EVALUATION OF CHEMICAL AND BIOLOGICAL SUPPRESSION PROGRAMS FOR PYTHIUM LEAK IN MICHIGAN POTATO PRODUCTION AND SUPRESSION ASSAY OF *PYTHIUM* SPP.

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ABSTRACT

Michigan leads the US in production of potatoes for chips. Pythium Leak is the leading cause of storage losses in US potato production. Wet and warm conditions promote germination of zoospores in the soil where after the harvesting processes cause wounds or bruises, creating entry points for *P. ultimum* infection post-harvest. Continued use of metalaxyl or mefenoxam against populations of *Pythium* spp. in potato rotations cause concern for resistant to these fungicides. Six treatments were evaluated using the cv. Silverton Russet in South-West Michigan. First year data supported statistically higher disease incidence with Tifi Max, *Trichoderma atroviride*, compared to plots treated with Ultra Flourish. After plot replication, year two data were unable to show any statistical differences between treatments on agronomic attributes or disease incidence. Further in-vitro *Pythium* spp sensitivity assays were conducted with these treatments. The Orondis Gold™ treatment resulted in the lowest EC50 which could not be calculated at the lowest level 0.01 ppm because it inhibited all *Pythium* spp. growth. Next, Ultra Flourish™ reduced half the *Pythium* spp. growth at 0.32ppm. The least effective treatments at reducing pathogen growth were Elumin™ or Previcur Flex™, both resulting with EC50 values of 1.9 ppm and 7.9 ppm respectively. Five days post transfer *Trichoderma atroviride* overtook the *Pythium* spp., providing support as a possible biological suppression tool.

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CHAPTER 1. LITERATURE REVIEW

1.1 INTRODUCTION

Potato (*Solanum tuberosum*) is one of the most widely used food crops with important vitamins, simple and complex sugars, and other nutrients (Bohl & Johnson, 2010). Potatoes are also the most widely grown non-grain commodity and the fourth largest commodity behind maize, rice, and wheat for yearly usage in the world (Bradshaw & Ramsay, 2009). Commercial potato production starts by planting what are known as daughter tubers. Daughter tubers are clones of mother plant material providing growers predictability in the offspring's characteristics. Progeny will have the same ability as the mother tuber to produce offspring with the same shape, color, texture, and taste. Starting with plant propagation in the green house, four growing years are needed to supply the number of daughter tubers needed for a commercial field. Home gardeners also benefit from using daughter tubers as seed because it provides varietal uniformity regardless of the seed source.

North American and European growers led the world's potato production until the early 2000s (Navarre & Pavek, 2014). In the last 20 years Europe's potato exports have diminished, resulting in a reduction in planted hectares and a 25% (from 60% to 35%) reduction in world production (Navarre & Pavek, 2014). Furthermore, Navarre discusses from 1990 to 2010, China and Russia rapidly expanded production efforts with their vast land base aiding Asia's move to the top of world production. In Asia, much of the population is becoming urbanized (Yamashita, 2017) while at the same time a dietary shift away from cereals such as rice to more dairy, fruit, and vegetables like potatoes is occurring (Scott & Suarez, 2012). China's increased production efforts still cannot fulfill its needs and imports a large amount of potato products

(Navarre & Pavek, 2014). Some of the imported potato supply is being produced by and exported from the western half of the United States: Washington, Oregon, Idaho, and California. High west coast regional exports created the need for other areas like the Midwest to supply the continental US with potatoes.

Supporting the increase in need, Michigan potato growers' ability to store their crop after harvest for 11 months has created a stable supply for demand. To meet this increased demand for potatoes, large seeding rates are utilized to increase production (0.55 metric tons of seed tubers per hectare). Large amounts of seed, in turn, increased the need for a local seed supply. Michigan currently has 19,100 hectares of potato production, of which seed production contributes 1,000 hectares (America., 2018).

Potato seed production starts in a greenhouse. Small petiole leaflets are cut off disease free plants then placed into agar until they grow roots and transform into plantlets (Frost et al., 2013b). These small plants will produce daughter mini tubers (small pea to dime size tubers) that are then planted at seed farms. Four to five years of tuber increase at a seed farm are needed to produce enough seed for commercial growers (Bohl & Johnson, 2010). Seed growers are strategically placed in isolation away from much of the commercial production in a state. Isolation assists in keeping the plants disease-free before commercial planting (Frost et al., 2013a). Michigan seed growers are spread across the northern upper peninsula as well as, northern counties in the lower peninsula. Seed produced in Michigan is shipped and planted across the Midwest and east coast from Michigan to Florida.

In total cwt (count weight or hundred wight) produced, Michigan is eighth in the country behind: Idaho, Washington, Wisconsin, North Dakoda, Oregon, Colorado, and

Minnesota (Services, 2017). Western states have higher yield potential with a longer growing season and conducive climates through lower night temperatures promoting more starch synthesis (Bohl & Johnson, 2010). While US potato exports remain stable around 1 million metric ton from 2015-2019 (USA, 2020) any production overages in the western US drives potato pricing down, offsetting transportation cost for processers to move potatoes into eastern markets. Jason Walther the CEO of Walther Farms explained that Michigan has been able to increase table stock production because of increasing freight costs from western regional supply. All the while, the growing consumer demand for locally grown produce has also stimulated an annually increase in table market supply for Michigan growers (Walther, 2020). He further expounded upon new service opportunities for Michigan table stock potatoes. Michigan growers can pack potatoes with same-day delivery to one-half of the US population, while western supply is requiring four to five days for delivery. Although eighth in total production, Michigan produces the most chip processing potatoes in the US. On average, 25% of the US potato chips produced are from Michigan potatoes (Michigan, 2019).

Potatoes have fewer fine root hairs and shallow rooting structures, requiring 50-60 cm of precipitation over the growing season (Navarre & Pavek, 2014). On average Michigan growers receive 20-30 cm of rainfall within a growing season and irrigation in required to supplement crop needs between rains (Po et al., 2010). Increasing the need for irrigation, potatoes are primarily grown on well drained sand avoiding tuber rot caused by saturated soils after heavy rains. Abundant water reserves and pockets of well drained sandy soils have aided Michigan growers to become a reliable locally supply of potatoes for processers in the east. The investment cost for irrigation can reach upwards of $2,500 to $3,700 dollars per hectare (Rain, 1999). To recoup this investment costs, growers adjust crop rotations adding high-value row crops and vegetables into their crop rotations such as dry beans, carrots, and sugar beets. When growers focus on growing the same high-value crops with shorter rotations difficulties with soil-borne diseases may occur (Bohl & Johnson, 2010).

1.2 CROP ROTATION DISEASES

Sclerotinia sclerotiorum, the cause of White Mold, is a necrotrophic ascomycete fungal pathogen that causes serious, yield-reducing disease in many different dicotyledonous crops. Even in a semi-arid macroclimate of Eastern Colorado or Western Nebraska, white mold creates difficulties for potato growers in rotation with beans, peas, and other dicot crops as a result of the need to irrigate (Blad, 1978). Ćosić et al., demonstrate the persistence and viability of *S. sclerotiorum* sclerotia germination, surviving for up to 3 years or more in soil (Ćosić et al., 2012). Figure 1 shows sclerotia can germinate hyphae to directly infect at ground level or produce apothecia, cup like structures. Each apothecium holds thousands of asci containing ascospores. When apothecia are mature, their ascospores can be ejected out a few centimeters and further spread by rain and irrigation.

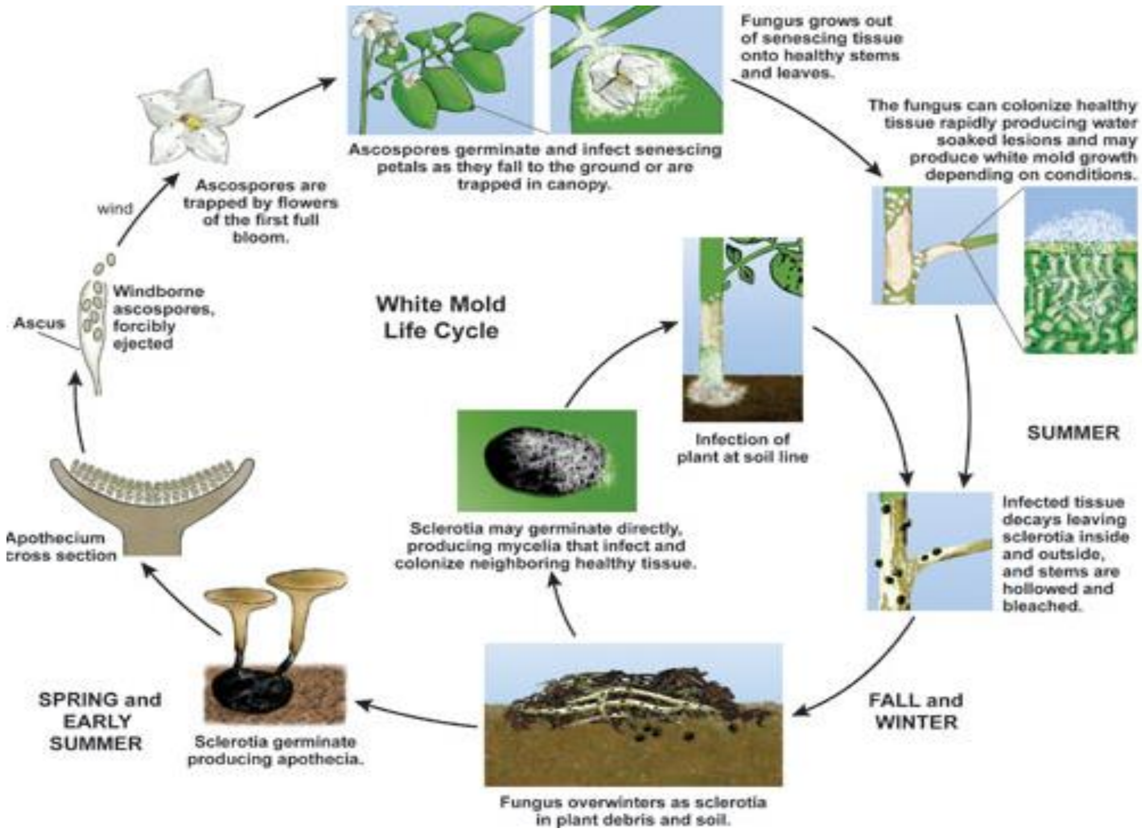

Figure 1. Life cycle of S. *sclerotiorum* (Kirk, Michigan State University Extension)

Figure 2. Picture showing White Mold water-soaked lesion resulting from S. *scleroitiorum* ascospores collected on a potato flower then discarded into the mid canopy causing disease under favorable climate (Brice Stine, Walther farms)

Sclerotinia ascospores land on flower petals that serve as an infection court for this pathogen. The petals fall into the cool and moist microclimate of the canopy where they serve as a food supply for the pathogen aiding in the infection process of the germinating ascospores. *Sclerotinia* commences its destruction at night or early morning when the mid canopy of the host crop has cool temperatures and very high humidity (Schwartz, 1989). Figure 2 shows the result of a flower covered with ascospores incubating on a potato leaf in the mid canopy for some time. The spores germinate and hyphae can produce enzymes to soften the cuticle and then grow into the host tissue. Flowers also fall to the bottom canopy or the ground where it is much easier for hyphae to infect shaded senescing chlorotic leaves or necrotic tissue. After infection *S. sclerotiorum* will produce an abundance of white fluffy mycelium that looks like a white mold on infected tissue. *S. sclerotiorum* mycelium is unable to overwinter on plant material as mycelium, at the end of the cropping season mycelium will generate specialized overwintering structures called monilioid cells, creating hard overwintering sclerotia inside the stems of infected plants (Li & Chen, 2011).

Another sclerotium-producing fungus affecting potatoes and its rotational crops is *Rhizoctonia solani*. This pathogen affects over 500 species of plants, including many vegetables like sugar beets, carrots, and potatoes (Uchida, 2011). R. *solani* causes a damping off or canker, girdling the stem or stolons of potato plants. When R. *solani* is left unchecked in a potato crop, the pathogen can cause delayed emergence or reduce the number of plant stems to directly affect yield (Banville, 1989). *Rhizoctinia* is a basidiomycete, but its telemorphic stage is very rare and no asexual spores are produced. In addition to forming black sclerotia, a notable characteristic is 90 degree angle branching of septate hypha without clamp connections

(Stalpers & Andersen, 1996). Figure 3 shows the life cycle of *R. solani*. The sclerotia of *R. solani* are modified monilioid cells surrounded with woven hyphae producing a tough over wintering structure (Tu & Kimbrough, 1975).

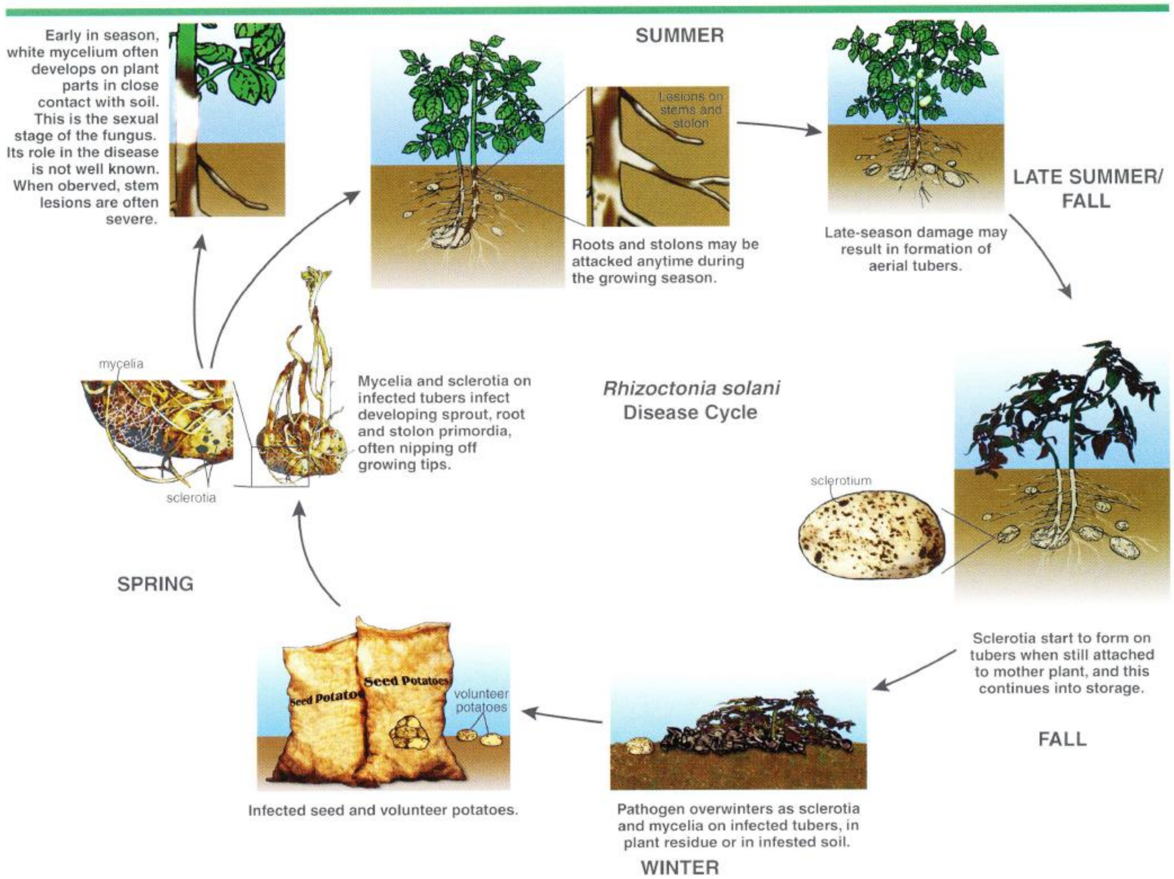

Figure 3. Showing life cycle of R *solani* on potato (MSU Extension Bulletin E-2994, 2007)

Figure 4. Photo shows R. *solani* sclerotia attached to the tuber periderm
(MSU Extension Bulletin E-2994, 2007)

Rhizoctonia solani is a major pathogen in potato production causing root rot, stem cankers and the formation of black sclerotia on the surface of daughter tubers (Gudmestad et al., 1979). *Rhizoctonia*, will produce sclerotia on the tuber so starting with clean disease-free potato seed or seed with less than 5% sclerotia coverage is the best way to avoid infections (Secor & Johnson, 2008). Figure 4 shows a daughter tuber with sclerotia covering more than 5% of the tubers surface. Seed this severally infested should be avoided, minimizing the risk of transferring the pathogen to daughter tubers in seed and commercial production. *Rhizoctonia solani* has been shown to cause aerial tubers after the pathogen killed off stolons of potato plants with canker formation. This results in a shift of source-sink relations causing tubers to form on leaf axils rather than on stolons (Banville et al., 1996). The Azoxystrobin class of fungicides used in-furrow or banded over the seed can reduce infection from seed borne sclerotia. Damping off caused by *R. solani* is most common in cool wet soils, so planting date of potatoes is an important piece of the puzzle to consider. Cankers can girdle stems, causing the plant to use more seed piece energy to regrow sprouts under the lesion. This extra required energy weakens plant vigor causing a loss of valuable days in the growing season.

Similar to *Rhizoctonia solani*, *Pythium* spp. cause a dampening off diseases of a wide host rang. *Pythium* species are not true fungi but oomycetes commonly known as water molds (Schumann & D'Arcy, 2006). *Pythium* spp. can reproduce asexually producing sporangium that can directly infect hosts or the sporangia can produce infectious zoospores. Zoospores have flagella on the front and back propelling it through water (Fry & Grünwald, 2010). The persistence in soil of *Pythium* spp. results from sexual reproduction and the formation of oospores (Ayers & Lumsden, 1975). Shown in figure 5, oospores can cause infections themselves or produce sporangium to infect. *Pythium* spp. sporangia can also produce asexual zoospores that can infect the plant. Figure 5 also depicts *Pythium* spp. paragynous characteristics. The antheridium is attached to the side of the oogonium on adjacent hyphae just prior to fertilization. The close proximity of antheridium and oogonium increase the likelihood of sexual reproduction. Paragynous attributes aid the oomycete the ability to be homothallic, easily mating with itself producing overwintering oospores. The thick cell walled oospores can withstand extreme climate changes providing inoculum many years after initial infection (Fry & Grünwald, 2010). Infections can occur with sporangia in the splash zone on the soil surface near wounds and natural plant openings or underground on hair roots and root tips in soil solution.

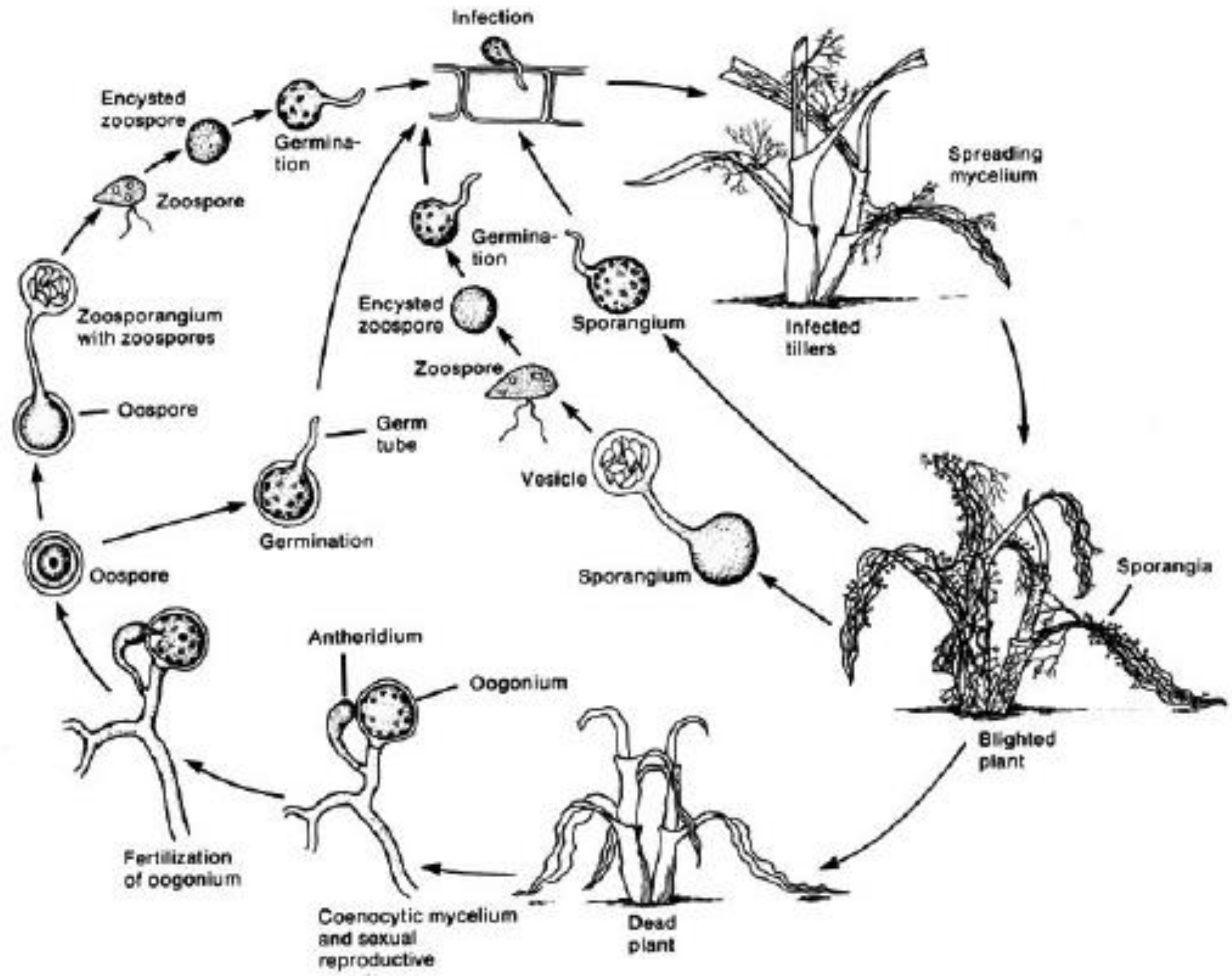

Figure 5. Life cycle of P. ultimum (Beckerman, Purdue Extension Bulletin BP-181-W, 2011)

Pythium aphanidermatum has been isolated from damped off plant roots but mainly resides in warm dry desert soils (Olsen, 1998). *P. ultimum* predominantly causes most problems with dampening off diseases for cropping systems in Michigan (Johnson & Halloin, 2001). Damping off most genially occurs when soils become saturated after heavy spring rains causing pre or post emergence disease (Zitnick-Anderson et al., 2015). Oospores germinate under high soil moisture conditions producing sporangia or zoospores that directly infect roots or stems of susceptible plants (Groves & Smith, 2013).

1.3 INCREASED INCIDENCE OF PYTHIUM LEAK

Pythium u*ltimum* has hosts across the US. This pathogen has been found from California in pumpkin productions (Tompkins et al., 1939) to Michigan in greenhouse ornamentals (Del Castillo Múnera et al., 2019). *Pythium* spp. move by water, wind, or people moving soil. Poorly sanitizing equipment or moving contaminated rootstock are ways for this pathogen to move into new cropping systems. Persistence in soil as oospores and a vast host range has made *Pythium* species impossible to eradicate once established.

Changes in weather patterns in recent years favor *P*. *ultimum* growth in Michigan. Mild winters have not promoted adequate ground frost needed to kill hosts left in fields post-harvest. Potato tubers have been shown to survive until temperatures drop below -2C for a period of 24 hrs (Olsen et al., 2001). Under mild-winter conditions, the Great Lakes do not freeze over providing an abundant source of lake effect snow covering the soil surface. Snow acts as insolation, preventing the ground from freezing when air temperatures are conducive for ground frost. Although *P*. *ultimum* oospores will survive extremely cold weather, utilizing cold weather to freeze out and kill volunteer potatoes eliminates a continuous host to survive on and potentially could lower the pathogen population in the soil the following year.

During the growing season, violent weather patterns move quickly across regions and can drop an abundance of precipitation. In the state of Michigan, average precipitation in June has increased by 0.76 cm over the last century (MDOT, 2011). Most of the increase has occurred in just the last decade. Well drained soils are becoming saturation or flooding more often due to these storms depositing 7cm to 12cm over night (MDOT, 2011).

Selection by plant breeders and industry leaders for yield with smaller size profiles possibly amplifies problems with tuber diseases. Higher producing varieties tend to take longer to mature, forcing producers to lengthen their growing season. Adding days to the growing season will come at the expense of shortening the harvest season. Abbreviated harvest seasons reduce the days tubers have to naturally set the skin on daughter tubers prior to harvest. Thinner periderms surrounding the tuber increases the risk of storage disease. During harvest, thin peridium slips much easier allowing pathogens direct access into flesh to cause infection and disease post-harvest. Continued selection of new varieties strictly on agronomic characteristics without considering disease suppression impacts disease incidence. Potato cultivars differ in their abilities to deposit suberin aiding in the thickening of tuber periderm (Ezekiel et al., 2004). Line selections of the same cultivars have shown variation in potential to produce and thicken tuber periderm before harvest (Thangavel et al., 2016).

1.4 PYTHIUM LEAK SUPPRESSION PRACTICES

Once *Pythium* spp. become established in production settings, some cultural practices can suppress disease caused by this pathogen. Reducing disease-conducive environments greatly limits exposer of diseases caused by *Pythium* spp. (Groves & Smith, 2013). For example, avoiding low lying areas or installing drainage tile, thereby increasing soil drainage for cropping systems, can reduced the severity of Pythium diseases. In potato production, cut seed tubers should be allowed to heal at least 48 hours before planting. Suberization of freshly cut surfaces adds to the protection against infection of *Pythium* spp. (Jones, 1935). Limiting late season nitrogen also promotes naturally maturing of the crop, assisting to stimulate periderm set on daughter tubers, thus lowering the likelihood of tuber injury and infection at harvest. Calcium fertilizers are used in strengthening cell walls of tubers, providing added defense against hyphae penetration and tuber decay (Simmons et al., 1988). Variety selection could similarly limit disease incidence in russet production after harvest (Hollingshead et al., 2020). Salas showed no differences in post-harvest incidence of *P*. *ultimum* infection among the russet and red varieties but provided evidence of differences in round white varieties. Incidence of Pythium Leak was highly significant (P = 0.001) and supported advantages with growing the round white cv Snowden, 33% incidence of infection, compared to Pike, 95% incidence of infection (Salas et al., 2003).

Growers have also adopted chemical protection for Pythium Leak management. In the past mercuric chloride was used on fresh cut seed to stop pathogen growth into the tuber (Jones, 1935). Although effective, the use of heavy metals like mercury is no longer approved for use. Jones's data showed success with treatments using copper sulfate to control infection, but the seed pieces showed damage due to copper toxicity. Metalaxyl provided control in the early 1980s against *Pythium* spp. in many different cropping systems across the United States. In the Pacific North West (PNW), metalaxyl and mefenoxam were used in cereal production protecting against dampening off caused by *Pythium* sp. and was used in potato production to aid in control of Pythium Leak (Porter et al., 2009). Porter et al. reported that continual use of this chemical year after year in multiple cropping systems raised concerns that mefenoxam resistant populations of *Pythium* spp. were developing. Research on efficacy with new chemicals such as Orondis™(Syngenta) or Elumin™(Valent) could be used to suppress Pythium Leak in potato production but more research is needed to find their fit for resistance management.

Knowing what chemicals are available for production agriculture and their efficacy is extremely important for producers. In addition, the EPA has reformed chemical registration, and they now look closely at environmental impacts new chemistries as well as efficacy. Pesticides targeting specific characteristics of pathogens or insects lower the amount of chemical needed for effective suppression (Damalas & Eleftherohorinos, 2011). Chemicals showing suppression against certain oomycetes like *Phytophthora* spp. are sometimes ineffective on other oomycetes such as *Pythium* spp. resulting in negligible suppression of Pythium Leak in potato production. Johnson et al provided evidence that phosphoric acid products like Phostrol™ (NuFarm) displayed activity on *Phytophthora* species such as P. *infestans* causing potato late blight and P. *erythroseptica* causing pink rot. However, Phostrol™ provided no protection against Pythium Leak (Johnson et al., 2004). Revus™ and Bravo™ are two products from Syngenta offering protection against *P. Infestans* in potato production but neither show suppression of *Pythium* spp. on their labels.

Pythium Leak is a major storage problem that needs more research (Porter et al., 2009). *Pythium* u*ltimum* needs an open wound or lenticel to penetrate the tuber so little to no field infection occurs during the growing season. Having little to no infection in fields during the growing season gives growers a false sense of security going into storage. During harvest, abrasive sand and tumbling on conveyers produce superficial skinning or cuts to the periderm. In these areas of the tuber, soil and pathogen spores will collect on the tuber flesh where infections can start. Tubers are normally harvested at temperatures between 9 ^{o}C to 21 ^{o}C which fall within the growth range of *P*. *ultimum* of 4 ^{o}C to 40 ^{o}C (Tompkins et al., 1939). Heavier tuber infections occur at harvest temperatures near 21 ^{o}C, closer to *P*. *ultimum's* optimal growth temperature of 25 ^{o}C to 28 ^{o}C (Tompkins et al., 1939). Under ideal conditions Pythium Leak symptoms can occur within 48 hours (Jones, 1935).

At harvest, tubers are placed in bulk climate-controlled storages. Throughout the storage season tubers are cooled to a temperature between 3 ^{o}C to 9 ^{o}C, depending on tuber purpose. Cooling tubers during the storage season aids in minimizing the risk of spoilage over time from storage pathogens such as *Pectobacterium, Pythium* spp.*, Phytophthora* spp. and *Fusarium* spp. In the first seven to ten days of the storage season, growers stabilize pile temperatures near 10 ^{o}C with 95%+ humidity. Artschwager demonstrated with high humidity, no differences in new suberin layers existed in wounded areas of different varieties when stored above 10 ^{o}C (Artschwager, 1927). While storage temperatures were under 7 ^{o}C , suberin layer formation was delayed 3 days on the variety Irish Cobler and more than 6 days in the other varieties. Artschwager's data indicated that deposition of new suberin layer across wounded areas occurs best at temperatures at or above 10 ^{o}C. The suberization layer is important for host defense against pathogens (Yang & Bernards, 2007). Although growers try to maintain high humidity with specialized equipment, suberization is also important to prevent dehydration of tubers while in storage. When passing cooler air containing less humidity over the surface of tubers cooling the pile, moisture is taken out of the tuber by concentration gradients. The newly formed periderm also provides a barrier from the environment slowing dehydration over the storage season.

1.5 PYTHIUM LEAK SYMPTOMOLOGY

P. ultimum penetrates host tissue poorly and will not normally cause tuber infection without wounding or enlarged lenticels (Tompkins et al., 1939). When soils become saturated in late summer or fall, tuber lenticels swell open. Tuber lenticels allow for gas exchange of carbon dioxide out of the tuber and oxygen in, but they also allow an entry point for pathogens. Although some infection can take place in the field through lenticels, most infection occur after wounds are created during the harvest. *P*. *ultimum* hyphae will grow under a wide range of temperatures, 4 ^{o}C to 40 ^{o}C, with 25 ^{o}C to 28 ^{o}C being the most optimum for stimulating growth (Tompkins et al., 1939).

Once established in the host, *P*. *ultimum's* hyphae produce enzymes that degrade cell walls and facilitate pathogen spread throughout the tuber. Most tubers will show symptoms within 48 hours of infection (Jones, 1935). *P*. *ultimum* removes sterols from host cell membranes as it infects, and this causes membrane damage and the leakage of cytoplasmic material (Schlösser & Gottlieb, 1968). *P. ultimum* is a necrotroph, the pathogen begins to feed on the host cell once dead. The disease name Pythium Leak refers to the excretion of fluids from infected tubers while under slight external pressure (Salas et al., 2003). After being cut open, the infected tissue will turn to a smoky or light black color and have a watery sheen look. There is no smell with genuine Pythium leak. According to Gudmestad, pathogens like

Pectobacterium begin to flourish in the infected tuber with the abundant free moisture created by Pythium Leak symptoms allowing secondary infections and a foul smell to infected tubers (Gudmestad, 2019).

Once infections take place, Pythium Leak cannot be reversed or cured. After tubers are harvested and placed into storage, infected tubers will eventually breakdown and rot (Olsen & Thornton, 2002). Figure 6 shows surface symptoms of infected tubers. One can see slight mycelium growth and free moisture in the infected areas of the tuber. Figure 7 shows tubers from Figure 6 cut in half. The infection originated on the outsides of tubers in wounds. Over time Pythium Leak progressed nearly through the entire tuber, causing tissue to turn smoky gray and excrete liquid. A suberization layer is also important to prevent storage diseases like bacterial soft rot and Pythium Leak from spreading to surrounding heathy tubers in storage. As Pythium Leak tubers breakdown, fluids are excreted due to pressure from surrounding tubers. In this fluid, P. *ultimum* zoospores as well as, bacteria coat adjacent tubers. Excess fluid covering adjusted tubers causes damp storage conditions and micro-climates around pockets of Pythium Leak infections. In these conditions, secondary pathogens gain access through wounds or lenticels to cause more infection, perpetuating storage diseases (Boyd, 1972).

Figure 6. image of Silverton Russet tubers infected with P. *ultimum* post-harvest. (Brice Stine, Michigan State University)

Figure 7. Image of Figure 6 tubers cut in half showing internal disease symptoms of Pythium Leak. (Brice Stine, Michigan State University)

The objective of this research is to evaluate multiple products for their ability to suppress Pythium Leak, as well as any additional agronomic benefit they may provide in potato production. The current standard chemical management of mefenoxam in-furrow seems to be diminishing and questions of resistance are being asked by growers (Ritchie, 2020). Each product trialed presented a different mode of action, potential providing growers with additional chemicals for resistance management. Biologic suppression of soil-borne diseases have been documented in other cropping systems (Aydin, 2019, Whipps et al., 1993) therefore, *Trichoderma atroviride* was trialed as a possible biological option against Pythium Leak.

A topic typically not discussed by growers is the effectiveness of their delivery system for chemical suppression in a changing environment. What growers see as potential chemical resistance as a result of more disease at the end of season might not be resistance but actually, the result of many different inhibitory factors working against the chemical efficacy throughout the growing season. In the third chapter, resistance to mefenoxam is evaluated and possible reasons for efficacy loss are discussed. As environmental factors change in growing regions, cultural practices and chemical delivery methods that worked in the past may not currently provide most effective means of disease suppression. New chemistry proves alternate modes of action but, it can sometimes be added to current chemistry for synergistic effects.

CHAPTER 2. EVALUATION OF MANAGEMENT PROGRAMS FOR PYTHIUM LEAK IN MICHIGAN POTATO PRODUCTION

2.1 ABSTRACT

Michigan leads the US in potato chip production. One out of every four potatoes converted into chips are produced in Michigan. Pythium Leak is the leading cause of storage losses in US potato production. Pythium Leak is caused by *Pythium ultimum* in temperate growing regions like Michigan. *Pythium* spp. are efficient saprophytes living off dead and decaying plant material and overwintering via oospores in the soil in the absence of a plant host. Wet and warm conditions promote germination of zoospores in the soil. Harvesting processes that cause wounds or bruises create entry points for *P. ultimum* infection post-harvest. FRAC group 4 fungicides historically have been used to control *Phytophthora erythroseptica* and *P. ultimum* in many cropping systems including potatoes, wheat, and corn. Continued fungicide use has slowly allowed specific populations of *Pythium* spp. to gain fungicide resistance. In furrow application programs using chemical and biological products are effective on other oomycetes like *P. infestans* and *P. erythroseptica*. Six treatments were tested using the Pythium leak susceptible cv. Silverton Russet in South-West Michigan where mefenoxam resistant populations of *Pythium* spp. have been reported. Five FRAC groups (4, 21, 22, 28, 49) and one biological fungicide (with the active ingredient *Trichoderma atroviride*) were evaluated. First year data did not show statistical differences in disease incidence between the non-treated control and treated plots. Statistically greater yield was recorded in plots treated with Tifi Max (*T. atroviride*) as compared to plots treated with Orondis Gold. Tifi Max treatments had greater incidence (%) of infection post-harvest compared to Ultra Flourish.

After treatment replication, year two data were unable to show any statistical differences between treatments on agronomic attributes or disease incidence.

2.2 INTRODUCTION

Potatoes are a complete and nutritious food source providing more protein and food energy compared to cereals (Douches, 2015 January). The harvested portion of a potato crop is located underground, lighter sandy soils with low organic levels are chosen for production to reduce crop loss after heavy rains. Michigan's well drained sandy soils and cool nights produce high gravity potatoes desired by potato chip manufacturers. Michigan grows roughly 19,000 hectares annually with one out of every four potatoes converted into chips coming from the region (Michigan, 2020). Michigan rakes fifth behind Idaho, Washington, Wisconsin, and Oregon for potato tons produced in 2019 (Service, 2020).

An integral part of Michigan potato production is storage capability following harvest. Shipping potatoes out of storge during the winter months, bridges a gap from freshly dug field potatoes. Fresh crop potatoes are dug and shipped to processors right out of the field after being washed and foreign materials are sorted out. Stored potatoes exclusively supply potato needs from early November through March when southern production regions are able to fresh dig the new crop once again. Michigan storage potatoes, reside in climate-controlled facilities over the storage period, provide a protected supply for processors as compared to newly harvested potatoes from southern regions that subject to daily environmental fluctuations. Michigan storage supply is utilized by processors throughout the winter months up to late June or early July.

Increasing precipitation and rising temperatures in Michigan's growing region favor soil pathogens like *Pythium ultimum* which increase the incidence of post-harvest tuber disease. More importantly, warming autumn harvest temperatures produce the greatest risk for P. *ultimum* (Winkler et al., 2018). Warm autumn soil temperatures during potato harvest correlates with *P. ultimum's* optimal growth temperature of 25-28^{o}C (Hollingshead et al., 2020). Potatoes harvested from warmer soils have an elevated risk of infection and Pythium Leak symptoms due to resulting warmer storage temperatures and high humidity. Pythium Leak causes substantial losses in storge facilities in US potato production (Taylor et al., 2008). P. *ultimum* generally cannot penetrate the tuber's periderm naturally, requiring openings such as lenticels or wounds created at harvest to invade past the tubers outer defenses (Taylor et al., 2004).

*P*ythium *ultimum* rapidly consumes the host once infection is established. For example, Tompkins et al. reported complete fruit collapse in pumpkins after six to ten days post fruit inoculation (Tompkins et al., 1939). Pythium Leak symptoms in tubers can be observed within 6 days post-harvest (Salas et al., 2003). The aggressiveness of *Pythium* threatens the storability and longevity of all storage potatoes. The shortened storage longevity risks recently developed markets for Michigan potatoes due to higher transportation costs and ability to provide year-round same day supply for half the U.S population (Walther, 2020).

Declining Pythium Leak suppression in Michigan potato production and a need to extend the storage season to meet processor needs, products from six different FRAC groups were evaluated on a commercial farm in Southwest Michigan to determine effectiveness when applied early in the growing season. Disease suppression was evaluated by evaluating percent incidence after harvest to determine the best options for protection against Pythium Leak. Agronomic differences were also evaluated between the treatments for added incentive for growers to change current practices.

2.3 MATERIAL AND METHODS

Field trials were established 22 May 2019 in a commercial potato field near Dowagiac, MI (42.018462, -86.073213) and 5 April 2020 (41.946526, -85.570887) near Three Rivers, MI evaluating in-furrow fungicides for post-harvest Pythium Leak suppression (Table 1-3). US#1 'Silverton Russet' tubers were mechanically cut into seed pieces weighing approximately 56-grams on 15 of April 2019 and 20 of March 2020 and allowed to heal for 2 weeks before planting. Trials were conducted using potato cultivar Silverton Russet due to known susceptibility to Pythium Leak and commercial use throughout the state of Michigan. A randomized complete block design with five replications was used for the experiment with each plot consisting of four 15-meter-long rows spaced 86 cm apart and seed pieces planted 25 cm apart. Plots were planted in 2019 with a Checchi & Magli F300L 2 row carousel planter. Due to university COVID-19 restrictions in 2020, Mid-Michigan Agronomy assisted with plot planting, treatment application, and harvest. Second-year plots were planted with a Lockwood 2 row pick planter. Treatments were applied with a CO_2 backpack boom sprayer in an open furrow. In-furrow treatments were sprayed over the seed pieces then covered with soil by using a hilling cultivator to close the furrow. In-furrow at-planting applications of fungicide were delivered with a hand-held R&D spray boom delivering 93.5 L/ha (345 kPa) and using one XR8002VR nozzle per row.

Two treatments (Ranman™ and Elumin™) required a post emergence application. Each treatment was mixed with water and dribbled over the top of hills at 50-100% potato emergence with a hand-held R&D spray boom delivering 93.5 L/ha (345 kPa) using one TP002-SS (by TEEJET) nozzle per row. Fertilizer was spread as needed for the commercial field surrounding the plot pre-plant and based soil tests taken the previous autumn. Additional nitrogen (N 56kg/ha) was applied to the growing crop with irrigation 45 DAP (days after planting).

A non-treated control was compared with 6 different treatment programs to evaluate efficacy in suppression of Pythium Leak in storage after harvest. Protectant fungicides such as Bravo WS™ (Syngenta) and Penncozeb™ (Nufarm) were applied at labeled rates to the commercial field and plot on a seven-day interval starting at 14 DAE until vine kill two weeks prior to harvest. A total of 10 applications were made by airplane for foliar disease control in year one of the study and 12 applications in year two. In 2020 Italpollina reformulated Tifi™ to Tifi Max™ doubling the cfu (colony-forming units) concentration per kg. Continuing the treatment with equal cfus as plots in 2019, Tifi Max™ was applied at 0.09 kg/ha in 2020.

A pre-emerge herbicide blend of Dual 8E™ (Syngenta) at 2.1 L/ha 10 DAP and Metribuzin75DF™ (MANA) at 280 gram/ha were applied at 10 days after planting (DAP) to the commercial field and plot area. A post-emerge herbicide application at 45 DAP of Metribuzin 75DF ™ at 280 gram/ha and Matrix™ (Dupont) at 105 gram/ha was applied to the commercial field and plot area for additional weed control. Insects were controlled with Admire Pro™ (Bayer) at 490 ml/ha at planting, Baythroid xL™ (Bayer) 105 ml/ha 45 DAP, and Reveal Endurx™ (Innvictis) 210 ml/ha 70 DAP. Plots were irrigated with overhead sprinkler irrigation in both 2019 and 2020 to achieve daily crop needs with an accumulative irrigation and precipitation total of 1.3 cm per 4-day period. In 2019 a vine kill application with Reglone™ (Syngenta) at 1.4 L/ha was made by a commercial ground rid applicator on 27 August, 104 DAP, and 2 September, 110 DAP, completely desiccating the crop 10 days prior to harvest. In 2020 a vine kill application with Reglone™ at 1.4 L/ha was made by a commercial ground rid applicator 18 August, 142 DAP, and 23 August, 147 DAP, completely desiccating the crop 9 days prior to harvest.

Plots (1 row x 7.5 meter of row) were machine-harvested on 12 September 2019 at 120DAP with individual treatments graded by size, weighed, bagged and transported to Clarksville Research Center. In 2020, plots (1 row x 7.5 meter of row) were lifted out of the ground with a bulb lifter. Tubers were manually separated into size A and B with harvest weights recorded. After weighing, size A and B samples were combined and stored at Mid-Michigan Agronomy storage facility in Dewitt Michigan. For both years, tuber pulp temperatures were 22-24^{o}C during the harvest periods. Soil moisture at harvest time was determined to at 80-85% by hand ball method in 2019 and 75-80% in 2020. Once at the storage site, all bag samples were opened, tubers from individual plots were counted and placed into plastic crates, and tubers stored on shelving at 18^{o}C for three weeks to avoid storage effects on treatments. After the storage period of three weeks, each tuber was evaluated for symptoms of Pythium Leak and counted to compile percent incidence of Pythium Leak.

2.4 RESULTS

Environmental variables were measured in 2019 with the nearest Campbell weather station located in the Dowagiac, MI (table 4). Average daily air temperatures (^{o} C) were 14, 21, 23, 21 and 17 (May, June, July, August, and through 13 September respectively) and the number of days with temperatures >32 °C over this period was 0 for each month except 6 days in July. Average daily soil temperature at 10 cm depth (°C) over this period was 16, 24, 27, 26 and 21 for months May through September respectively. Monthly precipitation totals (cm) over the same period were 11.3, 4.3, 5.0, 8.8 and 2.8 (May through September respectively).

In 2020 environmental variables were measured with the nearest Campbell weather station located in the Mendon, MI (table 4). Average daily air temperatures (°C) were 7, 14, 22, 24 and 22 (April, May, June, July, August, respectively) and the number of days with temperature >32 °C were 8 in June, 10 in July, and 1 in August. Average daily soil temperature at 10cm depth (°C) this period was 8, 14, 21, 24 and 22 (April through August respectively). Monthly precipitation totals (cm) during this period were 7.2, 17.2, 7.5, 11.8 and 9.3 (April through August respectively).

The 2019 plots showed uniform emergence 89-95% across all treatments (table 1). Although lower in 2020 at 80-83%, plant emergence was uniform as well (table 2). Tables 1 and 2 also show Orondis Gold™ (Syngenta) plus Ultra Flourish™ (Nufarm) with the lowest percent emergence in 2019 at 89% and one of the lowest in 2020 at 80.7%. In 2020 stem per plant data were collected to determine differences between treatments. The yield potential and number of tubers per plant is directly related to number of stems produced. Lemaga and Caesar reported an increase in emerged stems per plant will result in an increased production of tubers and potentially overall yield of the plant (Lemaga & Caesar, 1990). No statistically significant differences were observed with any agronomic data or disease suppression in the second year of the study.

Tubers per plant data in 2019 showed that the grower standard of Ultra Flourish was statistically better than the Elumin treatment with 7.8 vs 6.7 tubers per plant respectively (Tabel 1). Data by Lemaga and Caesar supports a negative relationship between tuber count per plant and individual tuber weight (Lemaga & Caesar, 1990).

Potatoes are categorized by size, anything over 6.4 cm diameter is considered as size A and anything under 6.4 cm diameter is deemed size B. 2019 yield data correlated back to the number of tubers per plant. The Ultra Flourish™ treatment with more tubers per plant resulted in several smaller tubers not developing to size A potatoes by the end of the season. The Ultra Flourish™ treatment had statistically higher size B yield at 23.5 tons per hectare compared to all treatments, except for the Tifi™ treatment. With the increased number of tubers per plant for both the Tifi™ and Ultra Flourish™ treatments, size B yield for the two were not statistically different. Tifi™ size B yield was 21.5 tons per hectare and Ultra Flourish™ size B yield was 23.5 tons per hectare at (Table 1). No significant differences for size A yield were observed in 2019. As a result of a statistically higher size B yield, the Tifi™ treatment had a significantly higher total yield compared to the Orondis Opti™ plus Ultra Flourish™ treatment 39.2 and 34.2 ton per hectare respectively (Table 1).

In 2020 no statistical differences were seen in size A or size B yield among treatments. The Ranman™ (Summit Agro) treatment resulted in more B size tubers with 182.0 total tubers harvested as compared to the non-treated control at 152.8 (Table 2). Having more size B tubers resulted in Ranman treatment having a higher B size yield 22.9 tons per hectare compared to 18.0 tons per hectare in the control (Table 3). Similar trends were observed in these two treatments with size A tubers harvested in the non-treated and the Ranman™ treatment. The non-treated control had 99.2 size A tubers harvested compared to Ranman's™ 69.2 tubers resulting in the non-treated control having a higher size A yield at 31.9 tons per hectare compared to Ranman's™ 23.7 tons per hectare (Table 3).

Very low Pythium leak incidence occurred in the storage trial for both plot years. Plots were placed in commercial fields where Pythium Leak was historically present and caused storage issues for the grower, so post-harvest inoculations were not conducted for this study. In 2019 the grower standard of Ultra Flourish in-furrow showed statistically lower incidence in tuber infection compared to the non-treated control and the Tifi treatments, 0%, 0.49% respectively (Table 1). Although numerical differences existed between treatments, no statistical differences in disease incidence in 2020 were detected. The greatest differences appeared between Ultra Flourish™ with the highest incidence at 0.44% and the lowest incidence with Ranman™ at 0% (Table 2)

Table 1. Evaluation of fungicide treatments on suppression of Pythium Leak in storage of potatoes in 2019.

Treatment and rate[a]	Percent Emergence	Total Tubers Harvested	Tubers per Plant	Yield As	Yield Bs	Yield Total	% Incidence Tuber Infection
1. Non-Treated	94.5 a	195.4 ab	6.9 ab	17.0 a	17.7 c	34.7 ab	.17 ab
2. Ultra Flourish 146 ml/ha (A) Admire Pro 281.3 g ai/ha (A)	93.4 a	218.6 a	7.8 a	14.5 a	23.5 a	38.0 ab	0 b
3. Ranman 65 ml/ha (A) Admire Pro 281.3 g ai/ha (A) Ranman 31 ml/ha (B)	91.4 a	193.2 ab	7.1 ab	17.9 a	18.9 bc	36.8 ab	.24 ab
4. Elumin 90ml/ha (A) Admire Pro 281.3 g ai/ha (A) Elumin 90 ml/ha (B)	94.8 a	189.8 b	6.7 b	16.0 a	18.9 bc	34.9 ab	.18 ab
5. Tifi 0.18 kg/ha (A) Admire Pro 281.3 g ai/ha (A)	91.4 a	204.2 ab	7.4 ab	17.7 a	21.5 ab	39.2 a	.49 a
6. Orondis Gold 200 73 ml/ha (A) Ultra Flourish 108 ml/ha (A) Admire Pro 281.3 g ai/ha (A)	89 a	189.2 b	7.08 ab	14.9 a	19.3 bc	34.2 b	.17 ab
7. Previcur Flex 215 ml/ha (A) Admire Pro 281.3 g ai/ha (A)	91.6 a	187.4 b	6.8 ab	17.9 a	17.7 c	35.6 ab	.12 ab

[a] Application time; A= In-furrow at planting; B= Emergence dribble over row.

[b] Means followed by same letter are not significantly different at $P = 0.05$ (Fishers LSD).

[c] Yield in tons per hectare

Table 2. Evaluation of fungicide treatments on suppression of Pythium Leak in storage of potatoes in 2020.

Treatment and rate[a]	Percent Emergence	Stems per Plant	Tubers per Plant	Total Tubers Harvested	Size A Tubers	Size B Tubers	% Incidence Tuber Infection
1. Non-Treated	80 a	2.56 a	5.26 a	252 a	99.2 a	152.8 a	.22 a
2. Ultra Flourish 146 ml/ha (A) Admire Pro 281.3 g ai/ha (A)	80.3 a	2.53 a	5.34 a	258.2 a	78.4 a	179.8 a	.44 a
3. Ranman 65 ml/ha (A) Admire Pro 281.3 g ai/ha (A) Ranman 31 ml/ha (B)	83.3 a	2.61 a	5.02 a	251.2 a	69.2 a	182.0 a	0 a
4. Elumin 90ml/ha (A) Admire Pro 281.3 g ai/ha (A) Elumin 90 ml/ha (B)	83.6 a	2.51 a	4.66 a	234.4 a	89.2 a	145.2 a	.28 a
5. Tifi Max 0.09 kg/ha (A) Admire Pro 281.3 g ai/ha (A)	82.3 a	2.61 a	5.20 a	256 a	83.6 a	172.4 a	.16 a
6. Orondis Gold 200 73 ml/ha (A) Ultra Flourish 108 ml/ha (A) Admire Pro 281.3 g ai/ha (A)	80.7 a	2.62 a	5.14 a	248.2 a	89.2 a	159.0 a	.14 a
7. Previcur Flex 215 ml/ha (A) Admire Pro 281.3 g ai/ha (A)	81 a	2.71 a	5.22 a	253.4 a	81.8 a	171.6 a	.35 a

[a] Application time; A= In-furrow at planting; B= Emergence dribble over row.

[b] Means followed by same letter are not significantly different at P = 0.05 (Fishers LSD).

Table 3. Yield evaluation of fungicide treatments on suppression of Pythium Leak in storage of potatoes in 2020.

Treatment and rate[a]	Yield Size A	Yield Size B	Total Yield
1. Non-Treated	31.9 a	18.0 a	49.9 a
2. Ultra Flourish 146 ml/ha (A) Admire Pro 281.3 g ai/ha (A)	26.2 a	21.6 a	47.8 a
3. Ranman 65 ml/ha (A) Admire Pro 281.3 g ai/ha (A) Ranman 31 ml/ha (B)	23.7 a	22.9 a	46.6 a
4. Elumin 90ml/ha (A) Admire Pro 281.3 g ai/ha (A) Elumin 90 ml/ha (B)	29.4 a	18.7 a	48.1 a
5. Tifi Max 0.09 kg/ha (A) Admire Pro 281.3 g ai/ha (A)	28.1 a	19.0 a	47.1 a
6. Orondis Gold 200 73 ml/ha (A) Ultra Flourish 108 ml/ha (A) Admire Pro 281.3 g ai/ha (A)	30.5 a	21.4 a	51.9 a
7. Previcur Flex 215 ml/ha (A) Admire Pro 281.3 g ai/ha (A)	29.3 a	21.2 a	50.5 a

[a] Application time; A= In-furrow at planting; B= Emergence dribble over row.

[b] Means followed by same letter are not significantly different at $P = 0.05$ (Fishers LSD).

[c] Yield in tons per ha

Table 4. Environmental Data collected from plot locations both in 2019 and 2020.

Month	2019 Avg Air Temperature	2020 Avg Air Temperature	2019 Avg Soil Temperature	2020 Avg Soil Temperature	2019 Monthly Precipitation	2020 Monthly Precipitation
April	9.0	7.3	10.4	8.5	11.5	7.3
May	14.3	14.6	16.2	13.9	11.3	17.3
June	21.5	22.3	24.4	20.8	4.3	7.5
July	24.0	24.4	26.7	24.4	5.0	11.8
August	21.7	21.9	25.8	22.4	8.8	9.3
September	18.1	19.7	20.9	21.0	2.8	0.8

[a] Avg air temperature and Avg 5 cm soil temperature in °C

[b] Monthly precipitation in cm.

2.5 DISCUSSION

Due to abnormally wet spring conditions in 2019, the plot was planted 15 to 20 days later than most of the commercial crop in the Southwest Michigan. Potato crops in southwest Michigan are typically planted March 25th through May 7th and vine-killed to promote skin set prior to harvest between August 20th through September 15th. This growing region typically has 100 "green days" or days of growth after plants emergence until killing the crop two or three weeks prior to harvest. Because of the late planting in 2019, the plot was only able to capture 85 green days. The reduction in growing season directly impacted the 2019 tuber bulking period as demonstrated by decreased size A yields and higher size B yields in 2019 as compared to 2020.

April 2019 excess soil moisture before planting resulted in a more time needed for soils to dry, pushing the planting date back until early May. Due to later planting, soils warmed faster after planting in 2019 accelerating sprout growth and lowered the time for emergence. In 2020 pre-emergence precipitation was similar to 2019, but soil temperatures were much cooler. As a result, time required for plants to emerge lengthened in 2020 as compared to 2019. This 7-day difference, 28 days in 2019 vs 35 days in 2020, assisted in lowering plant emergence in 2020. In 2020 random short digs were done outside the plot in the surrounding commercial field that was planted the same day and decayed seed pieces were observed. Every day that the stems remain under ground is another day the plant relies on the seed piece for energy further increasing the risk of crop loss due to seed rot. Not wanting to destroy any plots, seed loss counts were not conducted in plot rows but a small reduction in plant stand was observed in 2020 compared to 2019. Another factor contributing to lower final plant stands in 2020 was the use of a mechanical pick planter. In 2019 handplanting with the carousel planter ensured a viable seed piece was deposited every 25 cm within the row. Evaluating the seed, riders can pick out small or blind seed pieces increasing the likelihood for higher plant stands as seen in 2019. Mechanical pick planters use little nails stabbing every seed piece it comes into contact with, selecting slivers of seed that do not have eyes or enough energy reserved to push a sprout out of the ground. Picks depositing seed into the planter also create wounds in tuber periderm and flesh, and this potentially provides an entryway for pathogens like Pectobacterium that can cause seed rot in wet conditions.

Leaching rains are one of the leading causes for loss of effectiveness for water soluble chemicals like metalaxyl and mefenoxam. A study by (Sharom & Edgington, 1982) found metalaxyl was easily leached through columns of sandy soil containing less than 6% clay. Using white rust of radish, researchers found that 20 cm of simulated precipitation resulted in only 50% control. Disease suppression of 75% was observed when 10 cm of simulated rainfall was applied to soil when compared to the treated control with no precipitation. Sharom and Edgington related metalaxyl's water solubility to atrazine, one the most detected pesticides in the United States ground water supply ((Wehtje et al., 1983); (Wilson et al., 1987)). Sharom discovered that metalaxyl moved completely through the soil column, and they detected metalaxyl in the leachate when 20 cm of simulated precipitation occurred. Atrazine with the same precipitation amounts only moved through three quarters of soil column and remained concentrated in the top 10 cm of soil. Thus, metalaxyl was much more mobile in the soil profile after large rain events. Sharom and Edgington explained that metalaxyl may be substantially leached through sandy soils due to the lack of adsorption properties of the sandy soil and the intensity of rainfall.

None of the 2019 plots received any precipitation amounts over 3 cm post fungicide applications however, plots did in 2020. Five weeks post planting in 2020, there was 10 cm of precipitation over a 5-day period, May 14th to May 18th. At this crop stage, 5 days after 100% emergence, expected root development would be centered around the seed piece and up to 10 cm deep. Sharom and Edgington's study provides cause to suspect some pesticides were leach out of the root's profile by the amount of rainfall. Lower amounts of pesticides in rooting zones, reduce the root's ability to adsorb the product and bring it into the plant for tuber protection. This 10 cm of precipitation event would have pushed a portion of some pesticides like metalaxyl down at least 20 cm, well below expected rooting depths of 10 to 15 cm. This would explain the lack of differences in percent incidence among treatments in 2020 at the end of the storage trial.

Another reason for the lack of differences in disease incidences could have been low field inoculum of P. *ultimum*. Considering the history of Pythium Leak in tubers from the plot areas in these commercial fields, harvest temperatures were chosen to optimize P. *ultimum* infection probability. As described by Tompkins with *Pythium* spp. in pumpkins, optimal growth with P. *ultimum* is 25^{o}C to 28^{o}C (Tompkins et al., 1939). Harvest temperature was also described as playing an important role by Hollinghead in post-harvest potato infection rates (Hollingshead et al., 2020). In both years harvest began late morning as temperatures started to rise, resulting in a tuber pulp temperature of 22^{o}C to 24^{o}C during harvest. These temperatures would be on the very upper threshold of industry acceptability but were needed for optimal infection by the pathogen in plots. Unfortunately for both years, the inoculum pressure was not high enough to separate out differences in treatments from the non-treated controls. Future studies should consider spraying a spore suspension of 10,000 per mL and 0.1 ml per tuber as described by Gachango (Gachango, 2011). Gachango demonstrated spore suspensions in a water solution could be sprayed onto injured tubers using a R&D XR11003VS spray nozzle at a rate of 1L/ton at 344.7 KPa. Tuber periderms were scraped using wire brush creating wounds prior to being sprayed with the spore suspension. Injuries causes by abrasive sand rubbing skin off or mechanical cuts act as an entry point for the zoospores that are sprayed on post-harvest to cause better infection rates in storage.

Several variables need to be considered when evaluating differences among treatments for soil-borne pathogens. Soil variability has a large effect on pathogen development and survival. Multiple replications are needed in a plot to ensure treatment placement is in an area of the pathogen. The absence of pathogen in a plot area will also lead to more variation in results and sample error increasing the difficulty to statistically separate treatments from one another. Spraying a known amount of pathogen on samples to guarantee the host and pathogen are joined followed by manipulating storage environments with high humidity and temperature for infection is one example of improving infection occurrence. Environmental conditions, application types, application timing, and varietal genotypes will also have some impact on efficacy of disease suppression and will be discussed further in the next chapter.

CHAPTER 3. CHEMICAL AND BIOLOGICAL SUPPRESSION ASSAY OF SOUTHERN MICHIGAN *PYTHIUM* SPP.

3.1 ABSTRACT

Pythium ultimum survives well as a saprophyte throughout soil profiles by feeding on a wide range of plant material. Primarily *Pythium* spp. oospores germinate under high humidity on wounded tubers just after harvest causing storage disease, but oospores can also germinate on the surface of open lenticels after heavy rains resulting in field infections. Metalaxyl is used to help manage *Pythium spp.* in potato production. However, due to the wide host range of *Pythium* spp., metalaxyl may be used within the same field every year on different crops. Repeated field use can result in the development of fungicide resistance. Providing growers with effective chemical options for rotation will prolong pathogen suppression. In this study four FRAC groups (4, 22, 28, 49) and one biological (*Trichoderma atroviride*) were evaluated for in vitro suppression of *Pythium* spp.. The Orondis Gold™ (Frac 4 and 49) treatment resulted in the lowest EC50 which could not be calculated at the lowest level 0.01 ppm because it inhibited all *Pythium* spp. growth. Next, Ultra Flourish™ (Frac 4) reduced half the *Pythium* spp. growth at 0.32ppm. The least effective treatments at reducing pathogen growth were Elumin™ (Frac 22) or Previcur Flex™ (Frac 28), both resulting in EC50 values of 1.9 ppm and 7.9 ppm, respectively. Five days post transfer *Trichoderma atroviride* had overtaken the *Pythium* spp. on nonamended V8 media, providing support as a possible biological suppression tool.

3.2 INTRODUCTION

Fusarium spp. and *Pythium* spp. are two major soilborne pathogens causing storage rots in Michigan potato production (Gachango et al., 2012b). Applications of the benzimidazole fungicides (e.g., thiabendazole (TBZ)), released into potato production in the early 1970s, were the main suppression measure against fusarium dry rot disease (Leach & Nielsen, 1975). Benzimidazole fungicides are thought to bind to tubulin molecules preventing microtubules from forming and stopping cell growth (Kawchuk et al., 1994). Thiabendazole applications were an effective approach versus Fusarium dry rot for almost 20 years before reports of fungicide resistance started surfacing (Staub, 1991).

Potato tuber surveys by Desjardins et al (1993) presented possible explanations of how TBZ resistance moved so quickly throughout North America. Their data support a single gene transfer in the sexual phase due to a 4:4 progeny ratio (TBZ sensitive to TBZ resistant) when TBZ sensitive and TBZ resistance parents were crossed (Desjardins et al., 1993). Because potato seed is moved and planted across North America, it was originally thought that high genetic diversity would be present with *Fusarium* spp. in potato fields. Desjardin's data showed mating types are compatible suggesting this resistance gene was transferred throughout a very large population. With widespread use, TBZ functioned as a selection agent in field populations resulting in a drift toward more resistant populations. Desjardin et al reported no fitness cost with growth rates or virulence for *Fusarium* spp. having the TBZ resistance phenotype (Desjardins et al., 1993). Therefore, once resistance phenotypes were introduced and continually selected for, TBZ efficacy diminished in potato production.

Tuber surveys from Michigan seed growers in 2009 and 2010 revealed that *Fusarium oxysporum* and *F*. *equiseti* (Gachango et al., 2012a) were the predominant pathogen causing the dry rot symptoms on storage potatoes. *F*. *sambucinum* was the third most collected isolate in the survey but showed the most virulence. Fungicide assays by Gachango et al. provided evidence of resistance to the widely used post-harvest fungicide fludioxonil in 20% and 9% of the *F*. *oxysporum* and *F. sambucinum* populations collected, respectively. Isolate sensitivity to difenoconazole was also discovered. Difenoconazole was not used in Michigan seed production prior to this survey so isolates were not thought to be previously exposed (Gachango et al., 2012a), thus contributing to difenoconazole's efficacy against *Fusarium* spp. in this survey.

The ability of P. *ultimum* to suppress or manipulate tuber defenses (Tata et al., 2014) results in host susceptibility and requires reliance on chemicals for suppression of Pythium Leak disease. *Pythium* spp. have the ability of to overcome host defenses including natural antimicrobial compounds thus this pathogen can also evolve resistance to crop protection chemistries. Continuous exposure of the pathogen over multiple crop rotations aids in the selection of resistance to the most used *Pythium* spp. chemical suppression classes including metalaxyl in the FRAC 4 group.

Within the first two years of metalaxyl's use, resistance was reported in *Pseudoperonospora cubensis* of cucumbers (Reuveni et al., 1980). Early United States reports of *Pythium* spp. resistance to metalaxyl in turfgrass blight occurred just a few years later (Sanders, 1984). In the Pacific Northwest United States, small grains and vegetables are often grown in rotation with potatoes thus exposing soil-borne pathogens like *Pythium* spp. repeatedly to group FRAC 4 fungicides. In small grains and vegetables, metalaxyl-M is used to mitigate damping off (Pscheidt & Ocamb, 2002) and used in potato production for pythium leak (Porter et al., 2009) thus annually subjecting Pythium spp. to metalaxyl and selecting for resistant populations.

Metalaxyl resistance has widened from a local issue to a multi-state challenge covering Idaho, Oregon, and Washington. Porter et al., (2009) described metalaxyl resistant populations in the Pacific Northwest (PNW) covering 312 potato cropping systems and discovered that nine species of *Pythium* were moderately resistant (MR) to metalaxyl-M and able to grow out on amended auger. Of those MR isolates, *P. ultimum* was collected and could cause issues in potato production with Pythium Leak disease.

Growers believe similar circumstances have unfolded in southwest Michigan (Ritchie, 2020). Cucumber production adopted mefenoxam/metalaxyl seed treatments aiding in protection against soil pathogens such as *Phytophthora capsici* (Lamour & Hausbeck, 2003). In addition to cucumbers, many corn and soybean acres adopted metalaxyl seed treatments to protect against seedling diseases. In growth chamber and field studies (Griffin, 1990) demonstrated the benefits of metalaxyl reducing seedling disease severity in soybean production caused by *P. ultimum.* Furthermore (Radmer et al., 2017) validated both soybean and corn susceptibilities to *Pythium* spp as well as the pathogen's relative sensitivity to common seed treatment fungicides in the Midwest. As a result of increased metalaxyl use in rotation crops, more Pythium Leak symptoms occur in potato production for the southwest Michigan region compared to the prior decade (Ritchie, 2020).

Biological controls have been documented for host protection against soil-borne diseases (Wilhelm, 1973)(Whipps et al., 1993). Biologicals could be utilized in organic cropping systems or even paired with conventionally used chemicals to prevent diseases. *Pythium oligandrum* displayed success at increasing plant stands of sugar beets when used against *P. ultimum* (Whipps et al., 1993). *Trichoderma* spp. (Aydin, 2019) as well as arbuscular mycorrhizae (Niemira et al., 1996) have proved to be successful antagonists against *Fusarium sambucinum* in potatoes. Under ideal conditions replied cut seed treated with *T. harzianum* had statistically similar disease incidence and severity as the chemical control (fludioxonil + mancozeb) on fusarium dry rot (Wharton & Kirk, 2014).

3.3 MATERIAL AND METHODS

Silverton russet tubers showing symptoms of Pythium Leak were collected in 2019 from non-treated areas of a Pythium Leak plot in the Three Rivers, MI. Small pieces (5 x 5 mm) of infected tissue from tubers were transferred to water agar containing ampicillin (100 ug/ml) then grown at 18^{o}C to 20^{o}C for 5 days in the dark. Purified isolates were then transferred by placing 5 mm diameter plugs of water agar with mycelium onto modified V8 (100ml 5% V8 juice filtered through four layers of cheesecloth, 900 ml distilled H_2O and 15g Bacto-Agar) (Taylor et al., 2002) and grown 3 days in the dark at room temperature (18-20^{o}C). To confirm that the cultures were *Pythium,* DNA was extracted from four isolates: T01, T03, T05, and T07 using DNeasy® PowerMax® soil DNA extraction kit. Samples were then amplified using primers Pu1F1 (5' GACGAAGGTTGGTCTGTTG 3') and Pu2R1 (5' CAGAAAAAGAAAGGCAAGTTTG 3') in a BioRad PCR (Cullen et al., 2007). PCR program consisted of 7 steps: I.) 94 ^{o}C for 3 minute, II.) 94^{o}C for 1 minute, III.) 55 ^{o}C for 3 minutes, IV.) 72^{o}C for 2 minutes, V.) repeat steps II.-IV. for 25 cycles, VI.) 72^{o}C for 10 minutes, and finally VII.) hold at 4^{o}C. Isolates were confirmed as *Pythium* spp. after running an electrophoresis gel with two known *Pythium* spp. markers *Pythium* *aphanidermatum* (PA) and *Pythium ultimum (*Gold) the DNA product size explained by Cullen was to be 307 bp (Cullen et al., 2007).

3.4 FUNGICIDE SENSITIVITY TESTING

Stock solutions of each fungicide: Ultra Flourish (mefenoxam) labeled UF, Orondis Gold (oxathiapiprolin + mefenoxam) labeled OG, Elumin (ethaboxam) labeled EL, and Previcur Flex (propamocarb) labeled PF, were prepared by dissolving commercial-grade fungicide into acetone for UF, OG, and PF or sterile DI water for EL fungicide. Sterile V-8 agar cooled to 37 C° was dispensed into beakers containing stock solutions of fungicides. Final concentrations of fungicides in the media were of 0.0, 0.01, 0.1, 1.0, 10, and 100ppm. Amended agar was then pipetted into dishes, cooled for 6 hours, and stored at 4 to 5^{o}C until use. A completely randomized design was used during experiments. Two replications, per concentration, for each fungicide were used.

Table 5. Fungicide products, active ingredient, Frac Group, and concentrations used in sensitivity study.

Products	Active Ingredients	Frac	Concentrations (PPM)
Ultra Flourish	Mefenoxam	4	0.01, 0.1, 1.0, 10, 100
Orondis Gold	Mefenoxam, Oxathiapiprolin	4, 49	0.01, 0.1, 1.0, 10, 100
Elumin	Ethaboxam	22	0.01, 0.1, 1.0, 10, 100
Previcur Flex	Propamocarb hydrochloride	28	0.01, 0.1, 1.0, 10
Tifi Max	Trichoderma atroviride		

Isolate T03 grew modestly covering the V-8 agar plate with mycelium in about five days. Isolate T05 and T07 covered the V-8 agar plate within three days and growth was considered too fast for the initial experiment. T01 mycelium grew the slowest of the isolates and was not used in the initial sensitivity study. For these reasons, T03 was the P*ythium* spp. isolate chosen for sensitivity testing.

Using a #3 cork borer, seven-day old pure cultures of isolate T03 *Pythium* spp. grown on V-8 agar were transferred into the center of amended media plates. After transfer, plates were incubated in the dark at room temperature for three-days, and mycelial growth evaluated at each fungicide concentration (replicated twice). Vertical and horizontal perpendicular lines were drawn on the bottom of plates, through the center of the growing culture. Relative radial growth data was collected using a digital caliper averaging the four perpendicular radii of outer growth from the pure culture plug.

Sensitivities were recorded as relative growth expressed as the ratio of growth in the presence of fungicide vs. fungal growth without the presence of fungicide times 100%. Sensitivity was shown in mg/liter (ppm) determined by approximating the EC_{50}. EC_{50} values were calculated by interpolation of the 50% intercept based on the regression of arcsine for relative growth on the $\log_{10}$- transformed fungicide concentration. Sensitivity was grouped based upon mean EC_{50} values (ppm) according to Taylor et al. (Taylor et al., 2002): sensitive (<1), intermediate sensitivity (1-100), and resistant (>100).

Tifi Max was obtained from Italpollina (Anderson, IN). One gram of Tifi Max spore mixture was added to five milliliter of sterile water with one milliliter of the mixture pipetted onto modified V8 auger then placed into dark storage at room temperature for five days. A #3 cork borer was used to transfer isolated *Trichoderma atroviride* onto modified V8 media then placed back into dark storage at room temperature.

For the Tifi Max treatment a duel culture technique was used (Aydin, 2019). A #3 cork borer was used to place a two-day old pure culture plug of *Trichoderma atroviride* on one side of unamended modified V8 media plate. A two-day old pure culture plug of the T03 isolate was placed in the same plate on the other side, expecting a relationship to occur in the center of the plate. Observations were made two, three, and five days later (figures 13-15).

3.5 RESULTS

Figure 8 shows gel electrophoresis results for the *Pythium* isolates collected from nontreated tubers in the 2019 field plot. The ladder was located in Lane 1, lane 2 was a check for *Pythium aphanidermatum*, the check for *Pythium ultimum* Gold was in lane 3, lanes 4-7 were isolates collected from infected tubers (T01, T03, T04, T05, T07, respectively). The expected product sizes were 307 base pairs for both *P. aphanidermatum* and *P. ultimum* Gold. Both T01 and T03 lined up very close to the same product size of both checks but T05 and T07 were slightly smaller product sizes.

Figures 9-12 show T03 mycelium growth on the control plate, left, and fungicide amended media at increasing concentrations from 0.01ppm increasing by 10-fold steps up to 100 ppm. Mycelium nearly covered the control media plates across all treatments after 48 hours. The Orondis Gold amended media inhibited mycelium growth at even the lowest concentration tested (0.01 ppm) as shown in figure 10. Therefore, an EC50 could not be calculated. For Ultra Flourish the mycelium growth was inhibited at the second concentration of 0.1 ppm shown in figure 9. The Elumin concentration did not inhibit mycelium growth until the

10ppm concentration as shown in figure 10. Lastly the Previcur Flex amended media was unable to completely inhibit mycelium growth at any concentration tested. However, it was able to reduce growth 50% which allowed determination of an EC_{50} value. EC_{50} for OG was below 0.01ppm but was not calculated due to the inhibition of growth at the lowest concentration of 0.01ppm. The calculated EC50s were as follows: 0.318ppm for Ultra Flourish, 1.9ppm for Elumin, and 7.9ppm for Previcur Flex. Table 5 show how each treatment compares with one another.

Contrary to poor field-study results in 2019 and 2020, Orondis Gold was effective at reducing *Pythium* growth in amended media. Ultra Flourish was effective at reducing the growth rate of this isolate at low concentrations as well. Elumin effectiveness on *Pythium* growth was low in the current sensitivity study and poor in the previous field studies. The worst treatment for reduction of mycelium growth in the current study was the use of Previcur Flex.

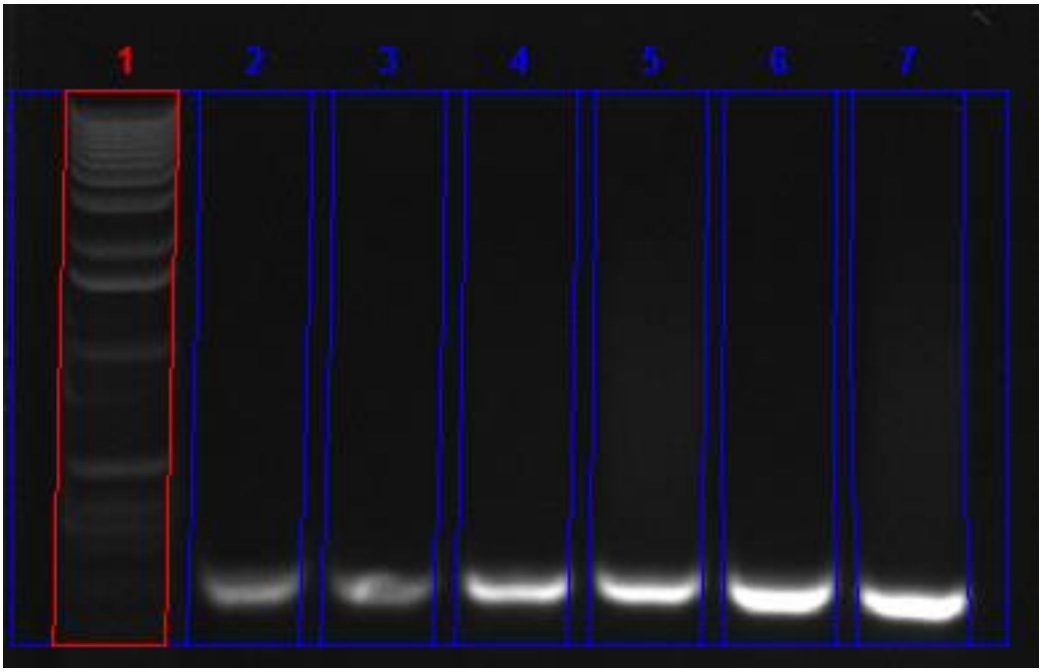

Figure 8. Gel electrophoresis analysis of DNA extracted from isolates T01 (lane 4), T03 (lane 5), T05 (lane 6), and T07 (lane 7). Markers *Pythium aphanidermatum* (PA) in lane 2 and Pyhtium *ultimum* Gold in lane 3. Isolates T01, T03, T05, T07, *Pythium aphanidermatum,* and *Pythium ultimum* Gold product size expected to be about 307 bp

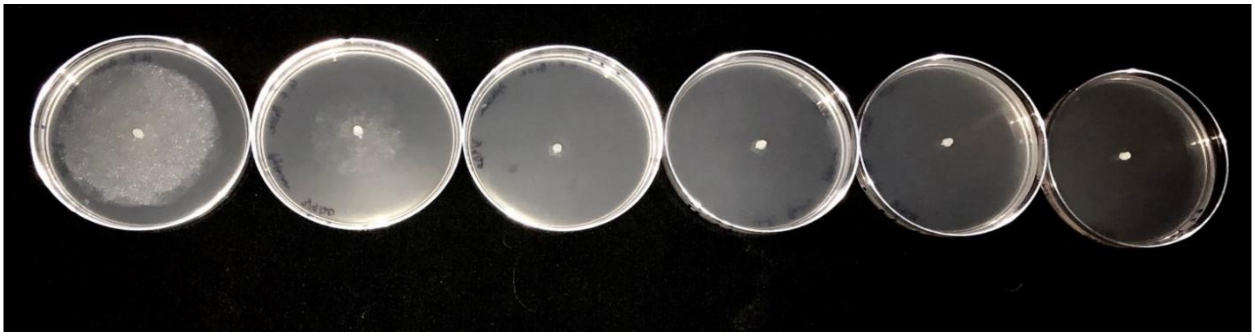

Figure 9. *Pythium* spp. mycelial growth of isolate T03 on Ultra Flourish amended modified V8 media. From left to right- plate 1 control (0ppm) then .01, .1, 1, 10, 100 ppm. All doses are ppm of ai (active ingredient). Reduction of growth on .01ppm and no growth on .1ppm

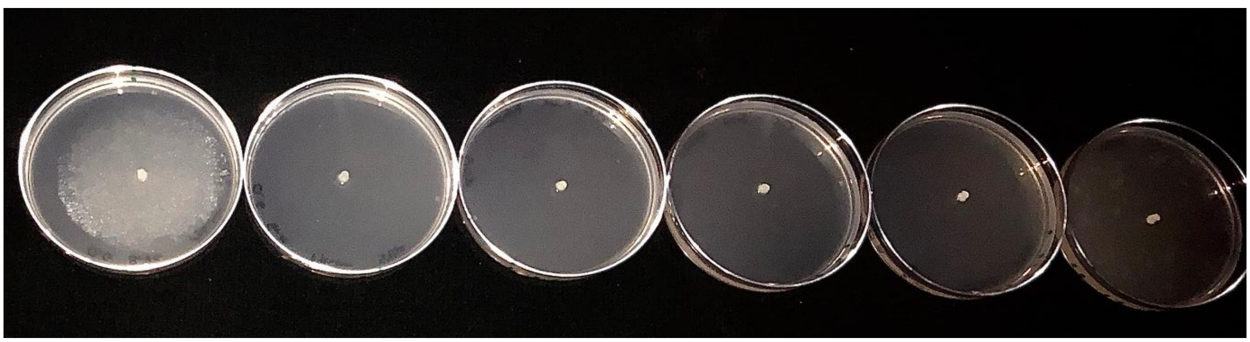

Figure 10. *Pythium* spp. mycelial growth of isolate T03 on Orondis Gold amended modified V8 media. From left to right- plate 1 control (0ppm) then 0.01, 0.1, 1, 10, 100 ppm All doses are ppm of ai (active ingredient). No growth on 0.01ppm

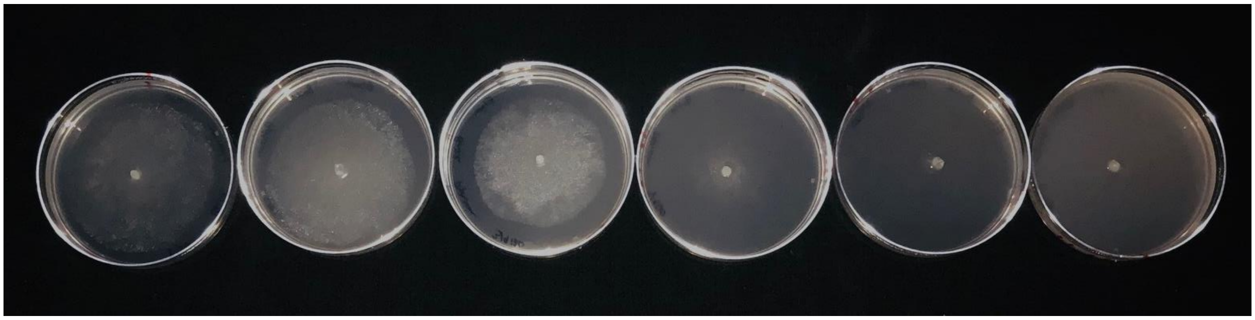

Figure 11. *Pythium* spp. mycelial growth of isolate T03 on Elumin amended modified V8 media. From left to right- plate 1 control (0ppm) then 0.01, 0.1, 1, 10, 100 ppm All doses are ppm of ai (active ingredient). Slight reduction of growth on 0.1ppm, reduction on 1, no growth on 10ppm

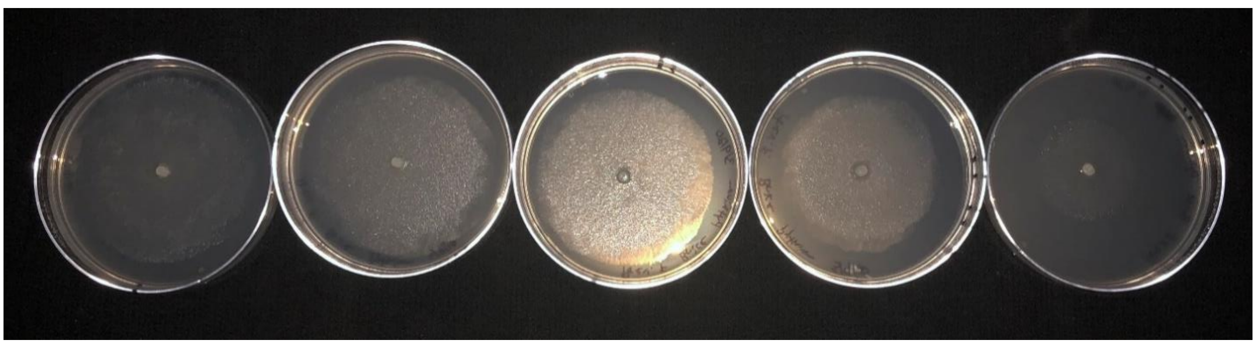

Figure 12. *Pythium* spp. mycelial growth of isolate T03 on Previcur Flex amended modified V8 media. From left to right- plate 1 control (0ppm) then .01, 0.1, 1, and 10 ppm. All doses are ppm of ai (active ingredient). Slight reduction of growth on 1ppm and reduction on 10ppm

Tifi Max, (*Trichoderma atroviride)* was able to overgrow the *Pythium* spp. isolate T03 after four days of growth. At two days post transfer, the *Trichoderma* spp. and *Pythium* spp. were just beginning to touch in the center of the plate shown in figure 13, so observations were made again at 3 days and 5 days post transfer. Figure 14 shows the *Trichoderma* spp. changing from a white to green pigmented mycelium at 3 days but no differences in growth were noted from 24 hours prior. At 5 days the *Trichoderma* spp. had taken over the plate shown in figure 15, eliminating nearly all the *Pythium* spp. mycelium.

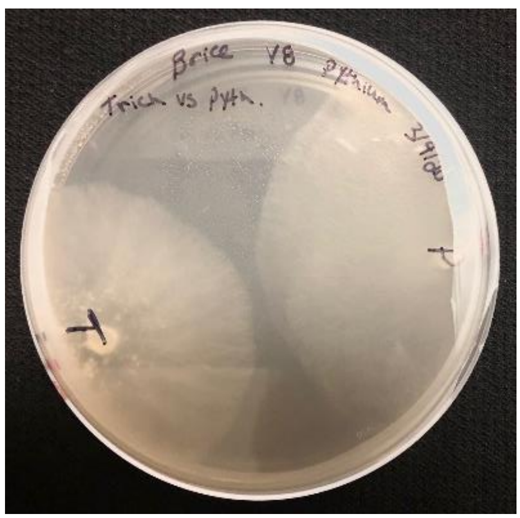

Figure 13. Tifi Max (T) and Pythium (P) mycelium growth on unamended modified V8 media two days after plating

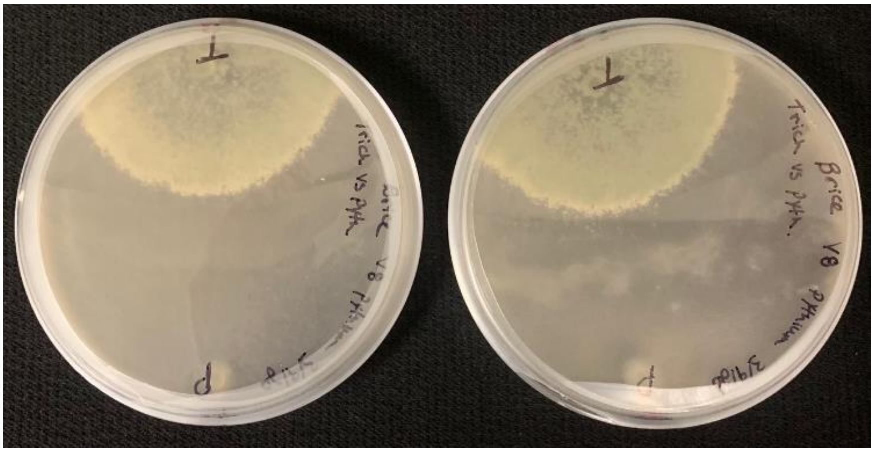

Figure 14. Tifi Max (T) turning green after three days no effect on Pythium (P) mycelium growth yet

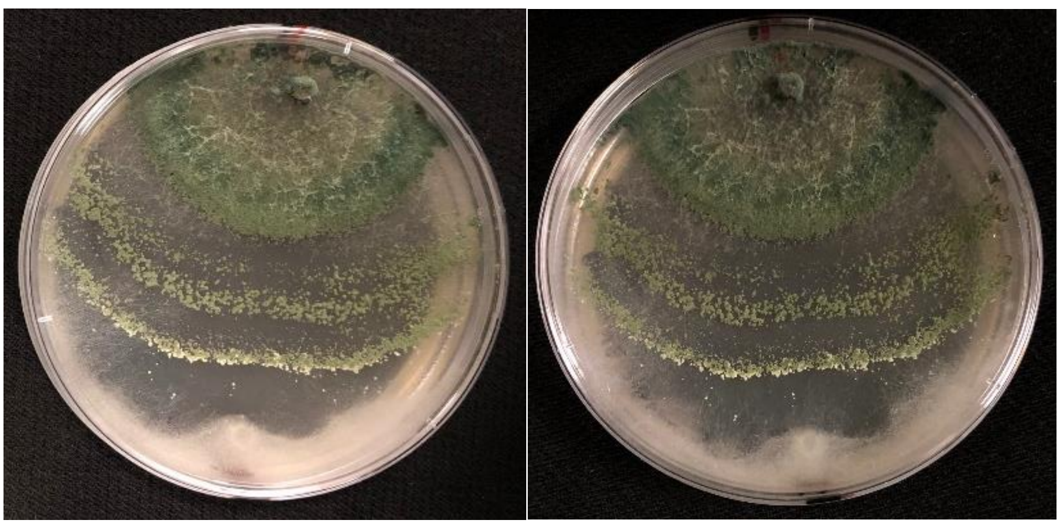

Figure 15. Tifi Max (green) mycelium over taking plate, reducing Pythium (white) mycelium growth after five days

Table 6. Comparison of EC_{50} for all 4 fungicides on single isolate (T03) tested, EC 50s are ppm ai (active ingredient).

Treatment	EC 50 (ppm)
1. Ultra Flourish	.1-1
2. Orondis Gold	>.1
3. Elumin	1-10
4. Previcur Flex	1-10

3.6 DISCUSSION

Due to Covid 19 restrictions and the university shutdown, this trial could not be repeated with multiple *Pythium spp.* isolates. Following the findings of Taylor et al., chemical effectiveness grouping for this isolate tested would be considered sensitive to Orondis Gold™ and Ultra Flourish™ with intermediate sensitivity to Elumin™ and Previcur Flex™ (Taylor et al., 2002).

3.5.1 BIOLOGICAL SUPPRESSION

Trichoderma spp. aggressive growth characteristics may support usage as a biological means for suppressing *Pythium spp.* The challenge with using a biological control in commercial production agriculture is restricting the damage of the beneficial fungi while limiting the activity of other detrimental fungi with fungicide applications. EC 50 studies should be done on traditional potato production fungicides to see impacts to specific *Trichoderma* spp. being implemented as an antagonistic fungus.

Co-colonization in the same area within the potato hill is another obstacle to overcome for biologicals to suppress the target pathogens. This *in vitro* colonization study did not take into consideration environmental factors like temperature and soil moisture levels that would be present in a potato field. To locate the pathogen in the field, DNA could be extracted from soil samples periodically throughout the growing season. In the *Trichoderma* spp. treatment areas, DNA could be extracted to see how far this fungus grows out from the application band and how much of the potato hill is colonized. In order for the *Trichoderma spp.* treatment to be effective at suppressing Pythium Leak, it must colonize the entirety of the hill so it can out compete and suppress *Pythium* spp. late in the growing season. It is unlikely this fungus would have the ability to grow throughout the entirety of the hill because of the vast differences in soil temperature and moisture from the top of the hill to the bottom.

3.5.2 CHEMICAL SUPPRESSION

Current study data support Orodis Gold (oxathiapiprolin + mefenoxam) as an effective chemistry for *Pythium* spp. suppression. Data from previous field studies in 2019 and 2020 did not separate Orondis Gold as being statistically better in vivo at Pythium Leak suppression caused by *Pythium* spp. A portion of Orodis Gold is mefenoxam and we would expect similar sensitivity results as the Ultra Flourish (also mefenoxam) in this study. As the combination of these two chemicals resulted in better efficacy than mefenoxam alone, a synergistic effect between Orondis (oxathiapiprolin) and Gold (mefenoxam) should be examined. Straight Orondis was unavailable for this trial, and therefore we could not examine efficacy on *Pyhtium* spp. of the oxathiapiprolin component. Timing of application or application type may be the determining factor for the Orondis Gold (oxathiapiprolin + mefenoxam) efficacy in the field.

To better understand diminished efficacy in recent years, multiple factors should be investigated in Michigan potato production. Current data provides evidence of mefenoxam sensitivity in *Pythium* spp. isolates in southwest Michigan. The isolate tested in this experiment was slower to grow and although could be a minority in the population was still in the population after multiple selections with mefenoxam. A tuber survey should be completed from all potato growing regions of Michigan, collecting a representative sample of the *Pythium* spp. population. Once a representative sample of isolates are collected, a sensitivity study can be conducted on a larger Michigan population. Results of the more encompassing study would give supporting evidence for discussions on reduced effectiveness being caused by mefenoxam resistance within Michigan populations or if the delivery mechanism is less effective in recent years, resulting in withered chemical suppression.

Weather patterns are deviating from moderate precipitation events to more unpredictable and volatile systems that deposit larger precipitation amounts in shorter times (MDOT, 2011). Soil applied applications of water-soluble chemicals are expected to adhere to a low CEC sandy soil so a shallow rooting crop like potatoes can continually uptake the chemical over an entire growing season. Leaching is a very possible explanation why chemical suppression of Pythium Leak has weakened over time. Experiments testing efficacy of application timing associated with precipitation volume post application could determine if products are being leached out prior to root interception.

3.5.3 CHEMICAL PROPERTIES

Modes of actions and movement of active ingredients throughout the plant are equally important factors when considering effectiveness of suppression. Mefenoxam and Metalaxyl are phenylamides, categorized by FRAC as a group 4 fungicide that targets the RNA polymerase I site, work by inhibiting endogenous nuclear RNA polymerase (Davidse et al., 1988). Davidse et al., also describe metalaxyl’s efficacy in vitro against *Phytophthora infestans* as a result of its very high water solubility (7100 ug/ml) compared to other chemicals trialed in their study. Potato tubers collect nutrients from the parent plant via transport of sugars and other nutrients from leaves and water from root uptake. Transport of foliar applied Metalaxyl to potato tubers was shown to have inconsistent efficacy against *Phytophthora infestans* causing tuber Late Blight in potato (Bruin et al., 1982). Photodegradation, thick cuticles, and transport mechanism work against moving applied chemicals from leaf surfaces down into tuber tissues. To provide protection against tuber pathogens like *Pythium ultimum* causing infections near the tuber surface, the active chemical must then diffuse from the vascular tissue of the tuber to the outer edges.

Orondis's active ingredient is oxathiapiprolin (OXPT), classified by FRAC as a group 49 lipid homeostasis and transfer protein targeting site, has medium to high risk expected for resistance being a single target site (FRAC, 2021). Oxathiapiprolin has been shown to inhibit zoospore release and reduce zoospore or sporangia germination in oomycetes (Cohen et al., 2018). Miao et, al. determined within the plant, OXPT was to be systemic in cucumber leaves when petiole-dip bioassays were performed as well as, detecting OXPT translocation from the roots of cucumber plants to stems and leaves (Miao et al., 2018). Miao et, al. also recorded the OXPT leaf concentration increasing over the experiment while these plants were grown under continuous light in growth chambers, suggesting this fungicide is moved throughout the plant with water during evapotranspiration.

When Mefenoxam and OXPT are combined in a tank mix, (such as Orondis Gold™) the risk for resistance management is thought to be reduced by having multiple modes of action in one application. In addition to lowering the risk of resistance to these two single site modes of action, tank mixing both chemistries can provide a synergistic reaction delivering better suppression of disease than either one chemical could by itself. Cohen et al., demonstrated the synergistic relationship between these chemicals. When comparing chemical suppression of mefenoxam resistant *P. infestans* isolates, the blighted leaf surface area was less when OXPT was combined with mefenoxam than OXPT by itself (Cohen et al., 2018). This relationship was shown to not only persist with foliar applications of the two but soil drench applications as well.

Because the isolates were mefenoxam resistant, mefenoxam had little to no effect on *P. infestans* itself. Cohen et al,. concluded the mefenoxam formulation in the tank mixture increased the mobility in the plant when combined with OXPT and therefore resulted in increased suppression of blighted leaf area.

3.5.4 APPLICATION TIMING

Pythium Leak is primarily a post-harvest disease and timing of application should also be considered as a source of efficacy differences. Water-soluble chemicals cannot be expected to remain in a low CEC soil for the duration of season. Greenhouse studies show mefenoxam increasing in soil concentration at lower soil depths as irrigation amounts increased (Wheeler et al., 2007). Broadbent suggests that oxathiapiprolin products are very systemic, moving throughout the plant with water in the xylem (Broadbent, 2021). Because OXTIs are water soluble, any large in-season rain events could flush the product down below the shallow rooting potato crop. A later application with Orondis™ (oxathiapiprolin) plus Revus™ (mandipropamid) packaged as Orodis Ultra™ either foliar or chemigation could provide protection against *Phytophthora infestans* as well as, protection at the end of season when *Pythium* spp. infections take place post-harvest.

Translocation of chemicals is also affected by timing. Early applications of oxathiapiprolin or mefenoxam before tuber initiation may be taken up by the plant but will likely be at much lower concentrations later on when tubers begin to form. One of the best times for chemicals to translocate to the tuber is during early bulking approximately 45-50 days after plant emergence. During this time period, plants are healthy with large rooting systems up taking water and nutrients for photosynthesis then translocating sugars and any systemic chemistries to daughter tubers.

3.4.5 APPLICATION TYPE

Application type is an efficacy variable needing evaluation. Many products today are applied via aerial application with low volume amounts of carrier. Chemigation, the application of products through irrigation systems, is becoming more popular in potato production. Chemigation allows growers to apply fertilizer or apply chemicals more frequently minimizing the risk of product loss via leaching (van der Gulik et al., 2007). Questions should be asked concerning whether there are any differences in fungicide coverage or efficacy when applied aerially versus chemigation, and whether products applied are moved to the area of interest within a plant differently with these application methods? Product concentrations could be measured not only in different areas of the potato plant but areas within the tuber as well.

3.4.6 POTATO GENOTYPIC DIFFERENCES

Many genetic differences exist among cultivated potato varieties, ranging from presence or absence of flowers, to leaf size, and above ground or root biomass. Nutrient use efficiency comparisons have also been done among varieties to advance productivity by selecting varieties with greater nutrient efficiencies in countries with limited resources (Hailu et al., 2017). Potato varieties have also been shown to have different abilities to remove compounds such as cadmium out of soil and translocating it into tubers (McLaughlin et al., 1997). Ashrafzadeh et al.(2017) was able show a two to four-fold difference in varietal abilities to adsorb and translocate cadmium and other micronutrients into daughter tubers (Ashrafzadeh et al., 2017). They explained differences in tuber cadmium concentrations for each variety was due to the variety's ability to bring in specific micronutrients into the plant and the relative antagonistic relationship of those micronutrients with cadmium. Varietal differences in rooting could also explain some differences among fungicide efficacy between varieties. Lower tuber concentrations of systemic chemistries like mefenoxam may result in differences in efficacy of less robust rooted plants. Susceptibility or a variance of symptoms could be caused by the smaller root mass of the plant bringing in less water and chemistry into the plant via mass flow, thus reducing transpiration rates within the plant while decreasing photosynthesis resulting in less carbohydrates and chemistry translocated down to the tuber.

Variety selections conducted by the potato industry are perpetuating storage diseases including Pythium Leak. A drive to increase productivity and yield has resulted in growers selecting for higher tuber counts per plant, diluting the concentrations of protective fungicides over more tubers within a plant. Chip manufactures focusing on minimizing peel waist have selected thinner skinned varieties more prone to harvest injury causing increased storage shrink via dehydration, pressure bruise, and opportunistic diseases like fusarium (Ritchie, 2020). Consumer demand even plays a part in increasing storage diseases with variety selection by shifting markets with higher demands for thin-skinned specialty varieties (petites and yellows) while desiring less amounts of the resilient thick-skinned russet varieties (USA, 2021).

My in vitro study supports chemical suppression as means to combat *Pythium* spp. in Michigan soils. Oxathiapiprolin plus mefenoxam shows inhibitory properties against our *Pythium* spp. isolate at very low concentrations in addition to mefenoxam applied individually. The challenge remains however as to how can a delivery system be adjusted to increase efficacy against Pythium Leak in Michigan potato production. Environmental factors including photodegradation, leaching from the soil profile, and physical adsorption within the soil all impact the efficiency of applied chemistries. Even with negative environmental factors minimized, the timing and means of application are critically important aspects affecting how chemistries are taken up into the plant or translocated to the target site of the pathogen. Lastly genotypical elements of varieties impact how chemistries are translocated within the plant. For instance, differences in root structure impacts how well chemistries are taken out of the soil water solution and brought into the plant or variations in metabolic properties of certain varieties will influence how much fungicide is needed impacting efficacy of disease suppression.

FUTURE DIRECTIONS

- Study varietal differences for susceptibility to Pythium Leak in chip cultivars
- *Pythium* spp. diversity surveys in potato production
  - Compare newer ground populations to ground with multiple potato crops and mefenoxam selection
  - Characterization of *Pythium* spp. causing Pythium Leak in potato production interpret effects of rotation crops on *Pythium* spp. in potato crop
- Compare delivery mechanisms for best efficacy with climate changes Michigan is experiencing. Evaluate in-furrow vs foliar via airplane vs ground sprayer, early season chemigation- 20DAE (days after emergence) vs later season chemigation (55DAE)

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