The Influence of Biopesticides on Verticillium Wilt in Potatoes

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Nicholas M. Vincent

Major Professor: Michael K. Thornton, Ph.D.

Committee Members: Amber Moore, Ph.D.; Phillip S. Wharton, Ph.D.

Department Administrator: Paul McDaniel, Ph.D.

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Authorization to Submit Thesis

This thesis of Nick Vincent, submitted for the degree of Master of Science with a Major in Plant Science and titled "The Influence of Biopesticides on Verticillium Wilt in Potatoes," has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

Major Professor: _____ Date: _____

Michael K. Thornton, Ph.D.

Committee Members:

_____ Date: _____

Amber Moore, Ph.D.

_____ Date: _____

Phillip S. Wharton, Ph.D.

Department _____ Date: _____

Administrator: Paul McDaniel, Ph.D.

Abstract

Potato growers in Southern Idaho are searching for alternative ways to control diseases that are both environmentally sustainable and cost effective. The need for alternative approaches to chemical fumigants is critical, given the recent restrictions on chemical fumigants mandated by regulatory agencies. Application of biopesticides is an approach that is becoming more popular in the agricultural industry, but has not been evaluated extensively in potato production systems. A field and greenhouse experiment was conducted over a two-year period at the University of Idaho Parma Research and Extension Center in 2016/2017 to evaluate how these products might impact Verticillium wilt in potato. The field trial consisted of two biopesticide treatments (*Bacillus subtilis* and *Trichoderma asperellum*/*Trichoderma gamsii*) were applied at 4 different rates/timings. *Bacillus subtilis* rates consisted of 4.7 l/ha for in-furrow, 4.7 l/ha for low rate chemigation, and 9.4 l/ha for high rate chemigation. *Trichoderma asperellum*/*Trichoderma gamsii* rates consisted of 350.8 ml/ha for in-furrow, 2.8 kg/ha for low rate chemigation, and 5.6 kg/ha for high rate chemigation. The in-furrow treatment was applied at planting and the chemigation treatments were applied four times throughout the growing season. Soil and stem samples obtained from each of the treatments before the first chemigation and after all the treatments were completed were analyzed using a real-time polymerase chain reaction (qPCR) to measure the amount of *Verticillium dahliae* and *Colletotrichum coccodes* present in the soil and stem tissue. Symptoms of early die were rated at two week intervals and values were calculated using the relative area under the disease progress curve (RAUDPC). There were no significant treatment effects of *Bacillus subtilis*, *Trichoderma*

asperellum/*Trichoderma gamsii* or fumigation with metam sodium on visual symptoms of early die, total tuber yield or pathogen populations in stem tissue or soil at harvest when compared with the non-treated control. The greenhouse study consisted of four biopesticides including *Bacillus subtilis*, *Trichoderma asperellum*/*Trichoderma gamsii*, *Trichoderma virens*, and *Reynoutria sachalinensis* plant extract. Field soil was amended with sphagnum peat moss to establish three levels of organic matter. There were no significant differences among the treatments that would suggest that biopesticides can effectively control Verticillium wilt in potatoes. More research is needed to understand the efficacy of these products on potato pathogens.

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I want to thank my committee members Dr. Amber Moore and Dr. Phillip Wharton on their knowledge and input throughout this process.

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Dedication

I dedicate this thesis to my parents Mike and Lori Vincent, who always encouraged me to follow my heart and pursue a career in the agricultural industry. Without them, I would not be where I am at today. I love you and thank you.

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CH. 1 INTRODUCTION

1.1 BACKGROUND

Potatoes (*Solanum tuberosum* L.) are an important crop, ranking 3rd in world in total production behind rice and wheat as of 2012 (Birch et al., 2012). In 2014, 381,682,144 metric tonnes of potatoes were produced worldwide on 19,000,000 hectares (FAO, 2014). The United States is 4th in total production behind China, Russia, and India as of 2014.

Approximately 50% of all the potatoes produced in the world are dedicated for fresh consumption, while the other 50% of potatoes produced are used for processing, animal feed, or seed as of 2012 (Birch et al., 2012). Potatoes are high in water and nutritional content containing approximately 80% water by weight on average, with a 142 g potato providing 50% of the daily vitamin C requirements (Stark and Love, 2003).

Potatoes originated in the Andes mountains of South America (Stark and Love, 2003). The cultivation of potatoes has been documented as far back as 7,000 years ago (Stark and Love, 2003). Potatoes arrived in Europe from South America in 1570 AD and reached North America by the early 1700's (Stark and Love, 2003).

The Irish Potato Famine occurred in 1845 and lasted until 1849. Late blight (*Phytophthora infestans*) spread throughout Ireland causing reductions in quality and yield, which led to a mass starvation throughout the country. About 1 million people starved to death during this famine and about 1.5 million people migrated to other countries, mostly the United States and Canada. The Irish Potato Famine is known to be the birth of plant pathology (Schumann, 1991).

There are many potato diseases that cause production issues throughout world. The Compendium of Potato Diseases lists six bacterial and twenty-six fungal diseases for the U.S. (Franc et al., 2001). Among the most common or economically important are late blight (*Phytophthora infestans*), bacterial ring rot (*Clavibacter michiganensis*), Verticillium wilt (*Verticillium dahliae*), black dot (*Colletotrichum coccodes*), and pink rot (*Phytophthora erythroseptica* (Franc et al., 2001).

1.2 POTATO EARLY-DYING COMPLEX

Potato early-dying complex (PED) is an important disease in Idaho. It is known as a complex because there are thought to be many pathogens involved in it. The most commonly cited casual agents include *Verticillium dahliae* Kleb, *Colletotrichum coccodes*, root lesion nematodes (*Pratylenchus penetrans* and *Pratylenchus neglectus*), *Rhizoctonia solani*, *Erwinia carotovora*, and *Spongospora subterranea* (Saeed et al., 1997; Johnson and Dung, 2010; Powelson and Rowe, 1993; Johnson and Miliczky, 1993; Rowe and Powelson, 2002). The most common visual symptom of early-die is premature vine senescence, leading to decreases in yield and overall quality (Franc et al., 2001). In some cases, yield losses as high as 50% have been reported (Rowe and Powelson, 2002).

Black dot, which is caused by the fungus *Colletotrichum coccodes*, used to be considered a minor disease in the potato industry. More recently, it has been reported that this fungus could be a major component of PED (Lees and Hilton, 2003; Johnson and Miliczky, 1993). *Colletotrichum coccodes* interacts with *V. dahliae* by increasing wilting symptoms and reducing tuber quality (Davis et al., 2001). Although the role of the root lesion nematode (*Pratylenchus penetrans*) in PED has been studied more extensively, the exact role

of *P. penetrans* in PED is still unknown, but there is a synergistic relationship with *V. dahliae* and possibly other pathogens (Rowe et al., 1985). However, it is known that the role of *P. penetrans* in PED is not from feeding sites creating openings for *V. dahliae* to infect (Rowe and Powelson, 2002). Nematode feeding sites could potentially increase root exudates or increase root branching to stimulate microsclerotia germination (Rowe and Powelson, 2002). Bowers et al. (1996) showed that there was an increase in vascular colonization by *V. dahliae* in the presence of *P. penetrans* versus *V. dahliae* alone. This could mean that there is physiological change associated with the two pathogens (Rowe and Powelson, 2002).

While there are several pathogens associated with the PED complex, the general consensus is that *V. dahliae*, and black dot caused by *C. coccodes* are two of the primary pathogens involved.

1.3 VERTICILLIUM WILT

Verticillium dahliae Kleb. is in the fungal phylum Ascomycota, subphylum Pezizomycontina, and class Sordariomycetes (Fradin and Thomma, 2006). *Verticillium dahliae* has been classified further into different vegetative compatibility groups (VCGs) with VCG 4A the most virulent and aggressive towards potatoes (Berlanger and Powelson, 2000). *Verticillium albo-atrum* Reinke & Bertheir is another important pathogen that can cause Verticillium wilt. However, this pathogen is favored in cooler regions and is uncommon in areas where soil temperatures exceed 25^{o} C (Rowe and Powelson, 2002). The temperate climate in the Pacific Northwest favors the growth of *V. dahliae*, but pathogen growth can be inhibited at 30^{o} C (Rowe and Powelson, 2002). *Verticillium dahliae* has a large host range of over 200 dicotyledonous species (Johnson and Dung, 2010). *Verticillium dahliae* overwinters

in the soil as microsclerotia, which is different from *V. albo-atrum* that overwinters as melanized hyphae (Johnson and Dung, 2010). *Verticillium dahliae* distribution throughout fields is known to be in clustered patterns (Steere et al., 2016; Xiao et al., 1997). Microsclerotia of *V. dahliae* will not move through the soil profile and are mainly distributed through cultivation practices (Berlanger and Powelson, 2000). Taylor et al. (2005) found that most of the *V. dahliae* population was concentrated in the top 10 cm of the soil.

1.3.1 Life Cycle

The life cycle of *V. dahliae* is monocyclic and starts with microsclerotia germinating in the presence of root exudates. *Verticillium dahliae* hyphae colonize the root tip and cortex. The pathogen then penetrates the stele and colonizes the xylem (Johnson and Dung, 2010) *Verticillium dahliae* then produces mycelia, which will continue to grow upward through the xylem and colonize the plant over the course of the growing season (Johnson and Dung, 2010). The optimum temperature for *V. dahliae* growth in the plant is between 13^{o} to 24^{o} C (Strand, 2006).

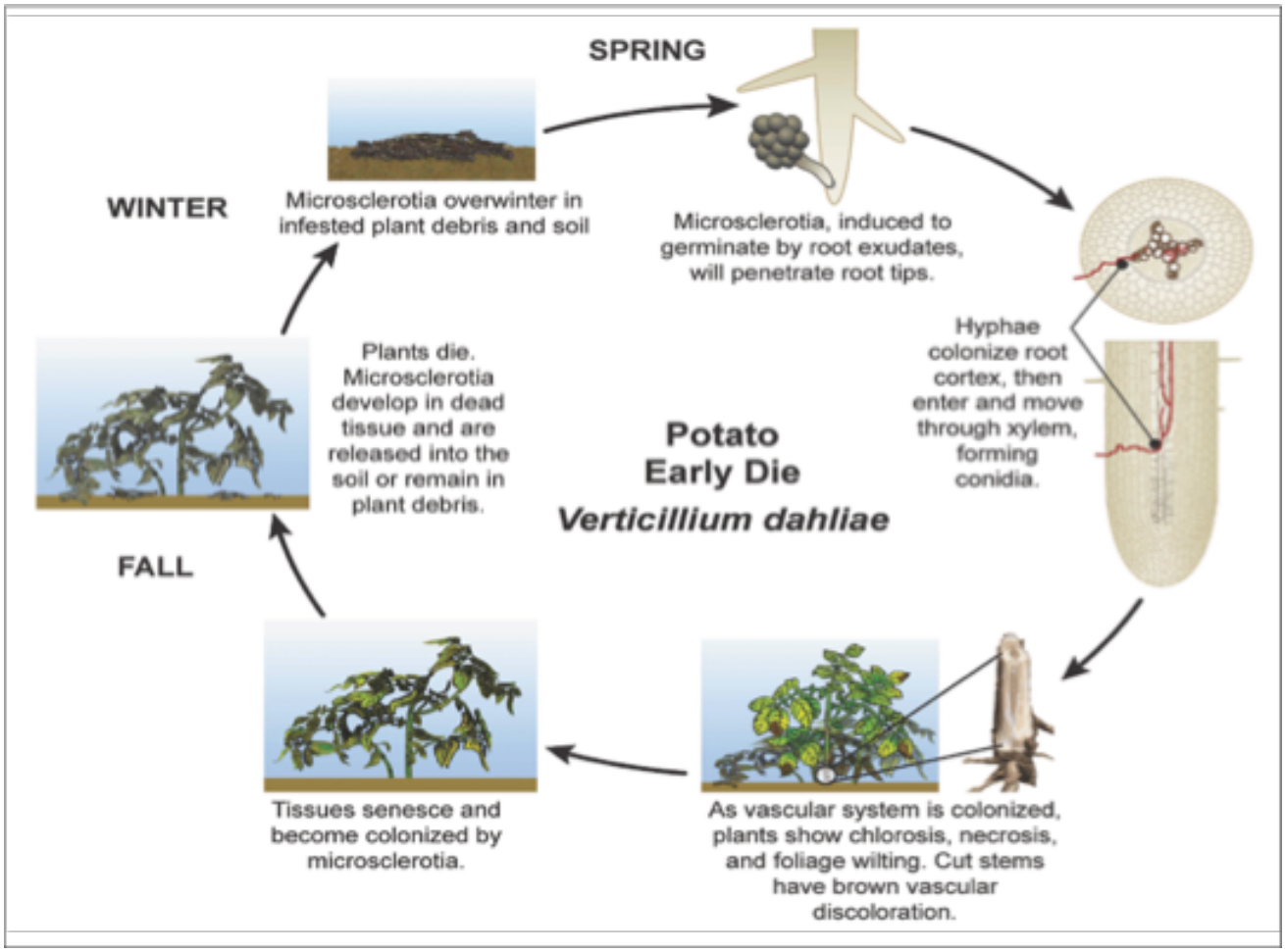

Figure 1.1: *Verticillium dahliae* life cycle.

Courtesy of Steere et al., 2016.

The fungus disrupts the movement of water and nutrients throughout the plant. When the plant begins to senesce, *V. dahliae* forms microsclerotia inside the desiccating stems of the plant. Eventually the plant dies and the microsclerotia are released back into the soil during decomposition, where they remain dormant until root exudates stimulate germination. One infected potato plant can release as many as 90,000 microsclerotia into the soil (Johnson and Dung, 2010). Microsclerotia of *V. dahliae* have been documented to remain viable for up to 13 years (Xiao et al., 1997).

1.3.2 Symptoms

Symptoms will first develop on the lower leaves, progressing upwards toward the top of the plants (Franc et al., 2001). Common symptoms include wilting, chlorosis, and necrosis. Progression of the symptoms leads to total vine senescence in severe cases. It is common to observe that half the leaves on a petiole develop wilting symptoms while the other half remain fine. Flagging is a symptom in severe cases and consist of the plants standing erect above other potato plants in the field (Strand, 2006). Stem and tuber vascular discoloration is another common symptom that is identified with Verticillium wilt, but physiological stresses and other pathogens can cause similar symptoms (Berlanger and Powelson, 2000). Therefore, it can be difficult to differentiate Verticillium wilt from natural senescence, or other diseases such as grey mold.

1.3.3 Control Measures

The most commonly used practices to control Verticillium wilt include crop rotation, planting resistant varieties, sanitation practices, eliminating volunteer potatoes, fumigation and maintaining proper fertility and irrigation levels.

Crop rotation with non-potato crops is a common practice used throughout potato production to reduce potato disease pressure. However, it can be challenging when trying to control *V. dahliae* because the microsclerotia can remain viable in the soil for many years. Typically, growers will use rotations of 3 or 4 years between potato crops. Recommended rotation crops to use to decrease *V. dahliae* inoculum include peas, sugar beet, corn and onions (Strand, 2006). Green manure crops have been effective in reducing the inoculum levels of *V. dahliae*, but the impacts on disease symptoms have been inconsistent (Strand,

2006; Davis et al., 1996; Larkin et al., 2011). Eliminating volunteer potatoes between rotations can be used to avoid increasing inoculum levels (Strand, 2006).

Use of resistant varieties has been shown to be an effective management tool for controlling *V. dahliae*. Resistant varieties include Bannock Russet, Payette Russet, Clearwater Russet, and Chipeta. Moderately resistant varieties include Ranger Russet, Dakota Russet, Atlantic, and Umatilla Russet. Susceptible varieties include Russet Norkotah, Yukon Gold, and Shepody (Johnson and Dung, 2010). Even the most resistant varieties will develop symptoms if the inoculum levels are high enough under the proper conditions (Strand 2006).

Cultural practices such as sanitation, proper fertilization and irrigation are all methods that can be used to help control *V. dahliae*. Sanitation practices include cleaning equipment to avoid moving inoculum between fields (Strand, 2006). If a field has a historical record of having a high incidence of Verticillium wilt, it is important to plant and harvest that field after the other non-infected fields have been planted and harvested, because the microsclerotia could be transferred from one field to another. Tare dirt present on seed tubers containing microsclerotia is another potential source of infection (Johnson and Cummings, 2015).

Controlling fertility and moisture levels may be the most effective way for preventing Verticillium wilt issues. Certain cultivars may not express symptoms even if infected with *V. dahliae* until stressed (Strand, 2006). Nitrogen deficiency has been shown to increase the severity of the symptoms of Verticillium wilt (Davis and Everson, 1986). Over irrigation early in the season prior to tuber initiation and deficient irrigation later in the season also have been linked to increase severity of the disease (Cappaert et al., 1992).

Soil organic matter has been shown to have an impact on the severity of Verticillium wilt in potatoes. Davis et al. (2001) showed that there was a correlation with increasing soil organic matter from 0.8 to 2.3% and decreasing wilting symptoms from 63.4 to 0.0%. Soil organic matter has been linked to improved soil fertility, soil structure, and cation exchange capacity, which can improve nutrient uptake and water retention (Carter et al., 2004; Bot and Benites, 2005). Increasing soil organic matter levels increases the activity of microorganisms, which are important for mineralization (Bot and Benites, 2005). Also, some microorganisms have been shown to be effective against soil borne pathogens (Harman, 2006; Whipps et al., 2008; Choudhary and Johri, 2009; Kloepper et al., 2004; Berg, 2009).

Fumigation, primarily with metam sodium (sodium N-methyl dithiocarbamate), has been the most commonly used approach for controlling Verticillium wilt in potatoes (Pasche et al., 2014). Metam sodium breaks down to methyl isothiocyanate (MITC), which is toxic to soil borne pathogens (Triky-Dotan et al., 2007). Numerous studies have shown that metam sodium is effective in controlling *Verticillium dahliae* in potato (Hamm et al., 2003; Rowe and Powelson, 2002; Saeed et al., 1997). However, recently the Environmental Protection Agency (EPA) has been re-registering fumigants and adding restrictions on application, making it difficult to rely on this method of control (EPA, 2005). Fumigants have been linked to causing damage to the environment and non-target organisms (Sande et al., 2011; Yates et al., 2002; Macalady et al., 1998). Methyl bromide, which was a popular fumigant that was used throughout the agricultural industry has been banned for causing ozone depletion (EPA, 2017).

1.4 BLACK DOT

Colletotrichum coccodes (Wallr.) S. Hughes is widely distributed throughout all potato regions across the world (Lees and Hilton, 2003). *Colletotrichum coccodes* can infect all parts of the potato plant including stems, tubers, roots and foliage (Kirk et al., 2012). The fungus has been viewed in the past as a secondary pathogen, but recent literature suggests that the pathogen may be more important than previously understood (Lees and Hilton, 2003). Black dot gets its name from the tiny black microsclerotia that are formed on the tuber. The pathogen has a wide host range including other plants in the Solanaceae family (Kirk et al., 2012). These include tomato, pepper, eggplant, and weeds like hairy nightshade (Kirk et al., 2012). Black dot microsclerotia has been reported remain viable in the soil for up to 2 years (Kirk et al., 2012). Soil borne and seed borne inoculum have both been reported to contribute to disease incidence, but soil borne inoculum has been shown to be the primary source of infection (Lees and Hilton, 2003).

1.4.1 Life Cycle

The life cycle of *C. coccodes* is polycyclic and the pathogen overwinters as microsclerotia. Acervuli are produced in the spring on infected host plant debris, potato tubers, and soil (Kirk et al., 2012). Spores are dispersed through air, wind, or rain to neighbouring plants. *Colletotrichum coccodes* spore formation increases in high moisture environments and is favored by temperatures that are between 7 and 35 ^{o}C (Kirk et al., 2012). Spores germinate and infect through the epidermis or through wounds. Underground plant parts can become infected at all stages of the potato plant, but microsclerotia production increases as the temperature increases (Kirk et al., 2012). As the plants start to

senesce, microsclerotia are produced and will overwinter until the following year on infected tubers, host plant debris, and in soil.

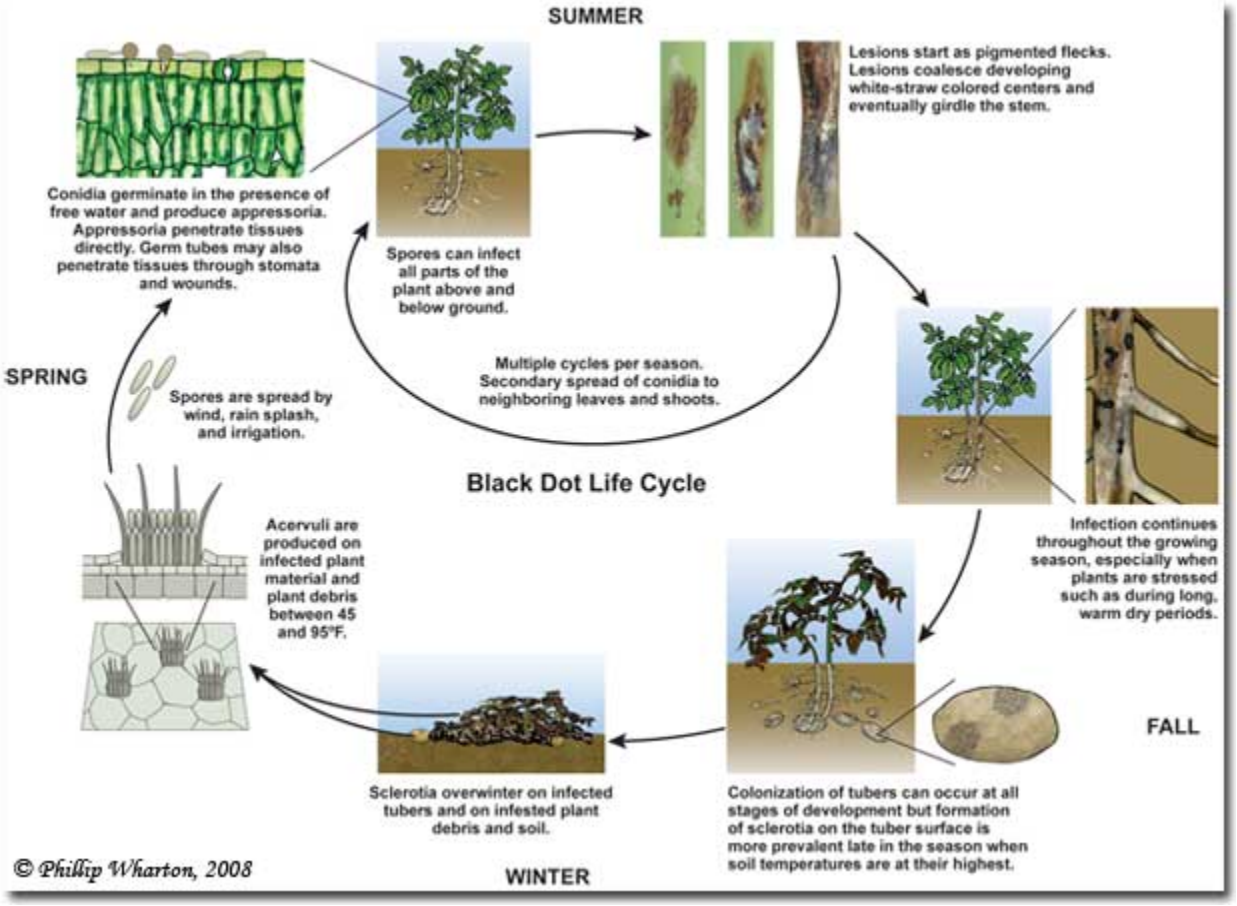

Figure 1.2: *Colletotrichum coccodes* life cycle.

Courtesy of: P. Wharton

1.4.2 Symptoms

Symptoms associated with black dot include early vine senescence and tuber blemishes. Tiny black dots, the microsclerotia, are produced on the tubers, stems, and vines (Kirk et al., 2012). These microsclerotia become visible as the plant starts to senesce and are often visible with the naked eye. The tuber blemishes can sometimes be mistaken for silver scurf, but can be differentiated by using a hand lens to view the microsclerotia of *C. coccodes*

(Kirk et al., 2012). The blemishes visible on the tubers can cause issues with processors and fresh pack producers (Lees and Hilton, 2003). Black dot can also cause lesions on the stolons and stems, which can contribute to early plant senescence (Kirk et al., 2012).

1.4.3 Control Measures

Control measures for black dot are similar to Verticillium wilt, and include crop rotation, using certified seed, fungicides, fumigation and proper irrigation and fertility management.

Crop rotation has been shown to be effective in controlling black dot because the microsclerotia can remain viable for about 2 years (Kirk et al., 2012). A rotation with non-host crops between potatoes of 4 to 5 years will reduce the amount of soilborne inoculum that is present in the field (Kirk et al., 2012). Potential crops to use to reduce black dot soil inoculum include soy bean, corn, and small grains (Kirk et al., 2012). Using certified seed can be effective in controlling black dot. Johnson et al. (1997) reported greater stem infections from plants grown from seed tubers infected with *C. coccodes* versus seed tubers not infected with the pathogen. There are no varieties that are resistant to black dot at this time. However, reports have shown that later maturing varieties are more susceptible to infection (Lees and Hilton, 2003). There are limited number of fungicides that can be used to control black dot. Azoxystrobin has been reported to reduce black dot on stems, but fungicide effectiveness reports have been variable (Kirk et al., 2012). Fumigation is another possible option to reducing soil inoculum of *C. coccodes*. A study in South Africa reported 41% black dot incidence in unfumigated soil versus 1% of black dot incidence in soil fumigated with

methyl bromide. However, this method of control might not be economically viable (Lees and Hilton, 2003).

1.5 NEED FOR ALTERNATIVES

Due to a focus on improved sustainability in the potato industry, recently there has been an required emphasis on finding alternatives to fumigation. In 2005, potato production in the U.S. required 19,050,879 kg of fumigants, with the next closest crop tomatoes at 8,890,410 kg (EPA, 2005). Another issue with fumigation in that it results in a higher amount of active ingredient applied per unit area compared to other chemicals used in agriculture production. For example, in 2002, almost 25 million kg of metam sodium active ingredient was used in the U.S., compared to 17 million kg of imidacloprid (a commonly used insecticide) (Gianessi and Reigner, 2006). Farmers are also interested in alternatives to fumigation because it is expensive, costing growers around $112.14 per hectare on average in Southwest Idaho in 2015 (Eborn and Paterson, 2015).

With the possibility of other fumigants being banned for agricultural use, cost of application, and a movement towards sustainability, researchers are searching for effective alternatives to control PED in potatoes.

1.6 BIOPESTICIDES

Biopesticides could potentially be an alternative to fumigation that is more sustainable. The Environmental Protection agency (EPA) defines biopesticides as, “certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals.” (EPA, 2017). There are three types of biopesticides, microbial, plant-incorporated protectants (PIPs), and biochemical (EPA, 2017). Microbial biopesticides consist

of microorganisms such as bacteria, fungi, viruses, or protozoa as the biocontrol agents. Plant-incorporated protectants (PIP) consist of genetic material added to the plant that creates pesticidal activity (EPA, 2017). An example of a PIP would be J.R. Simplot Company's Innate potato that has late blight resistance. Biochemical biopesticides are substances that occur naturally, but are non-toxic to the target pests. For example, insect pheromones are used in apple production to disrupt the mating of the codling moth insect (Judd et al., 1997). Another example is using plant extracts, like giant knotweed (*Reynoutria sachalinensis*), to stimulate plant defenses against pests and pathogens (Tamm et al., 2011).

Biopesticides have been used in agriculture for hundreds of years. One of the earliest uses of biopesticides dates to the 17th century when nicotine was used to control plum beetles (BPIA, 2017). In the 20th century, research on biopesticides continued at a low level because of the adoption of cheaper and more effective synthetic chemicals (BPIA, 2017). In the 21st century, there has been a movement towards sustainable agriculture that has resulted in an increase in research and development of these products. The United States is the biggest market for biopesticides in the world, accounting for 44% of the total market. (Bailey et al., 2010).

1.7 MICROBIAL BIOPESTICIDES

Microbial biopesticides may have multiple modes of action including antibiosis, parasitism, direct competition, plant growth promotion, and induced resistance (EPA, 2017; University of Connecticut, 2017). There have been numerous studies that have shown microbial biopesticides being effective against soil borne pathogens (Harman, 2006; Whipps

et al., 2008; Choudhary and Johri, 2009; Kloepper et al., 2004; Berg, 2009). Larkin, (2007) showed that the fungus *Trichoderma virens* reduced the incidence of black scurf (*Rhizoctonia solani*) in potatoes. *Trichoderma asperellum* was also shown to be effective in suppressing *Rhizoctonia solani* in cucumber (Trillas et al., 2006). Varo et al. (2016) showed that the combination of *Trichoderma asperellum* and *Trichoderma gamsii* was effective in controlling *Verticillium dahliae* in olive trees.

Bacteria have also been used in various soil borne pathogen studies. *Bacillus subtilis* has been shown to be effective against pink rot (*Phytophthora erythroseptica*) in potatoes (Wharton et al., 2012).

However, many of the studies on soil borne pathogens have been conducted *in vitro*, and there is not a lot of data demonstrating efficacy of these products under field conditions. Therefore, we decided to carry out a study under field and greenhouse conditions that would determine if biopesticides could provide economical and consistent control on the early-die complex in potatoes.

The objective of the Ch. 1 study was to determine the efficacy of commercial formulations of *Trichoderma asperellum/Trichoderma gamsii* and *Bacillus subtillis* on the potato early-dying complex in a field environment. In-furrow and chemigation applications were used to determine if the timing and method of application has an impact on effectiveness. The objective of the Ch. 2 study was to determine how four different biopesticides (*Trichoderma asperellum/Trichoderma gamsii*, *Bacillus subtilis*, *Reynoutria sachalinensis* and *Trichoderma virens*) impacted the potato early-dying complex in a greenhouse environment. This study included subplots with different levels of organic

matter to evaluate how organic matter could potentially impact the efficacy of these products.

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CH. 2 INFLUENCE OF BIOPESTICIDES ON VERTICILLIUM WILT IN POTATOES

2.1 ABSTRACT

Potato growers are searching for alternative ways to control diseases that are both environmentally sustainable and cost effective. The need for alternative approaches to chemical fumigants is critical, given the recent restrictions on chemical fumigants mandated by regulatory agencies. Application of biopesticides is an approach that is becoming more popular in the agricultural industry, but has not been evaluated extensively in potato production systems. A two-year field experiment was conducted at the University of Idaho Parma Research and Extension Center in 2016 and 2017 to evaluate how these products might impact Verticillium wilt (*Verticillium dahliae)* and black dot (*Colletotrichum coccodes*) in potato. Two biopesticide treatments (Serenade ASO - *Bacillus subtilis*) and (Bio-Tam - *Trichoderma asperellum*/*Trichoderma gamsii*) were applied at 4 different rates/timings. There were no significant differences in either year that would suggest that these two biopesticides could effectively control the potato early-dying complex. More research is needed to understand the efficacy of these products on potato pathogens.

2.2 INTRODUCTION

Many studies using biopesticides have looked at how these products impacted a particular pest, but there hasn't been a lot of research conducted on how application timing might impact effectiveness. Wharton et al., (2012) applied Serenade ASO (*Bacillus subtilis*) as an in-furrow application and reported a reduction in pink rot incidence and severity. Jordan and Gevens, (2013) reported a decrease in black scurf (*Rhizoctonia solani*) incidence when Regalia (*Reynoutria sachalinensis* plant extract) was applied as a seed treatment, in-furrow, and chemigated at the 4-6 leaf rosette plant stage. This same study showed an increase in black scurf incidence compared to the untreated check when Serenade ASO was applied as an in-furrow application only. More research is needed to understand if the method and frequency of application has an impact on disease control.

The objective of this study was to evaluate the efficacy of commercial formulations of Serenade ASO (*Bacillus subtilis* - Bayer CropScience, Research Triangle, NC) and Bio-Tam (*Trichoderma asperellum*/*Trichoderma* gamsii – Marrone Bio Innovations, Davis, CA) on the potato early-dying complex in a field environment. Different in-furrow and chemigated applications were used to evaluate if the timing and method of the application had an impact on effectiveness.

2.3 MATERIALS AND METHODS

Field trials were conducted in 2016 and 2017. Due to the fact that natural inoculum was used in 2016, while the field was inoculated with *Verticillium dahliae* in 2017, the results are presented and analyzed separately.

2.3.1 2016 TRIAL

The experiment was performed at the Parma Research and Extension Center in a Greenleaf silt loam soil (pH 8.2 and organic matter of 2.25%) in a field where the previous crop was wheat (2015). It should be noted that potatoes were last grown four years prior in 2011. Before the field was tilled in fall of 2015, the soil contained 10 ppm NO_3-N and 3 ppm NH_4-N to a depth of 0-30 cm (2015). The field was fertilized in the fall of 2015 with 47 kg N/ha, 224 kg P_2O_5/ha, and 112 kg K_2O/ha. There was an additional topdress application made at hilling on 10 May 2016. This consisted of 202 kg N/ha as Environmentally Smart Nitrogen (ESN) (Agrium, Inc., Calgary, Alberta, Canada).

The trial was planted using cut certified Russet Norkotah seed on 26 April 2016 when the soil temperature was 9.8^{o} C at the 10 cm depth. Russet Norkotah was chosen as the cultivar for this experiment because the literature has shown that this cultivar is susceptible to Verticillium wilt (Jansky and Miller, 2010). The average seed piece size was 62 g +/- 21 g. The seed was treated with mancozeb immediately after it was commercially cut at a rate of 0.5 kg per 45 kg of seed potatoes (Bonide Products, Oriskany, NY). Seed pieces were planted in 91.4 cm rows with 25.4 cm in-row spacing. Individual plots were 6 rows wide (5.5 meters) by 10.7 meters long. Fumigation was carried out in the fall prior to planting on 13 November 2015 using a shank injection method with metam sodium at a rate of 374 l/ha (Vapam - AMVAC Chemical Corporation, Newport Beach, CA). Treatments were arranged in a randomized complete block design with four replications. Pesticide applications for control of weeds and insects followed the University of Idaho guidelines (University of Idaho, 2017).

The fields were irrigated to maintain a minimum of 65-70% available soil moisture using a solid-set sprinkler system.

Two different biopesticides, Serenade ASO (*Bacillus subtilis* – Bayer CropScience, Research Triangle, NC) and Bio-Tam (*Trichoderma asperellum*/*Trichoderma gamsii* – Marrone Bio Innovations, Davis, CA) were applied at 4 different rates per timing. The applications consisted of in-furrow, in-furrow plus chemigated low rate, chemigated low rate, and chemigated high rate. In-furrow applications were applied at planting using a CO_2 sprayer with two XR TeeJet 80015 nozzles operated at 172.4 kPa to treat two rows at a time (TeeJet, Wheaton, IL). The application spray volume was 1.04 liters per plot. Chemigation treatments were applied four times during the growing season using a gasoline powered sprayer attached to a boom with eight flood jet nozzles (TeeJet, Wheaton, IL) spaced 61 cm apart operated at 172.4 kPa. This method is designed to stimulate a circle pivot irrigation with 0.63 cm/ha. The application dates were 7 June (53 DAP), 22 June (68 DAP), 7 July (83 DAP), and 20 July (96 DAP). Tuber stages at each application date are labeled in the appendix. The treatments were as follows:

1- Non-treated check
2- Fumigated check
3- Bio-Tam in-furrow
4- Bio-Tam chemigated (low rate)
5- Bio-Tam in-furrow + chemigated (low rate)
6- Bio-Tam chemigated (high rate)
7- Serenade Soil in-furrow
8- Serenade chemigated (low rate)
9- Serenade in-furrow + chemigated (low rate)
10- Serenade chemigated (high rate)

Bio-Tam rates were 350.8 ml/ha for in-furrow, 2.8 kg/ha for low rate chemigation, and 5.6 kg/ha for high rate chemigation. Serenade ASO rates consisted of 4.7 l/ha for in-furrow, 4.7 l/ha for low rate chemigation, and 9.4 l/ha for high rate chemigation. These rates were consistent with the label recommendation. Bio-Tam was premixed with water and soaked 24 hours prior to each application.

Stand counts were taken on 3 June 2016 by counting the number of fully emerged potato plants in the middle two rows; for a distance of 3 meters. Plant heights were measured on the first and last application date by randomly selecting ten plants from each plot to see if the treatments had an impact on plant vigor.

Soil and stem samples were taken prior to the first chemigation application on 7 June 2016 to see if the microbial biopesticides would decrease the pathogen levels and again after all the applications were made on 23 August 2016. Soil samples consisted of twelve soil cores taken at a depth of 0-30 cm in an X pattern across the entire plot. The twelve cores were combined and mixed, and a subsample of 50 g (moist weight basis) was used for determination of pathogen populations. Stem samples consisted of two stems per plant from five total plants on each date. The stem and soil samples were analyzed using real-time polymerase chain reaction (qPCR) to measure the amount of *Verticillium dahliae* and *Colletotrichum coccodes* present. qPCR analysis methodology for soil and stem samples were adapted from Woodhall et al., (2012).

2.3.2 Soil DNA extraction

All soil samples were placed in a cooler at 4.4 ^{o}C for 60+ days, prior to DNA extraction and qPCR. Soil was air-dried for two days and then 50 g of soil was transferred into a

Nalgene wide mouth bottle containing six 25 mm stainless steel ball bearings, 100 ml of extraction buffer (120 mM sodium phosphate buffer pH 8.0, 2% cetrimomium bromide [CTAB], 1.5 M sodium chloride) and 3 ml of Antifoam B. To homogenise the samples, the Nalgene bottles were placed in a Red Devil 5990 multisize paint shaker (Radia, North Plymouth, MN) for 3 minutes. Forty ml of the homengate was then transferred to a 50 ml screw-cap centrifuge tube and centrifuged in a Sorvall Legend XT centrifuge (Thermo Fisher Scientific, Waltham, MA) at 5,000 x g for 5 minutes. The supernatant was recovered and was added to a clean 50 ml screw-cap centrifuge tube with 2 ml of 5 M potassium acetate. The tubes were incubated on ice for 10 minutes and then centrifuged at 12,000 x g for 5 minutes. The supernatant was recovered and added to a clean 50 ml screw-cap centrifuged tube with 15 ml of isopropanol and 1 ml 100% silicon dioxide suspension. The tubes were incubated at room temperature for 20 minutes and then centrifuged at 12,000 x g for 5 minutes. The supernatant was discarded and silicon dioxide pellets were air dried in a laminar flow hood for 30 minutes. After 30 minutes, silicon dioxide pellets were re-suspended by adding 2 ml of Buffer A from the Wizard Food Kit (Promega). The tubes were incubated in a shaking water bath for 10 minutes at 65 ^{o}C and then centrifuged at 12,000 x g for 10 minutes. One ml of Buffer A was then processed using the Wizard Food Kit DNA extraction method in conjunction with a Kingfisher ML (ThermoFisher). The resulting DNA samples were stored at -20 ^{o}C until qPCR analysis.

2.3.3 STEM DNA EXTRACTION

All stem samples were placed in a cooler at 4.4 ^{o}C prior to DNA extraction and qPCR. The stem samples were washed and weighed prior to DNA extraction. A masticating juicer

(Omega J8006) was used to extract the sap from the stem samples into BioReba tissue bags (BioReba Ag, Reinach, Switzerland). Ten ml of CTAB extraction buffer [1l: 100ml of M Tris-HCl, 280 ml of 5 M NaCl, 40 ml of 0.5 M EDTA, and 400 ml of 5% CTAB solution] was added to each tissue bag and 750 µl was extracted from each tissue bag into a 1.7 microcentrifuge tube. The tubes were placed in a 65 ^{o}C water bath for 1 to 2 h. The tubes were centrifuged at 16,000 g for 10 minutes and 500 µl of the supernatant was transferred into a clean 1.7 ml microcentrifuge tube. An equal volume of phenol:chloroform:isoamyl alcohol was added to each tube and centrifuged at 16,000 g for 10 minutes. Approximately 350 µl of the supernatant was transferred into a clean 1.7 ml microcentrifuge tube with an equal volume of chloroform:isoamyl alcohol. The tubes were centrifuged at 16,000 g for 10 minutes and 250 µl of supernatant was transferred into a clean 1.7 ml microcentrifuge tube. An equal amount of cold isopropanol (-20 ^{o}C) was added to each tube and were stored for 1 h at -20 ^{o}C. The tubes were centrifuged at 16,000 g for 10 minutes and the supernatant was discarded. The resulting pellet was washed with 200 µl of cold 70% ethanol and centrifuged at 16,000 g for 5 minutes. The pellet was air dried until completely dry and then 50 µl of 1X TE buffer at 37 ^{o}C was added to the pellet. The tubes were placed at 37 ^{o}C for 30 minutes and then the DNA samples were stored at -20 ^{o}C until qPCR analysis.

Quantitative polymerase chain reactions were performed using 1 µl of DNA in a 15 µl reaction. The primers and probes used for *Verticillium dahliae* and *Colletotrichum coccodes* were adapted from Bilodeau et al., (2012) and Cullen et al., (2002) respectively.

Soil samples were taken in the prior fall on 9 November 2016 and early spring as well on 20 April 2016, but the samples were taken by reps 1-4 and were not subjected to

individual plots at the time of sampling. There was only one positive *Verticillium dahliae* result for each test indicating low natural inoculum levels.

Visual symptoms of Verticillium wilt (*Verticillium dahliae)* were rated every two weeks until symptoms were noticed and then ratings occurred weekly throughout June, July, and August. Ten random plants were chosen on each evaluation date and visual ratings were based on a 0-5 scale that was based on severity of chlorosis. A visual representation of the scale is presented in the appendix. The scale was adapted from the method of Zhang et al., (2015) where:

0 = No symptom

1 = <25% chlorotic/necrotic leaves

2 = 25-50% chlorotic/necrotic leaves

3 = 50-75% chlorotic/necrotic leaves

4 = > 75% chlorotic/necrotic leaves

5 = Complete defoliation or plant death

Disease severity over the season was measured by calculating the relative area under the disease progress curve (RAUDPC) using the method of Kirk et al., (2001). A rating of three or higher was considered diseased and was used to calculate the RAUDPC.

Plots were treated with diquat dibromide (Reglone – Syngenta Crop Protection, Greensboro, NC) on 8 September at a rate of 2.3 l/ha to desiccate the plants and were subsequently harvested on 22 September. For each plot the middle 2 rows by 7.6 meters were mechanically harvested. After harvest, tubers were graded by classifying the tubers meeting the U.S. No. 1 grade standards into different size categories, which were 0-113 g,

114-170 g, 171-283g, 284-454 g, and >455 g. Tubers not meeting the U.S. #1 standards were classified as U.S. #2's (5-10% total tuber loss due to defects) and (>10% total tuber loss due to defects) categories. After the tubers were graded, percentages greater than 171 g and 284 g size class tubers were calculated to view tuber weight distribution and the total yield was calculated. Specific gravity was determined on a twenty tuber sample that ranged in size from 114-283 g. The samples were washed, surface dried, and weighed for specific gravity using the weight in air and water method (Kleinschmidt et al., 1984).

2.3.4 2017 TRIAL

This trial was conducted at the Parma Research and Extension Center on Greenleaf silt loam soil (pH of 8.6 and an organic matter percentage of 3.51%) where the previous crop was wheat (2016), with potatoes being grown three years prior in 2013. The soil contained 5 ppm NO_3-N and 6 ppm NH_4-N and was fertilized the prior fall with 19 kg N/ha, 90 kg P_2O_5/ha, and 112 kg K_2O/ha (2016). There was an additional topdress application made at hilling on 31 May 2017, which consisted of 168 kg N/ha as Environmentally Smart Nitrogen (ESN) (Agrium, Inc., Calgary, Alberta, Canada). The relatively lower level of nitrogen application compared to 2016 was made in an attempt to increase the symptoms of Verticillium wilt.

The trial was planted using cut certified Russet Norkotah strain TX 278 on 4 May 2017 when soil temperature was 12.4^{o} C at a depth of 10 cm. The average seed piece size was 62.4 g +/- 21 g. The seed was treated with mancozeb and penfluen (Emesto Silver-Bayer CropScience, Research Triangle, NC) at a rate of 9.2 ml per 45 kg of seed potatoes immediately after it was commercially cut. Seed pieces were planted in 91 cm rows with 25.4 cm in-row spacing. Individual plots were 6 rows wide (5.5 meters) by 12.2 meters long.

Fumigation was carried out in the fall prior to planting on 11 November 2016 using a shank injection method with metam sodium at a rate of 374 l/ha (Vapam - AMVAC Chemical Corporation, Newport Beach, CA). The treatments were arranged in a randomized complete block design with four replications. Pesticide applications and irrigation management were as described above for 2016.

To increase *Verticillium dahliae* populations, the field trial was inoculated prior to planting and again prior to hilling. The isolate for the first inoculation was obtained from an infected potato plant in Idaho. The second inoculation used an isolate obtained from a mint plant in Idaho. *Verticillium dahliae* inoculum was produced on barley as follows. Barley grain was autoclaved twice before being inoculated with *Verticillium dahliae*. The first autoclave was done in disposable aluminum roaster pans. Each pan was 29.9 cm by 23.5 cm by 6.4 cm and held 2,000 cc of barley grain. The pans were covered in aluminum foil and autoclaved for 40 minutes on the fast exhaust setting. After cooling, 1,500 cc from each pan was transferred into a sterilizable Air Flow Spawn bag (Fungi Perfecti LLC, Shelto, WA). Each spawn bag received 500 ml of distilled water and the barley was mixed to evenly disperse the water throughout the bag. Bags were sealed with a heat sealer and left to sit overnight at room temperature. The second autoclave occurred the following day for 35 minutes on the slow exhaust setting. Each bag was mixed prior to being autoclaved to distribute the water evenly throughout the bag after it settled on the bottom overnight. After the spawn bags cooled down they each received 10 plugs of *Verticillium dahlia*e from cultures that were started at least 7-10 days prior. The bags were sealed after inoculation and were mixed weekly for three weeks. After three weeks, the inoculum was placed onto a tray and air

dried for 3-4 days. The inoculum was then ground in preparation for field inoculation. Prior to field inoculation, the inoculum was stored in paper bags and placed in a refrigerator.

The first inoculation occurred on 3 May 2017 when soil temperature was 11.0 ^{o}C at a depth of 10 cm and consisted of 50 g of blended barley per plot. The second inoculation occurred on 31 May 2017 (27 DAP) when soil temperature was 18.9 ^{o}C at 10 cm depth and consisted of 450 g. of blended barley per plot. On both dates, the inoculum mixture was spread onto each plot using a hand-held fertilizer spreader and mechanically incorporated.

Treatments, application rates, and application methods were identical to 2016. The chemigation application dates were 23 June 2017 (50 DAP), 7 July 2017 (64 DAP), 21 July 2017 (78 DAP), and 4 Aug 2017 (92 DAP). Tuber stages at each application date are labeled in the appendix.

Stand counts and plant heights were measured as described for 2016. Stem and soil sample collection followed a similar protocol as in 2016, but the soil from the soil probe was divided into two separate samples. Stem and soil samples were collected on 21 June 2017 prior to the first chemigation and after all the treatments were made on 16 August 2017. The top 15 cm of the soil probe was one sample and the other sample was the bottom 15 cm of the soil probe. Dividing the samples into two separate sections was done to help determine how *Verticillium dahliae* is distributed through the soil profile. Visual estimates of early die symptoms were as outlined above.

The plots were treated with diquat dibromide (Reglone – Syngenta Crop Protection, Greensboro, NC) on 28 August 2017 at a rate of 2.3 l/ha to desiccate the plants; and were subsequently harvested on 12 September 2017. For each plot the middle 2 rows by 6.1

meters were mechanically harvested. Grading, yield and specific gravity determination were as outlined for the 2016 trial.

2.4 STATISTICAL ANALYSIS

Data for 2016 and 2017 trials were analyzed separately by Analysis of Variance (ANOVA) using the Proc GLM SAS statistical program (SAS Institute 9.2, 2008). When the F-test was significant the means were separated using Fisher's protected least significant difference (LSD) at the 5% level. Real-time polymerase chain reaction (qPCR) results were cube root transformed prior to analysis to meet normality assumptions for ANOVA. Single degree of freedom orthogonal contrasts were used to conduct planned comparisons as follows: biopesticide treatment versus check, chemigation versus in-furrow application, and Bio-Tam versus Serenade ASO.

2.5 RESULTS

2.5.1 2016 RESULTS

2.5.2 PLANT HEIGHT AND STAND COUNT:

Stand counts average above 90%, and were similar among all the treatments (Tables 1 and 2). The single degree of freedom contrasts indicated that the check tended to have lower stands than the biopesticide treatments (93.8 vs 96.6, P = 0.0744). Likewise, in-furrow treatments as a group tended to have lower stand counts compared to the chemigation treatments (95.6 vs 97.6, P = 0.0527). Bio-Tam and Serenade ASO treatments had similar stands.

Plant heights ranged from 22 to 33 cm on 6 June 2016, and from 58 to 62 cm on 19 July 2016; and were similar among all the treatments (Tables 1 and 2). Single degree of

freedom contrast identified a significant difference between the non-treated check and biopesticide treatments in plant height on both the 6 June 2016 and 19 July 2016 evaluation dates. The non-treated check had taller plants on the 6 June 2016 evaluation, but had shorter plants compared to the biopesticides on 19 July 2016.

2.5.3 Visual Symptoms:

The plants did not show strong visual symptoms of disease development throughout the experiment. The relative area under the disease progress curve (RAUDPC) ranged from 2.4 to 5.2 and were not significantly different among the treatments or the control (Tables 3 and 4). Single degree of freedom contrast indicated that as a group the Bio-Tam treatments had lower RAUDPC values compared to Serenade ASO (3.1 vs 4.9, P = 0.0234).

2.5.4 Pathogen populations:

The values from the qPCR analysis were presented in pico gram (pg) of *Verticillium dahlia*e and *Colletotrichum coccodes* per g of soil/stem. *Verticllium dahliae* populations were highly variable ranging from 0 to 36,570 pg/g and *C. coccodes* populations ranged from 0 to 17,000 pg/g (Tables 5 and 6). The soil and stem samples for both *V. dahliae* and *C. coccodes* increased in pathogen levels from pre-treatment to post-treatment, but were not significantly different among the treatments at any sampling date (Tables 5 and 6).

2.5.5 Yield and Specific Gravity

Yield ranged from 69.2 to 76.6 t/ha and were not significantly different among the treatments (Tables 7 and 8). Specific gravity was similar among the treatments ranging from 1.073 to 1.076 (Tables 9 and 10).

2.5.6 2017 Results

2.5.7 Plant Height and Stand Count:

Stand counts average above 85%, and were similar among all the treatments on 9 June 2017 (Tables 11 and 12). The single degree of freedom contrasts indicated that Serenade ASO as a group had higher stand counts versus Bio-Tam (91.7 vs 87.2, P = 0.0107).

Plant heights ranged from 37 to 41 cm on 21 June 2017, from 43 to 48 cm on 11 July 2017, from 44 to 50 cm on 20 July 2017, and 39 to 47 cm on 3 August 2017 and were similar among the treatments (Tables 11 and 12).

2.5.8 Visual Symptoms:

The plants in 2017 showed more disease symptoms compared to the previous year's study. The relative area under the disease progress curve (RAUDPC) ranged from 17.4 to 25.5 and were not significantly different among the treatments or the control (Tables 13 and 14).

2.5.9 Pathogen populations:

The values from the qPCR analysis were presented in pg/g of *Verticillium dahlia*e and *Colletotrichum coccodes* per g of soil/stem. The soil and stem samples for both *V. dahliae* and *C. coccodes* increased in pathogen levels from pre-treatment to post-treatment, but were not significantly different among the treatments at any sampling date (Tables 15, 16, 17 and 18). The values were highly variable ranging from 313,335 to 3,917,565 pg/g in the stem tissue post-treatment for *V. dahliae*.

2.5.10 YIELD AND SPECIFIC GRAVITY:

Yield ranged from 57.8 to 62.0 t/ha and were not significantly different among the treatments (Tables 19 and 20). Specific gravity was similar among the treatments ranging from 1.075 to 1.079 (Tables 21 and 22).

2.6 DISCUSSION

The objective of this study was to determine if biopesticides could effectively control the potato early-dying complex (PED). Based on the results over a two-year period, there was no evidence that biopesticides significantly reduced visual symptoms or pathogen populations in soil or stem tissue when compared to the non-treated check under the conditions of this study. 2016 had relatively low initial pathogen populations, while 2017 had moderate to high pathogen populations and there were still no differences observed between the treatments. There was also no impact on plant growth as shown by plant stand, height, yield, grade and specific gravity. In contrast, Varo et al. 2016 and Wharton et al. 2012 both showed that these biopesticides were effective against soil borne pathogens, but that was not observed in this study. There are various reasons for why biopesticicides were not shown to be effective in this study, which includes lack of efficacy on controlling *Verticillium dahliae*, other pathogens in PED involved, initial soil microbial populations, and soil organic matter levels.

Timing and rate of application in both studies didn't show a significant difference. Jordan and Gevens, (2013) reported similar results with a decrease in black scurf (*Rhizoctonia solani*) incidence when Regalia (*Reynoutria sachalinensis* plant extract) was applied as a seed treatment, in-furrow, and chemigated at the 4-6 leaf rosette plant stage.

That study also showed an increase in black scurf incidence compared to the untreated check when Serenade ASO was applied as an in-furrow application only. Thus, more research needed to understand how the timing and rate of application impacts the efficacy of biopesticides.

Fumigation with metam sodium also did not have an impact on disease development in 2016 or 2017. These results contrast with Hamm et al. 2003 and Taylor et al 2005 which showed that metam sodium was effective at reducing *V. dahliae* populations. Hamm et al. 2003 also reported that metam sodium was effective at reducing other pathogens including *Fusarium* spp and *Pythium* spp. These results suggest the possibility that pathogens other than *V. dahliae* and *C. coccodes* were involved in observed disease symptoms in this study.

Verticillium dahliae and *Colletotrichum coccodes* DNA values were highly variable among all the treatments for both years. Pasche et al. 2014 showed a high variability in *V. dahliae* concentration in the stem tissue. The pathogen populations ranged from 104,756 to 434,712 mg/g of stem tissue. The 2016 study had higher *C. coccodes* in both the stem and the soil samples when compared with *V. dahliae*. These differences suggest that the relatively low symptoms that were observed in 2016 could have been attributed to *C. coccodes*. In contrast, the 2017 study had higher *V. dahliae* and *C. coccodes* populations in stem tissue, which may explain the higher RAUDPC values in that year. However, *Verticillium dahliae* had higher DNA levels in the stem tissue when compared to *C. coccodes*, suggesting that *V. dahliae* could have been the main pathogen causing disease symptoms in 2017. Pathogen population distributed by depth was highly variable for *V. dahliae* and there wasn't a difference. Taylor et al. 2005 and Pasche et al. 2014 showed contradicting results with the

highest concentration of *V. dahliae* at the 0-10 cm versus the 10-20 cm depth. *Colletotrichum coccodes*, however, showed similar results with population numbers the highest at 0-15 cm versus the 15-30 cm depth.

In both years, the levels of *V. dahliae* and *C. coccodes* measured in specific treatments did not correlate with observed disease symptoms in those treatments. These results suggest that *V. dahliae* and *C. coccodes* populations in the soil and stem tissue do not always correlate with disease symptoms. A lack of relationship between pathogen populations and disease symptoms agree with studies reported by Jansky and Miller (2010) and Frost et al., (2007).

There have been studies that have reported a positive correlation of *V. dahliae* and wilting symptoms, but this has not been reported for C*. coccodes*. However, there are studies that have reported a positive correlation between *C. coccodes* levels and tuber blemishes (Lees et al., 2010). There may be a need for more studies to identify the correlation of wilting and *C. coccodes*.

The plants did show disease symptoms that are associated with PED, but previous literature states that there are many pathogens involved in PED (Saeed et al., 1997; Johnson and Dung, 2010; Powelson and Rowe, 1993; Johnson and Miliczky, 1993; Rowe and Powelson, 2002). The fumigated check didn't effectively control *V. dahliae* or *C. coccodes* in 2016 or 2017.The disease symptoms that were observed could have been associated with pathogens that were not evaluated in this experiment. For example, grey mold, caused by *Botrytis* species have been isolated from potatoes in Idaho showing what are thought by most growers to be early die symptoms (P. S. Wharton, personal communication). When

comparing the fumigated check with the non-inoculated fumigated check, there wasn't much difference for any of the measurements that were used in this study. The two treatments had similar disease symptoms and DNA levels on *V. dahliae* and *C. coccodes*, which suggests that the inoculation with *V. dahliae* was not a factor in observed symptoms.

Davis et al. (2010) reported that there are many factors that can contribute to disease susceptibility. For example, soil organic matter and soil microbial activity are negatively correlated with Verticillium wilt incidence. These factors will be highly variable between different fields and even years. These uncontrollable factors could be one of the main reasons that these biopesticides have been inconsistent in efficacy. It's possible that these biopesticides will only be effective under specific conditions.

Future studies are needed to better understand the specific conditions under which these biopesticides will be effective in controlling PED. Combining bacterial biopesticides with fungicide products is a study that is being evaluated in Idaho. *In-vitro* studies for these biopesticides against strains of *Verticillium*, *Colletotrichum*, and other pathogens involved in PED are needed. More controlled tests are needed to understand at what levels of pathogens are these biopesticides effective and is there a threshold in their effectiveness. It is also important to understand how each of these pathogens impact PED. To achieve this more studies are needed on *C. coccodes* involvement in PED. Finally, learning how these microbial biopesticides colonize potato roots and their interactions with other microorganisms is important.

Based off the results that were presented over a two-year period, there were no differences among the treatments that would suggest that biopesticides can effectively

control the potato early-die complex. However, there are various reasons presented in this chapter for why there were not effective in this study. With the Environmental Protection Agency (EPA) reregistering many products that are used in potato production, these biopesticides could be an important need in the future. There are more studies needed to understand biopesticides and their potential in the agricultural industry.

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Table 2.1: Analysis of variance of the effect of fumigation and biopesticide treatments on stand count percentage and plant height in Russet Norkotah potatoes grown at Parma, ID during 2016. Significance is indicated by P ≤ 0.05.

	Stand Count %	Plant Height (cm) 6 Jun	Plant Height (cm) 19 Jul
	P-value	P-value	P-value
Treatment	0.0535	0.3427	0.1062
Planned Contrasts			
Non-Treated vs Biopesticides	0.0744	0.0490	0.0016
In-furrow vs chemigation	0.0527	0.7977	0.6163
Bio-Tam vs Serenade	0.1408	0.9591	1.0000

Table 2.2: Effect of fumigation and biopesticide treatments on stand count percentage and plant height in Russet Norkotah potatoes grown at Parma, ID during 2016. Means are of 4 replications.

Treatment		Stand Count %	Plant Height (cm) 6 Jun	Plant Height (cm) 19 Jul
Non-treated	1	93.8	31	58
Fumigated check	2	96.8	33	61
Bio-Tam in-furrow (IF)	3	93.8	24	60
Bio-Tam chemigation - low rate (CL)	4	98.9	23	60
Bio-Tam IF + CL	5	97.9	23	61
Bio-Tam chemigation - high rate (HL)	6	98.9	25	62
Serenade ASO in-furrow (IF)	7	93.8	24	60
Serenade ASO chemigation - low rate (CL)	8	94.8	24	61
Serenade ASO IF + CL	9	96.8	22	61
Serenade ASO chemigation - high rate (HL)	10	97.9	24	60
LSD		ns	ns	ns
Contrasts^				
Non-treated		93.8	31	58
Biopesticides		96.6	24	61
In-furrow		95.6	23	60
Chemigation		97.6	24	61
Bio-Tam		97.4	24	61
Serenade ASO		95.8	24	61

^Single degree of freedom contrast comparing the following treatments means:

Non-treated (1) vs biopesticides (3,4,5,6,7,8,9, and 10)
In-furrow (3,5, 7, and 9) vs chemigated (4, 6, 8, 10)
Bio-Tam (3, 4, 5, and 6) vs Serenade ASO (7, 8, 9, and 10)

Table 2.3: Analysis of variance of the effect of fumigation and biopesticide treatments on the relative area under the disease progress curve (RAUDPC) in Russet Norkotah potatoes grown at Parma, ID during 2016. Significance is indicated by P ≤ 0.05.

	RAUDPC
	P-value
Treatment	0.4487
Planned Contrasts	
Non-Treated vs Biopesticides	0.7508
In-furrow vs chemigation	0.5780
Bio-Tam vs Serenade ASO	0.0234

Table 2.4: Effect of fumigation and biopesticide treatments on the relative area under the disease progress curve (RAUDPC) in Russet Norkotah potatoes grown at Parma, ID during 2016. Means are of 4 replications.

Treatment		RAUDPC
Non-treated	**1**	3.6
Fumigated check	**2**	3.3
Bio-Tam in-furrow (IF)	**3**	3.0
Bio-Tam chemigation - low rate (CL)	**4**	2.4
Bio-Tam IF + CL	**5**	4.0
Bio-Tam chemigation - high rate (HL)	**6**	3.1
Serenade ASO in-furrow (IF)	**7**	5.2
Serenade ASO chemigation - low rate (CL)	**8**	3.9
Serenade ASO IF + CL	**9**	4.6
Serenade ASO chemigation - high rate (HL)	**10**	5.8
LSD (0.05)		**ns**
Contrasts^		
Non-treated		3.6
Biopesticides		4.0
In-furrow		4.2
Chemigated		3.8
Bio-Tam		3.1
Serenade ASO		4.9

^Single degree of freedom contrast comparing the following treatments means:

Non-treated (1) vs biopesticides (3,4,5,6,7,8,9, and 10)
In-furrow (3,5, 7, and 9) vs chemigated (4, 6, 8, 10)
Bio-Tam (3, 4, 5, and 6) vs Serenade ASO (7, 8, 9, and 10)

Table 2.5: Analysis of variance of the effect of fumigation and biopesticide treatments on soil and stem tissue DNA concentration pre and post-treatment in Russet Norkotah potatoes grown at Parma, ID during 2016. Significance is indicated by P ≤ 0.05.

	V. dahliae Soil Pre Trt (pg/g)	*V. dahliae* Soil Post Trt (pg/g)	*C. coccodes* Soil Pre Trt (pg/g)	*C. coccodes* Soil Post Trt (pg/g)	*V. dahliae* Stem Pre Trt (pg/g)	*V. dahliae* Stem Post Trt (pg/g)	*C. coccodes* Stem Pre Trt (pg/g)	*C. coccodes* Stem Post Trt (pg/g)
	P-value	P-value	P-value	P-value	P-value	P-value	P-value	P-value
Treatment	0.7190	0.4505	0.0899	0.8786	0.4612	0.4217	0.5672	0.4668
Planned Contrasts								
Non-Treated vs Biopesticides	0.7298	0.8040	0.4489	0.6278	1.0000	0.2354	0.6043	0.3401
In-furrow vs chemigation	0.1651	0.3788	0.1980	0.3446	1.0000	0.3375	0.9163	0.2212
Bio-Tam vs Serenade	0.3634	0.4260	0.5662	0.5438	1.0000	0.0566	0.9163	0.4874

Table 2.6: Effect of fumigation and biopesticide treatments on soil and stem DNA concentrations pre and post-treatment in Russet Norkotah potatoes grown at Parma, ID during 2016. DNA values were determined by qPCR analysis, are the means are of 4 replications.

Treatment		*V. dahliae* Soil Pre Trt (pg/g)	*V. dahliae* Soil Post Trt (pg/g)	*C. coccodes* Soil Pre Trt (pg/g)	*C. coccodes* Soil Post Trt (pg/g)	*V. dahliae* Stem Pre Trt (pg/g)	*V. dahliae* Stem Post Trt (pg/g)	*C. coccodes* Stem Pre Trt (pg/g)	*C. coccodes* Stem Post Trt (pg/g)
Non-treated	1	200	320	865	11100	0	0	0	249
Fumigated check	2	54	120	568	13175	125	0	0	548
Bio-Tam in-furrow (IF)	3	120	360	818	16003	0	0	0	145
Bio-Tam chemigation - low rate (CL)	4	15	210	738	8998	0	0	0	380
Bio-Tam IF + CL	5	8	220	4770	5215	0	2	0	559
Bio-Tam chemigation - high rate (HL)	6	30	36570	1748	11743	0	5	0	495
Serenade ASO in-furrow (IF)	7	120	550	1265	7220	0	1	0	167
Serenade ASO chemigation - low rate (CL)	8	30	320	1253	8880	0	31	0	193
Serenade ASO IF + CL	9	108	280	2508	9773	0	1	0	535
Serenade ASO chemigation - high rate (HL)	10	23	190	695	17000	0	30	0	2325
LSD (0.05)		ns	ns	ns	ns	ns	ns	ns	ns
Contrasts^									
Non-treated		200	320	865	11100	0	0	0	249
Biopesticides		57	4838	1724	10604	0	9	0	600
In-furrow		89	353	2340	9553	0	1	0	352
Chemigated		24	9323	1109	11655	0	16	0	848
Bio-Tam		43	9340	2019	10490	0	2	0	395
Serenade ASO		70	335	1430	10718	0	16	0	805

^Single degree of freedom contrast comparing the following treatments means:

Non-treated (1) vs biopesticides (3,4,5,6,7,8,9, and 10)

In-furrow (3,5, 7, and 9) vs chemigated (4, 6, 8, 10)

Bio-Tam (3, 4, 5, and 6) vs Serenade ASO (7, 8, 9, and 10)

Table 2.7: Analysis of variance of the effect of fumigation and biopesticide treatments on yield, #2's, and culls in Russet Norkotah potatoes grown at Parma, ID during 2016. Significance is indicated by P ≤ 0.05.

	Yield 0-113 g t/ha	Yield 114-170 g t/ha	Yield 171-283 g t/ha	Yield 284-454 g t/ha	Yield >455 g t/ha	Yield #2s t/ha	Yield Culls t/ha	Total Yield t/ha
	P-value	P-value	P-value	P-value	P-value	P-value	P-value	P-value
Treatment	0.8458	0.4043	0.6287	0.6435	0.8643	0.5235	0.9028	0.7123
Planned Contrasts								
Non-Treated vs Biopesticides	0.4544	0.5135	0.2666	0.1505	0.7350	0.0574	0.8139	0.7830
In-furrow vs chemigation	0.9815	0.9704	0.8382	0.4476	0.8709	0.6870	0.9829	0.6656
Bio-Tam vs Serenade ASO	0.4452	0.0659	0.1596	0.7266	0.7350	0.7297	0.2986	0.5981

Table 2.8: Effect of fumigation and biopesticide treatments on yield, #2's, and culls in Russet Norkotah potatoes grown at Parma, ID during 2016. Means are of 4 replications.

Treatment		Yield 0-113 g t/ha	Yield 114-170 g t/ha	Yield 171-283 g t/ha	Yield 284-454 g t/ha	Yield >455 g t/ha	Yield #2s t/ha	Yield Culls t/ha	Total Yield t/ha
Non-treated	1	2.0	5.0	17.4	20.0	27.0	1.7	0.9	73.9
Fumigated check	2	2.2	5.2	17.7	22.7	26.5	0.5	1.1	75.9
Bio-Tam in-furrow (IF)	3	2.4	4.9	16.9	20.5	28.9	0.9	0.7	75.1
Bio-Tam chemigation - low rate (CL)	4	1.8	4.5	17.5	23.4	26.3	0.9	1.0	75.4
Bio-Tam IF + CL	5	2.4	3.5	15.1	23.5	22.1	1.1	1.5	69.2
Bio-Tam chemigation - high rate (HL)	6	2.5	4.0	15.9	22.2	27.4	1.0	1.4	74.5
Serenade ASO in-furrow (IF)	7	2.0	4.8	14.2	23.7	29.8	0.8	1.1	76.6
Serenade ASO chemigation - low rate (CL)	8	2.6	5.2	14.3	21.8	24.2	1.4	0.9	70.5
Serenade ASO IF + CL	9	2.7	5.1	15.8	24.6	23.0	1.0	0.6	72.8
Serenade ASO chemigation - high rate (HL)	10	2.6	4.7	15.0	21.2	24.3	1.0	0.6	69.4
LSD (0.05)		ns	ns	ns	ns	ns	ns	ns	ns
Contrasts^									
Non-treated		2.0	5.0	17.4	20.0	27.0	1.7	0.9	73.9
Biopesticides		2.4	4.6	15.6	22.6	25.8	1.0	1.0	72.9
In-furrow		2.4	4.6	15.5	23.1	26.0	0.9	1.0	73.4
Chemigation		2.4	4.6	15.7	22.2	25.5	1.0	1.0	72.4
Bio-Tam		2.2	4.2	16.4	22.4	26.2	1.0	1.2	73.6
Serenade ASO		2.5	4.9	14.9	22.8	25.3	1.0	0.8	72.3

^Single degree of freedom contrast comparing the following treatments means:

Non-treated (1) vs biopesticides (3,4,5,6,7,8,9, and 10)

In-furrow (3,5, 7, and 9) vs chemigated (4, 6, 8, 10)

Bio-Tam (3, 4, 5, and 6) vs Serenade ASO (7, 8, 9, and 10)

Table 2.9: Analysis of variance of the effect of fumigation and biopesticide treatments on yield percentage greater than 171 and 284 grams and specific gravity in Russet Norkotah potatoes grown at Parma, ID during 2016. Significance is indicated by P ≤ 0.05.

	Percent >171 g	Percent >284 g	Specific Gravity
	P-value	P-value	P-value
Treatment	0.8704	0.9655	0.3237
Planned Contrasts			
Non-Treated vs Biopesticides	0.8355	0.4594	0.5258
In-furrow vs chemigation	0.8651	0.7272	0.8188

Table 2.10: Effect of fumigation and biopesticide treatments on yield percentage greater than 171 and 284 grams and specific gravity in Russet Norkotah potatoes grown at Parma, ID during 2016. Means are of 4 replications.

Treatment		Percent >171 g	Percent >284 g	Specific Gravity
Non-treated	1	87.3	63.5	1.074
Fumigated check	2	88.3	64.8	1.075
Bio-Tam in-furrow (IF)	3	87.8	65.0	1.076
Bio-Tam chemigation - low rate (CL)	4	89.3	65.8	1.076
Bio-Tam IF + CL	5	87.5	65.5	1.075
Bio-Tam chemigation - high rate (HL)	6	88.3	66.8	1.073
Serenade ASO in-furrow (IF)	7	88.5	70.3	1.074
Serenade ASO chemigation - low rate (CL)	8	85.3	64.5	1.074
Serenade ASO IF + CL	9	87.0	65.3	1.073
Serenade ASO chemigation - high rate (HL)	10	87.3	65.8	1.074
LSD (0.05)		ns	ns	ns
Contrasts^				
Non-treated		87.3	63.5	1.074
Biopesticides		87.6	66.1	1.074
In-furrow		87.7	66.5	1.074
Chemigation		87.5	65.7	1.074
Bio-Tam		88.2	65.8	1.075
Serenade ASO		87.0	66.4	1.074

^Single degree of freedom contrast comparing the following treatments means:

Non-treated (1) vs biopesticides (3,4,5,6,7,8,9, and 10)
In-furrow (3,5, 7, and 9) vs chemigated (4, 6, 8, 10)
Bio-Tam (3, 4, 5, and 6) vs Serenade ASO (7, 8, 9, and 10)

Table 2.11: Analysis of variance of the effect of fumigation and biopesticide treatments on stand count percentage and plant height in Russet Norkotah potatoes grown at Parma, ID during 2017. Significance is indicated by P ≤ 0.05.

	Stand Count %	Plant Height 21 Jun (cm)	Plant Height 11 Jul (cm)	Plant Height 20 Jul (cm)	Plant Height 3 Aug (cm)
	P-value	P-value	P-value	P-value	P-value
Treatment	0.1375	0.8801	0.5210	0.2924	0.6118
Planned Contrasts					
Non-Treated vs Biopesticides	0.5337	0.8386	0.7035	0.5277	0.5394
In-furrow vs chemigation	0.1614	0.2487	0.3107	0.7375	0.4438
Bio-Tam vs Serenade ASO	0.0107	0.5424	0.3701	0.2245	0.7584

Table 2.12: Effect of fumigation and biopesticide treatments on stand count percentage and plant height in Russet Norkotah potatoes grown at Parma, ID during 2017. Means are of 4 replications.

Treatment		Stand Count %	Plant Height 21 Jun (cm)	Plant Height 11 Jul (cm)	Plant Height 20 Jul (cm)	Plant Height 3 Aug (cm)
Non-treated	1	91.0	39	45	44	44
Fumigated check	2	91.8	40	48	50	47
Bio-Tam in-furrow (IF)	3	85.8	37	44	45	45
Bio-Tam chemigation - low rate (CL)	4	88.8	41	44	45	39
Bio-Tam IF + CL	5	87.8	40	43	44	42
Bio-Tam chemigation - high rate (HL)	6	86.5	40	45	45	41
Serenade ASO in-furrow (IF)	7	93.0	39	44	46	43
Serenade ASO chemigation - low rate (CL)	8	88.8	38	44	46	46
Serenade ASO IF + CL	9	96.0	38	46	46	42
Serenade ASO chemigation - high rate (HL)	10	89.0	40	46	47	39
Non-inoculated fumigated check	11	93.8	38	46	46	47
LSD						
Contrasts^		ns	ns	ns	ns	ns
Non-treated		91.0	39	45	44	44
Biopesticides		89.4	39	44	45	42
In-furrow		90.6	38	44	45	43
Chemigation		88.3	40	45	46	41
Bio-Tam		87.2	39	44	45	42
Serenade ASO		91.7	39	45	46	42

^Single degree of freedom contrast comparing the following treatments means:

Non-treated (1) vs biopesticides (3,4,5,6,7,8,9, and 10)

In-furrow (3,5, 7, and 9) vs chemigated (4, 6, 8, 10)

Bio-Tam (3, 4, 5, and 6) vs Serenade ASO (7, 8, 9, and 10)

Table 2.13: Analysis of variance of the effect of fumigation and biopesticide treatments on the relative area under the disease progress curve (RAUDPC) in Russet Norkotah potatoes grown at Parma, ID during 2017. Significance is indicated by P ≤ 0.05.

	RAUDPC
	P-value
Treatment	0.8290
Planned Contrasts	
Non-Treated vs Biopesticides	0.7793
In-furrow vs chemigation	0.1145
Bio-Tam vs Serenade ASO	0.7166

Table 2.14: Effect of fumigation and biopesticide treatments on the relative area under the disease progress curve (RAUDPC in Russet Norkotah potatoes grown at Parma, ID during 2017. Means are of 4 replications.

Treatment		RAUDPC
Non-treated	**1**	18.9
Fumigated check	**2**	17.9
Bio-Tam in-furrow (IF)	**3**	17.4
Bio-Tam chemigation - low rate (CL)	**4**	21.8
Bio-Tam IF + CL	**5**	18.4
Bio-Tam chemigation - high rate (HL)	**6**	20.1
Serenade ASO in-furrow (IF)	**7**	17.9
Serenade ASO chemigation - low rate (CL)	**8**	19.3
Serenade ASO IF + CL	**9**	18.3
Serenade ASO chemigation - high rate (HL)	**10**	25.5
Non-inoculated check	**11**	18.6
LSD (0.05)		**ns**
Contrasts^		
Non-treated		18.9
Biopesticides		19.8
In-furrow		18.0
Chemigated		21.7
Bio-Tam		19.4
Serenade ASO		20.3

^Single degree of freedom contrast comparing the following treatments means:

Non-treated (1) vs biopesticides (3,4,5,6,7,8,9, and 10)
In-furrow (3,5, 7, and 9) vs chemigated (4, 6, 8, 10)
Bio-Tam (3, 4, 5, and 6) vs Serenade ASO (7, 8, 9, and 10)

Table 2.15: Analysis of variance of the effect of fumigation and biopesticide treatments on stem tissue DNA concentration pre and post-treatment in Russet Norkotah potatoes grown at Parm a, ID during 2017. Significance is indicated by P ≤ 0.05.

	V. dahliae Stem Pre Trt pg/g	*V. dahliae* Stem Post Trt pg/g	*C. coccodes* Stem Pre Trt pg/g	*C. coccodes* Stem Post Trt pg/g
Treatment	0.3487	0.9924	0.1593	0.9650
Planned Contrasts				
Non-Treated vs Biopesticides	0.7746	0.9144	0.7160	0.6883
In-furrow vs chemigation	0.1974	0.6086	0.0967	0.4998
Bio-Tam vs Serenade ASO	0.6567	0.4498	0.6947	0.3459

Table 2.16: Effect of fumigation and biopesticide treatments on stem DNA concentrations pre and post-treatment in Russet Norkotah potatoes grown at Parma, ID during 2017. DNA values were determined by qPCR analysis, are the means are of 4 replications.

Treatment		*V. dahliae* Stem Pre Trt pg/g	*V. dahliae* Stem Post Trt pg/g	*C. coccodes* Stem Pre Trt pg/g	*C. coccodes* Stem Post Trt pg/g
Non-treated	1	1	1012648	106	37905
Fumigated check	2	1	1674898	198	28743
Bio-Tam in-furrow (IF)	3	0	105813	23	35865
Bio-Tam chemigation - low rate (CL)	4	8	313335	360	24425
Bio-Tam IF + CL	5	35	728965	1051	36970
Bio-Tam chemigation - high rate (HL)	6	0	316183	318	28793
Serenade ASO in-furrow (IF)	7	1	822023	62	27080
Serenade ASO chemigation - low rate (CL)	8	0	3917565	4268	29523
Serenade ASO IF + CL	9	23	1547560	43	35150
Serenade ASO chemigation - high rate (HL)	10	3	660380	166	13533
Non-inoculated check	11	0	3845730	185	51078
LSD (0.05)		ns	ns	ns	ns
Contrasts^					
Non-treated		1	1012648	106	37905
Biopesticides		9	1051478	786	28917
In-furrow		15	801090	295	33766
Chemigated		3	1301866	1278	24069
Bio-Tam		11	366074	438	31513
Serenade ASO		6	1736882	1135	26322

^Single degree of freedom contrast comparing the following treatments means:
Non-treated (1) vs biopesticides (3,4,5,6,7,8,9, and 10)
In-furrow (3,5, 7, and 9) vs chemigated (4, 6, 8, 10)
Bio-Tam (3, 4, 5, and 6) vs Serenade ASO (7, 8, 9, and 10)

Table 2.17: Analysis of variance of the effect of fumigation and biopesticide treatments on soil DNA concentration pre and post-treatment in Russet Norkotah potatoes grown at Parma, ID during 2017. Significance is indicated by P ≤ 0.05.

	V. dahliae Soil Pre Trt Top 15 cm pg/g	*V. dahliae* Soil Pre Trt Bottom 15 cm pg/g	*V. dahliae* Soil Post Trt Top 15 cm pg/g	*V. dahliae* Soil Post Trt Bottom 15 cm pg/g	*C. coccodes* Soil Pre Trt Top 15 cm pg/g	*C. coccodes* Soil Pre Trt Bottom 15 cm pg/g	*C. coccodes* Soil Post Trt Top 15 cm pg/g	*C. coccodes* Soil Post Trt Bottom 15 cm pg/g
Treatment	0.3347	0.0819	0.8327	0.3128	0.1748	0.3169	0.9949	0.7222
Planned Contrasts								
Non-Treated vs Biopesticides	0.9630	0.8528	0.6804	0.4758	0.6957	0.1700	0.7302	0.4741
In-furrow vs chemigation	0.4994	0.8272	0.0731	0.8655	0.0364	0.5703	0.6644	0.9393
Bio-Tam vs Serenade ASO	0.1310	0.4002	0.6717	0.0441	0.4231	0.4908	0.9444	0.6234

Table 2.18: Effect of fumigation and biopesticide treatments on soil DNA concentrations pre and post-treatment in Russet Norkotah potatoes grown at Parma, ID during 2017. DNA values were determined by qPCR analysis, are the means are of 4 replications.

Treatment		*V. dahliae* Soil Pre Trt Top 15 cm pg/g	*V. dahliae* Soil Pre Trt Bottom 15 cm pg/g	*V. dahliae* Soil Post Trt Top 15 cm pg/g	*V. dahliae* Soil Post Trt Bottom 15 cm pg/g	*C. coccodes* Soil Pre Trt Top 15 cm pg/g	*C. coccodes* Soil Pre Trt Bottom 15 cm pg/g	*C. coccodes* Soil Post Trt Top 15 cm pg/g	*C. coccodes* Soil Post Trt Bottom 15 cm pg/g
Non-treated	1	0	0	25	3	380	37	1218	405
Fumigated check	2	2	36	609	10	628	210	1305	446
Bio-Tam in-furrow (IF)	3	0	0	101	39	173	148	4388	1190
Bio-Tam chemigation - low rate (CL)	4	1	0	773	5	863	285	1455	503
Bio-Tam IF + CL	5	0	0	8	2	83	210	1960	338
Bio-Tam chemigation - high rate (HL)	6	0	0	800	1	166	29	3938	913
Serenade ASO in-furrow (IF)	7	1	2	11	3820	150	133	2423	1443
Serenade ASO chemigation - low rate (CL)	8	0	0	588	661	588	274	4373	2258
Serenade ASO IF + CL	9	2	0	0	0	65	28	5970	290
Serenade ASO chemigation - high rate (HL)	10	1	0	525	1345	358	180	2948	614
Non-inoculated check	11	0	0	114	132	175	199	3630	635
LSD (0.05)		ns	ns	ns	ns	ns	ns	ns	ns
Contrasts^									
Non-treated		0	0	25	3	380	37	1218	405
Biopesticides		1	0	351	734	305	161	3432	943
In-furrow		1	0	30	965	117	130	3685	815
Chemigated		1	0	671	503	493	192	3179	1072
Bio-Tam		0	0	420	12	321	168	2935	736
Serenade ASO		1	0	281	1457	290	154	3929	1151

^Single degree of freedom contrast comparing the following treatments means:

Non-treated (1) vs biopesticides (3,4,5,6,7,8,9, and 10)

In-furrow (3,5, 7, and 9) vs chemigated (4, 6, 8, 10)

Bio-Tam (3, 4, 5, and 6) vs Serenade ASO (7, 8, 9, and 10)

Table 2.19: Analysis of variance of the effect of fumigation and biopesticide treatments on yield, #2's, and culls in Russet Norkotah potatoes grown at Parma, ID during 2017. Significance is indicated by P ≤ 0.05.

	Yield 0-113 g t/ha	Yield 114-170 g t/ha	Yield 171-283 g t/ha	Yield 284-454 g t/ha	Yield >455 g t/ha	Yield #2s t/ha	Yield Culls t/ha	Total Yield t/ha
	P-value	P-value	P-value	P-value	P-value	P-value	P-value	P-value
Treatment	0.0795	0.1702	0.3309	0.9001	0.6289	0.0072	0.7602	0.9986
Planned Contrasts								
Non-Treated vs Biopesticides	0.0984	0.8480	0.1186	0.5851	0.9784	0.2022	0.1864	0.9449
In-furrow vs chemigation	0.8336	0.3779	0.4311	0.7061	0.1530	0.9621	0.9870	0.7333
Bio-Tam vs Serenade ASO	0.4270	0.4170	0.6408	0.5859	0.3970	0.6022	0.7083	0.8957

Table 2.20: Effect of fumigation and biopesticide treatments on yield, #2's, and culls in Russet Norkotah potatoes grown at Parma, ID during 2017. Means are of 4 replications.

Treatment		Yield 0-113 g t/ha	Yield 114-170 g t/ha	Yield 171-283 g t/ha	Yield 284-454 g t/ha	Yield >455 g t/ha	Yield #2s t/ha	Yield Culls t/ha	Total Yield t/ha
Non-treated	1	8.2	9.9	19.5	16.1	4.5	0.7	0.6	59.5
Fumigated check	2	8.6	11.7	25.4	11.9	3.5	0.5	0.3	62.0
Bio-Tam in-furrow (IF)	3	5.6	8.6	23.3	16.3	4.5	0.8	0.5	59.6
Bio-Tam chemigation - low rate (CL)	4	7.2	10.1	22.8	13.0	4.8	0.8	0.4	59.0
Bio-Tam IF + CL	5	7.7	10.0	23.1	15.1	2.5	0.7	0.0	59.0
Bio-Tam chemigation - high rate (HL)	6	6.0	9.0	20.2	16.6	4.9	1.7	0.3	58.6
Serenade ASO in-furrow (IF)	7	7.0	11.1	22.7	14.6	4.5	1.7	0.3	61.9
Serenade ASO chemigation - low rate (CL)	8	6.9	9.5	22.6	13.3	6.7	0.6	0.2	59.7
Serenade ASO IF + CL	9	7.3	10.2	21.0	14.4	4.1	1.0	0.3	58.2
Serenade ASO chemigation - high rate (HL)	10	7.1	9.1	21.0	15.3	4.1	1.2	0.1	57.8
Non-inoculated fumigated check	11	9.0	11.9	21.2	14.4	3.7	0.6	0.0	60.8
LSD (0.05)		ns	ns	ns	ns	ns	ns	ns	ns
Contrasts^									
Non-treated		8.2	9.9	19.5	16.1	4.5	0.7	0.6	59.5
Biopesticides		6.8	9.7	22.1	14.8	4.5	1.1	0.3	59.2
In-furrow		6.9	10.0	22.5	15.1	3.9	1.1	0.3	59.7
Chemigation		6.8	9.4	21.7	14.5	5.1	1.1	0.3	58.8
Bio-Tam		6.6	9.4	22.3	15.2	4.2	1.0	0.3	59.1
Serenade ASO		7.0	9.9	21.8	14.4	4.9	1.1	0.2	59.4

^Single degree of freedom contrast comparing the following treatments means:

Non-treated (1) vs biopesticides (3,4,5,6,7,8,9, and 10)

In-furrow (3,5, 7, and 9) vs chemigated (4, 6, 8, 10)

Bio-Tam (3, 4, 5, and 6) vs Serenade ASO (7, 8, 9, and 10)

Table 2.21:Analysis of variance of the effect of fumigation and biopesticide treatments on yield percentage greater than 171 and 284 grams and specific gravity in Russet Norkotah potatoes grown at Parma, ID during 2017. Significance is indicated by P ≤ 0.05.

	Percent >171 g	Percent >284 g	Specific Gravity
	P-value	P-value	P-value
Treatment	0.7109	0.6498	0.8997
Planned Contrasts			
Non-Treated vs Biopesticides	0.4427	0.6705	0.3392
In-furrow vs chemigation	0.8453	0.5242	0.4655
Bio-Tam vs Serenade ASO	0.4875	0.8552	0.6359

Table 2.22: Effect of fumigation and biopesticide treatments on yield percentage greater than 171 and 284 grams and specific gravity in Russet Norkotah potatoes grown at Parma, ID during 2016. Means are of 4 replications.

Treatment		Percent >171 g	Percent >284 g	Specific Gravity
Non-treated	1	67.0	34.0	1.075
Fumigated check	2	65.8	24.8	1.079
Bio-Tam in-furrow (IF)	3	73.8	37.8	1.079
Bio-Tam chemigation - low rate (CL)	4	68.5	29.5	1.077
Bio-Tam IF + CL	5	68.5	29.0	1.076
Bio-Tam chemigation - high rate (HL)	6	70.8	36.8	1.075
Serenade in-furrow (IF)	7	67.5	30.3	1.079
Serenade ASO chemigation - low rate (CL)	8	71.0	33.3	1.078
Serenade ASO IF + CL	9	67.8	31.5	1.077
Serenade ASO chemigation - high rate (HL)	10	69.0	33.0	1.076
Non-inoculated fumigated check	11	64.0	29.5	1.077
LSD (0.05)		ns	ns	ns
Contrasts^				
Non-treated		67.0	34.0	1.075
Biopesticides		69.6	32.7	1.077
In-furrow		69.4	32.2	1.078
Chemigation		69.8	33.2	1.077
Bio-Tam		70.4	33.3	1.077
Serenade ASO		68.8	32.0	1.078

^Single degree of freedom contrast comparing the following treatments means:

Non-treated (1) vs biopesticides (3,4,5,6,7,8,9, and 10)

In-furrow (3,5, 7, and 9) vs chemigated (4, 6, 8, 10)

Bio-Tam (3, 4, 5, and 6) vs Serenade ASO (7, 8, 9, and 10)

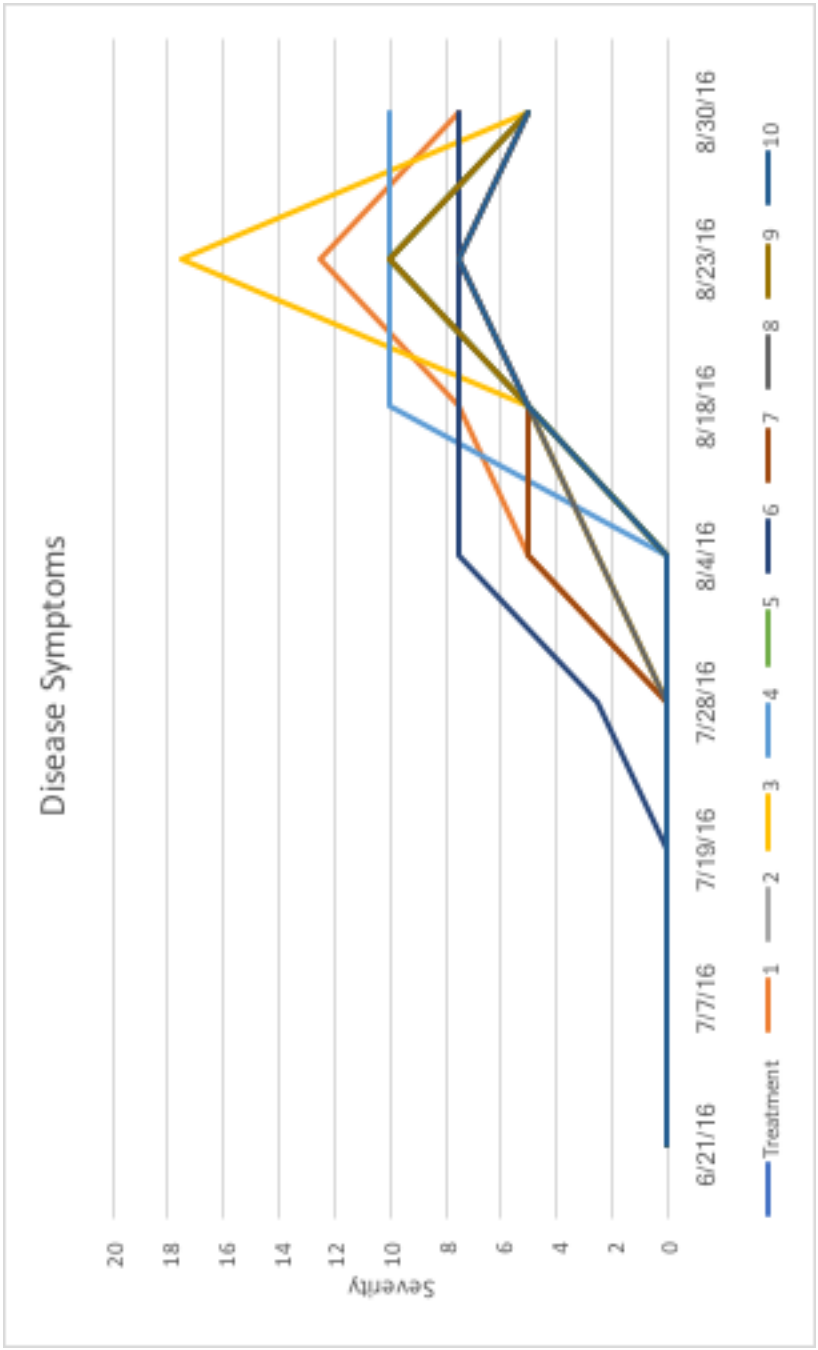

Figure 2.1: Effect of fumigation and biopesticide treatments on the severity of disease symptoms in Russet Norkotah potatoes grown at Parma, ID during 2016. Means are of 4 replications.

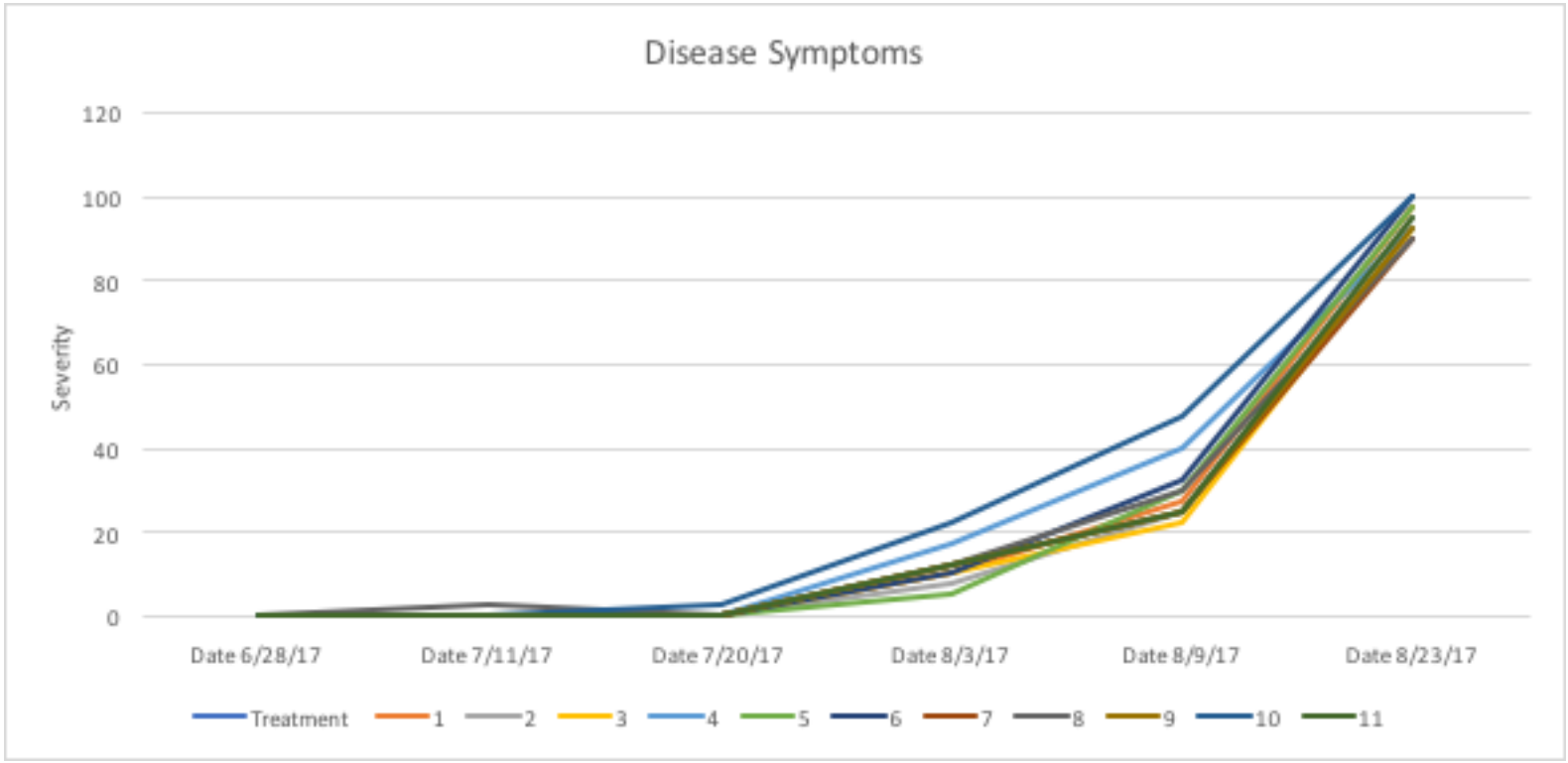

Figure 2.2: Effect of fumigation and biopesticide treatments on the severity of disease symptoms in Russet Norkotah potatoes grown at Parma, ID during 2017. Means are of 4 replications.

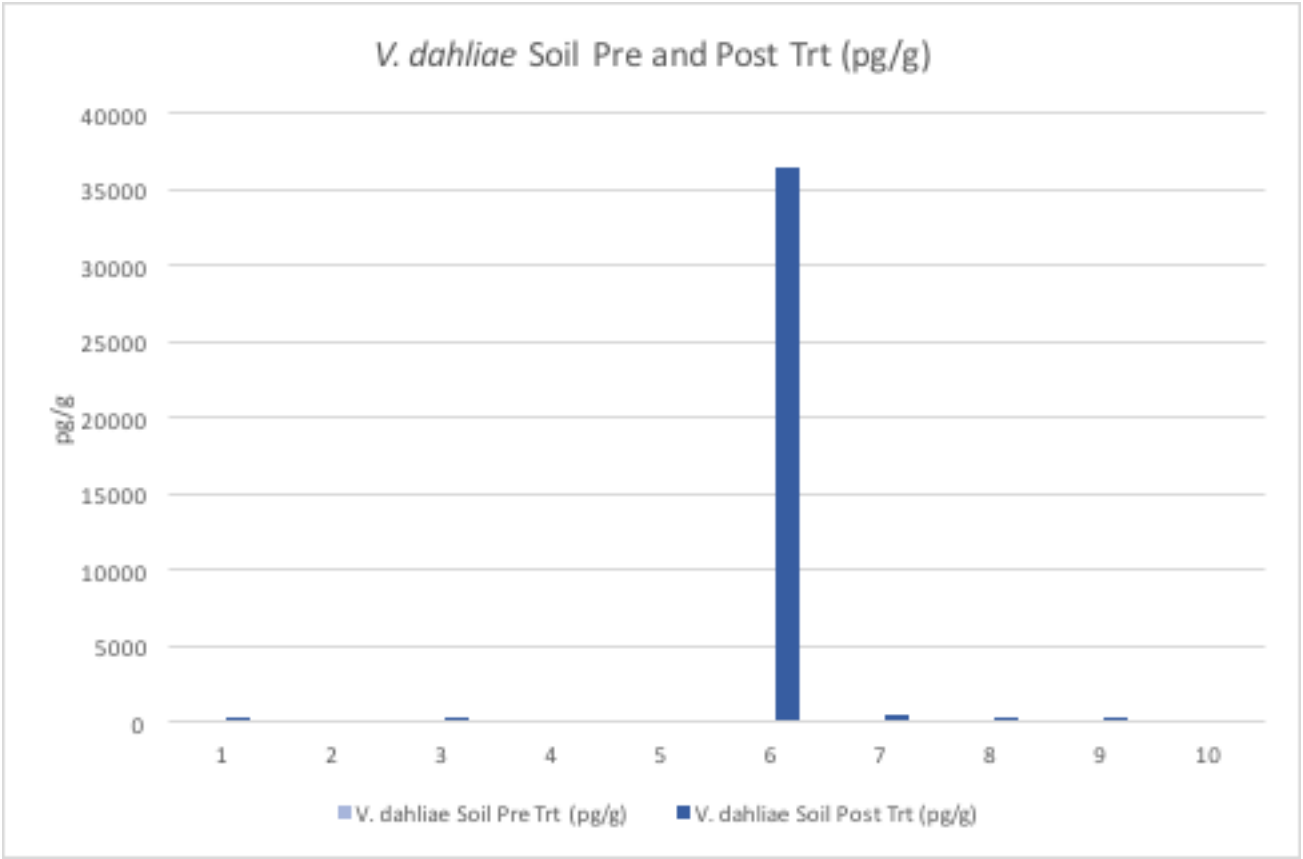

Figure 2.3: Effect of fumigation and biopesticide treatments on soil DNA concentrations pre and post-treatment in Russet Norkotah potatoes grown at Parma, ID during 2016. DNA values were determined by qPCR analysis, are the means are of 4 replications.

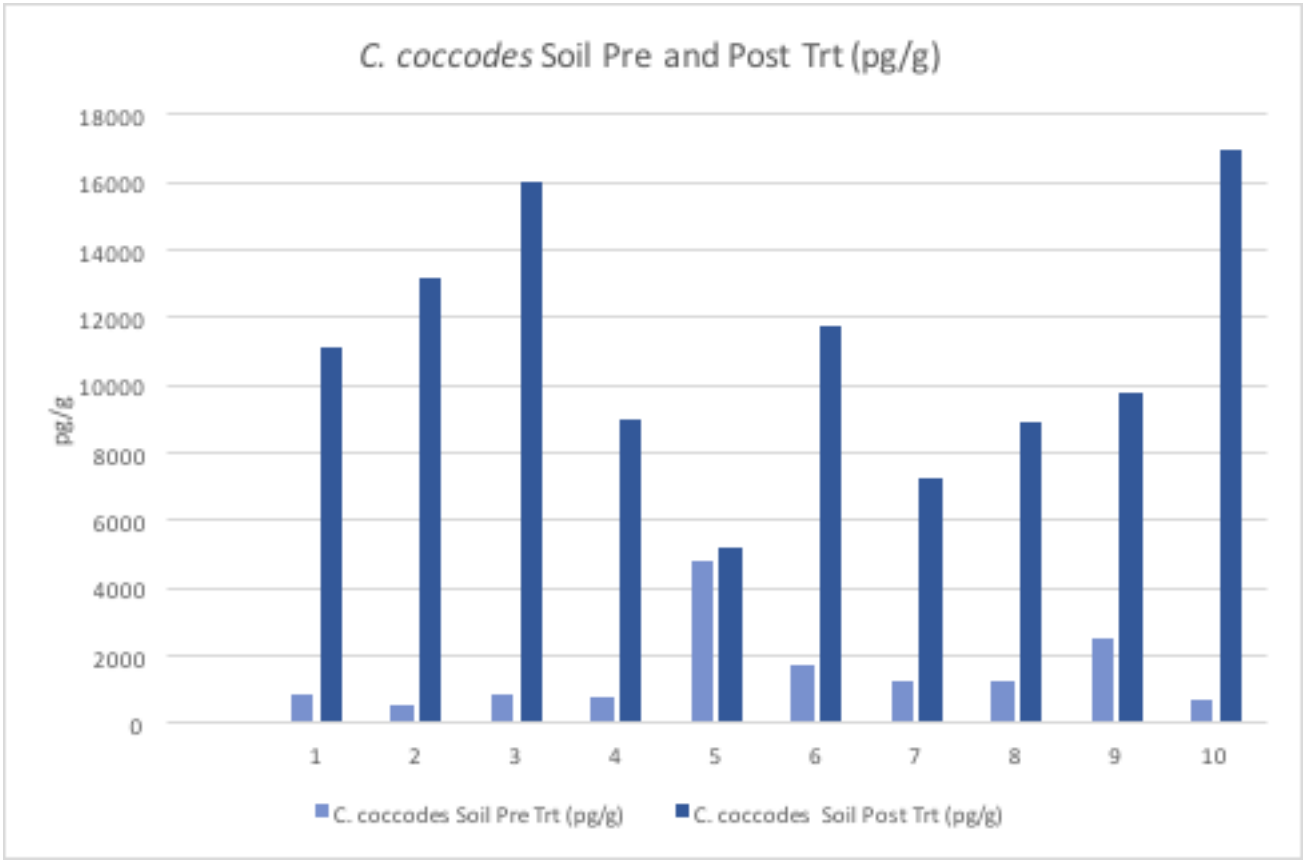

Figure 2.4: Effect of fumigation and biopesticide treatments on soil DNA concentrations pre and post-treatment in Russet Norkotah potatoes grown at Parma, ID during 2016. DNA values were determined by qPCR analysis, are the means a re of 4 replications.

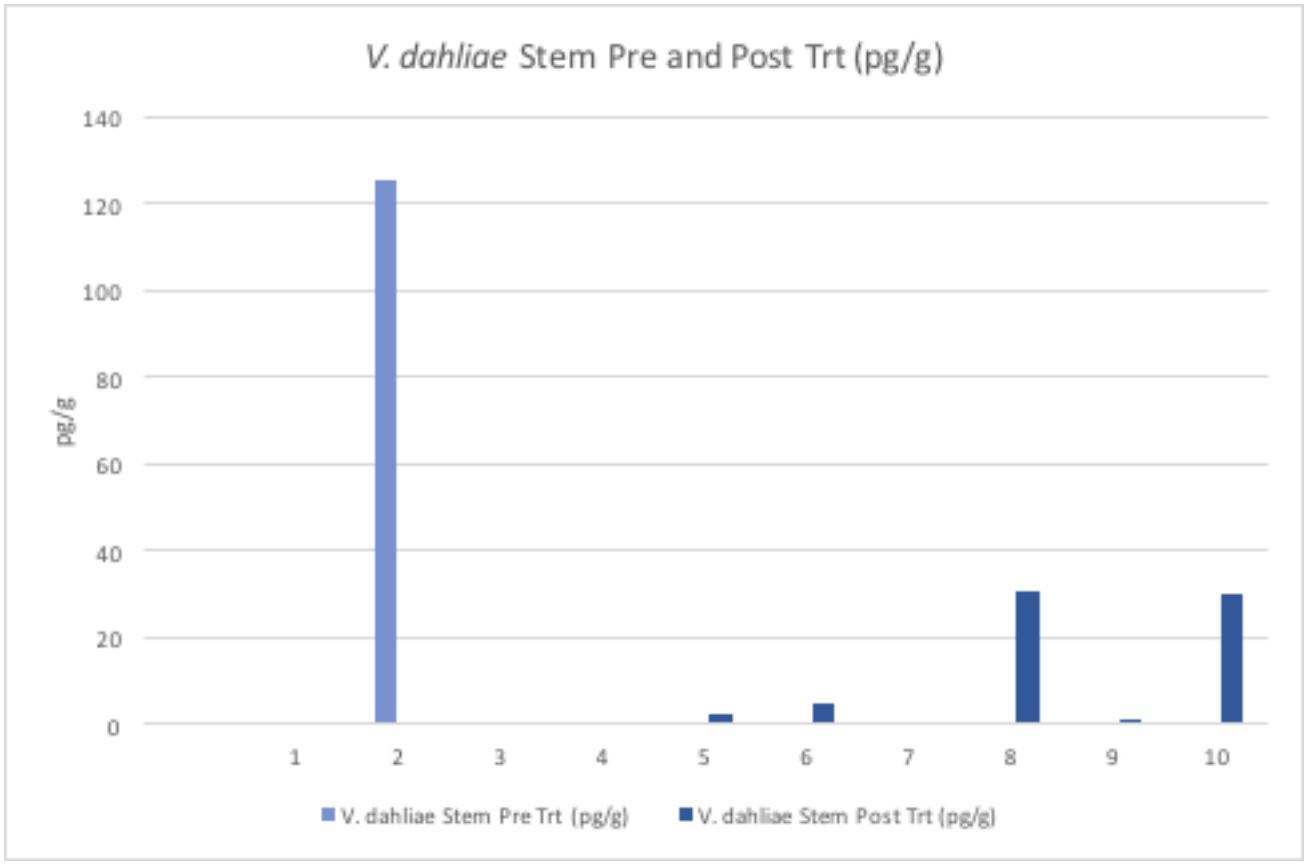

Figure 2.5: Effect of fumigation and biopesticide treatments on stem DNA concentrations pre and post-treatment in Russet Norkotah potatoes grown at Parma, ID during 2016. DNA values were determined by qPCR analysis, are the means are of 4 replications.

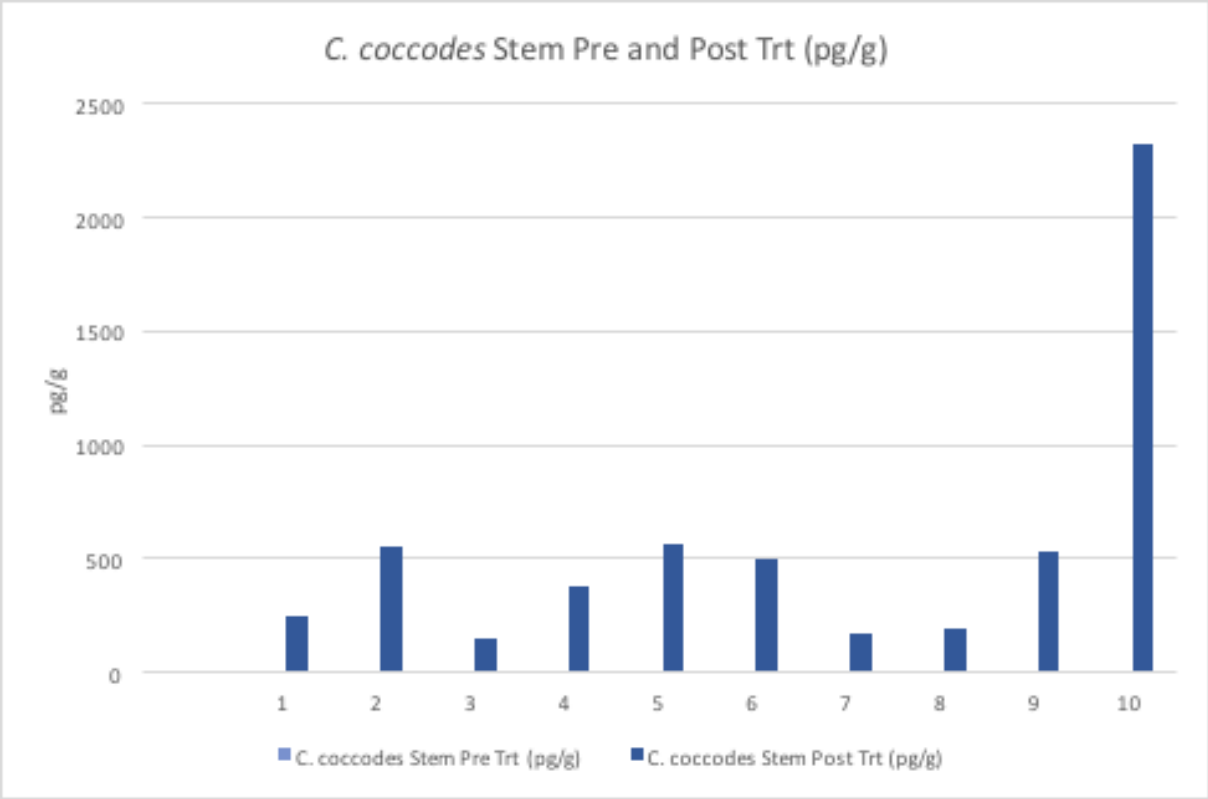

Figure 2.6: Effect of fumigation and biopesticide treatments on stem DNA concentrations pre and post-treatment in Russet Norkotah potatoes grown at Parma, ID during 2016. DNA values were determined by qPCR analysis, are the means are of 4 replications.

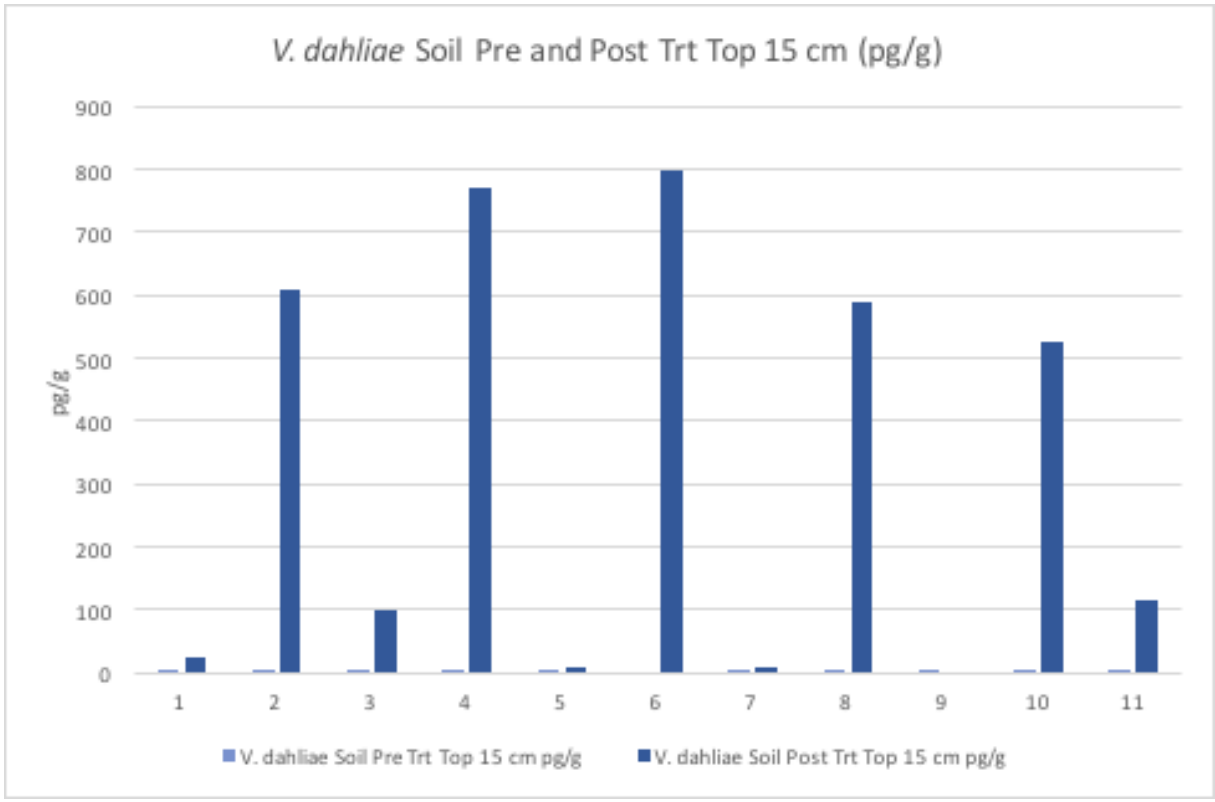

Figure 2.7: Effect of fumigation and biopesticide treatments on soil DNA concentrations pre and post-treatment in Russet Norkotah potatoes grown at Parma, ID during 2017. DNA values were determined by qPCR analysis, are the means are of 4 replications.

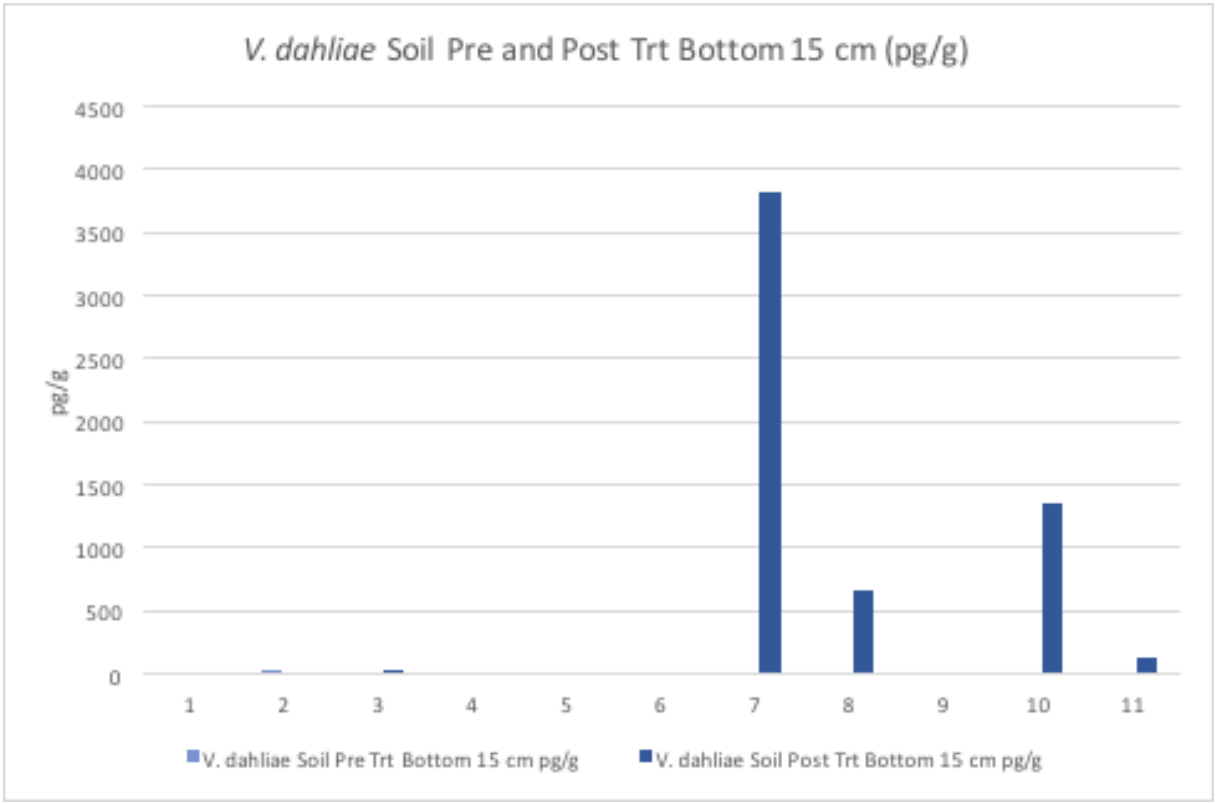

Figure 2.8: Effect of fumigation and biopesticide treatments on soil DNA concentrations pre and post-treatment in Russet Norkotah potatoes grown at Parma, ID during 2017. DNA values were determined by qPCR analysis, are the means are of 4 replications.

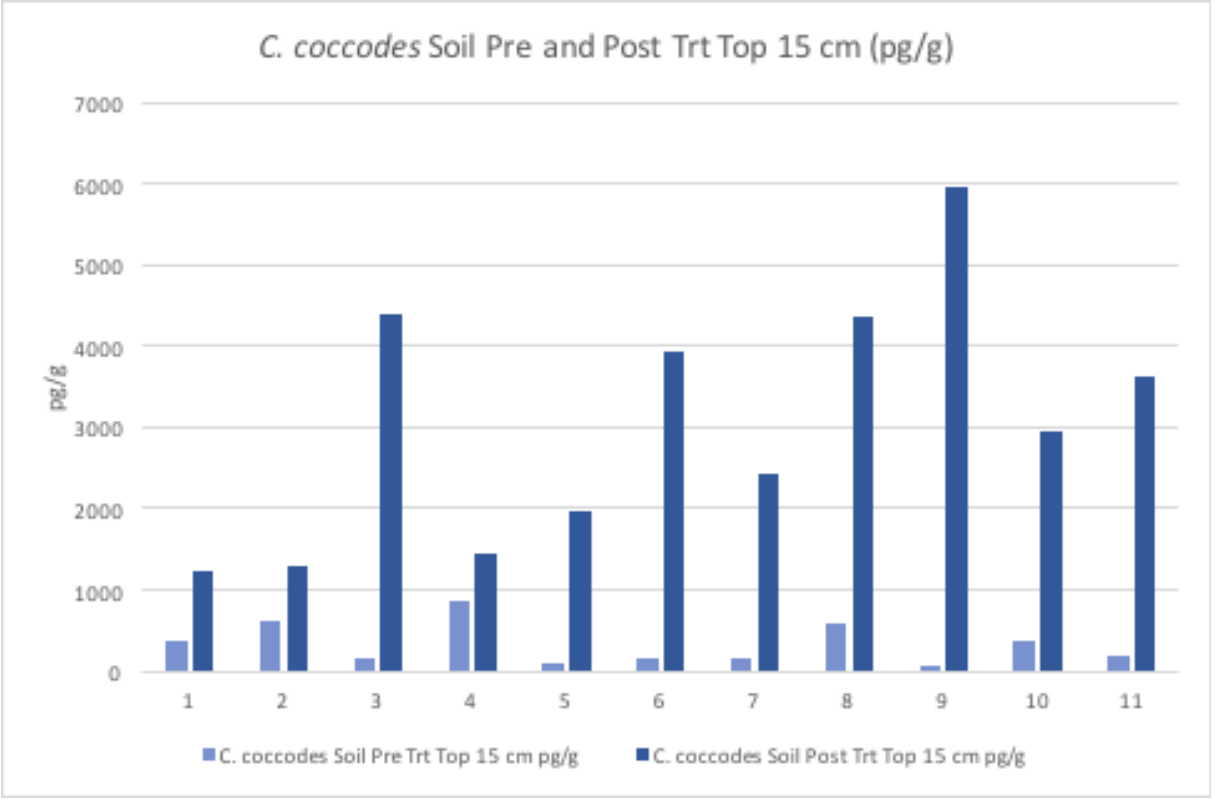

Figure 2.9: Effect of fumigation and biopesticide treatments on soil DNA concentrations pre and post-treatment in Russet Norkotah potatoes grown at Parma, ID during 2017. DNA values were determined by qPCR analysis, are the means are of 4 replications.

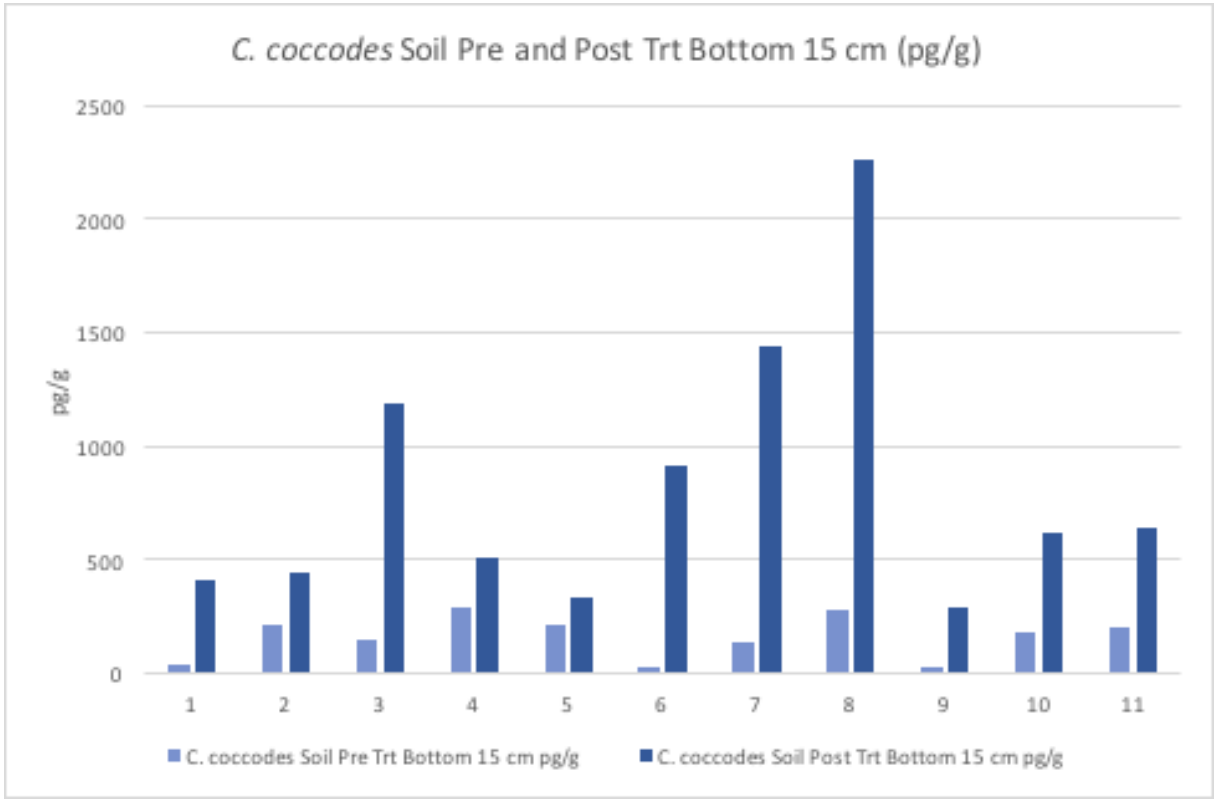

Figure 2.10: Effect of fumigation and biopesticide treatments on stem DNA concentrations pre and post-treatment in Russet Norkotah potatoes grown at Parma, ID during 2017. DNA values were determined by qPCR analysis, are the means are of 4 replications.

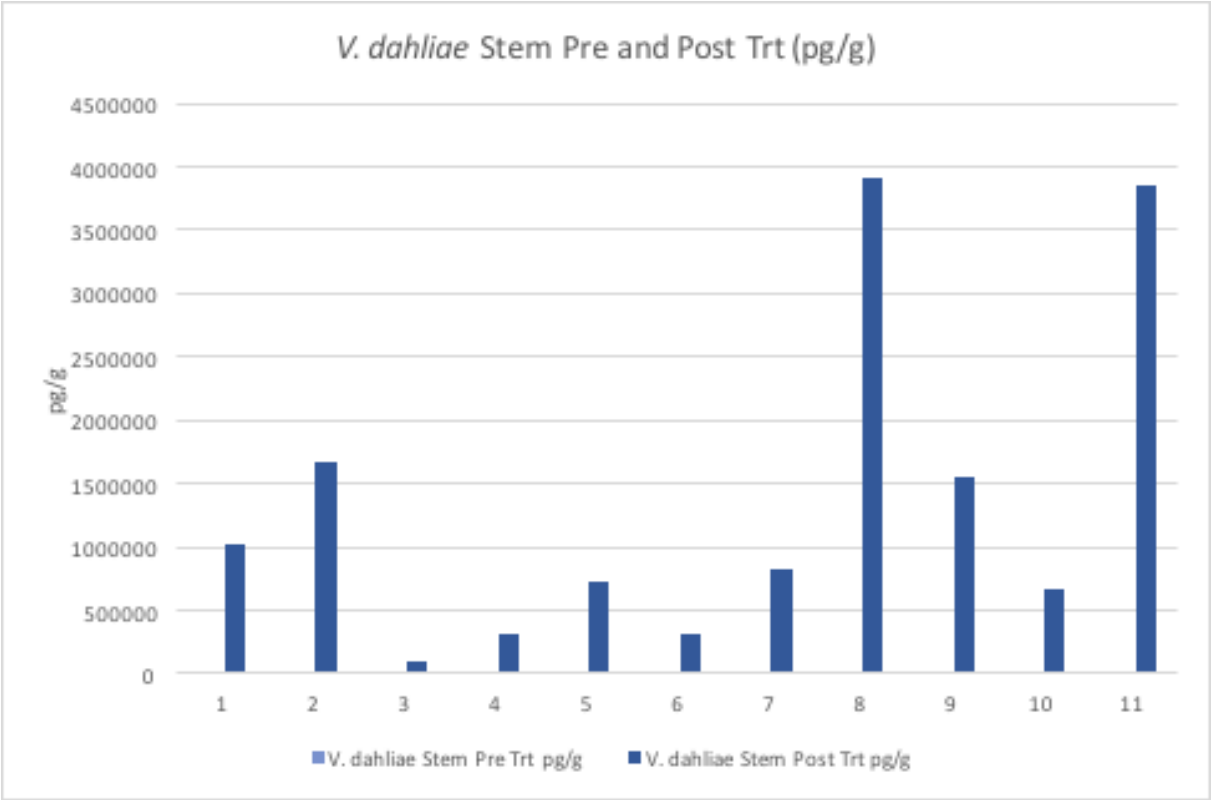

Figure 2.11: Effect of fumigation and biopesticide treatments on stem DNA concentrations pre and post-treatment in Russet Norkotah potatoes grown at Parma, ID during 2017. DNA values were determined by qPCR analysis, are the means are of 4 replications.

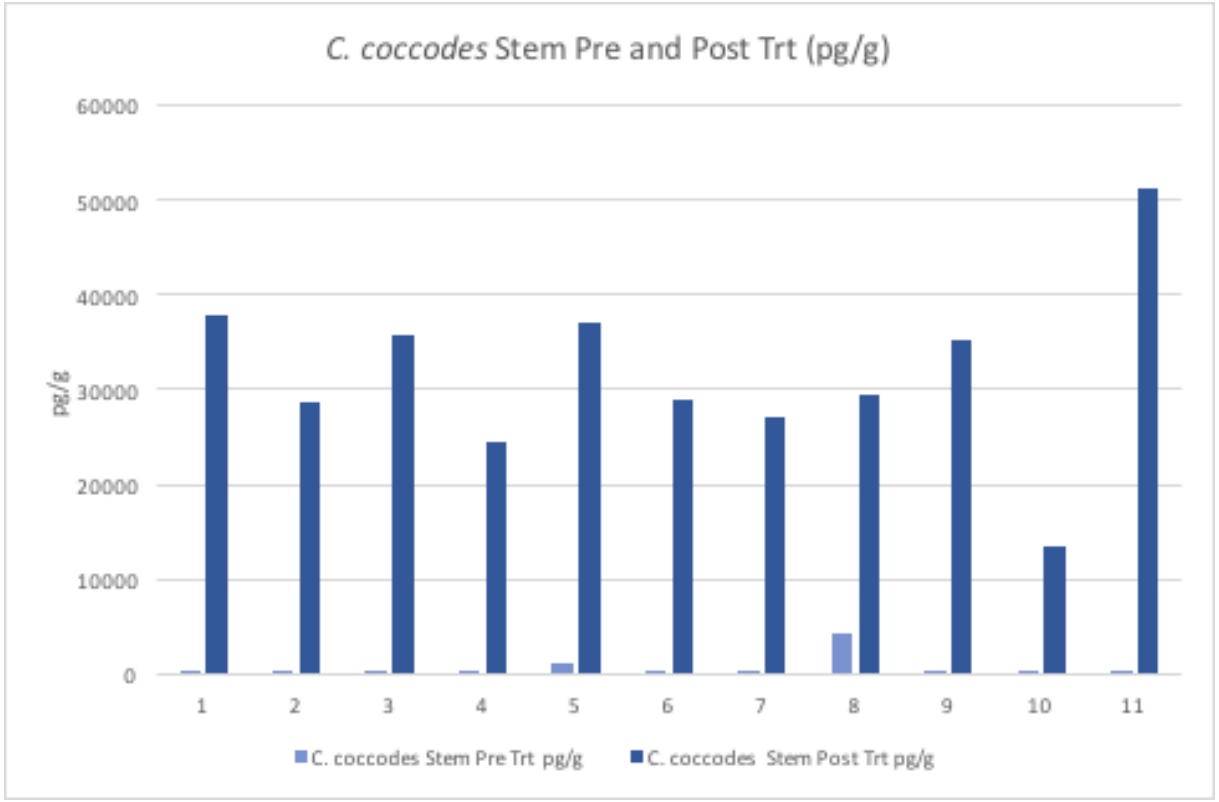

Figure 2.12: Effect of fumigation and biopesticide treatments on stem DNA concentrations pre and post-treatment in Russet Norkotah potatoes grown at Parma, ID during 2017. DNA values were determined by qPCR analysis, are the means are of 4 replications.

CH. 3 INFLUENCE OF FOUR BIOPESTICIDES AND SOIL ORGANIC MATTER CONTENT ON VERTICILLIUM WILT UNDER GREENHOUSE CONDITIONS

3.1 ABSTRACT

Biopesticides based on microbial agents may provide an alternative to fumigation for the control of the potato early die complex caused by *Verticillium dahliae* Kleb and *Colletotrichum coccodes*. Levels of soil organic matter have been shown to influence efficacy of chemical pesticides, and may be even more critical for biopesticides due to microorganisms rely on soil organic matter for survival. Four biopesticides including Serenade ASO (*Bacillus subtilis*), Bio-Tam (*Trichoderma asperellum*/*Trichoderma gamsii*), SoilGard (*Trichoderma virens*), and Regalia (*Reynoutria sachalinensis* plant extract) were evaluated for control of Verticillium wilt under greenhouse conditions. Field soil was amended with sphagnum peat moss to establish three levels of organic matter. There were no significant differences among the treatments that would suggest that biopesticides can effectively control Verticillium wilt in potatoes.

3.2 INTRODUCTION

Biopesticides have been shown to be effective against many different plant pathogens, but the lack of consistency of biopesticides has been the most concerning issue. There are many different possibilities for why biopesticides have shown inconsistent results. One is that the pathogen populations and environment under field conditions is much more variable versus a laboratory or greenhouse. Field conditions can present variable initial microbial populations and soil organic matter that could impact the efficacy of these products. There is also the possibility that these biopesticides are competing with initial beneficial microorganisms that are impacting pathogen levels.

Bot and Benites (2005) define soil organic matter as, “any material produced originally by living organisms (plant or animal) that is returned to the soil and goes through the decomposition process.” Soil organic matter has been shown to have an impact on the efficacy of pesticides and fungicides used in an agricultural system. Higher soil organic matter content can also restrict the movement of chemicals and cause enhanced adsorption. These chemicals can be broken down by microorganisms to a form that is no longer effective (Bots and Benites, 2005; Nelson, 1996). High soil organic matter can restrict the movement of fumigants, which can lead to a two to three fold increase in application rate for effective control (Hafez and Sundararaj, 2009).

Microorganisms have shown the ability to be effective against many soil-borne pathogens (Bot and Benites, 2005; Harman, 2006; Whipps et al., 2008; Choudhary and Johri, 2009; Kloepper et al., 2004; Berg, 2009). Soil organic matter has been shown to be a factor in

disease severity of PED. Davis et al., (2001) reported a negative correlation between soil organic matter and wilt symptoms under field conditions in Southern Idaho.

It is important to point out that increasing soil organic matter content of soil has many beneficial impacts on soil characteristics that have nothing to do with suppression of pathogens or interactions with pesticides. These impacts include increases in soil levels of organic nutrients, structure, water holding capacity, aeration and microbial biodiversity (Carter et al., 2004; Bot and Benites, 2005). Soil organic matter increases the total soil microbial activity. Microorganisms are essential for mineralization, which is the process of converting organic elements to the inorganic plant available form (Bot and Benites, 2005). Microorganisms use soil organic matter as food and release the surplus of nutrients back to the soil (Bot and Benites, 2005).

A number of commercial biopesticides have been studied against a range of pathogens, however, they have not been evaluated for control of PED in potatoes. A greenhouse environment was chosen for this study to allow conditions to be controlled more efficiently, and inoculum levels of *Verticillium dahliae* to be more uniform. In a field environment, distribution of *Verticillium dahliae* is known to be in clustered patterns (Steere et al., 2016; Xiao et al., 1997). Likewise, a controlled environment allowed soil organic matter content to be adjusted while compensating for impact on nutrient content of the soil.

The objective of this study was to determine how four different biopesticides (*Trichoderma asperellum/Trichoderma gamsii*, *Bacillus subtilis*, *Reynoutria sachalinensis* plant extract and *Trichoderma virens*) impacted PED in a greenhouse environment. This

study included subplots with different levels of organic matter to evaluate how that factor impacts the efficacy of these products.

3.3 MATERIALS AND METHODS

A greenhouse experiment was performed during the winter of 2016/2017 at the Parma Research and Extension Center. Soil obtained from a commercial field in Wilder, ID at a depth of 0-30 cm, previously cropped to potatoes was used as the growing media. The soil texture was Feltham fine sandy loam with a pH of 6.0, and contained 70 ppm NO_3-N and 9 ppm NH_4-N. The soil was not autoclaved or otherwise sterilized prior to the experiment in an effort to preserve the inherent pathogen populations and microbial communities found in a common potato production soil.

This trial was planted using cut certified Russet Norkotah mini tubers on 6 September, 2016. Russet Norkotah was the chosen cultivar in this experiment because literature has shown that this cultivar is susceptible to Verticillium wilt (Jansky and Miller, 2010). Mini tubers were hand planted individually into separate greenhouse pots. Each pot had a height of 26.7 cm and diameter of 23.8 cm, for a total volume of 11,872.3 cm^3. Temperature in the greenhouse was set to 24 ^oC during the day and 13 ^oC at night. Twelve hours of artificial light was provided each day. Irrigation was hand applied sparingly until emergence to prevent seed piece decay. After emergence, a drip irrigation system was set up as follows. Two self-cleaning drippers were located at each pot, which applied a total of 63 ml of tap water per minute. Frequency and duration of irrigation increased based on plant stage to maintain 65-70% soil moisture. The treatments were arranged in a randomized complete block design that consisted of three replications.

To increase *V. dahliae* populations, the pots were inoculated prior to planting. The protocol to prepare the inoculum followed the same procedure outlined in chapter 2. Prior to inoculating, a triplicate sample of the field soil was analyzed for *V. dahliae* and *C. coccodes* using qPCR and there was only one positive test for both pathogens. The inoculum was mixed into the individual pots using a cement mixer. The inoculum rate was 369 g per pot and occurred the same day as planting.

Four different biopesticides, Serenade ASO (*Bacillus subtilis* - Bayer CropScience, Research Triangle, NC) and Bio-Tam (*Trichoderma asperellum*/*Trichoderma* gamsii – Marrone Bio Innovations, Davis, CA), SoilGard (*Trichoderma virens* – Certis USA, Columbia, MD), and Regalia (*Reynoutria sachalinensis* plant extract – Marrone Bio Inovations, Davis, CA) were applied four times throughout the growing season. The application dates were 11 October 2016 (35 DAP), 25 October 2016 (49 DAP), 8 November 2016 (63 DAP), and 22 November 2016 (77 DAP).

Serenade ASO was applied at a rate of 4.7 l/ha, SoilGard at 4.5 kg/ha, Bio-Tam at 2.8 kg/ha, and Regalia at 4.7 l/ha in a total spray volume of 200 ml as a soil drench. These rates were consistent with the label recommendation. Bio-Tam was soaked in water 24 hours prior to application. An application of imidacloprid (Admire Pro) occurred on 8 November 2016 at a rate of 636 ml/ha (Bayer CropScience, Research Triangle, NC) to control insects.

This study included subplots to determine if organic matter levels impact the efficacy of these biopesticides. The subplots include non-amended (base level); low rate organic matter, and high rate organic matter above the base level. The low and high rates were calculated to give an 1% and 2% increase in soil organic matter, respectively. Sphagnum peat

moss was used to increase the levels of organic matter. Based on the tests from University of Idaho and SoilTest laboratories in Moses Lake, WA, we determined the organic matter percentages of both the soil and sphagnum peat moss. The soil had an organic matter of 0.91% and the sphagnum peat moss had 0.16 kg organic matter per 1 kg. This made our target organic matter levels for the low and high rates 1.82% and 2.73%, respectively. Soil was mixed with sphagnum peat moss using a cement mixer. Based off the soil test reports, 0.012 kg of peat moss was needed per 1 kg of soil and 0.023 kg of peat moss was needed per 1 kg of soil for the low and high rates. Soil samples were taken after mixing to confirm changes in soil organic matter content and are presented in Table 25.

Visual symptoms of Verticillium wilt (*Verticillium dahliae)* were evaluated every two weeks until symptoms were present, and then weekly throughout November and December. The visual ratings were based on a 0-5 scale that increased based on increased chlorosis. The scale was adopted from Zhang et al. (2012) where:

0 = No symptom

1 = <25% chlorotic/necrotic leaves

2 = 25-50% chlorotic/necrotic leaves

3 = 50-75% chlorotic/necrotic leaves

4 = > 75% chlorotic/necrotic leaves

5 = Complete defoliation or plant death

Disease severity was evaluated over the season by calculating the relative area under the disease progress curve (RAUDPC) using the method of Kirk et al. (2001).

The pots were harvested on 15 December, 2016 (100 DAP). For each pot, two stem sections at the soil line and 100 grams of soil were collected. The samples were analyzed for pathogen populations using a real-time polymerase chain reaction (qPCR). Real-time polymerase chain reaction (qPCR) was used following the same protocol that was outlined in the 2016 and 2017 field trial described in Chapter 2. Tubers were collected and total weight and tuber number per pot were determined.

3.4 STATISTICAL ANALYSIS

Data was analyzed as a two-way factorial Analysis of Variance (ANOVA) using the Proc GLM SAS statistical program. When the F-test was significant the means were separated using Fisher's protected least significant difference (LSD) at the 5% level. Real-time polymerase chain reaction (qPCR) results were cube root transformed prior to analysis to meet normality assumptions for ANOVA.

3.5 RESULTS

3.5.1 VISUAL SYMPTOMS:

The plants did show strong visual symptoms of disease development throughout the experiment. The values presented in the table are calculated from the relative area under the disease progress curve (RAUDPC) to present the rate of disease development over the course of the growing season with a single value. RAUDPC values ranged from 30.0 to 37.0 and there were no significant differences among the biopesticide treatments, or between the inoculated and non-inoculated checks (Tables 23 and 24). Amendment of soil with organic matter also did not significantly impact RAUDPC. However, there was a trend for treatment and soil organic matter with the inoculated check/low organic matter level with

the lowest RAUPDC and Bio-Tam/high organic matter level the highest (22.8 vs 48.3, P = 0.0521) (Figure 1).

3.5.2 Pathogen populations:

The values from the qPCR analysis are presented in pg of *V. dahlia*e and *C. coccodes* per g of soil/stem. *Verticillium dahliae* populations were highly variable in both the stem and soil samples and ranged from 0 to 25,532,371 pg/g. There were no significant differences among the biopesticide treatments, or between the inoculated and non-inoculated checks (Tables 23 and 24). Likewise, amendment of soil with organic matter had no impact on pathogen populations in stem or soil tissue.

Colletotrichum coccodes populations in soil and stem samples were also highly variable and ranged from 6 to 1,424,951 pg/g. There were no significant differences among the biopesticide treatments, or due to amendment of soil with organic matter (Tables 23 and 24).

3.5.3 Yield and Tuber Count:

Total yield did show some differences among the treatments with non-inoculated check having the lowest yield and SoilGard with the highest yield (173.9 vs 217.4, P = 0.0519) (Tables 23 and 24). Amendment with organic matter did significantly impact yield with the low organic matter rate the highest and the high organic matter rate the lowest (204.1 vs 178.5, P = 0.0376). Tuber count was similar among the treatments, ranging from 6 to 8 with no significant difference (Tables 23 and 24).

3.6 DISCUSSION

The first objective of this study was to determine if biopesticides could effectively control the potato early-dying complex (PED) in potato under controlled conditions in a greenhouse environment. The results of this study provided no evidence that biopesticides effectively control PED based on either visual disease symptoms or pathogen populations determined via qPCR. These results contradict the work done by Jordan and Gevens 2013, Wharton et al. 2012, Trillas et al. 2006, and Varo et al. 2016, who have reported that these biopesticides have been effective against soil borne pathogens in a field environment.

Verticillium dahliae and *C. coccodes* DNA values were highly variable among all the treatments and the subplots. Soil that was used in this study was obtained from a commercial field in Wilder, ID. With potatoes grown on the field the year prior we expected *V. dahliae* and *C. coccodes* levels to be high. However, a triplicate sample of the soil was taken and analyzed through qPCR. The results showed one positive test for *V. dahliae* and one positive test for *C. coccodes* at very low levels. Based on these results and the results from the 2016 field study, artificial inoculation with *V. dahliae* seemed practical. Soil results post inoculation indicated higher pathogen levels of *V dahliae*, but were highly variable ranging from 0 to 2,329,920 pg/g (Table 25). The non-inoculated check had less disease symptoms and less *V. dahliae* and *C. coccodes* in the stem tissue when compared with the inoculated check. However, these differences did not correlate with total yield as the inoculated check had higher yield. SoilGard had the fewest disease symptoms when compared with the other treatments, but had the second highest *V. dahliae* concentrated in the stem tissue. These results suggest that increasing *V. dahliae* and *C. coccodes* in the soil

and stem tissue does not always correlate with disease symptoms. These results would agree with studies reported by Jansky and Miller (2010) and Frost et al., (2007) that pathogen level does not always correlate with disease symptoms. The non-inoculated check had the highest levels of *C. coccodes* in the soil samples, but the other treatments had low levels of *C. coccodes*. The field soil was uniformly mixed when the experiment began so the large fluctuations in *C. coccodes* numbers are difficult to explain.

The plants did show disease symptoms that are associated with PED, but previous literature states that there are many pathogens involved in PED (Saeed et al., 1997; Johnson and Dung, 2010; Powelson and Rowe, 1993; Johnson and Miliczky, 1993; Rowe and Powelson, 2002). The disease symptoms that were observed could have been associated with different pathogens that were not evaluated in this experiment. Commercial field soil was chosen to simulate a field experiment under greenhouse conditions, but this could have created too many unknown variables. Possibly the field soil and the sphagnum peat moss should have been sterilized prior to being inoculated with *V. dahliae* to prevent the possibility of other pathogens causing disease symptoms to the plants. Using this method, however, would eliminate *C. coccodes* from being associated with disease symptoms. The non-inoculated check and inoculated check were similar in disease symptoms so it suggests that the inoculation with *V. dahliae* was not a major factor in observed symptoms.

Literature has shown organic matter to have an impact on PED in potatoes (Davis et al., 2001), therefore the second objective was to determine how different levels of organic matter impacted the efficacy of biopesticides. In this study, there was no evidence that

organic matter levels had an impact on the efficacy of the biopesticide treatments. Likewise, there was no evidence that higher soil organic matter suppressed disease.

Deficiencies in moisture and fertility levels have been reported to increase levels of PED (Strand, 2006). The sphagnum peat moss that was used in this study was tested for nutrient content to ensure that there weren't any unknown impacts on soil fertility in the amended treatments that could have impacted the results. The soil that was obtained from the commercial field was tested for nutrient analysis and showed high levels of nitrogen. These results indicated that fertility levels shouldn't have aggravated the levels of disease symptoms.

Due to the abnormally cold winter that occurred during this experiment, there were large fluctuations in the temperature levels in the greenhouse. These temperature fluctuations could have impacted the amount of water that was needed daily causing the soil to become saturated for long periods. Under anaerobic conditions, denitrification can occur and could have been the cause of some of the wilting symptoms that were observed (Radersma and Smit, 2011). Potato plants require root respiration for growth so the increasing moisture levels could have impacted the wilting symptoms observed (Strand, 2006). Increasing soil organic matter levels can increase the water holding capacity of soil, which could have caused the high organic matter subplots to retain more water versus the other subplots (Carter et al., 2004; Bot and Benites, 2005). This could have been the reason why the high organic matter subplots had the lowest yield, but the visual symptoms did not correlate with this observation.

Overall, there was no evidence that the biopesticides in these studies effectively control PED or that soil organic matter impacts the efficacy of these products. Future studies are needed to better understand how soil organic matter impacts biopesticides, positively or negatively. Eliminating pathogens from the soil or organic amendment through sterilization would focus on how soil organic matter impacts biopesticides. Also, controlling moisture and temperature levels is important to eliminate these factors from impacting the results. Finally, *in vitro* testing to evaluate how organic matter impacts the movement of these biopesticides is something to consider in future studies.

Biopesticides were not shown to effective in reducing symptoms of the potato early-die complex when compared to the check, but they have shown to be effective in various other studies used to control potato pathogens. There are numerous reasons as presented in this chapter for why biopesticides have been inconsistent in effectiveness, which leads to more research needed to understand these various biopesiticides. With the agricultural industry becoming more sustainable, biopesticides could be important in the future and more studies will determine if we can rely on biopesticides to effectively control various pathogens in potato production.

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Table 3.1: Analysis of variance of the effect of organic matter and biopesticide treatments on relative area under the disease progress curve (RAUDPC), soil and stem DNA concentrations, total yield, and tuber number in Russet Norkotah potatoes grown at Parma, ID during 2016. Significance is indicated by P ≤ 0.05.

	RAUDPC	*V. dahliae* Soil (pg/g)	*C. coccodes* Soil (pg/g)	*V. dahliae* Stem (pg/g)	*C. coccodes* Stem (pg/g)	Total yield (g)	Tuber Number
	P-value	P-value	P-value	P-value	P-value	P-value	P-value
Treatment	0.9307	0.6156	0.5011	0.1391	0.2331	0.0519	0.7776
Subplot	0.6194	0.4653	0.5792	0.2448	0.2684	0.0376	0.7432
Treatment X Subplot	0.0521	0.3313	0.2445	0.4704	0.4477	0.1642	0.6462

Table 3.2: Effect of organic matter and biopesticide treatments on relative area under the disease progress curve (RAUDPC), soil and stem DNA concentrations, total yield, and tuber number in Russet Norkotah potatoes grown at Parma, ID during 2017. Means are of 3 replications.

Treatment		RAUDPC	*V. dahliae* Soil (pg/g)	*C. coccodes* Soil (pg/g)	*V. dahliae* Stem (pg/g)	*C. coccodes* Stem (pg/g)	Total yield (g)		Tuber Number
Non-inoculated check	10	31.2	19	847	962551	34687	173.9		8
Inoculated check	20	32.7	22	8	20246376	78092	184.9		7
Serenade ASO	30	32.1	167	7	8519481	1424951	184.0		7
SoilGard	40	30.0	44	19	20265279	85476	217.4		7
Bio-Tam	50	37.0	0	6	25532371	241882	197.9		6
Regalia	60	31.1	20	6	1247474	183109	200.1		7
LSD		ns	ns	ns	ns	ns	ns		ns
F-Test		0.9307	0.6156	0.5011	0.1391	0.2331	0.0519		0.7776
		0.6194	0.4653	0.5792	0.2448	0.2684	0.0376		0.7432
Subplot									
Non-Amended	1	33.8	105	8	3847994	138976	196.6	ab	7
Low Rate	2	29.6	15	425	6445749	73633	204.1	a	7
High Rate	3	33.6	28	14	28093023	811489	178.5	b	7
LSD		ns	ns	ns	ns	ns	19.886		ns

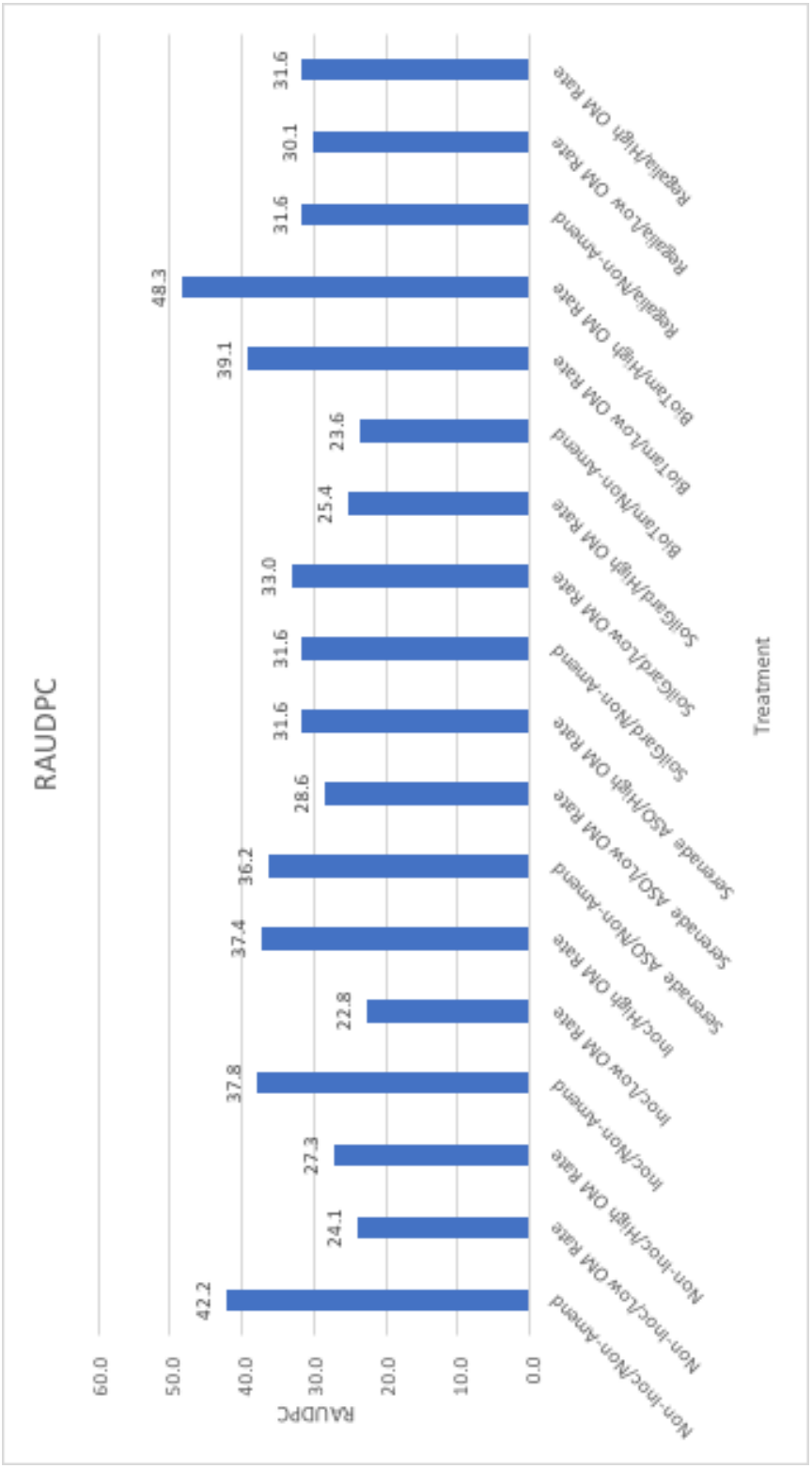


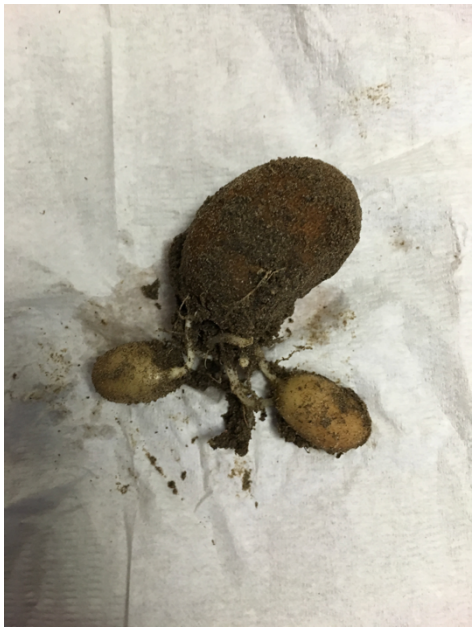
Figure 3.1: Effect of organic matter and biopesticide treatments on the relative area under the disease progress curve (RAUDPC) in Russet Norkotah potatoes grown at Parma, ID during 2016. Means are of 3 replications.

Table 3.3: Effect of amendments with sphagnum peat moss and *V. dahliae* on end concentrations in percentage and pg.

	Organic Matter %
Non-Amended	0.77
Low Rate OM	1.7
High Rate OM	2.2
	***V. dahliae* soil pg/g**
Soil plus inoculum rep #1	44130
Soil plus inoculum rep #2	2329920
Soil plus inoculum rep #3	0
Soil plus inoculum rep #4	50

APPENDIX 1: LITTLE TUBER SYNDROME AND OBSERVED PATHOGENS

Little Tuber Syndrome from Greenhouse Study



Other pathogens observed in Greenhouse Study

APPENDIX 2: 2016 PLANT STAGES

7 June (53 DAP)

22 June (68 DAP)

7 July (83 DAP)

20 July (96 DAP)

APPENDIX 3: 2017 PLANT STAGES

23 June (50 DAP)

7 July (64 DAP)

21 July (78 DAP)

4 August (92 DAP)

APPENDIX 4: VISUAL ASSESSMENT OF DISEASE RATINGS (0-5)

1 = <25% chlorotic leaves **2 = 25-50% chlorotic leaves** **3 = 50-75% chlorotic leaves**

4 = >75% chlorotic leaves **5 = Complete plant death**