THE EFFECT OF VARIABLE SEED-BORNE INOCULUM LOAD OF DICKEYA DIANTHICOLA ON PERFORMANCE AND INFECTION OF FIELD GROWN 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 at Live Oak, Florida, and Rhodesdale, Maryland, to evaluate the effect of variable seed-borne inoculum load of *Dickeya dianthicola* on potato plant emergence, plant growth throughout the growing season, disease prevalence in the field throughout the growing season, yield and grade, and transmission of *D. dianthicola* to progeny tubers. No statistically significant differences in emergence, plant height, or disease incidence were observed at either location. Statistically significant differences were observed in yield at the Florida location; all inoculated treatments had significantly lower yield than the non-inoculated control. No significant differences in yield were observed among treatments at the Maryland location. No significant differences in grade were observed at the Florida location. Significant differences in one tuber profile category were observed at the Maryland location. No significant differences in transmission of *D. dianthicola* to progeny tubers were observed at the Florida and Maryland locations.

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INTRODUCTION

Pathogens in the genus *Dickeya* are becoming increasingly prevalent in the potato blackleg disease complex in the US. Prior to 2005, species that are now considered to be in the genus *Dickeya* were classified in the genus *Pectobacterium* along with other bacteria that would become the genus *Brenneria*. The genus *Dickeya* is now comprised of nine distinct species: *D. aquaticus, D. chrysanthemi, D. dadantii, D. dianthicola, D. dieffenbachiae, D. paradisiaca, D. solani, D. fangzhondai* and *D. zeae. Dickeya* species have a wide range of host plants including banana, maize, tomato, chrysanthemum, and of particular importance, the potato (Toth et al. 2011). When compared to *Pectobacterium*, *Dickeya* species grow at warmer temperatures and are more aggressive than *Pectobacterium*. *Dickeya* survival in soil is limited, with survival ranging from one week to six months depending on the environmental conditions (Czajkowski et al 2011). Despite limited survivability in the soil, *Dickeya* spp. have been isolated from surface water in lakes and rivers (Potrykus et al. 2016).

Dickeya was first documented in potato in 1972 and continued to be a cause of soft rot throughout Europe since its discovery (Maas Geesteranus, 1972). Bacterial soft rot is a symptom of bacterial infection of plants that is characterized by the enzymatic maceration and breakdown of plant tissues. Blackleg caused by *Dickeya* was not a serious cause of stand and yield loss in the United States until the summer of 2015 when an outbreak of blackleg on potato caused by *D. dianthicola* occurred in Maine (Jiang et al. 2016). *Dickeya*-caused blackleg from infected seed potatoes continued to increase in subsequent years, with substantial losses reported when conditions favorable to disease development occurred, especially in the eastern United States (Jiang et al. 2016). *Dickeya dianthicola* is a serious pathogen of potato, and with global temperatures predicted to continue rising, it is likely to become more serious as climates become

conducive to its development. *Dickeya dianthicola* is a rod shaped, non-spore forming, facultative anaerobe that has peritrichous flagella in the family *Pectobacteriaceae.* It has an optimal growth temperature of greater than 25C and has been found throughout potato producing regions of the world including North America, Europe, Russia, and the Middle East. Much remains unknown about the disease including pathogen infection of the host, and expression of disease in the field. The objective of this project was to determine if variable seed-borne inoculum loads of *D. dianthicola* effect emergence of plants, plant growth, disease incidence, yield and grade, and transmission of the pathogen to progeny tubers.

OBJECTIVES

Hypothesis: Seed borne inoculum concentration of *Dickeya dianthicola* impacts potato plant development and tuber quality with low concentrations being asymptomatic visually in the plant, but affect tuber yield and grade.

Objectives:

1. Determine the effects of initial seed borne inoculum concentration of *D. dianthicola* on stand and blackleg incidence during the growing season.
2. Determine the effects of initial *D. dianthicola* seed borne inoculum concentration on plant height.
3. Determine the effects of initial *D. dianthicola* seed borne inoculum concentration on yield and grade of harvested tubers.
4. Determine the effects of initial *D. dianthicola* seed borne inoculum concentration on transmission of *D. dianthicola* to progeny tubers.

LITERATURE REVIEW

The potato

The potato (*Solanum tuberosum L.)* is an annual herbaceous dicot in the Solanaceae family, which also includes important crops such as peppers, tomatoes, eggplant, and several nightshade weeds. Potato plants possess branched stems that emerge from eyes in the seed piece that when planted that may reach over a meter in length, depending on the cultivar. Leaves of the potato plant are compound; they are comprised of multiple leaflets attached to a single petiole. Petioles are arranged alternately along the length of the stem. The leaflets borne on the petioles are generally oval shaped with a pointed tip. Flowers of the potato plant have five petals with variable color. The reproductive structures (anthers and stigma) are generally yellow. The fruit of the potato plant is a firm, green berry approximately two to three centimeters in diameter, filled with true potato seed. While the fruit are of little use in commercial production, they are used in breeding programs to facilitate the development of new cultivars. The tubers of the potato plant are modified stem tissue that serve as nutrient storage for the plant, particularly carbohydrates as starch. Tuber characteristics vary widely depending on the cultivar.

The potato has been in cultivation for over 7000 years, first being domesticated somewhere between 8000 and 5000 BC in Peru and northwestern Bolivia, which are considered to be the center of origin (Spooner et al. 2005). The potato is a staple food in the diets of many people around the world. Potatoes are the fourth most important crop grown worldwide following only rice, maize, and wheat, respectively (FAOSTAT 2019). The introduction of the potato to other areas of the world began when the Spanish introduced the potato to Europe sometime in the late 16th century while returning from the Andes where it had long been in cultivation by the native population (Ames and Spooner 2008). The potato was subsequently

introduced to other European, Asian, and African countries in the following years. The first documented instance of the potato being cultivated in the United States occurred in the year 1719 in Nutfield, New Hampshire, when a group of Scotch-Irish families immigrated to the area and planted the first documented crop of potatoes in the new world (Parker 1974).

Today, potatoes are grown throughout the world with over 388,191,000 tons produced in 2017 (FAOSTAT 2019). The top five potato producing countries in the world today are China at 99.2 million tons, India at 48.6 million tons, the Russian Federation at 29.6 million tons, the Ukraine at 22.2 million tons, and the United States at 22 million tons (FAOSTAT 2019) (USDA 2019). In the United States during the 2018 growing season, 5.3 million tons of potatoes were produced for table stock, 14.5 million tons for processing, 1.2 million tons for seed, and the remaining 1 million tons for feed and other uses (USDA 2019). During the 2018 growing season approximately one million acres of potatoes were planted, with the top five potato producing states being Idaho at 7 million tons, Washington at 5 million tons, Wisconsin 1.4 million tons, Oregon at 1.3 million tons, and North Dakota at 1.2 million tons; which accounted for 71 percent of potato production in the US (USDA 2019).

The potato is a nutritionally valuable crop with a wide variety of processing options in addition to the fresh market. Potatoes are an excellent source of vitamin C, potassium, iron and a litany of micronutrients, with approximately 147 calories in a single medium sized potato (USDA 2019). The high diversity of nutrients and relative ease of potato cultivation has been a driving force for its production in many countries throughout the world, especially those with limited access to similarly nutritious food.

Potatoes are exposed to a nearly constant barrage of pests and pathogens that if left unchecked can substantially reduce yield and quality of harvested tubers. On an annual basis

approximately 65 million tons, or 22% percent of potatoes are lost as a result of pathogens and pests (Czajkowski et al. 2011). Multiple pathogens impact potatoes such as bacteria, fungi, nematodes, viruses, and plasmodiophoromycetes. For example, potatoes are plagued in the field by *Phytophthora infestans*, a devastating oomycete that is capable of causing complete crop failure, as seen during the Irish potato famine from 1845-1849 (Donnelly 2013). *Ralstonia solanacearum*, a causal agent of bacterial wilt in potato can reduce yield from 33 to 90 percent (Yuliar et al. 2015). Insect pests can also pose a serious threat to potato production. The most serious of these insect pests on potato is the Colorado potato beetle, which is capable of defoliating entire commercial production fields if controls are not implemented (Alyokhin et al 2014).

The pathogen

One of the most common bacterial diseases of potato is soft rot that causes seed decay, stem soft rot (blackleg), and storage decay. This disease affects all plant parts, and can be present at any growth stage of the potato (Gary Secor personal communication 2019). Soft rot bacteria have a wide host range and are a frequent cause of soft rot decay of all fleshy fruits and vegetables. Soft rot bacteria accomplish this through use of plant cell wall degrading enzymes. The primary enzymes are pectinases, but other enzymes are present including cellulases, xylanases, and proteases that result in plant cell necrosis, leaky membranes, and tissue maceration which frequently results in the death of the host (Abbott and Boraston 2008). The pectinases function by degrading the middle lamella between the plant cells, resulting in the aforementioned symptoms, since the primary function of pectin is to cross-link cellulose and hemicellulose fibers in order to provide rigidity to the cell wall (Abbott and Boraston 2008). *Dickeya* species are able to multiply rapidly when conditions are favorable in order to move from

the mother seed tuber through the soil through use of peritrichous flagella, or through the vascular system of the potato plant which can result in symptoms such as blackleg, wilting, and premature plant death (Czajkowski et al. 2010).

Pectolytic bacteria such as *Pectobacterium*, *Pseudomonas*, and *Dickeya* can be detrimental not only to potatoes in the field, but can also cause soft rot decay of stored potatoes and can result in serious losses. Since bacteria require a wound for entry to the host, infection by bacteria causing soft rot decay in storage often follow infection by another disease such as late blight, *Fusarium* dry rot, or pink rot, providing a means of entry by damaging the plant cell wall. In instances of soft rot caused by *Pectobacterium,* soft rot will continue to develop in storage conditions. Soft rot caused by *Dickeya* spp. is not common in storage conditions because temperatures are too cool for the bacteria to be active (Gary Secor personal communication 2019).

Prior to its final delineation into the current genus, *Dickeya* underwent several reclassifications, leading to confusion even today when discussing bacteria causing blackleg of potato. In order to understand the taxonomic place of *Dickeya* in the phylogeny of bacteria, a brief history of the conception of the genus must be discussed. The genus *Erwinia* was formed in 1920, and it encompassed all plant pathogenic bacteria that were known at the time (Winslow et al 1920). This genus was not well received by members of the scientific community due to a lack of diagnostic characteristics for the bacteria that belonged to it. For instance, all plant pathogenic bacteria were included in this genus at its formation, regardless of morphological differences. This lack of appropriate taxonomic characteristics lead to an extremely variable genus. In 1942 the first effort was made to separate the genus *Erwinia* into four different genera under the proposed family Erwineaceae by Waldee (Waldee 1942). This was largely ignored by the

scientific community despite the fact that Waldee's findings were later corroborated by Brenner in 1973 whose work was also largely ignored (Brenner et al 1973). *Dickeya* was first characterized when the pathogen was determined to be causing hollow stalk of *Chrysanthemum morifolium* in 1953 by Burkholder (Burkholder et al. 1953). Over the next several years other similar bacteria were discovered from various diseased plants. This group of similar bacteria were grouped together into a single "species" within the genus *Erwinia* called *E. chrysanthemi.*

Subsequent to its 1953 classification, *E. chrysanthemi* was separated into five pathovars in 1984; pv. *chrysanthemi,* pv. *dieffenbachiae,* pv. *parthenii,* pv. *zeae,* and pv. *paradisiaca* (Lelliott and Dickey 1984). Both Brenner's and Waldee's proposals to separate the genus *Erwinia* continued to be ignored until 1998. In 1998, Hauben proposed the genera *Pectobacterium* and *Brenneria*, which encompassed the species belonging to the *Erwinia chrysanthemi* complex (Hauben et al. 1998). The pathovar *paradisiaca* was transferred to the genus *Brenneria,* while the remaining pathovars were transferred to the genus *Pectobacterium* (Hauben et al. 1998). In 2005, Sampson and colleagues proposed the novel genus *Dickeya* for the remaining species, named after the late Robert Dickey whose work on the *Erwinia chrysanthemi* complex was instrumental to its characterization and classification (Dickey et al. 1984) (Dickey 1978) (Dickey 1980). Based on a series of assays assessing DNA-DNA hybridization, serology, phenotypic characteristics, and 16s rRNA; bacteria which now belong to the genus *Dickeya* were shown to have higher degrees of relatedness to one another than to the bacteria they were classified with at the time (Sampson et al. 2005).

In 2016, the entire Enterobacteriaceae family was restructured to form seven distinct families; Enterobacteriaceae, Erwiniaceae, Pectobacteriaceae, Yersiniaceae, Hafniaceae, Morganellaceae, and Budviciaceae (Aduolu et al 2016). This restructuring relied upon a more

thorough analysis of proteins and comparisons of entire genomes, while previous works had primarily relied on 16s rRNA comparisons. The genus *Dickeya* along with *Pectobacterium* were transferred to the family Pectobacteriaceae.

Today there are nine recognized species of *Dickeya: D. aquatica, D. chrysanthemi, D. dadantii, D. dianthicola, D. dieffenbachiae, D. paradisiaca, D. solani, D. fangzhondai* and *D. zeae* (Sampson et al. 2005) (Tian et al 2016) (Parkinson et al 2014) (Van der Wolf et al 2014). Although *D. solani* is the most aggressive and devastating to potato of the bacteria in the genus, and has caused considerable losses of up to 30% in Europe and Israel (DAERA 2018). *D. solani* is of less concern in the United States as *D. solani* has not been found in North America. The most concerning of the *Dickeya* species in the United States is *D. dianthicola* because it is the prevalent species.

Disease cycle

Symptoms caused by *Dickeya* are very similar phenotypically to those caused by *Pectobacteria*. However, there are some distinctions that can be used to separate the causal agent responsible. *Dickeya* infections can be differentiated from infections caused by *Pectobacterium* by smell. Infections caused by *Dickeya* have a mild, earthy odor or none at all, while infections caused by *Pectobacterium* will have a putrid odor (Gary Secor; personal communication 2019). Visual inspection can also be used to differentiate the two. Stems exhibiting blackleg caused by *Dickeya* will generally be hollow or in the process of hollowing with a macerated pith, while blackleg infections caused by *Pectobacteria* instead rot from the outside in and have a comparatively wet and slimy cortex.

The disease cycle for *Dickeya* is typical for a bacterium. It lacks the ability to directly penetrate host tissues, as many fungal pathogens do that are equipped with an appressorium.

Therefore the bacterium relies on wounds caused by hail, insect feeding, and wind damage or natural openings such as lenticels, stomata, and hydathodes in order to gain access to its host. The bacteria can be transferred to new plants through rain splash, or through sufficiently saturated soil that allow the bacteria to swim to a new host plant. Interestingly, *Dickeya* species have been shown to be vectored and spread by aphids which can act as an alternate host themselves (Grenier et al. 2006). In the study, *D. dadantii* in particular was shown to be highly pathogenic to aphids.

Dickeya has limited survivability in the soil, and survives in crop debris only one week to six months, depending on environmental conditions such as temperature and available free water (Czajkowski et al. 2011). Some species of *Dickeya* such as *D. aquaticus* have been shown to persist in bodies of water, which demonstrates that surface water could potentially be serving as a source of inoculum for other *Dickeya* species.

Blackleg caused by *Dickeya* is frequently a seed-borne disease. Plants and seed tubers infected with *Dickeya* can exhibit seemingly latent infection with no observable disease symptoms. *Dickeya* species have been shown to be able to colonize the vascular system of a potato host plant without eliciting symptoms or effecting the transpiration rate of the plant (Ansermet et al. 2016). Infected plants can subsequently pass the bacteria on to the progeny tubers. Once the bacteria is inside the roots of the host plant it will multiple within the intracellular spaces, continuing to move through the vascular system of the host, or if sufficient free water is available, moving through the soil to the roots and tubers of nearby plants (Czjkowski et al. 2010).

Dickeya is known to produce N-acyl homoserine lactones (AHLs) that impacts virulence when the bacterial population reaches a sufficient density (Crepin et al. 2012). Research has

shown that the critical cell density *in planta* for expression of cell wall degrading enzymes is 10^7-10^8 cfu/ml (Pérombelon and Salmond 1995). In addition to concentration of AHLs, severity of disease in the field is known to be impacted primarily by two environmental factors: availability of free water, and temperatures above 25C (Gugino 2016). Other factors have been assessed for their contribution to the development of disease; however, more research is required as development seems to depend upon a large number of factors (Gill et al 2014).

Management

Management options for blackleg of potato are largely preventative. Due to the bacterial nature of the causal agent and the annual nature of the crop curative practices are not feasible to implement. Additionally, they are largely unavailable, making preventative measures the best strategy to manage *Dickeya.*

Cultural control practices serve as the best option for management of blackleg caused by *Dickeya.* A range of options exist, the most effective of which is planting seed that is free of *Dickeya*. This is accomplished through seed certification programs, which vary in stringency from region to region. Currently, there is no tolerance established for blackleg in US seed certification programs regardless if the cause is *Dickeya* or *Pectobacterium*. Some certification agencies in the US will print estimated blackleg incidence on certification documents, but it does not act as an official parameter for certification eligibility. Conversely, certification agencies in Scotland have a zero tolerance policy regarding *Dickeya* in seed lots according to SASA. In addition to seed certification, quarantine and disposal of infected seed lots can be an effective means of reducing disease incidence, but this requires laboratory testing. Proper sanitization of machinery and apparatuses used in the cultivation and production process is an option that applies not only to blackleg, but many other diseases as well (Agrios 1988). Despite the

controversy surrounding the seeming inability of *Dickeya* to spread during the seed cutting process (Secor, unpublished data), sanitization of equipment between seed lots is still a worthwhile precaution. Water management is another important aspect to consider when managing potato blackleg. *Dickeya* flourishes in wet and oversaturated ground. Failure to avoid favorable conditions can greatly exacerbate disease since the bacteria are capable of moving through the soil if sufficient water is present. Since blackleg caused by *Dickeya* is not capable of surviving in the soil for more than six months even with favorable conditions, crop rotation is unnecessary (though still a sound practice), unless the growing season is long enough to facilitate more than one cropping cycle in the same field (Czajkowski et al 2011).

Chemical control practices are limited, but some options do exist. One potential means of managing potato blackleg is through use of chemical disinfectants on seed prior to planting. In a study conducted by (Czajkowski et al. 2013), several chemical disinfectants were assessed for their ability to suppress blackleg development under favorable conditions and for their phytotoxicity to the host. Of the eight agents tested sodium hypochlorite and MennoClean, a benzoic acid based sanitizer, were shown to be effective at reducing incidence of disease and were non-phytotoxic to the host (Czajkowski et al. 2013).

Biological control of *Dickeya* species has been shown to be possible via antagonistic bacteria. A study published in 2016 elucidated 58 bacterial strains that are capable of inhibiting the growth of *Dickeya* species in lab trials, most of which belonged to the *Pseudomonas* and *Bacillus* genera (Essarts et al. 2016). The study revealed that when three of these biocontrol agents: *Pseudomonas putida* PA14H7, and *Pseudomonas fluorescens* PA3G8 and PA4C2, when used in conjunction with one another in a greenhouse study were able to not only reduce the severity of blackleg symptoms of potato plants, but also reduce the rate of transmission of

Dickeya to the progeny tubers (Essarts et al. 2016). A study conducted at the University of Cukurova in Turkey similarly demonstrated that antagonistic bacteria and fungi can be used to reduce disease severity of *Erwinia chrysanthemi* (*D. dadantii)*. The study assessed the ability of seventy-one candidate antagonists, of which eight were shown to reduce disease development between 33% and 89% (Aysan et al. 2003). In addition to control with antagonistic bacteria and fungi, a recent publication has indicated that bacteriophages show promise in the management of *Dickeya* (Kabanova et al 2019). In the study, Kabanova et al. demonstrated that the PP35 phage in the *Ackermannviridae* family had high specificity for *D. solani* and was able to significantly reduce bacterial populations. Antagonistic organisms represent a more environmentally friendly means of controlling *Dickeya* populations, compared to antibiotic and chemical control methods. Additionally, microbiome interactions with the pathogen could prove to be an important area of study in understanding *Dickeya* pathogenesis in the future.

It is not known if the concentration of *Dickeya dianthicola* in seed potatoes (bacterial load) can result in sub-lethal infection that reduces potato plant performance. Experiments were conducted at two field locations to determine the effects of initial seed borne inoculum concentration of *D. dianthicola* on stand and blackleg incidence during the growing season, its effect on plant height, its effects on yield and grade of harvested tubers, and its effects on transmission of *D. dianthicola* to progeny tubers.

MATERIALS AND METHODS

Experimental design

The experiment was arranged in an RCBD with four replications, six treatments with five bacterial concentrations and a water control. The bacterial concentrations were 10^9 cfu/ml, 10^7 cfu/ml, 10^6 cfu/ml, 10^5 cfu/ml, and 10^4 cfu/ml. Sterilized water was used for the water inoculated control treatment. At both locations Atlantic tubers were used for the experiment. Each plot consisted of two rows planted with 25 seed pieces per row. The treatments are separated on each side by two border rows having a total of four border rows between two individual treatments. Treatments were separated vertically from one another with five Red Norland (RN) tubers. The within row spacing was ten inches (25.4 cm), and the between row spacing was thirty four inches (86.36 cm). All planting was done by hand, but furrows were mechanically opened and closed. Spacing was accomplished using a set of studded metal wheels towed behind a tractor. The studs put depressions in the ground every ten inches in which the seed pieces were placed. The Florida location was planted February 14th and harvested May 20th 2019 and the Maryland location was planted April 4th and harvested July 19th 2019. Irrigation, fertilization, and chemical applications were typical of a commercial field at both locations. The trial design was the same at both locations (Figure 1).

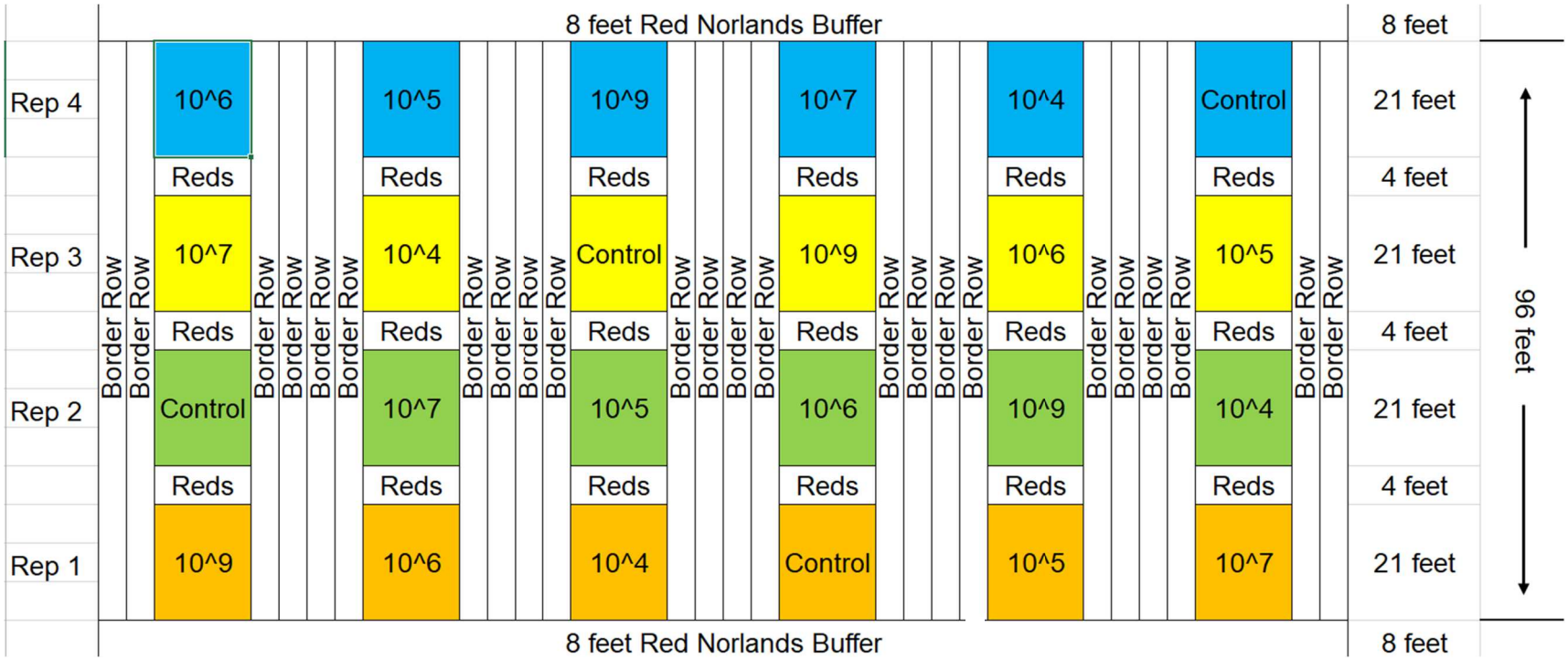

Figure 1: Field design at Live Oak, Florida and Rhodesdale, Maryland. Colored rectangles indicate a two row plot. Bacterial concentration (cfu/ml) is given for each plot.

Because of the motile nature of *D. dianthicola,* soil samples were collected before planting from both the Florida location and the Maryland location for mechanical analysis by the NDSU soil testing lab. The soil at the Florida location was a sand type and the soil and the Maryland location was a loamy sand (Table 1).

Table 1: Soil analysis for Live Oak, Florida and Rhodesdale, Maryland

Sample ID	Sampling depth (inches)	Mechanical Analysis			Soil Texture
		Percent Sand	Percent Silt	Percent Clay	
LiveOak 19	0-6	94.5	4.9	.6	Sand
Rhodesdale 19	0-6	77.6	19.3	3.1	Loamy Sand

Weather data was collected from the nearest airport weather stations at both locations because tillage and chemical application equipment prevented in-field weather stations. Weather data for the Florida location was collected at the Valdosta Georgia regional airport weather station located 59 miles from the trial site (Table 2). Weather data for the Maryland location was collected at the Salisbury-Ocean City Wicomico regional airport station located 29 miles from the trial site (Table 3).

Table 2: Weather data for Live Oak, Florida, Valdosta Georgia regional airport. Temperatures are given in degrees Celsius.

Live Oak FL 2019	Average High	Average Low	Overall Average	Planting to Harvest Days	Disease Favorable Days
Feb (14^{th}-)	23.5	11.4	17.0	96	55
March	21.4	5.0	14.8		
April	24.0	10.2	19.1		
May (-20^{th})	30.2	18.3	23.8		

Table 3: Weather data for Rhodesdale, Maryland, Salisbury-Ocean City Wicomico regional airport. Temperatures are given in degrees Celsius.

Rhodesdale MD 2019	Average High	Average Low	Overall Average	Planting to Harvest Days	Disease Favorable Days
April (4^{th} -)	21.7	10.7	15.9	106	62
May	27.3	11.6	20.1		
June	27.6	17.2	23.5		
July (-19^{th})	32.2	21.6	26.6		

Inoculum preparation

Dickeya dianthicola isolate ME23, the type strain for this species (Ma et al. 2019), was transferred to plastic Petri plates with nutrient agar prepared according to the manufacturer's directions (Sigma-Aldrich, St. Louis, Missouri) and incubated at 30C for two days. After incubation, bacteria were scraped from the agars surface and diluted with sterile water to concentrations 10^9, 10^7, 10^6, 10^5, and 10^4 cfu/ml. Ten liters of each concentration were prepared.

Tuber inoculation protocol

Atlantic tubers (USDA, Beltsville Maryland) sourced from Black Gold Farms, Forest River, North Dakota were used for this experiment. The seed lot the tubers were sourced from had been tested and found to be free of *Dickeya* species. Prior to inoculation the tubers were washed to remove soil and debris from the surface. The tubers were inoculated by vacuum infiltration separately with each bacterial concentration (10^9, 10^7, 10^6, 10^5, and 10^4 cfu/ml) and a water control. Infiltration was accomplished by submerging approximately eight tubers in one liter of the bacterial solution into a desiccator coupled to a Gast vacuum pump that generated 600mmHg of vacuum pressure for five minutes. Every fourth cycle of infiltration fresh inoculum was used. Two hundred and ten tubers per treatment were infiltrated to allow for an extra ten tubers per treatment. The desiccator was sterilized between treatments as to avoid contamination

between treatments. After inoculation, the tubers were allowed to air dry, placed into mesh bags, and stored at 10 degrees Celsius until planting.

Field data collection protocol

In both the Florida and Maryland locations, plant heights were measured three times and were visually assessed for blackleg two, four, and six weeks after 100% emergence of the control treatment. Stand was assessed at the time of the first measurement. The third, sixth, ninth, twelfth, and fifteenth emerged plant from each row in each treatment rep was flagged for subsequent height measurements of the same plants. Plant height was measured in centimeters from ground level to the longest point of the vine at the time of measurement not including the petiole. At the Florida location measurements were taken March 24th, April 8th, and April 20th of 2019. At the Maryland location measurements were taken May 17th, May 31st, and June 14th of 2019.

Harvest protocol

Prior to harvest, two tubers from each flagged plant were collected for subsequent *Dickeya* testing by PCR. From each tuber a single core was extracted using a #5 coring tool inserted approximately one centimeter into the stem end of the tuber, and the peel samples measuring approximately two centimeters in length were collected from the stem end using a conventional potato peeler. After sampling, the tubers were placed back into their original hill and covered with soil to facilitate accurate yield and grade measurements. These samples were then transported back to the lab for testing via PCR for the presence of *Dickeya*. After sampling, a single row digger was used to lay the tubers out on top of each plot and the tubers were collected by hand and placed into labeled burlap bags for yield and grade measurement.

Yield and grade protocol

Yield was determined for each treatment by weighing the tubers using a Rubbermaid 4010 commercial scale. Grading was accomplished using a grading table fitted with five separate sizing plates. The tubers were split into five categories based on the smallest sizing plate that they could pass through freely. The potatoes were sized into the following groups: tubers < 3.81 centimeters in diameter, >3.81- 4.76 centimeters, >4.76-6.99 centimeters, >6.99-8.89 centimeters, and >8.89 centimeters. These sizing categories were selected because they represent the Frito-Lay standard sizing profiles. Upon completion of yield and grade, the tubers were disposed of.

DNA extraction protocol

Cores and peels that were collected from the field tubers were placed into maceration bags and macerated with a ceramic pestle by hand. Four milliliters of Ringer's solution was then added to each maceration bag and incubated at 30 degrees Celsius for a period of 24 hrs. After the incubation period, DNA was extracted using the Power Plant™ Pro DNA Isolation Kit (Qiagen) by the following procedure: 2 ml from each sample was collected in a 2ml Eppendorf tube and centrifuged for 10 minutes at 14,000 rpm and the supernatant was discarded. At this point 500 µl of bead solution, 40 µl of phenolic separation solution, 40 µl of solution SL and 3 µl of RNase A solution were added to the Eppendorf tube along with the pellet and vortexed. Samples were then placed in a water bath to homogenize for a period of 30 minutes and then centrifuged at 13,000 rpm for two minutes. After centrifugation 5 µl of the supernatant was transferred into a clean 2 ml collection tube along with 200 µl of Solution IR. The sample was then vortexed, incubated at 4 degrees Celsius for a period of five minutes, and then centrifuged at 13,000 rpm for two minutes. At this point 600 µl of supernatant was transferred to a new 2ml

collection tube along with 600 µl of solution PB and 600 µl of ethanol. At this point 600 µl of lysate was loaded into the MD spin column and centrifuged at 10,000 rpm for 30 seconds. The flow through was discarded and the spin filter was placed back into the collection tube so that the process could be repeated two more times in order to filter all of the lysate. The second and third centrifugation of the lysate through the spin filter differed from the first only in that they were centrifuged at 12,000 rpm as opposed to 10,000 rpm. After filtration of the lysate 500 µl of CB solution was added to the filter and centrifuged at 10,000 rpm for 30 seconds with the flow through being discarded and the filter being placed back into the collection tube. 500 µl of ethanol was then added to the spin filter and centrifuged at 10,000 rpm for 30 seconds with the flow through being discarded and the filter being placed back into the collection tube. The spin filter and collection tube were then centrifuged at 15,000 rpm for 30 seconds in order to remove any residual alcohol that was still in the filter. The filter was then placed into a new collection tube at which point 125 µl of EB solution was loaded into the filter and incubated at room temperature for a period of five minutes before being centrifuged for 30 seconds at 10,000 rpm. The spin filter was then discarded and the collection tube was labeled with the sample ID and placed into storage at -20 degrees Celsius.

PCR protocol

Conventional PCR was conducted on each sample to detect *Dickeya dianthicola* DNA utilizing the *pelADE* primer pair and a Bio-Rad PCR machine (Nassar et al. 1996). Reagents added along with the sample were 5 µl of 5x Green GoTaq™ Reaction Buffer, 2.5 µl of MgCl2, 2.5 µl of Sigma water, and 5 µl of DNA. Denaturation was accomplished by subjecting the sample to a temperature of 94 degrees Celsius for two minutes. After denaturation 30 PCR cycles were run. These cycles consisted of a denaturation period at 94 degrees Celsius for 45 seconds,

an annealing period at 62 degrees Celsius for 45 seconds, and an extension period at 72 degrees Celsius for one minutes and 30 seconds. After thermocycling was completed, the samples were held at 72 degrees Celsius for 10 minutes and then held at 4 degrees Celsius.

Statistical analysis

Statistical analysis was performed for all data sets excluding the data sets for transmission of *D. dianthicola* to progeny tubers using SAS 9.4 (Statistical Analysis Software, version 9.4. SAS Institute Inc. 100 SAS Campus Dr. Cary, NC 27513). Data was analyzed by location and presented separately. ANOVA was used to find significance (P=.05). Duncan's multiple range test was used to separate treatment means if significance was found. Analysis for assessing transmission of *D. dianthicola* to progeny tubers was accomplished by Kruskal Wallis one way ANOVA.

RESULTS AND DISCUSSION

Emergence

At both the Florida (Figure 2) and Maryland (Figure 3) locations no statistically significant differences were observed in emergence among any of the treatments.

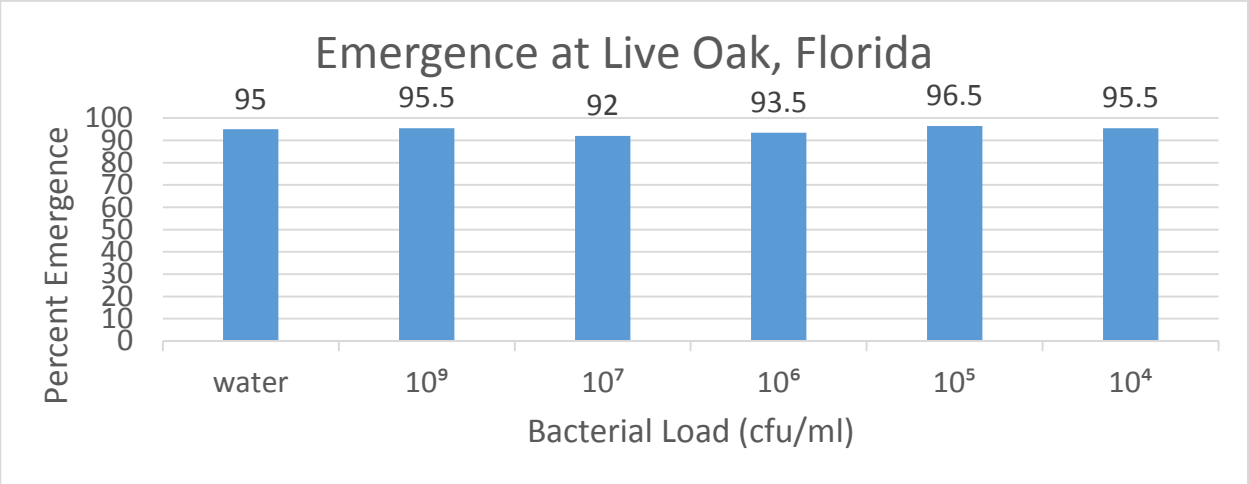

Figure 2: Percent emergence of potato plants at Live Oak, Florida. Bacterial Load indicates the number of colony forming units present in each milliliter of inoculum. Percent emergence is mean percent of plants that emerged through the soil in each treatment.

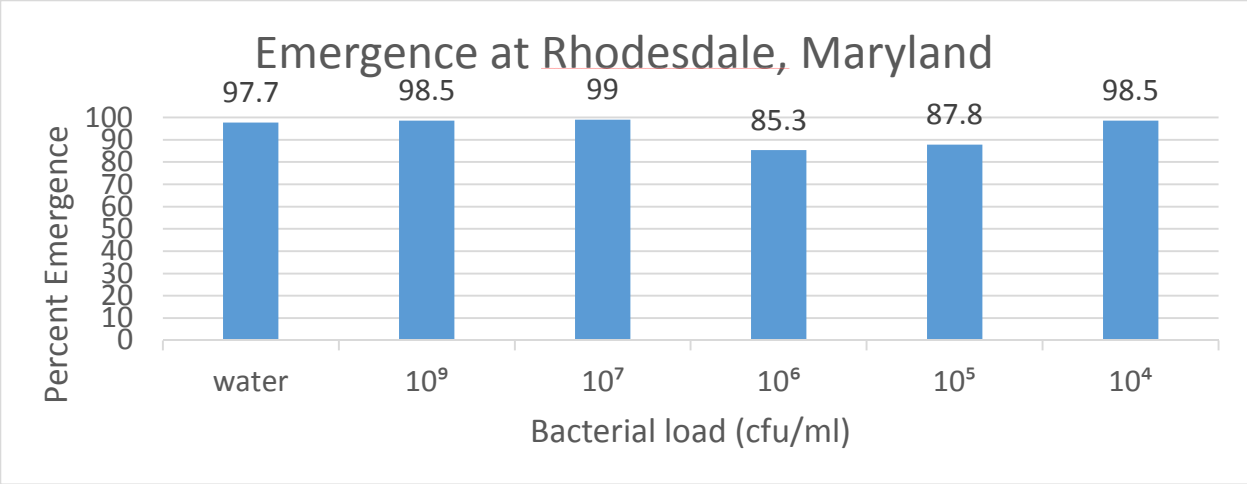

Figure 3: Percent emergence of potato plants at Rhodesdale, Maryland. Bacterial Load indicates the number of colony forming units present in each milliliter of inoculum. Percent emergence is mean percent of plants that emerged through the soil in each treatment.

Statistically significant differences in emergence were not observed at the Florida location (Figure 2) or the Maryland location (Figure 3). The data collected in this study indicates that the amount of seed borne inoculum of *D. dianthicola* appears to play little role in the emergence of potato plants whose mother tubers were inoculated with the bacteria. The average temperature at the Florida location during February when the trial was planted was 17 degrees Celsius (Table 4). The average temperature at the Maryland location during April when the trial was planted was 15.9 degrees Celsius (Table 5). At both locations the average temperature was well below 25 degrees Celsius which is the temperature at which disease development for *Dickeya* spp. is favored (Gugino 2016). The non-favorable temperatures after planting at both locations likely played a role in the ability of the bacteria to cause disease and impact plant emergence in this study.

Plant growth

At both the Florida location (Figure 4) and the Maryland location (Figure 5) no significant difference in average plant height among any of the treatments were observed throughout the growing season.

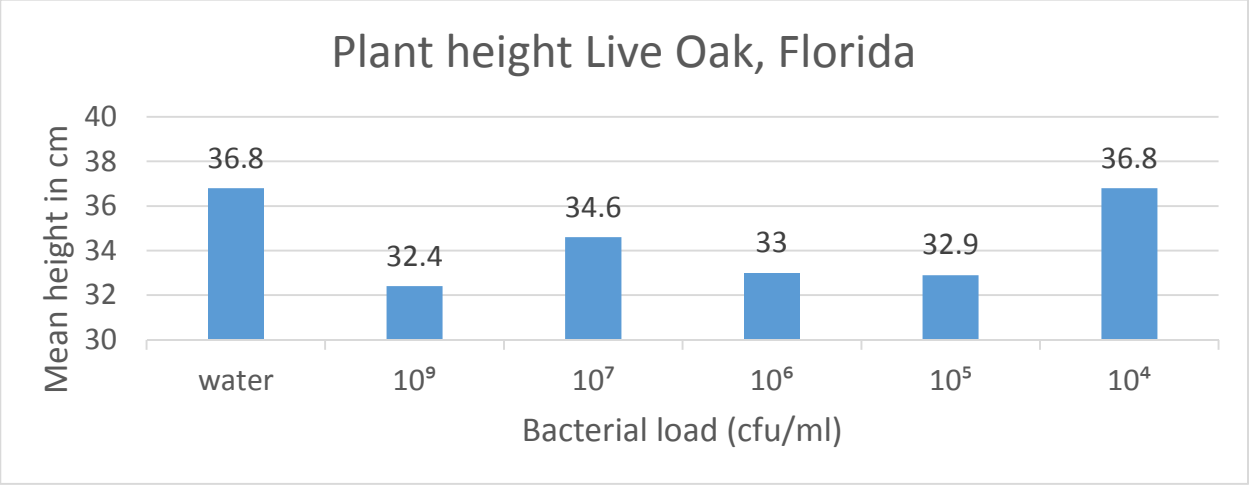

Figure 4: Average potato plant height at Live Oak, Florida. Bacterial Load indicates the number of colony forming units present in each milliliter of inoculum.

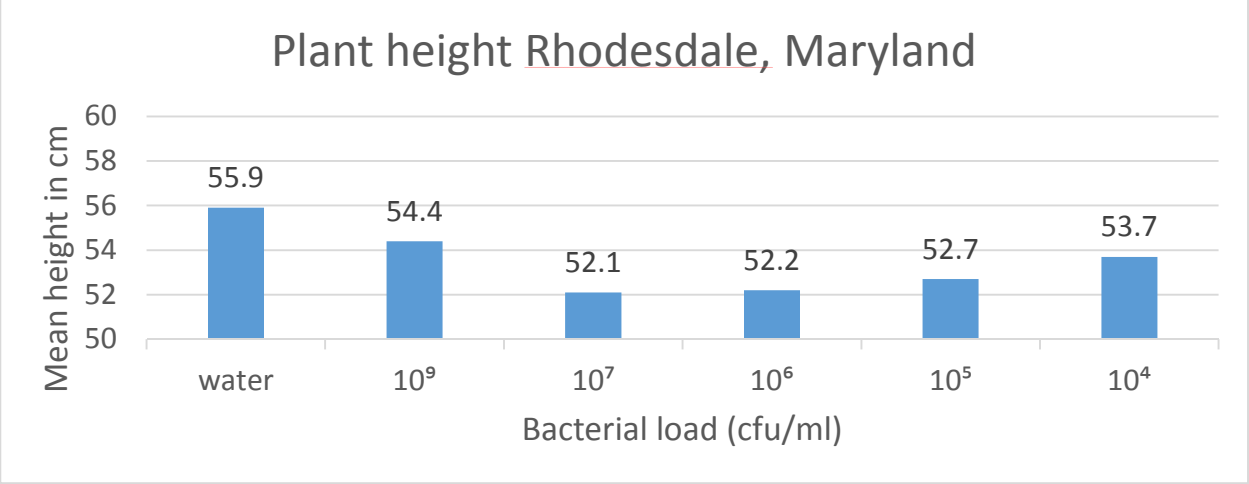

Figure 5: Average potato plant height at Rhodesdale, Maryland. Bacterial Load indicates the number of colony forming units present in each milliliter of inoculum.

Plant height was not impacted by the level of seed-borne inoculum in this study. Plant height data collected at the Florida location revealed no significant differences among treatments

(Figure 4). Plant height data collected at the Maryland location did not show significant differences among any of the treatments (Figure 5). The non-significant observations at both sites suggest that initial quantity of seed borne inoculum levels played little role in plant growth throughout the growing season in this study.

Blackleg incidence

No significant difference was observed in the expression of blackleg between any of the treatments at either location. Since no disease was observed in any treatments at either location statistical analysis was not performed.

The quantity of initial seed-borne inoculum of *D. dianthicola* appears to play little role in the development of disease over the growing season in this study as no disease was observed at either location. The lack of *Dickeya* related disease observed at both locations is not unique to this study. Other researchers have experienced issues in eliciting disease symptoms in potato seed inoculated with *Dickeya* species (Van der Wolf et al. 2016) (Steve Johnson personal communication 2019) (Amy Charkowski personal communication 2019). This highlights the lack of understanding in disease development of soft rot caused by *Dickeya* species.

Yield and grade

At the Florida location all treatments inoculated with bacteria had significantly lower yield when compared to the water control. Mean yields at the Florida location are shown below (Figure 6).

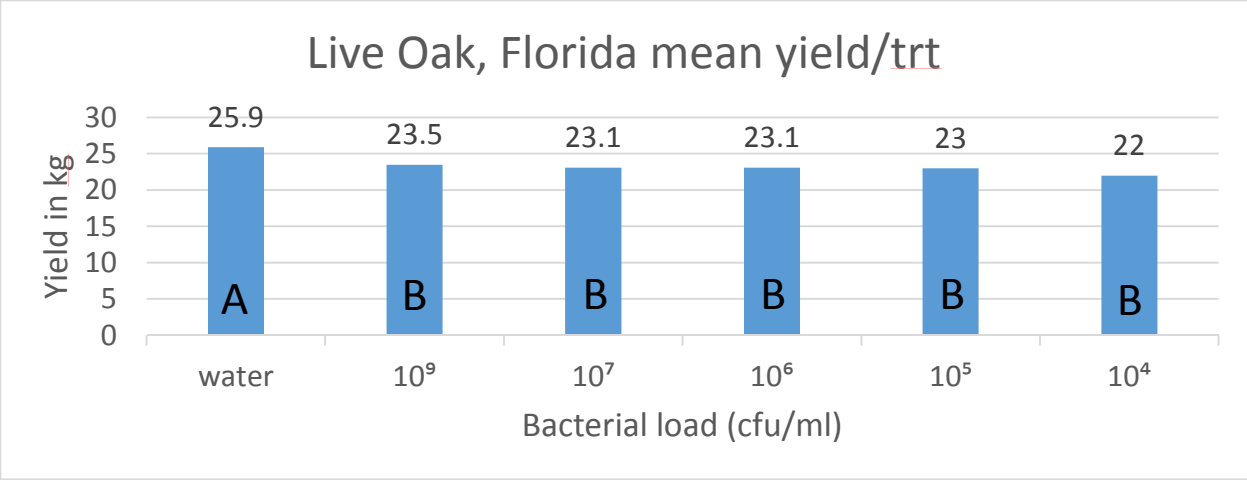

Figure 6: Yield at Live Oak, Florida. Bacterial Load indicates the number of colony forming units present in each milliliter of inoculum.

Yields at the Florida location were reduced if they were inoculated with any quantity of *D. dianthicola*, but treatments inoculated with bacteria did not differ significantly (Figure 6). The highest bacterial load treatment, 10^9 cfu/ml, had the highest average yield of the bacterially inoculated treatments at 25.9 kilograms, while the lowest bacterial load treatment, 10^4 cfu/ml, had the lowest average yield of the treatments at 22 kilograms. These observations suggest that higher levels of seed borne inoculum do not correlate to a proportionally higher yield reduction when compared to seed inoculated with lower levels of inoculum.

At the Florida location, no significant differences in mean tuber yield of each profile were observed among the treatments (Figure 7).

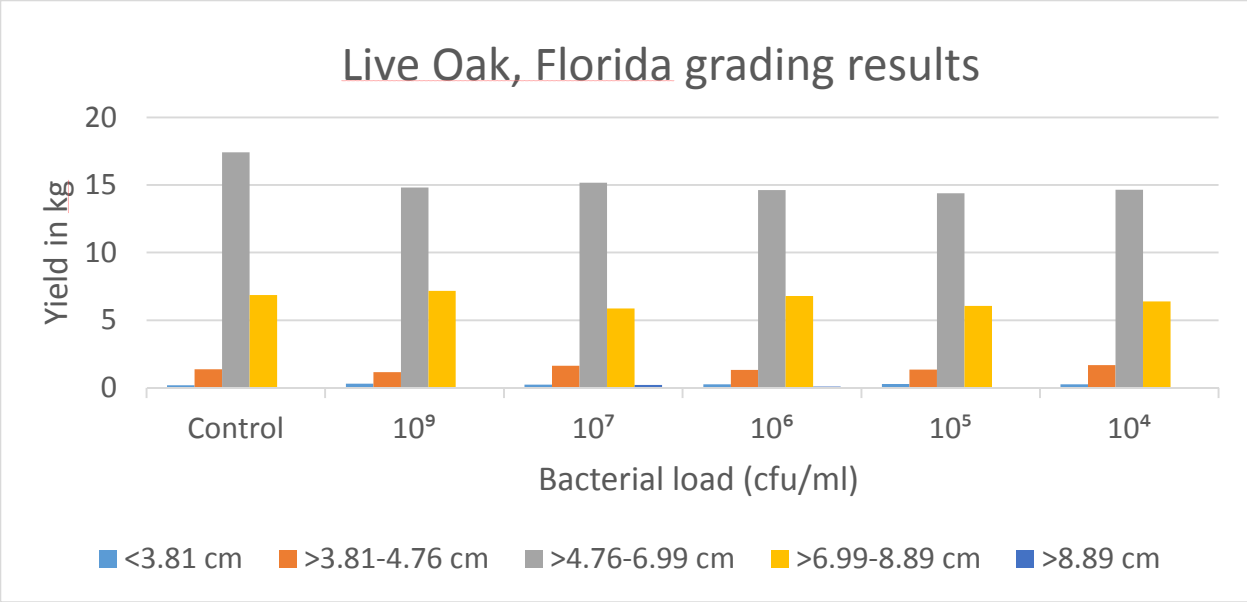

Figure 7: Grade at Live Oak, Florida. Yield for each tuber profile is shown in kilograms for each treatment. Bacterial Load indicates the number of colony forming units present in each milliliter of inoculum

This data furthers the argument that the starting quantity of inoculum in the seed piece does not contribute to differences in yield of tuber profiles. Though not statistically significant, mean yield reduction was observed in the >4.76-6.99 cm profile for all treatments inoculated with *D. dianthicola* when compared to the water control. The losses observed in this category account for the bulk of the total yield loss observed in overall yield at this trial location.

At the Maryland location no significant differences in yield were observed among any of the treatments (Figure 8).

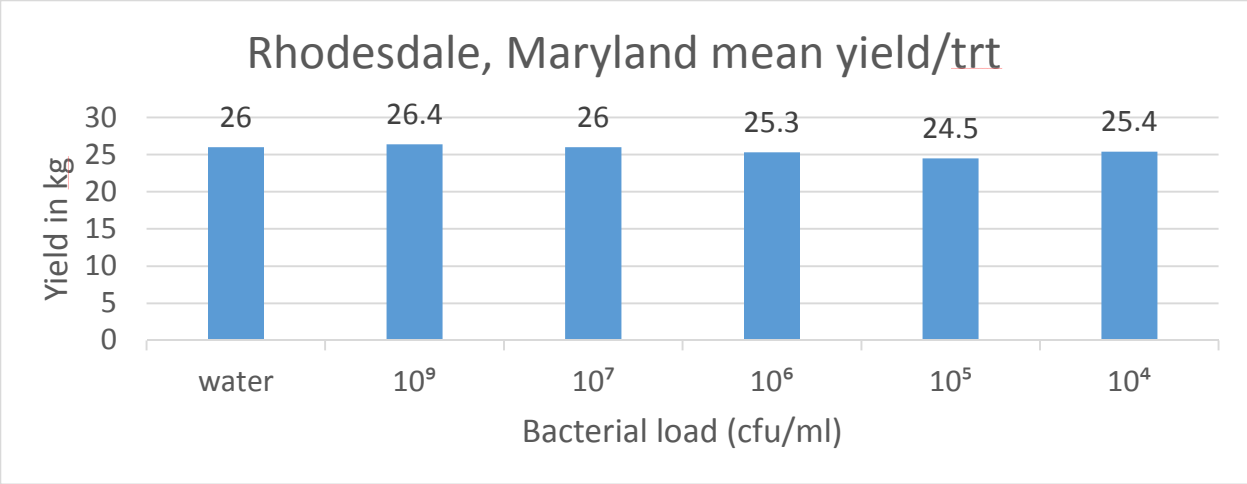

Figure 8: Yield at Rhodesdale, Maryland. Bacterial Load indicates the number of colony forming units present in each milliliter of inoculum.

Yields for treatments at the Maryland location did not significantly differ among any of the treatments (Figure 8). Although not significantly different than the other treatments at the Maryland location, the highest yielding treatment on average was the highest bacterial load treatment, 10^9 cfu/ml, at 26.4 kilograms. The observations at the Maryland location also indicate that the starting amount of seed borne *D. dianthicola* inoculum contributes little to reduction of yield and that other unknown environmental factors are at work.

At the Maryland location significant differences in >3.81-4.76 cm tuber profile category were observed. Mean yields of the five tuber size categories for the Maryland location are shown below (Figure 9).

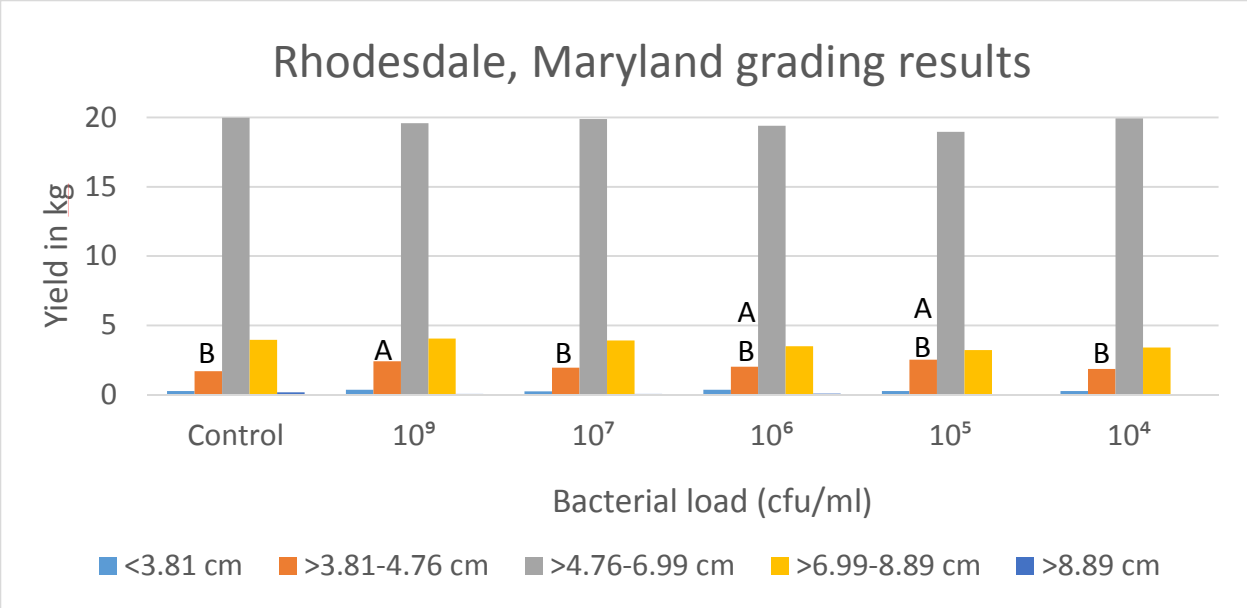

Figure 9: Grade at Rhodesdale, Maryland. Yield for each tuber profile is shown in kilograms for each treatment. Bacterial Load indicates the number of colony forming units present in each milliliter of inoculum.

Significant differences in yield were observed in the >3.81-4.76 cm category at the Maryland location but no other tuber profile category (Figure 9). These differences are likely not meaningful as they follow no pattern, and the overall yields of the treatments at the Maryland location were not significantly impacted because the yield in this profile constituted very little of the overall yield.

It is interesting to note that the Florida location had reduced yield for all treatments inoculated with *D. dianthicola*, despite having cooler temperatures through the majority of the growing season when compared to the temperatures at the Maryland location over that trials growing season (Table 2) and (Table 3). Additionally, the Florida location experienced fewer disease favorable days when compared to the Maryland location (Table 2) and (Table 3). Since both trials were grown under irrigated conditions, and the Florida location experienced conditions less conducive to disease development, other factors must be responsible for the

differences observed in yield between the control treatments and the treatments inoculated with *D. dianthicola* at the Florida location. Similarly, other factors must be responsible for the lack of yield reduction observed among treatments at the Maryland location, despite the fact the Maryland location had more disease favorable days than the Florida location.

Transmission to progeny tubers

At the Florida location no treatments were found to differ significantly from one another in transmission of *D. dianthicola* to progeny tubers. Transmission was observed in all treatments, including the control.

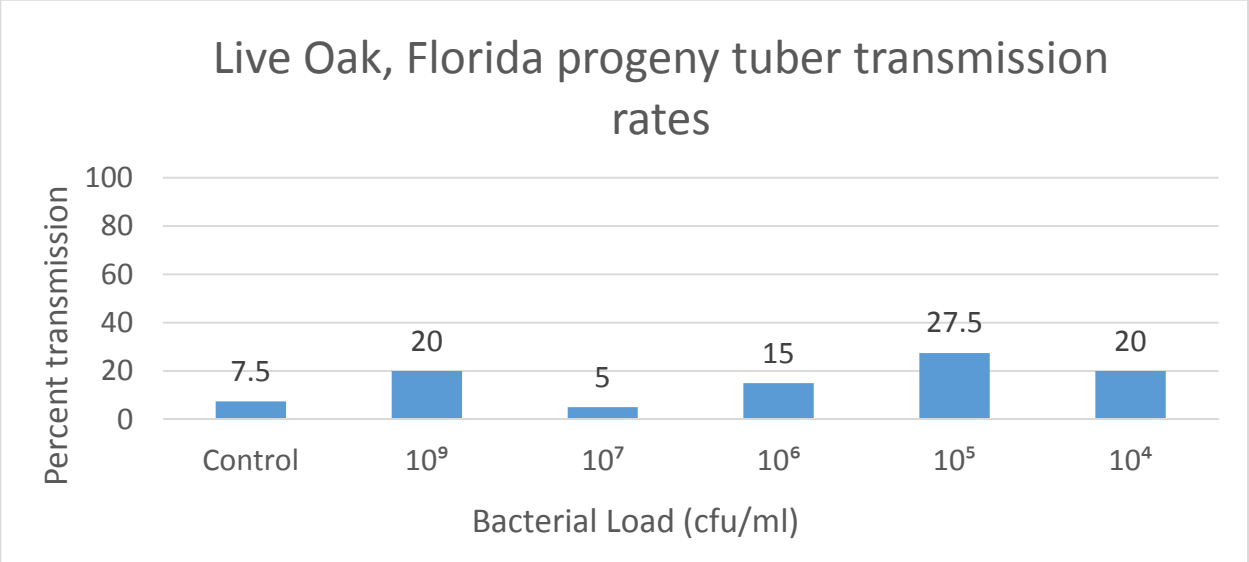

Figure 10: Percent transmission of *D. dianthicola* to progeny tubers in Live Oak, Florida. Bacterial Load indicates the number of colony forming units present in each milliliter of inoculum.

At the Maryland location no significant differences were observed among any of the treatments. While not significant, transmission of *D. dianthicola* was observed in the 10^9 cfu/ml treatment and the 10^7 cfu/ml treatment. Transmission of the bacteria was not observed in any other treatments at the Maryland location. Figure 11 shows the percent transmission rate to progeny tubers at the Maryland location.

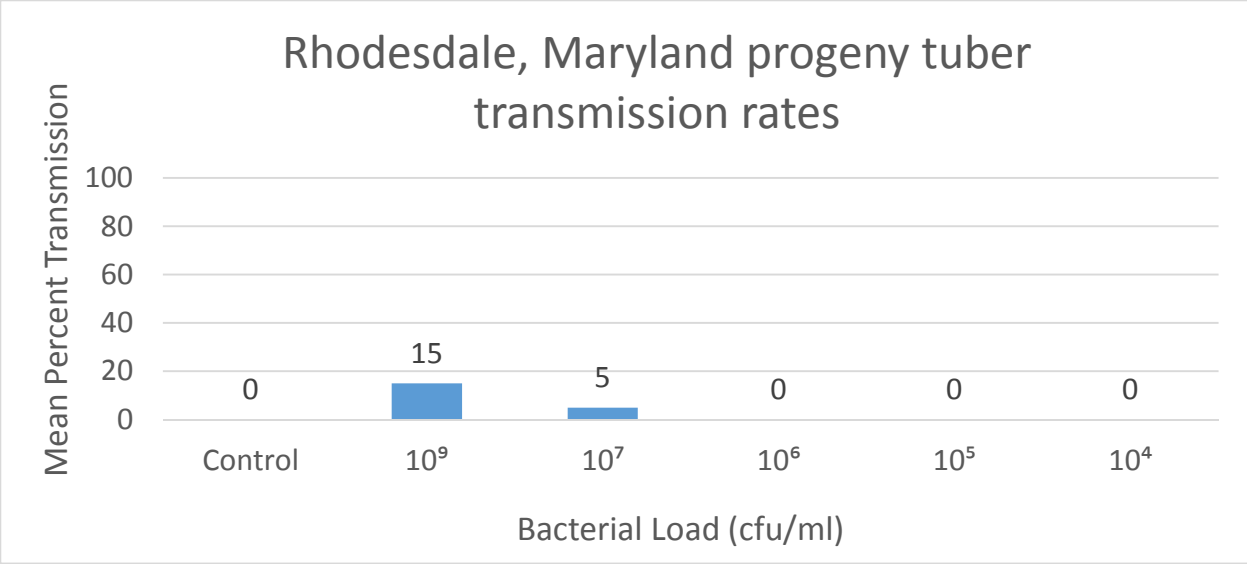

Figure 11: Percent transmission of *D. dianthicola* to progeny tubers in Rhodesdale, Maryland. Bacterial Load indicates the number of colony forming units present in each milliliter of inoculum.

In this study varying levels of seed-borne inoculum did not result in statistically significant differences in the rate of transmission of the bacteria to progeny tubers. At the Florida location, transmission to progeny tubers was observed in all treatments including the control, but did not follow a pattern in regard to the concentration of the bacteria the treatments were inoculated with (Figure 10), nor did any of the treatments differ significantly from one another. In the Maryland location transmission of *D. dianthicola* to progeny tubers was only observed in the 10^9 cfu/ml and 10^7 cfu/ml treatments (Figure 11), and none of the treatments were found to differ significantly from one another. The increased transmission in the Florida location can likely be explained by the sand soil type at the research site (Table 1). The sandy soil has an increased porosity due to the large size of the individual particles compared to the particles typical of clay or silt. This increased porosity would likely facilitate easier movement of the bacteria from the infected mother tuber, through the free water in the soil, and into adjacent tubers. This would also help to explain the transmission of the bacteria to the non-inoculated

control plots. The Maryland location had a loamy sand soil type (Table 1) and transmission to progeny tubers was only observed in the two highest bacterial concentrations (Figure 11). The 10^9 cfu/ml treatment had a 15% rate of transmission of the bacteria to the progeny tubers while the 10^7 cfu/ml treatment had a 5% rate of transmission of the bacteria to the progeny tubers. Based on this data in this study it appears that the starting quantity of seed borne inoculum plays little role in the of transmission *D. dianthicola* to progeny tubers, but other factors such as water table height, soil type, and microbiome interactions could play an important role as evidenced by the data collected from the Florida location.

CONCLUSION

Field experiments were conducted at Live Oak, Florida, and Rhodesdale, Maryland, to assess the effect of variable seed-borne inoculum load of *Dickeya dianthicola* on potato plant emergence, plant growth throughout the growing season, disease prevalence in the field throughout the growing season, yield and grade, and transmission of *D. dianthicola* to progeny tubers. Based on our data, it would appear that the quantity of seed borne inoculum plays little role in emergence, disease development, plant height throughout the growing season, and transmission of the pathogen to progeny tubers, as no statistically significant differences were found between any of the treatments.

At the Florida location, yield was significantly reduced in all treatments that were inoculated with *D. dianthicola* when compared to the control, however there was no difference among the inoculated treatments. At the Maryland location no differences in yield were observed among any of the treatments. At the Florida location no significant differences in grade were observed. At the Maryland location significant differences in grade were observed in the 3.81-4.76 centimeter size category, but the differences observed followed no observable pattern.

Based on the data collected in this study, the quantity of initial seed borne inoculum plays little role in the rate of transmission of *D. dianthicola* to progeny tubers. Environmental conditions are likely more important. Our data demonstrates that visually asymptomatic plants grown from seed inoculated with any amount of *D. dianthicola* can have significantly reduced yields despite not manifesting disease symptoms, but this is not always the case.

The emerging field of microbiome interactions could prove to be useful in understanding disease development with *Dickeya* species. Plant disease caused by a synergy of multiple organisms and environmental factors such as the Potato Early Die Complex are not uncommon

(Saeed et al. 1997) (Powelson 1985). Additionally, work done on tomato pith necrosis has shown that *D. chrysanthemi* when paired with other bacterial species can greatly enhance the severity of disease compared to infection by any one of the bacteria species individually (Lamichhane and Venturi 2015). Given the discrepancy between the recorded environmental conditions and the data collected, along with seemingly erratic disease expression of *Dickeya* observed by other researchers, it is not unreasonable to hypothesize that *Dickeya* species are interacting with other organisms in ways that are not yet understood. Research highlighted previously in this paper has shown that a range of options for the biological control of *Dickeya* through the use of various antagonistic organisms. Microbiome assessments of soil in fields with serious losses due to *Dickeya* outbreaks could offer insight into the mechanics of disease development and elucidate potential means of controlling the pathogen.

LITERATURE CITED

Abbott, D. W., and Boraston, A. B. 2008. Structural Biology of Pectin Degradation by Enterobacteriaceae. Microbiology and Molecular Biology Reviews. 72:301–316 Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2415742/.

NASS, Washington, DC: National Agricultural Statistics Service, Agricultural Statistics Board, U.S. Dept. of Agriculture. Acreage

Adeolu, M., Alnajar, S., Naushad, S., and Gupta, R. S. 2016. Genome-based phylogeny and taxonomy of the 'Enterobacteriales': proposal for Enterobacterales ord. nov. divided into the families Enterobacteriaceae, Erwiniaceae fam. nov., Pectobacteriaceae fam. nov., Yersiniaceae fam. nov., Hafniaceae fam. nov., Morganellaceae fam. nov., and Budviciaceae fam. nov. International Journal of Systematic and Evolutionary Microbiology. 66:5575–5599 Available at: http://pdfs.semanticscholar.org/b34f/c060d9ab8b42dc472be42a65db9070011b06.pdf.

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

Alyokhin, A., Mota-Sanchez, D., Baker, M., Snyder, W. E., Menasha, S., Whalon, M., et al. 2014. The Red Queen in a potato field: integrated pest management versus chemical dependency in Colorado potato beetle control. Pest Management Science. 71:343–356 Available at: https://www.ncbi.nlm.nih.gov/pubmed/24817433.

Ames, M., and Spooner, D. M. 2008. DNA from herbarium specimens settles a controversy about origins of the European potato. American Journal of Botany. 95:252–257 Available at: https://bsapubs.onlinelibrary.wiley.com/doi/pdf/10.3732/ajb.95.2.252.

Ansermet, M., Schaerer, S., Kellenberger, I., Tallant, M., and Dupuis, B. 2016. Influence of seed- borne and soil- carried inocula of Dickeya spp. on potato plant transpiration and symptom expression. European Journal of Plant Pathology. 145:459–467.

Aysan, Y., Karatas, A., and Cinar, O. 2003. Biological control of bacterial stem rot caused by Erwinia chrysanthemi on tomato. Crop Protection. 22:807–811.

Blackleg of potato (Dickeya solani). 2018. DAERA. Available at: https://www.daera-ni.gov.uk/articles/blackleg-potato-dickeya-solani [Accessed March 8, 2020].

Brenner, D. J., Steigerwalt, A. G., Miklos, G. V., and Fanning, G. R. 1973. Deoxyribonucleic Acid Relatedness Among Erwiniae and Other Enterobacteriaceae: The Soft-Rot Organisms (Genus Pectobacterium Waldee). International Journal of Systematic Bacteriology. 23:205–216 Available at: https://www.microbiologyresearch.org/content/journal/ijsem/10.1099/00207713-23-3-205 [Accessed September 28, 2019].

Burkholder, W. R., McFadden, L. A., and Dimock, E. W. 1953. A bacterial blight of Chrysanthemums. Phytopathology. 43:522–526 Available at: https://www.cabdirect.org/cabdirect/abstract/19541101119 [Accessed September 28, 2019].

Crépin, A., Beury-Cirou, A., Barbey, C., Farmer, C., Hélias, V., Burini, J.-F., et al. 2012. N-Acyl Homoserine Lactones in Diverse Pectobacterium and Dickeya Plant Pathogens: Diversity, Abundance, and Involvement in Virulence. Sensors. 12:3484–3497 Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3376598/ [Accessed June 11, 2019].

Czajkowski, R., Pérombelon, M. C. M., Veen, J. A. V., and Wolf, J. M. V. D. 2011. Control of blackleg and tuber soft rot of potato caused by Pectobacterium and Dickeya species: a review. Plant Pathology. 60:999–1013 Available at:

https://onlinelibrary.wiley.com/doi/full/10.1111/j.1365-3059.2011.02470.x [Accessed June 11, 2019].

Czajkowski, R., Boer, W. J. D., Velvis, H., and Wolf, J. M. V. D. 2010. Systemic Colonization of Potato Plants by a Soilborne, Green Fluorescent Protein-Tagged Strain of Dickeyasp. Biovar 3. Phytopathology. 100:134–142 Available at: https://www.ncbi.nlm.nih.gov/pubmed/20055647.

Czajkowski, R., Boer, W. J. D., and Wolf, J. M. V. D. 2013. Chemical disinfectants can reduce potato blackleg caused by 'Dickeya solani.' European Journal of Plant Pathology. 136:419–432 Available at: https://www.researchgate.net/publication/257558655_Chemical_disinfectants_can_reduce_potato_blackleg_caused_by_'Dickeya_solani' [Accessed June 11, 2019].

Dickey, R. S. 1978. Erwinia chrysanthemi: A Comparative Study of Phenotypic Properties of Strains from Several Hosts and OtherErwiniaSpecies. Phytopathology. 69:324 Available at: https://www.apsnet.org/publications/phytopathology/backissues/Documents/1979Articles/Phyto69n04_324.PDF.

Dickey, R. S. 1981. Erwinia chrysanthemi:Reaction of Eight Plant Species to Strains from Several Hosts and to Strains of OtherErwiniaSpecies. Phytopathology. 71:23 Available at: https://www.apsnet.org/publications/phytopathology/backissues/Documents/1981Articles/Phyto71n01_23.pdf.

Dickey, R. S., Zumoff, C.H., Uyemoto, J.K. 1984. Erwinia chrysanthemi: Serological Relationships Among Strains from Several Hosts. Phytopathology. 74:1388 Available at: https://www.apsnet.org/publications/phytopathology/backissues/Documents/1984Articles/Phyto74n11_1388.pdf.

Donnelly, J. S. 2013. *The Great Irish potato famine*. Stroud: The History Press.

Essarts, Y. R. D., Cigna, J., Quêtu-Laurent, A., Caron, A., Munier, E., Beury-Cirou, A., et al. 2016. Biocontrol of the Potato Blackleg and Soft Rot Diseases Caused by Dickeya dianthicola. Applied and Environmental Microbiology. 82:268–278 Available at: https://aem.asm.org/content/aem/82/1/268.full.pdf [Accessed June 11, 2019].

FAOSTAT. Available at: http://www.fao.org/faostat/en/#data/QC [Accessed June 12, 2019].

Gill, E. D., Schaerer, S., and Dupuis, B. 2014. Factors impacting blackleg development caused by Dickeya spp. in the field. European Journal of Plant Pathology. 140:317–327 Available at: https://www.researchgate.net/publication/265476947_Factors_impacting_blackleg_development_caused_by_Dickeya_spp_in_the_field [Accessed June 11, 2019].

Grenier, A.-M., Duport, G., Pages, S., Condemine, G., and Rahbe, Y. 2006. The Phytopathogen Dickeya dadantii (Erwinia chrysanthemi 3937) Is a Pathogen of the Pea Aphid. Applied and Environmental Microbiology. 72:1956–1965 Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1393189/ [Accessed June 11, 2019].

Gugino, B. K., and Gugino, B. K. 2016. Best Management Practices for Soft Rot Associated with Dickeya in Potatoes. Penn State Extension. Available at: https://extension.psu.edu/best-management-practices-for-soft-rot-associated-with-dickeya-in-potatoes [Accessed June 11, 2019].

Hauben, L., Moore, E. R., Vauterin, L., Steenackers, M., Mergaert, J., Verdonck, L., et al. 1998. Phylogenetic Position of Phytopathogens within the Enterobacteriaceae. Systematic and Applied Microbiology. 21:384–397 Available at: https://www.ncbi.nlm.nih.gov/pubmed/9779605.

Jiang, H. H., Hao, J. J., Johnson, S. B., Brueggeman, R. S., and Secor, G. 2016. First Report of Dickeya dianthicola Causing Blackleg and Bacterial Soft Rot on Potato in Maine. Plant Disease. 100:2320–2320 Available at: https://apsjournals.apsnet.org/doi/full/10.1094/PDIS-12-15-1513-PDN.

Kabanova, A. P., Shneider, M. M., Korzhenkov, A. A., Bugaeva, E. N., Miroshnikov, K. K., Zdorovenko, E. L., et al. 2019. Host Specificity of the Dickeya Bacteriophage PP35 Is Directed by a Tail Spike Interaction With Bacterial O-Antigen, Enabling the Infection of Alternative Non-pathogenic Bacterial Host. Frontiers in Microbiology. 9 Available at: https://www.frontiersin.org/articles/10.3389/fmicb.2018.03288/full.

Lamichhane, J. R., and Venturi, V. 2015. Synergisms between microbial pathogens in plant disease complexes: a growing trend. Frontiers in Plant Science. 06 Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4445244/.

Lelliott, R.A. and Dickey, R.S. (1984) Erwinia. In: Krieg, N.R. and Holt, J.G., Eds., Bergey's Manual of Systematic Bacteriology, Phytopathology Journal, 1, 469-476.

Ma, X., Perna, N. T., Glasner, J. D., Hao, J., Johnson, S., Nasaruddin, A. S., et al. 2019. Complete Genome Sequence of Dickeya dianthicola ME23, a Pathogen Causing Blackleg and Soft Rot Diseases of Potato. Microbiology Resource Announcements. 8 Available at: https://mra.asm.org/content/ga/8/7/e01526-18.full.pdf.

Maas Geesteranus, H.P. 1972. Natrot en zwartbenigheid bij aardappelen. Bedrijfsontwikkeling 3:941-945.

McGrath, M. T. 2017. Dickeya: A New Disease Causing Major Impact. Potato Country. Available at: https://potatocountry.com/2017/04/28/dickeya-new-disease-causing-major-impact/ [Accessed June 11, 2019].

Nassar, A. Darrase, A.Lamattre., Kotoujansky, A., Dervin, C., Vedel, R., & Bertheau, Y. 1996. Characterization of Erwinia chrysanthemi by pectinolytic isozyme polymorphism and restriction fragment length polymorphism analysis of PCR-amplified fragments of pel genes. Appl. Environ.

Parker, E. L. 1974. *The history of Londonderry*. Londonderry, NH: Town of Londonderry.

Pérombelon, M.C.M. & Salmond, G.P.C. (1995). Bacterial soft rots In: U.S Singh, R.P. Singh, K. Kohmoto, (eds) Pathogenesis and host specificity in plant diseases: Histopathological, biochemical, genetic and molecular bases, Volume 1 edn. (1-20) Great Britain: Pergamon.

Parkinson, N., Devos, P., Pirhonen, M., and Elphinstone, J. 2014. Dickeya aquatica sp. nov., isolated from waterways. International Journal Of Systematic And Evolutionary Microbiology. 64:2264–2266 Available at: https://www.microbiologyresearch.org/content/journal/ijsem/10.1099/ijs.0.058693-0.

Potrykus, M., Golanowska, M., Sledz, W., Zoledowska, S., Motyka, A., Kolodziejska, A., et al. 2016. Biodiversity of Dickeya spp. Isolated from Potato Plants and Water Sources in Temperate Climate. Plant Disease. 100:408–417 Available at: https://apsjournals.apsnet.org/doi/pdf/10.1094/PDIS-04-15-0439-RE [Accessed June 11, 2019].

Powelson, M. L. 1985. Potato early dying disease in the Pacific Northwest caused byErwinia carotovora pv.carotovora andE. carotovora pv.atroseptica. American Potato Journal. 62:173–176 Available at: https://link.springer.com/article/10.1007/BF02852974.

Saeed, I. A. M., Macguidwin, A. E., and Rouse, D. I. 1997. Synergism of Pratylenchus penetrans and Verticillium dahliae Manifested by Reduced Gas Exchange in Potato. Phytopathology. 87:435–439.

Samson, R., Legendre, J. B., Christen, R., Fischer-Le Saux, M., Achouak, W., and Gardan, L. 2005. Transfer of Pectobacterium chrysanthemi (Burkholder et al. 1953) Brenner et al. 1973 and Brenneria paradisiaca to the genus Dickeya gen. nov. as Dickeya chrysanthemi comb. nov. and Dickeya 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 Systematic And Evolutionary Microbiology. 55:1415–1427 Available at: https://www.microbiologyresearch.org/content/journal/ijsem/10.1099/ijs.0.02791-0 [Accessed September 30, 2019].

Spooner, David M.; McLean, Karen; Ramsay, Gavin; Waugh, Robbie; Bryan, Glenn J. 29 September 2005. A single domestication for potato based on multilocus amplified fragment length polymorphism genotyping. PNAS. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1253605/ [Accessed June 12, 2019].

Tian, Y., Zhao, Y., Yuan, X., Yi, J., Fan, J., Xu, Z., et al. 2016. Dickeya fangzhongdai sp. nov., a plant-pathogenic bacterium isolated from pear trees (Pyrus pyrifolia). International Journal of Systematic and Evolutionary Microbiology. 66:2831–2835 Available at: https://www.microbiologyresearch.org/docserver/fulltext/ijsem/66/8/2831_ijsem001060.pdf?expires=1582569525&id=id&accname=guest&checksum=0E02A1F6137D33D930D6C51DA87126AB.

Toth, I. K., Wolf, J. M. V. D., Saddler, G., Lojkowska, E., Hélias, V., Pirhonen, M., et al. 2011. Dickeya species: an emerging problem for potato production in Europe. Plant Pathology. 60:385–399 Available at: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1365-3059.2011.02427.x [Accessed June 11, 2019].

USDA.gov. Potatoes. SNAP Education Connection. Available at: https://snaped.fns.usda.gov/seasonal-produce-guide/potatoes [Accessed February 27, 2020].

USDA . Available at: https://downloads.usda.library.cornell.edu/usda-esmis/files/fx719m44h/7p88cv74d/b5645489z/pots0919.pdf.

Waldee, E. L. Comparative studies of some peritrichous phytopathogenic bacteria. Iowa State College. Available at: https://lib.dr.iastate.edu/cgi/viewcontent.cgi?article=15149&context=rtd [Accessed September 28, 2019].

Winslow, C. E., Broadhurst, J., Buchanan, R. E., Krumwiede, C., Rogers, L. A., and Smith, G. H. 1920. The Families and Genera of the Bacteria: Final Report of the Committee of the Society of Bacteriologists on Characterization and Classification of Bacterial Types. Journal of Bacteriology. :191–229 Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC378870/?page=1 [Accessed September 28, 2019].

Wolf, J. M. V. D., Nijhuis, E. H., Kowalewska, M. J., Saddler, G. S., Parkinson, N., Elphinstone, J. G., et al. 2014. Dickeya solani sp. nov., a pectinolytic plant-pathogenic bacterium isolated from potato (Solanum tuberosum). International Journal Of Systematic And Evolutionary Microbiology. 64:768–774 Available at: https://www.microbiologyresearch.org/content/journal/ijsem/10.1099/ijs.0.052944-0.

Yuliar, Nion, Y. A., and Toyota, K. 2015. Recent Trends in Control Methods for Bacterial Wilt Diseases Caused by *Ralstonia solanacearum*. Microbes and Environments Microbes and environments. 30:1–11 Available at: https://www.ncbi.nlm.nih.gov/pubmed/25762345.

APPENDIX A. ANOVA TABLES OF PERCENT STAND OF *D. DIANTHICOLA* INOCULATED SEED AT LIVE OAK, FLORIDA AND RHODESDALE, MARYLAND

Florida

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	153.3333333	17.0370370	1.30	0.2682
Error	38	497.3333333	13.0877193		
Corrected Total	47	650.6666667			

R-Square	Coeff Var	Root MSE	emergence Mean
0.235656	3.821509	3.617695	94.66667

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Trt	5	106.6666667	21.3333333	1.63	0.1756
rep	3	34.6666667	11.5555556	0.88	0.4586
row	1	12.0000000	12.0000000	0.92	0.3443

Maryland

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	2251.18533	250.13170	0.72	0.6911
Error	37	12929.92105	349.45733		
Corrected Total	46	15181.10638			

R-Square	Coeff Var	Root MSE	emergence Mean
0.148289	19.80630	18.69378	94.38298

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trt	5	1538.677812	307.735562	0.88	0.5037
rep	3	696.949939	232.316646	0.66	0.5790
row	1	15.557580	15.557580	0.04	0.8340

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trt	5	1509.226234	301.845247	0.86	0.5145
rep	3	701.908216	233.969405	0.67	0.5762
row	1	15.557580	15.557580	0.04	0.8340

APPENDIX B. ANOVA TABLES OF AVERAGE PLANT HEIGHT OVER THE GROWING SEASON AT LIVE OAK, FLORIDA AND RHODESDALE, MARYLAND

Florida

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	3642.1661	280.1666	0.76	0.7079
Error	703	260722.7070	370.8716		
Corrected Total	716	264364.8731			

R-Square	Coeff Var	Root MSE	score Mean
0.013777	55.99580	19.25803	34.39191

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trt	5	2308.263560	461.652712	1.24	0.2863
rep	3	1042.921500	347.640500	0.94	0.4221
row	1	90.174401	90.174401	0.24	0.6221
plant	4	200.806597	50.201649	0.14	0.9693

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trt	5	2298.258609	459.651722	1.24	0.2888
rep	3	1042.031843	347.343948	0.94	0.4225
row	1	89.636242	89.636242	0.24	0.6231

Source	DF	Type III SS	Mean Square	F Value	Pr > F
plant	4	200.806597	50.201649	0.14	0.9693

Maryland

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	2161.2917	166.2532	1.05	0.4032
Error	706	112055.9028	158.7194		
Corrected Total	719	114217.1944			

R-Square	Coeff Var	Root MSE	score Mean
0.018923	23.59126	12.59839	53.40278

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trt	5	1294.394444	258.878889	1.63	0.1494
rep	3	660.250000	220.083333	1.39	0.2457
row	1	82.688889	82.688889	0.52	0.4707
plant	4	123.958333	30.989583	0.20	0.9409

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trt	5	1294.394444	258.878889	1.63	0.1494
rep	3	660.250000	220.083333	1.39	0.2457
row	1	82.688889	82.688889	0.52	0.4707

Source	DF	Type III SS	Mean Square	F Value	Pr > F
plant	4	123.958333	30.989583	0.20	0.9409

APPENDIX C. ANOVA TABLES OF YIELD AT LIVE OAK, FLORIDA AND RHODESDALE, MARYLAND

Florida

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	830.581875	92.286875	3.50	0.0031
Error	38	1001.391250	26.352401		
Corrected Total	47	1831.973125			

R-Square	Coeff Var	Root MSE	yield Mean
0.453381	9.932923	5.133459	51.68125

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Trt	5	320.5693750	64.1138750	2.43	0.0523
rep	3	175.5772917	58.5257639	2.22	0.1015
row	1	334.4352083	334.4352083	12.69	0.0010

Maryland

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	122.911667	13.656852	0.52	0.8498
Error	38	995.307500	26.192303		
Corrected Total	47	1118.219167			

R-Square	Coeff Var	Root MSE	yield Mean
0.109917	9.057459	5.117842	56.50417

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Trt	5	93.04416667	18.60883333	0.71	0.6193
rep	3	28.53416667	9.51138889	0.36	0.7800
row	1	1.33333333	1.33333333	0.05	0.8227

APPENDIX D. ANOVA TABLES FOR GRADE AT LIVE OAK FLORIDA, AND RHODESDALE, MARYLAND

Florida

Tuber Profile: <3.81 centimeters

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	0.50750000	0.05638889	0.81	0.6099
Error	38	2.64500000	0.06960526		
Corrected Total	47	3.15250000			

R-Square	Coeff Var	Root MSE	A Mean
0.160983	44.90691	0.263828	0.587500

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Trt	5	0.35750000	0.07150000	1.03	0.4155
rep	3	0.08250000	0.02750000	0.40	0.7573
row	1	0.06750000	0.06750000	0.97	0.3310

Tuber Profile: >3.81- 4.76 centimeters

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	14.42354167	1.60261574	2.08	0.0558
Error	38	29.21625000	0.76884868		
Corrected Total	47	43.63979167			

R-Square	Coeff Var	Root MSE	B Mean
0.330514	27.85462	0.876840	3.147917

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Trt	5	7.64604167	1.52920833	1.99	0.1025
rep	3	3.72729167	1.24243056	1.62	0.2017
row	1	3.05020833	3.05020833	3.97	0.0536

Tuber Profile: >4.76-6.99 centimeters

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	489.923333	54.435926	1.59	0.1524
Error	38	1298.436667	34.169386		
Corrected Total	47	1788.360000			

R-Square	Coeff Var	Root MSE	C Mean
0.273951	17.47521	5.845459	33.45000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Trt	5	248.2100000	49.6420000	1.45	0.2280
rep	3	145.3800000	48.4600000	1.42	0.2524
row	1	96.3333333	96.3333333	2.82	0.1013

Tuber Profile: >6.99-8.89 centimeters

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	165.8900000	18.4322222	1.46	0.1979
Error	38	479.4566667	12.6172807		
Corrected Total	47	645.3466667			

R-Square	Coeff Var	Root MSE	D Mean
0.257056	24.69581	3.552081	14.38333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Trt	5	49.2966667	9.8593333	0.78	0.5694
rep	3	1.2733333	0.4244444	0.03	0.9916
row	1	115.3200000	115.3200000	9.14	0.0045

Tuber Profile: >8.89 centimeters

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	1.50750000	0.16750000	0.79	0.6267
Error	38	8.05166667	0.21188596		

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Corrected Total	47	9.55916667			

R-Square	Coeff Var	Root MSE	E Mean
0.157702	356.3696	0.460311	0.129167

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Trt	5	1.30416667	0.26083333	1.23	0.3137
rep	3	0.06250000	0.02083333	0.10	0.9605
row	1	0.14083333	0.14083333	0.66	0.4200

Maryland

Tuber Profile: <3.81 centimeters

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	0.72583333	0.08064815	0.97	0.4812
Error	38	3.16666667	0.08333333		
Corrected Total	47	3.89250000			

R-Square	Coeff Var	Root MSE	A Mean
0.186470	45.28237	0.288675	0.637500

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Trt	5	0.57750000	0.11550000	1.39	0.2513
rep	3	0.00750000	0.00250000	0.03	0.9929
row	1	0.14083333	0.14083333	1.69	0.2014

Tuber Profile: >3.81- 4.76 centimeters

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	12.96604167	1.44067130	1.61	0.1488
Error	38	34.10375000	0.89746711		
Corrected Total	47	47.06979167			

R-Square	Coeff Var	Root MSE	B Mean
0.275464	21.54082	0.947347	4.397917

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Trt	5	11.05354167	2.21070833	2.46	0.0499
rep	3	0.74062500	0.24687500	0.28	0.8430
row	1	1.17187500	1.17187500	1.31	0.2603

Tuber Profile: >4.76-6.99 centimeters

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	67.4660417	7.4962269	0.38	0.9368
Error	38	746.7537500	19.6514145		
Corrected Total	47	814.2197917			

R-Square	Coeff Var	Root MSE	C Mean
0.082860	10.24920	4.432992	43.25208

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Trt	5	30.16854167	6.03370833	0.31	0.9056
rep	3	3.79729167	1.26576389	0.06	0.9784
row	1	33.50020833	33.50020833	1.70	0.1995

Tuber Profile: >6.99-8.89 centimeters

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	91.1241667	10.1249074	1.06	0.4145
Error	38	363.5483333	9.5670614		
Corrected Total	47	454.6725000			

R-Square	Coeff Var	Root MSE	D Mean
0.200417	38.24503	3.093067	8.087500

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Trt	5	22.62750000	4.52550000	0.47	0.7940
rep	3	38.09583333	12.69861111	1.33	0.2798
row	1	30.40083333	30.40083333	3.18	0.0826

Tuber Profile: >8.89 centimeters

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	1.24416667	0.13824074	0.87	0.5615
Error	38	6.05500000	0.15934211		
Corrected Total	47	7.29916667			

R-Square	Coeff Var	Root MSE	E Mean
0.170453	309.0401	0.399177	0.129167

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Trt	5	0.70666667	0.14133333	0.89	0.4993
rep	3	0.20416667	0.06805556	0.43	0.7347
row	1	0.33333333	0.33333333	2.09	0.1563