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The Potential for Dickeya dianthicola to be Vectored by Two Common Insect Pests of Potatoes

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THE POTENTIAL FOR *DICKEYA DIANTHICOLA* TO BE VECTORED BY TWO COMMON INSECT PESTS OF POTATOES

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An Abstract of the Thesis Presented
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Dickeya dianthicola (Samson) causing blackleg and soft rot was first detected in potatoes grown in Maine in 2014. Previous work has suggested that insects, particularly aphids, may be able to vector bacteria in this genus between plants, but no conclusive work has been done to confirm this theory. In order to determine whether insect-mediated transmission is likely to occur in potato fields, two model potato pests common in Maine were used: the Colorado potato beetle (*Leptinotarsa decimlineata* Say) and the green peach aphids (*Myzus persicae* Sulzer). Olfactometry and recruitment experiments evaluated if either insect discriminates between infected and uninfected foliage. To determine whether other insect species may display discriminatory recruitment, pitfall traps and adhesive trap cards were set up beside infected and uninfected plants placed in the field. In the laboratory, beetles and aphids were fed plants infected with *Dickeya dianthicola* and then transferred onto uninfected plants to determine if bacteria would be transmitted between plants. Both plants and insects were sampled and tested using polymerase chain reaction (PCR) for *Dickeya* spp. In 2017 and 2018, a single potato field was divided into five rows of four plots with randomly assigned insecticide treatments to

selectively suppress aphids and/or Colorado potato beetles. Disease spread was monitored among plants in 2017, and tubers were harvested in both years to test for *Dickeya*.

Neither Colorado potato beetles nor green peach aphids were attracted to infected foliage in either olfactometry or recruitment experiments. To the contrary, the presence of 2,3-butanediol, which is product of *Dickeya* fermentation, significantly reduced beetle attraction to the odor of potato foliage. Green peach aphids preferred uninfected foliage, but only when conspecifics were present. Flea beetle (Coleoptera: Chrysomelidae: Alticinae) captures by adhesive cards were affected by the infection status of provisioned potato plants, but the effect was inconsistent between the dates of trap deployment. Neither Colorado potato beetles nor green peach aphids acquired and transmitted *D. dianthicola* through feeding on infected plants in the laboratory. In the field, neither insect's abundance correlated significantly with the spread of this disease.

This study did not find indications that *D. dianthicola* is vectored by either Colorado potato beetles or green peach aphids. Therefore, controlling these pests is unlikely to prevent blackleg outbreaks in potato fields. Instead, the efforts to limit spread of this disease should focus on sanitation, water management, and seed screening.

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CHAPTER 1

EPIDEMIOLOGY OF *DICKEYA DIANTHICOLA* AND ITS POTENTIAL INSECT VECTORS

The soft rot phytopathogen *Dickeya dianthicola* (Samson) has been observed causing disease among cultivated potatoes (*Solanum tuberosum* L.) grown in Maine and neighboring states (Jiang et al., 2016; Johnson, 2018). With some *Dickeya* strains having been blamed for yield losses as high as 30% in the Netherlands and 25% in Israel (Golanowska & Lojkowska, 2016), *D. dianthicola*'s appearance in North America is a cause for alarm. Unfortunately, there is the lack of information on the biology and ecology of individual pectolytic erwiniae species. In part, this can be attributed to the relative ambiguity of historic records concerning the taxonomy of these bacteria (Ma et al., 2007).

After rice and wheat, potatoes are the third most important food crop in the world and are capable of producing two to four times more food per hectare than either grain and at seven times greater water efficiency (Scott & Suarez, 2012; Devaux et al., 2014; CIP, 2018). In 2016, US farmers harvested approximately 19.4 million metric tons of potatoes for both processing and direct human consumption, making this country the 5th largest producer of cultivated potatoes in the world (NPC, 2018). China and India are the world's top producers of potatoes at over 72 and 40 million tons per year, respectively (Scott & Suarez, 2012; Singh & Joshi, 2016). Here and elsewhere in the world, potatoes have been taken up by local peoples to add variety, vitamin C, and important dietary minerals to traditionally grain-based diets. Thus, introduction of potato cultivation has been looked at by both local and international groups as a way to reduce levels of chronic hunger and malnutrition while simultaneously providing a source of employment and

income where chronic poverty and landlessness are persistent problems (Devaux et al., 2014; Scott & Suarez, 2012).

A member of the nightshades, family Solanaceae, the modern cultivated potato (*Solanum tuberosum* L.) descends from a complex of wild, tuber-bearing plants collectively referred to as *S. brevicaule* (Bitter) that are distributed between Peru and Argentina (Spooner et al., 2005). The indigenous peoples of the Andes are believed to have begun domesticating regional *S. brevicaule* varieties roughly 10,000 years ago (Ovchinnikova et al., 2011). The region remains a hotbed of potato genetic diversity and efforts have been made in Peru to study and preserve the approximately 5,000 traditional cultivars for future use in future selection efforts (Glendinning, 1979; FAO, 2008). This is important because the majority of potato cultivars grown outside of the Andes are genetically impoverished tetraploids derived from a small number of cultivars brought to Europe in 1570 and 1590 (Hawkes, 1979).

Among the challenges which face large scale potato production efforts are soft rot and blackleg disease caused by *Dickeya* spp. (Samson) and some strains of *Pectobacterium* spp. (Waldee). These two genera of pectolytic, necrotrophic bacteria belong to the family Enterobacteriaceae. Members of this family are all Gram negative, non-spore forming, bacilliform bacteria (Borman et al., 1944). The family includes many medically important bacteria such as *Escherichia* (Castellani & Chalmers), *Klebsiella* (Trevisan), and *Salmonella* (Lignières) (Borman et al., 1944; Toth et al., 2003), as well as many plant pathogens which were formerly lumped together under the genus *Erwinia* (Winslow). All of these phytopathogens undergo anaerobic fermentation, convert sugars into acids, and have peritrichous arrangements of their flagella. However, the use of 16S rDNA analyses demonstrated that the genus was actually composed of several disparate parts, species which were related but distinct enough to

be removed from *Erwinia*. While some species were reassigned to the preexisting genera, such as *Enterobacteria* (Hormaeche & Edwards), others required new genera. These new genera include *Pantoea* (Gavini), *Brenneria* (Hauben), *Pectobacterium*, and *Dickeya* (Hauben et al., 1998; Kado, 2006; Ma et al., 2007; Toth et al., 2011). As a result of this early waste-basket treatment of phytopathogenic enterobacteria and their subsequent revision, most historic records concerning *Erwinia* bacteria cannot be accurately mapped to currently recognized species (Ma et al., 2007).

In potatoes, *Dickeya* and *Pectobacterium* are the causes of blackleg, aerial stem rot, and slow wilt (Ma et al., 2007). Several species of *Dickeya* have spread across much of Europe and Israel in recent decades, resulting in increased crop losses (Cappaert et al., 1988, Laurila et al., 2008; Toth et al., 2011). This development has been unexpected because most *Dickeya* species had been believed restricted to tropical and subtropical conditions (Czajkowski et al., 2011, Toth et al., 2011) as supported by their higher optimum growth temperatures than the more temperate *Pectobacterium* (Toth et al., 2011). The only exception prior to 2005 had been strains of *D. dianthicola* isolated from Western Europe but were unknown in the United States (Czajkowski et al., 2011; Toth et al., 2011). As *Dickeya* spp. are proving to be tolerant of more temperate growing conditions, there is a worry that their spread and concomitant crop losses will increase, especially as the planet's climate warms (van der Wolf et al., 2007; Toth et al., 2011).

The visible symptoms of blackleg disease include the development of watery, darkly discolored lesions on the stems, roots, and tubers of potatoes, wilting leaves, and stunted plant growth (Czajkowski et al., 2011; Laurila et al., 2008; Tsror et al., 2009). The damage caused by *Dickeya* can be seen not only in the field during the growing season, but also while tubers are kept in storage, where it causes spoilage. The problem is particularly serious in large scale storage facilities that do not employ refrigeration (Laurila et al., 2008). When the necrosis

originates from below the soil, such as through damaged roots or via an infected mother tuber, a creeping, dark green-to-black discolored lesion develops at the stem base and the pith tissue within the stem hollows, a condition known as “blackleg” (Cappaert et al., 1988; Czajkowski et al., 2010a, 2010b). When *Dickeya* cells enter through above-ground wounds or scars, the lesions begin in the stems and migrate both up and down within the host’s tissues. This is referred to as “aerial stem rot” (Cappaert et al., 1988; Czajkowski et al., 2010a, 2011). These soft rot symptoms are usually observed when growing conditions are very wet and temperatures are above 25°C. Unlike the pre-existing *Pectobacterium* established in Maine, *D. dianthicola* possesses a higher degree of virulence and a lower minimum infective dose, capable of establishing and eliciting disease symptoms within a host tuber from as few as 10 founding cells (Toth et al., 2011). However, under drier, cooler conditions, host plants manifest infections as a chlorotic “slow wilt” of the leaves and stems with gradual desiccation (Czajkowski et al., 2011).

Recent work strongly suggests that water is critical in the movement and symptomology of *Dickeya* infections. First, *D. dianthicola* cells move using flagella and require water films to move through soil and plant tissues. When the soil is wet, bacteria are capable of migrating as far as 10 meters in search of a new host (Toth et al., 2003). Once such a host is found, entrance occurs via tuber lenticels that expand when soils are inundated, or by way of wounds found on tubers or roots (Czajkowski et al., 2010a). Once inside a plant, bacteria reside within interstitial spaces of the parenchyma, where they remain largely asymptomatic until experiencing suitable environmental conditions. When temperatures rise and water is present in excess, the host plant’s oxygen-dependent chemical defenses are reduced and subsequently overcome by the bacteria. At such time, *D. dianthicola* cells secrete several different pectinases, which macerate the host’s tissues by destroying their structural integrity. The subsequent rupture of the host’s cells liberates

nutrients that were locked up within the plant's tissues (Toth et al., 2003). With the immediate environment now saturated with free carbohydrates, the bacteria rapidly multiply and migrate through the host's vascular tissues. As they spread, the bacteria metabolize the available nutrients into fermentative byproducts, such as common volatile compounds 2,3-butanediol and succinate (Effantin et al., 2011). As the host dies, bacterial cells are shed into the environment through run-off, which in turn allows for infection of new hosts (Czajkowski et al., 2010a).

The goal of this investigation was to evaluate the potential of insect pests of potatoes to serve as potential vectors for *D. dianthicola*. Previously, it has been well established that contaminated soil can serve as a medium for the transmission of blackleg bacteria (Czajkowski et al., 2010a). Recent work also suggests that surface water, both stagnant and running, may serve as sources of inoculum for erwiniae bacteria, including *Dickeya*, in both Europe and North America (Cappaert et al., 1988; Laurila et al., 2008; Potrykus et al., 2016). However, there has been little work to look at whether insects may be capable of transmitting *Dickeya* between host plants.

The idea that such a relationship may be present stems from the observation of other erwiniae bacteria. *Pantoea* (*Erwinia) stewartii* (Margaert) is known to exploit the corn flea beetle, *Chaetocnema pulicaria* (Melsheimer), as a means for dispersal between corn hosts (Menelas et al., 2006). Two species of *Erwinia*, *E. tracheiphila* (Smith) and *E. amylovora* (Burrill), also exploit insects in order to vector between hosts (cucumbers and apples, respectively) (Sasu et al., 2010; Johnson & Stockwell, 1998). Similarly, the closely related *Pectobacterium (Erwinia) carotovorum* (Jones) can be transmitted by drosophilid flies between infected and uninfected potatoes under both laboratory and field conditions (Kloepper et al., 1981; Molina et al., 1974). *P. carotovorum* appears to infect its vectors as opposed to being

mechanically transmitted on contaminated body parts, as evidenced by its eliciting a unique, systemic immune response in *Drosophila melanogaster* (Meigen) following ingestion of *Pectobacterium* cells by larvae during regular feeding (Basset et al., 2000).

With *Dickeya*, concerns over a possible insect vector-phytopathogen relationship grew when it was discovered that several erwinia species are capable of shifting from a phytopathogenic lifestyle to that of an entomopathogen when consumed by the pea aphid, *Acyrthosiphon pisum* (Harris). Virulence was seen to be quite variable, with *D. dadantii* (Samson) proving to be the most virulent species, while *P. carotovorum* was among the least virulent species (Grenier et al., 2006). When *D. dadantii* cells are ingested by a pea aphid, the bacteria multiply within the gut lumen and eventually escape into the coelomic cavity. The resulting septic infection can spread quickly throughout the aphid's body to penetrate most of its somatic tissues and those within any developing embryos that are present (Costechareyre et al., 2012). Emergence from the gut lumen into the coelomic cavity is achieved with the aid of Cyt toxins homologous to those produced by *Bacillus thuringiensis* (Berliner) (Grenier et al., 2006; Costechareyre et al., 2012). These genes are likely the result of horizontal gene transmission by way of a gram positive donor bacterium, and are not found within *Pectobacterium*, explaining, at least in part, the difference in virulence between these sometimes co-occurring bacteria (Grenier et al., 2006; Costechareyre et al., 2012).

Once the infection enters a septic stage, pea aphids quickly turn black and die, either falling to the ground, where their bodies may retain viable bacteria for up to a week, or hanging from the host plant by their embedded rostra (Grenier et al., 2006; Costechareyre et al., 2012). However, *D. dadantii*'s entomopathic ability is not expressed universally across all insect species, as inoculations of *Drosophila melanogaster* (Meigen), *Spodoptera littoralis* (Boisduval),

and *Sitophilus oryzae* (L.) do not result in bacterial infection as seen in pea aphids. Interestingly, the lethality of *D. dadantii* was also not uniform among all life stages of pea aphids. Young alate individuals were less sensitive to the impacts of infection than apterous adults (Grenier et al., 2006). This suggests the possible existence of a co-evolutionary relationship, where bacteria are less virulent to winged aphids that can potentially serve as its vectors as they disperse. Overall, it is reasonable to suggest that insects are able to vector *D. dianthicola* between potato plants. However, no experimental evidence exists to address this suspicion.

In the state of Maine, several species of aphids are pests of potatoes, with the green peach aphid, *Myzus persicae* (Sulzer), among the most common (UMCE, 2016). Green peach aphids are an especially economically important species not simply because of the mechanical and physiological damage that they cause to immature and growing plants, but because they have been linked to the transmission of over 100 viral diseases across 30 economically important plant species (van Emden et al., 1969; Capinera, 2017). They exhibit a high degree of polyphagy, utilizing species across 50 plant families (Silva et al., 2012), which may stem from their selection of suitable hosts based on the target's physiological condition rather than taxonomic affiliation, favoring either growing or mature foliage depending on the species of host (van Emden et al., 1969). Green peach aphids also have a high propensity for pesticide resistance development due to both their rapid generational turn over (with up to 20 generations per year) and multiple, independent, insensitivity-mediating physiological mechanisms to overcome both plant and artificial chemical defenses (Capinera, 2017; Silva et al., 2012).

Green peach aphids display a variably heterocyclic, multigenerational annual cycle where it alternates between herbaceous secondary host plants during the growing season, and feeds on *Prunus* spp. (L.) during the end and very beginning of the year. At temperate latitudes, spring

and early summer populations of green peach aphids consist of parthenogenic, viviparous females feeding on potatoes, citrus, beets, beans, brassicas, and many other plants (Capinera, 2017; Dixon, 1977). These "viviparae" can multiply very rapidly, producing 30-80 nymphs over the course of their lives (van Emden et al., 1969). Should they become over crowded or should the current host plant's condition deteriorate, the apterous viviparae will produce offspring that mature into alate viviparae and disperse to new plants, where they give birth to new apterous viviparae. As photoperiods decrease in late summer and fall, viviparae give birth to gynoparae, special parthenogenic viviparae whose offspring will mature into oviparous alate females ("oviparae") and alate males. These sexual alates move to overwintering hosts and mate, after which oviparae seek out bark cracks and buds of *Prunus* spp. (L.) in which they deposit their cold tolerant eggs. The eggs endure the winter months in a state of diapause, which terminates synchronously with the host's spring bud crack (van Emden et al., 1969).

Due to their competence as vectors of plant viruses, their complex seasonal life cycles, and the aforementioned polyphagy, green peach aphids are able to transmit pathogens between different plant species (Manachini et al., 2007). This is important as at least some *Dickeya* strains are known to attack other plants such as maize and brassicas (Ma et al., 2007) and highlights the need to evaluate this insect's competence as a vector of this bacterium. Even so, there are other insects within potato fields that may be capable of acting as vectors for *Dickeya*. Decomposing plants are readily visited by *Drosophila melanogaster* (Meigen) and other dipterans, which have been previously shown to be able to carry and disperse *Pectobacterium* (Kloepper et al., 1981; Molina et al., 1974). Similarly, the anthomyiid fly *Delia radicum* (L.) has been shown to transmit a *Pectobacterium* to crucifers (Baur et al., 1998). It and other *Delia* spp. (Robineau-Desvoidy)

have been recently suggested to play a role in the transmission of this and other soft rot bacteria in potato fields (Rossmann et al., 2018).

In addition to aphids, potato fields support many species of insect pests, some of which might be capable of transmitting *Dickeya*: thrips (Fathi et al., 2008), members of the click beetle genus *Agriotes* (Eschscholtz) (Parker & Howard, 2001), the flea beetle *Epitrix cucumeris* (Harris) (Boiteau, 1983), plant bugs of the genus *Lygus* (Hans) (Boiteau, 1983), the potato leaf hopper *Empoasca fabae* (Harris) (Radcliffe, 1982), and the moths *Ostrinia nubilalis* (Hübner) (Kennedy, 1983) and *Phthorimaea operculella* (Zeller) (Baggen & Gurr, 1997) are some examples. However many beneficial insects also live within managed potato fields. These can include predatory true bugs such as *Nabis* (Latreille) and *Geocoris* (Fallén) (Boiteau, 1983; Chang & Snyder, 2004; Koss et al., 2005), parasitoid wasps (Boiteau, 1983), aphidophagous syrphid flies (Tschumi et al., 2016), and carabid beetles which can prey either on insect pests (Alvarez et al., 2013) and the seeds of weed plants (Gaines & Gratton, 2010) depending on the species. While pest insects are what take immediate attention when investigating the potential for a pathogen to be spread by insects, it's important not to neglect the presence of predators and parasitoids. Predators and parasitoids can reduce the abundance of an insect vector, thus reducing transmission. However, the presence of a predator or parasitoid can also increase dispersal of an insect vector, potentially increasing disease spread (Jeger et al., 2011). This, while a predator or parasitoid may not directly transmit a plant pathogen like *D. dianthicola*, it may have an indirect role in its spread.

Another important and abundant pest of potatoes is the Colorado potato beetle, *Leptinotarsa decemlineata* (Say). Originally restricted to the southwestern North America, the Colorado potato beetle is an oligophagous folivore of Solanaceae (Hare, 1990), originally

feeding on species native within its range such as buffalobur (*S. rostratum* Dunal) and silverleaf nightshade (*S. eleagnifolium* Cav.) (Weber & Ferro, 1997). A shift to cultivated potatoes was not observed until 1859 and came at great cost to farmers (Alyokhin, 2009). Outbreaks of this insect can develop rapidly, and, if untreated, result in complete plant defoliation and total loss of crop (Hare, 1990). Since that change, the beetle has extended its range across North America, Europe, northern Africa, and western Asia (Weber & Ferro, 1997). Over this area, Colorado potato beetles have adapted to tolerate a wide range of habitat conditions, and display variability in their life histories, host preferences, and pesticide resistances.

In general, Colorado potato beetles are capable of producing one to three generations per year, with more generations where the growing seasons are longer and winters are mild. Development from egg to adult can be completed in as little as 21 days at 25-30°C. Adults and larvae feed on the same host plants, and females deposit their eggs on the undersides of leaves. A single female mates multiple times, and can produce 500-800 (in extreme cases, even up to 4,000) eggs in 20-60 masses over the course of her life time (Weber & Ferro, 1997; Hare, 1990). Eclosion occurs simultaneously, and the larvae go through four instars within a span of 10-20 days before abandoning the host plant to burrow into the surrounding soil (Hare, 1990). Following an approximately 10-day pupation, teneral adults emerge from the ground, locate a suitable host plant, and feed, mate, and reproduce.

In temperate latitudes, environmental stimuli such as declining food quality and decreasing photo periods stimulate Colorado potato beetles to abandon their host plants and burrow 20-50 cm into the ground and undergo hibernal diapause. Those living in milder, more equatorial latitudes may remain on the soil surface but still undergo hibernal diapause (Hare, 1990; Weber & Ferro, 1997). The ability to diapause is a second characteristic that make

Colorado potato beetles so difficult to combat, especially in northern latitudes. While many beetles come out of hibernal diapause the following spring, the length of a beetle's diapause and the sensitivity to termination cues can vary greatly between individuals, allowing it to last as long as two or more years (Weber & Ferro, 1997; Tauber & Tauber, 2002). Beetles are also capable of undergoing multiple diapauses, as declines in food quality and in temperatures can stimulate aestival diapause as well as hibernal diapause. Due to this variation, each generation can add to a bank of dormant beetles buried 20-50 cm below the surface (Weber & Ferro, 1997). After hibernal diapause is broken, males and females emerge and locate suitable host plants.

Colorado potato beetles show ability for rapid evolution of resistance to a wide range of pesticides within only a few generations. This species has developed behavioral and physiological resistances to 52 different insecticides from every major class, although resistances are not uniformly distributed across all populations or life stages (Alyokhin et al., 2008; Mota-Sanchez et al., 2006). It's been consistently shown that within one to three years after a pesticide enters the market that populations of CPB are observed to express resistance to the pesticide in question (Mota-Sanchez et al., 2006). This capacity for resistance-adaptation combined with the previously discussed factors together make Colorado potato beetles globally recognized as among the most significant potato pests (Alyokhin et al., 2008; Tauber & Tauber, 2002).

There is at present no evidence connecting Colorado potato beetles with the transmission of potato diseases; however, this may be due to a lack of direct investigation. As a chrysomelid leaf beetle, the Colorado potato beetle is phylogenetically related to known erwiniae vectors, such as the alticine *C. pulicaria* (the vector of *P. stewartii*), and the galerucines *Acalymma vittatum* (Fabricius) and *Diabrotica undecipunctata howardi* (Barber) (the vectors for *E. tracheiphila*). Thus, it is possible Colorado potato beetle may be capable of acting as a vector for

bacterial diseases. As mentioned previously, their feeding is also highly injurious to their host plants and can lead to complete defoliation (Hare, 1990). Thus, both the physiological stress and the mechanical wounds created may make affected plants more susceptible to successful infection by *D. dianthicola*.

Large quantities of 2,3-butanediol produced by *Dickeya* (and *Pectobacterium*) during fermentative metabolism may be attractive to certain insects. Some insect include 2,3-butanediol within their pheromones: Male medflies, *Ceratitis capitata* (Wiedemann), secrete traces of 2,3-butanediol as a component of the male sex pheromones used to attract females for mating or other males when forming a lek (Sollai et al., 2018). Other species are attracted to 2,3-butanediol as an environmental cue: The sorghum chafer, *Pachnoda interrupta* (Olivier) is a pest of fruits and grains such as papaya, bananas, and sorghum. Adult *P. interrupta* detects 2,3-butanediol with other volatiles released by flowers and ripe fruits as an indicator of a potential food source (Bengtsson et al., 2009). The responses of potato pests such as green peach aphids or Colorado potato beetles to *Dickeya* metabolites have not been tested and may provide insight into the dynamics which take place within potato fields between competing consumers regardless of the outcome.

In order to determine whether insects play a significant role in *D. dianthicola* transmission in Maine, this study pursues four lines of inquiry: (1) Do green peach aphids or Colorado potato beetles discriminate between infected and uninfected foliage and if so, what is their response? (2) Can green peach aphids or Colorado potato beetles transmit *D. dianthicola* between potato plants? (3) Does control of insect pests in the field, whether by chemical or mechanical means, influence the scale of blackleg outbreaks? (4) Is there a difference in the community composition of the insects which visit potato plants that have active blackleg

infections compared with plants which are not infected? Each of these questions has significant implications with regard to how researchers, producers, and policy makers move forward in addressing the on-going problems posed by *D. dianthicola*. Furthermore, in the course of conducting this research, the study also demonstrates that *D. dianthicola* can in fact reside asymptomatically within daughter tubers, adding evidence to the hypothesis that transport of asymptomatic seed tubers can serve as a source for *D. dianthicola* geographic dispersal.

CHAPTER 2

INSECT ATTRACTION TO *DICKEYA DIANTHICOLA*

2.1 Introduction

Since at least 2014, potato production industries in the Eastern U.S. have been forced to cope with sudden and growing outbreaks of an adventive bacterial phytopathogen, *Dickeya dianthicola* (Samson) (Jiang et al., 2016, Johnson, 2018). For the fifth largest producer of potatoes internationally, the sudden appearance and spread of a member of a bacterial genus responsible for crop losses as high as 30% in Europe has been a cause for alarm (Scott & Suarez, 2012; NPC, 2018; Golanowska & Lojkowska, 2016). Within the field, the symptomatic expression of infections by this and other *Dickeya* spp. is variable, but typically presents as either a blackened, watery stem lesion (referred to as "blackleg") when moisture availability is high, or as a gradual foliar wilt and stem desiccation (known as "slow wilt") when field conditions are dry (Tsror et al., 2009). Although research efforts have been able to rapidly illuminate several key aspects of *D. dianthicola*'s epidemiology within the last 15 years, efforts to understand this pathogen's biology in the face of wide spread crop outbreaks were delayed due to early taxonomic ambiguity of *Dickeya* and other soft-rot bacteria (Ma et al., 2007).

Dickeya spp. (Samson) are generally unable to survive within soil longer than six months without suitable host plants, and instead are understood to be dependent on latently infected seed tubers for reintroduction to cultivated fields each growing seasons (Czajkowski et al., 2011; Pérombelon, 2000). Previous work has shown that contaminated farm equipment, windborne aerosols kicked up by rain or mechanical flailing, and contaminated surface waters used for irrigation can all play a role in transmission (Czajkowski et al., 2011; Skelsey et al., 2016;

Laurila et al., 2008; Pérombelon, 1979). Work by Grenier et al. (2006) has shown that one species, *Dickeya dadantii* (Samson), elicits a rapidly developing and lethal entomopathic septicemia which is both species specific toward the pea aphid (*Acythosiphon pisum* Harris) and is targeted toward apterous morphs, with elates suffering half the levels of mortality seen among apterous pea aphids. This has been interpreted by some authors to suggest that some insects, such as pea aphids, may be able to transmit certain species of *Dickeya* between host plants (Reverchon & Nasser, 2013). However, pea aphids are not known to feed on solanaceous crop plants.

Several species of herbivorous insects have been observed feeding on cultivated potatoes, sometimes to the degree as to be considered serious crop pests. These include the onion thrips, *Thrips tabaci* (Lindeman) (Fathi et al., 2008), *Agriotes* wireworms (Eschscholtz) (Parker & Howard, 2001), *Epitrix* flea beetles (Foudras in Mulsant) (Boiteau, 1983; Boavida & Germain, 2009), the tuber worm moth *Phthorimaea operculella* (Zeller) (Radcliffe, 1982), the potato leafhopper *Empoasca fabae* (Harris) (Radcliffe, 1982), and the psyllid *Bactericera cockerelli* (Šulc) (Munyaneza et al., 2007). Predators and parasitoids, such as aphidophagous syrphid flies like *Sphaerophoria* spp. (Lepeletier & Serville) and the parasitoid wasp *Aphidius nigripes* (Ashmead), may also influence disease transmission by increasing the rates of dispersal among disease-vectoring prey species trying to avoid predation (Belliure et al., 2011; Boiteau, 1983; Hodge et al., 2011; Tschumi et al., 2016). Several families of flies associated with decomposing matter, such as Scatopsidae, Drosophilidae, and Muscidae, can also be found within and around potato fields and may be capable of acquiring *Dickeya* during foraging (Boiteau, 1983; Kloepper et al., 1981; Rossmann et al., 2018)

Dickeya derives energy through fermentation of plant-derived carbohydrates released during host tissue necrosis and a primary metabolite resulting from this is 2,3-butanediol

(Effantin et al., 2011). While it hasn't been evaluated within the context of *Dickeya*-insect relationships, 2,3-butanediol is known to play a minor and recurrent role as a component of floral- and decompositional volatiles that various insects used to locate sources of food (Bengtsson et al., 2009). This organic volatile is one of several which African sorghum chafers (*Pachnoda interrupta* Olivier) use to locate the ripening grains, fruits, and flowers on which they feed (Bengtsson et al., 2009). Drosophilid flies feed and oviposit on decaying plant matter located by following the 2,3-butanediol-containing volatile blends released by yeasts responsible for fruit fermentation (Becher et al., 2012). The association between drosophilids and 2,3-butanediol-containing volatile blends is evidently so strong that several species of *Ceropegia* (L.) secrete similar volatile blends which contain traces of 2,3-butanediol in order to lure these and other decay-associated flies in for the purpose of pollination (Heiduk et al., 2017).

This attraction by flies to volatile blends containing 2,3-butanediol is interesting as the flies *Drosophila melanogaster* (Meigen) and *Delia* spp. (Robineau-Desvoidy) have been shown to transmit strains of *Pectobacterium* to potatoes and other plant species under both laboratory and field settings (Kloepper et al., 1981; Molina et al., 1974; Rossmann et al., 2018). *Pectobacterium* (Waldee) is a closely related genus of phytopathogenic enterobacteria related to *Dickeya* which also elicit blackleg in potatoes and release 2,3-butanediol as a major metabolite during symptomatic infections (Effantin et al., 2011; Toth et al., 2011). It seems, therefore, entirely plausible that the release of 2,3-butanediole could serve as a mechanism by which *Dickeya* (and conversely *Pectobacterium*) can attract potential insect vectors.

The volatiles released by phytopathogenic enterobacteria have been evaluated before in regard to whether they play a role in vector-mediated transmission. Though it has been previously observed that *Drosophila melanogaster* can acquire *Pectobacterium* spp. and transmit

them to uninfected potato plants (Kloepper et al., 1981; Molina et al., 1974), Brewer et al. (1980) observed no significant preference from *D. melanogaster* for either infected and uninfected potato tissue. However, *Delia antiqua* (Meigen) has been shown to oviposit preferentially on onions exhibiting bacterial infections associated with conspecific feeding (Hausmann & Miller, 1989), and Rossmann et al. (2018) showed that several species of *Delia* were among the most frequent carries of *Pectobacterium*. The causal agent of bacterial wilt in cucurbits, *Erwinia tracheiphila* (Smith), was shown by Shapiro et al. (2012) to reduce floral volatile production while dramatically increasing the concentrations of foliar volatiles released by symptomatic leaves. They demonstrated that by doing this, *E. tracheiphila* manipulates its plant host to attract the bacterium's vector, the stripped cucumber beetles, *Acalymma vittatum* (Fabricius). The beetles are preferentially attracted to the higher volatile concentrations released by symptomatic leaves, but prefer to use uninfected flowers as a location for mating. As *E. tracheiphila* can enter host plants through feeding damage and floral nectaries, this manipulation promotes the transmission of the bacterium, which is spread from the beetles through the frass left behind within cucurbit flowers (Mitchell & Hanks, 2009; Shapiro et al., 2012).

Of the insects common in potato fields, two species are of particular interest with regard to this study. The green peach aphid (*Myzus persicae* Sulzer) and the Colorado potato beetle (*Leptinotarsa decimlineata* Say) are two of the world's most important insect pests of potatoes (Alyokhin, 2009; Godfrey & Haviland, 2008; Radcliffe, 1982). Both species have shown themselves to be both behaviorally and physiologically adaptable, such that they readily evolve resistance toward pesticides (Alyokhin et al., 2008; Silva et al., 2012). Colorado potato beetles and green peach aphids both exhibit high fecundity and are able to build up in number rapidly within a single growing season if left unmanaged (Capinera, 2017; Hare, 1990). What's more,

both insects share confamilial taxonomic relationships with known vectors of other phytopathogenic bacteria, such as *Pseudomonas syringae* (Van Hall) which can be transmitted by the previously mentioned pea aphid, *A. pisum,* and the *Dickeya*-relative *Pantoea stewartii* (Smith) which is vectored by the corn flea beetle, *Chaetocnema pulicaria* (Melsheimer) (Nadarasah & Stavrinides, 2011; Stavrinides et al., 2009).

To find out whether *Dickeya* influences the behavior of insects found within potato fields, two lines of experimentation were used in this study. The first focused on the ability of green peach aphids and Colorado potato beetles to detect and discriminate infected and uninfected host plants through the use of olfactometry and choice assays. The second line of experimentation took into account that a diverse assemblage of insect species live within and around cultivated potato fields by determine whether provision with either individual infected or uninfected plants causes a community level response in either insect richness of abundances in proximity to the provided plants. The determination of whether the presence of *Dickeya dianthicola* within potato plants influences insect behavior is an important step in understanding whether insects actually serve as vectors for blackleg disease or not with northern Maine's potato fields.

2.2 Materials & Methods

2.2.1 Maintenance of insects and plants

The management of insects used in this research follows protocols used by Galimberti & Alyokhin (2018). Colorado potato beetles, *Leptinotarsa decemlineata* (Say) were reared in a colony from wild-caught adults collected from potato fields at the Aroostook Experimental Farm in Presque Isle, ME, and supplemented annually with individuals from that location to maintain

genetic diversity. Colorado potato beetles were kept within 50 cm x 50 cm x 90 cm wood and fine mesh screen insect cages within a greenhouse. Potted potato plants ('Superior') grown from seed tubers provided by the Aroostook Experimental Farm were planted in green plastic azalea pots (ca. 20 cm) with potting mix (Fafard #2 Suchine #8 Grower Mix, Griffin Greenhouse Supplies, Inc., Tewksbury, MA) and dry blended fertilizer (Cavendish Agri Services, Dieppe, Canada). Dry fertilizer and potting mix were measured out and mixed, by volume, in ratio of ca. 250 cubic centimeters fertilizer for 5 azalea pots of potting mix. These plants served as the staple food for both insect species and were maintained in the same greenhouse as the Colorado potato beetles. Entire potted plants were placed in the insect cages for beetles to feed and oviposit on. Beetle's eggs were collected in 100 mm X 15 mm plastic Petri dishes (Fisher Scientific, Hampton, NH) and stored in an incubator (Series 33 Controlled Environment Chamber, Percival Scientific Inc., Perry, IA) at 25°C under a photoperiod of 16:8 (L:D) h until eclosion. Plants were placed inside insect cages and newly hatched larvae were then transferred to the foliage of the caged plants using a small paint brush

Similar to the Colorado potato beetles, the green peach aphids, *Myzus persicae* (Sulzer), were obtained from a laboratory colony reared from wild-caught individuals originating from the same fields in Presque Isle, ME. Green peach aphids were raised on excised potato foliage ('Superior') inserted in vials of water taped to the floors of colony enclosures built from clear plastic jars (ca. 1.9 L) and deli cups (1 L). The enclosures had holes cut in them to provide ventilation. Enclosures were kept at 25°C and a 16:8 (L:D) h photoperiod within the same incubator (Series 33 Controlled Environment Chamber, Percival Scientific Inc., Perry, IA) as the Colorado potato beetle eggs. During warm summer months, green peach aphid colonies were also maintained outside of the incubator on a laboratory shelf at room temperature. Light was

provided with two 1.2 m fluorescent lights (White Ceiling Fluorescent Shop Light, model HBSL-16, Commercial Electric Products, Cleveland, OH) and operated on a 16:8 L:D photoperiod using an electric timer (Intermatic TN311, Intermatic Incorporated, Spring Grove, IL).

2.2.2 Culturing *Dickeya dianthicola*

To prepare liquid inoculum, 100 µL of liquid-suspended bacterial culture of *Dickeya dianthicola* ME30 (Jiang et al., 2016) was cultured in 30 mL of tryptic soy broth (TSB) (Sigma-Aldrich, St. Louis, MO), for 24 hours at 30°C on an incubating orbital shaker (VRW International, Radnor, PA). While centrifuge tubes were preferred for this purpose, 50 mL clean, autoclave-sterilized glass flasks (Pyrex, Greencastle, PA) sealed with aluminum foil (Reynolds Consumer Products LLC, Lake Forest, IL) were used identically when supplies of centrifuge tubes were limited.

To isolate bacteria for long term storage, 100 µL of the aforementioned liquid inoculum was applied to 100 mm x 15 mm polystyrene Petri dishes (Fisher Scientific, Hampton, NH) containing crystal violet pectin (CVP) medium. CVP medium was made following the protocols by Hélias et al. (2011). The liquid applied to the media was spread evenly using a flame-sterilized glass cell spreader. Each plate was then sealed using Para-film (Bemis, Neenah, WI) and placed within an incubator (Series 33 Controlled Environment Chamber, Percival Scientific Inc., Perry, IA) set to either 28 or 30° C with 16:8 (L:D) h photoperiod for 48 hours (Czajkowski et al., 2010a, 2015; Hao, 2017, personal communication). After incubation, each plate was inspected for discrete colonies which caused cavitation of the medium. A single colony was selected and added to liquid TSB medium and incubated at 30°C for 24 hours to create inoculum. One mililiter of the inoculum was added to 2 mL microcentrifuge tubes with attached snap caps

(Global Scientific Supply, Wilmington, NC) together with 1 mL of 50% glycerin. The suspension was then homogenized for 5 seconds on a digital pulsing vortex machine (VWR International, Radnor, PA), labelled, and placed in -80°C storage until needed.

All work involving a sterile environment was carried out in a sterile transfer hood (SterileGard III Advanced, The Baker Company, Sanford, ME). That included, but was not limited to, pouring media plates, plating bacteria, or transferring bacteria to TSB medium to make inoculum.

2.2.3 Plant inoculation

Mechanical injuries, such as through flailing (Czajkowski et al., 2011), have been previously implicated as potential routes of entry for *Dickeya*. Therefore, a method of trauma-based inoculation based on Czajkowski et al. (2010b) was used over the course of this research to artificially infect plants with *D. dianthicola*.

Potato plants were grown as described above within 25.4 cm green plastic azalea pots from seed tubers ('Superior') produced by the Aroostook Research Farm in Presque Isle, ME. When potato stems were at least 25 to 30 cm tall and possessed mature leaves, wounds were made using sterile pipette tips (one tip per plant) in either the stem or petioles by repeated puncturing (stem) or scratching (petiole). Stems too small to support such wounding were removed. Either 10 or 100 µL of liquid was injected into the wound of each stem. Liquid used was either non-sterile distilled water (for control treatments) or TSB-based inoculum of *D. dianthicola* ME30. The volume of liquid used depended on the protocols, but the change from the initial 10 to 100µL was adopted when attempts to inoculate plants with visible soft rot lesions proved difficult. Later, incubation temperature was identified as the probable source of

complication, but to ensure continued success, the volume of liquid applied to wounds was maintained at 100 µL.

Following injection, each stem was wrapped with Para-film (Bemis, Neenah, WI) to seal the wounds and to slow down desiccation. Depending on the experimental protocol, plants were either allowed to incubate at room temperature to induce slow wilt (used when plants needed to be utilized over several weeks) or incubated at 28 or 30°C in an incubator (Series 33 Controlled Environment Chamber, Percival Scientific Inc., Perry, IA) set to a photoperiod of 16:8 (L:D) h (used when rapid development of symptoms was needed). Plants were incubated at 28 to 30°C for a maximum of seven days, but were removed to continue incubating as room temperature if the disease had caused the plant's stems to collapse before the end of the incubation period had been reached. That approach was needed to ensure that useable foliage was available for experiments. During incubation, plants were placed in plastic planter saucers (Duraco Products, Inc., Streamwood, IL) and watered excessively from bellow over the course of their incubation in order to induce physiologic stress upon the plants, a factor which benefits *Dickeya* (Czajkowski et al., 2011).

Unless otherwise specified, infected plants were inoculated through injection to the stem as it was observed during an initial investigation into how to best inoculate plants found that infections of the petiole typically resulted in abscission and poor translocation of the bacteria within the plant. This observation was found in support of results from work by Czajkowski et al. (2010a).

2.2.4 Molecular confirmation of infection

Depending on the source of tissue samples, several methods were used to retrieve and extract bacterial DNA. Samples were collected from potato stems and were processed according to *Dickeya* detection protocols drafted on February 22, 2016 by the Maine Potato Board. Stem tissue was excised from the bottom 2 cm of one to three stems per plant. Tissue expressing lesions was sampled preferentially. When no lesions were present on plants which had received injections, the sampled tissue included the site of injection. Sampling was done using a knife sterilized with 70% ethanol and flame-treated between samples with an ethanol burner. Excised tissue was placed in ca. 3 mL of sterile distilled water for at least five minutes to allow bacteria to stream from the cut ends of the stem tissue. The 200 µL of water with suspended bacteria was then sampled and put through the DNA extraction process following the instructions and supplies contained within the MP Bio FastDNA Kit (MP Biomedicals, Santa Ana, CA). All infected plant material was autoclaved after use and then discarded.

After tissue sampling and extraction of DNA following manufacturer's directions (MP Biomedicals, Santa Ana, CA), samples were analyzed by PCR using known genus-level primers, pelADE1 (GATCAGAAAGCCCGCAGCCAGAT) and pelADE2 (CTGTGGCCGATCAGGATGGTTTTGTCGTGC) (Nasser et al., 1996). Once DNA was extracted, diluted aliquots were made between 20 and 30ng/µL. Higher concentrations were used to check the validity of results if samples came up negative. Once aliquots were made, 1 µL of the diluted DNA was added to 24µL of master mix within 0.2 mL polypropylene 8-strip tubes with flat strip caps (Olympus Scientific Solutions America, Waltham, MA). The master mix was comprised of 17.9 µL sterile molecule-grade water, 5.0 µL 5x colorless GoTaq reaction buffer (Promega, Madison, WI), 0.5 µL 10mM dNTP mix (Promega, Madison, WI), 0.25 µL 10 mM

ADE1, 0.25 µL 10 mM ADE2, and 0.1 µL GoTaq DNA polymerase (Promega, Madison, WI) for each sample. The resulting 25 µL mixtures were gently flicked to mix the ingredients and were then spun through a mini centrifuge (Cat# 05-090-100, Fisher Scientific, Hampton, NH) to remove air bubbles. Amplification was done using a T100 thermocycler (Bio-Rad Laboratories, Hercules, CA) with the following program (Hao, Morangoni & Ge, personal communications):

Table 2.1. The thermocycler program used for DNA amplification using pelADE primers.

Step	Temp.	Duration
1	94°C	5:00
2	94°C	0:40
3	56°C	0:40
4	72°C	0:40
5	Goto Step 2	X42
6	72°C	10:00
7	12°C	0:00

2.2.5 Insect response to foliar volatiles from *Dickeya*-infected plants

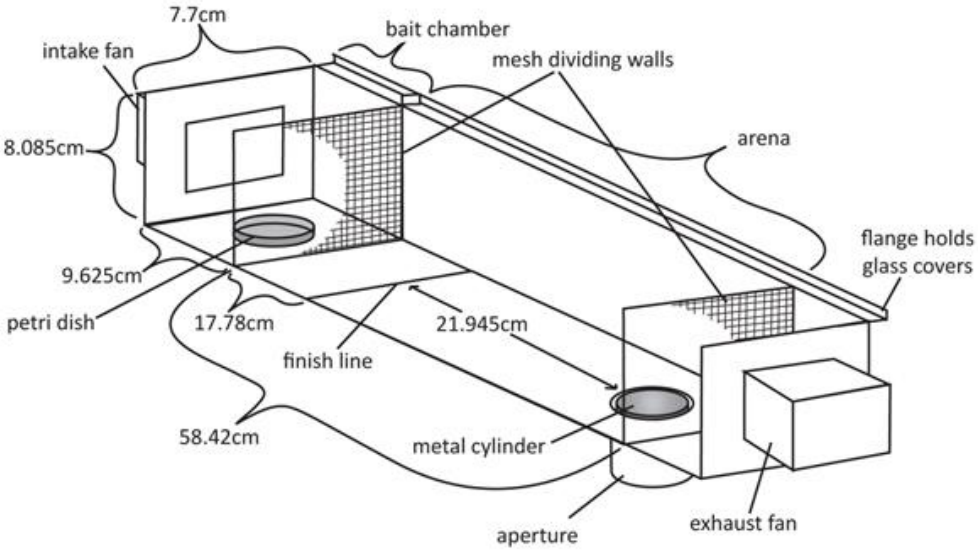
All infected foliage in olfactometery work was taken from plants which were incubated at room temperature to induce slow wilt, so that plants would be able to provide foliage over several weeks. Plants were replaced as remaining foliage became unsuitable for use in the experiment.

2.2.5.1 Colorado potato beetle olfactometry

To test the influence of various odor baits on adult Colorado potato beetle behavior, a custom-built wind tunnel was utilized. The wind tunnel, which measured ca. 8 cm high by 7.7 cm wide by 1 m long, was comprised of a two-chamber system, with an anterior chamber measuring 9.5 cm long for the bait and a tunnel measuring 58.42 cm long. The floor at the end of the tunnel opposite of the bait chamber contained an aperture through which insects were

introduced. A mesh screen separated the two chambers from one another. Wind speeds could be adjusted with a dial from 0.8 m/s to a maximum of 1.2 m/s, as measured using a flow meter (Kestrel 1000, Kestrel Instruments). The lowest speed (0.8 m/s) was always used to reduce the impact of wind speed on insect behavior. Two glass panels covering the top of the tunnel and the bait chamber allowed for observations and easy access to the interior for retrieval of beetles and bait, as well as for cleaning. During operation, the wind tunnel remained within an active, ventilated fume hood (Labconco Corp, South Kansas City, MO) to prevent odors from lingering between trials.

Fig. 2.1. A simplified diagram of the wind tunnel used to test the responses of beetles to different olfactory cues.



Adult Colorado potato beetles were tested individually for their response to one of several types of odor-baits. Beetles were given 30 minutes to cross a line 22 cm from the starting point. Failure to reach this line within the time limit was recorded as being “unresponsive,” while crossing the line ended the trial and was recorded as “responsive.” Between the trials, the interior

of the wind tunnel was cleaned with acetone that was allowed to evaporate before the beginning of the next trial. No individual beetle was released in the wind tunnel more than once.

The first olfactometer experiment tested the responses of 80 Colorado potato beetles, 20 per type of bait. The following baits were tested: 10 mL of 2,3-butanediol (Sigma-Aldrich, St. Louis, MO), 10 mL of distilled water, an excised potato leaflet from an uninfected plant which received no injections, and an excised leaflet to which 10 mL of 2,3-butanediol was applied. The organic volatile 2,3-butanediol is a major fermentative byproduct of *Dickeya* metabolism (Effantin et al., 2011) and was being tested as a proxy for infected foliage while methods to infect plants in a controlled manor were under investigation. The second experiment was conducted using only two odor baits: an excised leaflet from an infected potato plant, and an excised leaflet from an uninfected, uninjected plant. Again, 20 adult Colorado potato beetles were tested per type of bait, for a total of 40 individuals. Bait was placed in a glass Petri dish (Pyrex, Greencastle, PA) and was cleaned and swapped with a new Petri dish between trials. Both experiments controlled for the sex of the insects, testing equal numbers of males and females with each type of bait.

Results of this experiment were quantified and analyzed using the χ^2 test with a threshold of 0.05 in R (R Core Team, Vienna, AT).

2.2.5.2 Green peach aphid olfactometry

To test whether green peach aphids respond to volatiles released by infected foliage, a Y-tube olfactometer (2-port Humidifying Air Delivery System, model #OLFM-HADS-2AFM2C, Analytical Research Systems, Gainesville, FL) was used because adult aphids proved too small to use with the wind tunnel designed for Colorado potato beetles. Apterous adult aphids were

tested individually, given the choice between an air current baited with the scent of foliage or an unbaited air current with no foliar volatiles. Between each trial, all glass equipment was rinsed with acetone to remove any odor residues. Aphids were given 60 minutes to walk half way up one of the two arms, at which time the trial ended and the results were recorded. If the aphid traveled up the baited arm, it was interpreted as having a "positive response." If the aphid traveled up the unbaited arm, it was interpreted as having a "negative response."

Two experiments were run using this system. In the first experiment, aphid responses to foliar volatiles were compared between excised foliage taken either from plants that had been given traumatic injections of *D. diathicola* ME30 or from uninfected plants which had received no injections at all. In the second experiment, aphids were exposed to foliage that had been taken either from plants infected using traumatic injection with *D. dianthicola* ME30 or from plants which had been given traumatic injections of sterile distilled water. This second experiment was done to determine whether aphids were responding to volatiles released by plants in association to previous mechanical damage and not specifically to volatiles associated specifically with *Dickeya* infections. The results of this experiment were analyzed using a χ^2 test in R (R Core Team, Vienna, AT).

2.2.6 Insect recruitment to infected foliage in artificial arenas

2.2.6.1 Colorado potato beetles

Three outdoor enclosures were constructed at the Aroostook Research Farm in Presque Isle, ME (Booth and Alyokhin, 2016). Each enclosure measured 4 m x 3 m x 8 m and was constructed of wood and aphid mesh screen. A latched door allowed for access to the interior, where a false bottom made of purple insulation foam was covered in field soil to simulate the

conditions of local fields. Two holes spaced 1 m apart were present in the false bottom where potted plants were inserted. One plant had been inoculated with *D. dianthicola* ME30 using traumatic stem injections and incubated for seven days at room temperature. The other was an uninfected, uninjected plant. After placing plants in the cages, the pots were covered with soil and hidden, while 10 beetles (5 of each sex) were released from a Petri dish (Fisher Scientific, Hampton, NH) equidistant between the two plants. After 24 hours, each cage was checked and the number of beetles on each plant was counted. Collected data were analyzed using Welch's two sample t-test in R (R Core Team, Vienna, AT).

2.2.6.2 Green peach aphids

To determine if green peach aphids would choose between infected and uninfected host foliage when presented with both simultaneously, a choice assay was designed. Using a clean 100 mm X 15 mm Petri dish (Fisher Scientific, Hampton, NH) as an enclosure, a brown paper towel was cut, laid on the floor of the dish, and lightly sprayed with water to help maintain humidity during the experiment. Two excised leaflets, one from a plant inoculated with *D. dianthicola* ME30 via traumatic injection and one leaflet from an uninfected plant which received no injections, were placed within opposite ends of the dish. The position of the infected leaflet was marked on the underside of the Petri dish using a permanent felt-tipped marker (Sharpie, Oak Brook, IL).

In the first experiment, ten apterous adult green peach aphids were placed in the center of the dish, equidistant between the two excised leaflets. The closed Petri dish was then sealed with Para-film (Bemis, Neenah, WI) to prevent escape and desiccation and left for 24 hours in an incubator set to 30°C and a 16:8 (L:D) h photoperiod. After 24 hours, the dish was opened and

the number of aphids on each leaflet was tallied. The experiment was replicated 30 times in sets of 10 trials.

The second experiment followed the same protocol, but one apterous adult aphid instead of ten was released in each dish to control for possible communication among the released aphids. Data from both grouped and individual aphid trials were analyzed using the Wilcoxon Test in R (R Core Team, Vienna, AT).

2.2.7 Insect recruitment to *Dickeya*-infected plants in the field

To determine whether the presence of a *Dickeya* infection within a potato plant attracts members of the adjacent insect community, insects were sampled in the field using infected and uninfected plants as bait. Twelve potted potato plants produced using the previously discussed method received randomly assigned traumatic injection treatments: six plants were injected with 100 µL/stem of TSB-based *D. dianthicola* ME30 inoculum and 6 plants were injected with 100 µL/stem of nonsterile distilled water. Injection sites were wrapped in parafilm, and the plants were incubated at 30°C for 48 hours in an incubator (Series 33 Controlled Environment Chamber, Percival Scientific Inc., Perry, IA). Following incubation, plants were placed randomly in an untreated potato plot on Aroostook Research Farm. For ease of retrieval, plants were left in their pots which were buried in the rows of the field.

One pitfall trap was placed ca. 30 cm in front of each experimental plant, and one yellow adhesive trap card was placed ca. 30 cm behind each experimental plant. Each trap card (IPM corn rootworm traps, Great Lakes IPM, Inc., Westburg, MI) was stapled 46 cm above the ground to a 61 cm wooden stake. The adhesive cards were positioned with their adhesive surfaces perpendicular to the field rows.

Each pitfall trap was assembled from one 0.5 L deli cup and one 1 L deli cup. The 1 L deli cup was buried to its rim at the top of the row to reduce the amount of soil filling the trap during inclement weather. A layer of 4-5 cm of tap water was added to the 1 L cup, along with sodium chloride (Morton Canning & Pickling Salt, Morton Salt, Chicago, IL) and a couple drops of scentless dish detergent (Ultra Palmolive Pure + Clear, Colgate-Palmolive, New York City, NY). A hole measuring approximately 2.5 cm by 1.3 cm was previously cut into the floor of the 0.5 L cup along the wall, and the 0.5 L cup was placed into the 1 L cup. This arrangement allows fallen insects to follow the wall of the 0.5 L cup until they find and fall into the hole to the bottom of the 1 L cup, thus reducing (though not eliminating) vertebrate bycatch as well as reducing the amount of soil present in the accumulated samples. A lawn flag marked the location of each pitfall trap.

Both plants and insect traps were placed in the field on August 3rd, 2018. After one week, traps were serviced. Trap cards were removed from the wooden stakes and covered with a sheet of saran wrap (GLAD ClingWrap, The GLAD Products Company, Oakland, CA) to prevent loss of specimens and to allow for transport and storage. With pitfall traps, the accumulated contents of each trap was collected using fine-mesh aquarium dip nets (WECO, Long Beach, CA) and placed within an individual 0.7 L Nasco whirl-pack (BioQuip, Rancho Dominguez, CA) with enough 70% ethanol to cover the sample. On the day that traps were serviced (August 10th, 2018), new plants were placed at the same locations with new, randomly assigned injection treatments in order to carry out the second replication.

Each trap card possessed a seven-by-nine box grid on their adhesive side. Most insects caught within this grid were identified visually to family and marked with a wet erase marker (Sharpie, Oak Brook, IL) to prevent accidental recounts. During counting, insects were identified

and quantified to family level, except for three taxonomic groups, two of which came about as a result of time and labor constraints. Thrips (order Thysanoptera; families Thripidae and Aeolothripidae) were quantified at the level of order due in large part to their exceptionally high abundance. The second exception was the families of parasitoid wasps because their high diversity combined with often obscure family-specific anatomical characteristics compounded accurate quantification from the adhesive trap cards. Parasitoid wasps were thus pooled together under the paraphyletic infraorder "Parasitica". Despite the pooling, representatives of the different morphospecies belonging to these two groups were sampled and identified to family. While gall wasps (Cynipidae) were present in the study field, the majority of wasps belonged to one of at least eleven families of arthropod-targeting parasitoids: Aphelinidae, Brachonidae, Ceraphronidae, Diapriidae, Dryinidae, Encyrtidae, Ichneumonidae, Megaspilidae, Mymaridae, Platygastridae, and Pteromalidae. Thus, by pooling parasitoid wasps together, their data can be looked at as analogous to the treatment of spiders (which will be discussed), as insect predators that may play an indirect role in *Dickeya* transmission by influencing the behavior of more likely insect vectors (Hodge et al., 2011). Lastly, due to their abundance in potato fields and importance as defoliating pests, adult Colorado potato beetles were quantified separately from other chrysomelids. In all family-level statistical analyses, the abundances of Colorado potato beetles were added together with that of the other chrysomelids to match the treatment of the other taxa.

All immature insects were excluded from the data because the morphological differences between instars were a complicating factor in accurate identification. When possible, vouchers were collected either by cutting pieces of the sticky card with the specimen attached and pinning through the sticky card material, or by tearing the saran wrap and gently working the specimen

free using vegetable oil (Hannaford Brothers Company, Scarborough, ME) as a lubricant. Insects retrieved using the latter method were affixed to pieces of botanical paper (BioQuip, Rancho Dominguez, CA) and mounted on insect pins.

Some trap cards displayed such high abundances of either thrips or aphids that accurate quantification of their total abundance in a timely manner was impossible. To remedy this, an abundance threshold was used. As mentioned, the trap cards had a seven-by-nine box grid. The 5^{th} column of boxes located at the center of the grid possessed a crease down that allowed the trap cards to be folded around the wooden stakes. This created a "front" and a "back" side with different abundances. To count thrips and aphids, nine boxes were selected using a random number generator (Google, Mountain View, CA) to generate random x,y coordinates. The abundance of thrips and aphids were counted across four "front" boxes, four "back" boxes, and one "middle" box from the 5^{th} column. If the abundance of either insect was greater than 20 individuals in each of the four boxes from either the "front" or the "back" side, counting abundance for that insect would stop after the nine random boxes. The recorded abundances were totaled, and this value was multiplied by seven (for each row of the grid) to get an estimated abundance. Should neither side meet this threshold, the thrips and aphids would continue being counted across the entire sticky card.

To quantify the insects collected by the pitfall traps, captured individuals from each trap were rinsed off with tap water at the laboratory to remove residual salt, detergent, and fine soil. The insects were then placed in a 100 mm x 15 mm polystyrene petri dish (Fisher Scientific, Hampton, NH) and submerged in 70% ethyl alcohol. Insects were sorted out into morphospecies and subsequently identified to family for the purpose of measuring approximate taxonomic richness. Just as with the trap cards, however, thrips were pooled by order (Thysanoptera) and

parasitoid wasps were pooled by infraorder (Parasitica) in order to match the data collected from the cards. Similar to sticky cards, Colorado potato beetles were also recorded separately from other chrysomelids so their abundances could be analyzed separately. However, for family-level analyses, Colorado potato beetle abundances were added together with that of the other chrysomelid beetles. All immature insects collected by pitfall traps were ignored. With both types of traps, the abundances of four groups of non-insect arthropods were recorded: spiders (order Araneae), harvestmen (order Opiliones), centipedes (class Chilopoda, order Lithobiomorpha), and millipedes (class Diplopoda, order Polydesmida) were quantified at the level of order.

Both mites (subclass Acari) and springtails (class Collembola) were omitted from the data due to methodological short comings of this research. While both mites and springtails were present within the field, the sampling methods used were quite damaging toward smaller invertebrates. Early attempts to quantify springtails within a preliminary trial proved futile due to a combination of their sudden exceptionally high abundances and the damage that they incurred during the sample processing. Several species of mites known to attack plants are also serve as vectors of plant viruses (Oldfield, 1970; Rodrigues & Childers, 2013) and can disperse great distances by wind, insect-, or human-mediated passive transmission (Duffner et al., 2001; Boykin & Campbell, 1984; Navia et al., 2006). However, the small size of many such mites was reasoned to be beyond the ability of the equipment used in sampling and processing to accurately reflect the abundance of mites present within the study field, and so they were omitted from the data. Despite the omission of these two arthropod groups, it should not be interpreted to imply that these two groups of non-insect arthropods can be ruled out from serving as potential vectors

of soft rot bacteria. However, to include mites and springtails would require additional sampling and preservation methods which were not within this research's scope.

Specimen identifications were checked against the University of New Hampshire Entomology Collection (located in Durham, NH), and voucher specimens were deposited with the Maine Forest Service Entomology Collection (Augusta, MA). The subsequent data were organized by which trapping method was used. To decrease skewness of the data, a threshold was established to isolate and remove "rare" taxa whose appearance within the data was the result of transient individuals and not indicative of an active population within the study field. Any taxon whose total abundance was less than 10 individuals was removed from data before analysis. One pitfall trap sample that had a very low yield comprised of only a single aphid and three chrysomelid beetles, was treated as an outlier and omitted from the data. The distribution of the remaining data was investigated using the Shapiro-Wilk test. Treatment effects were analyzed using two-way ANOVA in R (R Core Team, Vienna, AT), with a threshold of 0.05 and plant infection status and the date of deployment in the field as the two independent factors.

2.3 Results

2.3.1 Insect response to foliar volatiles from *Dickeya*-infected plants

2.3.1.1 Colorado potato beetle olfactometry

When testing 2,3-butanediol as a proxy for *Dickeya*-infected foliage, foliage with 2,3-butanediol elicited the least response from Colorado potato beetles: Only two (10.00%) responded to that bait. By comparison, 15 beetles (75.00%) responded to butanediol on its own and 12 (60.00%) responded to water. Control foliage elicited the strongest response with 18

beetles (90.00%) responding to it. There was significant variation in beetle response among the four treatments (χ^2 = 12.24, df = 3, *P* = 0.0066).

In comparing infected and uninfected foliage, Colorado potato beetles did not display a significant ability to discriminate between infected and uninfected foliage (χ^2 = 0.46, df = 1, *P* = 0.4996). Out of the 20 beetles tested against each of the two baits, 15 beetles (75.00%) responded to uninfected foliage, while 12 beetles (60.00%) responded to infected foliage.

2.3.1.2 Green peach aphid olfactometry

When the plant from which control foliage was collected did not receive any injections, the number of aphids moving upwind did not differ significantly whether infected or uninfected foliage was placed in the olfactometer (χ^2 = 0, df = 1 , *P* = 1). Out of 20 individuals tested against each type of foliage, 12 individuals (60%) responded to infected foliage from injected plants while 13 individuals (65.00%) gave a response to the smell of uninfected foliage from uninjected plants. Among responsive aphids, nine green peach aphids (45.00%) showed a positive response toward infected foliage, while six green peach aphids (30.00%) showed a positive response to uninfected foliage. As with the response frequency, the difference between positive and negative responses was not statistically significant (χ^2 = 1.1, df = 1, *P* = 0.2881).

Similarly, green peach aphids did not display significant discrimination between infected and uninfected foliage when both types of foliage came from plants which had received traumatic injections: out of 20 trials for each type of foliage, 15 green peach aphids (75.00%) were responsive toward infected foliage, while 14 green peach aphids (70.00%) were responsive toward the uninfected foliage (χ^2 = 0, df = 1, *P* = 1). Of the 15 green peach aphids that responding to infected foliage, 6 (40.00%) gave a positive response. Of the 14 green peach

aphids that responded to uninfected foliage, 6 (42.86%) gave a positive response ($\chi^2 < 0.0001$, df = 1, $P = 1$).

2.3.2 Insect recruitment to infected foliage in artificial arenas

2.3.2.1 Colorado potato beetles

When presented with a choice of infected or uninfected host plants, Colorado potato beetle did not display a significant preference for either one of them (t = -0.22, df = 16, $P = 0.8267$). On average, 2.22 ± 0.71 beetles (mean ± SE) were found on uninfected plants, while 2.44 ± 0.70 beetles were counted on the infected plants.

2.3.2.2 Green peach aphids

Among the individually tested green peach aphids there was a statistically significant preference for *Dickeya*-infected foliage: when given a choice between infected and uninfected foliage, 27 individuals chose foliage from infected potato plants while 13 individuals chose foliage from uninfected plants ($\chi^2 = 4.9$, df = 1, $P = 0.02686$). This trend reversed when aphids were released in groups of 10: green peach aphids were more likely to settle on foliage from plants which were injected with distilled water (67.68%, mean = 6.13 ± 2.53, SE = 0.40) than foliage from an infected plant (32.32%, mean = 2.93 ± 2.36, SE = 0.37). Using the Wilcoxon test, the choices made by aphids in groups show strong statistical significance (Z = -8.1, df = 1, $P < 0.0001$). The difference between aphid choices when expressed as a percentage differed significantly between isolated individuals and those in groups ($\chi^2 = 23$, df = 1, $P < 0.0001$).

2.3.3 Insect recruitment to *Dickeya*-infected plants in the field

Before removing any of the taxa represented by ten individuals or fewer, pitfalls accumulated 40 taxa while adhesive trap cards collected 48 taxa. Between both trap types, a total

of 59 taxa were collected and counted, of which 55 were insects. After removal of the "rare" taxa, pitfalls had collected 25 taxa while adhesive trap cards had collected 27. A total of 32 taxa were removed due to having total abundances of fewer than 10 individuals (mean = 2.41 ± 2.03, SE = 0.36, range = 7) (see Table 2.2). Of the remaining taxa which were quantified, thrips were the most abundant insects, with total captures from both trap types totaling 33,390 individuals. This was followed by aphids with 27,448 individuals, leafhoppers (Cicadellidae) with 2,204 individuals, and root maggot flies (Anthomyiidae) with 927 individuals. Spiders (Araneae) were represented by 60 individuals and were the most abundant non-insect arthropods quantified in this study.

The composition and abundances of the counted arthropods collected by pitfall traps and by yellow adhesive trap cards differed from one another. Sticky cards collected a total of 67,474 individuals of the arthropod groups of interest, of which 67,445 were insects. Pitfall traps had much lower yields, having collected a total of 865 individuals, of which 831 individuals were insects. Diptera were the most family-rich order of insects collected by sticky cards, with representative from 19 families. Coleoptera were second with 10 families, and Hemiptera came in third with eight families. In abundance, thrips were the most prolific insect group recorded on adhesive trap cards, with a total of 33,374 individuals counted across all cards. Aphids (Hemiptera: Aphididae) were second with 27,427 individuals, followed by leafhoppers (Hemiptera: Cicadellidae) with 2,195 individuals.

Pitfall traps collected more beetles than any other order, with 12 families represented within the data. Diptera were second with 11 families, and Hemiptera were third with six families. In terms of abundance, root-maggot flies had the greatest abundance within pitfall traps, with 228 individuals collected. Chrysomelid beetles were the second most abundant family, with

120 individuals collected. The third most abundance family was the carabids, with 119 individuals collected in pitfall traps. Unfortunately, because parasitoid wasp families were pooled during sticky card counting, the richness of hymenopterans from each sampling method is uncertain. However they were most prevalent on trap cards, with 1,298 individuals collected using this method versus the 37 collected by pitfall traps. All four groups of target non-insect arthropods were collected in the pitfalls samples, with 31 spiders, one lithobiomorphan centipede, one polydesmidan millipede, and one harvestman. With respect to trap cards, spiders, with 29 individuals counted, were the only target non-insect arthropod collected.

Table 2.2. Taxa removed from the data due to their small abundances. *Accurate family level identification was not possible with these taxa due to the fragmentary condition of the specimens. For these taxa, identification was been carried out to the lowest possible level.

Taxon	Treatment	Date	Total Abundance (Trap cards)	Total Abundance (Pitfall traps)
Hemiptera: Coccididae	Infected	10-Aug	1	0
		17-Aug	6	0
	Uninfected	10-Aug	1	0
		17-Aug	0	0
Hemiptera: Delphacidae	Infected	10-Aug	2	0
		17-Aug	0	0
	Uninfected	10-Aug	1	0
		17-Aug	0	0
Hemiptera: Derbidae	Infected	10-Aug	0	0
		17-Aug	0	0
	Uninfected	10-Aug	0	0
		17-Aug	1	0
Hemiptera: Pachygronthidae	Infected	10-Aug	0	0
		17-Aug	0	0
	Uninfected	10-Aug	0	2
		17-Aug	0	0
Diptera; Ceratopogonidae	Infected	10-Aug	2	0
		17-Aug	0	0
	Uninfected	10-Aug	2	0
		17-Aug	4	0

Table 2.2 Continued

Diptera: Dolichopodidae	Infected	10-Aug	1	0
		17-Aug	0	0
	Uninfected	10-Aug	0	0
		17-Aug	0	0
Diptera: Fanniidae	Infected	10-Aug	1	0
		17-Aug	0	0
	Uninfected	10-Aug	0	0
		17-Aug	0	0
Diptera: Lauxaniidae	Infected	10-Aug	0	0
		17-Aug	0	0
	Uninfected	10-Aug	2	0
		17-Aug	0	0
Diptera: Muscidae	Infected	10-Aug	0	2
		17-Aug	0	0
	Uninfected	10-Aug	0	1
		17-Aug	0	0
Diptera: Odiniidae	Infected	10-Aug	1	0
		17-Aug	0	0
	Uninfected	10-Aug	0	0
		17-Aug	0	0
Diptera: Sarcophagidae	Infected	10-Aug	0	3
		17-Aug	1	0
	Uninfected	10-Aug	0	0
		17-Aug	1	0
Diptera: Scatopsidae	Infected	10-Aug	0	0
		17-Aug	1	0
	Uninfected	10-Aug	0	0
		17-Aug	1	0
Diptera: Simuliidae	Infected	10-Aug	0	0
		17-Aug	1	0
	Uninfected	10-Aug	0	0
		17-Aug	1	0
Ephemeroptera: Baetidae	Infected	10-Aug	0	0
		17-Aug	1	0
	Uninfected	10-Aug	0	0
		17-Aug	0	0
Hymenoptera: Apidae	Infected	10-Aug	0	0
		17-Aug	0	0
	Uninfected	10-Aug	0	0
		17-Aug	1	0
Hymenoptera: Formicidae	Infected	10-Aug	0	2
		17-Aug	0	0
	Uninfected	10-Aug	0	2
		17-Aug	0	0
Hymenoptera:	Infected	10-Aug	0	0

Table 2.2 Continued

Halictidae		17-Aug	1	0
	Uninfected	10-Aug	0	1
		17-Aug	1	0
Coleoptera: Anthicidae	Infected	10-Aug	0	0
		17-Aug	0	1
	Uninfected	10-Aug	0	0
		17-Aug	0	0
Coleoptera: Catheridae	Infected	10-Aug	0	0
		17-Aug	0	1
	Uninfected	10-Aug	0	0
		17-Aug	0	1
Coleoptera: Corylophidae	Infected	10-Aug	1	0
		17-Aug	0	0
	Uninfected	10-Aug	0	0
		17-Aug	0	1
Coleoptera: Ptiliidae	Infected	10-Aug	3	0
		17-Aug	0	0
	Uninfected	10-Aug	0	1
		17-Aug	2	0
Lepidoptera: Hesperiidae	Infected	10-Aug	0	0
		17-Aug	0	0
	Uninfected	10-Aug	0	0
		17-Aug	1	0
Neuroptera: Chrysopidae	Infected	10-Aug	0	0
		17-Aug	0	1
	Uninfected	10-Aug	0	0
		17-Aug	0	0
Neuroptera: Hemerobiidae	Infected	10-Aug	0	0
		17-Aug	0	0
	Uninfected	10-Aug	0	0
		17-Aug	1	0
Orthoptera: Acrididae	Infected	10-Aug	0	0
		17-Aug	0	0
	Uninfected	10-Aug	0	0
		17-Aug	0	1
Orthoptera: Gryllidae	Infected	10-Aug	0	0
		17-Aug	0	1
	Uninfected	10-Aug	0	0
		17-Aug	0	0
Ephemeroptera: Baetidae	Infected	10-Aug	0	0
		17-Aug	1	0
	Uninfected	10-Aug	0	0
		17-Aug	0	0
Trichoptera*	Infected	10-Aug	1	0
		17-Aug	0	0

Table 2.2 Continued

	Uninfected	10-Aug	0	0
		17-Aug	0	0
Psocoptera: Psocomorpha*	Infected	10-Aug	0	0
		17-Aug	2	0
	Uninfected	10-Aug	2	0
		17-Aug	1	0
Lithobiomorpha	Infected	10-Aug	0	1
		17-Aug	0	0
	Uninfected	10-Aug	0	0
		17-Aug	0	0
Polydesmida	Infected	10-Aug	0	0
		17-Aug	0	0
	Uninfected	10-Aug	0	0
		17-Aug	0	1
Opiliones	Infected	10-Aug	0	0
		17-Aug	0	0
	Uninfected	10-Aug	0	0
		17-Aug	0	1

Richness of taxa captured by pitfall traps was not affected by the date of sample collection ($F = 0.25$, df = 1, 19, $P = 0.624$), plant injection treatment ($F = 0.62$, df = 1, 19, $P = 0.439$), or by the interaction effect between date and treatment ($F = 1.3$, df = 1, 19, $P = 0.262$). The same was true for the taxonomic diversity, where date ($F = 2.1$, df = 1, 19, $P = 0.168$), treatment type ($F = 0.059$, df = 1, 19, $P = 0.811$), and the interaction effect ($F = 1.3$, df = 1, 19, $P = 0.276$) showed no statistical significances. The normality assumptions of both pitfall richness (W = 0.96831, $P = 0.6487$) and pitfall diversity (W = 0.92738, $P = 0.096$) were confirmed using with the Shapiro-Wilk Test. The abundance data from the pitfall traps, however, was unable to pass this assumption (W = 0.88942, $P = 0.0154$). Following logarithmic transformation, the abundance data collected from the pitfall traps suggested that the date of sample collection had a significant effect ($F = 4.6$, df = 1, 19, $P = 0.0452$). The plant injection treatment ($F = 0.51$, df = 1, 19, $P = 0.4830$) and the interaction effect between date and treatment ($F = 0.31$, df = 1, 19, $P = 0.5874$) were not statistically significant.

Table 2.3. Total arthropod mean abundance, richness, and diversity sampled by pitfall traps on Aroostook Research Farm.

Treatment	Date	Abundance	Richness	Diversity
Infected	10-Aug	8.444 ± 2.884	20	2.205531
	17-Aug	5.370 ± 1.609	19	2.390806
Uninfected	10-Aug	9.481 ± 3.725	18	2.042414
	17-Aug	7.815 ± 2.612	20	2.30194

Adhesive trap cards showed a significant relationship between the date of sample collection and both the abundance ($F = 50$, df = 1, 20, $P < 0.0001$) and richness ($F = 4.9$, df = 1, 20, $P = 0.0396$) of quantified taxa. Taxa diversity did not show a significant effect by date ($F = 1.7$, df = 1, 20, $P = 0.2089$). The type of injection treatment each plant was exposed to was not a statistically significant factor in the abundance ($F = 0.027$, df = 1, 20, $P = 0.871$), richness ($F = 0.070$, df = 1, 20, $P = 0.7944$), and diversity ($F = 0.001$, df = 1, 20, $P = 0.9726$) of taxa collected by adhesive trap cards. There was also a lack of any statistically significant relationships between either abundance ($F = 2.9$, df = 1, 20, $P = 0.103$), richness ($F = 2.2$, df = 1, 20, $P = 0.1501$), or diversity ($F = 3.5$, df = 1, 20, $P = 0.0762$) and the interaction between the sampling date and treatment type. The normality assumptions of the two-way ANOVA's run on adhesive trap card abundance (W = 0.93266, $P = 0.1118$), richness (W = 0.94455, $P = 0.2059$), and diversity (W = 0.98481, $P = 0.9656$) were all confirmed by the Shapiro-Wilk Test.

Table 2.4. Total arthropod mean abundance, richness, and diversity sampled by adhesive trap cards on Aroostook Research Farm.

Treatment	Date	Abundance	Richness	Diversity
Infected	10-Aug	854 ± 541.886	27	1.096129
	17-Aug	380.037 ± 245.01	26	1.251463
Uninfected	10-Aug	1019.37 ± 657.316	25	1.020882
	17-Aug	243.704 ± 175.586	25	1.246521

When taxa were analyzed individually, no taxon appeared to experience a significant change in abundance due to the treatment condition of the plant, although the sap beetles (Nitidulidae) collected by pitfall traps (F = 3.8, df = 1, 20, P = 0.0658), as well as the hover flies (Syrphidae) (F = 3.8, df = 1, 20, P = 0.0624) and minute scavenger beetles (Latridiidae) (F = 3.7, df = 1, 20, P = 0.0673) collected from adhesive trap cards came close. Date had a significant effect on the abundances of four taxa (Thysanoptera, Aphididae, Carabidae, and Chrydomelidae) and marginal significance on the abundance of spiders (Araneae) caught in pitfall traps. Among adhesive trap cards, sampling date was a significant factor in the abundances of 11 taxa (Thyrsanoptera, Aphididae, Cicadellidae, Anthochoridae, Cecidomyiidae, Chrysomelidae, Coccinellidae, Nitidulidae, Staphylinidae, Tineidae, and Araneae).

The interaction effect of both sampling date and plant treatment was marginally significant for spiders (Araneae) collected from pitfall traps (Table 2.6) and was statistically significant in the abundances of leafhoppers (Cicadellidae) and leaf beetles (Chrysomelidae) (Table 2.5) collected with adhesive trap cards. However, t-tests conducted separately on each day of the experiment did not detect any difference between the treatments in spider abundance either on August 10th (t = 1.4, df = 7.2, P = 0.213) or on August 17th (t = -2.0, df = 6.2, P =

0.0937). The same was also true for leaf hoppers present on adhesive trap cards retrieved on August 10th (t = -1.9, df = 6.3, *P* = 0.1104) and on August 17th (t = 2.6, df = 8.6, *P* = 0.2999). Leaf beetles, to the contrary, were more abundant on adhesive cards deployed next to infected plants on August 10th (t = 3.2, df = 9.8, *P* = 0.0104) but the reverse was true on cards collected on August 17th (t = -2.7, df = 8.9, *P* = 0.0254) (see Table 2.5).

Table 2.5. The mean abundances and two-way ANOVA results of arthropod taxa collected using yellow adhesive trap cards. In each ANOVA, df=1, 20.

Taxon	Treatment	Date	Mean Abundance	Effect (Date)	Effect (Treatment	Effect (Date & Treatment)
Thysanoptera	Infected	10-Aug	1761.5 ± 347.5	*P*=0.0054 *F*=16.0	*P*=0.9071 *F*=0.014	*P*=0.3390 *F*=0.96
		17-Aug	1046.7 ± 127.38			
	Uninfected	10-Aug	1958.17 ± 251.29			
		17-Aug	796 ± 91.92			
Hemiptera: Aphididae	Infected	10-Aug	1776.33 ± 422.06	*P*<0.0001 *F*=29.0	*P*=0.785 *F*=0.077	*P*=0.203 *F*=1.7
		17-Aug	416.33 ± 340.96			
	Uninfected	10-Aug	2311.17 ± 395.88			
		17-Aug	67.33 ± 8.11			

Table 2.5 Continued

Hemiptera: Cicadellidae	Infected	10-Aug	95.5 ± 11.51	*P*=0.0008 *F*=16.0	*P*=0.3951 *F*=0.76	*P*=0.0162 *F*=6.9
		17-Aug	71.83 ± 10.15			
	Uninfected	10-Aug	158.17 ± 31.72			
		17-Aug	40.33 ± 6.67			
Hemiptera: Anthochoridae	Infected	10-Aug	28.83 ± 4.8	*P*=0.00045 *F*=18.0	*P*=0.8602 *F*=0.032	*P*=0.2861 *F*=1.2
		17-Aug	11.5 ± 1.98			
	Uninfected	10-Aug	25.83 ± 2.57			
		17-Aug	15.67 ± 3.03			
Hemiptera: Miridae	Infected	10-Aug	3.67 ± 2.35	*P*=0.582 *F*=0.31	*P*=0.389 *F*=0.77	*P*=0.165 *F*=2.1
		17-Aug	1.83 ± 0.6			
	Uninfected	10-Aug	2.5 ± 1.73			
		17-Aug	6.67 ± 2.92			
Hemiptera: Psyllidae	Infected	10-Aug	6.67 ± 1.43	*P*=0.0862 *F*=3.3	*P*=0.0862 *F*=3.3	*P*=0.4909 *F*=0.50
		17-Aug	8.5 ± 2.38			
	Uninfected	10-Aug	2.5 ± 0.81			
		17-Aug	6.67 ± 1.65			
Diptera: Anthomyiidae	Infected	10-Aug	31.0 ± 4.98	*P*=0.577 *F*=0.32	*P*=0.261 *F*=1.3	*P*=0.671 *F*=0.19
		17-Aug	31.5 ± 4.34			

Table 2.5 Continued

	Uninfected	10-Aug	25.17 ± 1.74			
		17-Aug	28.83 ± 2.69			
Diptera: Calliphoridae	Infected	10-Aug	3.17 ± 0.7	*P*=0.659 *F*=0.20	*P*=0.883 *F*=0.022	*P*=0.557 *F*=0.36
		17-Aug	2.0 ± 1.48			
	Uninfected	10-Aug	2.33 ± 1.05			
		17-Aug	2.5 ± 1.09			
Diptera: Chironomidae	Infected	10-Aug	5.33 ± 1.5	*P*=0.704 *F*=0.15	*P*=0.871 *F*=0.027	*P*=0.361 *F*=0.87
		17-Aug	3.33 ± 2.19			
	Uninfected	10-Aug	4.17 ± 1.28			
		17-Aug	5.0 ± 0.73			
Diptera: Chloropidae	Infected	10-Aug	3.0 ± 0.93	*P*=0.281 *F*=1.3	*P*=0.489 *F*=0.50	*P*=0.411 *F*=0.71
		17-Aug	3.67 ± 1.36			
	Uninfected	10-Aug	2.67 ± 1.05			
		17-Aug	7.5 ± 4.56			
Diptera: Cecidomyiidae	Infected	10-Aug	0.33 ± 0.21	*P*=0.0323 *F*=5.3	*P*=0.5517 *F*=0.37	*P*=0.9048 *F*=0.015
		17-Aug	1.83 ± 0.75			
	Uninfected	10-Aug	0.67 ± 0.33			
		17-Aug	2.33 ± 1.09			

Table 2.5 Continued

Diptera: Hybotidae	Infected	10-Aug	0.67 ± 0.67	*P*=0.488 *F*=0.5	*P*=0.173 *F*=2.0	*P*=1.000 *F*=0.0
		17-Aug	1.0 ± 0.63			
	Uninfected	10-Aug	0 ± 0			
		17-Aug	0.33 ± 0.21			
Diptera: Mycetophilidae	Infected	10-Aug	4.17 ± 4.17	*P*=0.330 *F*=1.0	*P*=0.330 *F*=1.0	*P*=0.369 *F*=0.84
		17-Aug	0.17 ± 0.17			
	Uninfected	10-Aug	0.17 ± 0.17			
		17-Aug	0 ± 0			
Diptera: Phoridae	Infected	10-Aug	13.5 ± 2.35	*P*=0.9655 *F*=0.002	*P*=0.6976 *F*=0.16	*P*=0.0743 *F*=3.5
		17-Aug	9.83 ± 0.95			
	Uninfected	10-Aug	9.17 ± 2.06			
		17-Aug	12.67 ± 1.96			
Diptera: Sciaridae	Infected	10-Aug	22.17 ± 3.64	*P*=0.220 *F*=1.6	*P*=0.121 *F*=2.6	*P*=0.550 *F*=0.37
		17-Aug	16.0 ± 2.21			
	Uninfected	10-Aug	14.83 ± 4.23			
		17-Aug	12.67 ± 2.73			
Diptera: Syrphidae	Infected	10-Aug	1.17 ± 0.65	*P*=0.1469 *F*=2.3	*P*=0.0624 *F*=3.9	*P*=0.5681 *F*=0.34
		17-Aug	2.67 ± 1.15			

Table 2.5 Continued

	Uninfected	10-Aug	0.17 ± 0.17			
		17-Aug	0.83 ± 0.54			
Hymenoptera: "Parasitica"	Infected	10-Aug	52.17 ± 9.17	*P*=0.174 *F*=2.0	*P*=0.947 *F*=0.005	*P*=0.350 *F*=0.92
		17-Aug.	56.67 ± 8.24			
	Uninfected	10-Aug	42.0 ± 10.61			
		17-Aug	65.5 ± 11.39			
Coleoptera: Carabidae	Infected	10-Aug	0.17 ± 0.17	*P*=0.329 *F*=1.0	*P*=0.329 *F*=1.0	*P*=0.329 *F*=1.0
		17-Aug	0 ± 0			
	Uninfected	10-Aug	0 ± 0			
		17-Aug	0 ± 0			
Coleoptera: Chrysomelidae	Infected	10-Aug	2.17 ± 0.4	*P*<0.0001 *F*=26.0	*P*=0.7188 *F*=0.13	*P*=0.0007 *F*=16.0
		17-Aug	2.67 ± 0.42			
	Uninfected	10-Aug	0.5 ± 0.34			
		17-Aug	4.67 ± 0.61			
Coleoptera: Coccinellidae	Infected	10-Aug	1.83 ± 0.65	*P*=0.492 *F*=0.49	*P*=0.921 *F*=0.01	*P*=0.378 *F*=0.81
		17-Aug	2.0 ± 1.1			
	Uninfected	10-Aug	2.5 ± 0.85			
		17-Aug	1.17 ± 0.65			

Table 2.5 Continued

Coleoptera: Cryptophagidae	Infected	10-Aug	3.17 ± 1.08	*P*=0.835 F=0.045	*P*=0.533 *F*=0.4	*P*=0.835 *F*=0.045
		17-Aug	2.83 ± 0.87			
	Uninfected	10-Aug	2.5 ± 0.43			
		17-Aug	2.5 ± 0.62			
Coleoptera: Curculionidae	Infected	10-Aug	0.17 ± 0.17	*P*=0.155 *F*=2.2	*P*=0.155 *F*=2.2	*P*=0.263 *F*=0.018
		17-Aug	0.33 ± 0.33			
	Uninfected	10-Aug	0.33 ± 0.21			
		17-Aug	1.67 ± 0.92			
Coleoptera: Latridiidae	Infected	10-Aug	7.33 ± 1.2	*P*=0.0953 *F*=3.1	*P*=0.0673 *F*=3.7	*P*=0.2450 *F*=1.4
		17-Aug	4.67 ± 1.17			
	Uninfected	10-Aug	4.5 ± 0.56			
		17-Aug	4.0 ± 0.37			
Coleoptera: Nitidulidae	Infected	10-Aug	15.17 ± 3.85	*P*=0.0015 *F*=14.0	*P*=0.6828 *F*=0.17	*P*=0.7591 *F*=0.097
		17-Aug	4.33 ± 3.16			
	Uninfected	10-Aug	14.83 ± 4.03			
		17-Aug	2.0 ± 0.58			
Coleoptera: Staphylinidae	Infected	10-Aug	2.67 ± 1.36	*P*=0.0199 *F*=6.4	*P*=0.3097 *F*=1.1	*P*=0.7689 *F*=0.089
		17-Aug	5.17 ± 1.58			

Table 2.5 Continued

	Uninfected	10-Aug	1.17 ± 0.54			
		17-Aug	4.33 ± 0.61			
Leptidoptera: Tineidae	Infected	10-Aug	0.5 ± 0.34	*P*=0.0078 *F*=8.8	*P*=0.1396 *F*=2.4	*P*=0.0911 *F*=3.2
		17-Aug	1.33 ± 0.49			
	Uninfected	10-Aug	0.33 ± 0.33			
		17-Aug	3.67 ± 1.23			
Aranea	Infected	10-Aug	0.67 ± 0.33	*P*=0.0225 *F*=6.1	*P*=0.5079 *F*=0.46	*P*=0.8245 *F*=0.051
		17-Aug	1.5 ± 0.22			
	Uninfected	10-Aug	0.83 ± 0.4			
		17-Aug	1.83 ± 0.48			

Table 2.6. The mean abundances and two-way ANOVA results of arthropod taxa collected using pitfall traps. In each ANOVA, df=1, 20.

Taxon	Treatment	Date	Mean Abundance	Effect (Date)	Effect (Treatment	Effect (Date & Treatment)
Thysanoptera	Infected	10-Aug	0.17 ± 0.17	*P*=0.0478 *F*=4.4	*P*=0.3044 *F*=1.1	*P*=1.000 *F*=0
		17-Aug	0.83 ± 0.31			
	Uninfected	10-Aug	0.5 ± 0.22			
		17-Aug	1.17 ± 0.48			
Hemiptera: Aphididae	Infected	10-Aug	1.5 ± 0.56	*P*=0.0339 *F*=5.2	*P*=0.6051 *F*=0.28	*P*=0.8627 *F*=0.031
		17-Aug	0.5 ± 0.22			
	Uninfected	10-Aug	1.33 ± 0.71			
		17-Aug	0.17 ± 0.17			
Hemiptera: Cicadellidae	Infected	10-Aug	0.33 ± 0.21	*P*=0.737 *F*=0.12	*P*=0.737 *F*=0.12	*P*=0.319 *F*=1.0
		17-Aug	0.5 ± 0.22			
	Uninfected	10-Aug	0.5 ± 0.34			
		17-Aug	0.17 ± 0.17			
Hemiptera: Anthochoridae	Infected	10-Aug	0.33 ± 0.33	*P*=0.139 *F*=2.4	*P*=0.139 *F*=2.4	*P*=0.614 *F*=0.26
		17-Aug	1.0 ± 0.52			
	Uninfected	10-Aug	0 ± 0			
		17-Aug	0.33 ± 0.21			

Table 2.6 Continued

Hemiptera: Miridae	Infected	10-Aug	0.33 ± 0.21	*P*=0.452 *F*=0.59	*P*=1.000 *F*=0	*P*=0.141 *F*=2.4
		17-Aug	0.17 ± 0.17			
	Uninfected	10-Aug	0 ± 0			
		17-Aug	0.5 ± 0.34			
Hemiptera: Psyllidae	Infected	10-Aug	0.17 ± 0.17	*P*=1.000 *F*=0	*P*=1.000 *F*=0	*P*=0.173 *F*=2.0
		17-Aug	0 ± 0			
	Uninfected	10-Aug	0 ± 0			
		17-Aug	0.17 ± 0.17			
Diptera: Anthomyiidae	Infected	10-Aug	9.67 ± 8.12	*P*=0.408 *F*=0.71	*P*=0.426 *F*=0.66	*P*=0.974 *F*=0.001
		17-Aug	5.17 ± 1.58			
	Uninfected	10-Aug	13.67 ± 5.64			
		17-Aug	9.5 ± 2.25			
Diptera: Calliphoridae	Infected	10-Aug	0.17 ± 0.17	*P*=0.819 *F*=0.054	*P*=0.260 *F*=1.3	*P*=0.495 *F*=0.48
		17-Aug	0 ± 0			
	Uninfected	10-Aug	0.33 ± 0.21			
		17-Aug	0.67 ± 0.67			
Diptera: Chironomidae	Infected	10-Aug	0.17 ± 0.17	*P*=1.000 *F*=0	*P*=0.173 *F*=2.0	*P*=1.000 *F*=0
		17-Aug	0.17 ± 0.17			

Table 2.6 Continued

	Uninfected	10-Aug	0 ± 0			
		17-Aug	0 ± 0			
Diptera: Chloropidae	Infected	10-Aug	0.17 ± 0.17	*P*=0.724 *F*=0.13	*P*=0.296 *F*=0.15	*P*=0.724 *F*=0.13
		17-Aug	0.17 ± 0.17			
	Uninfected	10-Aug	0.33 ± 0.33			
		17-Aug	0.5 ± 0.22			
Diptera: Cecidomyiidae	Infected	10-Aug	0 ± 0	*P*=0.329 *F*=1.0	*P*=0.329 *F*=1.0	*P*=0.329 *F*=1.0
		17-Aug	0 ± 0			
	Uninfected	10-Aug	0.17 ± 0.17			
		17-Aug	0 ± 0			
Diptera: Hybotidae	Infected	10-Aug	0 ± 0	*P*=0.329 *F*=1.0	*P*=0.329 *F*=1.0	*P*=0.329 *F*=1.0
		17-Aug	0 ± 0			
	Uninfected	10-Aug	0 ± 0			
		17-Aug	0.17 ± 0.17			
Diptera: Mycetophilidae	Infected	10-Aug	0 ± 0	*P*=0.5421 *F*=0.39	*P*=0.5421 *F*=0.39	*P*=0.0776 *F*=3.5
		17-Aug	0.33 ± 0.21			
	Uninfected	10-Aug	0.17 ± 0.17			
		17-Aug	0 ± 0			

Table 2.6 Continued

Diptera: Phoridae	Infected	10-Aug	2.17 ± 1.38	*P*=0.164 *F*=2.1	*P*=0.558 *F*=0.36	*P*=0.453 *F*=0.59
		17-Aug	1.5 ± 0.62			
	Uninfected	10-Aug	3.5 ± 1.12			
		17-Aug	1.33 ± 0.56			
Diptera: Sciaridae	Infected	10-Aug	3.83 ± 1.1	*P*=0.149 *F*=2.3	*P*=0.767 *F*=0.09	*P*=0.379 *F*=0.81
		17-Aug	4.83 ± 0.95			
	Uninfected	10-Aug	2.83 ± 1.49			
		17-Aug	6.83 ± 2.6			
Hymenoptera: "Parasitica"	Infected	10-Aug	1.17 ± 0.48	*P*=0.0755 *F*=3.5	*P*=0.5874 *F*=0.30	*P*=0.7442 *F*=0.11
		17-Aug	2.33 ± 0.92			
	Uninfected	10-Aug	0.5 ± 0.34			
		17-Aug	2.17 ± 1.05			
Coleoptera: Carabidae	Infected	10-Aug	7.33 ± 2.06	*P*=0.00287 *F*=12.0	*P*=0.3552 *F*=0.90	*P*=0.4776 *F*=0.52
		17-Aug	1.17 ± 0.31			
	Uninfected	10-Aug	7.67 ± 1.82			
		17-Aug	3.67 ± 1.15			
Coleoptera: Chrysomelidae	Infected	10-Aug	6.0 ± 1.47	*P*=0.0412 *F*=4.8	*P*=0.3075 *F*=1.2	*P*=0.9313 *F*=0.008
		17-Aug	2.0 ± 0.37			

Table 2.6 Continued

	Uninfected	10-Aug	8.17 ± 2.96			
		17-Aug	3.83 ± 1.89			
Coleoptera: Coccinellidae	Infected	10-Aug	0.17 ± 0.17	*P*=0.724 *F*=0.13	*P*=0.724 *F*=0.12	*P*=0.296 *F*=1.2
		17-Aug	0.5 ± 0.22			
	Uninfected	10-Aug	0.33 ± 0.33			
		17-Aug	0.17 ± 0.17			
Coleoptera: Cryptophagidae	Infected	10-Aug	0.17 ± 0.17	*P*=0.173 *F*=2.0	*P*=1.000 *F*=0	*P*=1.000 *F*=0
		17-Aug	0 ± 0			
	Uninfected	10-Aug	0.17 ± 0.17			
		17-Aug	0 ± 0			
Coleoptera: Curculionidae	Infected	10-Aug	0 ± 0	*P*=0.195 *F*=1.8	*P*=0.660 *F*=0.2	*P*=0.660 *F*=0.2
		17-Aug	0.17 ± 0.17			
	Uninfected	10-Aug	0 ± 0			
		17-Aug	0.33 ± 0.33			
Coleoptera: Latridiidae	Infected	10-Aug	0 ± 0	*P*=0.329 *F*=1.0	*P*=0.329 *F*=1.0	*P*=0.329 *F*=1.0
		17-Aug	0.17 ± 0.17			
	Uninfected	10-Aug	0 ± 0			
		17-Aug	0 ± 0			

Table 2.6 Continued

Coleoptera: Nitidulidae	Infected	10-Aug	0.17 ± 0.17	*P*=0.7012 *F*=0.15	*P*=0.0658 *F*=3.8	*P*=0.2566 *F*=1.4
		17-Aug	0 ± 0			
	Uninfected	10-Aug	0.33 ± 0.21			
		17-Aug	0.67 ± 0.33			
Coleoptera: Staphylinidae	Infected	10-Aug	1.33 ± 0.49	*P*=0.180 *F*=1.9	*P*=0.387 *F*=0.78	*P*=0.709 *F*=0.14
		17-Aug	2.5 ± 0.99			
	Uninfected	10-Aug	1.0 ± 0.45			
		17-Aug	1.67 ± 0.56			
Aranea	Infected	10-Aug	2.67 ± 0.99	*P*=0.0518 *F*=4.3	*P*=0.6835 *F*=0.17	*P*=0.0518 *F*=4.3
		17-Aug	0.17 ± 0.17			
	Uninfected	10-Aug	1.17 ± 0.48			
		17-Aug	1.17 ± 0.48			

Colorado potato beetles were common in pitfall traps, but only two individuals, one from each plant treatment, were caught by adhesive cards. The large size and hard, rounded bodies appeared to allow Colorado potato beetles to dislodge themselves from the adhesive traps (personal observation). Most of the remaining chrysomelids were identified as belonging to multiple species of flea beetles (subfamily Alticinae).

Table 2.7. The mean abundances of leaf beetles (except Colorado potato beetles) captured on Aroostook Research Farm.

Taxon	Treatment	Date	Abundance (Trap cards)	Mean Abundance (Pitfall traps)
Leptinotarsa decemlineata	Infected	10-Aug	0 ± 0	5.17 ± 1.22
		17-Aug	0.17 ± 0.17	1.67 ± 0.42
	Uninfected	10-Aug	0 ± 0	8.17 ± 2.96
		17-Aug	0.17 ± 0.17	3.67 ± 1.91
Other Chrysomelidae	Infected	10-Aug	2.17 ± 0.4	0.83 ± 0.4
		17-Aug	0.5 ± 0.34	0.33 ± 0.21
	Uninfected	10-Aug	2.5 ± 0.43	0 ± 0
		17-Aug	4.5 ± 0.56	0.17 ± 0.17

2.4 Discussion

Dickeya dianthicola displayed an experimentally detectable capacity to modify insect behavior. However, its effects were small and sporadic. Therefore, while insect vectoring of *D. dianthicola* cannot be completely ruled out, it is unlikely that changes observed in the present study will translate to a significant increase in the transmission of this bacterium between potato plants under field conditions.

Colorado potato beetles, which are the most prominent defoliators in most potato agroecosystems, did not discriminate between foliage from infected and uninfected plants in either olfactometry or recruitment experiments. This may benefit *D. dianthicola* in that Colorado potato beetles do not preferentially destroy infected plants, thus depriving the bacteria of their

hosts. However, it also diminishes the importance of Colorado potato beetles as vectors for *D. dianthicola*.

While 2,3-butanediol alone did not affect Colorado potato beetle behavior, its combination with potato foliage elicited a strong negative response. Colorado potato beetles are attracted to specific blends of volatiles emitted by potato plants rather to individual chemicals (Dickens, 2000). Earlier studies also showed differential Colorado potato beetle attraction to potato plants depending on plant age, mechanical damage, herbivory, and treatments with volicitin and methyl jasmonate (Bolter et al., 1997, Landolt et al., 1999). Therefore, change in behavior following an alteration in blend composition is not surprising. The volatile combination of potato foliage and 2,3-butanediol may mimic the odor of potato vegetation in the late stages of infection, where the health of the host is rapidly depleted due to maceration of the vascular tissues by *D. dianthicola*. If this is true, then Colorado potato beetles avoid plants or parts of plants where significant wet necrosis and decomposition are present. As the exudates from such macerated tissues are sources of inoculum (Czajkowski et al., 2010a), avoidance by beetles of these tissues may limit the likelihood of Colorado potato beetles serving as vectors of *D. dianthicola*.

Colorado potato beetle behavior observed in the present study is different from behaviors reported for several other insect species. Some fermentative bacteria, including both *Dickeya* spp. and *Pectobacterium* spp., and yeasts, such as *Saccharomyces cerevisiae* (Hansen), have been found to produce 2,3-butanediol as a component of volatile blends that result from the fermentation of host carbohydrates (Effantin et al., 2011; Becher et al., 2012; Davis et al., 2013). Drosophilid flies have been shown to be strongly attracted to such fermentative volatile blends released by yeasts growing on the decaying fruits these flies feed and reproduce on (Becher et

al., 2012). Two species of plants, *Ceropegia crassiflora* (Schlechter) and *Arum palaestinum* (Boissier), have even independently evolved an ability to secrete 2,3-butanediol or the chemically-related volatile 2,3-butanediol acetate as means of deceptively attracting drosophilids to serve as pollinators (Stökl et al., 2010; Heiduke et al., 2017). A few other insects are also known to respond to this volatile, such as the sorghum chafer, *Pachnoda interrupta* (Olivier), which seeks out 2,3-butanediol alongside other volatiles as indicators of the location of the fruits and ripening grains it feeds on (Bengtsson et al., 2009). This volatile is also employed by male medflies, *Ceratitis capitata* (Wiedemann), who secrete it as a component of their aggregation pheromones during lekking (Sollai et al., 2018).

However, this study demonstrates that Colorado potato beetles do not display a significant, positive attraction toward 2,3-butanediol. In fact, when combined with the odor of potato foliage, 2,3-butanediol appeared to repel them. This is likely due in part to the ecological differences between Colorado potato beetles and the insect species that are discussed above. These species are attracted to 2,3-butanediol because the chemical serves as an indicator of potential sources of nourishment, suitable oviposition sites, or the presence of conspecifics (Davis et al., 2013). Since Colorado potato beetles feed on live potato foliage and not fermenting plant material where 2,3-butanediol-excreting fungi and bacteria are found, signs of decay are likely to indicate poor quality host plants. Presence of a strong additional odor may have also masked the odors of green leaf volatiles otherwise attractive to Colorado potato beetles (Hilker & McNeil, 2008).

Exposure to 2,3-butanediol also activates physiological defense pathways within plants, a condition which other work has shown Colorado potato beetles to find attractive in potato plants (Landolt et al., 1999). Applications of 2,3-butanediol to *Arabidopsis thaliana* (L.), tobacco

(*Nicotiana tabacum* L.), and creeping bentgrass (*Agrostis stolonifera* L.) have been shown to increase the resistances of these plants toward viral, bacterial, and fungal pathogens respectively (Kong et al., 2018; Han et al., 2006; Cortes-Barco et al., 2010). However, Landolt et al. (1999) showed that potato plants experiencing physiological stress, including those treated with methyl jasmonate, were more attractive to Colorado potato beetles than unstressed, untreated plants. Among the resistance responses activated by 2,3-butanediol are those mediated by jasmonic acid (Kong et al., 2018; Cortes-Barco et al., 2010). However, direct negative effects of 2,3-butanediol odor apparently outweighed positive effects of inducing the jasmonic acid defense pathway.

Green peach aphids differed in their discrimination ability between the recruitment and the olfactometry experiments. In the olfactometry experiment, no significant discrimination was observed. Thus, the tested green peach aphids were responding from a distance to the presence of potato odor in general and not to the condition of the plant from which the provided foliage had originated. This may reflect the importance of visual, gustatory and tactile cues during host selection (Hori, 1999; Pelletier, 1990). Orientation and host selection by aphids follows an orderly series of investigatory behaviors, the initial stages of which rely on visual and olfactory cues to locate potential host plants (Pickett et al., 1992; Powell et al., 2006; Smith & Chuang, 2014). Once a prospective host has been found, an aphid will settle upon the plant to obtain host-specific information from several sources including tactile stimuli (Powell et al., 1999), close-quarter visual cues (Pelletier, 1990), and gustatory information collected through exploratory stylet insertion and fluid ingestion (Powell et al., 2000, 2006). Probing by a stylet has been shown to be especially important, as it occurs on both host and non-host plant species (Montgomery & Arn, 1974; Powell et al., 2000), and is often followed by either flight from a rejected plant or acceptance and subsequent feeding (Giordanengo et al., 2010; Powell et al.,

2006). Since aphids used in the olfactometry experiment were unable to make physical contact with the provided foliage, this suggests that foliar olfactory cues are not sufficient for aphids to discriminate between plants with and without *D. dianthicola* infections.

In the recruitment experiment, the difference in feeding preferences between aphids acting alone and in groups may be due to an interaction between plant susceptibility to insect pests and social facilitation. It has been shown that plants which have been previously infested by conspecifics support aphids better than those which have not been fed on before (Civolani et al., 2010; Dugravot et al., 2007; Takemoto et al., 2013). Adult aphids manipulate their plant hosts in several ways. For one, aphids must overcome many physiological defenses utilized by plants in order to feed without being harmed. Plant cells damaged during the navigation of the stylets toward the host's phloem and the subsequent feeding releases toxic oxygen radicals, such as hydrogen peroxide, that are not only harmful to aphids but also activate the calcium channels that signal initiation of sieve element occlusion (Giordanengo et al., 2010; Will et al., 2013). In addition, jasmonic acid- and salicylic acid-mediated pathways, which are responsible for the production of many insect- and pathogen-related defense chemicals, also respond to the presence of feeding aphids (Moran & Thompson, 2001; Giordanengo et al., 2010).

To defend themselves and their ability to feed, aphids employ a compositionally complex watery saliva that contains, among other substances, calcium-scavenging proteins, which prevent phloem sieve occlusion (Will et al., 2007), and detoxifying enzymes, such as peroxidase, which combat oxidative stressors (Giordanengo et al., 2010; Will et al., 2013). Several species, including the green peach aphid, have also been noted to selectively suppress genes relating to more efficient jasmonic acid-dependent chemical defenses, although less effective salicylic acid-dependent pathways remain uninhibited (Giordanengo et al., 2010; Moran & Thompson, 2001;

Walling, 2008). The nutritional quality of a plant's phloem may also be subject to modification by aphids, though this ability is not present within all species (Sandström et al., 2000). It has been shown, for example, that greenbug, *Schizaphis graminum* (Rondani), is able to increase the abundance of amino acids within the phloem sap of both wheat and barley (Sandström et al., 2000). This change is systemic within these plants, and strains of this aphid which are unable to induce this nutritional modification display reduced growth and reproduction (Dorschner et al., 1987; Sandström et al., 2000).

By manipulating their host plants, aphids can promote the development and reproduction of conspecifics (Takemoto et al., 2013). For example, potato aphids, *Macrosiphum euphorbiae* (Thomas) (Ameline et al., 2007), cowpea aphid, *Aphis craccivora* (Koch) (Pettersson et al., 1998), and the damson-hop aphid, *Phorodon humuli* (Schrank) (Campbell et al., 1993) preferred plants previously infested by conspecifics to undamaged plants. Also, pre-infestation by conspecifics resulted in longer periods of phloem ingestion in *M. persicae* (Civolani et al., 2010; Dugravot et al., 2007). As *D. dianthicola* metabolizes nutrients from the host plant and impairs the flow of phloem sap on which aphids feed through destruction of the host's vascular tissues (Effantin et al., 2011; Toth et al., 2003), such aphid-manipulated uninfected hosts may be preferable when conspecifics are present. However, in the absence of conspecifics, a host whose physiological defenses have been compromised by a bacterial infection may be more suitable for individual aphids. Similarly, modulation of aphid attraction to host plants in a density-dependent manner has been earlier demonstrated for the bird cherry – oat aphid, *Rhopalosiphum padi* (L.) (Pettersson et al., 1995), and for the cowpea aphid (Pettersson et al., 1998).

Within the field recruitment experiment, leaf beetles (family Chrysomelidae) caught by adhesive cards were the only insects to display any signs of being influenced by *D. dianthicola*'s

presence or absence from the provisioned bait plants. However, the response by leaf beetles was not consistent between the two sampling periods. Twice as many beetles were captured in the vicinity of infected plants than in the vicinity of uninfected plants on August 10, while the reverse was true on August 17 (Table 5). That may have been related to seasonal population trends among the constituent species.

Colorado potato beetles were rarely caught by adhesive cards, with only two individuals collected in this manner. Chrysomelid captures were dominated by several species of flea beetles (subfamily Alticinae), which were more abundant on the adhesive cards than in the pitfall traps. Thus, the apparent statistical response by chrysomelids to *D. dianthicola* infections was mostly due to the behavior of these species and not to Colorado potato beetles. However, due to the limited number of replications and the of lack species-specific abundance data, it cannot be deduced what the actual relationship between individual beetle species and infected plants actually is. Rather, all that can be concluded is that there simply exists some interaction between *D. dianthicola* and some member(s) of the subfamily Alticinae.

Several flea beetles belonging to the specious genus *Epitrix* have been recorded feeding on potatoes in the United States, with *E. cucumeris* (Harris) and *E. tuberis* (Gentner) being the most damaging (Boavida & Germain, 2009; Germain et al., 2013). Adult *Epitrix* flea beetles feed primarily on the foliage of solanaceous plants, creating numerous small holes in a pattern likened to shotgun fire, while the larvae feed below ground and can cause severe disfigurement of tubers (Boavida & Germain, 2009; Germain et al., 2013; Pernal et al., 1996). *Epitrix* flea beetles have been shown to be competent vectors of certain bacterial diseases, with adults having been documented spreading bacterial ring-rot, caused by *Clavibacter michiganensis* subsp. *sepedonicus* (Spieckermann & Kotthoff), and larvae serving as vectors of the common scab

bacterium *Streptomyces scabies* (Thaxter) (Christie et al., 1993; Schaal, 1934). Though it is currently not known if *Epitrix* or other flea beetles are capable of transmitting *Dickeya dianthicola*, there is a confamilial phytopathogic bacterium which does utilize flea beetles to spread between plants. Stewart's wilt in corn is a disease of North American origin caused by the soft rot enterobacterium *Pantoea stewartii* (Smith) (Roper, 2011). Infections manifesting as watery lesions and lethal wilting among seedlings and severe stunting and chlorosis in mature corn plants, and are now known to be spread primarily through the feeding activities of corn flea beetles, *Chaetocnema pulicaria* (Melsheimer), rather than through infected seed as had been believed (Block et al., 1998; Esker & Nutter, 2003; Menelas et al., 2006; Roper, 2011). What's more, *P. stewartii* can persist in the field between growing seasons by residing within the alimentary canals of adult flea beetles during their hibernal diapause (Esker & Nutter, 2003; Roper, 2011). While no work has been done to look at whether *Epitrix* spp. or other flea beetles found within potato fields can vector blackleg bacteria, the observed interaction effect from the field together with the known vectoring capabilities of flea beetles with other pathogens lends weight to the need for further investigations.

Though none of these experiments speak directly to whether insects are capable of transmitting *D. dianthicola* between potato plants, the evidence presented suggests that *D. dianthicola* does not elicit a strong attraction of potential insect vectors. In fact, the behavior of green peach aphids and of Colorado potato beetles suggests that at least some insect pests under certain conditions will actively avoid *D. dianthicola,* as seen when beetles encounter foliage laced with a primary *Dickeya* metabolite, 2,3-butanediol, and when aphids detect conspecific cues that guide them to more desirable uninfected hosts. As both species do not utilize dead plant matter for food, this makes sense, especially given the rapidity with which the disease caused by

this bacterium can progress under certain conditions. However, no statistically significant relationships were observed even among taxa associated with decaying plant matter, such as Sciaridae and Nitidulidae, or even the Anthomyiidae which have been previously implicated in the spread of bacterial soft rot in several crops, including potatoes (Rossmann et al., 2018; Johnson, 1930; Bonde, 1939). The only potential vectors identified in this study were flea beetles, but even their responses were inconsistent between the sampling dates.

Overall, there was no evidence of a strong directional response of studied insects to infected foliage. However, the absence of statistically significant effects of plant infection status on the attraction, abundance, and taxonomic richness of insects does not imply that the sampled insects are incapable of serving as vectors for *D. dianthicola*. Their movement in response to factors other than plant infection may still result in significant bacterial spread within potato fields. The possibility of such a situation was investigated in the next chapter.

CHAPTER 3

TRANSMISSION OF *DICKEYA DIANTHICOLA* BY INSECTS

3.1 Introduction

Several species of gram-negative bacteria of the enterobacteria genera *Dickeya* (Samson) and *Pectobacterium* (Waldee) are causal agents of soft rot diseases seen in potato production (Ma et al., 2007). In the field, these infections manifest in the development of wilting and wet, darkly discolored stem lesions sometimes referred to as "blackleg disease" (Toth et al., 2003; Samson et al., 2005). Disease caused by these bacteria can occur not only in the field but also during tuber storage, where a watery tissue necrosis can be observed when sufficiently low temperatures are not maintained (Laurila et al., 2008). The impact of soft rot bacteria, and of *Dickeya* in particular, has increased in recent years to become a significant problem for the potato production industries in both the US and Europe (Toth et al., 2011; Golanowska & Łojkowska, 2016; Jiang et al., 2016; Ma et al., 2018). A factor compounding efforts to combat bacterial soft rots in potatoes is the lingering uncertainty about the primary mode of transmission utilized by these bacteria.

Prior to infection, *Dickeya* is believed to reside within soils, surface waters, and as epiphytic aggregates on the external surfaces of plants (Toth et al., 2003). In these places, *Dickeya* obtains carbon as a benign saprophyte (Reverchon & Nasser, 2013). However, when the environment is sufficiently wet, these peritrichous bacteria can travel within water films and enter potato plants through pre-existing openings such as wounds or lenticels located on tubers (Czajkowski et al., 2010a). Once inside a host, *Dickeya* migrates within the vascular system and colonizes the interstices within the host's tissues. When growing conditions in the field are wet and temperatures are above 25°C, the bacteria secret large quantities of pectolytic enzymes into

the surrounding host tissues. This breaks down the cell walls of the plant, releasing the host's stored nutrients, resulting in rapid bacterial proliferation and development of characteristic watery lesions (Toth et al., 2011).

Most work investigating possible modes of transmission utilized by *Dickeya* have focused on factors such as movement of contaminated soil or formation of sap-derived aerosols by farming equipment (Czajkowski et al., 2010a; Skelsey et al., 2016) or surface waters used in irrigation (Cappaert et al., 1988; Laurila et al., 2008). The possibility that insects may be capable of transmitting *Dickeya* has been suggested before (Reverchon & Nasser, 2013); however, their role has never been firmly established. The presence of vector-mediated transmission is a widespread strategy seen across confamilial phytopathogenic bacteria. Species such as *Erwinia amylovora* (Burrill), *Pantoea stewartii* (Margaert), and even *Pectobacterium carotovorum* (Jones) have been confirmed to use insects for spreading to new hosts (Ordax et al., 2015; Nadarasah & Stavrinides, 2011; Kloepper et al., 1981).

Currently, however, only one species of *Dickeya* has been shown to have a significant interaction with an insect species, although whether this interaction actually facilitates transmission has not been yet demonstrated. Laboratory studies using *D. dadantii* (Samson) have shown it to be capable of penetrating the gut walls of pea aphids (*Acyrthosiphon pisum* Harris) and eliciting lethal septicemia due to the action of genes homologous to those used by *Bacillus thuringiensis* (Berliner) (Grenier et al., 2006; Costechareyre et al., 2012). The minimum infective dose was quite small, with septicemic infections occurring after ingestion of as few as 10 cells (Grenier et al., 2006). Furthermore, pathogenic effects were stronger against apterous individuals. Thus, there is suspicion that the expression of lower virulence toward alate aphids may indicate their involvement in the transmission of *D. dadantii* between plants (Grenier et al.,

2006). However, without directly showing that infected aphids can transmit the bacterium to uninfected plants, it is premature to conclude that those works definitively proved that *D. dadantii* or other species of *Dickeya* are spread between plants by pea aphids or other aphid species.

Potatoes are the world's third most important crop after rice and wheat due in part to their high yield, efficiency of water use, and tolerance for organically-poor soils and cool growing conditions which support few other crops (Scott & Suarez, 2012; CIP, 2018). Unfortunately, in 2014 *D. dianthicola* was isolated from potato fields in Maine and has subsequently spread across much of the northeastern USA (Johnson, 2018). While farmers in the United States for many years have had to cope with losses from *Pectobacterium*-related soft rots, the sudden appearance of *Dickeya* in the U.S. has been cause for alarm since several species, including *D. dianthicola*, have erupted in Europe, causing increased crop losses (Toth et al., 2011).

The previously mentioned studies with *D. dadantii* and the evidence pointing to an insect-mediate transmission of agriculturally impactful *Dickeya* species has spurred this study to focus its attention on two particularly damaging insect pests of potatoes. As pea aphids are not known to attack solanaceous crops, green peach aphids (*Myzus persicae* Sulzer) were utilized instead. Green peach aphids are adaptable pests, capable of rapidly evolving pesticide resistance, and are well known for their propensity to attack potato, being one of the more common aphid species found in fields (UMCE, 2016, Godfrey & Haviland, 2008, Silva et al., 2012). In addition, they are also well-documented disease vectors that are known to transmit over 100 species of plant viruses (van Emden et al., 1969). Facilitating this large array of diseases is aphid polyphagy, with their host range including representatives from 50 plant families, and their ability to transmit pathogens between species (Capinera, 2017; Srinivasan et al., 2006). As some

Dickeya spp. that are associated with potatoes are able to live on plant species such as corn and brassicas (Ma et al., 2007), it may be possible for green peach aphids to transmit *D. dianthicola* to potatoes from a reservoir host in climates where milder winters allow year-round persistence of the bacterium.

The second important insect pest of potatoes is the Colorado potato beetle (*Leptinotarsa decemlineata* Say). Originally restricted to wild solanaceous plants in the southwestern United States, this chrysomelid leaf beetle spread across North America, Europe, Western Asia, and Northern Africa following a host shift onto potatoes in the late 1850's (Alyokhin, 2009). There are several features of Colorado potato beetles which make them especially problematic as pests. Their populations can rapidly grow due to their high fecundity and polyvoltinism (Weber & Ferro, 1997; Hare, 1990). If left unmanaged, the beetles will completely defoliate fields and cause total crop loss (Hare, 1990). Like green peach aphids, Colorado potato beetles, show a great proclivity for evolving multiple pesticide resistances due at least in part to pre-existing physiological adaptations for coping with the high concentrations of toxins present within their *Solanum* (L.) host plants (Alyokhin et al., 2008). Although Colorado potato beetles are not currently known to act as disease vectors, this may be due to a lack of investigation rather than a true lack of ability. Colorado potato beetles share close affiliations with several species of beetles that are known to spread phytopathogenic enterobacteria, such as *Chaetocnema pulicaria* (Melsheimer) that spreads *Pantoea stewartii* (Margaert) among corn plants and both *Diabrotica undecimpunctata* (L.) and *Acalymma vittatum* (Fabricius) that spread *Erwinia tricheiphila* (Smith) among cucurbits (Nadarasah & Stavrinides, 2011). Thus, it is reasonable to evaluate Colorado potato beetles' potential to vector *D. dianthicola* between potato plants.

3.2 Materials & Methods

3.2.1 Laboratory transmission *of Dickeya dianthicola*

Due to the difference in size between Colorado potato beetles and green peach aphids, two different sets of protocols were devised to determine whether either insect is capable of transmitting *Dickeya* between potato plants. Both protocols centered utilized a basic two-stage system. During the first stage, two plants received one 100 µL injection in each stem, one with *Dickeya* inoculum and one with nonsterile distilled water. The injected plants, hereafter referred to as the "Stage One plants," were incubated for seven days at 30°C using the method previously described in Chapter 2. After incubation, the Stage One plants or their excised foliage were exposed to either Colorado potato beetles or green peach aphids for 48 hours to allow insects to feed and acquire any *Dickeya* cells present in the plant tissues.

After 48 hours, Stage One plants were removed, and both insects and tissues from Stage One plants were sampled to test for *Dickeya* using PCR as described in Chapter 2. Those insects not sampled were then placed onto uninfected plants, which marked the start of the second stage of the experiment. These plants, which received no prior injections, are hereafter referred to as "Stage Two plants." After allowing insects to feed and plants to incubate, tissue samples from Stage Two plants were collected to determine if *Dickeya* had been transmitted to them. The remaining insects and plant material were destroyed of at the end of each experiment by autoclaving. A total of 11 replications using green peach aphids and 13 replications using Colorado potato beetles were completed. Aphid mortalities were compared using an analysis of variance with R (R Core Team, Vienna, AT). Due to lack of measurable responses (see below), it was impossible to analyze other data.

3.2.1.1 Transmission by Colorado potato beetles

Stage One plants were placed into individual insect cages after their initial incubation. The cages were identical to those used to raise Colorado potato beetles and were located in the same greenhouse. Plastic water saucers (Duraco Products, Inc., Streamwood, IL) were provided to allow for excessive watering from below to maintain conditions which promote *Dickeya*. Ten adult Colorado potato beetles, five males and five females, were placed onto each Stage One plant and allowed to feed for 48 hours. Watering continued during that time.

After 48 hours, Stage One plants were removed for tissue sampling and were replaced with the Stage Two plants. Concurrently, Colorado potato beetles were transferred from the Stage One plants to the Stage Two plants. During transfer, two live male Colorado potato beetles and two live female Colorado potato beetles, along with any dead individuals, were removed from each trial to test if insects had acquired *Dickeya*. Lastly, dead foliage on the floor of the cages was removed and water saucers were replaced to prevent either from serving as reservoirs which could pass *Dickeya* on to the Stage Two plants.

The remaining Colorado potato beetles were allowed to feed on Stage Two plants for 48 hours, after which they and any eggs which had been laid were destroyed. Stage Two plants where then placed in an incubator (Series 33 Controlled Environment Chamber, Percival Scientific Inc., Perry, IA) set to 30°C and a photoperiod of 16:8 (L:D) and allowed to incubate for seven days to promote *Dickeya* growth. At the end of the incubation period, stem tissue was collected form all Stage Two plants in order to test for the presence of *Dickeya*.

Due to the amount of tissue available in a single Colorado potato beetle, it was possible to use two methods for testing whether Colorado potato beetles had acquired *Dickeya* cells. One

mL of sterile distilled water was placed within a 2.5 mL round-bottomed polypropylene microcentrifuge tube. Two adult Colorado potato beetles of the same sex were added to the tube and ground by hand using a flame-sterilized glass rod until thoroughly homogenized. Insects were not surface sterilized as the location of *Dickeya*, if present, was not viewed as a concern. Following this, 200 µL of the gut-suspension underwent DNA extraction following the protocols outlined by the MP Bio FastDNA Kit (MP Biomedicals, Santa Ana, CA). Extracted DNA was analyzed for Dickeya sequences using PCR as described in Chapter 2. Female and male beetles were sampled separately, and individuals which had expired before the end of the experiment were sampled individually for bacteria using the same method.

In addition to PCR, bacteria from Colorado potato beetles were cultured. Two hundred µL of the insect homogenate underwent serial dilution using sterile distilled water, until a final product of 2,000 µL of 10^{-5} g/L suspension was achieved. (That target concentration was chosen following preliminary testing of multiple concentrations.) One hundred µL of diluted suspension was applied to two CVP plates, spread using a flame sterilized glass cell spreader, sealed with Para-film, and incubated for 48 hours at 30°C in an incubator (Series 33 Controlled Environment Chamber, Percival Scientific Inc., Perry, IA). After 48 hours of incubation, plates were checked for colonies that exhibit media cavitation. Those colonies were sampled directly and run through the procedures outlined by manufacturer for the MP Bio FastDNA Kit (MP Biomedicals, Santa Ana, CA).

3.2.1.2 Transmission by green peach aphids

Preliminary tests proved that recovery of green peach aphids directly from plants was unreliable. Therefore, green peach aphids were placed on excised leaflets. Six leaflets were excised from each Stage One plant following stem injections and incubation. These leaflets and

60 apterous adult female green peach aphids were divided in half between two 100 mm x 15 mm polystyrene Petri dish (Fisher Scientific, Hampton, NH), resulting in two Petri dishes containing three leaflets and 30 green peach aphids for each Stage One plant. In each Petri dish, a moist paper towel cut to fit the floor of the dish was provided to prevent the leaflets from wilting. Each Petri dish was then sealed with parafilm to prevent escape and placed in an incubator (Series 33 Controlled Environment Chamber, Percival Scientific Inc., Perry, IA) used to incubate Colorado potato beetle eggs. Tissue samples from the stems of the Stage One plants were then collected for DNA testing.

Green peach aphids were allowed to feed for 48 hours on the excised foliage. After 48 hours, the number of dead aphids was counted. Dead aphids from both dishes were placed together into 1.5 mL polypropylene microcentrifuge tubes (Global Scientific Inc., Mahwah, NJ). At the same time, 10 live adult aphids (five per Petri dish) were collected into 1.5 mL microcentrifuge tubes. All aphid samples were then stored at -80° C before DNA extraction. The remaining live aphids were transferred onto Stage Two plants housed within individual insect cages identical to those used to rear Colorado potato beetles. Each Stage Two plant had a plastic water saucer and was watered excessively from below. Due to the inability to ensure that all green peach aphids could be removed from a plant and the consequent risk of green peach aphids traveling between plants housed together, Stage Two plants were not removed from their individual cages for incubation but instead remained within a greenhouse for nine days. After nine days, tissue samples were collected from Stage One plants and all remaining plant material and green peach aphids were destroyed by autoclaving.

Because of their small size, green peach aphids were not used for plating bacteria, but instead were placed into Fastprep cell lysing tubes (MP Biomedicals, Santa Ana, CA)

undergoing the DNA extraction protocols outlined by the MP Bio FastDNA Kit (MP Biomedicals, Santa Ana, CA). Live and dead green peach aphids were tested separately. The data concerning insect mortalities were analyzed using ANOVA in R (R Core Team, Vienna, AT).

3.2.2 Field transmission of *Dickeya dianthicola*

Two experiments were run using the same field located on the Aroostook Research Farm in Presque Isle, ME. One experiment was conducted during the 2017 season to investigate the spread of symptomatic infections between plants when either Colorado potato beetles or aphids were selectively suppressed by insecticides. The second experiment was run during the 2018 season and looked at the numbers of infected tubers on plots managed using the same pest suppression practices.

The field used for both experiments measured 152 m × 30 m and was broken into five replication blocks of four randomly arranged plots of four 15.24-meter rows each. Fifty seed tubers (‘Katahdin’) were planted, one per foot, in each row. After plants had germinated and reached approximately 25 cm in height, four plants per row (16 per plot) were randomly selected and received injections of 10 µL of TSB-based *Dickeya* inoculum in each stem using the traumatic injection method outlined previously. Following these initial injections, wounds were wrapped in parafilm to prevent desiccation and the position of each injected plant was marked using lawn flags.

After injections, each plot within each block received a different pest management prescription. Aphids were suppressed in one plot using pymetrozine (Fulfill, Syngenta Crop Protection Inc., Greensboro, NC), another plot was treated with spinosad (Blackhawk Naturalyte Insect Control, Dow AgroSciences, Indianapolis, IN) to suppress Colorado potato beetles, a third

plot was treated with both chemicals to suppress both aphids and Colorado potato beetles, and a fourth plot served as a control by receiving no insecticide applications. Treatments were randomized between replications, resulting in a randomized complete block design. Both years, plants were irrigated to adjust weekly precipitation to at least 2.54 cm and were top killed prior to harvest.

To ensure that the spinosad used to control beetles would not inhibit *Dickeya*, a Kirby-Bauer assay (Hudzicki, 2009) was run. Ten Petri dishes (Fisher Scientific, Hampton, NH) with CVP medium were inoculated with *D. dianthicola* ME30 by dipping a sterile cotton-tipped applicator (Medline Industries, Inc., Northfield, IL) (one per plate) into TSB-based inoculum and streaking uniformly across each plate twice to create perpendicular streaks which would result in a bacterial lawn. Six mm filter paper discs were made with a 3-hole hole-punch and were sterilized within a closed glass Petri dish (Pyrex, Greencastle, PA) using an autoclave. The paper discs were impregnated with 20 µL of either spinosad (Blackhawk Naturalyte Insect Control, Dow AgroSciences, Indianapolis, IN), 10% sodium hypochloride (Austin's A-1 Bleach, James Austin Company, Mars, PA), or sterile distilled water and allowed to dry. Once the bacteria had been applied to each CVP plate, one of each type of impregnated filter paper disc was placed on top of the medium equidistant of each other at the perimeter of each plate. The chemical treatment of each disc was marked on the underside of each Petri dish with a black permanent marker (Sharpie, Oak Brook, IL) to distinguish between the three discs as they otherwise looked identical. The plates were then marked with their replication number, the date of inoculation and sealed with Para-film. Plates were incubated for 24 hours at 28°C. After incubation, plates were observed for zones of inhibition and photographed.

In 2017, plants were injected twice, once on July 7th and again on July 20th due to a high rate of failure among plants to develop symptoms of the disease after one injection. Second injections were only given to those plants which were previously injected and failed to develop blackleg symptoms. The parafilm was not used during the second round of injections because it did not appear to have an impact on the success of infection. Each plot was inspected once a week for plants expressing signs of *Dickeya* infection, as well as to record the level of defoliation by pests and the abundances of Colorado potato beetles and aphids.

To document the abundance of aphids, ten flagged plants and ten unflagged plants were randomly chosen from each plot during weekly plot inspection. From each plant, the numbers of aphids found on three leaves (one near the bottom, one from the middle, and one at the top) of a single stem were recorded. The abundances of three potato-colonizing aphid species were recorded: potato aphids (*Macrosiphum euphorbiae* Thomas), green peach aphids (*Myzus persicae* Sulzer), and buckthorn aphids (*Aphis nasturtii* Kaltenbach) (UMCE, 2016). When analyzing the abundance of the insect pests, the aphid species were pooled together due to their low abundance and richness (only green peach aphids and potato aphids were present).

The procedure used for recording the abundance of Colorado potato beetles within each plot was based on those used by Alyokhin et al. (2005). As with aphids, Colorado potato beetles were recorded from ten randomly selected flagged plants and ten randomly selected unflagged plants from each plot, but their numbers were recorded from entire plants instead of from three leaves per plant. Abundances were recorded by life stage, with the numbers of egg masses, of egg masses that were actively hatching, of "small" (first and second instar) larvae, of "large" (third and fourth instar) larvae, and of adults being recorded from each plant. Finally, the level of

defoliation of each plot was documented as a visually-estimated percentage of the ground covered by the canopy of an entire plot.

Lawn flags were used to mark the locations of symptomatic plants. Those flags were labeled to identify the date symptoms were first observed. All flagged plants were harvested individually and tubers were bagged by plant with a plant-specific code designating whether the parent plant had received injections and whether the plant had displayed symptoms of blackleg disease. The remaining tubers were pooled together by plot, and five tubers from each pool were randomly collected and bagged for sampling. The result was that tubers fell into one of four categories based on their origin: from injected plants which displayed disease symptoms ("injected, symptomatic), from plants which had been injected with *D. dianthicola* but failed to develop the symptoms of blackleg disease ("injected, asymptomatic"), from plants which had not received any injections but still developed the symptoms of *Dickeya* infection ("uninjected, symptomatic"), and from plants which had neither received injections nor developed the symptoms of blackleg disease ("uninjected, asymptomatic").

When sampling DNA from tubers, protocols were based on those developed by Ge (personal communication, 2017). Tubers were gently brushed of excess soil, and samples of the skin were collected using a vegetable peeler which was wiped clean and sterilized with ethanol and a flame between samples. Peels were collected lengthwise, from stolon end to distal end. Depending on the size of the tuber, 30-50% of a tuber's skin was collected from each tuber. Tubers less than 5 cm in length could not be sampled with this method. Instead, such tubers were cut in half, proximal to distal end, and one half was placed into a sealable plastic sandwich bag (Hannaford Brothers Company, Scarborough, ME). The tuber piece was then laid, in its bag, on

the work surface and gently crushed by applying pressure with the hand. The remaining pieces of tubers were stored in paper bags as backup material should samples be lost.

Peels and crushed tuber pieces were placed in individual sealable plastic sandwich bags (Hannaford Brothers Company, Scarborough, ME) with just enough sterile distilled water to cover them (between 3-8 mL). The bags were sealed after being flattened to force out as much air as possible. Samples were suspended with a wooden clothes pin from the walls of a plastic 1 L rectangular Tupperware container, so that peals and water accumulated in the corner of each bag. Samples were incubated at 30°C for 24-48 hour.

In the lab, 128 tubers from 17 plants underwent DNA extraction and PCR to determine whether plants which had been injected acquired *Dickeya* within their daughter tubers, whether all daughter tubers from an infected plant were infected, and whether plants which did not display signs of infection produced infected tubers. Harvested tubers which had not been sampled for *Dickeya* were replanted in 2018 to observe whether the resulting plants expressed signs of infection. A total of 73 tubers from 10 plants were planted in plastic pots using methods and materials identical to the production of plants for use in insect rearing. The remaining tubers were planted in a field at the Aroostook Research Farm. The plant-back field was checked every other week and the numbers of symptomatic plants were recorded.

In 2018, plants were only injected once due to an observed higher rate of success in symptom development when compared with the previous year. The injections done during the 2018 season were carried out over the course of two consecutive days (July 17th and 18th) because inclement weather did not allow them to be completed on a single day. For consistency with the 2017 season, parafilm was still used to wrap wounds during both days of injecting in

2018. The main focus was placed on the infection of harvested tubers. Therefore, protocols on collecting field data were less detailed than in the previous year, and their main purpose was establishing the presence or absence of corresponding damage. Colorado potato beetle life stages were counted on July 3 and July 11. Defoliation data were collected on July 30, and aphid data were collected on August 10. Plants expressing black leg symptoms were counted on August 3. Timing for insect counts was selected based on the perceived peaks of their densities.

During the 2018 harvest, all symptomatic plants were harvested individually and their tubers were bagged individually. Those tubers were sampled for *Dickeya*. The remaining tubers in the field were harvested and pooled by plot. In the lab, ten tubers were selected at random from each of these 20 pools and tested for *Dickeya* infection following the same protocol as in 2017.

Normality of the distribution of field data was tested by Shapiro-Wilk test (PROC UNIVARIATE, SAS Institute, 2018). Non-normal data were transformed using rank transformations (PROC RANK, SAS Institute, 2018). Field data collected in 2017 were analyzed using repeated measure ANOVA (PROC MIXED, SAS Institute, 2018). Numbers of insects on symptomatic and asymptomatic plants were compared using Wilcoxon's test (PROC UNIVARIATE, SAS Institute, 2018). Stepwise multiple regression analysis was used to test a relationship between mean abundances of Colorado potato beetle life stages and the number of symptomatic plants within each plot. Field data collected in 2018 were analyzed using ANOVA (PROC GLM, SAS Institute, 2018).

3.3 Results

3.3.1 Laboratory transmission *of Dickeya dianthicola*

Neither green peach aphids nor Colorado potato beetles displayed any ability to transmit *Dickeya*, as no stage two plants were found to have acquired *Dickeya* following insect feeding and incubation. One Colorado potato beetle sample tested positive through PCR for *Dickeya*. However, that sample had been collected from a single dead male beetle from a control trial, and the beetle suspension from this individual failed to produce cavity-forming bacterial colonies on CVP medium. All other samples, including all the beetles feeding on infected plants, tested negative.

During the Colorado potato beetle transmission experiment, two beetles died: one male from a control trial and a female from an infected trial. As such, there does not appear to be any difference in mortality between infected and control treatments. With green peach aphids, a total of 167 individuals from infected trials died, while 121 green peach aphids died during the course of the control trials. An analysis of variance test shows that the difference in mortalities lacked statistical significance ($F = 1.16$, df = 1,20, $P = 0.295$). Plated Colorado potato beetle suspensions displayed no cavitation following incubation, indicating that no culturable pectolytic erwiniae were present within any of the samples.

3.3.2 Field transmission *of Dickeya dianthicola*

In the Kirby-Bauer assays, no inhibition was observed in *D. dianthicola* cultures across the ten replications due to exposure from either spinosad or water. In nine of the 10 replications, a visible zone of inhibition was created by bleach. In one replication no zones of inhibition were discernable around any of the three discs.

Insecticide treatments had an impact on Colorado potato beetles (Fig. 3.1). Due to beetle phenology, the differences depended on the dates of sampling, as evidenced by significant interactions between the treatment and the sampling date for egg masses (F = 3.46, df = 15,80, P = 0.0002), small larvae (F = 2.90, df = 15,80, P = 0.0011), large larvae (F = 19.60, df = 15,80, P < 0.0001), and adults (F = 3.50, df = 15,80, P = 0.0001). However, as expected, the overall observed effects were negative (Fig. 3.1). Defoliation followed beetle numbers in being higher on plots that were not treated with spinosad (F = 5.18, df = 9,64, P < 0.0001; Fig. 3.2). Aphid numbers were very low throughout the season (0.93 ±0.29 individuals per plant per date). Neither treatment effect (F = 0.20, df = 3,16, P = 0.8976) nor its interaction with the day of sampling were statistically significant (F = 0.70, df = 12,64, P = 0.7457). Based on Wilcoxon's tests, insect numbers were similar among the symptomatic and asymptomatic plants (P> 0.1).

In the field, no significant variation was observed between the numbers of symptomatic plants among the treatments in 2017(F = 0.22, df = 3,16, P = 0.8813). Slightly over half (55.63%) of injected plants across all field treatments acquired visible symptoms (8.9 ± 0.5). Of plants which had not been injected, only an average of 1.4 ± 0.5 plants per plot developed symptoms of blackleg disease. The difference in symptom expression between injected and uninjected plants was significant (F = 43.04, df = 1,127, P < 0.0001).

Stepwise multiple regression showed that only the abundance of adult Colorado potato beetles had a significant negative effect on the number of symptomatic plants (F = 9.45, df = 1,78, P = 0.0029). More adult beetles resulted in fewer symptomatic plants on a plot (Fig. 3.3). However, that relationship explained only 10% of the variation. Neither aphids nor larval Colorado potato beetles shared any significant relationship with the number of symptomatic plants over the 2017 field season.

In 2018, numbers of Colorado potato beetles were similar among the treatments regardless of the sampling date ($P > 0.1$) for all life stages, with 33.9 ± 3.1 egg masses, 179.7 ± 29.9 small larvae, 39.6 ± 7.8 large larvae, and 5.7 ± 0.7 adults found, on average, on a scouted plot. However, applications of spinosad dramatically reduced defoliation of the treated plots ($F = 19.66$, df = 3,16, $P < 0.0001$; Fig. 3.4). Also, significantly more aphids were encountered on plots treated with spinosad ($F = 35.72$, df = 3,16, $P < 0.0001$; Fig. 3.5).

Of the 73 tubers harvested in 2017 from injected plants and grown in greenhouse conditions, only three tubers failed to germinate: two tubers (one from a symptomatic plant and one from an asymptomatic plant) came from a field which had been treated against aphids only, while a third tuber came from a symptomatic plant from a control plot. When the remains of these tubers were tested using PCR, none of these tubers came back positive for *Dickeya*. Of the remaining 70 plants, none displayed any symptoms associated with *Dickeya* infections. Similarly, only two plants in the plant back field displayed symptoms. Both were grown from tubers harvested from fields where only aphids had been sprayed for.

Fig. 3.1. The abundances of Colorado potato beetle life stages by insecticide treatment in 2017.

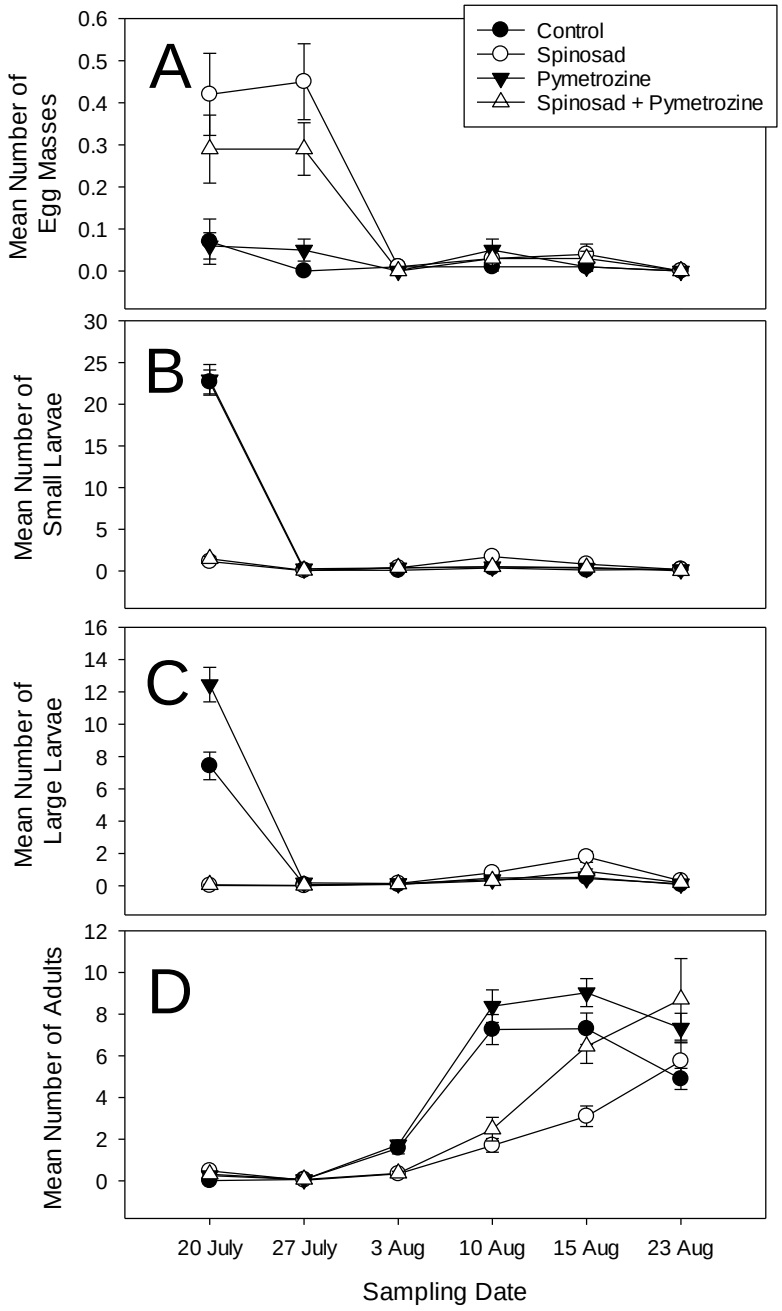


Fig. 3.2. The percentage defoliation affected by insecticide treatment in 2017.

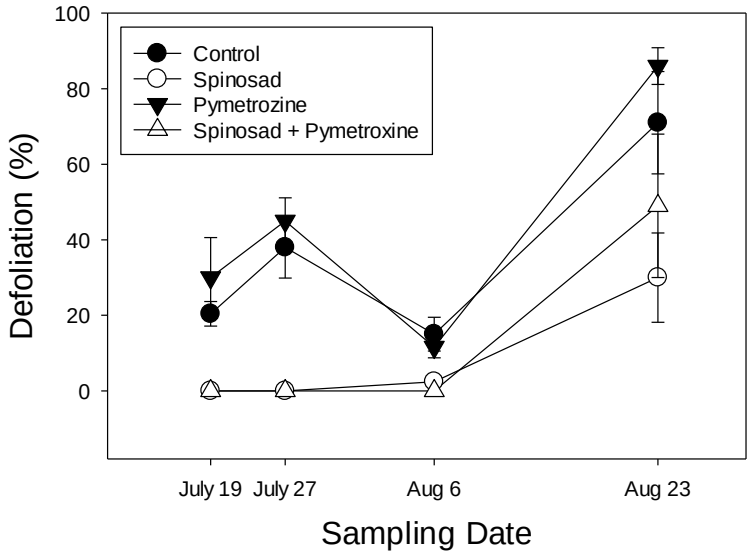


Fig. 3.3. The abundance of adult Colorado potato beetles versus the number of symptomatic potato plants recorded within each plot in 2017.

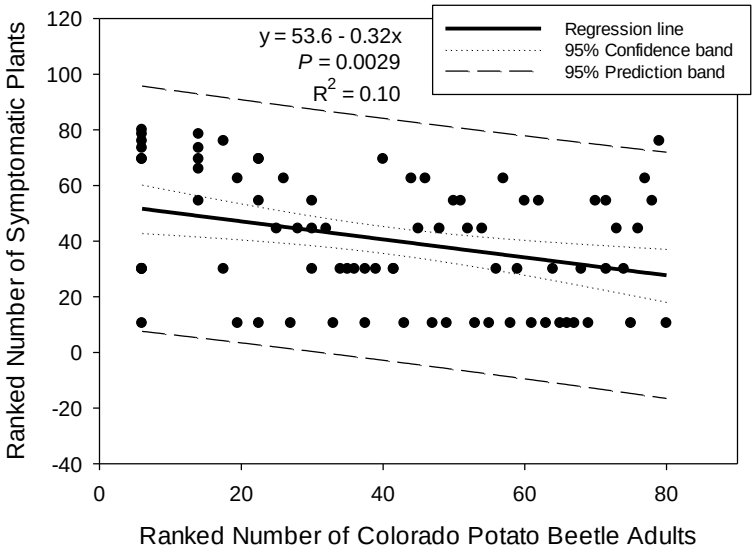


Fig. 3.4. The canopy defoliation by insecticide treatment type in 2018.

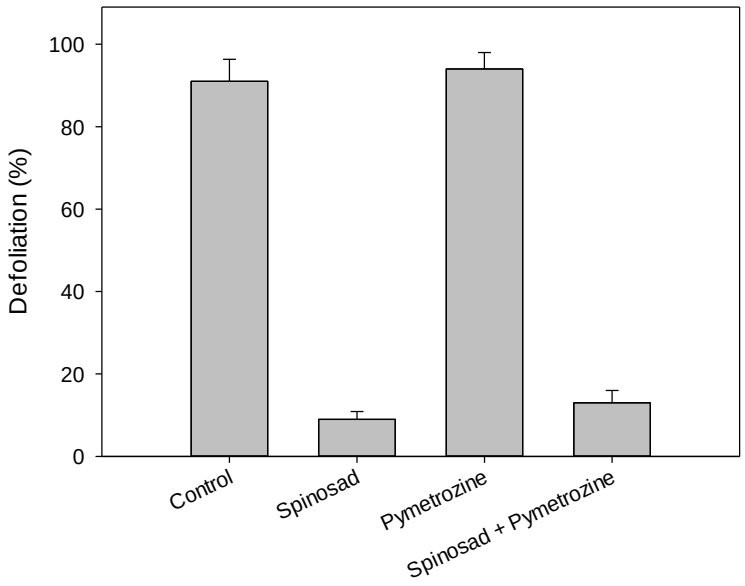
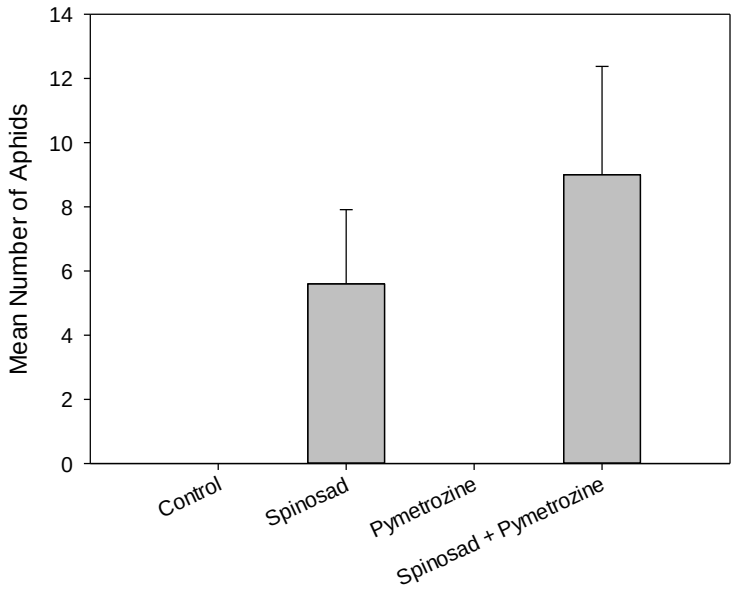


Fig. 3.5. The abundance of aphids affected by insecticide treatment in 2018.



In the field transmission experiment run in 2017, all four categories of plants yielded tubers which tested positive for *Dickeya*. Of the plants which had been injected with *D. dianthicola*, three of the 39 tubers (7.68%) from symptomatic plants tested positive for *Dickeya*, while three out of the 50 tubers (6.00%) from asymptomatic plants tested positive. Among plants which had not received prior injections, three out of 29 tubers (6.90%) from symptomatic plants were positive for *Dickeya*, and one of the 10 tubers (10.00%) harvested from asymptomatic plants was positive following PCR. In addition, *Dickeya* was not found to be present within all daughter tubers produced by infected plants, regardless of how infections had been acquired. Of the six plants which produced infected daughter tubers, only one or two tubers from each plant had tested positive for *Dickeya*.

Tubers harvested from the same field in 2018 yielded fewer positive results. A total of 213 tubers were tested, of which 200 came from unflagged plants and 13 came from flagged plants. Out of these tubers, PCR showed that only three were infected with *Dickeya*. Two of these tubers came from a single plot treated with spinosad. One of these tubers came from an injected plant while the other was from a plant that had not been flagged. The third tuber that tested positive for *Dickeya* originated from an injected plant from a control plot.

3.4 Discussion

Neither Colorado potato beetles nor green peach aphids demonstrated an ability to acquire and transmit *D. dianthicola* between potato plants during laboratory experiments. Although a single Colorado potato beetle sample tested positive while using PCR, the sample had come from a control trial. Furthermore, the sample's bacterial suspension failed to produce

cavities within CVP medium, indicating that it did not contain culturable pectolytic bacteria. Therefore, the reported PCR result is considered to be a false positive.

Dickeya dianthicola may be unable to survive within either Colorado potato beetles or green peach aphids. Bacteria and other microorganisms are faced with several kinds of barriers that may prevent them from establishing on or within the bodies of insects (Nyholm & Graf, 2012). These include behavioral (Evans & Spivak, 2010), physiological (Evans & Spivak, 2010; Boutros et al., 2002; Gerardo et al., 2010), and anatomical adaptations (Ohbayashi et al., 2015; Lanan et al., 2016) designed to avoid exposure to, screen, or suppress potential pathogens. Already-established endosymbionts can also confer protection against certain pathogens (Kambris et al., 2009; Scarborough et al., 2016). The absence of *D. dianthicola* from the sampled insects suggests that *D. dianthicola* may be unable to overcome these hurdles.

Neither insect species displayed a significant difference in mortalities between the individuals feeding on infected foliage versus those feeding on uninfected foliage. Given that the minimum infective dose of *D. dadantii* needed to elicit septicemia within pea aphids, *Acythosiphon pisum* (Harris), is just 10 cells (Grenier et al., 2006), it would be expected that if either insect were susceptible to infection by *D. dianthicola*, they would show a marked increase in mortality following consumption of infected foliage. However, that was not observed. These results corroborate the findings by Grenier et al. (2006), who showed that that D. *dadantii* is not a generalist entomopathogen. Of the insects tested, only the pea aphid displayed septicemia after ingesting *D. dadantii*, while the vinegar fly *Drosophila melanogaster* (Meigen), the cotton leaf-worm *Spodoptera oryzae* (Boisduval), and the rice weevil *Sitophilus oryzae* (L.) suffered no mortalities through ingestion. At the same time, several other species of phytopathogenic bacteria, such as *Psuedomonas syringae* (Van Hall) (Stavrinides et al., 2009), *Pantoea stewartii*

(Roper) (Stavrinides et al., 2010), *Erwinia aphidicola* (Harada) (Harada & Ishikawa, 1997), and even *Dickeya paradisiaca* (Fernandez-Borrero & Lopez-Duque) (Grenier et al., 2006), cause disease in pea aphids and do not all share close taxonomic affiliations within one another. As the interactions between phytopathogenic bacteria and insect vectors are often coevolved, species-specific relationships (Nadarasah & Stavrinides, 2011), we may be limited in our ability to extrapolate observations made on pea aphids and *D. dadantii* to other *Dickeya*-insect relationships.

Spinosad was shown to have no effect on the growth of *D. dianthicola* on CVP medium in the lab. Spinosyns are a family of insecticidal fermentative byproducts produced by the actinomycete bacterium *Saccharopolyspora spinosa* (Mertz & Yao) (Kirst, 2010). As the biochemical products synthesized by actinomycetes are also sources of many antibiotics (Waksman et al., 2010), it was important to demonstrate that the use of spinosad would not be a direct factor in the ability for *D. dianthicola* to spread between plants.

In the field, the abundances of Colorado potato beetles and potato-colonizing aphids did not differ significantly between infected and uninfected plants. This is in line with the pattern of behavior observed in laboratory experiments discussed in the previous chapter, which demonstrated that neither Colorado potato beetles nor green peach aphids discriminate between plants based on the presence or absence of *D. dianthicola*.

Very little disease spread was observed across all treatments during both seasons, even with the heavy defoliation caused by Colorado potato beetles. Observed spread occurred early in the season, with very little accumulation in the numbers of symptomatic plants observed later in the season. Wounds are known to serve as sites of infection by *Dickeya* (Czajkowski et al., 2011). Wounded plant tissues release jasmonic acid, along with other signaling molecules, to

initiate the launch of physiological defenses against herbivores and pathogens (Dammann et al., 1997; Moran & Thompson, 2001; Reymond et al., 2000). *Dickeya* cells display strong positive chemotaxis toward jasmonic acid, allowing them to locate sites of entry into potential hosts (Reverchon & Nasser, 2013). However, once inside a plant, *Dickeya* cells face several barriers to successful induction of systemic infection. Plants utilize a battery of physiological weapons, such as formation of phenol-derived reactive oxygen species that inhibit bacterial protein function, local alkalization of normally acidic intercellular spaces to impair bacterial acquisition of iron needed in enzymatic processes, and depositing lignin and suberin to create mechanical barriers against bacterial spread (Ngadze et al., 2012; Reverchon & Nasser, 2013).

The feeding activities of herbivorous insects can also elicit plant defense responses that can impair the growth of phytopathogens. Exposure of *Arabidopsis thaliana* (L.) to the larval regurgitates of the small cabbage white butterfly, *Pieris rapae* (L.), has been found to trigger localized salicylic-acid mediated defenses. The activation of these physiological pathways increased the resistance of injured leaves to infection by *Pseudomonas syringae* (Van Hall) and *Xanthomonas campestris* (Pammel) (de Vos et al., 2006). Feeding by the silverleaf whitefly, *Bemisia argentifolii* (Bellows & Perring), on tomato plants, *Lycopersicon esculentum* (L.), stimulated the production of defensive chitinase and peroxidase within foliage which had an inhibitory effect on the growth of powdery mildew, *Erysiphe cichoracearum* (DC.) (Mayer et al., 2002). Similarly, previous infestation of rice plants, *Oryza sativa* (L.), by the white-backed planthopper, *Sogatella furcifera* (Horváth), induced resistance within rice to the rice blast fungus *Magnaporthe grisae* (Hebert) (Kanno & Fujita, 2003; Kanno et al., 2005). Therefore, even if the damage caused by Colorado potato beetles could have increased the number of points of entry for *D. dianthicola*, additional factors such as the physiological response of plants to infection and

possible cross-resistance between anti-herbivore plant defenses and *D. dianthicola* may have complicated successful spread of the bacterium in the field. Thus feeding by Colorado potato beetles does not appear to facilitate the spread of *D. dianthicola* under field conditions.

During both field seasons, aphid abundances were exceptionally low. While the drought experienced in 2017 may account for the low abundance of aphids that year, the abundance of Colorado potato beetles was so great in 2018 that they had stripped all plants not treated with spinosad. This resulted in high abundances of aphids among spinosad-treated plots. Unfortunately, that low abundance of aphids coupled with the low frequency of infection among harvested tubers from all treatments (particularly in 2018) prevents any accurate conclusions from being made about the effect of aphids on *D. dianthicola* transmission in the field.

Feeding by green peach aphids has been shown to stimulate a reduced susceptibility of pepper plants, *Capsicum annum* (L.), to infection by *Xanthomonas axonopodis* (Hasse) and *Ralstonia solanacearum* (Smith). This may be due in part to activation of systemic jasmonic acid/ethylene- and salicylic acid-mediated chemical pathways and induced recruitment of mutualistic rhizosphere bacteria (Lee et al., 2012). Thus, it is possible that aphid feeding may also reduce the susceptibility of plants to *D. dianthicola*.

Regardless of insect activity, *D. dianthicola* did not appear to be highly contagious. In part, the low frequency of infection detected among tubers harvested during both years may be explained by dry conditions. These bacteria are reliant on water films to travel within soil and be taken up into roots and tuber lenticels (Toth et al., 2003; Czajkowski et al., 2010a), and symptom expression among plants which harbor *Dickeya* varies depending on the growing conditions (Czajkowski et al., 2011; Tsror et al., 2009). However, supplemental irrigation was used to bring up weekly precipitation to at least 25 mm; yet, the spread of infection between plants was still

low. Therefore, it is likely that and the low transmission rates could not be attributed to dry conditions alone.

During observation of plants for symptoms of infection, it appeared that *D. dianthicola* often had difficulty translocating within plants following stem injections. It was not unusual to see an area of apparently suberized brownish tissue surrounding the black necrotic lesion formed around the site of injection in the field. Similarly, Czajkowski et al., (2010b) found that foliar inoculations of potato plants under greenhouse conditions rarely results in the infection of daughter tubers, perhaps in part due to internal physiological barriers such as that posed by the junction between the petiole base and the plant's stem. Ongoing work by Hao et al. (2017) has also demonstrated that some potato cultivars have at least partial resistance to *D. dianthicola* ME30. While the potato cultivars chosen for these experiments ('Superior' and 'Katahdin') are known to be susceptible to infection (Hao et al., 2017; Buzza, and Alyokhin, 2017, personal communication), not all plants injected with *D. dianthicola* in the field acquired active, lethal infections. Therefore, it is possible that the plants did possess some limited resistance against *D. dianthicola* under the conditions of this study.

Based on these observations, there are at least two possible explanations for the low abundance of infected tubers produced between the two field seasons. First, as most disease spread occurred early in the year, it is possible that many of the plants which acquired *D. dianthicola* succumbed either from their infections or from the severity of defoliation by Colorado potato beetles before the plants had produced daughter tubers. This would help to explain the scarcity of tubers produced by flagged plants in 2018, as only 13 tubers were retrieved from flagged plants despite 320 plants having been injected with *D. dianthicola*. Second, the apparent poor mobility of *D. dianthicola* within and between the plants in the field

may have resulted in very few tubers acquiring or retaining the bacterium. This may be supported by the observation that, in 2017, only one or two tubers among those produced by plants which yielded infected tubers ever tested positive.

While this study did not definitively disprove that insect-mediated transmission occurs within *D. dianthicola* outbreaks, it appears likely that other media for transmission are more important. Initial introduction of this pathogen to a field often happens via planting of asymptomatic seed tubers (Pérombelon, 2000). After that, movement of soil, irrigation water, contaminated farming equipment, or aerosols formed by rain or flailing have been shown as factors responsible for in-field spread of *D. dianthicola* (Ansermet et al., 2016; Cappaert et al., 1988, Czajkowski et al., 2011; Skelsey et al., 2016; Laurila et al., 2008). It is thus important that management strategies focus on these factors in order to curtail outbreaks and losses caused by *D. dianthicola*.

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BIOGRAPHY OF THE AUTHOR

Jonas Klair Insinga was born on November 4th, 1988, in Framingham, MA. He grew up in Nashua, NH, and graduated from Nashua High School North in 2007. The same year he enrolled at the University of New Hampshire in Durham, NH. There, Jonas worked assisting in the curation of the University of New Hampshire Entomology Collection and conducted an undergraduate study on the relationship between selective tree harvest and mycophagous beetles under the guidance of Dr. Donald S. Chandler. Jonas graduated with a Bachelor of Arts in Zoology in 2012.

From 2013 through 2015, Jonas worked as an independent ceramics artist. At the same time, he also had seasonal employment as a field technician in a study utilizing the predatory wasp *Cerceris fumipennis* as a tool for monitoring the spread of invasive emerald ash borer, *Agrilus planipennis*. In 2015, Jonas worked as a substitute teacher with the Nashua Public School District while taking classes at the University of Massachusetts in Amherst.

In 2016, Jonas was accepted into the University of Maine to work on a research project funded by the Maine Potato Board. While here, Jonas served as the 2017 president of the Maine Entomology Student Organization, a graduate student club that seeks to promote public interest in insects through holding outreach and education events. Jonas is a candidate for the Masters of Science degree in Entomology from the University of Maine in December 2019.