of 26.0 (Table 9) (Appendix J).

Considering both significant amounts of stand loss and plant height reduction at Live Oak, FL samples, a higher amount of *Dickeya* may have been present in the Live Oak, FL, samples, or the environment may have been more conducive for the expression of the bacteria.

For example, average plant heights for samples from Oakes, ND, are numerically shorter than Live Oak, FL. The reason for this could be due to frost damage incurred on 8 Mar 18. Due to the frost damage, the plants from Oakes, ND were set back in growth and therefore had a lower average plant height (Table 9). The lack of disease expression in Oakes, ND samples also suggests that *Dickeya* infection may remain latent in potato tubers and may not result in disease expression after planting and through the growing season. Explanations for the lack of blackleg expression in both trials may be attributed to the minimally conducive environmental conditions at the beginning of 2018 (Table 10). At planting, average soil temperatures were approximately 18 ºC, and average temperatures were approximately 17 ºC with approximately 0.65 cm of irrigation. Air and soil temperatures suggest that conditions were minimally conducive for growth of *Dickeya* at planting. However, from 1 to 15 DAP average air and soil temperatures became more conducive. At 16 to 46 DAP average air temperatures dropped well below optimal temperatures for *Dickeya* spp. growth, but average soil temperatures were around 18 ºC.

Table 8. Average percentage stand of *Dickeya* infected seed potato samples and non-infected seed potato samples, harvested from two 2017 spread trial locations at 25, 41, and 77 DAP, planted at Live Oak, FL, in 2018

Treatment	Florida Progeny	North Dakota Progeny
Dickeya Infected	93.8 B[a]	98.8
Dickeya Non-infected	98.8 A	98.8

[a]Numbers followed by the same letter in a column are not significantly different according to an ANOVA procedure using a t-Test (LSD) analysis at P=0.05.

Table 9. Plant height of *Dickeya* infected seed potato samples and non-infected seed potato samples harvested from two 2017 spread trial locations, at 41 DAP, planted at Live Oak, FL, in 2018

Treatment	Florida Progeny Tubers	North Dakota Progeny Tubers
Dickeya Infected	23.9 A[a]	16.8
Dickeya Non-infected	26.0 B	15.7

[a]Numbers followed by the same letter in a column are not significantly different according to an ANOVA procedure using a t-Test (LSD) analysis at P=0.05.

Table 10. Climatic conditions at Live Oak, FL, through the growing season in 2018.

Year	Month	Days After Planting	Total Irrigation	Total Rainfall	Total Water	Average Soil Temperature	Average Air Temperature
			cm	cm	cm	°C	°C
2018	Feb	1-15	11.2	1.9	13.1	21.6	20.7
2018	Mar	16-46	24	2.5	27.1	18.4	14.9
2018	Apr	47-76	23.2	1.2	47.6	23.4	19.9
2018	May	77-100	14.4	12.6	27	26.3	24.5
Average			19.2	4.6	28.7	22.4	20
Total			76.8	18.2	114.8	NA	NA

Note- Adapted from Florida Automated Weather Network, University of Florida. 2017) ([y] Soil temperatures measured at a depth of 10 cm) ([z] Average air temperatures measured at two meters)

Another possible explanation for the lack of disease expression in Oakes, ND, samples could be low quantity of bacteria present inside of the tuber samples which tested positive.

Although a threshold for the number of bacteria that is needed to cause natural infection and disease is unknown, the quantity of bacteria which spread into progeny tubers may not have been enough to cause infection or to multiply to a population high enough to cause infection in the mother plant. Additionally, tissue breakdown associated with soft rot may not have been high enough to cause rotting of the seed piece, and thus subsequent infection and rotting of the mother plant. It is possible, that when conditions are conducive, bacterial populations grow slowly, but exponentially from growing season to growing season, and once populations become high enough and environmental conditions become conducive enough, infection and rotting occurs in both the seed piece and mother plant. In potato seed growing areas of the United States, infection from *Dickeya* commonly goes unseen until the seed is shipped for production in a conducive environment. Currently in the United States, blackleg is not considered during the seed certification process. Due to this, seed growers are only required to rogue out blackleg plants in potato seed fields. If one rogues out the diseased plant, the rotted seed piece may remain and bacterial multiplication may occur, leading to subsequent spread to surrounding plants. This spread may then lead to latent infection and only cause disease when bacterial populations are high enough and conditions are conducive.

GENERAL CONCLUSIONS

Both vacuum infiltration and stem-end inoculation were tested to develop an efficient inoculation method for *Dickeya dadantii* at Live Oak, FL, and Oakes, ND. Results demonstrated significant differences in percentage stand of *Dickeya dadantii* infected treatments, compared to non-inoculated, and water inoculated treatments at Live Oak, FL, but not at Oakes, ND. Results suggest that both vacuum infiltration and stem-end inoculation methods are effective inoculation methods for in-field studies in subtropical climates such as Live Oak, FL, but not in temperate climates such as Oakes, ND. Although, plant heights of *Dickeya dadantii* infected treatments were significantly shorter compared to non-inoculated and water treatments at Oakes, ND, results were not definitive enough to suggest the inoculation methods to be efficient.

To test if *Dickeya dianthicola* can spread during the handling and cutting of seed potatoes, trials were conducted at both Live Oak, FL, and Oakes, ND. Trials consisted of healthy whole or cut treatments that were rolled with *Dickeya dianthicola* infected tubers or not, and treated with firbark or not, and allowed to heal for three days, or not. Results demonstrated no significant reductions in mean percentage stands at Live Oak, FL. Although, significant reductions of mean percentages of stand were found among cut treatments compared to uncut treatments at Oakes, ND. However, of the significantly different treatments, two were inoculated with *Dickeya dianthicola* cut seed and two were not. Orthogonal comparisons between cut and whole seed demonstrated significant differences in mean stand percentage, but emerged plants in both cut and whole treatments did not express in-season blackleg symptoms. Again, results were not definitive enough to conclude *Dickeya dianthicola* can spread during the seed cutting and handling processes.

Assessment of the natural in-field spread of *Dickeya dadantii* was conducted at both Live Oak, FL, and Oakes, ND, by vacuum infiltrating tubers with *Dickeya dadantii* or water, planting them in the field, and planting non-infected tubers in front of, behind, and adjacent to each infected tuber. At the end of the growing season, at each trial location, four tubers from each surrounding plant were harvested and combined into one sample and tested for *Dickeya* in the stem-end core tissue and peel tissue, by PCR using *pelADE* primers. Positive and negative samples from both locations were then saved and planted into two separate trails at Live Oak, FL. Results from surrounding plants demonstrated significant differences between treatments of *Dickeya dadantii* and water at Live Oak, FL, but not at Oakes, ND. Significant differences were also demonstrated between stem-end core tissue positive samples and peel tissue positive samples at Live Oak, FL and Oakes, ND. Planting of the progeny tubers from the natural in-field spread trial demonstrated significant differences in percentage plant stand of *Dickeya* infected treatments compared to non-infected treatments at Live Oak, FL, progeny tubers, but not for Oakes, ND progeny tubers. A significant difference in plant height was also demonstrated between infected *Dickeya* treatments compared to non-infected treatments in Live Oak, FL progeny tubers, but not Oakes, ND, progeny tubers. No blackleg was observed in plants grown from progeny tubers in both trials. Overall, data suggests natural, in-field spread, can and will occur from infected mother seed pieces and infected mother plants to progeny tubers or surrounding plants at both Live Oak, FL and Oakes, ND. Data also suggests *Dickeya dadantii* can remain latent in progeny tubers and cease to express until conditions are conducive due to the lack of blackleg observed in both progeny trials.

These trials have provided further conclusion that *Dickeya dadantii* does not readily spread during the seed cutting and handling processes and have demonstrated that *Dickeya*

dadantii can spread to adjacent plants in the field and infections to adjacent plants can remain latent into the next growing season.

LITERATURE CITED

Agrios, G. 1988. Plant Pathology. Academic Press 3: 510-558.

Alyokhin, A., Mota-Sanchez, D., Baker, M., Snyder, W.E., Menasha, S., Whalon, M., Dively, G., and Moarsi, W.F. 2014. The red queen in a potato field: Integrated pest management versus chemical dependency in Colorado potato beetle control. Pest Management Science 71(3): 343-356.

Bissonnette, H., Preston, D., and Lamey, H. 1993. Potato production and pest management in North Dakota and Minnesota. North Dakota State University Extension Service Ext. Bul. 26.

Charkowski, A.O. 2007. The soft rot *Erwinia*. Plant-Associated Bacteria 423-505.

Charkowski, A.O., Lind, J., and Rubio-Salazar, I. 2014. Genomics of plant-associated bacteria: the soft rot *Enterobacteriaceae*. Genomics of Plant-Associated Bacteria 37-58.

Collmer A. and N.T. Keen. 1986. The role of pectic enzymes in plant pathogenesis. Annual Review of Phytopathology 24: 383-409.

Czajkowski, R., de Boer, W.J., van der Zouwen, P.S., Kastelein, P., Jafra, S., de Haan, E.G., van den Bovenkamp, G.W., and van der Wolf, J.M. 2013. Virulence of '*Dickeya solani'* and *Dickeya dianthicola* biovar-1 and -7 strains on potato (*Solanum tuberosum*). Plant Pathology 62(3): 597-610.

Czajkowski, R., de Boer, W., Velvis, H., and van der Wolf, J. 2010. Systemic colonization of potato plants by a soilborne, green fluorescent protein-tagged strain of *Dickeya* sp. biovar 3. Phytopathology 100(2): 134-42.

Czajkowski, R., Grabe, G., and van der Wolf, J.M. 2009. Distribution of *Dickeya* spp. and *Pectobacterium carotovorum* subsp. *carotovorum* in naturally infected seed potatoes. European Journal of Plant Pathology 125(2): 263-275.

Czajkowski, R., Perombelon, M.C.M., Jafra, S., Lojkowska, E., Potrykus, M., van der Wolf, J.M., and Sledz, W. 2014. Detection, identification, and differentiation of *Pectobacterium* and *Dickeya* species causing potato blackleg and tuber soft rot: a review. The Annals of Applied Biology 166(1): 18-38.

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

De Boer, S.H., and Rubio, I. 2004. Blackleg of potato. The Plant Health Instructor. DOI: 10.1094/PHI-I-2004-0712-01.

De Boer, S.H., Li, X., and Ward, L.J. 2012. *Pectobacterium* spp. associated with bacterial stem rot syndrome of potato in Canada. Phytopathology 102(10): 937-947.

de la Pasture, L. 2016. Blackleg in potatoes – rapid haulm destruction key to blackleg control. Crop Production Magazine. Retrieved from http://www.cpm-magazine.co.uk/2016/12/06/blackleg-potatoes-rapid-haulm-destruction-key-blackleg-control/

du Raan, S., Coutinho, T.A., and van der Waals, J.E. 2015. Cardinal temperature differences, determined in vitro, between closely related species and subspecies of pectinolytic bacteria responsible for blackleg and soft rot on potatoes. European Journal of Plant Pathology 144(2): 361-369.

Florida Automated Weather Network (FAWN). 2017. IFAS Extension, University of Florida. University of Florida- Gainesville, Florida, 32611.

Helias, V., Hamon, P., Huchet, E., Wolf, J. V. D., and Andrivon, D. 2012. Two new effective semi-selective crystal violet pectate media for isolation of *Pectobacterium* and *Dickeya*. Plant Pathology 61(2): 339-345.

Hooker, W.J. 1981. Compendium of Potato Diseases. American Phytopathological Society. 1-2.

Jiang, H., Hao, J., Johnson, S., Brueggeman, R., and Secor, G. 2016. First report of *Dickeya dianthicola* causing blackleg and bacterial soft rot on potato in Maine. The American Phytopathological Society 100 (11): 2320.

Ma, B., Hibbing, M., Kim, H., Reedy, R., Yedidia, I., Breuer, J., Breuer, J., Glasner, J., Perna, N., Kelman, A., and Charkowski, A. 2007. Host range and molecular phylogenies of the soft rot enterobacterial genera *Pectobacterium* and *Dickeya*. Phytopathology 97(9): 1150-1163.

Motyka, A., Zoledowska, S., Sledz, W., Lojkowska, E. 2017. Molecular methods as tools to control plant diseases caused by Dickeya and Pectobacterium spp: A minireview. New Biotechnology 39 (2017) 181-189.

Nassar, A. Darrase, A., Lamattre., Kotoujansky, A., Dervin, C., Vedel, R., and Bertheau, Y. 1996. Characterization of *Erwinia chrysanthemi* by pectinolytic isozyme polymorphism and restriction fragment length polymorphism analysis of PCR-amplified fragments of pel genes. Applied and Environmental Microbiology 62(7): 2228-2235.

North Dakota Agricultural Weather Network (NDAWN). 2017. North Dakota State University. Fargo, ND.

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

Pietraszko, M., Gryn, G., and Przewodowski, W. 2018. An effect of weather and soil conditions and their interaction on infection of leaves and tubers of potato with bacteria *Clavibacter michiganensis* subsp. *sependonicus*. American Journal of Potato Research 95(3): 278-285.

Potrykus, M., Sledz, W., Golanowska, M., Slawiak, M., Binek, A., Motyka, A., Zoledowska, S., Czajkowski, R., and Lojkowska, E. 2014. Simultaneous detection of major blackleg and soft rot bacterial pathogens in potato by multiplex polymerase chain reaction. Annals of Applied Biology 165(3): 474-487.

Raker, C., and Spooner, D. 2002. Chilean tetraploid cultivated potato, *Solanum tuberosum*, is distinct from the Andean populations: Microsatellite data. Crop Science 42(5): 1451-1458.

Raoul des Essarts, Y., Cigna, J., Quetu-Laurent, A., Caron, A., Munier, E., Beury-Cirou, A., Helias, V., and Faure, D. 2016. Biocontrol of the Potato Blackleg and Soft Rot Diseases Caused by *Dickeya dianthicola*. Applied and Environmental Microbiology 82(1): 266-278.

Samson R., Legendre, J.B., Christen, R., Fischer-Le Saux, M., Achouak, W. and Gardan, L. 2005. Transfer of *Pectobacterium chrysanthemi* (Berholder et al. 1953) Brenner et al. 1973 and *Brenneria paradisiaca* comb. nov. and delineation of four novel species, *Dickeya dadantii sp. nov*., *Dickeya dianthicola* sp. nov., *Dickeya dieffenbachiae* sp. *nov*. and *Dickeya zeae* sp. *nov*. International Journal of Systemic and Evolutionary Microbiology 55: 1415-1427.

Stark, J.C, and Love, S.L. 2003. Potato Production Systems. University of Idaho Agricultural Communications, Moscow Idaho. 1-7, 287-289.

Suarez, M.B., Feria, F.J., Martin-Robles, M.J., del Rey, F.J., Palomo, J.L. 2017. *Pectobacterium parmentieri* causing soft rot on potato tubers in southern Europe. Plant Disease 101(6): 1029-1029.

Toth, I.K., van der Wolf, J.M., Saddler, G., Lojkowska, E., Helias, V., Pirhonen, M., Tsror, L., and Elphinstone, J.G. 2011. *Dickeya* species: An emerging problem for potato production in Europe. Plant Pathology 60(3): 385-399.

Tsror, L., Erlich, O., Hazanovsky, M., Ben Daniel, B., Zig, U., and Lebiush, S. 2012. Detection of *Dickeya* spp. latent infection in potato seed tubers using PCR or ELISA and correlation with disease incidence in commercial field crops under hot-climate conditions. Plant Pathology 61(1): 161-168.

Turner, R. 2005. After the famine: Plant pathology, *Phytophthora infestans*, and the late blight of potatoes, 1845-1960. Historical studies in the physical and biological sciences 35: 341-370.

USDA-NRCS Soil Survey Division (USDA-NRCS). 2017. Official Soil Series Descriptions View by Name. Accessed January 18, 2018. http://soilseries.sc.egov.usda.gov.

van der Wolf, J.M., de Haan, E.G., Kastelein, P., Krijger, M., de Haas, B.H., Velvis, H., Mendes, O., Kooman-Gersmann, M., and van der Zouwen, P.S. 2017. Virulence of *Pectobacterium carotovorum* subsp. *Brasiliense* on potato compared with that of other *Pectobacterium* and *Dickeya* species under climactic conditions prevailing in the Netherlands. Plant Pathology 66(4): 571-583.

Wang, J., Fernandez-Pavia, S., Larsen, M., Garay-Serrano, E., Gregorio-Cipriano, R., Rodriguez-Alvarado, G., Grunwald, N., and Goss, E. 2016. High levels of diversity and population structure in the potato late blight pathogen at the Mexico centre of origin. Molecular Ecology 26(4): 1091-1107.

Wang, B., Ma, Y., Zhang, Z., Wu, Z., Wu, Y., Wang, Q., and Li, M. 2011. Potato viruses in China. Crop Protection 30(9): 1117-1123.

Yanetri Asi Nion, Y., and Toyota, K. 2015. Recent trends in control methods for bacterial wilt diseases caused by *Ralstonia solanacearum*. Microbes and Environments 30(1): 1-11.

Yoshida, K.J., Schuenemann, V.M., Cano, L.N., Pais, M.A., Mishra, B., Sharma, R., Lanz, C., Martin, F.N., Kamoun, S., Krause, J., Thines, M., Weigel, D., and Burbano, H.A. 2013. The rise and fall of the *Phytophthora infestans* lineage that triggered the Irish potato famine. ELife 2013(2).

Zaheer, K. and Akhtar, M. 2016. Potato production, usage, and nutrition- a review. Critical Reviews in Food Science and Nutrition 56(5): 711-721.

Zotarelli, L., Dittmar, P., Roberts, P., Noling, J., and Wells, B. 2017. Vegetable production handbook for Florida. IFAS Extension 14: 239-259.

APPENDIX A. ANOVA TABLE FOR PERCENTAGE STAND OF DICKEYA DADANTII INOCULATED SEED RESULTS CHART AT LIVE OAK, FL, AND OAKES, ND IN 2017 (TABLE 3)

Live Oak, FL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	10099.20	1442.74	37.31	<.0001
Error	12	464.00	38.67		
Corrected Total	19	10563.20			

R-Square	Coeff Var	Root MSE	STAND Mean
0.96	8.31	6.22	74.80

Source	DF	Anova SS	Mean Square	F Value	Pr > F
REP	3	80.00	26.67	0.69	0.58
Inocmethod	4	10019.20	2504.80	64.78	<.0001

Oakes, ND

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	151.20	21.60	0.75	0.64
Error	12	344.00	28.67		
Corrected Total	19	495.20			

R-Square	Coeff Var	Root MSE	STAND Mean
0.31	5.96	5.35	89.80

Source	DF	Anova SS	Mean Square	F Value	Pr > F
REP	3	124.00	41.33	1.44	0.28
Inocmethod	4	27.20	6.80	0.24	0.92

APPENDIX B. ANOVA TABLE FOR INOCULATION METHODS PLANT HEIGHT DIFFERENCES AT 45, 51, 60, AND 65 DAP, AT OAKES, ND IN 2017 (TABLE 4)

45 DAP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	9111.70	1301.67	30.03	<.0001
Error	52	2254.23	43.35		
Corrected Total	59	11365.93			

R-Square	Coeff Var	Root MSE	t45 Mean
0.80	14.09	6.58	46.97

Source	DF	Type III SS	Mean Square	F Value	Pr > F
rep	3	870.60	290.20	6.69	0.0007
trt	4	8241.10	2060.28	47.53	<.0001

51 DAP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	5888.83	841.26	26.98	<.0001
Error	52	1621.50	31.18		
Corrected Total	59	7510.33			

R-Square	Coeff Var	Root MSE	t51 Mean
0.78	9.03	5.58	61.83

Source	DF	Type III SS	Mean Square	F Value	Pr > F
rep	3	271.00	90.33	2.90	0.0438
trt	4	5617.83	1404.46	45.04	<.0001

60 DAP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	1477.48	211.07	5.51	<.0001
Error	52	1991.37	38.30		
Corrected Total	59	3468.85			

R-Square	Coeff Var	Root MSE	t60 Mean
0.43	9.04	6.19	68.45

Source	DF	Type I SS	Mean Square	F Value	Pr > F
rep	3	39.38	13.13	0.34	0.7945
trt	4	1438.10	359.53	9.39	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
rep	3	39.38	13.13	0.34	0.7945
trt	4	1438.10	359.53	9.39	<.0001

65 DAP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	532.70	76.10	1.74	0.1190
Error	52	2268.23	43.62		
Corrected Total	59	2800.93			

R-Square	Coeff Var	Root MSE	t65 Mean
0.19	10.18	6.60	64.87

Source	DF	Type III SS	Mean Square	F Value	Pr > F
rep	3	448.93	149.64	3.43	0.0236
trt	4	83.77	20.94	0.48	0.7502

APPENDIX C. ANOVA TABLE OF SEED TREATMENT PERCENTAGE STAND COMPARISONS AT OAKES, ND IN 2017 (TABLE 6)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	3472.00	347.20	5.78	0.0004
Error	21	1262.00	60.10		
Corrected Total	31	4734.00			

R-Square	Coeff Var	Root MSE	Standpercentage Mean
0.73	9.43	7.75	82.25

Source	DF	Anova SS	Mean Square	F Value	Pr > F
REP	3	314.00	104.67	1.74	0.1892
SeedTrt	7	3158.00	451.14	7.51	0.0001

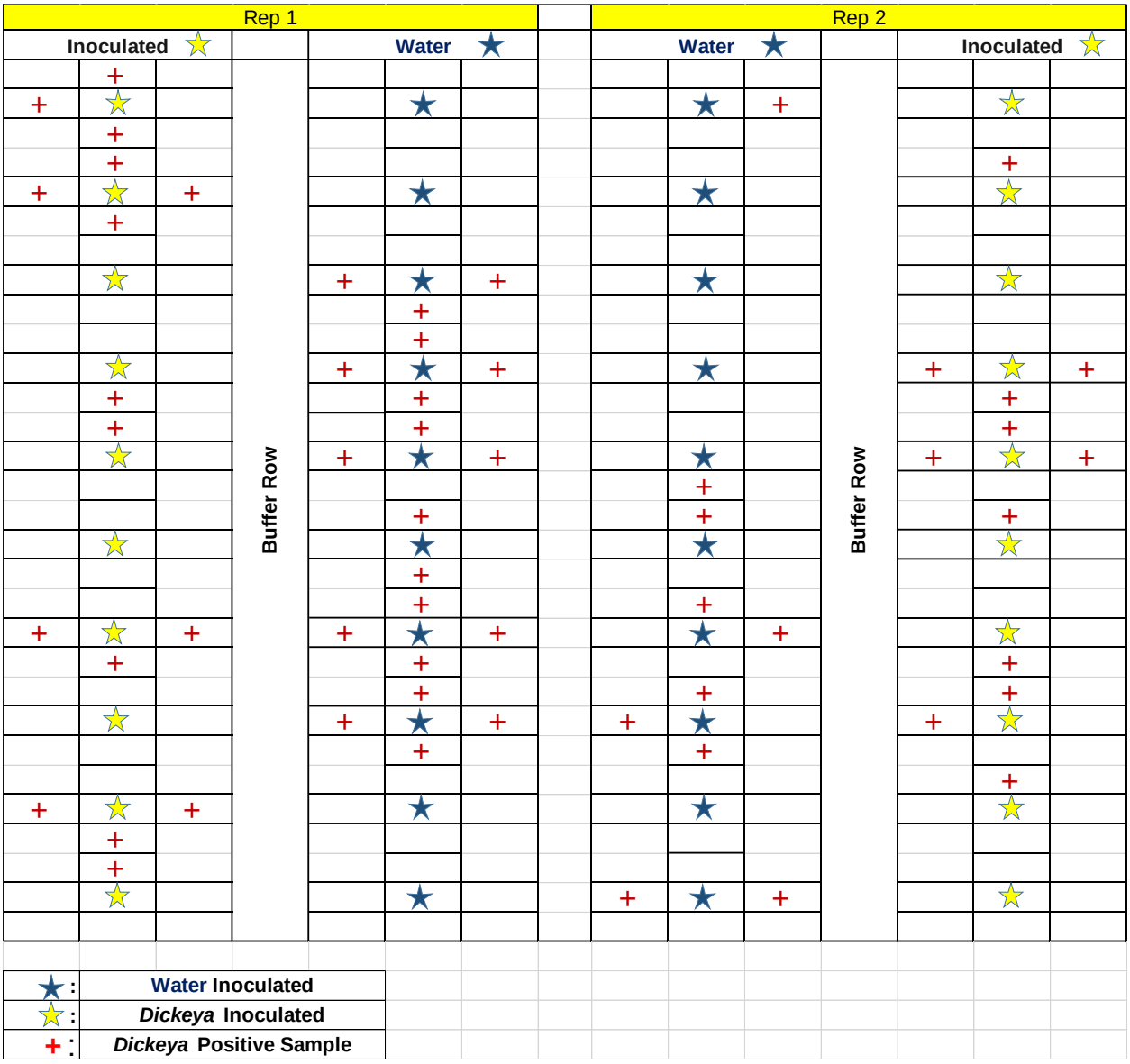
APPENDIX D. ANOVA TABLE OF ORTHOGONAL COMPARISONS FOR SEED TREATMENT TRIAL, BETWEEN CUT AND WHOLE SEED, AT OAKES, ND IN 2017

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	3472.00	347.20	5.78	0.0004
Error	21	1262.00	60.095		
Corrected Total	31	4734.00			

R-Square	Coeff Var	Root MSE	stand Mean
0.733	9.43	7.75	82.25

Source	DF	Type III SS	Mean Square	F Value	Pr > F
rep	3	314.00	104.67	1.74	0.1892
trt	7	3158.00	451.14	7.51	0.0001

APPENDIX E. DISTRIBUTION OF *DICKEYA* POSITIVE SAMPLES IN THE LIVE OAK, FL, SPREAD TRIAL IN 2017



Rep 3								Rep 4							
Water ★				Inoculated ☆				Inoculated ☆				Water ★			
	+								+						
	★			+	☆				☆				★		
					+								+		
	★			+	☆	+			☆				★		
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	★			+	☆	+			☆				★		
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	★	+			☆				☆	+			★		
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	★			+	☆	+			☆				★		
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	★			+	☆	+		+	☆	+			★		
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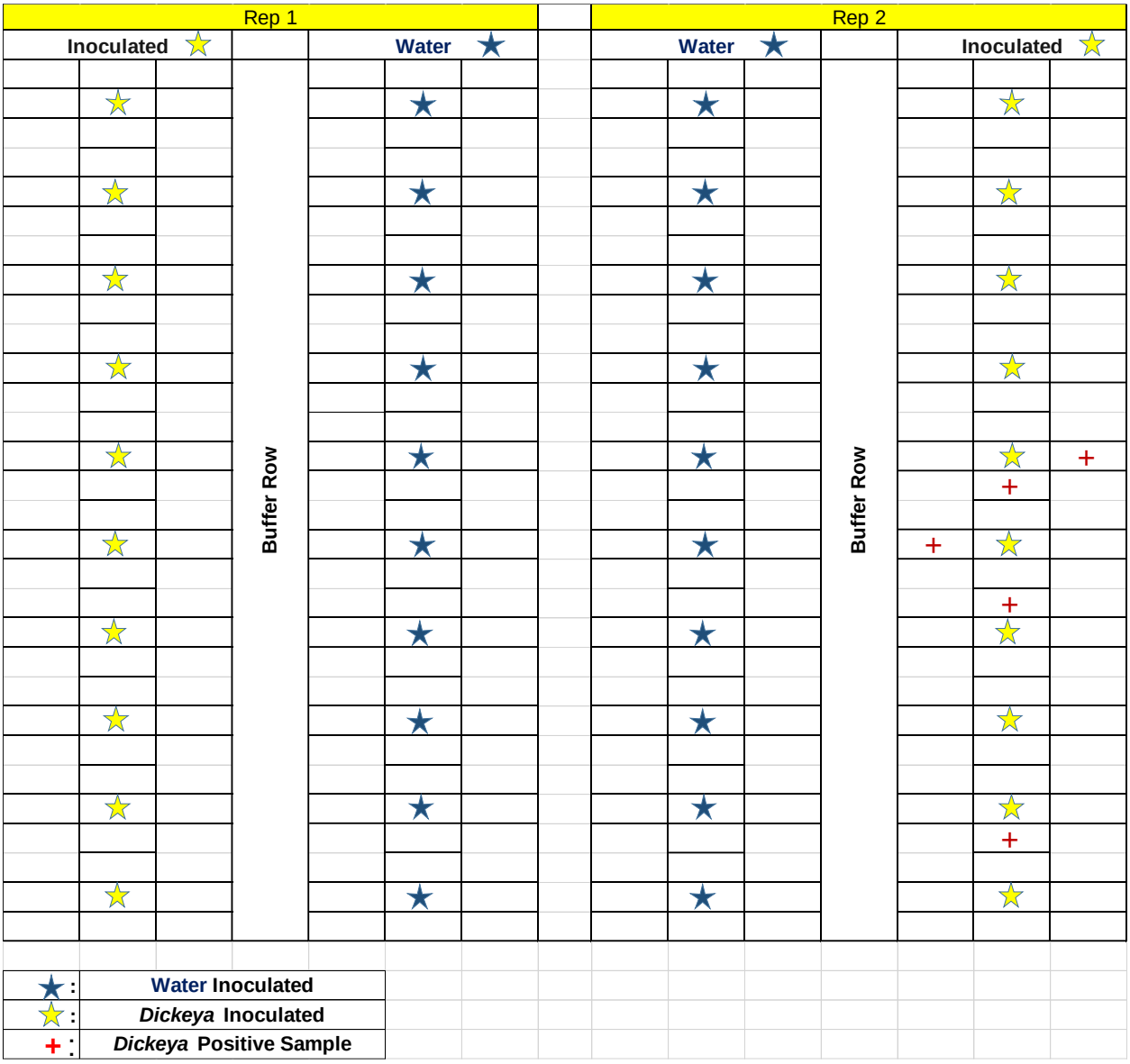
					+				+						

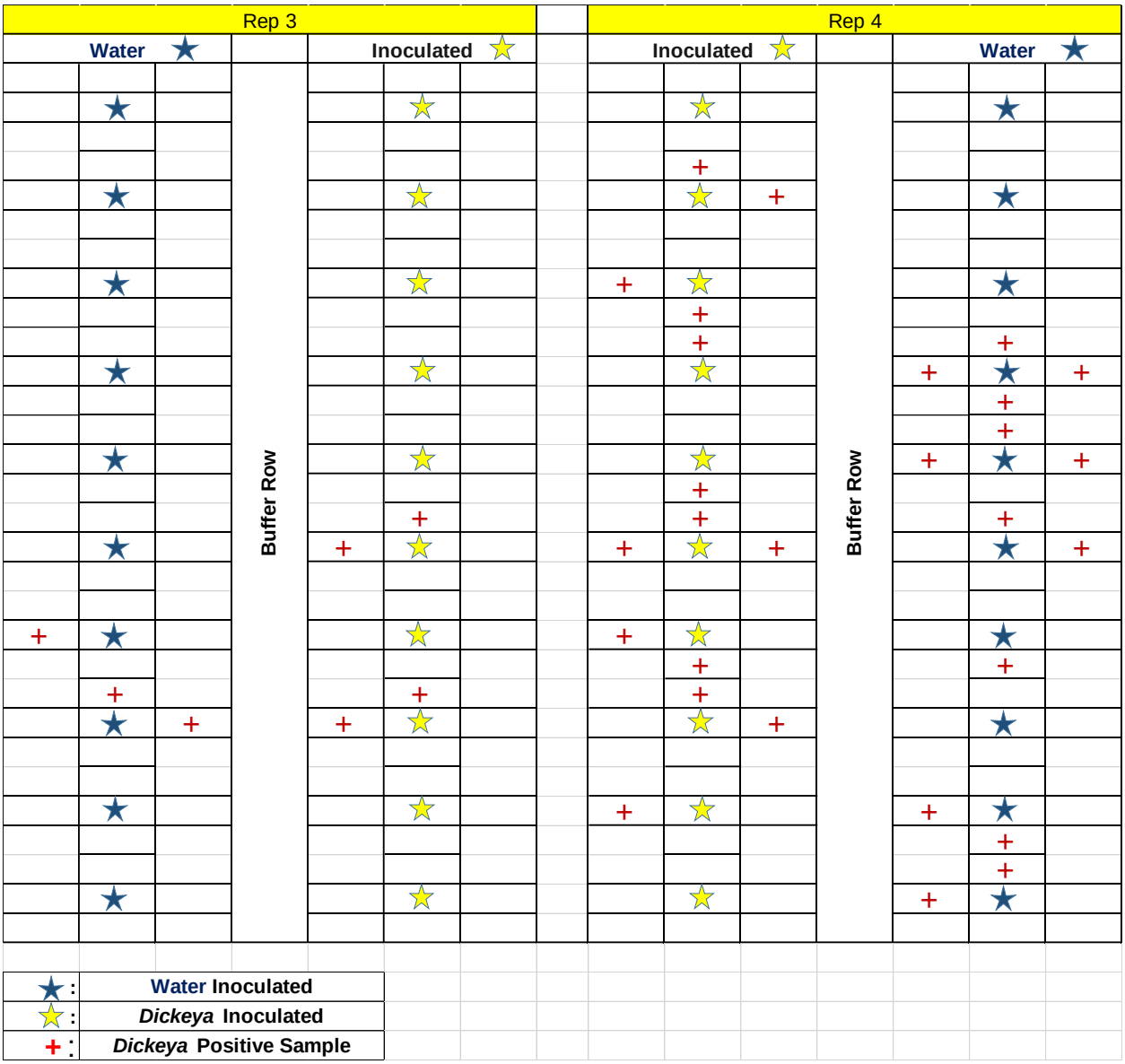
Buffer Row

Buffer Row

★ :	**Water Inoculated**
☆ :	***Dickeya* Inoculated**
+ :	***Dickeya* Positive Sample**

APPENDIX F. DISTRIBUTION OF *DICKEYA* POSITIVE SAMPLES IN THE OAKES, ND, SPREAD TRIAL IN 2017




Rep 3
Water
Inoculated
Buffer Row
Rep 4
Inoculated
Water
Buffer Row
: Water Inoculated
: Dickeya Inoculated
+ : Dickeya Positive Sample

APPENDIX G. ANOVA TABLE FOR DISTRIBUTION (%) OF *DICKEYA DADANTII* TESTING POSITIVE BETWEEN TREATMENTS AT LIVE OAK, FL, IN 2017 (FIG. 4)

Live Oak, FL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	23718.75	1824.52	1.95	0.0399
Error	66	61781.25	936.08		
Corrected Total	79	85500.00			

R-Square	Coeff Var	Root MSE	rot Mean
0.27	94.14	30.60	32.50

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Trt	1	5281.25	5281.25	5.64	0.0204
rep	3	4187.50	1395.83	1.49	0.2251
sample	9	14250.00	1583.33	1.69	0.1087

APPENDIX H. ANOVA TABLE FOR INCIDENCE (%) OF TUBER SAMPLE GROUPS TESTING POSITIVE FOR *DICKEYA* AT LIVE OAK, FL, AND OAKES, ND, IN 2017 (FIG. 6 AND 7)

Live Oak, FL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	99453.13	7650.24	5.59	<.0001
Error	626	856656.25	1368.46		
Corrected Total	639	956109.38			

R-Square	Coeff Var	Root MSE	infection Mean
0.10	202.35	36.99	18.28

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Trt	1	19140.63	19140.63	13.99	0.0002
rep	3	30921.88	10307.29	7.53	<.0001
sample	9	49390.63	5487.84	4.01	<.0001

Oakes, ND

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	38750.00	2980.77	5.86	<.0001
Error	626	318687.50	509.09		
Corrected Total	639	357437.50			

R-Square	Coeff Var	Root MSE	infection Mean
0.108411	380.0071	22.56292	5.937500

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Trt	1	18062.50	18062.50	35.48	<.0001
rep	3	16062.50	5354.17	10.52	<.0001
sample	9	4625.00	513.89	1.01	0.4308

APPENDIX I. ANOVA TABLE FOR STAND COMPARISONS AMONG TREATMENTS FROM LIVE OAK, FL, IN 2018 (TABLE 8)

Live Oak, FL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	28	4528.50	161.73	1.13	0.3100
Error	171	24487.00	143.20		
Corrected Total	199	29015.50			

R-Square	Coeff Var	Root MSE	infection Mean
0.156	12.43	11.97	96.25

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Trt	1	1250.00	1250.00	8.73	0.0036
rep	3	862.50	287.50	2.01	0.1147
sample	24	2416.00	100.67	0.70	0.8442

APPENDIX J. ANOVA TABLE FOR PLANT HEIGHT COMPARISONS OF SAMPLES FROM LIVE OAK, FL, IN 2018 (TABLE 9)

Live Oak, FL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	28	4624.03	165.14	1.92	0.0030
Error	722	61943.96	85.80		
Corrected Total	750	66567.99			

R-Square	Coeff Var	Root MSE	rot Mean
0.069	37.05	9.26	24.99

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Trt	1	852.73	852.73	9.94	0.0017
rep	3	415.25	138.42	1.61	0.1849
sample	24	3356.04	139.83	1.63	0.0296