INTEGRATED PEST MANAGEMENT STRATEGIES FOR CONTROL OF POTATO EARLY DIE IN MICHIGAN POTATO SYSTEMS

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ABSTRACT

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Root lesion nematodes (*Pratylenchus penetrans*) in conjunction with the fungal pathogen *Verticillium dahliae* create the disease complex, potato early die, which can drastically reduce potato (Solanum tuberosum L.) yields. In Michigan, this disease complex is often managed using broad-spectrum soil fumigants such as methyl bromide, metam sodium and 1,3–dichloropropene, which can be harmful to applicators as well as soil and environmental health. Since the phase-out of methyl bromide in 2005, alternative control tactics to soil fumigants have become increasingly important to potato growers. In this thesis I investigated the use of (1) manures and manure-based composts and (2) non-fumigant nematicides and fungal-based biocontrols and their efficacy in reducing potato early die incidence. In laboratory trials, poultry manure and a blend of poultry and dairy manure compost (Layer Ash Blend) provided significant control of root lesion nematodes with 0% survivorship at rates of 5% (vol/vol) or higher. In field trials, I did not observe significant ($P < 0.05$) reductions in nematode populations regardless of treatment but did see a reduction in germinating *V. dahliae* microsclerotia in plots treated with poultry manure. Of the nematicides tested, Salibro treatments significantly reduced root lesion nematode and *V. dahliae* populations. From this research, it is clear that non-fumigant alternatives are possible for Michigan potato growers.

Dedicated to my Grandma Cole
for fueling my love of nature
and having unwavering confidence
in me. Love you always Grandma

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applications (Salibro L + Vydate (x2), Velum and Movento (VelumMov), Velum and Serenade (VelumSer), Velum Serenade and Movento (VelumSerMov) and Vydate. All seed-pieces besides the Control No Trt were treated with CruiserMaxx Extreme seed treatment prior to planting. Yield was determined by harvesting one 15' row and translating the kilograms harvested to a per hectare scale. N.S. indicates no significance (Tukey HSD, α=.05).. 55

CHAPTER 1. POTATO EARLY DIE DEVELOPMENT AND CONTROL IN MICHIGAN AGROECOSYSTEMS

1.1 PEST OVERVIEW

1.1.1 Pratylenchus Penetrans – Biology and Identification

The root lesion nematode, *Pratylenchus penetrans,* is a widespread, migratory endoparasitic nematode documented on every continent except for Antarctica (Castillo and Vovlas, 2007). In United States potato systems, *P. penetrans* has been documented as a pest in key production states including Idaho, Wisconsin, Colorado, Washington, and Michigan (Bird and Warner, 2018; Florini and Loria, 1990; Hafez et al., 2010; Ingham et al., 2005; Omer et al., 2008). Because of the wide host range including over 400 species of ornamentals, grasses, fruit and field crops, *P. penetrans* poses a serious threat to crop production worldwide (Davis and MacGuidwin, 2000).

Pratylenchus penetrans is a transparent worm-like animal; with a body size ranging between 400 – 600 μm, and must be viewed under magnification to properly identify (Figure 1.1). The root lesion nematode is distinguishable by a hollow stylet in the head region which bears rounded basal knobs and functions to pierce root cells for feeding (Davis and MacGuidwin, 2000). Also located in the head region, are lips which consist of three annuals that are distinguishingly flattened and dark (Tarte and Mai, 1976). Other key diagnostic characters are the overlapping esophagus as well as a round spermatheca, ovaries that do not reach to the esophagus, and a rounded tail without striations. Sexual organs within the adults appear as a vulva and ovaries in females or a spicule and testes in males (Davis and MacGuidwin, 2000). In females, the vulva is positioned at about two-thirds the body length with an adjacent spermatheca used for sperm storage for future reproductive purposes. In males, the spicule serves as a distinguishing feature, located near the tail in a sickle shape (Tarte and Mai, 1976).

Figure 1.1. Example of adult female root-lesion nematode with diagnostic characteristics labeled.

Through sexual reproduction, a single female can produce up to 68 eggs in her lifetime (Mamiya, 1971; Thistlethwayte, 1970). Egg production increases directly as a function of temperature peaking at roughly 30°C, but dramatically declines at 33°C (Mamiya, 1971). When laying eggs the female is indiscriminate in locations – often depositing eggs in and out of the root (Mizukubo and Adachi, 1997). Nematode development is similar to hemimetabolous insects, with the juveniles resembling adults and multiple molting stages occurring before adulthood is reached. At each molting stage, the nematode will shed its outer layer, or cuticle, and stylet (Zunke, 1990). While still encased in the egg, the first stage juvenile (J1) will develop

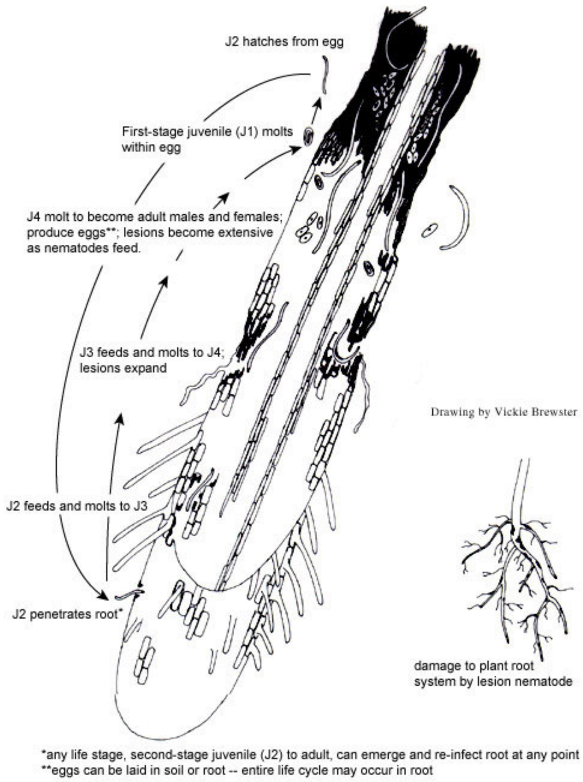

Figure 1.2. Visualization of the root lesion nematode's life cycle (Davis and MacGuidwin 2000)

and eventually emerge as the second stage juvenile (J2). J2 juveniles begin feeding on and traveling within roots and will molt into the third-stage juvenile (J3) and finally reach adulthood (Figure 1.2). Rate of development is strongly dependent on temperature, with more rapid development at higher temperatures. The optimal temperature range is between 22-30°C but reproduction and development is still possible at temperatures as low as 15°C (Acosta and Malek, 1979). In field conditions, the life cycle is typically completed within 4-8 weeks, allowing for multiple generations in a growing season and rapid population growth if not managed. (Davis and MacGuidwin, 2000).

Feeding occurs primarily on small, fine roots and is initiated by the nematode rubbing its lips on the exterior of the root presumably to prepare for cell piercing (Zunke, 1990). Once pierced the root cell is injected with pharyngeal gland secretions which increase pressure within the cell. Using the pressure differential the nematode then pumps out the cell contents (Zuckerman, 2012). When emptied, the nematode moves to another cell repeating the process (Zunke, 1990). This results in areas of necrosis (dead cells) along the root, reducing the plant's ability to uptake water and nutrients and ultimately causing symptoms such as wilting or chlorosis (Davis and MacGuidwin, 2000).

In field conditions, root lesion nematode survivorship is reduced in heavy soils such as those primarily composed of clay, or in soils with extremely low or high soil moisture levels (Mai and Kable, 1968). The nematode inhabits primarily soil pores and root tissues, moving via the water associated with soil particles (Nickle, 1991). Extremely dry soils, can lead to desiccation, or a state of quiescence known as anhydrobiosis where the nematode will suspend development until a return to favorable conditions (McSorley, 2003; Nickle, 1991; Sommerville and Davey, 2002). The length of time in which the nematodes will remain viable is variable depending on relative humidity and the speed of rehydration with higher humidity and slower rehydration periods favoring increased viability (McSorley, 2003). In ideal conditions *P. penetrans* typically remain viable up to 60 days, with remaining viable after a year or more of anhydrobiosis (Townshend, 1984). Other adverse environmental conditions such as high salinity, low oxygen, or low temperature can lead to other quiescence states including osmobiosis, anoxybiosis and cryobiosis, respectively (McSorley, 2003).

Distribution throughout the soil strata is strongly influenced by host crop root depth. The deeper the host crop roots travel into soil, the greater the vertical distribution of *P. penetrans*, but the greatest proportion is found in the top 0-30 cm of soil (Pudasaini et al., 2006).

1.1.2 *Verticillium dahliae*

Verticillium dahliae Kleb., is a soil borne fungal pathogen infecting a wide range of dicotyledonous crops. *Verticillium dahliae* can be distinguished via the production of septate, hyaline hyphae that become dark brown, and the development of black microsclerotia structures. Conidiophores are also branching forming a whorl (Smith, 1965). At the end of the whorls on separate branches lie single conidia which are dark in color and elliptical in shape (Smith, 1965). As a deuteromycete, *V. dahliae* does not reproduce sexually; instead, reproduction occurs asexually via the production of conidia. Microsclerotia serve as overwintering structures as well as primary inoculum the following season (Johnson and Dung, 2010; Mol and Termorshuizen, 1995). Microsclerotia remain dormant in the soil until a host or non-host is present where root exudates will stimulate the germination of microsclerotia resulting in hyphal growth. The hyphae will penetrate into the susceptible plant's roots and travel into the xylem. Conidia are produced along the way which travel and reproduce throughout the xylem vessels potentially infecting nearby cells further spreading infection (Figure 1.3). Eventually, this clogs the xylem and causes wilting (Wheeler et al., 2012). Microsclerotia are formed throughout plant senescence until the plant tissues contain low moisture levels where it then overwinters (Powelson and Rowe, 1993). It is important to note that this pathogen can also overwinter in plant residues as either mycelium or conidia, however the longevity is greatly reduced in these states (Mace, 2012). In the microsclerotia form, *V. dahliae* can survive in fallow fields for longer than a decade (Wilhelm, 1955). Moreover, *V. dahliae* has an extensive host range of over 300 primarily dicotyledonous plants spanning trees, fruits, vegetable, field crops, ornamentals, and weeds. Aside from potato, other agronomic crop hosts include mainly alfalfa and clover (Berlanger and Powelson, 2000). Because of the longevity and host range, management is difficult with growers often relying on fungicidal treatments.

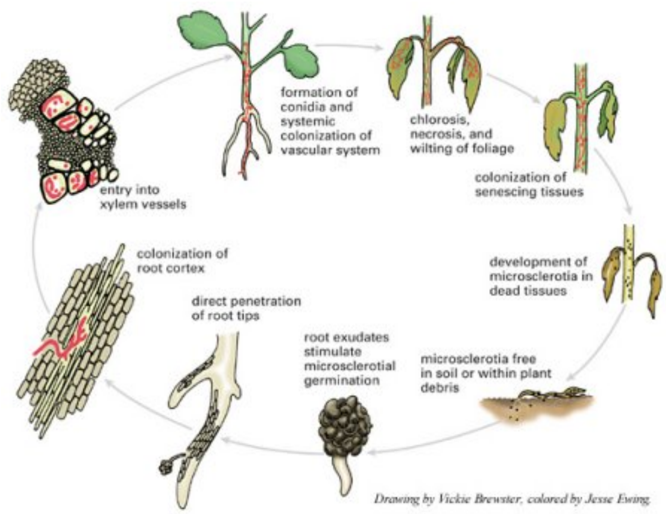

Figure 1.3. Life cycle of *Verticillium dahliae.*

Optimal growth occurs in moist soils with temperatures between 18- 29°C (Powelson and Rowe, 1993; Soesanto and Termorshuizen, 2001). Excessive through cycles of drying and wetting increases the levels of soil inoculum through *V. dahliae* sporulation (Cappaert et al., 1992). This has also been observed when there is an excess in available soil nitrogen (Cappaert et al., 1992; Sewell and Wilson, 1967; Sivaprakasam and Rajagopalan, 1974; Wheeler et al., 2012). The exact mechanism for increased *V. dahliae* in higher nitrogen soils is not perfectly clear however it is suspected that the excess nitrogen provides a more suitable substrate for *V. dahliae* (Isaac, 1957).

In potato, symptoms typically appear as chlorotic lower leaves that develop into a single wilted stem (Busch et al., 1978).These symptoms are exaggerated during periods of intense sunlight and heat but ultimately the severity of infection is directly related to the density of initial inoculum. In the field, diagnosis can be confirmed later in the growing season. When cut horizontally, infected stems will exhibit signs including a brown ring along the outer edges. Some infected tubers will also present browning within the vascular ring.

1.1.3 POTATO EARLY DIE

Potato early die (PED) results from the synergistic interaction between *P. penetrans* and *V. dahliae* (MacGuidwin and Rouse, 1990; Martin et al., 1982; Rowe et al., 1985; Saeed et al., 1998, 1997). Damage and yield loss from the two pathogens in concert is far greater than when alone indicating a synergistic rather than an additive effect. The complex ultimately leads to premature potato senescence, approximately 4-6 weeks early, hence the name "Potato Early Die". While the exact mechanism in which these two organisms interact to form a disease complex is not fully understood, it is hypothesized that nematode feeding may increase root exudates in the soil thereby increasing the germination of *V. dahliae* and subsequently increasing inoculum density (Bowers et al., 1996). Others have suggested that nematode feeding will increase root branching and therefore increase the area in which Verticillium hyphae can infect the plants (Rowe and Powelson, 2002a). The synergism, however, is not as simple as the nematode providing excess wound site thus allowing increased Verticillium infection. Moreover Saeed et al. (1998) found that even a single *P. penetrans* per cc of soil can induce synergism between *V. dahliae* and dramatically reduce potato yields. PED is worsened in fields with continuous potato, which promotes disease development over subsequent years and increases initial inoculum (Davis, 1985). Virgin potato fields typically experience limited PED expression, however without active management and prevention, inoculum and nematode populations increase overtime, worsening PED severity (Johnson and Dung, 2010).

Table 1.1. Nematode risk ratings based on nematode populations within root and soil as well as *Verticillium dahliae* populations in soil.

NEMA RISK	VERT RISK				
	1	2	3	4	5
1	1	2	3	4	5
2	1	3	4	5	5
3	3	4	4	5	5
4	4	5	5	5	5
5	5	5	5	5	5

Nematode Risk Rating (100cc soil + 1g roots)

1	<25
2	25-74
3	75-149
4	150-299
5	>=300

Verticillium Risk Rating propagules per 10 g air-dried soil

1	<=15
2	16-35
3	36-60
4	61-100
5	>100

The risk associated with PED has often been expressed as a matrix between root lesion nematode populations in 100 cc of soil + 1 gram of roots and *V. dahliae* propagules from 10g air dried soil (Table 1.1). The greater the populations of either root lesion nematode or *V. dahliae* the higher the risk for severe potato early die development. Overall, fields inflicted with PED can experience significant yield reductions of 20-50% (Rowe and Powelson, 2002b).

1.1.4 Potato Production

Potatoes originated in the Andean mountains and have since developed into an essential commodity for many cultures (Hawkes, 1992). Worldwide, potatoes are the 4th most consumed staple crop after corn, wheat and rice (FAO Stat, 2019). China, India, Russia and the Ukraine are the leaders in world production followed by the United States with average production of roughly 95.7, 43.4, 31.1, 21.7, and 20.0 million tonnes per year, respectively (FAO Stat, 2019). Within the United States, Michigan is the 6th largest producer of potatoes, harvesting an average of 46,000 acres worth approximately $173 million annually (NASS, 2016). Over 40 counties produce potatoes with Montcalm, St. Joseph, Bay, and Presque Isle counties leading potato production with nearly 70% of the potatoes harvested used for the chipping industry making Michigan the largest producer of chipping potatoes (NASS, 2016).

In US production, potatoes are propagated vegetatively via seed potatoes which are smaller tubers containing buds called “eyes” that germinate into the shoot. Seed pieces are planted in furrows approximately 6-12 inches apart with row spacing ranging from 30 – 36 inches. From planting to harvesting, the potato season will last roughly 90-110 days for many russet varieties. Depending on the soil temperature, emergence will occur in roughly two to four weeks. Once 8 – 12 inches tall, the plants are hilled to protect the roots and tuber from sun and heat, and aid in harvest (Bohl and Johnson, 2010). Vine killing occurs roughly two-weeks prior to the intended harvest date via broadcast herbicide applications. This is done for multiple reasons including: reduced vegetation during harvest, clean separation of tuber from stolon, adequate skin set on the tuber to reduce damage during harvest, and reduced storage weight loss (Zotarelli et al., 2003). After this two-week period, tubers will be harvested, typically with a mechanical potato harvester. Optimal growth occurs in well drained soils such as a sandy loam with a pH between 5.5 to 6.0 and temperatures between 50 – 86°F (Bohl and Johnson, 2010). To accommodate the potatoes lower temperature preference, growers often plant in late spring between April and May. Moreover, since Michigan temperatures often exceed the upper threshold for potatoes, irrigation is necessary to accommodate water loss via evapotranspiration and prevent negative physiological responses to changes soil water content which can reduce yields (Shock et al., 2007).

1.1.5 Crop Pests

A wide range of organisms including arthropods, fungi, oomycetes and bacteria are common pests of potatoes both during the growing season and storage. In Michigan, insect pests primarily affect the plant during growth stages. Commonly found chewing pests, which feed on leaf tissues, include Colorado potato beetles (*Leptinotarsa decemlineata*) and flea beetles (*Epirixcucumeris* spp.). Other pests include piercing sucking pests such as green peach aphid (*Myzus persicae*), potato aphid (*Macrosiphum euphorbiae*), and potato leafhoppers (*Empoasca fabae*) which pierce into plant tissues to feed on sap. Root feeders are typically immature insects which include white grubs (*Phyllophaga* spp.) and wireworms (Elateridae) (Johnson, 2000).

The majority of fungal, oomycete and bacterial pests are soil borne, causing damage to the developing plant, tuber or both. Fungal diseases most frequently found in Michigan potatoes include early blight (*Alternaria solani*), black scurf (*Rhizoctonia solani*), dry rot (*Fusarium sambucinum*), and Verticillium wilt (*Verticillium dahliae*).

Early blight is a polycyclic disease capable of infecting plants multiple times throughout the growing season. Infection results in bullseye shaped lesions on the leaves as well as necrotic areas along the tuber (Ding et al., 2019). Problems related to black scurf infection are typically reduced germination and seedling vigor as well as black patches, cracking or cankers on the tuber. The black patches are caused by masses of fungal structures that adhere to the tuber which results in low marketability (Brierley et al., 2016). Dry rot is a particular issue during the postharvest phase of potato production and infects tubers through tuber wounding. In Michigan many tubers are stored throughout the winter which invites opportunity for dry rot development. Damage often appears as large concentric rings on the tuber ultimately resulting in the tuber shrinking and becoming shriveled (Bojanowski et al., 2013). For all of these fungal pathogens, fungicide applications are standard.

Oomycete pathogens which are described as water molds and lack chitin in their cell walls, unlike fungi, are very damaging within potato systems usually causing tuber rot. Common pathogens include Pythium leak (*Pythium ultimum*) and late blight (*Phytophthora infestans*) (Johnson, 2000). Late blight appears on the leaves has brown or black patches, particularly near the tips of the leaves. In tubers, areas are shrunken and beneath the skin lies areas of rot that are brown and granular (Robinson et al., 2017). Pythium leak infection occurs in the the soil through tuber wounds but is a primary issue during storage. Infection initially leads to lesions on the tuber but ultimately results in starch breakdown and "liquidization" inside the tuber (Jones, 1935).

Bacterial pathogens can also be detrimental to potato production especially in post-harvest storage. Common bacterial pathogens include common scab (*Streptomyces* spp.) which results in scab like damage on the outside of the tuber and can dramatically reduce marketable yields depending on severity (Dees and Wanner, 2012).

In a 2018 survey, late blight, common scab, and PED ranked as the highest disease concerns within Michigan potato production (J. Willbur, personal communication, July 1, 2019). The same diseases also ranked in the top three diseases requiring critical management improvements among survey participants (J.Willbur, personal communication, July 1, 2019).

1.1.6 Chemical Control Measures

Control tactics, particularly for potato early die has historically involved frequent use of soil fumigants. Products such as methyl bromide, metam sodium (Vapam), and 1,3- dichloropropene (Telone®) have been the primary fumigants used in Michigan agriculture. Recently, the Environmental Protection Agency (EPA) has increased regulations for methyl bromide limiting product use due to the detrimental effects on the ozone layer in the upper atmosphere (EPA, 2005). Moreover, another commonly used fumigant, metam sodium, has been implicated in significant changes in the soil ecosystem as well as harmful health effects on human applicators (Macalady et al., 1998; O'Malley et al., 2005). These regulations and deleterious effects have dramatically reduced the use of fumigants and created a push for alternatives, such as nematicides. Nematicide chemistries vary in their mechanism, toxicity and selectively. Commonly used within the Michigan potato industry is Vydate (oxamyl) which is labeled with a danger-poison signal word indicating a high toxicity when ingested, inhaled and/or applied to skin or eyes. Vydate is a systemic carbamate pesticide that controls nematodes via inhibiting nerve impulses, but is also effective against insects (Chitwood, 2003). Newer chemistries such as fluensulfone and fluazaindolizine are more selective in their target-pests (Kearn et al., 2014; Lahm et al., 2017). Fluensulfone, is a fluoroalkenyl thioether chemical that inhibits nematode mobility, feeding and development without the detrimental of consequences as other products (Kearn et al., 2014). Fluazaindolizine is non-systemic within the plant and specifically targets plant-parasitic nematodes within soil pores, however the mode of action still isn't fully understood (Lahm et al., 2017). Outside of fumigant treatments, Verticillium control is limited. Curative fungicides are currently not an option. As of 2019, only one preventative fungicide is on the market for Verticillium control in potato which is Elatus (solatenol and azoxystrobin).

This product is applied in furrow at planting to prevent infection from various soil-borne diseases including Verticillium (Syngenta United States: http://www.syngenta-us.com/fungicides/elatus, 2019).

1.1.7 Biological Control

Biological control is the use of one organism, a natural enemy, to manage another organism, a pest, through predation, parasitism, competition or antibiosis. Implementation can take the form of classical, conservation or augmentative biological control. Classical control requires the introduction of a natural enemy into a non-native area for the control of a pest, that is usually invasive while conservation biological control requires the promotion of a landscape or habitat to promote populations of natural enemies. This can be paired with augmentative biological control where a vast quantity of natural enemies are released to control pests. Promoting a habitat for the newly released biological control agents can promot reproduction and extend the longevity of the released natural enemies (Flint, 2012). Typically, in agro-ecosystems growers employ augmentative biocontrol by supplementing their ecosystems. Currently, there are limited biological control options for *P. penetrans*. One option however that has shown potential is *Purpureiocillium lilacinum* strain 251, formerly known as *Paecilomyces lilacinus*, commercially available in the United States through Certis USA labeled as MeloCon® WG. *Purpureocillium lilacinum* (Thom) Samson, is a widespread hyphomycete soil fungus with important biological control functions. *Purpureocillium lilacinum* is characterized, when plated, by its colonies that become pink to light purple during the sporulation phase as well as branched, septate hyphae (Esser and El-Gholl, 1993; Luangsa-Ard et al., 2011). Optimal growth occurs between 25 and 30°C, however trials have shown adaptability to a range of climates (Cabanillas et al., 1989; Jatala, 1986).

Described as entomopathogenic or nematophagous, *P. lilacinum* produces hyphae capable of infesting and parasitizing eggs of many plant parasitic nematode genera including *Meloidognye*, *Tylenchus* and *Globodera* (Jatala, 1986; Khan et al., 2006; Kiewnick and Sikora, 2006). Infection occurs when hyphae encounter an egg mass and consequently penetrates them. Over the course of a few days the eggs will be completely filled and coated with mycelium (Jatala, 1986). Khan et al. (2006) obseved hyphal penetration directly into the cuticle of female nematodes as well as natural openings such as the vulva or anus suggesting that it is more than just an egg parasite. In the soil, *P. lilacinum* can be found colonizing the surfaces of plant roots, however it is not an endophytic fungus (Holland et al., 2003).

Numerous studies have evaluated the efficacy of *P. lilacinum* on various nematodes and crops. Singh et al. (2013), reported a 43% yield increase and 61% *Meloidognye*-induced gall reduction in *P. lilacinum* treated tomato plants when compared to the control. Kiewnick and Sikora (2006), reported similar yield responses in tomato. In potato, significant reductions in potato cyst nematode cysts and juvenile populations were observed (Hajji et al., 2017). Studies have suggested however that the level of efficacy is correlated to the concentration of the fungus when applied (Jatala, 1986; Oclarit and Cumagun, 2009). Kiewnick and Sikora (2006) studied the effect of MeloCon application rates on *Meloidogyne* control and found significant reductions in nematode populations were achieved at rates between 0.025 and 0.1 g/ 500cm^3 soil. There have not been studies focusing on the infectivity of *P. lilacinum* on *Pratylenchus penetrans* so the suitability of this organism as a biological control agent is relatively unknown.

1.1.8 Cultural Control

Cultural control options are essential to preventing the onset of PED but can have very little curative action. The first task in preventing potato diseases is purchasing certified diseasefree seeds. From here traditional cultural control tactics can be implemented such as using tolerant varieties and rotating to poor-host crops. Due to the wide host range of *P. penetrans*, rotating away from host crops is difficult. A few rotational crops to control *P. penetrans* have been identified including marigolds, forage pearl millet (*Pennisetum glaucum*), and sorghum sudangrass (*Sorghum × drummondii*) (Ball-Coelho et al., 2003; Everts et al., 2006; Sritharan et al., 2007a). Moreover, the longevity of *V. dahliae* microsclerotia allows this fungus to persist in field conditions for long periods of time without a host, making crop rotations effective in temporality reducing inoculum but ineffective at eliminating it. Breeding efforts have produced *V. dahliae* tolerant cultivars including russet varieties cv. Goldrush, cv. Ranger Russet, cv. and the chipping variety cv. Missaukee (Douches et al., 2010; Pavek et al., 1992). These cultivars do not prevent the infection of *V. dahliae* but prevent yield reductions due to disease. There are no current varieties available that are resistant to either root lesion nematodes or *V. dahliae*.

The utilization of green manures and/or bio-fumigant crops has shown promise in reducing potato early die incidence as well. Green manures are cover crops, typically ones with allelopathic properties such as those within the Brassicaceae family that are tilled into the soil (Kruger et al., 2016). The tilling process adds organic matter and in the case of bio-fumigant crops, toxic chemicals such as glucosinolate (Kruger et al., 2016). The incorporation of biofumigant green manures has been implicated in the reduction of nematode populations (Larkin et al., 2010; Ntalli and Caboni, 2017; Rahman and Somers, 2005). These results have not been consistent, however, with many other studies showing a lack of nematicidal effect (Korthals et al., 2014; Rudolph et al., 2019).

1.1.9 Compost

Compost is created through the breakdown of organic materials via controlled decomposition and is commonly used as a soil amendment to improve soil quality and nutrient availability (Azim et al., 2018; Wilson et al., 2019). Generally, compost is comprised of waste materials such as animal manures, plant materials, municipal wastes or a combination of base materials. A completed compost can be achieved either anaerobically or aerobically and contains an abundance of microorganisms that aide in the breakdown of solids.

In addition to improving soil health, composts and manures may aid in the control of nematodes and pathogens (Forge et al., 2016). The inherent diversity of compost means that all composts and manures are not equal in their pest control potential. For instance, Korthals et al. (2014) found that plant-based composts increased plant parasitic nematode and *V. dahliae* populations compared to an untreated control. On the other hand, Forge et al. (2016) observed layer manure with plant waste composts reduced root lesion nematode populations compared to the control and performed similar to fumigation in terms of nematode control.

Compost pest control mechanisms are not fully understood, but some possibilities have been explored. Volatile Fatty Acids (VFA) are present within composts and manures, particularly in liquid swine manure, and have been implicated in nematode reduction. A study completed by Mahran (2008) elucidated that acetic acid and capronic acid significantly reduced *P. penetrans* survivorship at low concentrations. Butyric acid has also been implicated as a source of nematicidal activity within composts as well as valeric acid which are produced by microbial communities during semi- or fully-anaerobic conditions of the composting process (Browning et al., 2006, 2004; Mahran et al., 2008; Plachá et al., 2013). VFAs are short lived as they serve as an energy store for future microbial activity but also are absorbed or degraded fairly quickly as well (Brinton, 1998). The detrimental effect of VFAs on nematodes is not fully understood, but it's been suggested that some prevent the uptake of materials such as phosphate, organic acids and amino acids (Mahran et al., 2008). Moreover, depending on the plant, levels of VFAs may induce phytotoxicity thereby stunting their growth (Brinton, 1998; Himanen et al., 2012).

Aside from pest control, the addition of composts aids in promoting soil health parameters such as porosity, aggregate stability, water retention, microbial diversity as well as improved nutrient availability through the addition of organic matter (Abawi and Widmer, 2000; Widmer et al., 2002). Soil structure is critical for optimal crop production, with the addition of composts or manures, macro-porosity has nearly doubled from 8.58% to 16.70% in field trials compared to an untreated control (Pagliai et al., 2004). Moreover, with improved pore structure, the soil is better equipped to retain moisture which ultimately reduces irrigation needs as well as nutrient leaching (Pagliai et al., 2004).

1.1.10 Summary

Potato early die complex consists of the synergistic interaction between the plant-parasitic nematode *Pratylenchus penetrans* and fungal pathogen *Verticillium dahliae*. Together these pests can cause significant damage to potato crops and reduce yields by 25% or more (Rowe and Powelson, 2002b). Current management strategies are heavily reliant on chemical programs often utilizing broad-spectrum soil fumigants during pre-plant preparations. Due to the ozone depleting nature of methyl bromide, the EPA began a phaseout of the product starting in 2005 which has since created the need for alternative management strategies. Cultural tactics such as crop rotation, green manures, cover cropping etc. provide temporary relief of pest pressures, but are not perfect in their pest control abilities. On the other hand, biological control options are sparse and rarely implemented by growers. Previous works have shown promise in the effects of composts and newly developed nematicide chemistries on reduction of potato early die incidence.

1.1.11 Objectives

The objectives of the studies presented in this thesis are to explore cultural, biological and chemical control tactics to reduce potato early die. The first objective was to determine the effect of commercially available manures and manure-based composts on *P. penetrans* and *V. dahliae* populations and yield. I hypothesized that adding manures or manure-based composts would significantly reduce P. penetrans and V. dahliae populations, reduce V. dahliae infection and increase yield compared to the control. The second objective was to evaluate nematicide chemical programs as well as potential biological control fungus and their impact on potato early die and potato yield. I hypothesized that compared to the untreated control the addition of nematicides or biological controls will significantly reduce *P. penetrans* populations as well as *V. dahliae* incidence while increasing mean yields.

CHAPTER 2. IMPACTS OF MANURES AND MANURE-BASED COMPOSTS ON ROOT-LESION NEMATODES (*PRATYLENCHUS PENETRANS*) AND *VERTICILLIUM DAHLIAE* IN MICHIGAN POTATOES

2.1 INTRODUCTION

Potatoes (*Solanum tuberosum* L.) are an important commodity within Michigan agriculture, contributing over $181 million to the state's agricultural value (NASS, 2018). Pest and disease pressures play a significant role in increasing production costs and reducing marketable yields. Disease complexes are difficult to manage due to the interaction between organisms – often from differing classes. Potato early die complex, in particular, is caused by the interaction between the root lesion nematode *Pratylenchus penetrans* (Tylenchida: Pratylenchidae) (Cobb, 1917) and the soil-borne fungal pathogen *Verticillium dahliae* (Glomerellaes: Plectosphearellaceae) (Klebhan, 1913). Root lesion nematodes feed on roots creating necrotic lesions while *V. dahliae* penetrates the roots with hyphae, traveling to the xylem and moving throughout the plant. Eventually, *V. dahliae* will clog the vascular tissues and cause wilting (Rowe and Powelson, 2002b). This synergistic interaction causes damage greater than if either pest were alone. Potato early die results in reduced nutrient uptake and biomass and premature plant senesce which leads to yield losses up to 50% (Rowe and Powelson, 2002b; Ruijter and Haverkort, 1999). With such substantial yield loss potential, growers must implement management strategies.

Management of potato early die can be difficult with cultural control tactics such as crop rotation or cover cropping due to the wide host range of both *P. penetrans* and *V. dahliae* (Kimpinski et al., 2000; Larkin et al., 2010; Woolliams, 1966). The overwintering microsclerotia of *V. dahliae* are also detrimental to management programs as they can persist in the soil for over ten years (Mace, 2012). Aside from cultural controls, preventative chemical control tactics do not offer effective management since symptoms do not appear until mid-season when the plant has already been infested by *V. dahliae* and experienced significant nematode feeding (Rowe and Powelson, 2002b).

Because of these hurdles, many growers turn to intense preventative chemical control programs often utilizing broad-spectrum soil fumigants. Historically, fumigants such as methyl bromide, metam sodium, and 1,3 - dichloropropene have been popular because of their wide spread efficacy in controlling soil borne pests (EPA, 2005). In 2005, the EPA began a phase out the use of methyl bromide, and in the last decade placed greater regulations on the use of other soil fumigants because of their danger to human and environmental health (https://www.epa.gov/ods-phaseout/methyl-bromide). This is not a US centric occurrence either; fumigation restrictions have been placed across Europe and other countries including Australia, Canada and Japan (Porter et al., 2010). Many areas with methyl bromide restrictions have turned to other forms of control including alternative chemical fumigation, solarization and biological control. Some countries have also implemented tactics such as soilless media, steaming, bio-fumigation, compost and mulching (FAO, 2001). Despite the availability of alternative chemical controls, many soil fumigants are still costly to apply and detrimental to soil health thereby calling for alternative management options to be explored (Ibekwe, 2004; O'Malley et al., 2005; South and Carey, 2000; South and Gjerstad, 1980).

Composts and manures are products commonly used within agricultural systems to improve nutrient availability and soil health and there is increasing interest in their pest control potential. A variety of composts and manures have been previously investigated as a potential control agent for potato early die. Previous work has demonstrated that the addition of composts or manures in forms such as liquid swine manure or dairy manure compost can potentially reduce populations of *P. penetrans* and *V. dahliae* (Conn et al., 2005; Forge et al., 2016; Molina et al., 2014). Moreover, manure-based composts have shown the greatest pest reduction ability, next to biofumigants, compared to other feedstocks such as agricultural waste, food waste or green manures (Korthals et al., 2014; Oka, 2010).

Although evidence has been found supporting the use of manures and composts to reduce potato early die, the inherent variability of composts both in their feedstocks and micro fauna populations make broad-spectrum recommendations difficult. Therefore, the objective of this study was to determine whether multiple commercially available compost and manure products available in Michigan could reduce *P. penetrans* and *V. dahliae* populations and enhance potato yields.

2.2 MATERIALS AND METHODS

To test the effects of composts and manures on potato early die, I first performed preliminary laboratory trials. The focus of the laboratory trials were *P. penetrans* survivorship when exposed to various products. The initial laboratory experiment exposed root lesion nematodes to 100% product which then lead us to test the best performing products at lower rates to mimic field applications. With knowledge gained from laboratory trials, I tested the best performing products in the field and monitored for changes in *P. penetrans* and *V. dahliae* populations as well as yield effects.

2.2.1 Compost Bioassays

2.2.1.1 *Nematode Culture*

Root-lesion nematodes (*Pratylenchus penetrans*) used for all lab experiments were from a laboratory colony (University of Wisconsin – Madison, Department of Plant Pathology). Nematodes were reared from a single female specimen collected from Wisconsin field soil, on

Gamborg's B-5 media with corn seedlings as the nutrition source. Inoculum was a mixture of male and female, adults and juveniles.

2.2.1.2 *Compost and Manure Selection*

Table 2.1. Name, description and nutrient composition of the products used in studies.

Compost/Manure	Composition	Nitrogen (TKN[1]) (kg/t)	Phosphate (P_2O_5) (kg/t)	Potash (K_2O) (kg/t)
Layer Ash Blend	Composted dairy cow and poultry manure amended with wood ash	17.7	20.3	21.1
Poultry Manure	Pure poultry manure	47.1	33.9	27.7
Dairy Doo w/ Spelt Hulls	Composted dairy cow manure amended with spelt hulls	9.3	13.3	7.4
Worm Castings	Castings from red-wriggler worms	13.3	10.5	8.8
Poultry Compost	Composted poultry manure	47.1	33.9	27.7
Dairy Doo	Composted dairy cow manure	9.6	5.3	12.5

[1]Total Kjeldahl Nitrogen (TKN) quantifies the total concentration of organic nitrogen, ammonia and ammonium

To determine which compost or manure had the most potential for nematode control, I compared four composts: layer ash blend, dairy doo, dairy doo with spelt, and poultry compost, and two manures: chicken manure and worm castings (composts and manures were sourced from Morgan Composting Inc., Sears, MI USA) (Table 2.1). Products were evaluated for effect on rate of nematode mortality. Approximately, 40 mL of each product was placed into 50 mL centrifuge tubes (Corning Inc., Corning, NY USA) to provide an application rate of 100%, while a 100% play sand treatment served as the control. Each treatment was replicated 5 times for lengths of exposure of 1, 2, 5, or 7 days for a total of 20 arenas per treatment. Replicates were inoculated on day 0 with approximately 200 *P. penetrans* nematodes and immediately irrigated with 5 mL of water and loosely capped to allow for airflow. After the specified exposure time, the compost or manure was placed onto a modified Baermann pan (Figure 2.1), consisting of a 100 x 25 mm petri dish and a 24mm, 0.64 cm mesh size hardware cloth disk (van Bezooijen, 2006). The hardware cloth disk was lifted off the bottom of the petri dish by folding a 7.0 x 1.5 cm piece of hardware cloth into a 45° angle, placing it in the middle of the dish and setting the hardware cloth disk on top. Kleenex® facial tissue (Kimberly-Clark Corporation, Neenah, WI USA) was then placed on the hardware cloth disk, and the overhanging edges of the tissue were twisted around the inside edge of the petri dish to prevent water from wicking out. The petri dish was filled with enough water to touch the tissue, allowing surviving nematodes to move through the tissue and collect in the water. The compost or manure mixture was then placed onto and spread evenly over the tissue. After 48 hours, the liquid in the petri dish was collected to determine the number of surviving nematodes.

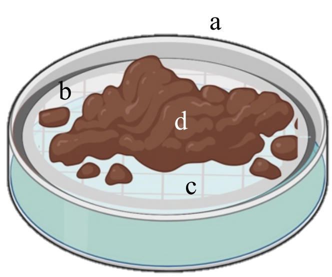

Figure 2.1.Example of a modified Baermann pan. Parts included: (a) 100 x 25mm petri dish, (b) hard ware cloth disk, (c) facial tissue, (d) manure/compost

2.2.1.3 ***Compost Rate Selection***

Two lab trials were conducted to determine how different rates of product influenced root-lesion nematode mortality. I tested layer ash blend, dairy doo, and poultry manure in both trials and a component of the layer ash blend – wood ash in the second trial. In Trial 1, products were tested at six rates: 5, 15, 30, 50, 75 and 100% compost or manure with a 0% product (100% sand) control. For Trial 2, products were tested at five rates: 0.1, 1, 5, 10, and 20% compost or manure with a 0% product (100% sand) control. Composts were homogenized with play sand on a volumetric ratio to achieve the desired rate of compost and obtain a total volume of 20 cm^3.

The mixtures were placed into 50 cm^3 centrifuge tubes, irrigated with 5mL of water, and inoculated with approximately 200 *P. penetrans* nematodes. The caps were placed on top of the tubes but not sealed to allow for airflow. Nematode survivorship was determined after 7 d, using the modified Baermann pan process described previously.

2.2.2 Field Trial

2.2.2.1 *Experimental Design*

A field trial was conducted at the Montcalm Research Center (Entrican, MI, USA; 43.352746, -85.170061) in summer 2018. Soil at this site was primarily characterized as a Tekenick-Elmdale loamy sand comprised of approximately 75.1% sand, 20.9 % silt, and 4.0% clay with roughly 1.46% organic matter in the top 30 cm of soil (USDA-NRCS Web Soil Survey, 2018) Previous cropping history in this plot included potato (2015), pearl millet (2016), and corn (2017).

The experiment consisted of a complete randomized block design with four experimental treatments and four blocks. Experimental plots were planted with potato (cv. Norkotah Russet) seed pieces in 15.25 x 3.66 m plots, representing 4 rows with 86 cm spacing. All compost (dairy doo and layer ash blend) and manure treatments (chicken manure) were applied at a high rate of 11.2 t/ha and a low rate of 2.8 t/ha. An untreated control was included and Vydate® C-LV, hereafter referred to as Vydate, (oxamyl, 19898, CAS no. 23135-22-0, DuPont de Nemours and Company, Wilmington, DE, USA) was selected as a positive control due to the widespread use in commercial Michigan potatoes. For this study, I did not include a fumigation control due to a lack of residual effects and rapid degradation. With such small plot sizes, there would have been increased risk of contamination in the fumigated fields.

Moreover, my goal with this study was to compare composts and manures to the grower standard non-fumigant treatment.

On 4 June 2018, composts and manures were applied using a top dresser (EcoLawn Applicator, 960 Leon-Trepanier, Sherbrooke, Quebec, Canada). At planting, fertilizers (28-0-0 and 10-34-0 NPK) were applied directly to the seed pieces at rates of 224.5 and 112.2 L ha $^{-1}$, respectively. The Vydate treatment was applied directly to seed pieces prior to row closure using a CO_2 powered backpack sprayer at the label recommended rate for potatoes, 4.7 L ha $^{-1}$. Throughout the season, herbicides and insecticides were applied as needed (S2).

2.2.2.2 ***Field Conditions***

Weather data was obtained from Michigan State University's Enviro-Weather taken from the Entrican field station located < 0.5 km from the field site. A total of 3269 Growing Degree Days (base 40), and 377.17 mm rainfall were accumulated over the course of the growing season.

2.2.2.3 ***Soil and Root Collection***

Soil was collected pre-plant (June 4), two-weeks post-plant (June 22), mid-season (August 15) and at harvest (September 24) by randomly taking 10 soil cores from the center two rows in each plot at a depth of approximately 10 cm. The cores from each plot were homogenized within a plastic bag to equal approximately 1 kg soil per plot. After collection, soil was stored in a cooler at 46° C until use for analyses. At mid-season only, a minimum of 1g of root was collected from each plot by up-rooting a plant, collecting the fine roots and placing them into a paper bag.

2.2.2.4 ***Nematode Extraction and Identification***

Nematodes were extracted from a 100 mL subsample of soil via the elutriation and centrifugal flotation method (Jenkins, 1964). Within two days of collection, roots were washed, dried and weighed to obtain 1 g fresh tissue. The roots were then cut into 1-2 cm pieces and placed into 150 mL Erlenmeyer flasks with 50mL of 1% bleach solution. The flasks were placed on a wrist-action shaker at 175 rpm for 48 hours (Bird, 1971). Since root-lesion nematodes can easily pass through a 100-mesh sieve we placed the solution directly into 50 mL centrifuge tubes to ensure accurate counts. Plant-parasitic nematodes were identified to the genus level using morphological characteristics observed using an inverted Nikon TMS microscope between x200 and x1000 magnification.

2.2.2.5 *Verticillium Analysis*

Soil collected two weeks post-plant and mid-season was analyzed for *V. dahliae* microsclerotia germination using the dilution plating method (Nicot and Rouse, 1987). For each sample, 5 g of soil was mixed with 40 mL of distilled water. I then pipetted a 2.5 mL aliquot including a large portion of soil particles onto four plates of Verticillium growth media containing 1000 mL distilled water, 15 g Bacto-agar, 10 mL 200 proof ethanol, and 0.4 g Streptomycin sulfate (Nadakavukaren and Homer, 1959). After two weeks in a dark environment, the number *of V. dahliae* colony forming units (CFU) was determined using morphological characteristics (Goud et al., 2003).

At mid-season, 10 stems, approximately 12 cm in length, were collected from each plot directly above the base of the soil. Stems were placed in paper bags and stored in a cooler (46° C) prior to analysis. Within a week of collection, a 2 cm slice of each stem was cut, surface sterilized with a 5% bleach solution and placed vertically on Verticillium growth media (Nicot and Rouse, 1987) A total of five stems were placed on each plate in a circular arrangement. I kept the plates in a completely dark environment at room temperature for two weeks and counted the number of stems with *V. dahliae* germination present.

2.2.2.6 *Yield*

On September 24, a 7-m row was harvested from each plot using a potato harvester. The potatoes were then washed, weighed and graded. The total yield and size distribution were determined. Size classifications were as follows: oversize- ≥ 8.3 cm, U.S. No. 1 - 4.8 ≥ 8.3 cm, and B - < 4.8 cm. Tubers with shape defects were culled and labeled as pick outs (PO).

2.2.3 Compost Organic Acid Composition

Dairy doo, layer ash blend, and poultry manure samples that were 6 months past maturation as well as layer ash blend, poultry manure and ash samples that were 1 month past maturation were sent to SDK Laboratories (Hutchinson, KS) to determine their concentrations of acetic, butyric, lactic, propionic and valeric acids.

2.2.4 Statistical Analysis

All statistical analyses were performed using R version 3.5.1 (R Core Team, 2018). Nematode counts from the lab trials and the field trial as well as Verticillium soil populations were analyzed using a generalized linear model with a negative binomial distribution. In the compost and manure selection and rate selection trials, many treatments recovered a mean of 0.0 nematodes and were therefore excluded from the model due to inability to predict with zeros. Verticillium stem presence was analyzed using a generalized linear model with a binomial distribution. Means separation was completed using Tukey's Honest Significant Difference (HSD) with the "emmeans" package (Lenth et al., 2019). Significant differences in yield between treatments were determined by using analysis of variance (ANOVA) with a Tukey HSD post-hoc test (α= .05).

2.3 RESULTS

2.3.1 Compost and Manure Selection

Treatment and duration of exposure significantly influenced *P. penetrans* survivorship and there was a significant interaction between the two effects ($X^2_{12,140}$ = 0.92, p = <.001). Over the course of 1, 2, 5 or 7 days zero *P. penetrans* were recovered for each replicate treated with layer ash blend or poultry manure. Dairy doo with spelt hulls, worm castings and poultry compost similarly reduced the number of recovered nematodes over the course of the trial with lower survivorship over increased exposure times. With only one-day exposure, recovery in the dairy doo (mean ± SEM; 29.6 ± 3.3) treatment was nearly double that of the control (15.0 ± 3.2) however at 2, 5 and 7 days after treatment (DAT), nematode survivorship in dairy doo treatments was similar to all other treatments (Figure 2.2).

2.3.2 Compost Rate Selection

2.3.2.1 *Trial One*

Nematode survivorship varied significantly among rate ($X^2_{6,90}$ = 22.5, p = <.001). Dairy doo treatments resulted in greater mean survivorship than the control for the 15 (100 ± 23.0%), 30 (121 ± 21.2%) and 75% (131 ± 18.6%) compost treatments. Only at 100% diary doo did survivorship dramatically decrease with a 50% decline compared to the control (Figure 2.3). Poultry manure steadily decreased survivorship with increasing rate ultimately reaching 0% with 50 and 75% manure treatments. Layer ash blend treatments significantly reduced nematode survivorship to 0.0 with just a 5% application rates and remained less than 5% for all rates.

2.3.2.2 *Trial Two*

Type and rate of amendment significantly influenced the survivorship of nematodes and there was a significant interaction between treatment and rate ($X^2_{5,100}$ = 20.5, p = <.001). In treatments with 0% compost, we recovered a mean ± SEM of 20.4 ± 1.5 *P. penetrans* nematodes (Figure 2.4). At the lowest rate of 0.1% product, ash, dairy doo and layer ash blend (LAB) performed similarly with an average decline in survivorship. Nematode survivorship significantly increased in poultry manure treatments with 38.2 ± 2.4 nematodes recovered compared to 19.6 ± 4.2 in dairy doo ($p=.008$), 16.6 ± 3.5 in layer ash blend ($p <.001$) and 15.8 ± 3.1 in Ash ($p<.001$) at the same rate (Figure 2.4). Increasing the rate of poultry manure from 0.1% to 1.0% decreased mean survivorship by 59.7%, while increasing the rate to 5% decreased survivorship by 99.5% compared to 0.1% application. Low survivorship continued for 10 and 20% poultry manure rates as well with 0.0 ± 0.0 and 0.2 ± 0.2 mean nematodes recovered, respectively (Figure 2.4).

The layer ash blend consistently decreased nematode survivorship with increasing application rate (Figure 2.4). Incorporating 5% layer ash blend, decreased the mean survivorship by more than 50% (7.4 ± 0.8) while adding 10% compost reduced survivorship by nearly 100% (0.6 ± 0.4). Rates of 5 ($p = .001$), 10 ($p < .001$) and 20 ($p<.001$) percent layer ash blend were significantly different from 0%. Ash performed similar the layer ash blend with rates greater than 1% showing a significant reduction in root lesion nematode survivorship ($p<.001$).

2.3.3 Field Trial

2.3.3.1 *Soil Nematode Populations*

Mid-season samples showed exceedingly low populations of root lesion nematodes with an average of fewer than 3.0 ± 0.4 *P. penetrans* per 100 mL of soil per plot. Because of this, the mid-season date was excluded from analyses to prevent extraneous results. Nematode populations varied significantly among treatments ($X^2_{6,120}= 17.07$, $p = .009$), however they were not significantly different across sampling dates ($p = 0.86$) (Figure 2.5). Over the course of the entire season, plots treated with poultry manure had significantly fewer *P. penetrans* per 100 mL of soil compared to the control (P = .0078) and dairy doo high (DD High) (P= .0073) treatments.

Two weeks after treatment, we observed declines in populations for the layer ash high (LAB High), layer ash low (LAB Low), poultry high and Vydate treatments. The greatest numerical declines were in layer ash high and Vydate treatments with a 50.4% and 61.8% decrease, respectively when compared to the initial samples (Figure 2.5). Control, dairy doo high, and dairy doo low (DD Low) all experienced increases in root lesion nematode populations after two-weeks. The dairy doo high treatments increased by 50.0%, while the control and dairy doo low treatments increased by and 6.0% and 34.6%, respectively (Figure 2.5).

At harvest, dairy doo low (DD Low), layer ash high (LAB High), poultry low, and Vydate treated plots had an average increase in root-lesion nematode populations compared to the initial and mid-season samples. Numbers in dairy doo low (DD Low) treatments increased on average by 50% from the start of the experiment to the harvest sampling. In dairy doo high (DD High) treated plots mean abundance decreased by 1.25 root lesion nematodes per 100cc soil between 2-week post planting and harvest but this was still and average of 3.5 root lesion nematodes higher on average than the initial counts.

2.3.3.2 *Root Nematode Populations*

Roots collected mid-season showed very low root lesion nematode populations with all treatments having fewer than 2.0 root lesion nematodes per gram of root on average with no significant differences among treatments (p = 0.92).

2.3.3.3 *Verticillium Stem Populations*

Stems collected midseason did not show significant differences among treatments (p = 0.75). Plots treated with layer ash low (LAB Low), however did have the lowest proportion of stems that showed *V. dahliae* germination with 37.5% on average while the Vydate treated plot showed the highest proportion a 60% germination rate (Figure 2.6).

2.3.3.4 *Verticillium Soil Populations*

The number of *V. dahliae* germinated propagules varied significantly among sampling date ($X^2_{6,64}$= 20.8, p=.002), and were only significantly different among treatments in soil sampled two weeks after planting. At two weeks post treatment, plots treated with layer ash blend at a low rate (LAB Low), had an average of 5.25 ± 2.0 germinated *V. dahliae* colonies per 10g soil per plot (Figure 2.7). This was not significantly different from the control (15.75 ± 3.2 colonies) but was statistically lower than the dairy doo high (p = 0.01) and poultry high (p = 0.03) treated plots which had an average of 26.25 ± 6.6 and 24.75 ± 10.7 germinated colonies per 10g soil per plot, respectively.

2.3.3.5 *Yield*

Yields were not significantly different among treatments and were much lower than grower standards (P= .078) (Figure 2.8). Average potato yields in Michigan have historically equaled roughly 46,450 kg ha $^{-1}$, while the average yield in this trial would have been equivalent to approximately 16,820 kg ha^{-1} therefore, yields observed were nearly 36% lower than grower standard.

2.3.4 Organic Acid Composition

Concentrations of volatile fatty acids were not detectable in dairy doo samples or the poultry manure six months past maturity. The greatest concentrations were found in the layer ash blends, poultry manure one-month past maturity and the wood ash. Butyric and acetic acids were found in the highest concentrations with lactic acid and valeric acid only being found in low concentrations for poultry manure one-month past maturity (Table 2.2).

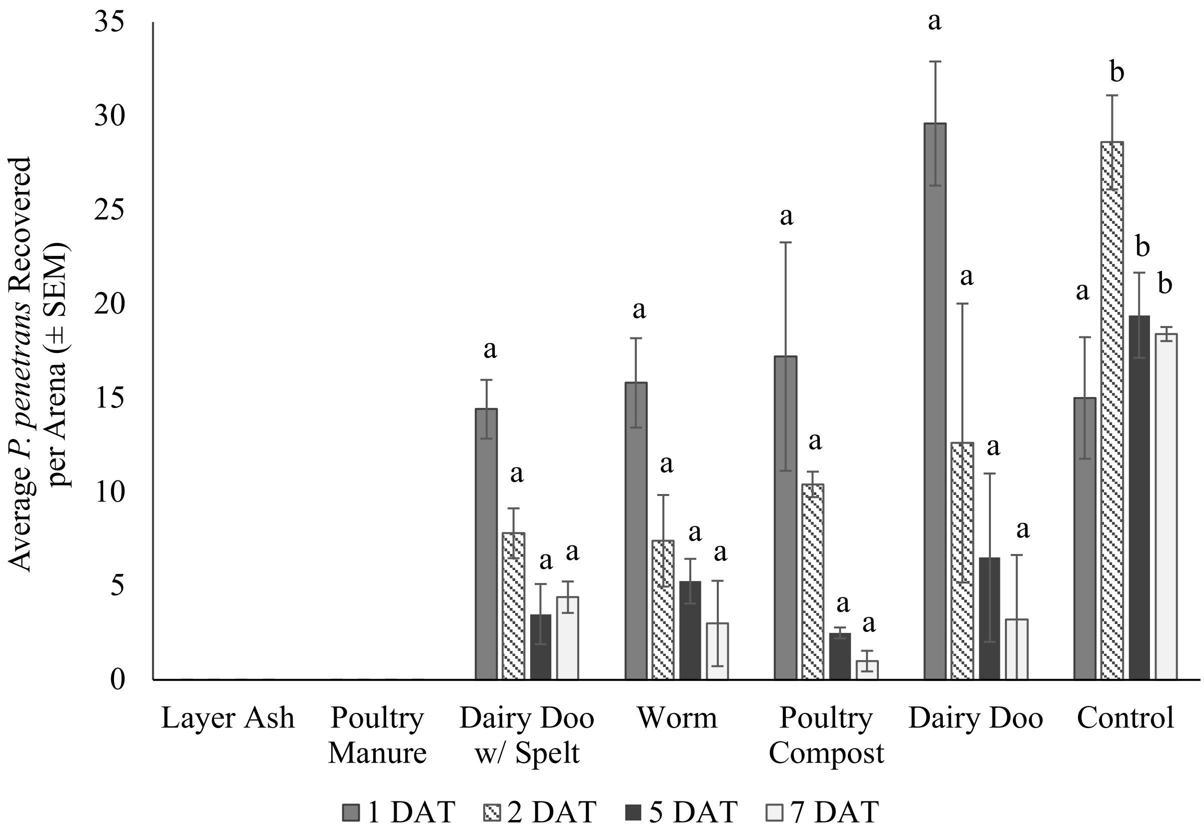

Figure 2.2. Mean ± SEM *P. penetrans* recovered out of 200 after exposure to dairy doo (DD), dairy doo with spelt hulls (DD w/ SH), poultry compost, worm casting, layer ash blend (LAB), and poultry manure for 1, 2, 5, or 7 days after treatment (DAT). Survivorship was determined by placing product on a modified Baermann pan for 48 hours and counting the number collected. LAB and poultry manure treatments were excluded from analyses because their survivorship was 0 for every replicate. Treatments labeled with different letters are significantly different within the DAT.

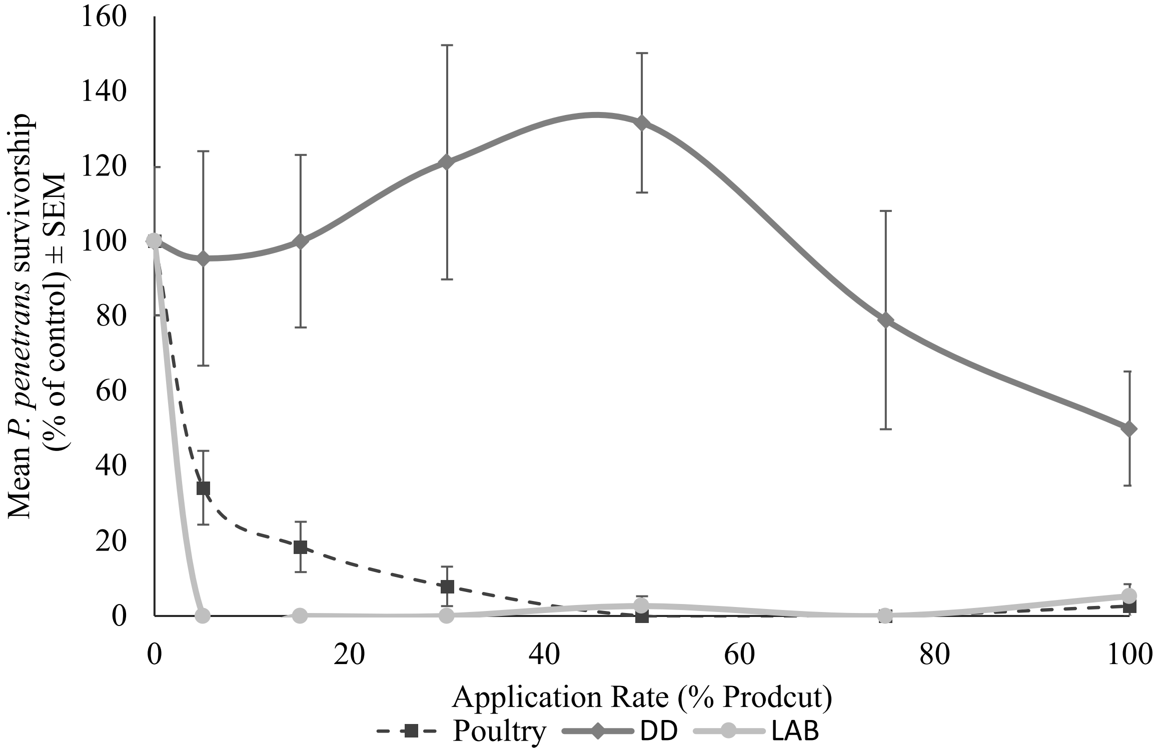

Figure 2.3. Mean *P. penetrans* survivorship as percent of control after 7-day exposure to poultry manure (Poultry), dairy doo (DD) or layer as blend (LAB) at rates of 0, 5, 15, 30, 50, 75 and 100 percent compost. Survivorship was determined by placing product on a modified Baermann pan for 48 hours and counting the number collected. Survivorship over 100% indicates greater survivorship than the control.

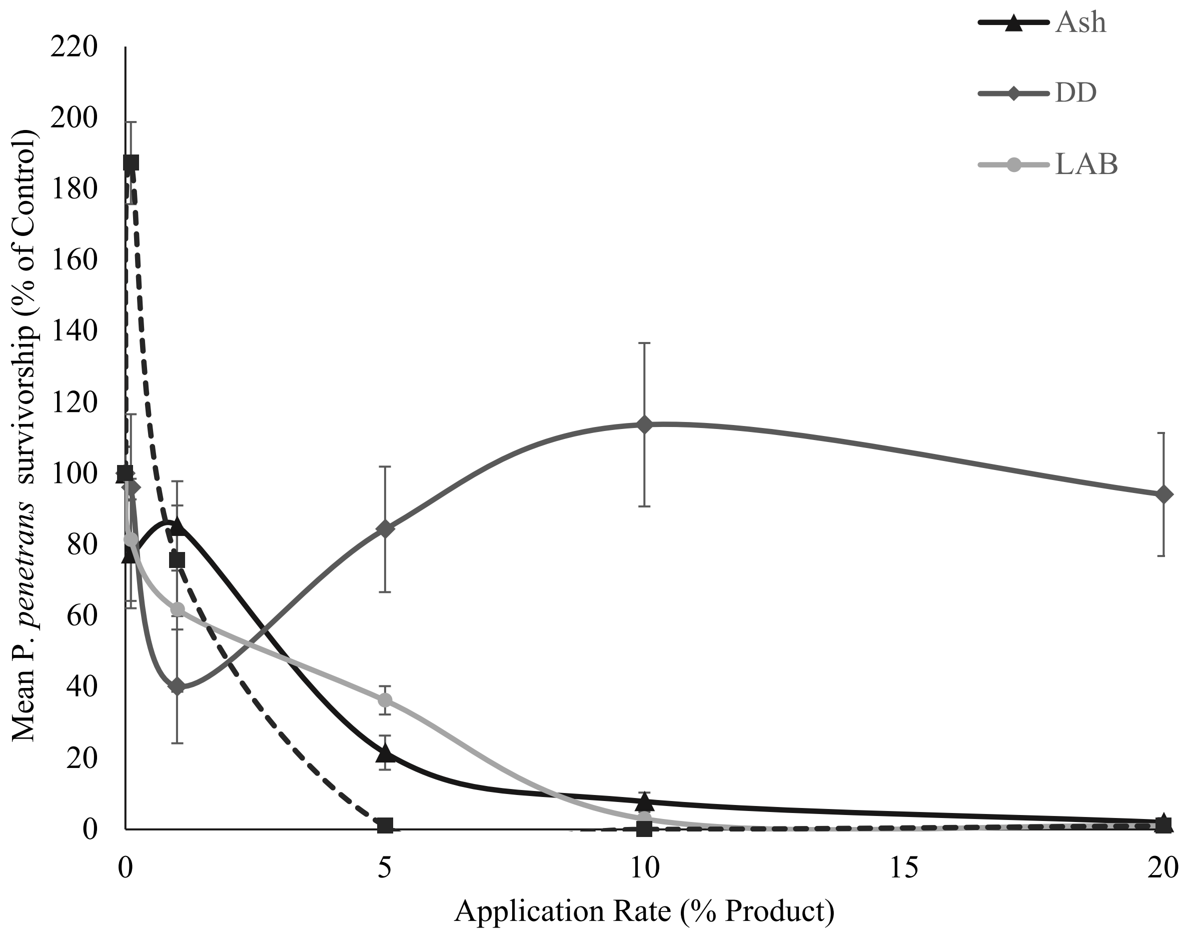

Figure 2.4. Mean ± SEM surviving nematodes collected after 7d exposure to Ash, Dairy Doo (DD), Layer Ash Blend (LAB) and Poultry Manure (Poultry) at rates of 0, 0.1, 1, 5, 10 and 20 percent product in a laboratory experiment. Rates based on a volumetric ratio of compost or manure homogenized with play sand. Living nematodes were collected using a modified Baermann pan.

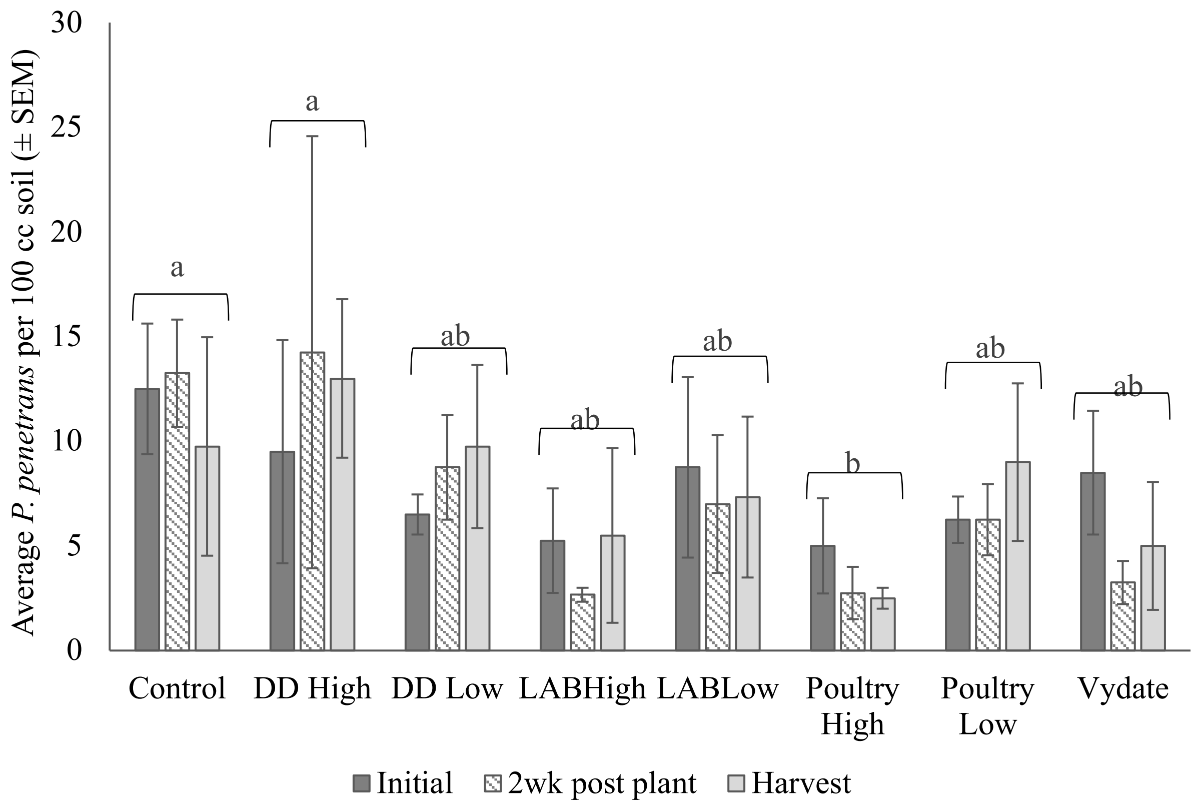

Figure 2.5. Mean ± SEM root lesion nematodes per 100 cc of soil at initial (June 4), 2-week post-planting (June 21) and harvest (September 24) samples dates. Treatments labeled with different letters are significantly different across all sampling dates (Tukey HSD, α = .05).

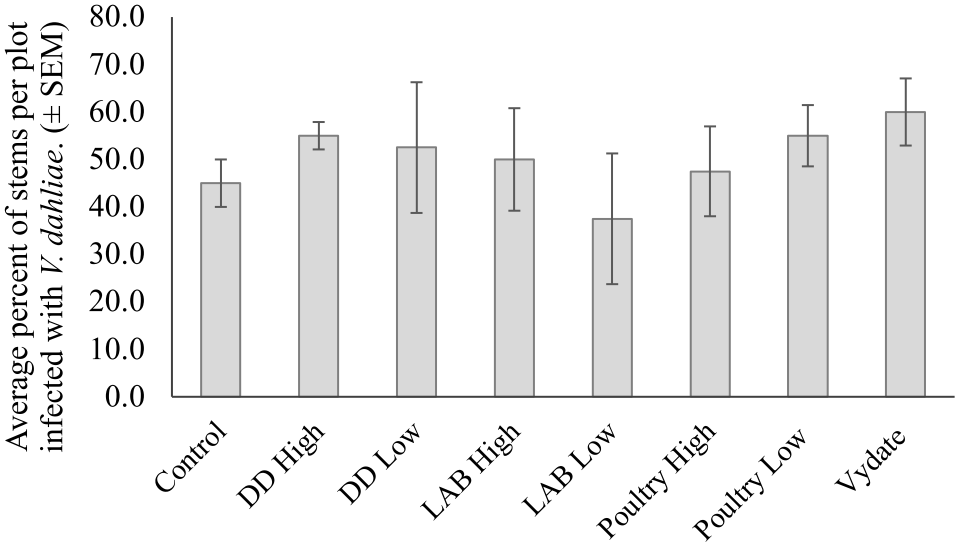

Figure 2.6. Mean ± SEM percent of stems per plot infected with *Verticillium dahliae* collected mid-season. Ten stem pieces per plot were incubated on Verticillium growth media to determine Verticillium infection. N.S.indicates no significance (α = .05).

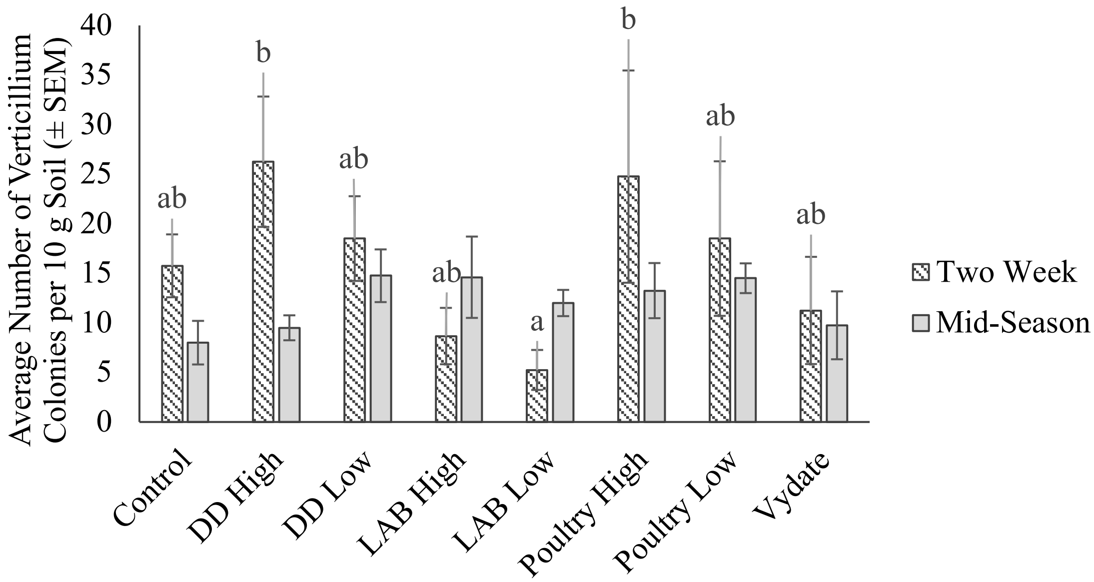

Figure 2.7. Average number of *Verticillium dahliae* colonies germinated per 10 g of soil per plot for control, Dairy Doo High (DD High), Dairy Doo Low (DD Low), Layer Ash High (LAB High), Layer Ash Low (LAB Low), Poultry High, Poultry Low and Vydate® treatments. Two-week samples were collected June 22, 2018 and the mid-season samples were collected August 14, 2018. Colony germination was determined via the dilution plating method. Treatments of the same date labeled with different letters are significantly different (Tukey's HSD, α = .05).

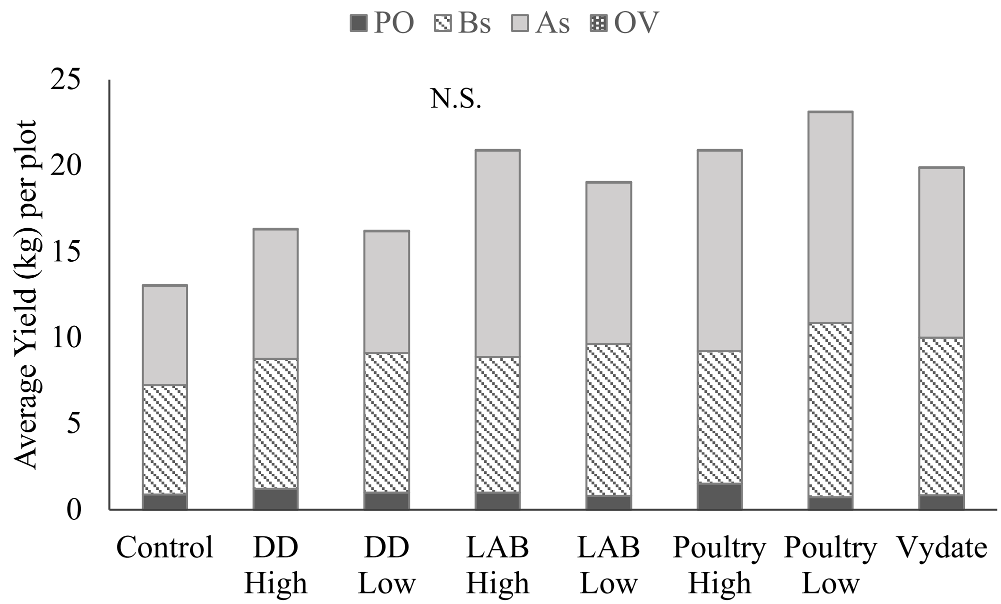

Figure 2.8. Average yield per plot separated into grade A (As), B (Bs), oversize (OV) and pick outs (PO) for control, Dairy Doo High (DD High), Dairy Doo Low (DD Low), Layer Ash High (LAB High), Layer Ash Low (LAB Low), Poultry High, Poultry Low and Vydate® treatments collected from one, 7 m row. N.S. indicates no significance (ANOVA, $\alpha = .05$).

Table 2.2. Volatile fatty acid concentration in one-month and six-month past mature compost and manure samples

	Volatile Fatty Acid				
	Lactic (%)	Acetic (%)	Butyric (%)	Propionic (%)	Valeric (ppm)
Layer Ash 1m[1]	<.01	0.68	0.17	0.02	<.01
Layer Ash 6m	<.01	0.02	<.01	<.01	<.01
Poultry Manure 1m	<.01	0.1	0.02	0.01	0.01
Poultry Manure 6m	<.01	<.01	<.01	<.01	<.01
Dairy Doo 6m	<.01	<.01	<.01	<.01	<.01
Wood Ash 1m	<.01	<.01	0.05	<.01	<.01

[1] 1m or 6m indicates that the product is 1 month or 6 months past maturity for composts or collection for other products.

2.4 DISCUSSION

In lab assays, nematode survivorship significantly decreased with increasing application rate for the layer ash blend and wood ash treatments as well as poultry manure at rates greater than 0.1%. In these treatments, butyric acid was detectable only in samples collected one-month past maturity with the highest concentrations in the layer ash blend (0.17%) followed by wood ash (0.05%) and poultry manure (0.02%). Butyric acid has nematicidal properties and can reduce plant parasitic nematode survivorship by more than 94% (Browning et al., 2004; Mahran et al., 2008). In the layer ash blend, butyric acid concentrations were 1.7 mg g^{-1} in the bioassay, providing a plausible mechanism for nematode mortality. Moreover, the one-month old layer ash blend had a concentration of 0.68% acetic acid which also had lethal effects on *P. penetrans* when extracted from liquid hog manure (Mahran et al., 2008). It is important to note that the concentrations of potentially lethal VFAs within the tested products depended on the age of the material; therefore, providing consistent nematode control over time could be problematic (Brinton, 1998). Most VFAs are not long-lived within composts or manures and are either broken down rapidly by microorganisms, degraded or absorbed, thus reducing the longevity of their antagonistic properties (Brinton, 1998; McBride et al., 2000).

In the field, composts and manures were applied one day prior to planting and at this time were less than one-month past maturity at rates of 0.125% and 0.5% compost per acre furrow slice for low and high rates, respectively. At the two-week sampling date, declines in nematode populations were observed in both layer ash treatments and the poultry high treatment, however significant differences in root lesion nematode populations were not detected among sampling dates, potentially due to the low initial populations. Although populations declined two weeks after treatment for the layer ash high and poultry low treatments, mean abundance reached or exceeded initial abundance by harvest. This may indicate that the nematicidal properties of the composts and manures dissipated over the course of the season allowing populations to recover through natural reproductive cycles. Contrary to our findings in the field, poultry manure caused significant declines in *P. penetrans* populations in different cropping systems over the course of one or more growing seasons (Conn and Lazarovits, 1999; Everts et al., 2006; Forge et al., 2016). Although we hypothesize that volatile fatty acid composition in the tested products contributed to the decline in nematode survivorship, other mechanisms may be responsible such as ammonia concentrations, antagonistic biological organisms or a combination thereof (Oka, 2010).

Surprisingly, wood ash may also play a critical role in reducing nematode populations. In the lab assay, wood ash alone performed similarly to the layer ash blend in reducing nematode survivorship. In previous studies, a comparable product, fly ash, significantly decreased root knot nematode populations when applied to soil (Ahmad et al., 2019). The mechanism behind wood ash's effects on plant parasitic nematodes, however, is still relatively unclear. Wood ash is characteristically nutrient dense with a high pH and adsorption potential (Demeyer et al., 2001). Abundant nutrients such as calcium, aluminum or potassium are not implicated in reduced root lesion nematode populations, but they may directly increase antagonistic microbial populations (Mkhabela and Warman, 2005; Nagy et al., 2004). It is plausible that the increase in antagonistic microbes could reduce root lesion nematode populations, but the rapid decline in nematode survivorship observed in the lab assays suggests the mechanism is likely more direct (Vestergård et al., 2018). High levels of adsorption which causes changes in osmotic pressure within the soil has little effect on root lesion nematodes (Zuckerman, 2013). Moreover, root lesion nematodes are highly resistant to high levels of pH in their environment (Willis, 1972). Alternatively, the ash may trigger the saponification of the lipid-dense epicuticle of the nematodes increasing their susceptibility to desiccation (Mkhabela and Warman, 2005). Perhaps there is a synergism occurring between multiple characteristics of the wood ash causing a decline in root lesion nematodes, but further studies such as examining the effect of a wood ash solution on nematode cuticle, are needed to fully understand the mechanism.

In the field, *V. dahliae* microsclerotia germination was significantly lower in soil collected from the layer ash low treatments two weeks post treatment compared to the control and poultry high treatments. All treatments except for the layer ash blend experienced mean decreases in *V. dahliae* germination in soil collected at mid-season. In the low layer ash blend we observed the lowest amount of microsclerotia germination in potato stems collected at mid-season. Volatile fatty acids have also been implicated in reduced *V. dahliae* germination in fields treated with liquid swine manure (Conn et al., 2005). Acetic acid in particular has inhibitory effects on germination when microsclerotia are exposed for longer periods of time (Tenuta et al., 2002).

Interestingly, *V. dahliae* germination from soil was greater in high rates of layer ash blend and poultry manure treated plots compared to their low rate counterparts. Additionally, the abundance of germinated microsclerotia increased over the course of the season for the layer ash treatments but decreased in the poultry manure treatments. Poultry manure is a high nitrogen, chemically unstable product resulting in the rapid decomposition of nitrogen into the soil after application (Robinson and Sharpley, 1995). High levels of nitrogen in forms such as ammonia, particularly with increased irrigation, amplifies *V. dahliae* proliferation perhaps explaining the increased populations of *V. dahliae* in poultry manure treatments (Conn and Lazarovits, 1999; Sewell and Wilson, 1967; Wheeler et al., 2012). Relative increases in *V. dahliae* abundance within the layer ash treatments between sampling dates may be attributed to the slower release of nitrogenous compounds associated with composts (Azim et al., 2018). This trend does not continue however within the dairy doo treated plots which showed a decrease in

V. dahliae abundance between sampling dates. This could be due to a lack of VFAs and TKN within the compost compared to the layer ash blend and poultry manure.

The average yields produced in this study were equivalent to approximately 16,820 kg ha^{-1}, exceptionally low compared to Michigan grower standards of roughly 46, 450 kg ha^{-1} making it difficult to determine if treatments are practical for grower implementation (NASS, 2019). Plots treated with poultry low had 51% higher total yields compared to the control (p= 0.065) and both layer ash blend high and poultry low treatments produced the highest proportion of grade A tubers with 57.7% and 54.8%, respectively. Although yields were not significant, using data collected in this and other Michigan potato field trials, Dyrdahl-Young et al. (in press), found a 61% and 56% mean increase in net revenues in plots treated with poultry manure and layer ash blend, respectively when compared to the control. The grower standard, Vydate, only averaged net returns 52% higher than the control.

Overall, lab assays indicate that poultry manure and the layer ash blend have the highest potential for nematode control. Variability in nematode pressure at fields sites prevented me from drawing clear conclusions about the effects of treatments on both nematode abundance and *V. dahliae* incidence. In conjunction with low nematode pressure, intensive potato management practices may have reduced pest populations, as well as prevented population recovery within the two-weeks between the first and second sampling dates. Intensive tilling for example, has been observed to reduce root-lesion nematode populations (Grabau et al., 2017; Schmidt et al., 2017). The previous cropping history of our plots may also explain low nematode pressure since pearl millet is not a suitable host for *P. penetrans* and is often used as a cover crop to reduce their populations (Ball-Coelho et al., 2003; Bélair et al., 2006; Sritharan et al., 2007b). Impacts of manures and compost in the future should be investigated in field plots with significant root lesion nematode and *V. dahliae* pressure with proper management to improve treatment effect detection on pests and yield. Our study was the first to indicate the potential for volatile fatty acids as a control for potato early die complex, however long-term control ability as well as effects on soil and environmental health still need to be investigated.

CHAPTER 3. EVALUATION OF NON-FUMIGANT NEMATICIDES AND BIOLOGICAL AMENDMENTS FOR THE CONTROL OF POTATO EARLY DIE.

3.1 INTRODUCTION

Soil fumigation has long been used to control soil dwelling pests including nematodes, weeds and pathogens. Despite their excellent ability to prevent pest incidence, some fumigants, such as methyl bromide or metam sodium, have non-target effects thereby killing an array of soil fauna and causing deleterious changes to the soil ecosystem. These changes can negatively impact soil health via a reduction in soil biodiversity, nutrient mineralization, and soil structure (Brussaard et al., 2007). Moreover, many soil fumigants are dangerous to human health, environmental quality and can be costly to apply (Collins et al., 2006; O'Malley et al., 2005; South and Carey, 2000). Because of this, methyl bromide and other fumigants have come under strict regulation by the Environmental Protection Agency (EPA). Despite being a key production tool, particularly in potatoes, the negative consequences of many soil fumigants require the development of alternative chemical and biological controls.

In Michigan potato systems, one of the driving factors for fumigation stems from the interaction of root-lesion nematode, *Pratylenchus penetrans* Cobb (Tylenchidae: Pratylenchidae) and the fungal pathogen *Verticillium dahliae* Kleb. As *P. penetrans* feeds throughout root systems, it creates necrotic lesions and depletes the plant of vital nutrients. Presence of *P. penetrans* in conjunction with the fungal pathogen *V. dahliae* hastens potato decline via the development of potato early die complex. Without proper management, yield loss can range from 20 to 50% thereby dramatically reducing economic returns (Rowe and Powelson, 2002b).

Since the phase-out of methyl bromide, many growers have turned to nematicides and biocides such as Vydate® (oxamyl), a carbamate pesticide. Aside from being grower standard within Michigan, numerous other studies have supported it's efficacy against *P. penetrans* in a variety of cropping systems including potato, raspberry, (Han et al., 2014; Kimpinski, 1986; Olthof et al., 1985; Zasada and Walters, 2016).

Several other nematicide chemistries have been developed within the last five years including: fluensulfone, fluopyram and fluazaindolizine, providing growers with an array of nematode control options. Fluensulfone, commercially available as Nimitz®, is in the group fluoroalkenyl thioethers and controls nematodes on contact however the exact mode of action still unknown. Observations have concluded that susceptible nematodes lose mobility and feeding ability when exposed to fluensuflone ultimately leading to mortality (Kearn et al., 2014; Oka and Saroya, 2019).

Fluopyram (Velum Prime®) in the group pyridinyl ethylbenzaimide, was initially marketed as fungicide but has since demonstrated nematicidal properties. It is suspected that the mode of action for fluopyram stems from its' succinate dehydrogenase inhibitory properties. Succinate dehydrogenase is essential for processes in the electron transport chain during ATP production (Rutter et al., 2010). Products with the same mode of action, however, have not exhibited the same nematicidal effects suggesting floupyram has other inhibitory effects on nematodes which are not fully understood (Oka and Saroya, 2019).

Finally, fluazaindolizine, is a relatively new product that is not yet on the market but is expected to be available in 2020 under the trade name Salibro through Dow DuPont. Similarly to fluopyram and fluensulfone, the exact mechanism for nematode control is still relatively unclear. Moreover, published research is currently limited because of the chemical's recent discovery.

One study in tomatoes however had indicated fluazaindolizine as effective in reducing root knot nematodes (Silva et al., 2019).

Alternatively utilizing biological organisms may provide long lasting nematode control. One such biological control agent is the naturally occurring fungal nematode parasite *Purpureocillium* lilacinum which has previously been implicated as an effective nematode biological control agent particularly against *Meloidogyne* eggs via hyphal infection (Khan et al., 2006). Observations also detected direct penetration into the cuticle of *Meloidogyne* juveniles by the hyphae suggesting it is capable of parasitizing other mobile nematodes (Khan et al., 2006). The range of nematode species susceptible to *P. liliacinum* infection, including *P. penetrans*, is not well described.

Understanding the efficacy of commercially available nematicides and biological control agents against root lesion nematodes has the potential to promote sustainable grower practices, increase economic returns and optimize potato production. In this study my objective was to (1) evaluate the potato early die control potential of five nematicides, and (2) determine if *P. liliacinum* is a viable biological control agent for *P. penetrans* in field studies.

3.2 MATERIALS AND METHODS

3.2.1 Field Trial Experimental Design

A field trial comparing the nematode control potential of five nematicides and a biological control to an untreated control and a seed treated control was conducted in 2018 at a potato farm located near Edwardsburg, Michigan, USA (41.818472, -86.093000). Soil at the field site was a Spinks Loamy-Sand, primarily comprised of 83.6% sand, 13.1% silt and 3.3% clay and 1.2% organic matter (. Nematicides were tested at multiple rates with combinations of fungicides and insecticides. Treatments are presented in Table 3.1. A total of fifteen treatments including an untreated control were evaluated in a complete randomized block design with four replicates of each treatment in each block. Blocks measured 15.3 x 24.4 m with individual replicate plots measuring 3.6 x 4.6 m. Prior to planting, potato (cv. Norkotah Russet) seed pieces, except for those for the untreated control, were treated with CruiserMaxx Extreme (a.is: thiamethoxam, fludioxonil, difenoconazole, CAS No. 153719-23-4; CAS No. 131341-86-1; CAS No. 119446-68-3, Syngenta Crop Protection, Greensboro, NC) at a rate of 0.18 mL kg^{-1}.

Potatoes were planted with a Checchi and Magli planter (Potato Seeder F300L, Via Guizzardi, 38 40054 Budrio, BO) with 86 cm row spacing. Treatments were applied in-furrow using a CO_2 powered back pack sprayer after planting. Two weeks after planting, granular Urea (46-0-0) was applied at a rate of 168 kg ha^{-1}. Throughout the season, foliar applications of insecticides and fungicides were applied as needed (S2a/b).

3.2.2 Nematode Collection and Identification

Soil samples were collected from each plot two-weeks after planting (May 22), mid-season (August 15) and harvest (September 24). Ten, 30-cm cores were randomly collected from each plot, homogenized in a plastic collection bag and subsequently stored in a cooler at 46°C until processing in the laboratory. To extract the nematodes, a 100 cm^3 subsample was processed using the elutriation and centrifugal flotation method (Jenkins, 1964). After processing, plant-parasitic nematodes were identified to genus while all other nematodes were identified to feeding group using morphological characteristics. Reproductive factor was calculated by dividing the final nematode counts from harvest sampling (P_f) by the two-week nematode counts (P_i) to determine changes in nematode populations over the course of the growing season. All identifications were completed using a Nikon TMS microscope between x20 – x100 magnification.

3.2.3 Verticillium Collection and Identification

The number of germinating microsclerotia propagules within the soil was determined from the soil samples collected two-weeks post treatment and at mid-season. Approximately 10 g of soil from each plot was homogenized with 40 mL of distilled water. A 2.5 mL aliquot of soil/water solution was pipetted onto a 100 cm diameter Verticillium growth plate (1000 mL distilled water, 15 g Bacto-agar, 10 mL 200 proof ethanol, and 0.4 g Streptomycin sulfate) (Nadakavukaren and Homer, 1959). A total of 4 plates were created from each homogenized mixture and this was repeated for each plot. The plates were covered and kept at room-temperature in a dark environment for two weeks. After two-weeks at the number of germinating microsclerotia was evaluated using morphological characteristics (Goud et al., 2003).

To determine the level infection within the potato plants, ten 12-cm stems were collected from each plot. A 3-cm piece of each stem was cut and placed on a Verticillium growth plate with a total of five pieces arranged in circle on each plate. Plates were kept in the same environmental conditions as previously mentioned and the number of stems with *V. dahliae* germination was recorded.

3.2.4 Yield

On September 24, one 4.8 m row was harvested from each plot. The harvested tubers were weighed and graded based on size with the resulting yields scaled to kg ha^{-1}. Grading sizes included: oversize- ≥ 8.3 cm, U.S. No. 1 - 4.8 ≥ 8.3 cm, and B - < 4.8 cm. Tubers with shape defects and/or rot were culled and labeled as pick outs (PO).

3.2.5 Cost-Benefit Analysis

To determine the potential net returns between treatment programs, the cost to apply each product per hectare was calculated and added to a mean cost of operation derived from UC Davis and University of Idaho materials (Wilson et al., 2015). The mean price earned per kg of #1 US potatoes was averaged from historic fresh market prices from 2011 to 2018 and multiplied by the mean and SEM of US #1 yields to obtain potential earnings (NASS, 2018). Net revenue was calculated by subtracting operating and product costs from the revenue.

3.2.6 Statistical Analyses

All analyses were completed using R version 3.5.1 (R Core Team, 2018). Nematode counts and soil Verticillium germination were analyzed using a generalized linear model with a negative binomial distribution using the package “MASS” (Ripley, 2019). Verticillium populations within collected stems were analyzed using a generalized linear model with a binomial distribution while yields were analyzed using ANOVA. Mean separation for all analyses was completed using Tukey’s Honest Significant Difference using the package “emmeans” (Lenth, 2019). Treatments were considered significantly different using an alpha value of 0.05.

Table 3.1. Rate, timing and signal words of treatments applied for nematode and Verticillium trials during the 2018 field season in Michigan potato plantings. All treatments listed were treated with CruiserMaxx Extreme prior to planting.

Treatment	Nematicide	Nematicide Active Ingredient	In-furrow application (units product per ha)	Additional nematicide applications	Signal Word	Additional product rate (units AI per ha)
Nimitz (Adama) NimHIgh	Nimitz	Fluensulfone	8.2 L	N/A	Caution	N/A
Nimitz (Adama) NimLow	Nimitz	Fluensulfone	5.8 L	N/A	Caution	N/A
Velum® Prime Serenade- ASO (Bayer) VelumSer	Velum Prime	Fluopyram	475 mL	N/A	Warning	2.34 L Serenade in furrow
Velum® Prime Serenade® ASO Movento® HL (Bayer) VelumSerMov	Velum Prime	Fluopyram	475 mL	N/A	Warning	2.34 L Serenade in furrow 182.6 Movento mL at hilling
Velum® Prime Serenade® ASO Luna® Tranquility (Bayer) VelumSerLu	Velum Prime	Fluopyram	475 mL	N/A	Warning	2.34 L Serenade in furrow 818.5 mL Luna Tranquility foliar
Velum® Prime Movento® HL (Bayer) VelumMov	Velum Prime	Fluopyram	500 mL	500 mL applied at hilling	Warning	370.6 mL Movento at hilling
[1]Salibro + Vydate® (DuPont) Salibro L + Vy	Salibro Vydate	Fluazaindolizine Oxamyl	1.13 L 2.34 L	N/A	[5]Unknown	N/A
Salibro + Vydate® (x2) (DuPont) SalibroL +Vy(x2)	Salibro Vydate	Fluazaindolizine Oxamyl	1.13 L 2.34 L	N/A 2.34 L applied at hilling	Unknown	N/A
Salibro + Vydate® (DuPont) Salibro H+Vy	Salibro Vydate	Fluazaindolizine Oxamyl	2.24 L 2.34 L	N/A	Unknown	N/A
Salibro + Vydate® (x2) (DuPont) SalibroH+Vy(x2)	Salibro Vydate	Fluazaindolizine Oxamyl	2.24 L 2.34 L	N/A 2.34 L applied at hilling	Unknown	2.34 L Vydate applied at hilling
Vydate® C-LV (DuPont)	Vydate	Oxamyl	4.68 L	2.34 L applied at hilling	Danger Poison	N/A
Mocap® EC (AMVAC)	Mocap	Ethoprop	4.68 L	N/A	Danger Poison	N/A
MeloCon® WG (Certis USA)	MeloCon	*Purpureocillium lilacinum* strain 251	10.01 kg	N/A	Caution	N/A

[1] Salibro is not commercially available (exp. 2020) therefore a full label with the signal word is unavailable.

3.3 RESULTS

3.3.1 Nematode Populations

There was not a significant treatment effect on root-lesion nematode populations at either the two-week nor mid-season sampling dates. *P. penetrans* abundance varied significantly among treatments ($\chi^2_{14,117} = 26.5$, p= .022) at harvest. Vydate treated plots had the lowest observed populations while Nimitz high had the most with a mean ± SEM of 2.8 ± 1.8 and 22.0 ± 8.9 100 cc^{-1} soil, respectively (Table 3.2). The changes in nematode populations over the course of the season, indicated by reproductive factor, was not significantly different between treatments (Table 3.2).

Similarly, root-lesion populations within 1 gram of sampled roots varied significantly among treatments ($\chi^2_{14,60} = 41.8$, p= <.001). The greatest abundance of *P. penetrans* was observed in the seed-treated control and Nimitz High with 101.3 ± 8.1 and 94.0 ± 9.5 nematodes per gram of root, respectively. Alternatively, all Salibro treatments except for the low rate with one Vydate application (SalibroL + Vy) had significantly fewer root-lesion nematodes than the aforementioned treatments (Figure 3.1).

3.3.2 *Verticillium dahliae* populations

Treatment and sampling date significantly impacted mean *V. dahliae* germination and there was a significant interaction between the two factors ($\chi^2_{14,89} = 1.5$, p= <.001). For all treatments, except for Salibro Low with one application of Vydate, the number of *V. dahliae* colonies germinated significantly decreased between two-week and mid-season sampling (Figure 3.2). The most dramatic decrease occurred in the untreated control and the Velum/Serenade/Luna Tranquility treatment with an 88% and 86% decrease, respectively.

The Salibro Low with two Vydate applications had the fewest germinating colonies at two-weeks post treatment, with a mean of 23.3 ± 7.3 colonies per 10 g soil while the untreated control had the most germinating colonies with 74.0 ± 16 colonies per 10 g soil. This is followed by Velum/Serenade/ Luna Tranquility (71.7 ± 4.1), Velum/Serenade (65.7 ± 12.3) and Nimitz Low (63.5 ± 0.5) (Figure 3.2). When sampled mid-season, soils treated with Salibro Low with one Vydate application had the greatest number of germinating microsclerotia, while Mocap had the fewest with a mean of 49.7 ± 3.9 and 6.0 ± 10.8 per 10g soil, respectively (Figure 3.2). Between sampling dates, all treatments experienced a significant decrease in *V. dahliae* germination except for the Salibro Low with one Vydate application treatment.

Treatment did not significantly impact the percentage of stems infected with *V. dahlia* with mean percentage of infection ranging between 60 – 100%. The seed treated control and MeloCon treatments (95%) had the highest rates and the Salibro High with one Vydate application treatments (62.5%) had the lowest (Figure 3.3).

3.3.3 Yield

Mean yield did not significantly differ between treatments, however the nontreated control did have the highest yields.

3.3.4 Cost-Benefit Analysis

The greatest revenues were observed in the untreated control (Control No Trt) with $1,659.17 ± 444 while both Nimitz treatments resulted in negative returns with a means loss of $1,449.11 ± 249 and $389.19 ± 471 for high and low treatments, respectively (Table 3.2). The seed treated control had mean net returns of $874.48 ± 337 which was about average compared to other treatments; Mocap, Salibro high with two Vydate applications, Salibro low with two Vydate applications, Velum/Movento and Velum/Serenade/Movento all had lower net returns than the seed treated control. In MeloCon treated plots, net returns were below average with a mean return of $576.69. Net returns for the Salibro treatments will be further reduced once the price point is determined.

Table 3.2. Mean ± SEM *P. penetrans* counts within soil at two-week (May 22), mid-season (August 8) and harvest (September 24) sampling dates. Reproductive factor calculated by harvest counts/two-week counts. Values followed by different letters within a column are significantly different Tukey HSD (α = .05). N.S. indicates no significant difference within that column.

Treatment	Two Week (P_i)	Mid-Season	Harvest (P_f)	Reproductive Factor (Pf/Pi)
Control – No Trt	12.75 ± 6.8	23.0 ± 7.7	3.5 ± 0.4 ab	0.27
Control – Seed Trt	9.75 ± 4.5	24.7 ± 8.8	12.0 ± 5.6 ab	1.23
MeloCon	9.5 ± 3.8	15.7 ± 7.1	21.3 ± 5.2 ab	2.25
Mocap	8.75 ± 5.2	12.7 ± 4.8	4.0 ± 1.0 ab	0.46
Nimitz High	9.25 ± 2.7	12.0 ± 4.9	10.0 ± 3.1 ab	1.08
Nimitz Low	5.75 ± 3.8	16.7 ± 8.9	22.0 ± 8.9 b	3.82
Salibro H + Vydate	12.75 ± 3.3	13.3 ± 9.9	6.5 ± 1.2 ab	0.51
Salibro H + Vydate(x2)	3.75 ± 1.9	5.0 ± 2.8	12.5 ± 5.3 ab	3.33
Salibro L + Vydate	4.75 ± 2.8	4.0 ± 1.3	11.0 ± 4.1 ab	2.32
Salibro L+ Vydate (x2)	11.0 ± 6.5	15.3 ± 11.1	12.3 ± 1.9 ab	1.11
Velum + Movento	3.75 ± 2.5	5.7 ± 2.8	4.0 ± 1.7 ab	1.07
Velum + Serenade	3.25 ± 1.1	2.5 ± 1.8	9.5 ± 5.9 ab	2.92
Velum + Serenade + Luna	18.0 ± 9.3	7.3 ± 3.2	9.3 ± 5.5 ab	0.52
Velum + Serenade + Mov.	7.75 ± 3.7	6.3 ± 2.5	6.0 ± 2.8 ab	0.77
Vydate	7.0 ± 2.8	13.3 ± 8.1	2.8 ± 1.8 a	0.39

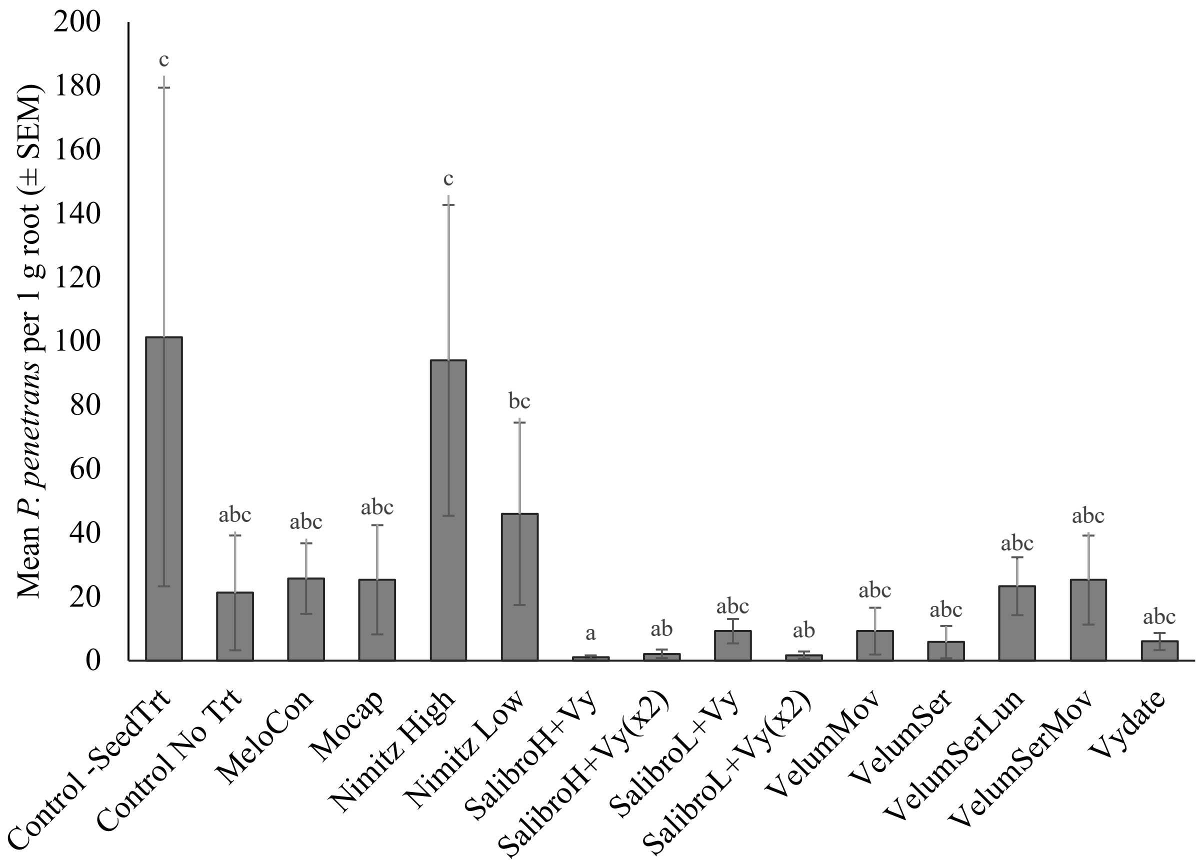

Figure 3.1. Mean ± SEM root lesion nematodes present within 1g root, extracted via the root-shaker method for treatments seed treated control (Control – SeedTrt), untreated control (Control No Trt) MeloCon, Mocap, Nimitz High, Nimitz Low, Salibro High with Vydate (Salibro H +Vy), Salibro High with two Vydate applications (Salibro H + Vy(x2), Salibro Low with Vydate (Salibro L + Vy), Salibro Low with two Vydate applications (Salibro L + Vydate (x2), Velum and Movento (VelumMov), Velum and Serenade (VelumSer), Velum Serenade and Movento (VelumSerMov) and Vydate. All seed-pieces besides the Control No Trt were treated with CruiserMaxx Extreme seed treatment prior to planting. Treatments labeled with different letters are significantly different (Tukey HSD, α=.05).

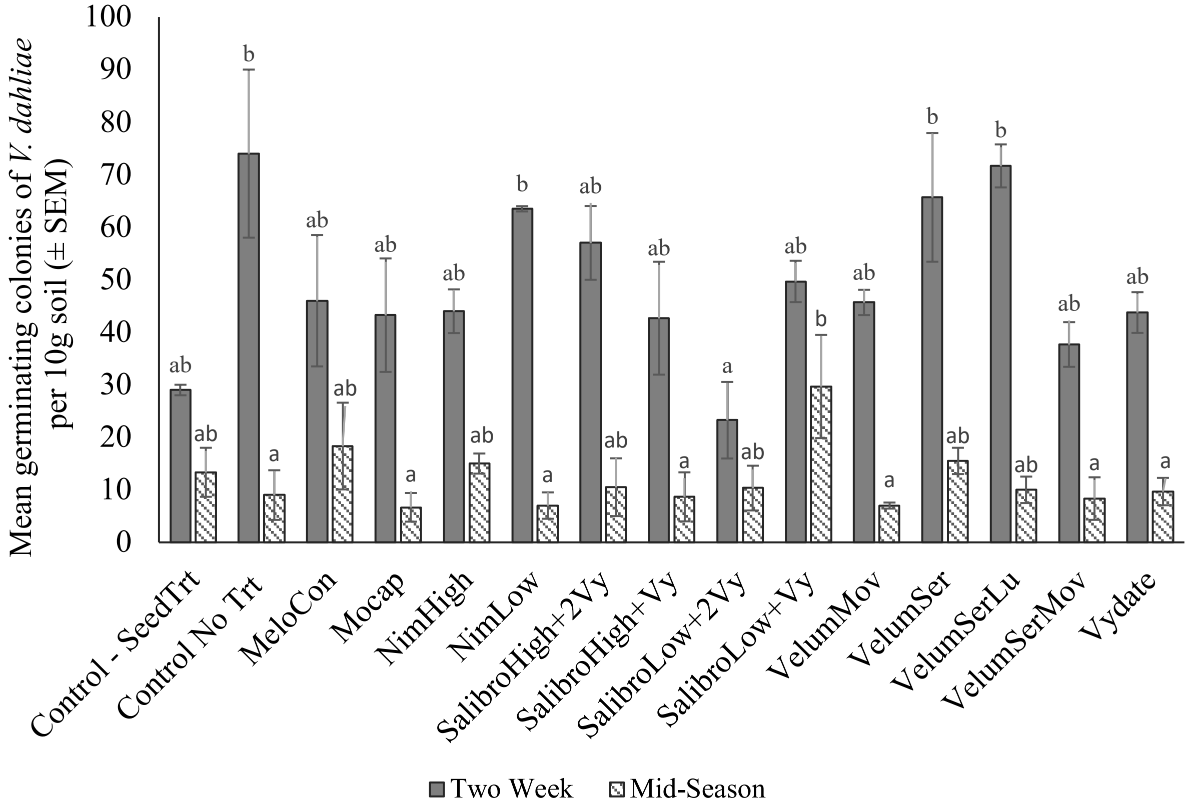

Figure 3.2. Mean SEM germinating *V. dahliae* colonies within soil collected two-week post treatment (May 22) and at mid-season (August 8) for treatments seed treated control (Control – SeedTrt), untreated control (Control No Trt) MeloCon, Mocap, Nimitz High, Nimitz Low, Salibro High with Vydate (Salibro H +Vy), Salibro High with two Vydate applications (Salibro H + Vy(x2), Salibro Low with Vydate (Salibro L + Vy), Salibro Low with two Vydate applications (Salibro L + Vydate (x2), Velum and Movento (VelumMov), Velum and Serenade (VelumSer), Velum Serenade and Movento (VelumSerMov) and Vydate. All seed-pieces besides the Control No Trt were treated with CruiserMaxx Extreme seed treatment prior to planting. Number of germinating colonies was determined by homogenizing 10g soil with 40mL distilled water, plating a 2.5mL aliquot onto Verticillium growth media, and counting germination after two weeks in a dark environment. Treatments within the same sampling period labeled with different letters are significantly different (Tukey HSD, α=.05). Between sampling dates, all treatments were significantly different except for Salibro Low + Vy.

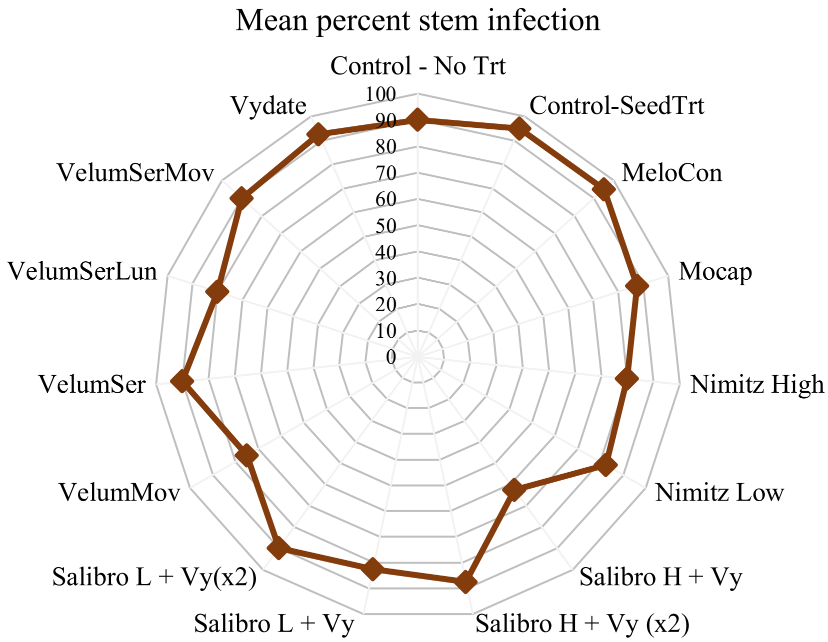

Figure 3.3. Mean percent of stems infected with *V. dahliae* for treatments: seed treated control (Control – SeedTrt), untreated control (Control No Trt) MeloCon, Mocap, Nimitz High, Nimitz Low, Salibro High with Vydate (Salibro H +Vy), Salibro High with two Vydate applications (Salibro H + Vy(x2), Salibro Low with Vydate (Salibro L + Vy), Salibro Low with two Vydate applications (Salibro L + Vydate (x2), Velum and Movento (VelumMov), Velum and Serenade (VelumSer), Velum Serenade and Movento (VelumSerMov) and Vydate. All seed-pieces besides the Control No Trt were treated with CruiserMaxx Extreme seed treatment prior to planting. Proportion of infection was calculated by assessing 10 stems from each plot with four plots per treatment for *V. dahliae* germination. N.S. indicates no significance (Tukey HSD, α=.05).

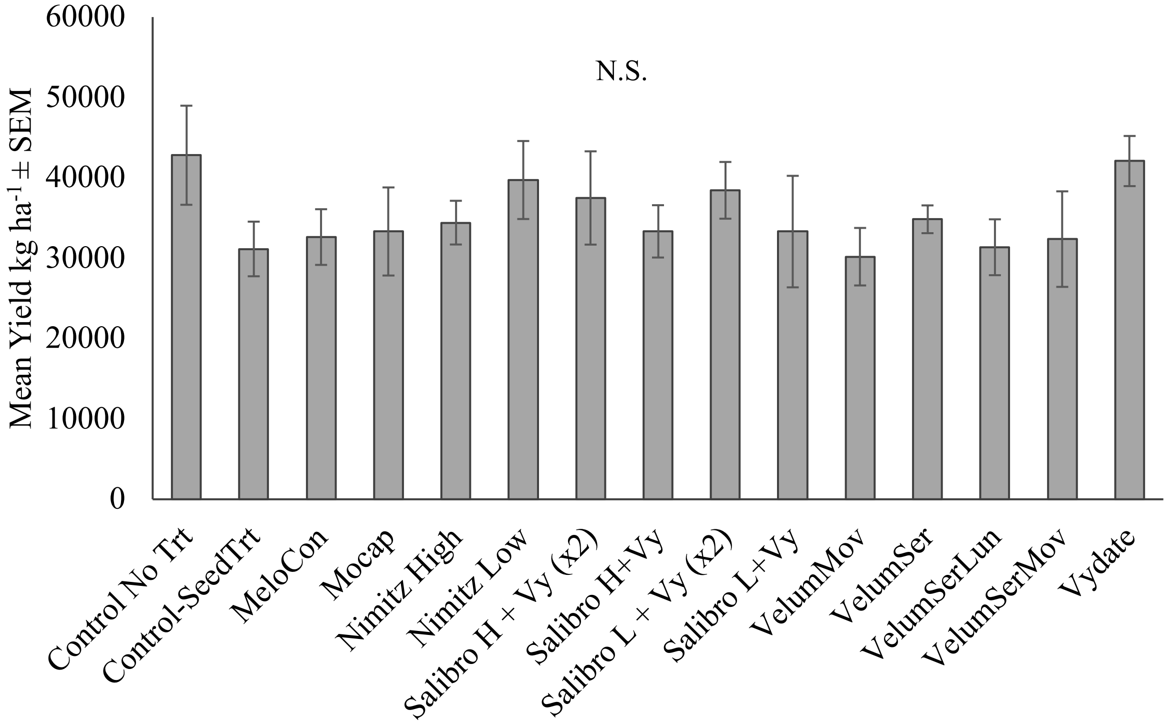

Figure 3.4. Mean SEM tuber yield in Kg ha^{1} for treatments: seed treated control (Control – SeedTrt), untreated control (Control No Trt) MeloCon, Mocap, Nimitz High, Nimitz Low, Salibro High with Vydate (Salibro H +Vy), Salibro High with two Vydate applications (Salibro H + Vy(x2), Salibro Low with Vydate (Salibro L + Vy), Salibro Low with two Vydate applications (Salibro L + Vydate (x2), Velum and Movento (VelumMov), Velum and Serenade (VelumSer), Velum Serenade and Movento (VelumSerMov) and Vydate. All seed-pieces besides the Control No Trt were treated with CruiserMaxx Extreme seed treatment prior to planting. Yield was determined by harvesting one 15’ row and translating the kilograms harvested to a per hectare scale. N.S. indicates no significance (Tukey HSD, α=.05).

Table 3.3. Cost benefit analysis of nematicide programs tested. Application cost per hectare is the cost of products within the program to a hectare of potatoes while the total cost per hectare factors in the operating costs including labor. Because Salibro is not yet on market we do not yet have a cost per hectare estimation, therefore the price of Salibro application is indicated by "X". For this purpose, a standard operating cost of $1040 per hectare was used for estimates. Revenue was estimated by averaging the past five years of historical potato prices for fresh market russet potatoes. Price used was $0.0875/kg which was multiplied by the average US #1 yield obtained from plots. Nematicide programs included: seed treated control (Control – SeedTrt), untreated control (Control No Trt) MeloCon, Mocap, Nimitz High, Nimitz Low, Salibro High with Vydate (Salibro H +Vy), Salibro High with two Vydate applications (Salibro H + Vy(x2), Salibro Low with Vydate (Salibro L + Vy), Salibro Low with two Vydate applications (Salibro L + Vydate (x2), Velum and Movento (VelumMov), Velum and Serenade (VelumSer), Velum Serenade and Movento (VelumSerMov) and Vydate. All seed-pieces besides the Control No Trt were treated with CruiserMaxx Extreme seed treatment prior to planting.

Treatment	Application Cost ($/ha)	Total Cost ($/ha)	Yield (kg/ha)	Revenue($/ha)	Net Revenue ($/ha; ± SEM)
Nimitz High	$2,379.50	3419.5	22519	$1,970.39	-$1,449.11 ± 249
Nimitz Low	$1,674.90	$2,714.90	26579	$2,325.71	-$389.19 ± 471
VelumSer	$480.00	$1,084.20	22405	$1,960.45	$876.25 ± 170
VelumSerMov	$49.43	$1,089.43	20808	$1,820.68	$731.25 ± 461.4
VelumSerLu	$142.00	$1,182.00	20985	$1,836.21	$654.21± 250
VelumMov	$25.88	$1,065.88	17350	$1,518.17	$452.29 ± 312
Salibro L + Vydate	X + $69.20	X + 1,109.20	22576	$1,975.36	$866.16–X ± 617
Salibro L + Vydate (x2)	X + $138.48	X + $1,178.40	27297	$2,388.44	$1,210.00–X ± 288
Salibro H + Vydate	X + $69.20	X + 1,109.20	21276	$1,861.68	$752.48–X ± 389
Salibro H + Vydate (x2)	X + $138.48	X + $1,178.40	24346	$2,130.24	$951.84–X ± 401
Vydate	$207.7	$1,247.70	27545	$2,410.19	$1,162.48 ± 201
Mocap	$34.65	$1,074.65	20730	$1,813.85	$739.20 ± 390
MeloCon	$81	$1121	19402	$1,697.69	$576.69 ± 316
Control No Trt	$0.00	$1,038.00[1]	30825	$2,697.17	$1,659.17 ± 444
Control SeedTrt	$0.00	$1,040.00	21880	$1,914.48	$874.48 ± 337

[1]Total cost was deducted $2.00 for the cost of seed treatment

3.4 DISCUSSION

Root lesion nematode soil populations were significantly affected by treatments, but only at the harvest sampling date. By the end of the season, Vydate (oxamyl; 2.8 ± 1.8) had the lowest abundance of root lesion nematodes followed by the untreated control (Velum/Movento (fluopyram; 4.0 ± 1.7) and Mocap (ethoprop; 4.0 ± 1.0). Vydate has been grower standard so it unsurprising that we observed population reductions with application. The efficacy of Vydate is also consistent with previous studies (Kimpinski et al., 2001; Zasada and Walters, 2016). Although not statistically significant, growth in nematode populations over the course of the season was lowest in the untreated control with a reproductive factor (P_f/P_i) of 0.27, a dramatic change compared to the 3.82 factor increase observed in the Nimitz Low (fluensulfone) treatment. At mid-season however, the untreated control had a mean root lesion nematode population of 23.0 ± 7.7 per 100 cc of soil. The dramatic change from mid-season to harvest could be related to sampling error such as leaving sampling bag in the sun for too long. Nimitz lacked significant control of *P. penetrans* at either rate tested, but has demonstrated nematicidal effects in previous trials (Oka, 2014; Watson and Desaeger, 2019).

In root tissues, nematode populations in the untreated control were similar to nearly all nematicide treatments including Vydate and Mocap and substantially lower than the seed treated control. The exact mechanism is uncertain for nematode population decline in the untreated control treatment, but it is likely linked to the lack of fungicides and neonicotinoids applied to seed pieces. Perhaps, the addition of fungicides inhibited natural fungal biological controls within the soil for seed treated treatments. It may be that, in the untreated seed piece control, the natural biological controls were allowed to flourish and reduce nematode populations. For instance, arbuscular mycorrhizal fungi which are obligate symbionts of many roots systems have also been implicated in plant parasitic nematode control including *P. penetrans* (Forge et al., 2001; Veresoglou and Rillig, 2012; Vos et al., 2012). More importantly, the active ingredient of the tested seed treatment, fludioxonil, can reduce the colonization of arbuscular mycorrhizal fungi (Cameron et al., 2017; Jin et al., 2013). It is important to also note, that some increases in nematode populations is expected because of their rapid reproduction. The goal when treating with nematicides however is to reduce the initial inoculum of nematodes to prevent damage early in the growing season.

The new-to-market product, Salibro (fluazaindolizine), also demonstrated adequate control of *P. penetrans* particularly in root tissues. Plots treated with the high rate of Salibro with one Vydate application (Salibro H + Vy), had the lowest abundance of root lesion nematodes with a mean of 1.1 ± 0.9 per 1 g root. The dual application of Vydate and Salibro however makes it difficult to discern the true effect of Salibro alone considering Vydate treatments also exhibited low nematode populations. Contrary to our findings, Salibro applied to Florida strawberries had little effect on *P. penetrans* populations in soil or roots suggesting there may be a crop or climate interaction in the efficacy of the product or that simply more trials are needed to see true effects.

Plots treated with Velum (fluopyram), exhibited low root lesion populations with a mean of fewer than 10.0 *P. penetrans* per 100 cc soil season long with the exception of the Velum/Serenade/Luna Tranquility (VelumSerLu) treatment. Within roots, Velum/Serenade (VelumSer) and Velum/Movento (VelumMov) had populations similar to Vydate and Salibro treatments with 5.8 ± 1.8 and 9.3 ± 2.4 *P. penetrans* per g root, respectively. Despite having fungicidal properties and being paired with additional fungicide treatments such as Luna Tranquility and Serenade, Velum treatments had no significant effect on *V. dahliae* infection.

This is the first study to investigate the effects of MeloCon (*P. lilacinum*) on *P. penetrans*. Based on our results, it appears as though MeloCon is not a practical biological control agent for *P. penetrans*. Over the course of the season soil populations rose by a factor of 2.25 from a mean abundance of 9.5 ± 3.8 two-weeks post treatment to 21.3 ± 5.2 at harvest while root populations (25.71 ± 5.5) were not significantly different from other treatments. In previous greenhouse studies, MeloCon has reduced the populations of root knot nematodes (*Meloidogyne* spp.) by 51% (Anastasiadis et al., 2008). In field studies however MeloCon has been underwhelming with little effect, particularly against root knot nematode (Baidoo et al., 2017). The addition of a biological control fungi into the complex soil web may impede establishment and proliferation of the beneficial fungi due to resource competition and predation by fungivores (Knudsen and Dandurand, 2014). Moreover, using MeloCon alongside traditional potato growing practices of fungicide seed treatment and frequent foliar fungicide applications likely counteracts *P. lilacinum* rendering it ineffective. Ultimately the mean net returns, were below average in this study with a mean return of only 571.69 per hectare.

Verticillium dahliae damage was evident in this field study and likely the main culprit of yield declines. In soil collected two-weeks post treatment, *V. dahliae* microsclerotia were abundant with a field-wide mean of 49.0 germinating colonies per 10 g soil. Among the treatments Salibro low with two Vydate applications (Salibro L + Vy(x2); 23.5 ± 7.3) had significantly fewer colonies compared to the untreated control (74.0 ± 16) it was not different from the seed treated control (29.0 ± 1.0). Treatments with fungicides applied in furrow (Velum/Serenade, Velum/Serenade/Luna Tranquility etc.) at planting exhibited no effect on Verticillium soil colonies two-weeks post planting. Across all treatments, a substantial decrease in *V. dahliae* colonies was observed. Interestingly, at the time the mid-season soil was sampled, potato stems were also collected to determine the percentage of plant infected. Across all treatments, infection ranged between 60-100% with the majority of treatments exhibiting greater than 80% infection. The observed decline in soil microsclerotia between the two sampling dates may be due to the germination of microsclerotia once potato roots formed and released exudates to stimulate germination, thereby reducing the number of viable microsclerotia in the soil over the course of the season. This seasonal decline trend has also been observed in previous studies across varying cropping systems including artichoke and potato (Berbegal et al., 2007; Joaquim et al., 1988). From this, it's clear that soil microsclerotia populations aren't necessarily an accurate measure of product efficacy, but rather disease incidence within the plant.

Ultimately, for growers the efficacy of the product is irrelevant if it does not translate to increased revenues. We projected substantial differences in net revenues, with both Nimitz treatments resulting in negative returns while the non-seed treated control had the greatest returns. In Michigan, at the time of this trial, Nimitz cost $288.55 per liter, with an application rate between 5.8 and 8.2 L per hectare, the cost becomes well over $1000 per hectare quickly diminishing potential returns. Moreover, the nematode control of Nimitz was not significantly lower than any of the treatments therefore, based on our results, it may be impractical for a potato grower to apply Nimitz to a field. Alternatively, despite the greatest net returns, it's likely that the ample production of tubers in the non-seed treated control is an anomaly and growers should not abandon pest management programs. The second-best potential net revenues are within the Vydate treated plots with a mean of $1,162.48 ± 201gained per hectare.

Overall, our study provides further evidence for the nematicidal effects of Vydate (oxamyl), Salibro (fluazaindolizine) and Velum (fluopyram) against root lesion nematodes. The lack of yield gain, however, puts the cost effectiveness of these products into question. But this may have been due to the relatively low abundance of root-lesion nematode populations in this field (<25 per 100 cc soil). In this study the greatest potential revenues were observed in the untreated control followed by Vydate and potentially Salibro, depending on the cost of application once on market. We also demonstrated a lack of nematicidal action in soils treated with MeloCon. Further studies are needed to fully understand relationship between nematode control tactics, potato early die development and the potential return on investment associated with these products.

CHAPTER 4. CONCLUSIONS AND FUTURE DIRECTIONS

The synergism between *Pratylenchus penetrans* and *Verticillium dahliae* creates potato early die complex and drives the need for both nematode and fungal pest management systems in Michigan potatoes (MacGuidwin and Rouse, 1990; Martin et al., 1982; Rowe et al., 1985). With increasing regulations on broad spectrum soil fumigants, the demand for environmentally and economically friendly management options is rising. The work described in this thesis explored the utilization of composts, manures, fungal-based biocontrols and non-fumigant nematicides to address the problems created from potato early die.

Composts and manure have significant nematode and *Verticillium dahliae* control potential based on previous works (Conn et al., 2005; Conn and Lazarovits, 1999; Forge et al., 2016; Molina et al., 2014). The efficacy, however, appears to be dependent on compost base materials as well as the maturity of the product. The research presented supports the use of composts and manures, specifically poultry manure and the layer ash blend which dramatically reduced nematode survivorship in lab assays and showed potential in field studies. In lab assays, rates of 5% (vol./vol.) or greater resulted in nematode survivorship of 0%. On the other hand, I did not observe a clear treatment effect on nematode populations in field studies. The root lesion nematode populations within the field site were low from the start with a mean of fewer than 20 nematodes per 100 mL of soil, making populations changes difficult to observe.

Volatile fatty acid concentrations could be responsible for the decline in observed root lesion nematodes and *V. dahliae* microsclerotia. I found concentrations of butyric and acetic acid equal to 0.17% and 0.68%, respectively within the layer ash blend. Butyric acid specifically has been implicated in significant nematode reductions both in lab and field testing when extracted from liquid swine manure (Browning et al., 2004; Mahran et al., 2008; Oka, 2010). Browning et.

al. (2004) found that concentrations of butyric acid as low as 0.088% reduced root lesion nematode survivorship by 94% or more. Since the concentrations present within the tested products were higher than the previously tested 0.088% it's feasible that butyric acid could be a main driver of the observed nematode population declines especially in lab assays. Furthermore, acetic acid is also implicated in the inhibition of *V. dahliae* microsclerotia germination (Tenuta et al., 2002). Two-weeks post treatment, the layer ash blend had significantly fewer germinating colonies and when evaluated mid-season for infection, had the lowest mean infection rate. The presence of acetic acid within the layer ash blend may be cause for the low rates of *V. dahliae* observed.

From the trials, VFAs could potentially serve as a stand-alone soil amendment or an additive to manures and composts to help ensure pest control. It has been suggested though that if applying VFAs as part of a manure mixture that it be added to dry, warm soils to prevent dilution (Conn et al., 2005). Moreover, high levels of certain VFAs could induce phytotoxicity to crops so determining the optimal balance between pest control and plant well-being is necessary. To determine if VFAs are the mechanism behind the observed pest control in this study, lab trials evaluating the specific effect of butyric and acetic acid on *P. penetrans* and *V. dahliae* at rates comparable to concentrations found the products tested should be completed. Despite, the potential for volatile fatty acids, further research is also needed to understand their longevity in pest control as well as their role in the soil ecosystem and how amending with larger quantities could impact the environment as a whole.

Aside from VFAs as a potential mechanism, other factors could play a role in the reduction of *P. penetrans* and *V. dahliae*. Such factors include, increased ammonia concentrations or the promotion of beneficial organisms. Oka et al. (2002) elucidated that when exposed to ammonia at rates of 75 g N per kg of soil, root knot nematode populations significantly declined. Numerous other studies have also linked pathogen death to ammonia within the soil (Akhtar, 1999; Rodriguez-Kabana et al., 1989; Rodríguez-Kábana, 1986; Tenuta and Lazarovits, 2002) Moreover, poultry manure in particular is known to emit fairly large quantities of ammonia when applied in agricultural settings (Salim et al., 2014; Zhao et al., 2016). Composts and manures can also promote populations of beneficial microorganisms which could potentially suppress populations of pests and diseases (Inbar et al., 2005; Serra-Wittling et al., 1996). Ultimately, the mechanism of the compost's efficacy on nematode and Verticillium suppression is likely a combination of chemical, physical and biological factors that will require testing to pinpoint the direct impact of compost or manure application.

Chapter 3 demonstrated that non-fumigant nematicides may be effective, but ultimately impractical due to the cost of application, especially in the case of Nimitz (fluensulfone). I observed significant nematode control in Salibro (fluazaindolizine), Vydate (oxamyl), and Velum (fluopyram) treatments, however little reduction in Verticillium infection was observed among treatments with the exception of Salibro Low with one Vydate application. Salibro is a relatively new product with limited field research, however, in Florida strawberries, applications of Salibro did not result in a significant reduction in *P. penetrans* populations or increase in yield (Watson and Desaeger, 2019). Since Salibro treatments were combined with Vydate, which had the best nematode control in this study, further studies are needed to determine the individual effects of Salibro on *P. penetrans* populations. Moreover, since Salibro is non-systemic, it would be advantageous to investigate the longevity of nematode control to determine the optimal frequency of applications (Lahm et al., 2017).

In terms of nematode control, both high and low rate Nimitz treatments had little effect on *P. penetrans* and saw a nearly four-fold increase in populations over the course of the season. Nimitz has shown efficacy in the control of other plant-parasitic nematodes such as the root-knot nematode (*Meloidogyne* spp.) (Morris et al., 2016; Oka, 2014; Oka and Saroya, 2019). The cost of Nimitz however, makes it a difficult option for growers to adopt. At nearly $1,000 per liter, the application cost quickly diminishes potential net returns.

To my knowledge this is the first study to evaluate the effect of MeloCon (*Purpureocillium lilacinum*) on *P. penetrans* and potato early die as a whole. The addition of MeloCon did not result in a significant decline in nematode populations, however, Verticillium abundance and yields were similar to many of the nematicides tested. Thus, MeloCon may not be an effective potato early die control tool. The majority of studies evaluating MeloCon have been conducted in growth chamber or greenhouse assays (Anastasiadis et al., 2008; Kiewnick and Sikora, 2006; Singh et al., 2013). Although these studies have found MeloCon effective in nematode control, further explorations into the direct effects of MeloCon in field settings and on *P. penetrans* specifically are needed. Moreover, the potential impacts of soil structural and ecological factors may be needed to determine if it is a practical root lesion nematode control agent.

The logical next step in determining the feasibility of the tested products are large field experiments with plots measuring a hectare or more. All of our field testing was completed in small plots where Verticillium and nematode pressures could change from plot to plot making trends difficult to distinguish. Additionally, increased attention to precise measurements of plant health, soil health and overall cost and revenues would improve product selection decision making. Ultimately, it is likely that no single treatment will be the “silver bullet” in potato early die management. Combining treatments to create a comprehensive Integrated Pest Management program may prove most effective. A program such as applying compost or manure prior to planting in order to reduce initial inoculum and prevent phytotoxic effects followed by in furrow nematicide applications at planting may be advantageous. Further testing of combined pest control tactics needs to be conducted to not only elucidate pest management programs but also move away from the perceived dogma that farming practices are either chemically intensive or ecologically focused (Sonnino and Marsden, 2006). By integrating pest management tactics, we can move forward towards creating optimized management systems for a sustainable future.

APPENDICIES

APPENDIX A: SUPPLEMENTAL TABLES

Product Code	Family	Product	Rate (per ha)
1	Adjuvant	Hasten (Modified Vegetable Oil)	2.3 L
2	Fertilizer	Starter Fertilizer (72-0-0 blended with 14-49-0; N-P-K Analysis)	336.7 L
3	Fertilizer	Urea 46-0-0	112 kg
4	Fungicide	Koverall seed treated control (Control – SeedTrt), untreated control (Control No Trt) MeloCon, Mocap, Nimitz High, Nimitz Low, Salibro High with Vydate (Salibro H +Vy), Salibro High with two Vydate applications (Salibro H + Vy(x2), Salibro Low with Vydate (Salibro L + Vy), Salibro Low with two Vydate applications (Salibro L + Vydate (x2), Velum and Movento (VelumMov), Velum and Serenade (VelumSer), Velum Serenade and Movento (VelumSerMov) and Vydate. All seed-pieces besides the Control No Trt were treated with CruiserMaxx Extreme seed treatment prior to planting. Fungicide (mancozeb, CAS No. 8018-01-7, FMC Corporation, Philadelphia, PA, USA)	2.2 kg
5	Fungicide	Bravo® (chlorothalonil, CAS No. 1897-45-6; Syngenta Crop Protection, Greensboro, NC, USA)	1.8 L
6	Fungicide	Equus 720 (chlorothalonil, CAS No. 1897-45-6, Makhteshim Agan of North America, Raleigh, NC, USA)	1.8 L
7	Fungicide	Echo® 720 (chlorothalonil, CAS No. 1897-45-6, SipcamAgro USA, Inc., Durham, NC, USA	1.8 L
8	Fungicide	Manzate® Pro Stick TM (mancozeb, CAS No. 8018-01-7, United Phosphorus, Inc., King of Prussia, PA, USA)	2.2 kg
9	Herbicide	Reglone® (diquat dibromide, CAS No. 85-00-7, Syngenta Crop Protection, LLC, Greensboro, NC, USA)	2.3 L
10	Herbicide	Linex® 4L (linuron, CAS No. 330-55-2, Tessenderlo Kerley Inc. Phoenix, AZ, USA)	2.3 L
11	Herbicide	Brawl II® (S-metolachlor, CAS No. 87392-12-9; naphthalene, CAS No. 91-20-3; benoxacor, CAS No. 98730-04-2, Tenkoz, Inc. Alpharetta, GA, USA)	1.2 L
12	Insecticide	Besiege® (lambda - cyhalothrin, CAS No. 91465-08-6; chlorantraniliprole, CAS No. 500008-45-7, Syngenta Crop Protection, LLC, Greensboro, NC, USA)	657 mL

Table S1a. Additional products applied to potato field trial conducted in Entrican Michigan at the Montcalm Research Center during the 2018 growing season. Refer to table S1b to translate product codes to dates of application. This table corresponds to data presented in Chapter 2 of this thesis.

Date Applied	Product Code
May 17	10, 11
June 4	2
June 14	4
June 21	3, 4
June 28	8
July 5	7
July 11	3
July 13	5
July 19	4
July 26	4
August 2	2, 12
August 24	1, 9
September 6	1, 9

Table S2b. Dates and product codes for additional products applied to potatoes during the 2018 field season conducted in Cassopolis Michigan. Refer to Table S2a to translate product code to product names and rates.

Date Applied	Product Code
June 12	4
June 15	5, 7, 16
June 19	1, 3, 4, 8
June 25	9, 12, 16, 19
July 3	4, 12, 17, 19
July 10	1, 3, 4, 10
July 17	3, 4
July 25	3, 4, 11
July 31	6, 5, 17
August 7	5, 19
August 15	5, 11, 12, 19
August 23	2, 5, 16, 18
August 27	13,14
September 1	13, 15

Table S1b. Dates and product codes for additional products applied to potatoes during the 2018 field season conducted in Entrican Michigan at the Montcalm Research Center. Refer to Table S1a to translate product codes to product names and rates of application.

Product Code	Family	Product	Rate (per ha)
1	Adjuvant	Velomax® (modified vegetable oil, petroleum hydrocarbons, alkyl phenol ethoxylate, Innvictis Crop Care LLC, Loveland, CO, USA)	292.3 m
2	Adjuvant	IVC® 5150 (Phosphatidycholine, methylacetic acid, polyoxyethylene ether, Innvictis Crop Care, LLC, Loveland, CO, USA)	116.9 ml
3	Fertilizer	8-24-0	23.4 L
4	Fungicide	Echo® 720 (chlorothalonil, CAS No. 1897-45-6, SipcamAgro USA, Inc., Durham, NC, USA	1.29 L
5	Fungicide	Koverall® Fungicide (mancozeb, CAS No. 8018-01-7, FMC Corporation, Philadelphia, PA, USA)	2.24 kg
6	Fungicide	Agri Tin (triphenyltin hydroxide, CAS No. 76-87-9, Nufarm Americas Inc., Burr Ridge, IL, USA)	140 g
7	Fungicide	Trevo® (azoxystrobin, CAS No. 131860-33-8, Innvictis Crop Care LLC, Loveland, CO, USA)	1.09 L
8	Fungicide	Revus Top® (difenoconazole, CAS No. 119446-68-3; mandipropamid CAS No. 374726-62-2, Syngenta Crop Protection, LLC, Greensboro, NC, USA)	511.5 ml
9	Fungicide	Omega 500F (fluazinam, CAS No. 79622- 59-6, Syngenta Crop Protection, LLC, Greensboro NC, USA)	584.6 ml
10	Fungicide	Luna Tranquility® (fluopyram, CAS No. 658066-35-4; pyrimethanil, CAS No. 53112-28-0; 1,2-propanediol, CAS No. 57-55-6, Bayer CropScience, Research Triangle PK, NC, USA)	803.9 ml
11	Fungicide	Tanos® (famoxadone, CAS No. 131807-57-3; cymoxanil, CAS No. 57966-95-7; sodium dioctyl sulfosuccinate, CAS No. 577-11-7, DuPont, Wilmington, DE, USA)	584.6 ml
12	Fungicide	Badge® Sc (copper oxychloride CAS No. 1332-40-7; copper hydroxide CAS No. 20427-59-2, Gowan Company, Yuma, AZ, USA)	584.6 ml
13	Herbicide	Reglone® (diquat dibromide, CAS No. 85-00-7, Syngenta Crop Protection, LLC, Greensboro, NC, USA)	1.17 L
14	Herbicide	Vida (naphthalene, CAS No. 91-20-3; pyraflufen-ethyl,CAS No. 129630-19-9, Gowan Company, Yuma, AZ, USA)	219.2 ml
15	Herbicide	Aim EC (carfentrazone-ethyl, CAS No. 128639-02-1; naphtha, CAS No. 64742-94-5; 2-methylnapthalene, CAS No. 91-57-6, 1- methylnapthalene, CAS. No. 90-12-0; n-butanol, CAS No. 71-36-3; naphthalene, CAS No. 91-20-3, 2- FMC Corporation Philadelphia, PA, USA)	146.2 ml
16	Insecticide	Ravage® (lambda-cyhalothrin, CAS No. 91465-08-6, Innvictis Crop Care LLC, Loveland, CO, USA)	219.2 ml
17	Insecticide	Reveal EndurX ® (bifenthrin, CAS No. 82657-04-3; naphthalene CAS No. 91-20-3, Innvictis Crop Care LLC, Loveland CO, USA)	476.7 ml
18	Insecticide	Beleaf 50 Sg Insecticide (flonicamid, CAS No. 158062067-0, FMC Corporation, Philadelphia, PA, USA)	196 g
19	Suspension Concentrate	AgroFuze Zinc (zinc oxide, CAS No. 1314-13-2, HydroGro, Scottsdale, AZ, USA)	584.6 ml

Table S2a. Additional products applied to entire potato field during 2018 product trial in Cassopolis, Michigan. Refer to Table S2b for dates in which products where applied. This table corresponds to data presented in Chapter 3 of this thesis.

APPENDIX B: RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included.

Voucher Number: 2019-05

Author and Title of thesis:
Emilie Cole
Integrated pest management strategies for control of potato early die in Michigan potato systems

Museum(s) where deposited:
Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Specimens:

Family	Genus-Species	Life Stage	Quantity	Preservation
Pratylenchidae	*Pratylenchus penetrans*	Adult	1 photo	Photograph

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