

EFFECT OF CULTIVAR AND SUBSTRATE ON EFFICACY OF BIOPESTICIDES TO
SUPPRESS *PYTHIUM* ON GREENHOUSE CROPS

BY

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ABSTRACT**EFFECT OF CULTIVAR AND SUBSTRATE ON THE EFFICACY OF BIOPESTICIDES TO
SUPPRESS *PYHTIUM* ON GREENHOUSE CROPS**

by

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Oomycetes, such as *Pythium*, are soil-borne plant pathogens that can cause significant losses in greenhouse crop production due to their swimming zoospores and wide host range. Additionally, the increasing use of substrates that lack microbial diversity in greenhouse production creates a “biological vacuum” that can reduce the substrate’s capacity to resist microbial invasion by soil-borne diseases. The lack of competition by a natural microbial community and the environmental conditions of greenhouse production creates an ideal situation for the use of biopesticides. Biopesticides are commercial products that use beneficial microorganisms (biocontrol agents) to suppress disease and promote plant health. Greenhouse producers can utilize commercial biopesticides in addition to chemical treatments to protect plants from soil-borne pathogens. One barrier to use of biopesticides is the variability of their performance which can be attributed to differences in environmental conditions, such as plant species and substrate materials. Few studies have evaluated the effect of plant cultivar and current substrates on the efficacy of biopesticides to suppress disease in horticulture crops. The objectives of this research were to 1) develop a greenhouse-based assay to study biopesticide suppression of *Pythium* root rot of greenhouse grown crops, 2) evaluate the effect of plant cultivar on biopesticide efficacy in a tomato system, and 3) evaluate the implications of propagation substrate on biopesticide efficacy in cucumber and calibrachoa systems. For each of

these systems, commercially available biopesticides were applied at the label rate twice during propagation. At transplant, plants were challenged with *Pythium* spp. or a water control. Root rot and root growth were evaluated at 21 days post infection. Findings reveal that the plant cultivars tested did not affect biopesticide efficacy, however a different cultivar panel with greater genetic diversity may affect biopesticide efficacy. There was a significant effect of propagation substrate on disease severity. Plants propagated in coconut coir had higher root disease than those propagated in Oasis[®]. These findings suggest that the chemical and physical properties of these substrates affect plant susceptibility to disease or pathogen activity, however further research is needed to evaluate this observation. Furthermore, there was a significant interaction between the effects of substrate and biopesticide on root rot severity in which biopesticide efficacy varied by substrate. This result suggests that substrate may affect biopesticide performance, but further research is needed to confirm these results and to understand the mechanisms behind this phenomenon. Finally, in all experiments, the commercial biopesticide Rootshield[®] WP suppressed root disease compared to the infested water control. These experiments provided initial data for determining the mechanisms driving variation in biopesticide performance and to improve on-farm performance and adoption.

CHAPTER 1

INTRODUCTION

1.1. Sustainable Agriculture

Sustainable agriculture relies on economically viable practices to meet society's food and textile needs without degrading the environment (Feenstra et al., 2019). Many agricultural practices, such as conservation tillage, cover cropping, precision agriculture, and crop rotation, can aid in creating a sustainable system (Eli et al., 2016). These practices promote soil health, minimize water use, and reduce runoff from excess synthetic chemicals (Feenstra et al., 2019). Implementation of sustainable practices will become increasingly important as agriculture will face new production challenges in the 21st century. With the world population expected to reach 9.6 billion in 2050 (Gerland et al., 2014), global food demand is forecasted to increase 100-110% from 2005 to 2050 (Tilman et al., 2011). And yet, climate change is expected to increase the intensity and frequency of severe environmental events (drought, flooding, high salinity) that act as stressors to many crop systems. The IPCC Report (2014) projects a 2% decline in the yield of the three major crops (wheat, rice, and maize) each decade until 2050, and thereafter the risk of severe impacts on yield increases. Impacts from climate change are already being experienced in places like California, where periods of extreme drought are depleting groundwater reservoirs and placing a strain on crop production which relies heavily on irrigation. This climate shift has led to a direct loss of 1.5 billion dollars to the California agriculture sector (Kerlin, 2014). Furthermore, losses due to insect pests, weeds, and pathogens have been estimated to be between 27-42% for major field crops and these numbers are increasing despite an increase in pesticide

use (Oerke, 2006). This is partially due to overexposure to chemicals with single site modes of action which can drive the development of pesticide-resistant strains, making pest and disease management more difficult, leading to devastating crop loss (Wilson, 1997). It is well documented that synthetic chemicals can be harmful to non-pest species, have adverse effects on human health, and become pollutants in the environment (Ekström and Ekbom, 2011; Pimentel, 2005). Integrated Pest Management (IPM) has developed to address these concerns by providing a systems approach to pest management that minimizes economic, health, and environmental risk. IPM is a science-based decision-making process that incorporates knowledge of pest biology along with cultural, biological, and chemical control strategies for pest and disease control (Ehler, 2006). The concept of pest control through the integration of biological and chemical control was introduced in 1959 by Stern et al. Initially, IPM primarily focused on insect pest management, but in the 1970s, the modern concept of IPM as a tool to manage insect pests, weeds, and pathogens was born (Ehler, 2006). Over the last 30 years, greenhouse growers have adopted IPM as an important tool to decrease pest and disease pressure (van Lenteren, 2000). The controlled environmental conditions and the high economic value of greenhouse-grown crops make greenhouse production an ideal system to incorporate IPM (Paulitz and Belanger, 2001).

1.2. Greenhouse Production of Horticultural Crops

Greenhouse production can range from simple structures used to start seedlings for field production in the spring to complex facilities that provide optimal growing conditions for production of fruits, vegetables, and floriculture crops year-round (Meier et al., 2013). High-tech modern greenhouses utilize automation and computer systems to achieve optimal temperature, airflow, carbon dioxide, light intensity, and photoperiod, allowing growers to optimize plant

growth and yield (Kime, 2016). Furthermore, growers can apply water, chemicals (fertilizers, pesticides), and beneficial insects directly to the plants, decreasing pollution and waste (Sonneveld and Voogt, 2009). In greenhouses, plants can be grown using several types of production systems such as containers/pots, ebb and flow tables, flood floors, substrate-based hydroponics (using slabs of substrate), or solution based hydroponics (nutrient film technique, floating rafts) (Lennard and Leonard, 2006; Stanghellini and Rasmussen, 1994). Plants can be irrigated through open systems, where the irrigation water runs through the substrate and is lost from the system or through closed systems where the water is captured and recirculated back to the crop (Sutton et al., 2006; Zappia et al., 2014).

1.2.1. Root Zone Management in Greenhouse Production

In modern greenhouse production, growers use soilless substrates to reduce the risk of soilborne disease that can plague field soils (Postma, 2004). Soilless culture is defined as growing plants without the use of soil as a rooting medium and where nutrients are supplied to plants via irrigation water (Agung Putra and Yuliando, 2015). For containerized floriculture crops, the primary component of most soilless substrate mixes is peat (Robbins and Evans, 2011a). The type of peat most commonly used is peat moss derived from sphagnum moss, mosses in the genus *sphagnum* (Schmilewski, 2009). Other common organic (containing carbon) substrates used in greenhouse production are coconut (coco) coir, pine-bark, wood fiber, and composted organic waste (Barrett et al., 2016; Drotleff, 2016) while common inorganic (lacking carbon) substrate components are perlite, vermiculite, sand, rockwool, and Oasis[®] foam (Robbins and Evans, 2011a). Many floriculture crops are grown with peat-perlite mixes or Oasis[®] (especially in propagation) while hydroponic vegetable crops are primarily grown in coco coir or rockwool (Robbins and Evans, 2011a). Coco coir is a waste product from the coconut

industry and contains fibers from the mesocarp of the fruit (Abad et al., 2002). Rockwool and Oasis[®] are sterile, synthetic substrates; rockwool is made of spun stone wool while Oasis[®] is made up of hydrophilic foam (Will and Faust, 2005).

While soilless culture has reduced losses due to soilborne plant pathogens, disease outbreaks still have a significant impact, even when conventional fungicides and water treatment technologies are used. Some closed irrigation systems have an increased risk of spreading water-borne plant pathogens (Postma, 2004; Zappia et al., 2014). This risk is a major reason for grower hesitation to adopt water recycling systems, particularly in high risk crops such as Cyclamen, which is highly susceptible to Fusarium wilt (Hong et al., 2001). There are certain plant pathogens that are well adapted to hydroponic and soilless systems and have become problematic in greenhouse production. Water-borne pathogens, such as *Pythium* and *Phytophthora*, have swimming spores, called zoospores, that can actively swim toward and infect roots (Postma et al., 2000; Stanghellini and Rasmussen, 1994). These pathogens survive in the irrigation water and infection is favored by the high water retention capacity of soilless substrates and other favorable environmental conditions of the greenhouse (Stanghellini and Rasmussen, 1994).

1.3. *Pythium* in Greenhouse Production

Pythium is a genus with over 200 species that can live in terrestrial and aquatic habitats world-wide (Moorman and May, 2019). Several species of soilborne plant pathogens are in the genus *Pythium* and are classified as Oomycetes, commonly known as water molds. Symptoms associated with *Pythium* infection are wilting, stunted growth, cankers on stems, root discoloration, and even plant death. *Pythium* spp. can cause crown and root rot by infecting through the root tip (Sabaratnam, 2016). On an infected root, some *Pythium* species, like *P. ultimum*, will produce hyphal swellings while other species will form a sporangium that will

release hundreds of swimming zoospores (Fry and Niklaus, 2010; Moorman and May, 2019) (Figure 1-1). The zoospores use chemotaxis, or directed movement toward exudates produced by plant roots, allowing them to find their host (Paulitz 1997). Once the zoospore reaches the root, it encysts, germinates, and colonizes the root tissue by producing hyphae (Sabaratnam, 2016) (Figure 1-1). Some *Pythium* species are heterothallic and require opposite mating types to reproduce sexually, however most species are homothallic and do not require an opposite mating type (Moorman and May, 2019). Sexual reproduction occurs with the production of the female gametangia (oogonium) and the male gametangia (antheridium) (Fry and Niklaus, 2010) (Figure 1-1). Once the oogonium is fertilized, it develops into a thick-walled oospore (Fry and Niklaus, 2010) (Figure 1-2). These oospores can become dormant and survive for many years in the soil, irrigation water, or plant debris (Hendrix and Campbell, 1973).

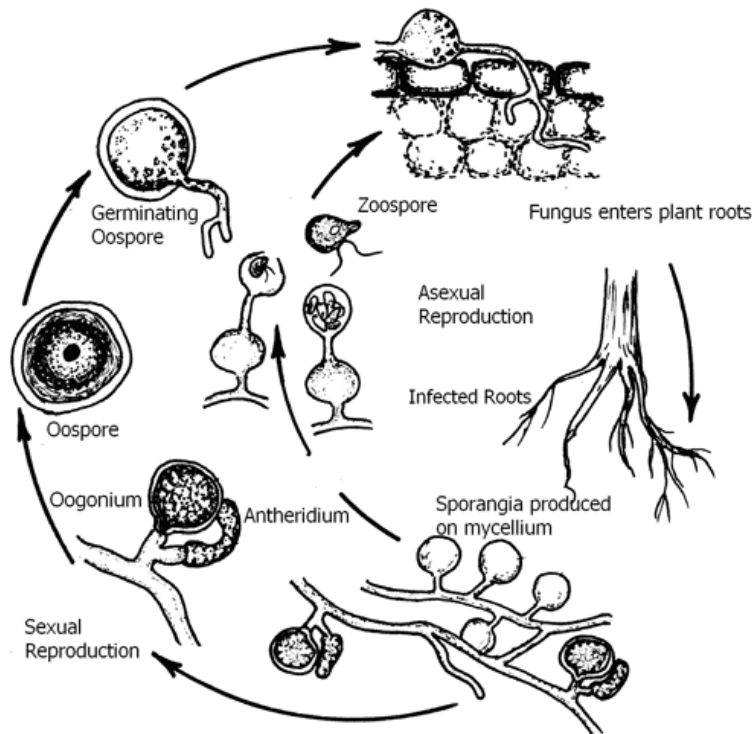


Figure 1-1. The life cycle of *Pythium* spp. (Sabaratnam, 2016).

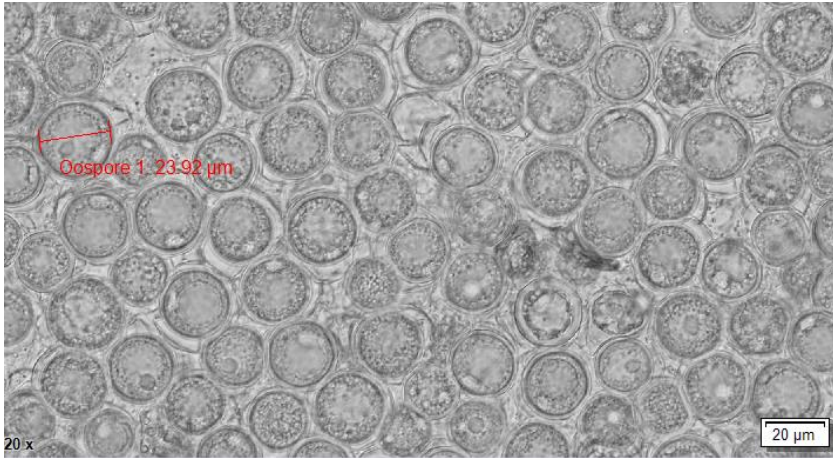


Figure 1-2. *Pythium* oospores under a compound microscope (Olympus Model CX43RF). Photo taken by Liza DeGenring using Microscope Digital Camera: Olympus LC30 (Olympus Soft Imaging Solutions, Munster, Germany).

Pythium has a world-wide distribution and a wide host range meaning that almost all greenhouse crops are susceptible (Moorman et al., 2002). The most common *Pythium* species found in greenhouses are *P. aphanidermatum* (Edson) Fitzp., *P. irregulare* Buisman, *P. ultimum* Trow, and *P. dissotocum* Drechsler (Del Castillo Múnera and Hausbeck, 2016; Howard et al., 1994; Moorman and Daughtrey, 2002). Even though soilless substrates are semi-sterile, pathogens can be introduced into the substrate where they persist and cause disease in the presence of a susceptible host. *Pythium* can be introduced into the greenhouse on workers' shoes, tools, equipment, through infected plant plugs, contaminated substrate, or irrigation water (Jarvis, 1992; Moorman et al., 2002). *Pythium* can also be spread by the movement of fungus gnats (*Bradysia spp.*) and shoreflies (*Scatella stagnalis*) (Moorman et al., 2002). *Pythium* species are ubiquitous in aquatic environments (Moorman and May, 2019), meaning that most greenhouses have *Pythium* in their water, however root rot primarily becomes a problem with poor water and root zone management. Managing *Pythium* root rot relies on sanitation and cultural controls, water treatment, chemical fungicides, and biological control. Unfortunately, the number of effective fungicides registered for growers is narrow and development of fungicide

resistance has rendered some fungicides ineffective (Del Castillo Múnera and Hausbeck, 2016; Moorman et al., 2002). Proper root zone management is key to managing *Pythium*. Overwatering of plants and stressors, such as extreme temperature, low dissolved oxygen, and high salts in the root zone, will dramatically increase the likelihood of infection (Martin and Loper, 1999).

Soilless substrates used in greenhouse production tend to have low microbial diversity and reduced capacity to resist an invasion by *Pythium* (Paulitz, 1997; Paulitz and Bélanger, 2001). Thus, if *Pythium* is introduced into a soilless substrate cropping system an epidemic can occur (Hendrix and Campbell, 1973; Paulitz, 1997; Stanghellini and Rasmussen, 1994). Under field soil circumstances *Pythium* is a poor competitor (Hendrix and Campbell, 1973; Rankin and Paulitz, 1994) but with the lack of microbial diversity of some soilless substrates and the high water content that favors the movement of zoospores, the pathogen can spread rapidly in a greenhouse (Howard et al., 1994; Paulitz and Belanger, 2001). Fortunately however, some of the conditions unique to greenhouses that favor disease, also provide ideal conditions for management with beneficial biocontrol microbes as part of an IPM strategy (Paulitz, 1997). In most modern greenhouses, variables such as temperature, soil moisture, and relative humidity can be tightly controlled to favor establishment of biological control agents. This offers an advantage over field production in which unfavorable conditions are considered to be a reason for control failure and/or inconsistency performance of biological controls (Paulitz and Belanger, 2001).

1.4. Biological Control

Biological control (or biocontrol) is defined as the “use of living organisms to suppress the population density or impact of another organism” that is pathogenic and damaging to the plant host (Eilenberg et al., 2001). Biocontrol can be used to suppress insect pests, noxious

weeds, and plant pathogens (Ehler, 2006). Biocontrol research is an established field of research encompassing the disciplines of ecology, entomology, weed science, soil ecology, and plant pathology. In plant pathology, the term is used to describe the use of microbes for suppression of plant diseases and weeds (Glare et al., 2012).

Microorganisms can suppress the activity of plant pathogens through one or more modes of action. Specifically, direct or indirect antagonism of the pathogen leads to suppression of the plant pathogen activity and disease symptoms (Baker, 1986; Whipps, 2001). Direct antagonism occurs when the biocontrol agent produces antibiotics that kill (or interfere with) the pathogen or through parasitism and predation of the pathogen (Table 1-1) (Belanger et al., 2012; Pal and McSpadden Gardener, 2006). Microorganisms can also be indirectly antagonistic to pathogens through competition for nutrients and space (Table 1-1) (Lugtenberg and Kamilova, 2009) and activation of induced systemic resistance (ISR) in the plant host (Kloepper et al., 2004; van Loon et al., 1998). ISR occurs when the plant's defense mechanisms are triggered by the beneficial microorganism, allowing the plant to be protected from a future attack (Compant et al., 2005; Pieterse et al., 2014). Biocontrol can occur in the phyllosphere (the aboveground portion of the plant) (Bulgarelli et al., 2013) or in the rhizosphere (the belowground portion of the plant). Much of the biocontrol research reported in the literature has focused on biocontrol in the rhizosphere (Chaparro et al., 2014; Kamilova et al., 2005; Mendes et al., 2013; Philippot et al., 2013; Whipps, 2001). Researchers have studied single microbial species/isolates that are suppressing disease in nature to understand the mechanisms of biocontrol (Stiling and Cornelissen, 2005). Some of the most well studied species are in the Genus *Bacillus*, *Pseudomonas*, *Trichoderma*, and *Streptomyces* (Paulitz and Belanger, 2001).

Table 1-1. Mechanisms of biocontrol (based on table from Pal and McSpadden Gardener, 2006 and enhanced with reviews from Lugtenberg and Kamilova, 2009 and Whipps, 2001).

Type of Antagonism	Mechanism of biocontrol	Example
Direct	Antibiotic	2,4-diacetylphloroglucinol Phenazines Volatiles
	Parasitism/Predation	Production of extracellular cell wall-degrading enzymes (chitinase or β -1,3 glucanase)
Indirect	Competition	Nutrients (exudates from roots) Niche space Iron through production of siderophores
	Induction systemic resistance	Detection of pathogen-associated, molecule patterns, such as flagella, salicylic acid, and siderophores
	Interference with pathogens	Inactivation of pathogen germination factors present in exudates Depredation of pathogenicity factors of pathogens (toxins) Signal interference

1.5. Biopesticides

Several biocontrol agents have been commercialized and sold as biopesticides for use in agriculture (Glare et al., 2012; Harman, 2000). These commercial biopesticides utilize modes of action of beneficial microorganisms that are unique from modes of action employed by chemical fungicides to suppress disease. Biopesticides are commercial products formulated with beneficial microorganisms (biocontrol agents) or microbial metabolites to suppress disease and promote plant health. Microorganisms and natural compounds are vigorously screened through in vitro, growth chamber, greenhouse, and field trials to determine their potential for commercialization (Fravel, 2005). The biopesticide market is the fastest growing segment of the crop protection market, with a market increase of over 200% from 2007 to 2012 (Alexander, 2014). Greenhouse production offers a unique niche for the use of biopesticide products (Paulitz and Belanger, 2001). The use of soilless substrates decreases biopesticide competition with indigenous

microbial communities found in field soils, and the controlled environmental conditions of the greenhouse (temperature and moisture) creates an ideal situation for the use of biopesticides (Paulitz, 1997). It is well established that biopesticides perform best when applied early in the crop production cycle when disease pressure is low to moderate, or before the pathogen has been introduced (Fravel, 2005; Harman, 2000). An ideal time to apply biopesticides is in propagation. This allows growers to give the plant an initial microbial boost and use less product as plants are grown in a small volume of substrate. Some chemical fungicides cannot be applied in propagation as they will harm the plants. As a result, some growers are cautious to apply any type of disease control product in propagation due to the perceived risk of phytotoxicity (Poleatewich, personal observation). Most all biopesticides however, are safe to use in propagation and even work best when applied at this early stage. The greatest benefit can be achieved by using biopesticides in rotation with synthetic fungicides. Because biopesticides suppress disease using modes of action that are different from chemical fungicides, the likelihood that a pathogen population will develop resistance is reduced (Xu et al., 2011). Biopesticides tend to be more expensive compared to chemical fungicides which is why some growers hesitate to use them. Biopesticides offer the most value when used as a rotational product to prolong the life of the few synthetic products available. Furthermore, crops produced in greenhouses have high economic value and therefore growers can better afford the cost of the biopesticides (Paulitz and Belanger, 2001).

Currently, there are 40 registered biopesticide products that are available to greenhouse growers in the United States (Lindberg and Arthurs, 2017). Most products are formulated with single fungal or bacterial agents. Some products are based on plant extracts or microbial metabolites (Belanger et al., 2012; Pal and McSpadden Gardener, 2006; Whipps, 2001). A few

examples of commercial biopesticides products are Rootshield[®], Cease[®], and Regalia[®] (Table 1-2).

Table 1-2. Commercial biopesticide products, their active ingredients (beneficial microorganism or natural compound), and the mode of action utilized to suppress disease.

Product	Active Ingredient	Mechanisms of Biocontrol	References
Cease [®]	<i>Bacillus subtilis</i>	Antibiotic production Production of volatiles Production of defense-related enzymes Competition ISR	Ongena and Jacques, 2008 Ryu et al., 2003 Chowdappa et al., 2013 Shafi et al., 2017 Kloepper et al., 2004
Rootshield [®] WP	<i>Trichoderma harzianum</i>	Production of defense-related enzymes ISR Predation/Parasitism Competition	Chowdappa et al., 2013 Martínez-Medina et al., 2013 Harman et al., 2004 Harman, 2000
Regalia [®]	Extract of <i>Reynoutria sachalinensis</i>	ISR	Daayf et al., 1997 Fofana et al., 2002

Barriers to widespread adoption of biopesticides include high cost of production, short shelf-life of the biocontrol agent, and variability in performance (Fravel, 2005). A significant challenge with biopesticides is an inability of researchers to get replicable results while examining biopesticide efficacy (Rankin and Paulitz, 1994) and variability with their on-farm performance (Fravel, 2005). Many researchers have suggested that this variability is due to the strong influence of the environment on the biocontrol agent's colonization, production of antibiotic compounds, and/or plant response. Specifically, inconsistencies have been attributed to environmental variables such as temperature, moisture, substrate, plant cultivar, and the interaction of the biocontrol agent, the pathogen, and the plant (Larkin and Fravel, 2002). Recently, several researchers have begun combining culture-based methods with molecular tools to study the effects of plant cultivar and substrate on rhizosphere microbial community composition.

1.6. Biocontrol in the Rhizosphere

Plants release compounds, such as sugars, amino acids, vitamins, and enzymes, from their roots that are known as exudates (Garbeva et al., 2004). In addition, root caps excrete polysaccharide mucilage and their border cells can slough off into the rhizosphere (Dennis et al., 2010). These excretions and exudates can be collectively referred to as rhizodeposits and can account for ~11% of the plant's net photosynthetically fixed carbon and 10-16% of total plant nitrogen (Bulgarelli et al., 2013). These nutrient rich rhizodeposits attract microorganisms and create a unique environment for each plant in the zone around the roots known as the rhizosphere (Philippot et al., 2013). Microbial inhabitants of the rhizosphere can include bacteria, fungi, oomycetes, nematodes, and protozoa (Mendes et al., 2013). These microorganisms play important roles in soil structure, decomposition of organic matter, toxin removal, and the cycling of nutrients (van Elsas and Trevors, 1997). It is thought that specific consortia of beneficial microbes are responsible for naturally suppressing plant disease, promoting plant resilience to stress, and increasing plant growth (Berendsen et al., 2012; Bonfante and Anca, 2009; Mendes et al., 2013, 2011; Nallanchakravarthula et al., 2014; Schlaeppli and Bulgarelli, 2015).

Microorganisms in the rhizosphere act as the first line of defense against soil-borne pathogens by inhibiting the growth or activity of plant pathogens through multiple antagonistic properties (Table 1-1) (Cook et al., 1995). The addition of beneficial microorganisms through the application of commercialized biopesticides can greatly reduce the risk of soil-borne diseases. However, research is greatly needed to understand how these biocontrol agents work under varying conditions in order to improve on-farm performance. Because each farm is unique, a one-size fits all approach is not ideal for biologically based management strategies. Research on

the effect of biotic and abiotic variables will help researchers develop best practices, and to support wider grower adoption.

1.7. Effect of plant cultivar and substrate on the microbial community

It is well documented that plant cultivar and substrate can affect microbial community composition (who is there) and function (what they are doing) (Berendsen et al., 2012; Berg and Smalla, 2009; Philippot et al., 2013). Some research suggests that plant species are important drivers of microbial community regardless of the type of soil that they have been grown in (Berg et al., 2006; Garbeva et al., 2008). The exudates released by plants vary between species (Garbeva et al., 2004), as well as between cultivars (Bakker et al., 2012), and thus harbor a unique rhizosphere microbial community (Bulgarelli et al., 2015; Peiffer et al., 2013). Peiffer et al. (2013) observed significant differences in the rhizosphere bacterial community composition across a collection of 27 modern maize cultivars. Bulgarelli et al. (2015) found similar results in barley, in which genotype accounted for 5.7% of the variance in microbial community. However, other studies suggest that substrate is more important to microbial community composition compared to plant species/genotype (Latour et al., 1996; Lundberg et al., 2012; Nallanchakravarthula et al., 2014). A substrate's texture and structure, organic matter, pH, and nutrients play a role in determining the type of microbial community present (Garbeva et al. 2004). Weinert et al. (2011) analyzed the rhizosphere bacterial communities of three potato cultivars grown at two field sites using PhyloChip technology and discovered that 40% of the operational taxonomic units were site specific, while only 4% of the operational taxonomic units were cultivar specific. Another study by Nallanchakravarthula et al. (2014) used high-throughput pyrosequencing to determine that soil type had a stronger effect on root inhabiting fungal community than strawberry cultivar. While these studies highlight influences of plant genotype

(cultivar) and soil type (substrate), it is also thought that these two factors interact and influence the rhizosphere microbial community and are usually interconnected. Soil type can influence which microorganisms are present in the soil and thus effect differences in cultivar accumulation of beneficial species in the root zone (Meyer et al., 2010). Bulgarelli et al. (2015) and Reinhold-Hurek et al. (2015) theorized that soil type has a stronger effect in the bulk soil while the effect of plant genotype increases with increasing proximity to the roots in the rhizosphere. These documented effects of plant cultivar and soil type on microbial community composition and function suggest that these same variables play an important role in the establishment of microbial biopesticides in the rhizosphere and their ability to suppress disease.

1.8. Effect of plant cultivar and substrate on biopesticides

While research shows that plant cultivar and substrate affect the native microbial community, little is known about how cultivar and substrate affect the efficacy of introduced biopesticides. Smith et al. (1999) reported differences in the growth of the biocontrol agent *Bacillus cereus* on tomato seed and its ability to suppress *Pythium torulosum* on recombinant inbred lines of tomato. Meyer et al. (2010) observed that a *Pseudomonas fluorescens* isolate was more effective at suppressing *Pythium* root rot on one wheat cultivar over the other cultivars tested. Some research suggests that differences in the efficacy of biopesticides could be related to the degree of resistance of each cultivar (King and Parke, 1993; Xue et al., 2014). Xue et al. (2014) found that biofungicide CLO-1, *Clonostachys rosea* strain ACM941, was more effective at suppressing Fusarium head blight on moderately resistant wheat cultivars. King and Parke (1993) also found that efficacy of the biocontrol *Pseudomonas cepacia* was related to the degree of susceptibility of the pea cultivars. However, Smith et al. (1997) found that differences in resistance to the pathogen and the efficacy of the biopesticides between in-bred tomato lines

were not correlated. Furthermore, Larkin and Fravel (2002) did not see an effect of cultivar on the efficacy of the biocontrol agents to control *Fusarium* wilt, even with tomato cultivars ranging in resistance to the pathogen.

Similarly, only a few studies have examined the effect of substrate on introduced biocontrol agents. Larkin and Fravel (2002) evaluated the efficacy of three non-pathogenic *Fusarium* species as biocontrol agents to control *Fusarium* wilt of tomato under different environmental conditions (temperature, light, soil type, pathogen isolate and race, and tomato cultivar). They observed that only one biocontrol agent was able to effectively suppress disease in all four soil types (Larkin and Fravel, 2002). Similarly, Krause et al. (2007) saw a significant effect of substrate on the efficacy of two biocontrol agents, *Chryseobacterium gleum* (C₂₉₉R₂) and *Trichoderma hamatum* 382, to reduce *Rhizoctonia* damping-off of radish and *Rhizoctonia* crown and root rot of Poinsettia. Composted pine bark mix consistently supported high populations of both the biocontrol agents and the compost's indigenous microbial community, and resulted in suppression of *Rhizoctonia* (Krause et al., 2001). Boehm and Hoitink (1992) found that *Pythium* root disease on poinsettia was correlated with the amount of decomposition of pest substrates where the least decomposed treatments (H₂ peat or composted pine bark amended mix) had the most microbial activity and the least amount of root disease. Several studies have revealed that the type of food (carbon) source found in the substrate influences the production of cell-wall degrading enzymes, such as β -glucanase and chitinase, that are essential for antagonism of fungal pathogens (de la Cruz et al., 1993; Windisch et al., 2017). Thus, while high energy reserves (cellulose) provide a food source for introduced biocontrol agents (Hoitink and Boehm, 1999), this type of food will repress the production of cell-wall degrading enzymes

(de la Cruz et al., 1993). Little research has been done evaluating newer substrates, such as coir and Oasis[®], and how they may affect biopesticide efficacy and disease suppression.

1.9. Research Objectives

The overall goal of this research was to better understand how variables like plant cultivar and substrate could affect the efficacy of commercial biopesticides to suppress soil-borne diseases, such as *Pythium*, in greenhouse crop production. Specific objectives were to;

- I.** Develop a greenhouse-based assay to study biopesticide suppression of *Pythium* root rot of greenhouse grown crops
- II.** Evaluate the effect of cultivar on efficacy of biopesticides to suppress root disease
- III.** Evaluate the effect of growing substrate on *Pythium* disease severity and the efficacy of biopesticides

A greenhouse-based assay was used to test the hypothesis that plant cultivar and substrate will differentially influence the ability of microbial biopesticides to suppress *Pythium* root rot. Outcomes of this research will provide preliminary insights on the effect of cultivar and commonly used substrates on *Pythium* root rot severity and biopesticide efficacy. The information gained from this research will highlight the ‘unknowns’ of this research area and what questions remain to be answered. The long-term goal of this research is to determine mechanisms driving variation in biopesticide performance and to improve on-farm performance and adoption. Increased utilization of biopesticides will decrease farmers’ dependence on synthetic pesticides and enhance the environmental sustainability of their production systems.

CHAPTER 2

DEVELOPMENT OF A GREENHOUSE-BASED ASSAY TO STUDY BIOPESTICIDE SUPPRESSION OF *PYTHIUM* ROOT ROT OF GREENHOUSE GROWN CROPS

2.1. Introduction

Oomycete pathogens, such as *Pythium*, are soil-borne diseases that can cause significant losses in greenhouse crop production due to their swimming zoospores and wide host range (Postma et al., 2000; Stanghellini and Rasmussen, 1994). This pathogen can survive in the irrigation water and infection is favored by the high water retention capacity of soilless substrates and other favorable environmental conditions of the greenhouse (Stanghellini and Rasmussen, 1994). Furthermore, the number of effective fungicides registered for use in greenhouses is narrow and development of fungicide resistance has rendered some fungicides ineffective (Del Castillo Múnera and Hausbeck, 2016; Moorman et al., 2002). Multiple *Pythium* isolates have already become resistant to mefenoxam, an isomer of the widely used fungicide metalaxyl (Moorman et al., 2002). Soilless substrates used in greenhouse production tend to have low microbial diversity and reduced capacity to resist an invasion by *Pythium* (Paulitz, 1997; Paulitz and Belanger, 2001). Thus, if *Pythium* is introduced into a soilless substrate cropping system an epidemic can occur with few effective fungicide treatments as options for management (Hendrix and Campbell, 1973; Paulitz, 1997; Stanghellini and Rasmussen, 1994). Fortunately however, some of the conditions unique to greenhouses that favor disease, also provide ideal conditions for management with beneficial microorganisms (Paulitz, 1997). In most modern greenhouses, variables such as temperature, moisture, and relative humidity can be tightly controlled to favor

establishment of biological control agents. Furthermore, crops produced in greenhouses have high economic value and therefore growers can afford the cost of the biopesticides (Paulitz and Belanger, 2001).

Researchers have extensively screened potential beneficial microorganisms for their antagonism and suppression of *Pythium* (Borrero et al., 2005; Gravel et al., 2005; Khalil and Alsanusi, 2010). Much of this research has been conducted in vitro or in growth chambers due to the lower cost and ability to screen larger numbers of candidate isolates in a short period of time. However, these studies are often poor predictors of efficacy (Köhl et al., 2011) and persistence of the biocontrol isolate (Fravel, 2005) in a production system. Essentially, these studies do not assess the ecological competence of candidate isolates and their ability to survive in varying environmental conditions. As a result, many isolates fail to meet the requirements for commercial use. Furthermore, some research has shown that there may not be a correlation between antagonism under in vitro conditions and in-planta (Knudsen et al., 1997). For example, Milus and Rothrock (1996) reported that bacteria showing the highest inhibition under in vitro testing, did not control *Pythium* root rot of wheat in the field. While in vitro and growth chamber studies are necessary to screen potential beneficial microorganisms, greenhouse trials are also important to determine the efficacy of these products in pre-commercial settings. Successful use of beneficial microorganisms depends on their efficacy to suppress disease in a production setting (Cook and Baker, 1983).

Environmental conditions, such as temperature, humidity, substrate, and cultivar, are known to effect microbial communities and could impact the suppressive activity of biocontrol microorganisms (Berg and Smalla, 2009; Fravel, 2005; Garbeva et al., 2004). Thus, greenhouse trials are necessary to understand how these conditions could impact efficacy and lead to

variability with performance. Larkin and Fravel (2002) reported that biocontrol agents' efficacy in controlling Fusarium wilt of tomato was varied under different temperatures, light, soil types, and cultivars. In modern research greenhouse systems, these environmental conditions can be tightly controlled to replicate 'commercial production' settings. Furthermore, there are many different types of production systems used in greenhouses, such as flood floors and ebb and flow tables, that could not be replicated in an in vitro or growth study trial. Thus, the use of a greenhouse-based assay is important to effectively evaluate the efficacy of biocontrol agents against realistic levels of *Pythium* disease pressure.

Several inoculation methods have been used to evaluate control methods against *Pythium* root rot (Calvo-Bado et al., 2006; Gravel et al., 2006; Lebreton et al., 2018; Rose et al., 2004). Inoculation of plants with *Pythium* is most commonly performed through a drench application of a spore suspension (Calvo-Bado et al., 2006; Gravel et al., 2006; Hausbeck and Glaspie, 2008). A variation of the drench method is to dip small plants in a spore suspension prior to transplanting (Vakalounakis, 1996), however dips require large quantities of *Pythium* inoculum which may be unrealistic for large scale experiments. Substrate-based inoculum methods have also been reported in the literature. For example a potato soil inoculum (Ko and Hora, 1971) is prepared by growing *Pythium* in sterilized loamy soil mixed with small pieces of sterilized potatoes. The mixture is dried, and the infested granules are added to the soil. Other substrate-based inoculum include oat grain (Ivors, 2015) and maize (Jayaraj et al., 2005).

The objective of this study was to develop and validate a greenhouse-based assay for evaluating suppression of *Pythium* root rot of greenhouse grown crops. Specific objectives were to (1) compare two *Pythium* species and three inoculation methods on tomato and cucumber and (2) evaluate seven tomato cultivars for susceptibility to *Pythium* spp. Through assessing the

susceptibility of these seven tomato cultivars, we will be able to create a cultivar panel with varying levels of susceptibility. This cultivar panel will then be used for evaluating the effect of cultivar on biopesticide efficacy to suppress *Pythium* root rot in greenhouse grown tomatoes (Chapter 3). The greenhouse-based assay developed in this research will be applied to future trials examining the effect of cultivar and substrate on biopesticide efficacy to suppress *Pythium* in a greenhouse system (Chapter 3 and 4).

2.2. Materials and Methods

2.2.1. Preparation of plant materials

Seven tomato cultivars consisting of heirlooms, hybrids, scions, indeterminate, and determinate plants were evaluated for their susceptibility to *Pythium* root rot. Tomatoes (*Solanum lycopersicum* L.) were seeded into rockwool plugs (22 mm x 27 mm, Cultilene, A.M.A. Plastics, Kingsville, Ontario). Plugs were pre-moistened with clear water before seeding and placed in trays (27.94 cm x 54.28 cm, To Plastics Inc, Clearwater, MN). The seeds were covered with vermiculite which is standard practice in tomato greenhouse production (McCullagh et al., 1996) (Figure 2-1). The trays were placed on benches equipped with under-bench heating in a propagation room at the University of New Hampshire's MacFarlane Greenhouses in Durham, NH.



Figure 2-1. Tomato plants germinating in rockwool plugs covered with vermiculite.

The plugs were overhead misted with clear water until germination, then fertilized with $100 \text{ mg}\cdot\text{L}^{-1}$ N of 17-4-17 NPK commercial water-soluble fertilizer by hand (Jack's Pure Water LX, JR Peters Inc, Allentown, PA). Temperatures in the propagation house were set to 24°C during the day and 23°C at night. Following the propagation period, seedlings were transported to a production greenhouse and transplanted into rockwool blocks (100 mm x 100 mm x 65 mm, Cultilene, A.M.A. Plastics, Kingsville, Ontario) at different times for experiment 1 and 2. Vermiculite was used to fill the space between the plug and the block to ensure a tight fit (Figure 2-2). During propagation and production, plants were exposed to a 16-hour photoperiod using 400-watt High Pressure Sodium (HPS) lights (PL Light Systems Inc., Beamsville, Ontario). The plants were fertilized through stackable 4-way driplines (Netafim Irrigation Inc, Fresno, CA) with $150 \text{ mg}\cdot\text{L}^{-1}$ N of 17-4-17 NPK commercial water-soluble fertilizer (Jack's Pure Water LX, JR Peters Inc, Allentown, PA). Plants were watered at 36.5 mL per minute 2-3 times per day depending on plant growth.

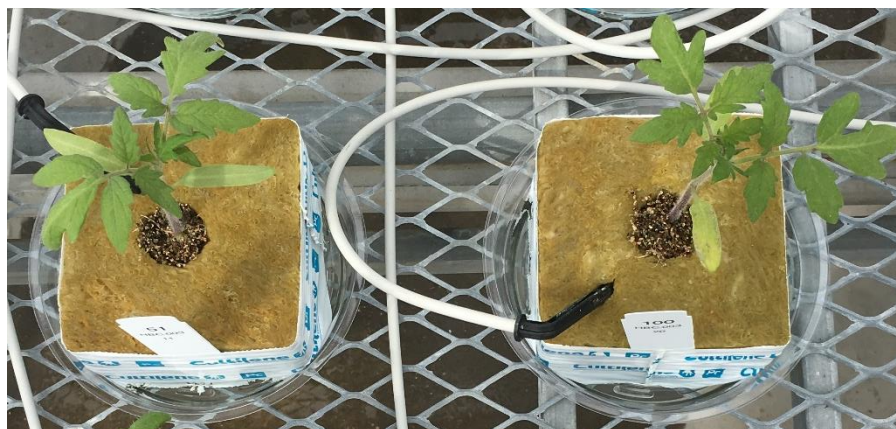


Figure 2-2. Tomato plants transplanted into rockwool blocks placed on saucers 14 days post seeding.

Plants were treated weekly with preventative applications of the *Steinernema feltiae* system (150,000-200,000 nematodes per plant) (BioBest, Westerlo, Belgium) to control fungus gnats. Swirskii-Breeding-System sachets (*Amblyseius swirskii*) (BioBest, Westerlo, Belgium)

containing predatory mites were placed on each plant to control whiteflies and thrips. Yellow sticky cards (BASF Corporation, Research Triangle Park, NC) were placed in the greenhouse, three per bench at plant level, to monitor pest populations.

2.2.2. *Source and preparation of pathogen isolates*

Two *Pythium* isolates, *Pythium aphanidermatum* (Edson) Fitzp. isolate KOP8 and *Pythium ultimum* isolate (Trow) NDT1-1, were used for these experiments. These pathogenic *Pythium* species were chosen as they are commonly found causing disease in greenhouse crops (Moorman et al., 2002; Moorman and Daughtrey, 2002). *P. aphanidermatum* readily produces swimming spores (zoospores) in high moisture substrates and is favored by high temperature (optimum temperatures of 35-40°C) while *P. ultimum* does not ordinarily produce zoospores and is favored by cool temperatures (optimum temperatures of 25-30°C) (Moorman and Daughtrey, 2002). Isolate KOP8 was isolated from wheatgrass seeds by Dr. M. Daughtrey, at Cornell University. The isolate was received at UNH in June 2017. Isolate NDT1-1 was isolated from cucumber plants infested with an isolate obtained from the University of New Hampshire Plant Diagnostic Lab in November 2017. The *Pythium* isolates were maintained for long-term storage as mycelial plugs in a sterile water storage as described by Dr. G. Moorman (<https://plantpath.psu.edu/pythium/module-2/cleaning-and-storing-isolates>). To prepare for storage, the isolates were grown on 1.5% water agar for 7 days. The colonized agar was cut into a grid using a sterile scalpel and 5-10 cubes were suspended in 10 mL of sterile tap water in a sterilized 15 mL capped test tube. The isolates were stored in the test tubes at room temperature.

To prepare the spore suspension inoculum, *Pythium* isolates were revived from storage by transferring colonized water agar cubes to 20% V8 (200 ml of clarified V8 vegetable juice, 15 g agar, and 2-3 g of CaCO₃ per liter of reverse osmosis (RO) water) media plates (100 mm x 15

mm, Fisher Scientific, Hampton, NH). After 4-7 days of growth, propagules were harvested in a laminar flow hood by flooding the plates with 20 mL of sterile RO water. A sterilized FisherBrand cell spreader (Fisher Scientific, Hampton, NH) was used to rub the top of the media to dislodge mycelia and propagules. The supernatant was drained from the petri dish and placed into a sterile beaker. The supernatant was then filtered using 3 layers of sterile cheesecloth to remove the mycelia. The number of propagules (oospores, zoospores) in the cell suspension were enumerated using a Hemocytometer (Hausser Scientific, Horsham, PA) under a compound microscope (Olympus Model CX43RF). The suspension was adjusted to 1×10^5 propagules/mL.

A potato soil inoculum (PSI) was prepared for each *Pythium* isolate as described by Ko and Hora with a few modifications (Ko and Hora, 1971). Five hundred mL of loamy soil was placed into a 1 L flask, followed by 50 g of peeled and finely chopped organic Yukon Gold potatoes (~0.5 cm cubes), and enough water to make the soil fairly wet but not muddy. The flask was closed with a cotton plug, covered with aluminum foil, and autoclaved at 121 °C, 15 psi for 1 hour on each of 2 consecutive days. The potato soil was then infested with 3 water agar disks (#9 cork borer) of a *Pythium* isolate taken from the colony edge. The *Pythium* grew for 1 week at room temperature, and the flask was gently shaken once during the middle of the week to distribute the colonized potato pieces throughout the soil (Figure 2-3). Once fully colonized, the potato soil inoculum was air-dried on paper towels in a laminar flow cabinet. The dried inoculum was sieved with 1- and 2-mm sieves and the 1-2 mm fraction was saved to be used as inoculum.



Figure 2-3. *Pythium* isolate colonizing potato soil in a 1L flask.

2.2.3. Experiment 1: Evaluation of tomato cultivars for susceptibility to *Pythium* root rot

An experiment was designed to screen tomato cultivars for susceptibility to *Pythium* root rot and to determine their potential use in a future cultivar panel. This experiment consisted of a 7 x 2 factorial with seven tomato cultivars and two disease treatments (infested with isolate NDT1-1 and a non-infested water control). Treatments were arranged on a greenhouse bench in a randomized complete block design with five blocks containing one replicate plant per treatment (five total replicate plants per treatment). Tomato cv. Komeett (De Ruiters Seeds, Oxnard, CA), cv. Rutgers (Burpee, Warminster, PA), cv. Ailsa Craig (Annie's Heirloom Seeds, Hudsonville, MI), cv. Trust (De Ruiters Seeds, Oxnard, CA), cv. Glamour (Stokes Seeds, Buffalo, NY), cv. Bonny Best (Stokes Seeds, Buffalo, NY), and cv. Wisconsin (Siskiyou Seeds, Williams, OR) were included in this cultivar panel. Komeett, Rutgers, Trust, and Wisconsin 55 have indeterminate growth habit, while Glamour and Ailsa Craig are determinate.

Approximately 21 days post seeding, the tomato seedlings were transplanted into rockwool blocks (100 mm x 100 mm x 65 mm, Cultilene, A.M.A. Plastics, Kingsville, Ontario) (Figure 2-2). After ten days, the blocks were placed in 1-gallon pots (Nursery Supplies, Griffin

Greenhouse Supplies, Tewksbury, MA) filled with a blend of 1:1 mix of coconut coir pith and medium size ($\frac{1}{2}$ " to $\frac{3}{4}$ ") coconut coir chips (Millenniumsoils Coir™ A Division of Vgrove Inc., Ontario). Using a 25 mL serological pipette, 30 mL of *P. ultimum* isolate NDT1 spore suspension was pipetted on the rockwool block, completing covering the top surface area, eleven days post-transplant. Thirty plants received the *Pythium* drench while 30 control plants received an equal volume of tap water. Disease assessments and root growth were measured through destructive sampling 21 days post-infestation.

2.2.4. Experiment 2: Comparison of *Pythium* inoculation methods in a greenhouse system

A greenhouse experiment was conducted to compare *Pythium* inoculation methods and identify a protocol that provided consistent development of root disease on tomato and cucumber. The tomato cv. Glamour and the cucumber (*Cucumis sativus L.*) cv. Straight eight (Burpee, Warminster, PA) were used in this assay. Cucumber plants were grown following the protocols described in section 2.2.1. This experiment consisted of a 2 x 3 x 3 factorial with two plant species (tomato and cucumber), three disease treatments (infested with isolate KOP8, infested with isolate NDT1-1, and a non-infested water control), and three inoculation methods (drench, wound-drench, and potato soil inoculum (PSI)). Treatments were arranged in a randomized complete block design with seven blocks containing one replicate per treatment (seven total replicate plants per treatment).

Approximately 14 days post seeding, the tomato and cucumber seedlings were transplanted into rockwool blocks (100 mm x 100 mm x 65 mm, Cultilene, A.M.A. Plastics, Kingsville, Ontario) (Figure 2-2). The blocks were pre-moistened and placed on 15.24 cm (6 inch) saucers (Curtis Wagner Plastics, Houston, TX). At transplant, plants were infested with one of the treatments. For the drench method, plugs were transplanted into the blocks and using a 25

mL serological pipette, 20 mL spore suspension of each *Pythium* isolate or water control was pipetted onto the rockwool block, completely covering the top surface area. For the wound and drench method, sanitized pruners were used to prune (wound) exposed roots on the outside of the rockwool plug, and the pruned plugs were placed into the blocks on saucers (Figure 2-4). Then, the root pruned plants were inoculated with isolates KOP8 and NDT1-1 by the drench method. For the PSI method (prepared as described above) plants were inoculated with 0.5 g/pot of KOP8 and NDT1-1 PSI. The PSI granules were placed under the rockwool plug immediately prior to transplantation.



Figure 2-4. Pruning exposed tomato roots on the outside of the rockwool plug prior to a drench inoculation with *Pythium* isolates.

2.2.5. Data Collection

At 21 days post infection, root rot disease severity was evaluated by rating each plant for percent diseased roots on a scale of 0-5 (0 = no root rot, 5 = roots completely rotted) for experiment 1, and percentage root rot (0% = no root rot, 100% = roots completely rotted) for experiment 2. Severity of root rot was evaluated by cutting the block in half and observing roots

inside and outside of the block. Roots that were browning with a cortex that could easily slough off were deemed 'high' root rot. Roots were also evaluated for growth where each plant was given a rating based on how much the roots had colonized the rockwool block (0 = no roots in the block, 5 = the block was fully colonized with roots) and a percentage rating was used for the cucumber trial in experiment 2 (0% = no roots in the blocks, 100% = the block was fully colonized). To confirm that symptomatic plants were infected with *Pythium*, roots were sampled from three plants per treatment using sterile forceps and stored in 15 mL falcon tubes at 4°C until processed. In a laminar hood, the root samples were surface washed by placing in sterile RO water in a glass petri dish. Four 1-cm root sections from the same plant were plated on the Oomycete semi-selective media PARP V8 (see Appendix A for recipe). The presence of *Pythium* growing from root segments was confirmed through examination of hyphae and sexual and/or asexual spores under a compound microscope (Olympus Model CX43RF). For experiment 2 (inoculation methods), plant height was collected by measuring each plant from the crown to the top leaf using a ruler and recorded in centimeters (cm). Aboveground biomass was also collected for experiment 2 by cutting all replicate plants at the crown, placing the aboveground biomass in an oven for 72 hours at 68°C, and weighing to 0.01 grams. Environmental data were collected using Argus Control Software Firmware Version 12.43 Build 00063 (Argus Control Systems Ltd., Surrey, BC).

2.2.6. *Statistical Analysis*

For experiment 1, disease severity and root growth data were analyzed for statistical significance using a One-Way Analysis of Variance (ANOVA) in JMP Pro 14 (SAS Institute, Cary, NC). The model statement was constructed to determine the effect of the independent variable (cultivar) on the dependent variables (disease severity and root growth) with block as

the random variable. For experiment 2, disease severity, root growth, height, and aboveground biomass data were analyzed for statistical significance using Two-Way ANOVA in JMP Pro 14 (SAS Institute, Cary, NC) for each plant species separately. The model statement was constructed to determine the effect of the independent variables (*Pythium* isolate and inoculation technique) and an interaction between the two on the dependent variables with block as the random variable. Statistical significance was assessed at $\alpha = 0.05$ and a Tukey Honest Significant Difference (HSD) Post-hoc test was used to separate the means.

2.3. Results

2.3.1. Experiment 1: Evaluation of tomato cultivars for susceptibility to *Pythium* root rot

A greenhouse experiment evaluated the susceptibility of seven tomato cultivars to *P. ultimum*. Susceptibility was assessed by measurement of root rot severity and root growth. The greenhouse compartment average day temperature was 22.3 °C (max: 26.6 °C; min: 10.2 °C) and average day relative humidity was 31.7% (max: 60.7%; min: 21.0%). The average night temperature was 20.1 °C (max: 26.1 °C; min: 8.1 °C) and average night relative humidity was 28.0% (max: 61.4%; min: 17.6%).

There was a significant interaction between the effects of cultivar and *P. ultimum* on root rot severity ($p = 0.0045$) (Figure 2-5). Trust had the highest mean root rot severity score (2.99 out of 5) and was significantly greater than Rutgers (1.4), Glamour (0.40), and Bonny Best (0.27) ($p < 0.0001$). In fact, the infested Bonny Best and Glamour plants had the same root disease severity as their respective non-infested controls. All the non-infested plants exhibited a low level of root rot with a mean severity of <1 except for Wisconsin 55 which had a mean rating of 1.03 and was not significantly different than any of the non-infested or infested plants ($p = 1.000$).

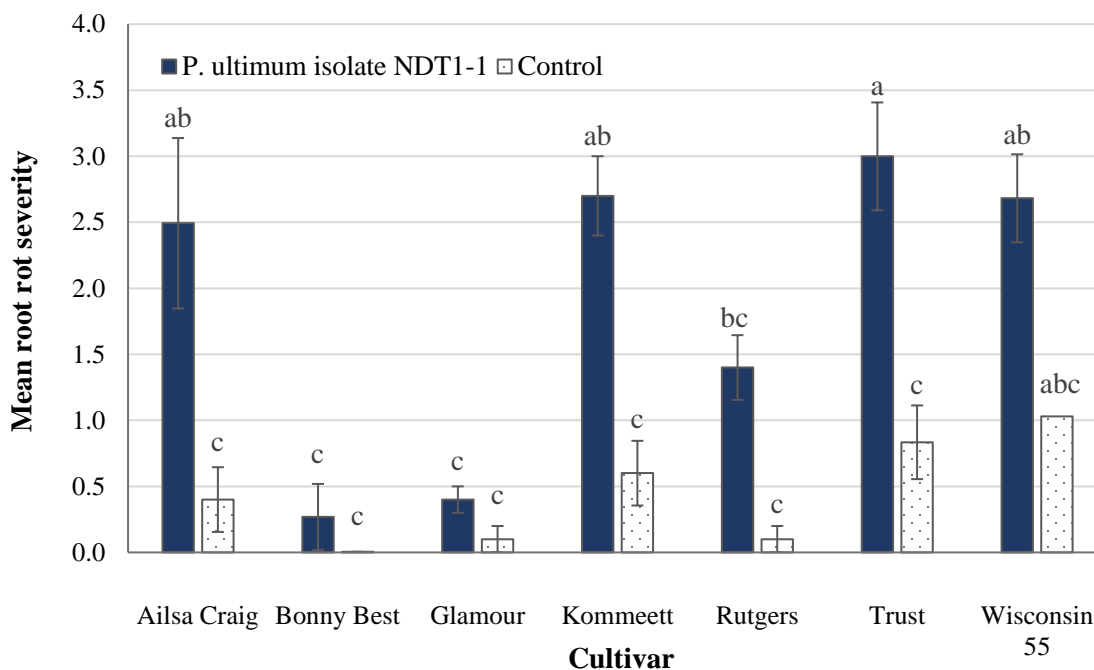


Figure 2-5. Mean root rot severity (0-5 scale) of tomato cultivars 21 days post inoculation with *P. ultimum* isolate NDT1-1 or control. Error bars represent the standard error from the mean (n=5). Means with the same letter are not significantly different ($\alpha = 0.05$) as determined by the Tukey HSD Post-hoc test.

The interaction between the effects of cultivar and *Pythium* on root growth was not significant ($p = 0.9970$). For the non-infested plants, there was a significant effect of cultivar on root growth ($p = 0.0002$). Glamour (5.00), Wisconsin 55 (5.00), and Bonny Best (4.83) had the greatest root growth, while Trust (2.67) and Ailsa Craig (2.00) had the least root growth (see Appendix B for data, Table A-1).

2.3.2. Experiment 2: Comparison of *Pythium* inoculation methods in a greenhouse system

An experiment was conducted in which a rockwool-based growing system was used to evaluate the most consistent and effective *Pythium* isolate and inoculation method. Greenhouse compartment average day temperature was 25.0 °C (max: 38.3 °C; min: 14.5 °C) and average day relative humidity was 48.6% (max: 83.5%; min: 20.5%). The average night temperature was 18.0 °C (max: 29.9 °C; min: 8.7 °C) and average night relative humidity was 69.4% (max: 92.6%; min: 26.8%).

For tomato, there was a significant interaction between the effects of isolate and inoculation method on root disease ($p = 0.0010$). All plants infested with *Pythium* exhibited greater mean root rot compared to the non-infested water control plants (root rot severity < 5%) (Figure 2-6). For isolate KOP8 drench and wound/drench inoculation methods resulted in significantly greater root rot, with the plants infested by wound and drench having 50% greater mean root disease compared to plants exposed to the other inoculation treatments ($p = 0.0010$) (Figure 2-6). The KOP8 PSI had the lowest root disease (24%) and each NDT1-1 inoculation had comparable results to the other NDT1-1 treatments (did not differ significantly).

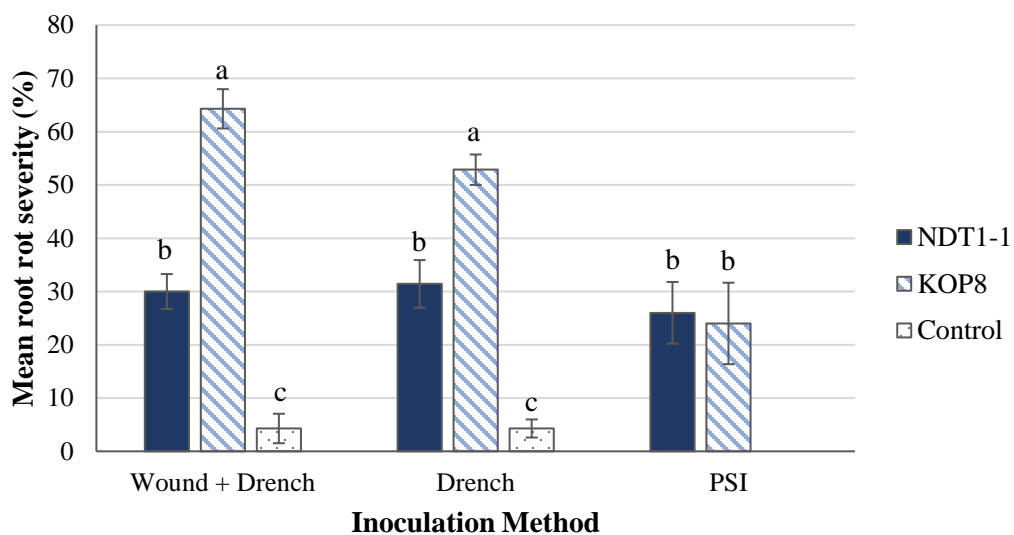


Figure 2-6. Mean percent root rot severity of tomato cv. Glamour, 21 days post inoculation with three *Pythium* treatments (NDT1-1, KOP8, and a water control) and three inoculation methods (wound and drench, drench, and potato soil inoculum (PSI)). Error bars represent the standard error from the mean ($n=7$). Means with the same letter are not significantly different ($\alpha = 0.05$) as determined by the Tukey HSD Post-hoc test.

There was a significant interaction between the effects of isolate and inoculation method on tomato root growth ($p = 0.0063$) (see Appendix B for data, Table A-2). The non-infested control plants had the greatest mean root growth with a rating of 4.2. The plants infested with KOP8 PSI had mean root growth of 3.1 and were not significantly different than the non-infested

control plants ($p = 0.0772$). Root growth was comparable between the other treatments. Plants inoculated with the KOP8 wound and dip method had the lowest root growth with a rating of 1.7. There was no effect of *Pythium* treatment ($p = 0.1071$) or inoculation method ($p = 0.7914$) on plant height. The *Pythium* isolate treatments had a significant effect on aboveground dry biomass weight ($p = 0.0100$). The control plants had the highest mean dry weight (4.54 g) while the plants inoculated with KOP8 had the lowest mean dry weight (2.57 g) ($p = 0.0040$). Plants inoculated with NDT1-1 had a mean dry weight of 3.58 g that differed significantly from both the control plants ($p = 0.0002$) and the plants inoculated with KOP8 ($p = 0.0100$). Inoculation method did not have an effect on the aboveground dry biomass of the tomato plants ($p = 0.9600$).

For cucumber, there was a significant interaction between the effects of isolate and inoculation method on root disease ($p < 0.0001$) (Figure 2-7). The non-infested control plants had low root disease (< 5%) while the plants infested with either *Pythium* isolate had moderate to high root disease (> 65%) ($p < 0.0001$). An exception to this was seen on plants infested with the isolate KOP8 PSI, which had a low mean root disease of 6%. Since this was also observed in the tomato trial, this inoculum was deemed not viable. After further investigation, the KOP8 PSI was found to be contaminated with bacteria which is known to reduce isolate pathogenicity. Therefore, plants treated with KOP8 PSI were considered controls. Apart from KOP8 PSI, there was no significant difference between isolates KOP8 and NDT1-1 and inoculation type combinations, however plants infested with KOP8 by the wound and drench method had the greatest mean root rot of 77% ($p = 0.7937$).

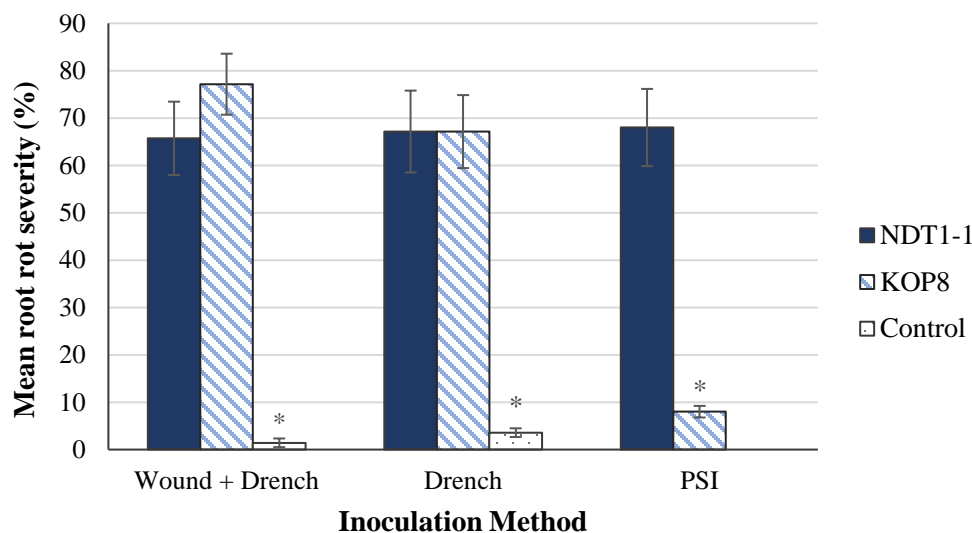


Figure 2-7. Mean percent root rot severity of cucumber cv. Straight eight, 21 days post inoculation with three *Pythium* treatments (NDT1-1, KOP8, and a water control) and three inoculation methods (wound and drench, drench, and potato soil inoculum (PSI)). Error bars represent the standard error from the mean (n=7). Means with an asterisk are significantly different than those without ($\alpha = 0.05$) as determined by the Tukey HSD Post-hoc test.

For cucumber root growth, the control plants (and those inoculated with the KOP8 PSI) had the greatest root growth (>77%) while plants infested with *Pythium* had significantly lower root growth (< 35%) ($p < 0.0001$) as expected (see Appendix B for data, Table A-3). Although not significant, cucumber plants infested with NDT1-1 PSI had the greatest root growth (32%) while the plants infested with KOP8 through the wound and drench method had the lowest root growth (17%) ($p = 0.2517$). This same trend was observed for plant height and aboveground dry biomass. Plants that received a control wound and water drench had the greatest plant height (30.6 cm) and weight (2.8 g) whereas the plants that received a KOP8 wound and drench inoculation were significantly shorter (19.9 cm) and weighed less (0.8 g) ($p = 0.0069$, $p = 0.0009$ respectively).

2.4. Discussion

During the cultivar susceptibility trials, the inoculated roots of cultivars Bonny Best, Glamour, and Rutgers exhibited low levels of root rot and were not significantly different from

their respective water controls. This suggests that these cultivars are more tolerant to *P. ultimum*. Since there was no interaction between the effects of cultivar and *P. ultimum* on root growth, this would suggest that root growth may contribute to the cultivar's susceptibility to root rot severity. We hypothesize that a more vigorous root system, like that seen in cv. Glamour and cv. Bonny Best, allows the plant to resist or tolerate root pathogens, while a less vigorous root system, like that seen in cv. Ailsa Craig and cv. Trust, is more susceptible to root pathogens. Ailsa Craig, Trust, and Glamour will be used for a future cultivar panel, utilizing a combination of cultivars that are susceptible and more resistant to *Pythium* to determine the effect of cultivar on efficacy of biopesticides to suppress root disease (Chapter 3).

Root disease severity was higher in tomato cv. Glamour infested with isolate KOP8 compared to isolate NDT1-1. This difference could be due to the difference in *Pythium* species (KOP8 is *P. aphanidermatum* and NDT1-1 is *P. ultimum*). *P. aphanidermatum* has been shown to be more aggressive on tomato plants, especially during warmer temperatures (Calvo-Bado et al., 2006; Sutton et al., 2006). Furthermore, on tomato, the wound-drench and drench treatments lead to more severe and consistent root rot compared to the PSI treatment. This could be due to inconsistencies with the PSI method, where the *Pythium* propagules may clump on the potato pieces and not evenly colonize the soil (personal communication with Postma, 2019). Future trials will examine the efficacy of oat soil inoculum and a pond water method for preparing a zoospore suspension. On cucumber, both *Pythium* isolates caused similar root disease severity and all the inoculation methods except for the KOP8 PSI were successful in initiating disease. It was determined after these trials that the KOP8 PSI was contaminated with bacteria which could have prevented the pathogen from properly infecting the plants. When the KOP8 isolate was free of contamination, a new batch of KOP8 PSI was effective at causing root rot. For future work,

tomato plants will be inoculated with isolate KOP8 using the wound and drench method (Chapter 3) and cucumber plants will be inoculated with the KOP8 PSI (Chapter 4).

CHAPTER 3

EFFECT OF CULTIVAR ON EFFICACY OF BIOPESTICIDES TO SUPPRESS ROOT DISEASE

3.1. Introduction

The development and integration of alternative disease management practices are crucial to creating a sustainable, productive food system. Synthetic chemicals can be harmful to non-pest species, have adverse effects on human health, and can become pollutants in the environment, contaminating water sources and negatively effecting wildlife (Pimentel, 2005). Furthermore, overexposure to synthetic chemicals drives the development of pesticide-resistant pathogen strains, making plant disease management more difficult (Wilson, 1997). An effective solution is the use of Integrated Pest Management (IPM) which combines cultural, biological, and chemical practices for pest and disease control (Ehler, 2006). A key strategy of IPM is to harness beneficial microbes (biopesticides) and their metabolites that can promote plant growth and suppress disease. Barriers to commercial use of biopesticides are high cost of production, short shelf-life, and the variability of their performance which can be influenced by environmental factors, including plant species and soil type (Fravel, 2005). Understanding how these environmental variables influence the efficacy of biopesticides will lead to improved performance, development of best practices, and support wider adoption by growers.

Plants influence the community of microorganisms that colonize their roots and surrounding soil through the excretion of exudates (Philippot et al., 2013). Exudates are compounds, such as sugars, amino acids, vitamins, and enzymes, that attract microorganisms and create a unique environment around the roots, known as a rhizosphere (Garbeva et al., 2004).

Plant-associated microorganisms play important roles in enhancing photosynthesis, nutrient uptake, and resistance to abiotic and biotic stress (van Elsas and Trevors, 1997). Additionally, these microbes can increase plant growth, suppress soilborne plant pathogens, and promote overall plant health in an agroecosystem (Berendsen et al., 2012; Bonfante and Anca, 2009; Mendes et al., 2013, 2011; Nallanchakravarthula et al., 2014; Schlaeppi and Bulgarelli, 2015). Research has focused on understanding how these benefits of plant-associated microbes change with different environmental conditions, such as plant species and soil type.

Plant species are important drivers of microbial community composition (who is there) and function (what they are doing) (Berg and Smalla, 2009; Smith and Goodman, 1999), regardless of the type of soil that they have grown in (Berg et al., 2006; Garbeva et al., 2008). Studies have found that specific plant species (Garbeva et al., 2004) and even plant cultivar harbor unique rhizosphere microbial communities (Bulgarelli et al., 2015; Weinert et al., 2011). Weinert et al. (2011) found that a significant portion of the microbial community in the potato rhizosphere was cultivar specific, and Andreote et al. (2010) found that bacterial community composition was mainly driven by potato cultivar. In another study, significant differences in the rhizosphere bacterial community composition were observed across a collection of 27 modern maize cultivars (Peiffer et al. 2013). Bulgarelli et al. (2015) found similar results in barley, in which genotype accounted for 5.7% of the variance in microbial community. Haney et al. (2015) observed that wild *Arabidopsis* accessions differed in their ability to support beneficial *Pseudomonas fluorescens* colonization of their root system. These differences were shown to affect plant health as accessions that were able to support *P. fluorescens* resulted in less disease when plants were challenged with the soilborne pathogen *Fusarium oxysporum* (Haney et al., 2015). Similar results were observed by Mazzola et al. (2004), in which specific strains of *P.*

fluorescens were preferentially increased in the rhizosphere of certain wheat cultivars, leading to improved disease control. In another study, enhanced apple seedling growth was observed when planted into fields previously cropped with wheat cultivars that were able to support *P. fluorescens*, leading to suppression of disease (Gu and Mazzola, 2003). These studies hint at the importance of linking specific plant genotypes with specific biocontrol agent genotypes, but little research has investigated this phenomenon and its implications for biocontrol. While research shows that cultivar affects the native microbial community composition and function, little is known about how cultivar affects establishment (and subsequent efficacy) of biocontrol agents applied to a system as a commercial biopesticide.

Several beneficial microorganisms and natural compounds that promote plant health and suppress disease have been commercialized and sold as disease control products called biopesticides. While researchers have investigated the effects of production variables on beneficial microbial strains, little is known about how strains in commercialized biocontrol products are affected by variables such as plant cultivar. Meyer et al. (2010) observed that a *P. fluorescens* isolate was more effective at suppressing *Pythium* root rot on one wheat cultivar compared to the other cultivars tested. Smith et al. (1999) saw differences in the growth of *Bacillus cereus* and its ability to suppress *Pythium torulosum* on inbred lines of tomato. Multiple studies have found that biopesticides are more effective on cultivars with higher resistance to disease than those that are more susceptible (King and Parke, 1993; Xue et al., 2014). Researchers theorize that disease development is slower on cultivars with a higher level of resistance, allowing for the biopesticide to be more effective (Xue et al., 2014). In contrast, Smith et al. (1997) found that differences in resistance to the pathogen and the efficacy of the biopesticides between in-bred tomato lines were not correlated. It is unknown how genetically

diverse cultivars with similar susceptibility to disease could affect efficacy of biopesticides. Much of the research is primarily done with strains of beneficial microorganisms that are not commercialized and therefore are not formulated with other ingredients, preservatives and food sources designed to increase the survival and stability of the microbial agent. Research is needed to determine if formulated biocontrol strains are affected by production variables (such as cultivar) in the same manner as non-formulated strains.

The objective of this research was to evaluate the effect of plant cultivar on microbial biopesticide efficacy. In this study, the *Pythium*-tomato pathosystem was used to test the hypothesis that tomato cultivars differentially influence the ability of commercially available microbial biopesticides to suppress *Pythium* root rot.

3.2. Materials and Methods

3.2.1 Experimental design

Two replicate experiments were conducted that consisted of a 4 x 3 factorial with four tomato cultivars (Glamour, Ailsa Craig, Trust, Maxifort) and three biopesticide treatments (Cease[®], Rootshield[®] WP, water control) (Table 3-1). Treatments were arranged in a randomized complete block design with five blocks containing four replicate plants per block (20 replicate plants total). In each treatment, half of the plants were infested with *Pythium* and half remained non-infested to observe any effects of the biopesticide on plant health and growth. This experiment was conducted in the summer of 2018 (6/27-7/18) and replicated in the fall of 2018 (10/18-11/8) at the University of New Hampshire's MacFarlane Greenhouses in Durham, NH.

3.2.2. Preparation of plant material

To evaluate the effect of cultivar on biopesticide efficacy, four tomato (*Solanum lycopersicum* L.) cultivars were selected. Tomato cv. Glamour (Stokes Seeds, Buffalo, NY) and

cv. Ailsa Craig (Annie's Heirloom Seeds, Hudsonville, MI) are determinate cultivars while cv. Trust (De Ruiter Seeds, Oxnard, CA) is an indeterminate scion and cv. Maxifort (De Ruiter Seeds, Oxnard, CA) is an indeterminate hybrid rootstock. Cultivars Trust and Maxifort are greenhouse cultivars. Maxifort is one of the most popular rootstock varieties used in hydroponic greenhouse production (Poleatewich, personal communication). Each tomato cultivar was seeded into rockwool plugs (22 mm x 27 mm, Cultilene, A.M.A. Plastics, Kingsville, Ontario). Plugs were pre-moistened with clear water before seeding and placed in trays (27.94 cm x 54.28 cm, To Plastics Inc, Clearwater, MN). The seeds were covered with vermiculite which is standard practice in tomato greenhouse production (McCullagh et al., 1996) (Figure 2-1). The trays were placed on benches equipped with under-bench heating in a propagation room at MacFarlane Greenhouse. The plugs were overhead misted with clear water until germination, then fertilized with $100 \text{ mg}\cdot\text{L}^{-1} \text{ N}$ of 17-4-17 NPK commercial water-soluble fertilizer by hand (Jack's Pure Water LX, JR Peters Inc, Allentown, PA). Temperatures in the propagation house were set to 24°C during the day and 23°C at night. Approximately 14 days post seeding, the tomato seedlings were transported to a production greenhouse and transplanted into rockwool blocks (100 mm x 100 mm x 65 mm, Cultilene, A.M.A. Plastics, Kingsville, Ontario) (Figure 2-2). The blocks were pre-moistened and placed on 15.24 cm (6 inch) saucers (Curtis Wagner Plastics, Houston, TX). Vermiculite was used to fill the space between the plug and the block to ensure a tight fit. During propagation and production, plants were exposed to a 16-hour photoperiod using 400-watt High Pressure Sodium (HPS) lights (PL Light Systems Inc., Beamsville, Ontario). The plants were fertilized through stackable 4-way driplines (Netafim Irrigation Inc, Fresno, CA) with $150 \text{ mg}\cdot\text{L}^{-1} \text{ N}$ of 17-4-17 NPK commercial water-soluble fertilizer (Jack's Pure Water LX,

JR Peters Inc, Allentown, PA). Plants were watered at 36.5 mL per minute 2-3 times per day depending on plant growth.

Plants were treated weekly with preventative applications of the *Steinernema feltiae* system (150,000-200,000 nematodes per plant) (BioBest, Westerlo, Belgium) to control fungus gnats. Swirskii-Breeding-System sachets (*Amblyseius swirskii*) (BioBest, Westerlo, Belgium) containing predatory mites were placed on each plant to control whiteflies and thrips. Yellow sticky cards (BASF Corporation, Research Triangle Park, NC) were placed in the greenhouse, three per bench at plant level, to monitor pest populations.

3.2.3. Biopesticide treatments

Two commercial biopesticides (representing a fungal and bacterial active ingredient) were used in this experiment to evaluate the effect of cultivar on their efficacy against *Pythium* root rot (Table 3-1). The biopesticide treatments were applied twice as a drench at the label rate (Table 3-1). Applications were made at 8 and 16 days post seeding as a 5 mL and 40 mL drench respectively. The water controls received an equal volume of water.

Table 3-1. Biopesticide products used, active ingredients, and the rate applied. Rates were based on the manufacturer recommendation.

Product	Active Ingredient	Guaranteed CFU/g	Rate
Cease®	<i>Bacillus subtilis</i> QST-713	1.0 x 10 ⁹	15 mL/L
Rootshield® WP	<i>Trichoderma harzianum</i> KRL-AG2	1.0 x 10 ⁷	0.4 g/L

3.2.4. Source and preparation of pathogen isolates

Pythium aphanidermatum (Edson) Fitzp. isolate KOP8 was used for these experiments (based on results discussed in Chapter 2). Isolate KOP8 was isolated from wheatgrass seeds by Dr. M. Daughtrey, at Cornell University. The isolate was received at UNH in June 2017. The KOP8 isolate was maintained for long-term storage as mycelial plugs in sterile water storage as

described by Dr. G. Moorman (<https://plantpath.psu.edu/pythium/module-2/cleaning-and-storing-isolates>). To prepare for storage, the isolate was grown on 1.5% water agar for 7 days. The colonized agar was cut into a grid using a sterile scalpel and 5-10 cubes were suspended in 10 mL of sterile tap water in a sterilized 15 mL capped test tube. The isolates were stored in the test tubes at room temperature.

To prepare spore suspension inoculum, *Pythium* isolate KOP8 was revived from storage by transferring colonized water agar cubes to 20% V8 (200 mL of clarified V8 vegetable juice, 15 g agar, and 2-3 g of CaCO₃ per liter of RO water) media plates (100 mm x 15 mm, Fisher Scientific, Hampton, NH). After 4-7 days of growth, propagules were harvested in a laminar flow hood by flooding the plates with 20 mL of sterile RO water. A sterilized FisherBrand cell spreader (Fisher Scientific, Hampton, NH) was used to rub the top of the media to dislodge mycelia and propagules. The supernatant was drained from the petri dish and placed into a sterile beaker. The supernatant was then filtered using 3 layers of sterile cheesecloth to remove the mycelia. The number of propagules (oospores, zoospores) in the cell suspension were enumerated using a Hemocytometer (Hausser Scientific, Horsham, PA) under a compound microscope (Olympus Model CX43RF). The suspension was adjusted to 1×10^5 propagules/mL (Figure 3-1).

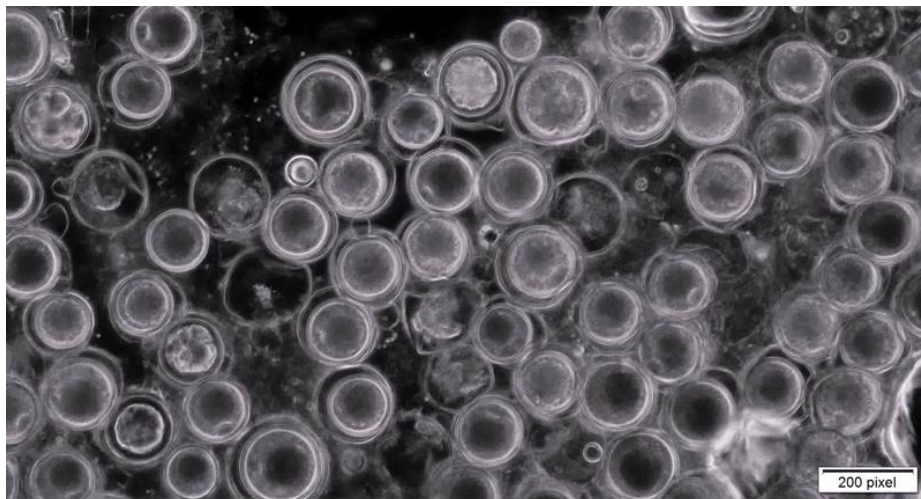


Figure 3-1. *P. aphanidermatum* isolate KOP8 oospores with a release of zoospores in upper left-hand corner. This was an undiluted spore suspension under a compound microscope (Olympus Model CX43RF). Photo taken by Liza DeGenring using Microscope Digital Camera: Olympus LC30 (Olympus Soft Imaging Solutions, Munster, Germany).

Tomato plants were infested with *P. aphanidermatum* isolate KOP8 just prior to transplanting into the rockwool blocks (14 days post seeding). A wound and drench method was utilized to infect the tomato plants. Sanitized pruners were used to prune (wound) exposed roots on the outside of the rockwool plug (Figure 2-4) and the pruned plugs were placed into the blocks on the saucers. Using a 25 mL serological pipette, 20 mL of *P. aphanidermatum* isolate KOP8 spore suspension was pipetted onto the rockwool block, completely covering the top surface area. Non-infested control plants received an equal volume of water.

3.2.5. Data collection

At 19 days post infestation, chlorophyll content of the leaves was measured to determine if the application of biopesticides affected photosynthesis and leaf greenness. Three measurements per plant were collected and averaged using a Soil-Plant Analyses Development (SPAD) unit of Minolta Camera Co. SPAD 502 Plus Chlorophyll Meter (Item 2900PDL, Spectrum Technologies, Aurora, IL) (Monje and Bugbee, 1992). Then, plant biomass, from five plants per treatment, was measured by harvesting the aboveground portion of each plant. The

aboveground portion was harvested by cutting the plant at the stem base and placing the material in paper bags. The bags were dried in a drying oven at 65°C for 48 hours to remove moisture. The aboveground biomasses were weighed to the nearest 0.01 grams.

At 20 days post infestation, root rot disease severity was evaluated by rating five plants per treatment for percent diseased roots on a scale of 0-100%. Severity of root rot was evaluated by cutting the block in half and observing roots inside and outside of the block. Roots that were brown to tan in color with a cortex that could easily slough off were deemed 'high' root rot (Figure 3-2). Roots were also evaluated for growth where each plant was given a rating based on root how much the roots had colonized the rockwool block (0 = no roots in the block, 5 = the block was fully colonized with roots). To confirm that symptomatic plants were infected with *Pythium*, roots were sampled from three plants in each treatment using sterile forceps and stored in 15 mL falcon tubes at 4°C until ready to be processed. In a laminar hood, the root samples were surface washed by placing in sterile RO water in a glass petri dish. Four 1-cm root sections from the same plant were plated on Oomycete semi-selective media PARP V8 (see Appendix A for recipe). The presence of *Pythium* growing from root segments was confirmed through examination of hyphae and sexual and/or asexual spores under a compound microscope (Olympus Model CX43RF). Greenhouse environmental data were collected using Argus Control Software Firmware Version 12.43 Build 000663 (Argus Control Systems Ltd., Surrey, BC).



Figure 3-2. *P. aphanidermatum* infected tomato root showing brown to tan discoloration and the cortex sloughing off (see arrow). The root rot rating for this plant was 50%.

Disease severity, root growth, SPAD readings, and dry biomass data, were analyzed for statistical significance using a Two-Way Analysis of Variance (ANOVA) in JMP Pro 14 (SAS Institute, Cary, NC). The model statement was constructed to determine the effect of the independent variables (cultivar and biopesticide) and the interaction between these variables on the dependent variables (disease severity, growth, SPAD, and biomass) with block as the random variable. All data were analyzed using an ANOVA for significant difference between non-infested and infested plants. Then, the data from non-infested and infested plants were analyzed separately. Statistical significance was assessed at $\alpha = 0.05$ and a Tukey Honest Significant Difference (HSD) Post-hoc test was used to separate the means.

3.3. Results

3.3.1. Greenhouse Environment

Temperature and humidity were notably different between the summer and fall replicate experiments (Table 3-2), as day temperatures tended to be higher in the summer compared to the fall.

Table 3-2. Greenhouse compartment environmental data for the summer 2018 (6/27-7/18) and fall 2018 (10/18-11/8) replicate experiments. Data were collected using Argus Control Software Firmware Version 12.43 Build 000663 (Argus Control Systems Ltd., Surrey, BC).

Experiment	Average Day Temperature (°C) (min-max)	Average Night Temperature (°C) (min-max)	Average Day Relative Humidity (%) (min-max)	Average Night Relative Humidity (%) (min-max)
Summer	30.1 (17.5-40.9)	22.2 (14.7-34.6)	54.6 (25.6-97.0)	75.5 (36.5-96.8)
Fall	23.6 (16.4-26.7)	22.2 (19.8-23.3)	43.9 (17.8-79.9)	44.2 (25.7-72.8)

3.3.2. Effect of cultivar on efficacy of biopesticide to suppress root disease

There was a significant difference between the two replicate experiments for root rot severity on infested plants ($p < 0.0001$), in which the summer 2018 experiment had 50% greater root rot severity than the fall 2018 experiment. This difference could be due to the high temperatures and humidity during the summer experiment (Table 3-2). These conditions may have caused plant stress and favored infection of *P. aphanidermatum* which is considered a “heat-loving” *Pythium*. The disease pressure caused three plants that were infested with *P. aphanidermatum* to wilt (Figure 3-3). While there was no significant interaction between the effects of cultivar and biopesticide on root disease severity for the plants infested with *Pythium* for either experiment, there was an effect of cultivar and biopesticide separately on root disease severity for the fall 2018 experiment (Table 3-3). For the fall experiment, cv. Maxifort had the highest root rot across biopesticide treatments compared to the other cultivars for both infested and non-infested plants (Table 3-4).



Figure 3-3. Wilted tomato plant nine days after inoculation with *P. aphanidermatum* isolate KOP8 in the summer 2018 experiment.

Table 3-3. Results from a Two-Way ANOVA (*p*-values) evaluating the effects of biopesticide (Cease[®], Rootshield[®] WP, and water control) and cultivar (Glamour, Ailsa Craig, Trust, and Maxifort) on root disease severity for plants infested with *Pythium* and the interaction between the effect of cultivar and biopesticide. Root disease severity was based on root rot percentage. Two replicate experiments were conducted once in summer 2018 (6/27-7/18) and another in fall 2018 (10/18-11/8).

Experiment	Main effects		Interactions
	Cultivar (Df=3)	Biopesticide (Df=2)	Cultivar x Biopesticide (Df=6)
Summer	0.3235	0.0900	0.1431
Fall	<0.0001	0.0029	0.1467

Table 3-4. Mean percent root rot of tomato cv. Maxifort, Glamour, Trust, and Ailsa Craig 21 days post-transplant for infested plants with *P. aphanidermatum* isolate KOP and non-infested plants for the fall 2018 replicate experiment (n=15). Means with the same letter are not significantly different ($\alpha = 0.05$) as determined by the Tukey HSD Post-hoc test.

Cultivar	Mean Root Rot (%) on Infested plants	Std Error	Mean Root Rot (%) on Non-Infested plants	Std Error
Maxifort	31.7 a	4.9	42.3 a	2.7
Glamour	13.7 b	2.8	18.3 b	3.7
Trust	15.7 b	3.7	19.3 b	2.6
Ailsa Craig	10.0 b	1.9	16.7 b	2.9

Tomato plants infested with *P. aphanidermatum* isolate KOP8 in the summer experiment had over 55% greater root rot than the non-infested water control plants ($p < 0.0001$). For the fall

experiment, the non-infested plants had higher root rot ratings (24.2 %) than the plants infested with KOP8 (18%), indicating that there were possible outside sources of root-rot pathogen(s) infecting the plants. It was confirmed through culture-based methods that some of the non-infested control roots were infected with *Pythium*. However, there were other fungi isolated from these roots making it unclear if *Pythium* was the initial cause of the root rot or if *Pythium* was a secondary pathogen.

For the summer experiment, there was no difference in root rot between infested plants treated with Cease[®], Rootshield[®] WP, or the water control ($p = 0.09$). Due to the differences between the two experiments, the fall 2018 data were analyzed separately. In the fall experiment, Rootshield[®] WP treated and infested plants had less root rot severity than the infested water control ($p = 0.0029$) (Table 3-5). The Rootshield[®] WP treated and non-non-infested plants also had lower root rot severity compared to the non-infested water control in the non-infested plants, although not significant (Table 3-5).

Table 3-5. Mean percent root rot of tomato plants infested with *P. aphanidermatum* isolate KOP8 and non-infested plants treated with Rootshield[®] WP, Cease[®], or a water control 21 days post-transplant for fall 2018 experiment (n=20). Means with the same letter are not significantly different ($\alpha = 0.05$) as determined by the Tukey HSD Post-hoc test.

Biopesticide	Mean Root Rot (%) on Infested plants	Std Error	Mean Root Rot (%) on Non-Infested plants	Std Error
Rootshield [®] WP	11.0 c	2.4	18.3 b	3.5
Cease [®]	18.7 b	3.8	29.5 a	3.1
Water control	23.4 ab	3.7	24.8 ab	3.6

3.3.3. Plant Health

Root growth, represented on a 0-5 scale of root colonization of the rockwool block, was analyzed for the non-infested plants to see if there was an effect of biopesticide. There was a significant effect of experiment on root growth, with the fall 2018 plants having greater root growth (3.67) compared to the plants in summer 2018 (2.95) ($p < 0.0001$), this may be due to

stress from exposure to high temperature and disease pressure observed in the summer experiment. Root growth was greater for cv. Glamour (3.48) and lowest for cv. Trust (3.21) ($p = 0.0336$). Plants infested with *Pythium* had less root growth (2.05) than those treated with water (non-infested) (3.31) ($p < 0.0001$). Cv. Maxifort had significantly lower SPAD measurements than the other cultivars ($p < 0.0001$) (Table 3-6).

Table 3-6. Mean SPAD measurements of tomato cv. Maxifort, Glamour, Trust, and Ailsa Craig 21 days post-transplant (n=60). Means with the same letter are not significantly different ($\alpha = 0.05$) as determined by the Tukey HSD Post-hoc test.

Cultivar	Mean SPAD on Infested Plants	Std Error	Mean SPAD on Non-infested Plants	Std Error
Glamour	49.5 a	0.6	42.2 bc	0.7
Trust	48.1 a	0.8	41.7 bc	1.0
Ailsa Craig	48.2 a	0.8	43.9 b	0.8
Maxifort	40.5 c	0.8	36.0 d	0.6

These lower SPAD measurements for Maxifort can be largely attributed to intumescence on the leaves, a type of abnormal water retention in the leaf, causing “blisters” on the underside of the leaves (Williams et al., 2015) (Figure 3-4). There was no effect of biopesticide on SPAD measurements ($p = 0.6150$). Plants infested with *Pythium* had lower SPAD measurements compared to those treated with water ($p < 0.0001$, Table 3-6). The fall 2018 experiment plants also had higher SPAD measurements (45.2) than those from the summer 2018 experiment (41.2) ($p < 0.0001$). Both the *Pythium* inoculation and the high temperatures from the summer of 2018 are stressors that could have decreased photosynthesis, thus decreasing SPAD measurements.



Figure 3-4. Intumescence on the underside of leaves of tomato cv. Maxifort.

3.3.4. Dry Biomass

There was no interaction between the effects of cultivar and biopesticide on aboveground dried biomass of healthy plants ($p = 0.7267$). Cv. Maxifort did have the greatest aboveground dried biomass (2.09 g) compared to the other cultivars (<1.71 g) ($p = 0.0002$). The water treated plants had greatest aboveground dried biomass (1.92 g) while Rootshield® WP had the least amount of aboveground biomass (1.55 g) ($p = 0.0018$). Plants infested with *Pythium* had average aboveground biomass weights 0.6 g lower than those treated with water control. There was also an effect of experiment on the aboveground biomass, with the fall of 2018 plants having twice as much aboveground biomass ($p < 0.0001$).

3.4. Discussion

In this study, there was no effect of cultivar on the efficacy of the biopesticides Rootshield® WP or Cease® on disease suppression or root growth. While this contradicts some of the recent literature (Meyer et al., 2010; Smith et al., 1999), other studies have found similar findings (Larkin and Fravel, 2002). Larkin and Fravel (2002) did not observe an effect of tomato cultivar on the efficacy of biocontrol agents to suppress Fusarium wilt. Other studies have found that soil type is a more important factor for microbial colonization in the rhizosphere than cultivar (Latour et al., 1996). Weinert et al. (2011) analyzed the rhizosphere microbial composition of two soil types using PhyloChip technology and discovered that 40% of the operational taxonomic units were site specific, while only 4% of the operational taxonomic units were cultivar specific. Studies have also suggested that biopesticides have greater efficacy when cultivars have more tolerance to disease (King and Parke, 1993; Xue et al., 2014). In preliminary work (chapter 2), Glamour was less susceptible to root rot compared to Ailsa Craig and Trust. However, in this trial, Glamour was more susceptible to root rot which may be primarily due to

the warmer temperatures in the greenhouse compartments during these trials. Regardless, the cultivar panel utilized for this experiment did not impact the efficacy of biopesticides. Larkin and Fravel (2002) evaluated eight different tomato cultivars with varying degrees of susceptibility to *Fusarium* wilt and they did not observe a cultivar effect, suggesting that susceptibility may not always play a role in biopesticide efficacy. However, a different cultivar panel with greater genetic diversity that includes heirloom varieties and wild relatives may show an effect on the efficacy of biopesticides to suppress root disease. A cultivar effect may be seen when testing more biopesticides since this study only tested two.

In the fall 2018 experiment, tomato plants treated with Rootshield® WP had significantly less root disease than the plants treated with Cease® and water. While there was no effect of biopesticide on disease severity of plants treated with *Pythium* for the summer 2018 experiment, the non-infested plants treated with Rootshield® WP and Cease® had lower disease severity than the water controls. This data suggests that when disease pressure is low to moderate (<50% disease severity), the biopesticides are able to suppress disease. Punja and Yip (2003), also observed a difference between seasons (and thus disease pressure) in the efficacy of biopesticide products against *P. aphanidermatum*. The effect of disease pressure on biopesticide efficacy is well documented (Harman, 2000; Rose et al., 2004). Thus, it is important to have biopesticides as one component of an integrated pest management system. The experiment run in summer 2018 characterized a combined set of circumstances that could cause a commercial grower to have major crop loss. In this experiment, the high temperatures and humidity, the size of the plants at transplant, and the inoculation of the plants with *Pythium* led to a high level of disease (mean root rot severity was 68.7%) causing multiple plants to damp off (Figure 3-4). However, the non-infested plants only had a root disease severity of 10.6%. This highlights the importance

of preventing root disease from establishing in plants, especially during periods of high stress when their roots are most vulnerable and when conditions are favorable for *Pythium* infection (Moorman and Daughtrey, 2002; Sutton et al., 2006). During these conditions, commercial growers should be routinely scouting and would benefit from rotating biopesticide and fungicide applications to prevent the *Pythium* from gaining a foothold (Fravel, 2005). Following a fungicide drench, the disease pressure on the plant would be significantly lower and a biopesticide application would be more effective at preventing disease and improving plant health (Harman, 2000; Rose et al., 2004).

Cv. Maxifort had consistently higher root rot severity compared to the other three cultivars tested. This is interesting due to the fact that Maxifort is a rootstock scion that has been shown to have higher resistance to several soil-borne pathogens, such as Fusarium wilt (Rivard and Louws, 2008). Our results suggest that Maxifort may not have the same resistance to *Pythium* root rot. Another possibility is that when Maxifort does not have a scion grafted on to it, the roots take-up more water than its leaves require, stressing the root system and increasing susceptibility to disease. The Maxifort plants exhibited intumescence in both replicates (Figure 3-4). Intumescence is commonly interchanged with edema; however, studies have shown that the lesions caused by edema are different than those caused by intumescence (Craver et al., 2013). Intumescence lesions protrude outward and increase in size and proliferate instead of rupturing or collapsing like the edema lesions (Craver et al., 2013; Williams et al., 2015). These lesions have traditionally been attributed to excess water retention in the plants that causes the epidermal cells to expand. Rud (2009) however, found that there was no correlating evidence between this disorder and moisture content of the plant or substrate. During the summer 2018 trial, originally it was thought that high temperatures and humidity may have been to blame for the lesions, but

the same was found in the fall of 2018 trial. Studies have found that exposure to UVB light decreased the development of intumescence on cv. Maxifort (Craver, 2014; Rud, 2009). There are many different suggested causes for intumescence and it is still unknown what plays the largest role (Williams et al., 2015). Regardless of the cause of the intumescence, it does alter physiological processes in the plant and decrease marketability of the tomato plants. Our SPAD results suggest that there was a decrease in photosynthetic activity in the cv. Maxifort. Roloff et al. (2004) found that with an increase of edema-like lesion on blueberry (*Vaccinium ashei* ‘Premier’ and ‘Climax’ and *V. corymbosum* ‘Bluecrisp’), there was a significant decrease of net CO₂ assimilation rate (NAR), correlating to a decrease in photosynthesis. Using cv. Maxifort, Wu et al. (2017) found that photosynthesis related genes were suppressed in leaves with intumescences. They also found that ethylene biosynthesis and its transduction pathway were more active in leaves with intumescence. A decrease in photosynthesis can stress the plant and can increase its susceptibility to *Pythium*, however, it is unknown how intumescence and a plant’s response could affect the plant’s susceptibility to root disease. A limitation of this study is that cv. Maxifort is used as a rootstock that has a scion grafted on to it in commercial greenhouse production. Thus, another study should examine root disease severity with a grafted Maxifort before conclusions are made about the susceptibility of this cultivar for commercial greenhouse production purposes.

Our data suggests that tomato cultivar does not affect the efficacy of a biopesticide to reduce root disease severity caused by *Pythium*. In future studies, a different cultivar panel representing greater genetic diversity will be utilized to further investigate the effect of cultivar on biopesticide efficacy. Additionally, more biopesticides will be tested to determine if specific isolates/species may be affected differently by cultivar. If our results continue to show that there

is no interaction between cultivar and the efficacy of a biopesticide to reduce root disease severity, then this will provide evidence that the variability of biopesticide performance may not be due to plant cultivars but rather other environmental conditions. However, there are some cultivars, like Maxifort, that are more susceptible to root rot disease and thus will have higher root disease severity across all treatment types. Furthermore, our data does suggest that under “normal disease pressure” (<50% root disease severity), Rootshield® WP does decrease root disease severity compared to a water control or Cease®. With further replications of this experiment, growers can make decisions on which biopesticide to integrated into their IPM program to improve their on-farm biopesticide performance.

CHAPTER 4

THE EFFECT OF GROWING SUBSTRATE ON *PYTHIUM* DISEASE SEVERITY AND THE EFFICACY OF BIOPESTICIDES

4.1. Introduction

Modern greenhouse production of floriculture and vegetable crops has moved away from growing in soil to the use of soilless substrates. One of the primary reasons for this transition was to avoid losses to soilborne plant pathogens and reduce the need for soil fumigation (Postma, 2004). Soilless culture is defined as growing plants without the use of soil as a rooting medium and where nutrients are supplied to the plants via irrigation water (Agung Putra and Yuliando, 2015). For containerized floriculture crops, the primary component of most soilless substrate mixtures is peat (Robbins and Evans, 2011a). The type of peat that is most commonly used is peat moss derived from sphagnum moss (Schmilewski, 2009). Other common organic (containing carbon) substrates used in greenhouse production are coconut (coco) coir, pine-bark, wood fiber, and composted organic waste (Barrett et al., 2016; Drotleff, 2016). Common inorganic (lacking carbon) substrate components include perlite, vermiculite, sand, rockwool, and other synthetic materials, such as Oasis[®] (Robbins and Evans, 2011a). Many floriculture crops are grown with peat-perlite mixes or Oasis[®] (especially in propagation) while vegetable crops are grown in coco coir or rockwool (Robbins and Evans, 2011a). Coco coir is a waste product from the coconut industry and contains fibers from the mesocarp of the coconut fruit (Abad et al., 2002). Rockwool and Oasis[®] are sterile, synthetic substrates that are completely inert; rockwool (or stone wool) is a man-made mineral fiber made of spun stone wool while Oasis[®] is water-absorbing product made from a phenolic foam (Will and Faust, 2005).

These organic and inorganic substrates vary widely in their chemical and physical properties which has implications for plant growth, nutrient uptake and microbial activity (Garbeva et al., 2004).

While soilless culture has reduced crop losses due to soilborne plant pathogens, disease outbreaks still have a significant impact, even when conventional fungicides and water treatment technologies are used. Certain pathogens are well adapted for survival and growth in soilless and hydroponic systems and have become problematic in greenhouse crop production. One reason for these outbreaks is the high water retention capacity of soilless substrates and the favorable environment of the greenhouse where temperatures and moisture regime are more constant (Stanghellini and Rasmussen, 1994). *Pythium* is a common plant pathogen causing damping-off and root rot in greenhouse crop production. Many *Pythium* species produce a swimming spore known as a zoospore, that uses chemotaxis to swim towards and infect its host (Postma et al., 2000; Stanghellini and Rasmussen, 1994). Substrates like rockwool and Oasis[®] are almost sterile at planting (containing little to no microbial community) which can create a “biological vacuum” (Paulitz, 1997; Paulitz and Belanger, 2001). Thus, if a pathogen, specifically one like *Pythium*, is present, an epidemic can occur due to the lack of competition and antagonism that would be found in natural soils with a more developed microbial community (Hendrix and Campbell, 1973; Paulitz, 1997; Stanghellini and Rasmussen, 1994). However, this lack of competition by the natural microbial community and the environmental conditions of soilless substrates, creates an ideal environment to apply biopesticides (Paulitz, 1997; Paulitz and Belanger, 2001). Biopesticides have been shown to be most effective when applied early in the crop production cycle before disease occurs (Fravel, 2005).

Biopesticides are disease control products formulated with beneficial microorganisms (biocontrol agents) to suppress disease and promote plant health. Several researchers have examined different biocontrol agents for suppression of root disease caused by *Pythium* spp. (Gravel et al., 2006, 2005; McCullagh et al., 1996; Rankin and Paulitz, 1994). Rankin and Paulitz (1994) were the first to show a reduction in *Pythium* disease with the use of *Pseudomonas corrugata* and *P. fluorescens* in a cucumber rockwool system. Since this initial research, there has been a significant increase in the number of commercially available biopesticides that use bacteria, fungi, or plant extracts to suppress disease (Fravel, 2005; Glare et al., 2012). While the biopesticide market is growing, consistency has been a barrier to widespread adoption by growers. Research trial results vary widely (Rankin and Paulitz, 1994) and on-farm performance had been met with variable success (Fravel, 2005). Recent advancements in soil microbiome research suggests that variability in biopesticide efficacy may be due to a number of environment variables, such as temperature, nutrient availability, plant species, plant cultivar, and substrate/soil type, that are known to effect microbial communities (Berg and Smalla, 2009).

Advances in ‘omics’ tools in recent years has led to a substantial increase in the amount of studies examining the effect of substrate/soil on the microbial community composition (who is there) and function (what they are doing) in growth chamber, greenhouse, and field studies (Berg and Smalla, 2009; Garbeva et al., 2004). These studies have shown that substrate influences microbial community composition, the health of the community, and the development of naturally suppressive soils (Mazzola and Freilich, 2017; Mendes et al., 2013). Some studies suggest that substrate is more important to microbial community composition than plant species/genotype (Latour et al., 1996; Nallanchakravarthula et al., 2014; Weinert et al., 2011),

however these results are not consistent between experiments. Latour et al. (1996) found that soil type was the most important influence on the diversity of fluorescent *Pseudomonas* species and Meyer et al. (2010) found that soil type affected the host's accumulation of 2,4-diacetylphloroglucinol-producing (an antimicrobial compound) *Pseudomonads* in the root interior. Windisch et al. (2017), determined that soil type influenced the biocontrol efficacy of *P. jessennii* and *Serratia plymuthica* in suppressing *R. solani* rot disease in lettuce. One study found that increasing the amount of sand in a peat:sand mix resulted in a decreased efficacy of antifungal metabolites, phenazine and sessilin, that are commonly produced by *Pseudomonas* species (Hua and Höfte, 2015). Furthermore, Koohakan et al. (2003) found differences in the microbial populations between coco fiber and rockwool in a tomato hydroponic system.

While research shows that substrate affects the native microbial community assembly and composition, little is known about how substrate affects establishment (and subsequent efficacy) of biocontrol agents applied to a system as a commercial biopesticide. Only a few studies have examined the effect of substrate on introduced biocontrol agents. Krause et al. (2007) examined three substrates (dark *Sphagnum* peat mix, light *Sphagnum* peat mix, and composted pine bark mix) on the efficacy of *Chryseobacterium gleum* (C₂₉₉R₂) and *Trichoderma hamatum* 382 to reduce *Rhizoctonia* damping-off of radish and *Rhizoctonia* crown and root rot of Poinsettia. A significant effect of substrate on the efficacy of C₂₉₉R₂ and 382 was observed. Composted pine bark mix consistently supported high populations of both biocontrol agents and the compost's indigenous microbial community, resulting in suppression of *Rhizoctonia* (Krause et al., 2001). Nelson et al. (1983) found that fresh (non-composted) hardwood bark was able to support higher populations of the biocontrol agent *T. harzianum* compared to composted hardwood bark, but the increase in colonization was not correlated with increased suppression of *Rhizoctonia solani*.

Boehm and Hoitink (1992) found that *Pythium* root disease correlated with the amount of decomposition of a substrate; the least decomposed peat or pine bark amendment had the most microbial activity and the least amount of root disease. Based on their studies, Hoitink and Boehm (1999) suggest that substrates amended with composted materials will increase the efficacy of biopesticides by providing a food source for the biological control agents. To explain this phenomenon, researchers have hypothesized that the type of food (carbon) source found in the substrate influences the production of cell-wall degrading enzymes, such as β -glucanase and chitinase, that are essential for the antagonism of fungal pathogens by *T. harzianum* (de la Cruz et al., 1993). It is hypothesized that these cell-wall degrading enzymes are repressed in the presence of cellulose, which is a food source that is more favorable to the fungus *T. harzianum* (de la Cruz et al., 1993; Hoitink and Boehm, 1999). This explains why *T. harzianum* does not suppress disease in non-composted bark substrates (with high cellulose content) compared to composted substrates (Chung et al., 1988). Follow-up studies showed that production of anti-fungal cell-wall degrading enzymes decreased with increasing carbon (glucose) concentration (Windisch et al., 2017). In a review article on biocontrol of soil diseases, Hoitink and Boehm (1999) suggest that substrates low in available food source for the introduced biocontrol agents cannot sustain disease suppression. Little research has been done evaluating newer substrates, such as coco coir and Oasis[®], and how they may affect biopesticide efficacy and disease suppression. Furthermore, little research has examined the effect of propagation substrate, which can vary greatly between production systems. Applying biopesticides in propagation gives the plants protection early, especially when growers don't want to risk phytotoxicity from fungicide drenches (van Lenteren, 2000). Moreover, an application during propagation will utilize significantly less biopesticide product, thus decreasing costs. Research is needed to better

understand how variables like substrate could affect the efficacy of biopesticides to suppress soil-borne disease such as *Pythium*.

The objective of this research was to evaluate the effect of propagation substrate on microbial biopesticide efficacy. Cucumber and calibrachoa cropping systems were used to test the hypothesis that propagation substrate will differentially influence the ability of microbial biopesticides to suppress *Pythium* root rot.

4.2. Materials and Methods

4.2.1. Experimental design

Two experiments were conducted to test the effect of propagation substrate on biopesticide efficacy. Experiment 1 was conducted using a vegetable crop (cucumber) and experiment 2 was conducted using a floriculture crop (calibrachoa). These two systems were used to develop a model to determine if similar effects occur in different production systems. Each experiment consisted of a 3 x 4 factorial with three substrates (Oasis[®], peat, coco coir) and four biopesticide treatments (Rootshield[®] WP, Cease[®], Regalia[®], and water). The four biopesticide treatments were chosen to represent the different classes of biocontrol agents (fungi, bacteria, and plant extract). Experiments were repeated twice.

All experiments were conducted at the University of New Hampshire's MacFarlane Greenhouse in Durham, NH. Treatments were arranged in a completely randomized design with 10 replicate plants per treatment (120 plants total). In each treatment, half of the plants were infested with *Pythium* and half remained non-infested to observe effects of the biopesticide on plant health and growth. The cucumber experiment was conducted in the fall of 2017 (11/14-12/4) and replicated in the fall of 2018 (10/11-11/1). The calibrachoa experiment was conducted in the winter of 2018 (2/19-4/9) and replicated in the fall of 2018 (11/2-11/30).

4.2.2. Plant Material and Propagation

Calibrachoa cv. Superbells ‘Lemon Slice’ (*Calibrachoa hybrid* Cerv.) (Pleasant View Gardens, Inc., Loudon, NH) were propagated in each of the three substrates (Oasis[®], peat, and coco coir, Table 4-1) (Figure 4-1). Calibrachoa cuttings were sprayed with CapSil 30 (Aquatrols Corp of America, Paulsboro, NJ) to decrease evapotranspiration while the cuttings produce roots. The Jiffy pellets were hydrated and the Oasis[®] cubes were pre-moistened with clear water and placed in trays (27.94 cm x 54.28 cm, To Plastics Inc, Clearwater, MN) before propagation. Cucumber cv. Straight Eight (*Cucumis sativus* L.) (Burpee, Warminster, PA) were seeded into each of the three substrates (Table 4-1). The seeds were covered with vermiculite which is standard practice in cucumber greenhouse production (McCullagh et al., 1996) (Figure 4-2).

Table 4-1. Product size and manufacture of the substrates used in propagation experiments.

Substrates	Size	Manufacturer
Oasis [®] Rootcube	3.81 cm	Smithers Oasis CO, Kent, OH
Jiffy-7 [®] peat pellet	30 mm	Jiffy Products of America, Lorain, OH
Jiffy-7C [®] coco coir	30 mm	Jiffy Products of America, Lorain, OH



Figure 4-1. Calibrachoa cv. Superbells ‘Lemon Slice’ cuttings stuck in Oasis[®] cubes (left), Jiffy-7[®] peat pellet (middle), and Jiffy-7C[®] coco coir (right).



Figure 4-2. Cucumber cv. Straight eight seed sown in Oasis[®] cubes (left), Jiffy-7[®] peat pellet (middle), and Jiffy-7C[®] coco coir (right).

After seeding or sticking, the trays were placed on benches equipped with under-bench heating in a propagation room at the MacFarlane Greenhouse. The cucumbers were overhead misted with clear water until germination. Temperatures in the propagation house were set to 24°C during the day and 23°C at night. Cucumber seeds typically germinated 4-5 days post seeding. Once the cucumber seeds were germinated and the calibrachoa rooted into the substrate, plants were fertilized with 100mg·L⁻¹ N of 17-4-17 NPK commercial water-soluble fertilizer by hand (Jack's Pure Water LX, JR Peters Inc, Allentown, PA). Calibrachoa flowers were routinely pinched off while in the germination room to promote root growth.

Fourteen days post seeding, the cucumber plants were transplanted into 15 cm (5.9 inch, The HC Companies, Middlefield, OH) pots and the calibrachoa was transplanted 17 days post sticking into 11.43 cm (4.5 inch, The HC Companies, Middlefield, OH) pots. Plants in both experiments were transplanted into a 1:1 mix of coco coir (70:30 blend fiber:chips, Fibre Dust LLC, Cromwell, CT) and sphagnum peat (ProMix BX General, Premier Tech Horticulture, Quakertown, PA). Pots were placed on open mesh benches in the greenhouse (Figure 4-3) under

a 16-hour photoperiod using 400-watt HPS lights (PL Light Systems Inc., Beamsville, Ontario). The cucumber plants were fertilized through stackable 4-way driplines (Netafim Irrigation Inc, Fresno, CA) with $200\text{mg}\cdot\text{L}^{-1}$ N of 17-4-17 NPK commercial water-soluble fertilizer (Jack's Pure Water LX, JR Peters Inc, Allentown, PA). The calibrachoa plants were fertilized through the driplines with $150\text{mg}\cdot\text{L}^{-1}$ N of 20-3-19 NPK commercial water-soluble fertilizer (Jack's Petunia FeED, JR Peters Inc, Allentown, PA). Plants were watered at 36.5 mL per minute 1-3 times per day depending on plant growth.



Figure 4-3. Cucumber cv. Straight eight plants transplanted into pots and placed on mesh benches in greenhouse compartment 14 days post seeding (left). Calibrachoa cv. Superbells 'Lemon Slice' in greenhouse compartment 21 days post infestation when disease assessments were done (right).

Plants were treated weekly with preventative applications of the *Steinernema feltiae* system (150,000-200,000 nematodes per plant) (BioBest, Westerlo, Belgium) to control fungus gnats. Swirskii-Breeding-System sachets (*Amblyseius swirskii*) (BioBest, Westerlo, Belgium) containing predatory mites were placed on each plant to control whiteflies and thrips. Yellow sticky cards (BASF Corporation, Research Triangle Park, NC) were placed in the greenhouse, three per bench at plant level, to monitor pest populations.

4.2.3. Biopesticide treatments

Three commercial biopesticides were evaluated (Rootshield® WP (BioWorks, Victor, NY), Cease® (BioWorks, Victor, NY), and Regalia® (Marrone Bio Innovations, Davis, CA)). The biopesticide treatments and a water control were applied twice as a drench at the manufacturer's label rate (Table 4-2). For the cucumber experiment, applications were made at 7 and 14 days post-seeding as a 10 mL and 25 mL drench. For the calibrachoa experiment, applications were made at 14 and 20 days post sticking as a 20 mL and 25 mL drench respectively. The water controls received an equal volume of water.

Table 4.2. Biopesticide products used, active ingredients, and the rate applied. Rates were based on the manufacturer recommendation.

Product	Active Ingredient	Guaranteed CFU/g	Rate
Cease®	<i>Bacillus subtilis</i> QST-713	1.0 x 10 ⁹	15 mL/L
Rootshield® WP	<i>Trichoderma harzianum</i> KRL-AG2	1.0 x 10 ⁷	0.4 g/L
Regalia®	Extract of <i>Reynoutria sachalinensis</i>		5 mL/L

4.2.4. Source and preparation of pathogen isolates

Two *Pythium* isolates, *Pythium aphanidermatum* (Edson) Fitzp. isolate KOP8 and *Pythium ultimum* (Trow) isolate NDT1-1, were used for these experiments. Isolate KOP8 was isolated from wheatgrass seeds by Dr. M. Daughtrey, at Cornell University. The isolate was received at UNH in June 2017. Isolate NDT1-1 was isolated from cucumber plants infested with an isolate obtained from the University of New Hampshire Plant Diagnostic Lab in November 2017. The *Pythium* isolates were maintained in long-term storage as mycelial plugs in a sterile water storage as described by Dr. G. Moorman (<https://plantpath.psu.edu/pythium/module-2/cleaning-and-storing-isolates>). To prepare for storage, the isolates were grown on 1.5% water agar for 7 days. The colonized agar was cut into a grid using a sterile scalpel and 5-10 cubes

were suspended in 10 mL of sterile tap water in a sterilized 15 mL capped test tube. The isolates were stored in the test tubes at room temperature.

For experiment 2 (calibrachoa) a spore suspension inoculum was prepared. *P. ultimum* isolate NDT1-1 was revived from storage by transferring colonized water agar cubes to 20% V8 (200 mL of clarified V8 vegetable juice, 15 g agar, and 2-3 g of CaCO₃ per liter of RO water) media plants (100 mm x 15 mm, Fisher Scientific, Hampton, NH). After 4-7 days of growth, propagules were harvested in a laminar flow hood by flooding the plates with 20 mL of sterile RO water. A sterilized FisherBrand cell spreader (Fisher Scientific, Hampton, NH) was used to rub the top of the media to dislodge mycelia and propagules. The supernatant was drained from the petri dish and placed into a sterile beaker. The supernatant was then filtered using 3 layers of sterile cheesecloth to remove the mycelia. The number of propagules (oospores, zoospores) in the cell suspension were counted using a Hemocytometer (Hausser Scientific, Horsham, PA) under a compound microscope (Olympus Model CX43RF). The suspension was adjusted to 1×10^5 propagules/mL. Using a 25 mL serological pipette, 20 mL of the *P. ultimum* isolate NDT1-1 spore suspension was pipetted onto the substrate of the calibrachoa pots seven days post-transplant, completely covering the top of the substrate. Control plants received an equal volume of water.

For experiment 1 (cucumber) the potato soil inoculum (PSI) method as described by Ko and Hora (1971) with a few modifications was used to prepare inoculum of *P. aphanidermatum* isolate KOP8. Five hundred mL of loamy soil was placed into a 1 L flask, followed by 50 g of peeled and finely chopped organic Yukon Gold potatoes (~0.5 cm cubes), and enough water to make the soil fairly wet but not muddy. The flask was closed with a cotton plug, covered with aluminum foil, and autoclaved at 121°C, 15 psi for 1 hour on each of 2 consecutive days. The

potato soil was infested with 3 water agar disks (#9 cork borer) of a *Pythium* isolate taken from the colony edge. The *Pythium* grew for 1 week at room temperature, and the flask was gently shaken once during the middle of the week to distribute the colonized potato pieces throughout the soil (Figure 2-3). Once the fully colonized, the potato soil inoculum was air-dried on paper towels in a laminar flow cabinet. The dried inoculum was sieved with 1- and 2-mm sieves and the 1-2 mm fraction was saved to be used as PSI. The cucumber plants were infested with 0.5 g/pot of PSI four days post-transplant. The PSI granules were buried 1 cm into the substrate at four points around the crown of the plant.

4.2.5. Disease Assessment

Root rot disease severity was measured 16 days post infestation for the cucumbers and 21 days post infestation for the calibrachoa. Disease severity was measured by giving each plant a root rot rating based on visual assessment of symptoms present on roots as seen by removing the root/soil mass from the pot and roots observed when the root/soil mass was pulled apart. For the cucumber experiment, the ratings were based on a 0-5 scale (0 = no root rot, 5 = roots completely rotted) and mid-point values were assigned when appropriate (Figure 4-4). For the calibrachoa experiment, the plants were rated based on percent root disease (0% = no root rot, 100% = roots completely rotted). Each plant was also assessed for root growth using a 5-point rating scale based on the degrees of root colonization of the pot (0 = no roots in the substrate, 5 = the substrate was fully colonized with roots). To confirm that symptomatic plants were infected with *Pythium*, root samples were collected from 3 replicate plants from each treatment using sterile forceps and stored in 15 mL falcon tubes at 4°C until ready to be processed. In the laminar hood, the root samples were surface washed by placing in sterile RO water in a glass petri dish. Four 1-cm root sections from the same plant were plated on Oomycete semi-selective media

PARP V8 (see Appendix A for recipe). The presence of *Pythium* growing from root segments was confirmed through examination of hyphae and sexual and/or asexual spores under a compound microscope (Olympus Model CX43RF).



Figure 4-4. A non-infested cucumber plant versus an infested cucumber plant with *P. aphanidermatum* water control treatment propagated in peat. The non-infested plant had a root rot rating of 0 and the infested root rot rating was 4.5.

Prior to disease assessment, chlorophyll content of the leaves was measured during the fall 2018 cucumber experiment to determine if the application of biopesticides affects plant health (i.e. photosynthesis). Three measurements per plant were collected and averaged using a Soil-Plant Analyses Development (SPAD) unit of Minolta Camera Co. SPAD 502 Plus Chlorophyll Meter (Item 2900PDL, Spectrum Technologies, Aurora, IL) (Monje and Bugbee, 1992). Environmental data were collected using Argus Control Software Firmware Version 12.43 Build 00063 (Argus Control Systems Ltd., Surry, BC).

4.2.6. Statistical Analysis

Disease severity and root growth data were analyzed for statistical significance using Two-Way Analysis of Variance (ANOVA) in JMP Pro 14 (SAS Institute, Cary, NC). The model statement was constructed to determine the effect of the independent variables (substrate and biopesticide) and the interaction between these variables on the dependent variables (disease

severity, growth, SPAD). All data were analyzed using an ANOVA for significant difference between non-infested and infested plants. Then, the data from non-infested and infested plants were analyzed separately. Statistical significance was assessed at $\alpha = 0.05$ and a Tukey Honest Significant Difference (HSD) Post-hoc test was used to separate the means.

4.3. Results

4.3.1. Effect of substrate on efficacy of biopesticide to suppress root disease of *Calibrachoa*

Plants infested with *Pythium* had 40% greater root disease than the non-infested plants ($p < 0.0001$). Plants infested with *Pythium* had root disease rating of 29.6% while the non-infested plants had a root disease severity of 11.9%. A significant interaction between the effects of substrate and biopesticide on root disease severity was observed on calibrachoa plants infested with *Pythium* ($p = 0.0505$) (Figure 4-5).

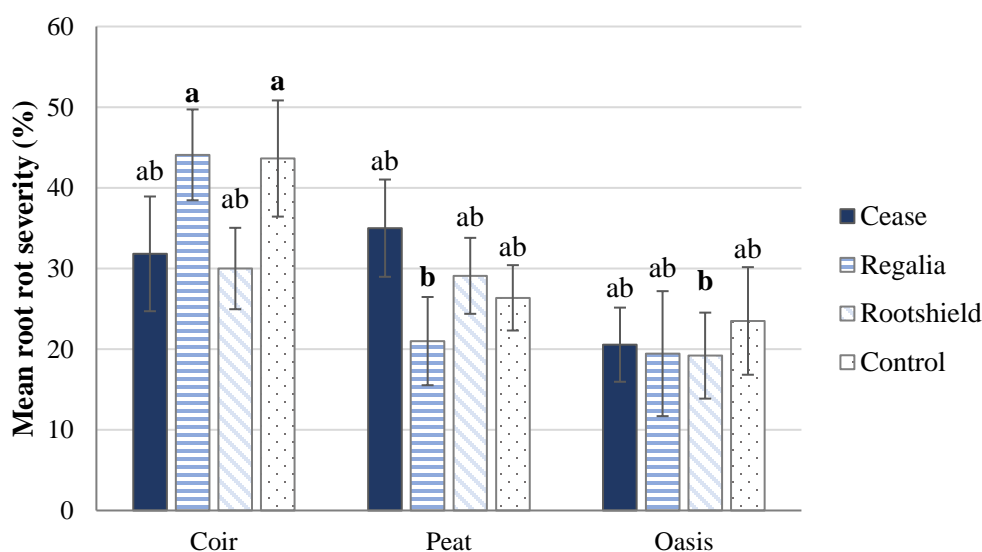


Figure 4-5. Mean root rot severity (%) of calibrachoa cv. Superbells ‘Lemon Slice’ propagated in three substrates (Jiffy-7C[®] coco coir, Jiffy-7[®] peat, and Oasis[®]) 21 days post-infestation with *P. ultimum* isolate NDT1-1 for both fall 2017 and fall 2018 replicates. Error bars are standard error (n=11). Means with the same letter are not significantly different ($\alpha = 0.05$) as determined by the Tukey HSD Post-hoc test.

The root rot severity of infested plants treated with biopesticides was not significantly different from the infested water control treatment (Table 4-3). Interestingly, disease severity was different among the propagation substrates tested (Table 4-3). Calibrachoa plants propagated in peat and treated with Regalia® tended to have lower root rot compared to plants treated with Regalia propagated in coco coir ($p = 0.0118$). Overall, calibrachoa propagated in coco coir had greater root disease severity (37.4%) compared to peat (28.0%) or Oasis® (20.7%) ($p < 0.0001$). Additionally, when root rot in the non-infested plants from both replicate experiments were compared, there were differences in severity between the three substrates (Table 4-4) where plants propagated in coco coir had greater root rot than those propagated in Oasis® or peat.

Table 4-3. Results from a Two-Way ANOVA (p -values) evaluating the effects of biopesticides (Cease®, Rootshield® WP, Regalia® and water control) and substrate (Jiffy-7C® coco coir, Jiffy-7® peat, and Oasis®) on root disease severity for plants infested with *Pythium* and the interaction between the effect of substrate and biopesticide. Root disease severity was based on root rot percentage.

Experiment ($p < 0.0001$)	Main effects		Interactions
	Substrate (Df=3)	Biopesticide (Df=2)	Substrate x Biopesticide (Df=6)
Winter 2018	0.0002	0.3539	0.0176
Fall 2018	0.0108	0.8687	0.0505

Table 4-4. Mean percent root rot of non-infested calibrachoa cv. Superbells ‘Lemon Slice’ plants propagated in Oasis® rootcube, Jiffy-7® peat pellet, or Jiffy-7C® coco coir 21 days post-transplant (n=44). Means with the same letter are not significantly different ($\alpha = 0.05$) as determined by the Tukey HSD Post-hoc test.

Substrate	Mean Root Rot (%)	Std Error
Oasis®	5.4 a	0.9
Peat	9.9 a	1.8
Coco coir	16.7 b	1.9

There was a difference in root disease severity between the two replicate experiments ($p < 0.0001$). The first replicate experiment (conducted in the winter (2/19-4/9)) had more than double the root rot than the second replicate experiment. Because the two replicate experiments were significantly different, the two were analyzed separately. When the winter 2018 replicate

experiment was analyzed independently, a significant interaction between the effects of substrate and biopesticide on root disease severity for plants infested with *Pythium* ($p = 0.0176$) was observed (Figure 4-6). In the plants propagated in coco coir and Oasis[®], there was a trend in which plants treated with Rootshield[®] WP had less root rot than the water control treatment, however this was not significant ($p = 0.1977$ for coco coir, $p = 0.8091$ for Oasis[®]).

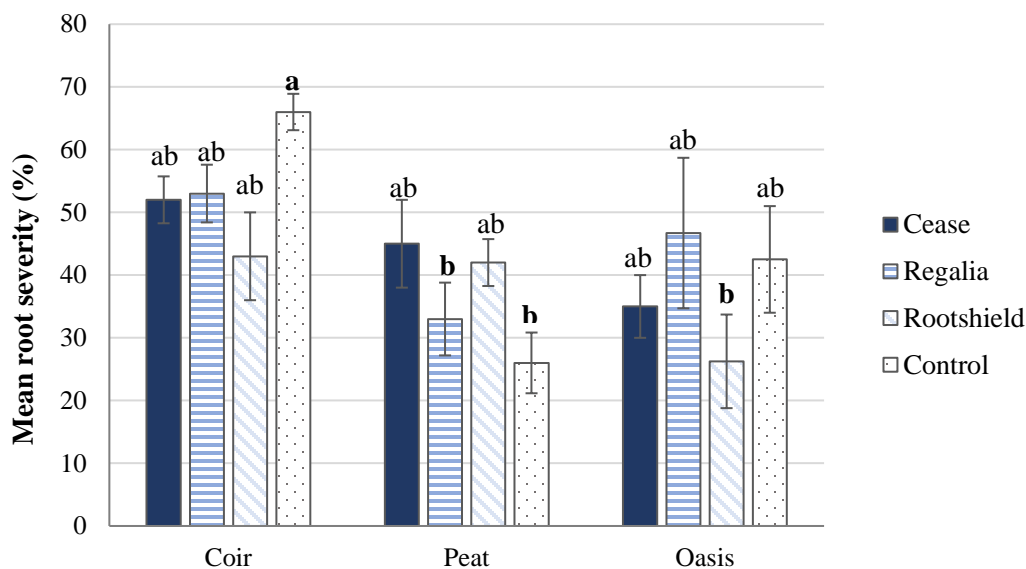


Figure 4-6. Winter 2018 mean root rot severity (%) of calibrachoa cv. Superbells ‘Lemon Slice’ propagated in three substrates (Jiffy-7C[®] coco coir, Jiffy-7[®] peat, and Oasis[®]) 21 days post-infestation with *P. ultimum* isolate NDT1-1. Error bars are standard error (n=5). Means with the same letter are not significantly different ($\alpha = 0.05$) as determined by the Tukey HSD Post-hoc test.

4.3.2. Effect of propagation substrate and biopesticides on Calibrachoa plant growth

The interaction between the effects of substrate and biopesticide on root growth for non-infested calibrachoa plants was not significant ($p = 0.8013$). Calibrachoa propagated in coco coir had significantly less root growth compared to plants propagated in peat or Oasis[®] ($p = 0.0002$) regardless of biopesticide treatment (see Appendix C for data, Table A-4). This could be due to the disease pressure on the plants that were propagated in the coco coir. Plants in the winter 2018 experiment had greater root growth ratings (3.85) than the fall 2018 experiment (3.07) ($p <$

0.0001). There were no significant differences in root growth rating among biopesticide treatments ($p = 0.1046$).

4.3.3. Effect of substrate on efficacy of biopesticide to suppress root disease of Cucumber

There was no interaction between the effects of substrate and biopesticide on root disease severity for cucumber plants infested with *Pythium* ($p = 0.6067$). There was, however, a significant effect of substrate on root disease severity ($p = 0.0102$). Cucumber propagated in Oasis[®] had 50% less root rot than plants propagated in coco coir and peat ($p = 0.0102$) (Table 4-5).

Table 4-5. Mean root rot severity (scale 0-5) of infested cucumber cv. ‘Straight eight’ propagated in Oasis[®] rootcube, Jiffy-7[®] peat pellet, or Jiffy-7C[®] coco coir 21 days post-transplant (n=40). Means with the same letter are not significantly different ($\alpha = 0.05$) as determined by the Tukey HSD Post-hoc test.

Substrate	Mean Root Rot	Std Error
Oasis [®]	0.89 a	0.18
Peat	1.69 b	0.22
Coco coir	1.68 b	0.23

Plants infested with *Pythium* had 30% more root disease than the non-infested plants ($p < 0.0001$). The plants infested with *Pythium* had a mean root disease rating (on a scale of 0-5) of 1.41 where the non-infested plants had a mean root disease rating of 0.42. There was a significant difference in root disease severity between the two replicate experiments. The fall 2018 experiment had greater average root rot (1.76) than the fall 2017 experiment (1.07) ($p < 0.0001$). Examining the fall 2017 experiment separately revealed that there was an interaction between the effects of substrate and biopesticide on root disease severity for plants infested with *Pythium* ($p = 0.0042$) (Figure 4-7). Cucumbers propagated in Oasis[®], regardless of the biopesticide treatment, had less root rot compared to plants grown in peat or coco coir. Additionally, plants propagated in coco coir had the greatest root disease, but there was a trend towards disease suppression by the biopesticides (Figure 4-7). When the plants propagated in

coco coir were analyzed separately, the Regalia[®] treatment had significantly less disease than the water control ($p = 0.0318$). Although not statistically significant, there was a trend towards reduced root disease severity on plants treated with Rootshield[®] WP compared to the other treatments in all substrates ($p = 0.0753$).

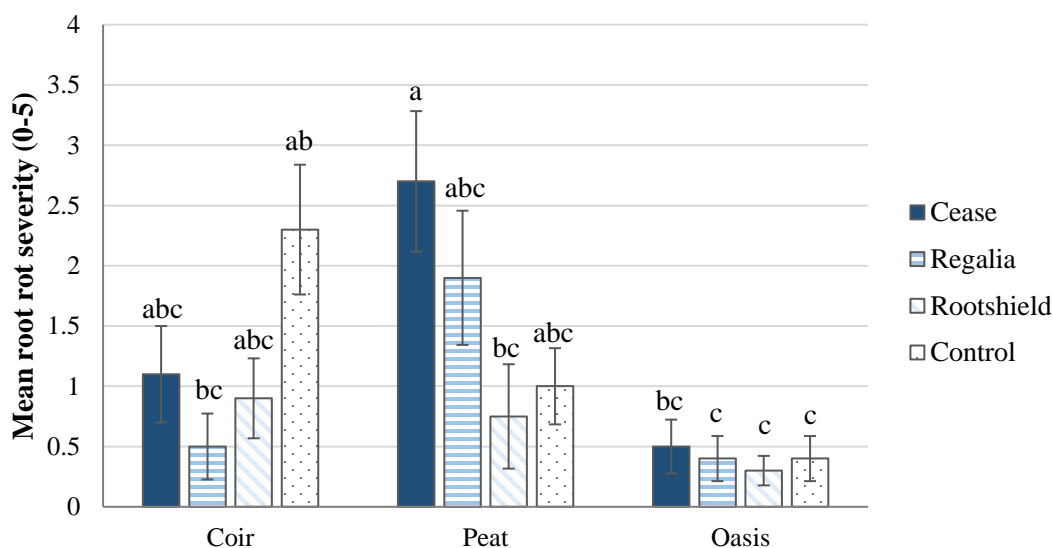


Figure 4-7. Fall 2017 mean root rot severity (scale 0-5) of cucumber cv. ‘Straight eight’ propagated in three substrates (Jiffy-7C[®] coco coir, Jiffy-7[®] peat, and Oasis[®]) 16 days post-infestation with *P. aphanidermatum* KOP8. Error bars are standard error (n=5). Means with the same letter are not significantly different ($\alpha = 0.05$) as determined by the Tukey HSD Post-hoc test.

4.3.4. Effect of propagation substrate and biopesticides on cucumber growth

There was no significant interaction between the effects of substrate and biopesticide on root growth for non-infested cucumber plants ($p = 0.3004$). Cucumber plants propagated in coco coir had significantly less growth than those that were propagated in peat ($p = 0.0337$) (see Appendix C for data, Table A-5). Plants in the fall 2018 experiment had greater root growth (4.55) than the fall 2017 experiment (3.83) ($p < 0.0001$). There were no significant differences in root growth ratings among biopesticide treatments ($p = 0.3220$). SPAD measurements, which were measured only for the fall 2018 experiment, were affected by biopesticide treatments (Table

4-6). Plants treated with Regalia[®] had significantly higher SPAD measurements than Rootshield[®] or the water control ($p = 0.0003$).

Table 4-6. SPAD measurements of non-infested cucumber cv. ‘Straight’ plants treated with Rootshield[®] WP, Cease[®], Regalia[®], or a water control 21 days post-transplant for fall 2018 (n=30). Means with the same letter are not significantly different ($\alpha = 0.05$) as determined by the Tukey HSD Post-hoc test.

Biopesticide	SPAD	Std Error
Regalia [®]	40.3 a	0.3
Cease [®]	39.5 ab	0.4
Rootshield [®] WP	38.1 b	0.5
Water Control	37.9 b	0.5

4.3.5. Greenhouse Environment

In experiment 1 (cucumber) conducted in fall 2017, greenhouse compartment average day temperature was 24.1 °C (max: 26.3 °C; min: 22.1 °C) and average day relative humidity was 35% (max: 60.0%; min: 18.7%). The average night temperature was 23.1 °C (max: 26.2 °C; min: 21.8 °C) and average night relative humidity was 30.4% (max: 53.7%; min: 30.4%). For the cucumber fall 2018 experiment, the greenhouse compartment average day temperature was 23.2 °C (max: 27.1 °C; min: 18.2 °C) and the average day relative humidity was 40.0% (max: 87.1%; min 15.9%). The average night temperature was 21.5 °C (max: 23.5 °C; min: 18.2 °C) and average night relative humidity was 42.6% (max: 91.5%; min: 26.4%). 20.6 °C (max: 29.9 °C; min: 13.7 °C).

In experiment 2 (calibrachoa) conducted in winter of 2018, the greenhouse compartment average day temperature was 20.6 °C (max: 29.9 °C; min: 13.7 °C) and average day relative humidity was 31.3% (max: 76.0%; min 12.3%). The average night temperature was 18.7 °C (max: 21.8 °C; min: 7.9 °C) and average night relative humidity was 37.8% (max: 69.1%; min: 18.3%). For the calibrachoa replicate experiment in the fall of 2018 experiment, the greenhouse compartment average day temperature was 23.0 °C (max: 26.7 °C; min: 19.4 °C) and the average day relative humidity was 42.7% (max: 73.1%; min 18.0%). The average night temperature was

21.9 °C (max: 22.8 °C; min: 18.2 °C) and average night relative humidity was 39.6% (max: 72.8%; min: 16.5%).

4.4. Discussion

Results of this study reveal that there was an effect of substrate on *Pythium* root rot severity. Cucumber and calibrachoa plants propagated in Oasis[®] consistently had less root rot regardless of biopesticide treatment, suggesting that the chemical and physical properties of Oasis[®] did not provide the ideal environment for disease development, may have affected plant susceptibility to disease, or affected pathogen activity. Furthermore, plants propagated in coco coir had greater root rot across treatments. The use of coco coir as a propagation substrate, may cause plants to have a higher susceptibility to root rot disease compared to the other substrates. Even the non-infested plants propagated in coco coir had higher root disease (Table 4-4), meaning that either the coco coir was making the plants more susceptible to root disease or that the substrate was coming in contaminated with pathogens. The fact that plants propagated in Oasis had less root rot while plants propagated in coco coir had greater root rot could be due to the physical and chemical properties of these two substrates. These properties were not measured in this experiment but will be incorporated into future studies to examine what properties could be correlated to disease severity.

The primary producers of coco coir are India, Sri Lanka, Philippines, and Mexico. Due to this large distribution of production, there is a lack of consistency in the quality of coco coir products (Robbins and Evans, 2011a). Abad et al. (2002) saw significant differences in chemical properties between coco coir products coming from different countries, and even between regions of production. One of the most significant differences between coco coir and peat is the EC (electrical conductivity), a measure of the overall concentration of ions in the substrate. Abad

(2002) used the saturation extract method and found an EC of peat of 0.21 mS cm^{-1} while the EC of coco coir ranged from 0.39 to 4.82 mS cm^{-1} . A typical range for substrate EC is 0.5 to 3.0 mS cm^{-1} , however the EC of an unused substrate should be less than 0.75 mS cm^{-1} because the addition of fertilizer will drive up the EC (Robbins and Evans, 2011b). The high EC of coco coir media is predominately because of the high concentration of potassium, phosphorus, sodium, and chloride ions (Abad et al., 2002; Carlile et al., 2015). The high salt content may be correlated to the high root disease observed in plants propagated in coco coir. High salt content can burn the root tips and cause them to be more susceptible to root disease. Many coco coir companies will pre-wash the substrate to remove these salts but variations in salt content between products still remain. Future studies will record the substrate EC, leachate, and ion concentration throughout the experiment in order to determine if there is a correlation between these chemical properties and plant disease.

Oasis[®] rootcubes are made from a sterile, synthetic material suggesting that it may not support microbial activity in the same way as in peat and coco coir. To our knowledge, there is no research examining the physical and chemical properties of Oasis[®] nor its ability to support microbial communities, thus the correlation between low root disease and Oasis[®] is largely unknown. There is research on the development of microbial communities in rockwool, which is similar to Oasis[®] in that it is a sterile, synthetic substrate. Research suggests that microbial communities are largely absent in rockwool until a plant is introduced and then the microbial community dramatically increases (Calvo-Bado et al., 2006; Carlile and Wilson, 1991). Postma et al. (2002) observed that with the addition of a plant, nutrient solution, and outside contaminants (such as air contamination), the microbial population in rockwool increased up to 10^7 CFU (colony forming units) mL^{-1} in 2 days. There is a possibility that a 'natural' microbial

community is forming in the Oasis[®] prior to the biopesticide application and this could explain why we are seeing disease suppression even in the infested water controls. It is possible that neither the biocontrol agent nor the pathogen was able to establish in this environment because of competition from the natural microbial community or due to low food (carbon) source found in the substrate (Hoitink and Boehm, 1999). However, there is also the possibility that physical and chemical properties of Oasis[®] are contributing to low disease. Future research should measure these properties and evaluate the microbial community that is present in each substrate.

While not significant, a trend was observed in which biopesticide treatments reduced the root rot severity of plants propagated in coco coir compared to the infested water control. There was also a trend that the Rootshield[®] WP treatment decreased root disease compared to the infested water control across propagation substrates. This data was supported by our previous research examining the effect of cultivar on biopesticide efficacy discussed in Chapter 3. Krause et al. (2001) saw suppression of *Rhizoctonia* crown and root rot by *Trichoderma* spp. due to large *Trichoderma* population counts in all three substrates. Evaluating population counts of our biopesticides in replications of this experiment will highlight if similar effects are happening in our research. In both of our experiments, there were low root rot ratings for plants that were infested, which could be part of the reason why there was not a stronger effect of biopesticide on disease suppression. Root rot ratings around 50% or 2.5 would be ideal for biopesticide evaluation experiments. In some of these experiments, the biopesticide treatment appears to be making the root disease worse. This could be due to many different environmental factors that were not measured in this study and is representative of the problems with biopesticide performance variability (Fravel, 2005).

In future studies, chemical, physical, and biological properties of the substrates will be measured throughout the experiment, such as EC, pH, and moisture content, microbial population, and biopesticide colonization data. Future studies will examine the effect of substrate throughout the duration of the production cycle, not just during propagation, where the propagation substrate is the same as the growing substrate (i.e. coco coir plug into coco coir pot). This will allow researchers to determine if producing plants in these different substrates enhances the effect of substrate or if it is primarily at propagation that substrate affects disease and biopesticide efficacy. Rockwool will be included in this study to determine if this substrate has similar lower disease ratings as Oasis[®]. Variability in biopesticide efficacy could be partially explained by propagation substrate as well as other environmental factors that are unknown at this time. Oasis[®] seems to have decreased root rot disease while coco coir has increased root disease caused by *Pythium*. Rootshield[®] WP tends to decrease root disease severity compared to a water control but this was not statistically significant in these experiments. Further replication will provide data to aid growers on making decisions on which biopesticide to integrate into their IPM to improve their on-farm performance and crop production.

CHAPTER 5

CONCLUSION

The overall goal of this research was to better understand how variables like plant cultivar and substrate, affect the efficacy of biopesticides to suppress soil-borne diseases in greenhouse production. A greenhouse-based assay was used to test the hypothesis that plant cultivar and substrate will differentially influence the ability of microbial biopesticides to suppress *Pythium* root rot. In this research, tomato cultivar did not affect biopesticide suppression of *Pythium* root rot. Although studies have suggested that biopesticide efficacy may be correlated with plant susceptibility (King and Parke, 1993; Xue et al., 2014), the cultivar panel utilized in this experiment did not impact the efficacy of biopesticides, regardless of their susceptibility to *Pythium* root disease. These findings are similar to Larkin and Fravel (2002), who evaluated eight tomato cultivars with varying degrees of susceptibility to Fusarium wilt and did not observe an effect of cultivar on the efficacy of biocontrol agents to suppress the disease. However, it is hypothesized that a different cultivar panel representing greater genetic diversity that includes heirloom varieties and wild relatives may show a cultivar effect on biopesticide efficacy similar to those reported for wheat (Meyer et al., 2010) and Arabidopsis (Haney et al., 2015).

Propagation substrate did affect *Pythium* root rot severity. Plants propagated in coconut coir had greater root disease than those propagated in Oasis[®], regardless of biopesticide treatment. These findings suggest that chemical and physical properties of these substrates affect disease severity. These properties may affect the pathogen directly by inhibiting growth, or indirectly by affecting the native microbial community. In the latter case, the substrate may

impact beneficial microorganism population structure and function (such as production of anti-fungal enzymes) leading to an effect on biopesticide efficacy. In a study comparing microbial population dynamics, Koohakan et al. (2004) found significant differences in the indigenous microorganism populations of an organic substrate (coco coir) and an inorganic substrate (rockwool). Specifically, they found that coco coir had a higher population density of fungi while rockwool contained higher populations of fluorescent pseudomonads. The authors did not discuss the implications for disease control. In this study, plants propagated in the inorganic substrate Oasis[®] had low root disease across the treatments, especially in the cucumber studies. This may be due to its semi-sterile nature (Calvo-Bado et al., 2006; Postma, 2004) or a lack of food (carbon) source may have prevented the pathogen and biocontrol agent from establishing (de la Cruz et al., 1993; Hoitink and Boehm, 1999), however, more research is needed to understand the mechanism(s) behind these results. In future studies, rockwool will be added as another inorganic substrate to determine if there is a similar effect on disease.

There are studies that suggest that plant cultivar is an important driver of microbial community (Berg and Smalla, 2009; Garbeva et al., 2008) while other studies reveal that substrate is more important (Latour et al., 1996; Lundberg et al., 2012; Nallanchakravarthula et al., 2014). Both plant cultivar and substrate interact and influence the rhizosphere microbial community and are interconnected. Substrate can influence which microorganisms are present and thus effect differences in cultivar accumulation of beneficial species in the root zone (Meyer et al., 2010). Cultivar and substrate are thought to impact microbial biopesticides similarly to how they affect microbial community composition and function. It is likely that in this research, both cultivar and substrate were impacting disease severity and biopesticide efficacy. Future

studies are needed where multiple cultivars are utilized in substrate studies to determine if there is an interaction between cultivar, substrate, and biopesticide in suppressing root disease.

In all experiments, the commercial biopesticide Rootshield® WP appeared to suppress root rot under “normal disease pressure” compared to the infested water controls. These findings are supported by Krause et al. (2001), who saw suppression of *Rhizoctonia* crown and root rot by *Trichoderma* spp. due to large *Trichoderma* population counts in three substrates. Evaluating population counts in replications of these experiments will highlight if similar effects are happening in our research. Multiple studies have shown that biopesticides are not effective under high disease pressure (Harman, 2000; Rose et al., 2004), thus it is critical in studies evaluating biopesticide efficacy to maintain a ‘medium’ (~50% root rot) level of disease pressure. In some of these experiments, the biopesticide treatment appeared to be making the root disease worse. This could be due to many different environmental factors that were not measured in this study and is representative of the problems with biopesticide performance variability (Fravel, 2005). Future research could incorporate more than the three biopesticides examined in these studies to examine if there are greater differences in efficacy between products, species, or isolates.

This research provided preliminary data on the effects of cultivar and substrate on *Pythium* root rot severity and biopesticide efficacy. In addition, this research has highlighted the ‘unknowns’ of this research area and what questions still remain unanswered. These experiments have provided new information that can be used in future research to determine the mechanisms driving variation in biopesticides performance. Continuation of this research will lead to improved on-farm performance and adoption of biopesticides, thus decreasing farmers’ dependence on synthetic pesticides and enhancing the environmental sustainability of their production system.

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APPENDIX A

PARP V8 Recipe:

To prepare 1 Litter

Ingredient	Amount
V8 (clarified or normal)	200 mL
Agar	15 g
dH ₂ O	800 mL
CaCO ₃	2 g – 3 g

1. Add 300 ml V8 juice to a centrifuge tube and spin for 10 minutes at 4000 xg to clarify to get 200 ml clarified V8.
2. Add 2 g CaCO₃ to the clarified V8 and stir for 10 minutes.
3. Be sure the pH is between (5 and 6) since CaCO₃ can sometimes make the pH higher than (6) which will slow or prevent *Pythium* from growing on the medium.
4. Add 800 mL of water and add 15 gm Agar and autoclave.

Ingredient	Amount
Pimaricin	10 mg (0.01 g)
Ampicillin	250 mg (0.25 g)
Rifampicin	10 mg (0.01 g)
PCNB (Pentachloronitrobenzene)	10 mL of stock solution (50 mg)

5. While waiting for the autoclave to be done, prepare the following stocks
 - Pimaricin (10 mg/L), In a falcon tube dissolve 10 mg pimaricin in 10 ml of dimethyl sulfoxide (DMSO) or Methanol and vortex until dissolved. Use 10 ml of the stock solution to make 1 L PARP V8. **Important:** Pimaricin is light-sensitive and degrades in solution rather quickly. It needs to be stored at 4C and replaced every 2 months.
 - Ampicillin (250 mg/1L): dissolve 250 mg in 10 ml water (dissolvable in water) but use autoclaved H₂O. Use all 10 ml to make 1 L of PARP V8. It can also be filtered using a syringe. The stock must be stored at 4C.
 - Rifampicin solution (10 mg/L): In a falcon tube dissolve 10 mg rifampicin in 10 ml of dimethyl sulfoxide (DMSO) or Methanol and vortex until dissolved. Use 10 ml to make 1 L of PARP V8. **Important:** Rifampicin is TOXIC to humans, light-sensitive, and degrades in solution rather quickly. It needs to be stored wrapped in foil at 4C and replaced every 2 months.

- Pentachloronitrobenzene (PCNB) (100 mg/ 1L). Prepare a stock solution by dissolving 2 g of PCNP in 400 ml of heated 95% Ethanol. Heat the Ethanol first for few minutes before adding the PCNB but add it slowly. leave the mixture for about 30 minutes in a water bath 60 C to 70 C to totally dissolve (may need to stir to completely dissolve). Use 10 ml of this stock to make 1 L of PARP V8 agar. The stock can be stored at room temperature.
6. Allow the basal medium to cool to 55°C
 7. Using a magnetic stick, stir in these antibiotics to the cooled V8 in the listed order
 8. Pour into plates, use small amounts that just cover the bottom of the plate
 9. Allow to cool in a protected place, away from the light
 10. Store in black crisper in the fridge

APPENDIX B

Plant Growth Data – Chapter 2

Table A-1. Mean root growth (0-5 scale) of non-infested tomato cultivars after 21 days of growth (n=5). Means with the same letter are not significantly different ($\alpha = 0.05$) as determined by the Tukey HSD Post-hoc test.

Cultivar	Mean Root Growth	Std. Error
Wisconsin	5.00 abcd	0.00
Glamour	5.00 a	0.00
Bonnie Best	4.83 ab	0.17
Rutgers	3.80 abc	0.73
Komeett	2.90 bcd	0.19
Trust	2.67 cd	0.40
Ailsa Craig	2.00 d	0.00

Table A-2. Mean root growth (0-5 scale) of tomato cv. Glamour, 21 days post inoculation with three Pythium treatments (NDT1-1, KOP8, and a water control) and three inoculation methods (wound and drench, drench, and potato soil inoculum (PSI) (n=7). Means with the same letter are not significantly different ($\alpha = 0.05$) as determined by the Tukey HSD Post-hoc test.

Pythium Treatment	Inoculation Method	Mean Root Growth	Std. Error
NDT1-1	Wound + Drench	2.5 bc	0.31
NDT1-1	Drench	3.0 b	0.19
NDT1-1	PSI	2.3 bc	0.30
KOP8	Wound + Drench	1.7 c	0.21
KOP8	Drench	2.0 bc	0.24
KOP8	PSI	3.1 ab	0.46
Control	Wound + Drench	4.2 a	0.10
Control	Drench	4.2 a	0.10

Table A-3. Mean percent root growth of cucumber cv. Straight eight, 21 days post inoculation with three Pythium treatments (NDT1-1, KOP8, and a water control) and three inoculation methods (wound and drench, drench, and potato soil inoculum (PSI)) (n=7). Means with the same letter are not significantly different ($\alpha = 0.05$) as determined by the Tukey HSD Post-hoc test.

Pythium Treatment	Inoculation Method	Mean Root Growth (%)	Std. Error
NDT1-1	Wound + Drench	22.9 a	4.06
NDT1-1	Drench	23.6 a	6.79
NDT1-1	PSI	32.0 a	7.52
KOP8	Wound + Drench	17.1 a	4.06
KOP8	Drench	27.9 a	5.55
KOP8	PSI	77.0 b	4.36
Control	Wound + Drench	85.0 b	1.09
Control	Drench	77.1 b	4.98

APPENDIX C

Plant Growth Data – Chapter 4

Table A-4. Mean root growth (0-5 scale) of non-infested calibrachoa cv. Superbells ‘Lemon Slice’ plants propagated in Oasis[®] rootcube, Jiffy-7[®] peat pellet, or Jiffy-7C[®] coco coir 21 days post-transplant (n=44). Means with the same letter are not significantly different ($\alpha = 0.05$) as determined by the Tukey HSD Post-hoc test.

Substrate	Mean Root Growth	Std Error
Oasis [®]	3.6 a	0.17
Peat	3.7 a	0.16
Coco coir	2.9 b	0.13

Table A-5. Mean root growth (0-5 scale) of non-infested cucumber cv. ‘Straight eight’ plants propagated in Oasis[®] rootcube, Jiffy-7[®] peat pellet, or Jiffy-7C[®] coco coir 21 days post-transplant (n=40). Means with the same letter are not significantly different ($\alpha = 0.05$) as determined by the Tukey HSD Post-hoc test.

Substrate	Mean Root Growth	Std Error
Oasis [®]	4.2 ab	0.12
Peat	4.3 a	0.08
Coco coir	4.0 b	0.13