INVESTIGATING SUSTAINABLE STRATEGIES TO MANAGE THE ROOT-LESION NEMATODE *PRATYLENCHUS PENETRANS* AND THE WILT-INDUCING FUNGUS *VERTICILLIUM DAHLIAE* IN POTATO PRODUCTION

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ABSTRACT

Plant-parasitic nematodes cause millions of dollars in economic loss each year in the state's $104.7 billion food and agriculture industry. In potato production, the root-lesion nematode, *Pratylenchus penetrans,* can synergistically interact with the wilt-inducing fungi *Verticillium dahliae*, causing a disease known as Potato Early Die (PED). This disease complex causes plants to senesce 4 to 6 weeks early, thereby causing yield losses between 30% to 50%. The industry standard for PED management is soil fumigation and applications of post-planting pesticides, nonetheless, current management practices are not sustainable, and PED remains one of the top industry priorities in Michigan potato production. Therefore, this Ph.D. dissertation investigates different sustainable management strategies to manage PED with a focus on manure-based amendments and biological control agents, emphasizing the importance of preserving soil health. For the first objective, the effectiveness of integrated management of PED with manure-based amendments and biological control agents was determined under field conditions. The results show that the most effective management alternatives for *P. penetrans* control are raw poultry manure, both standalone and in combination with a singular application of Vydate® or MeloCon® (*Purpureocilium lilacinum*), and Compost A in combination with a singular application of MeloCon®. It was also found that Compost A, a composite of composted raw poultry and cattle manure with wood ash, resulted in higher yields underscoring the necessity of selecting amendments based on their potential to mitigate pathogen prevalence or augment productivity. For the second objective, the effectiveness of the management of PED with non-fumigant nematicides, fungicides, and seed treatments was determined under field conditions. The results show that Vydate® is the most effective nematicide to control *P. penetrans*, while the treatment combination Velum®+Velum®+Movento®+Movento®+Vydate® showed a slight decrease of *V. dahliae* stem infection of 6%. As for the third objective, commercially available biological control agents for Michigan *Verticillium dahliae* management were identified. The results show that the active ingredients of Tenet® (*Trichoderma asperellum* and *T. gamsii*), Actinovate® (*Streptomyces lydicus*), and Elatus® (azoxystrobin and benzovindiflupyr) are highly antagonistic to the three evaluated *V. dahliae* isolates *in-vitro*. However, greenhouse trials suggest that Actinovate® delivers the most effective control of Michigan *V. dahliae* isolates. Finally, for the fourth objective, the influence of manure-based amendments on potato soil microbiome and its correlation with *P. penetrans* abundance was determined. The results from the greenhouse trials

showed that *P. penetrans* abundance in manure-treated soils was significantly lower than the control, independent of the manure amendments autoclaving process. The relative abundance of soil bacterial and fungal phyla and the α- and ß-diversity indices of bacterial and fungal species changed in response to both autoclaved and non-autoclaved manure amendments and trends were different between the two experiments. With the addition of manure amendments only in the second experiment did the relative abundance of the Firmicutes bacteria phyla significantly increase and negatively correlate with *P. penetrans* abundance. Disease complexes like PED are understudied and hence challenging to manage, but the evidence provided by these studies about management alternatives for *P. penetrans* and *V. dahliae* separately is essential due to their important roles in the PED disease complex. Disease complexes often require an integrated management approach to ensure maximum control of primary inoculum. Therefore, crop rotation with non-hosts, weed, irrigation, and nutrient management coupled with before planting soil incorporation of raw poultry manure, early season application of Vydate®, and applications of biological control agents like *P. lilacinum* and *S. lydicus* can mitigate the detrimental effects of PED, foster soil health and ensure sustainable agricultural practices.

This dissertation is dedicated to my sister, Paulina, and my grandmother, Amalia.

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TABLE OF CONTENTS

CHAPTER 1: LITERATURE REVIEW

Luisa M. Parrado and Marisol Quintanilla

1.1 PLANT PARASITIC NEMATODES AND PLANT DISEASE COMPLEXES

As the human population is expected to increase to 9.6 billion in 2050 and 10.9 billion by 2100, agriculture will need to increase by 60% to sustain such a population (Ristaino et al. 2021). However, plant pathogens represent an obstacle to this goal. A plant pathogen is considered to be an organism that possesses the ability to impair the functionality of the plant (pathogenicity), but the severity of the disease caused by the pathogen is defined by the degree of damage caused (virulence), which is known as "disease severity" (Sacristan and Garcia-Arenal, 2008). Some fungi, bacteria, nematodes, and viruses can invade plants, feed, and proliferate in them, causing symptoms like necrosis, wilting, blights, cankers, rots, scab, hypertrophy, hyperplasia, rusts, smut, and mildew (Nazarov et al. 2020).

Global yield losses due to plant pathogens are significant, ranging from 21% to 30% in different cropping systems, and sometimes greater in certain geographic areas (Savary et al. 2019). One may think the numbers above are not alarming, but plant diseases represent a big threat to food and financial security. Agriculture accounts for 4% of global gross domestic product but in some developing countries it can account for more than 25%. Therefore, yield losses in crops can result in detrimental economic impact, placing millions into poverty and hunger (The World Bank, 2023). Plant pathogens not only cause crop yield losses but can also be poisonous, representing a threat to human health. For instance, corn and peanuts are big sources of nutrition in Africa, which have been severely compromised by aflatoxins produced by *Aspergillus flavus*. When ingested, it can cause stunted growth in children and, in some cases, cancer (Mutiga et al. 2015). Thus, although not emphasized enough, control of plant diseases in agriculture is crucial for human sustainability.

Plant disease can be explained by the "disease triangle" (Stevens, 1960) (Figure 1). The latter explains that for disease to happen, three favorable conditions must happen. The pathogen must be pathogenic and virulent, must be within the economic threshold, and be compatible with the plant host. Likewise, the plant host must be susceptible to the pathogen and the environmental conditions must favor pathogen infection. For centuries, plant diseases have been documented, and management strategies have been developed; but although plant diseases are often caused by single organisms, some can be caused by the interaction of two or more plant pathogens from the

same or different phyla. This interaction is known as a "disease complex" and it can complicate the development of efficient management strategies (Lamichhane and Venturi, 2015).

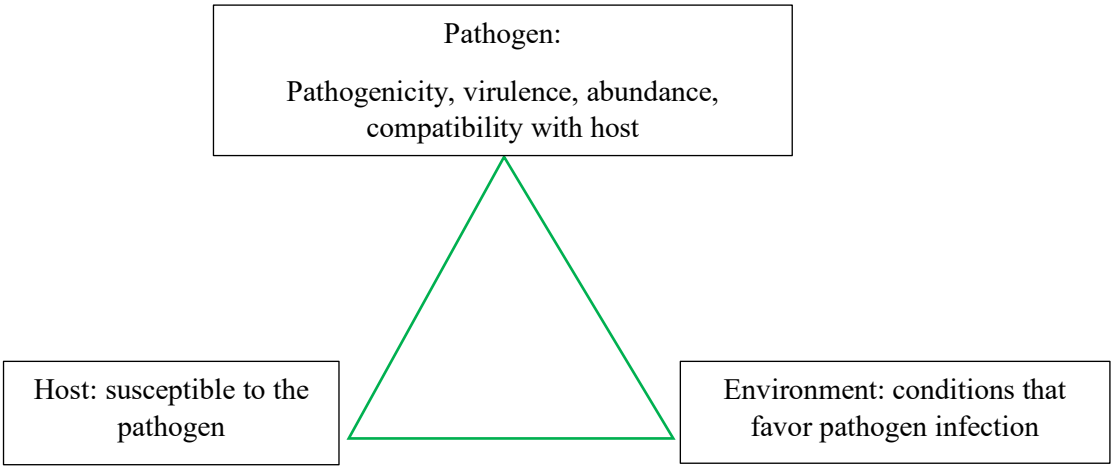

Figure 1. Conceptual representation of the disease triangle postulated by Stevens, 1960.

An example of disease complexes formed by organisms from different phyla are those between plant-parasitic nematodes (PPNs) and other microorganisms like fungi, bacteria, or viruses. PPNs are microscopic vermiform-like animals that possess a specialized organ called a "stylet", which they use to puncture plant cells and absorb nutrients. PPNs are a significant threat to high-value crops such as vegetables, fruits, field crops (*i.e.,* corn, soybean, cotton, wheat), ornamentals, and flowers. There are over 4,100 species of PPNs that have a major impact on global agriculture and horticulture, accounting for losses of US$173 billion every year (Phani et al. 2021).

PPNs can be classified based on their feeding behavior, and in this literature review, I will focus on PPNs that feed from roots only (Kumar and Yadav, 2020). The ectoparasitic PPNs remain outside the root. One example is *Xiphinema index* whose main host is the grapevine, and it is referred to as the only natural vector of the *grapevine fanleaf virus* (GFLV), which affects vineyards worldwide and can cause up to 80% yield losses (Andret-Link, et al. 2004). The semi-endoparasite PPNs partially penetrate the root tissue and feed occasionally. One example is *Rotylenchus reniformis,* which has about 350 host plants and can cause up to 50-60% yield losses in pineapple and banana crops, as well as up to 40% yield losses in cotton (Robinson et al. 1997; Davis et al. 2003; Mokrini et al. 2023).

The migratory endoparasites migrate through the root tissue while feeding on plant cells causing necrotic lesions along the way and represent the third most economically important group of

PPNs. The most representative species are *Pratylenchus brachyurus*, *P. coffeae*, *P. goodeyi*, *P. loosi*, *P. neglectus*, *P. penetrans*, *P. pratensis*, *P. scribneri*, *P. thornei*, *P. vulnus* and *P. zeae* (Jones and Fosu-Nyarko, 2014). For instance, *P. thornei* and *P. neglectus* can cause 62%, and 30% grain losses in wheat, respectively (Fanning et al. 2018).

Lastly, the sedentary endoparasites remain inside the root in a single spot where they establish highly specialized feeding sites. Root-knot and cyst nematodes have this feeding behavior and are the first and second most economically important PPNs, respectively. The most representative species of root-knot are *M. arenaria, M incognita, M. javanica, and M. hapla*, and they can infect almost every species of vascular plant, causing significant yield losses and representing a big challenge to management programs (Karssen et al., 2013). For the second group, the most important species of cyst nematodes are *Globodera pallida* and *G. rostochiensis* (potato cyst nematodes (PCN)), *Heterodera glycines* (soybean cyst nematode (SCN)), *H. avenae* and *H. filipjevi* (cereal cyst nematodes) and *H. schachtii* (sugar beet cyst nematode) (Lilley et al., 2005). For instance, SCN has caused yield losses of US$300 million in just Illinois, Iowa, and Missouri, while PCNs can reduce potato yields by up to 80% (Wang et al. 2003; Dandurand et al. 2019).

PPNs' feeding behavior, secretions, the type of cell they choose to feed from, population abundance, and time spent interacting with the plant cell define the physiological changes in the plant as well as its response. Above-ground symptoms caused by PPNs are often mistaken as nutrient deficiency or abiotic stress; only a few symptoms such as galls or knots can certainly be associated with a specific nematode infection (Kumar and Yadav, 2020). PPNs alone can be extremely detrimental to agriculture, however, some PPNs can interact with other plant pathogens, which leads to increased plant damage and adds a degree of complexity to the development of pest management programs. This section aims to highlight the roles of PPNs in disease complexes and discuss how insights into the mechanisms behind these interactions can provide tools for the development of efficient management strategies.

Plant-parasitic nematodes disease complexes in different cropping systems

Plants are host to an incredible number of microorganisms, some beneficial some pathogenic. The ecology of plant microbiome is rather complex and challenging to study, but with advances in molecular biology, new technologies have been developed that have allowed an understanding of the soil communities and their potential to be harnessed to improve plant and soil health, and

plant disease management (Afridi et al. 2022; Trivedi et al. 2022). In the plant microbiome, there can be associations or interactions, terms that according to Khan, 1993, are used interchangeably. According to the same author, in the study of plant disease, 'interaction' should be used under situations where there's a quantitative effect: either synergism or antagonism. For a disease to be considered to be caused by a complex of microorganisms, first, two or more organisms are involved, and second, there is a synergistic interaction between them. This means that the disease caused by the pathogens combined is greater than the sum of the disease caused by each pathogen alone (Powel, 1979). Plant-parasitic nematodes (PPNs) as part of the plant microbiome can either associate or interact with members of other communities, which can result in disease complexes.

PPNs are thought to be primary pathogens, that through their feeding behavior, favor the establishment of a secondary pathogen, which alone cannot infect the host. For instance, bacteria usually require natural openings or wounds in the plant tissue to cause infection. Under this scenario, PPNs feeding on the plant roots provide open wounds for secondary infections to happen. However, some pathogens possess the necessary pathogenic ability to cause disease, therefore, not requiring host physiological alteration by the nematode to cause infection. In this case, both pathogens possess the ability to cause disease separately, but when together, they can generate favorable changes in the host, which results in increased disease severity (Khan, 1993). Disease complexes that involve PPNs are thought to have two types of interactions. *i.* The expression of disease symptoms happens only if all pathogens are present, *ii.* Each pathogen causes disease, but PPNs presence enhances the other pathogen incidence. At the same time, these interactions happen depending on plant genotype, soil organic matter, nutrient content, and other microbes. Furthermore, PPNs can enhance infection by other soil-borne pathogens by either being vectors, wounding agents, or modifiers of plant biochemistry, physiology, or rhizosphere microbiome (Siddiqui et al. 2012; Zhang et al. 2020; Ravichandra, 2014).

For example, the first report of possible interaction between nematodes and bacteria was in 1901 by Hunger, when he observed that tomatoes planted in nematode-infested soil were infected with *Pseudomonas solanacearum*, while tomatoes remained bacteria-free in nematode-free soil (Hunger, 1901). Since then, a few PPNs-bacteria disease complexes have been described which has led to the understanding that the way PPNs benefit bacterial infections can be because they act as wounding agents and/or modifiers of plant biochemistry and physiology (Siddiqui et al.

2012). For instance, Lucas et al. used a tobacco variety resistant to *P. solanacearum* and found that when co-inoculated with *M. incognita*, the tobacco plants were infected with *P. solanacearum* and expressed bacterial wilt symptoms, indicating that PPNs can induce the production of host metabolites that favor the colonization of bacteria or break down metabolites involved in triggering plant resistance (Lucas et al. 1955). Additionally, there are some cases in which co-infection of PPNs and bacteria can also result in symptoms that neither pathogen causes alone. For example, infection of raspberry with *P. penetrans* and *Agrobacterium tumefaciens* is associated with the sudden decline symptom (Vrain and Coperman, 1987). Likewise, for the disorder in strawberries known as "cauliflower complex", both *Aphelenchoides ritzemabosi* and *Corynebacterium fascians* infection is required for disease expression (Parvatha Reddy, 2018).

In addition, there are also disease complexes between PPNs and fungi. The first evidence of PPNs-fungi interaction was recorded when the fusarium wilt of cotton was found to be more severe in the presence of *Meloidogyne* spp. by Atkinson, 1892, since then multiple disease complexes formed between *Meloidogyne* spp. and *Fusarium* spp. have been described affecting several crops including tomatoes and cotton (Khan and Sharma, 2020). In a disease complex involving *Meloidogyne* spp. and other plant-pathogenic fungi, Lamelas et al. 2020 determined that in coffee and tomato, the disease complex infection process is potentially mediated by compounds produced by associated bacteria communities, indicating that the rhizosphere microbiome also plays a role in PPNs disease complexes.

Most PPNs and fungi disease complexes occur between widespread pathogens, hence environmental conditions also aid in conducting disease complexes. For instance, root-knot and root-lesion nematodes, which can infect hundreds of different plant species under different environmental conditions, often form disease complexes with fungal pathogens like *Fusarium* spp., *Rhizoctonia* spp., *Phytophthora* spp., and *Verticillium* spp. (Zang et al. 2020). For example, some recent root-knot and fungi disease complexes comprise *M. incognita* and *M. javanica*, with mostly different species of *Fusarium* spp., followed by *R. solani* (Archana et al. 2023). Similarly, root-lesion nematodes like *P. thornei* can interact with the crown rot fungus *F. culmorus* in wheat, where the nematode downgrades plant resistance to the fungi (Laasli et al. 2022). Another example is a well-documented case of *P. penetrans* and *V. dahliae* disease complex, also known as potato early die, together these pathogens cause the potato plant to die 4-

6 weeks early, reducing yield up to 50% (Martin et al. 1982). Likewise, there are disease complexes between cyst-nematodes such as the soybean cyst nematode *H. glyciens* and the sudden death syndrome caused by *F. virguliforme*, which together cause the highest yield loss of soybeans in the United States, and most recently, it has been demonstrated that *H. glyciens* and *P. sojae* interact, causing more severe disease in soybeans (Xing and Wesphal, 2007; Chowdhury et al. 2022).

As previously mentioned, PPNs can serve as vectors in disease complexes, which is the case in those involving viruses; however, while the virus benefits from the PPN, is not clear how the PPN profits from the virus. The first report of PPNs being vectors of a plant virus was by Hewitt et al. in 1958, when they observed that the dagger nematode *Xiphinema index* was the vector of the *grapevine fanleaf virus*. Currently, it is known that 13 species of nepoviruses are transmitted by *Xiphinema*, while 10 are transmitted by 11 species of *Longidorus*. In addition, *Trichodorus*, and *Paratrichodorus* can also transmit other viruses, although fewer (Singh et al. 2020). These nematodes are ectoparasites, acquiring and transmitting the viruses through their feeding with their stylet, but also the ability of these nematodes to retain the virus particles in specific sites located in the esophagus (Singh et al. 2020).

PPN transmission of a virus starts with ingestion and is followed by the acquisition, adsorption, retention, release, and transfer, ending with the establishment of the virus in the host plants (Singh et al. 2020). Additionally, such steps involve different interactions. For example, ingestion involves nematode-virus-plant, while acquisition, adsorption, retention, and release involve nematode-virus interactions, and the establishment involves virus-plant. Therefore, it is important to consider such stages given that interruption of either part of the process could prevent the establishment of the virus.

It is also important to take into consideration that PPNs-virus interaction can be specific. For example, some nematodes, such as *X. index*, are specific vectors of *grapevine fanleaf virus*, while in other nematodes, like the trichondorids, various species can vector the same virus or different viruses can be vectored by the same nematode species. Such specificity could be explained by the composition of the virus and the nature of the compounds within the nematode that enable vector specificity (Schellenberger et al. 2011; Brown and Weischer, 1998). Hence, studying what triggers these interactions could provide foundational knowledge to develop tools to interfere with virus transmission.

Pathogens mechanisms of interaction as the key to the development of efficient management approaches

One of the limitations in the study of disease complexes is determining if there is a synergistic interaction between pathogens. As previously mentioned, for such interactions to take place, the environment plays a crucial role. Conventional experiments based on artificial inoculation under controlled conditions, may not provide the right environmental conditions, and pathogen-pathogen interaction might not take place. Therefore, it is important to mirror natural conditions as much as possible to accurately determine if there is a disease complex (Sikora and Carter, 1987). Another limitation in this same aspect is the use of correct statistics for the accurate establishment of possible interactions. For instance, to determine which pathogens thresholds trigger interactions, inoculations at different densities should be evaluated. For this case, multifactorial analysis rather than analysis of variance (ANOVA) provides a better analysis, given that factorial designs consider multiple densities of two or more organisms (Shoabib et al. 2023; Trivedi et al. 2022).

Another limitation is the lack of information about how to predict the damage and main driving factors of a disease complex, which ultimately can aid in the management practices decision-making process. Integration of advanced statistical methodologies into the analysis of data to determine interactions between PPNs, other plant pathogens, and other factors of an experiment such as crop type, growth stage, and environmental conditions, is vital to advance our foundational knowledge of disease complexes. For example, in a study published by Wheeler et al., 2019 they used machine learning methods to identify drivers of wilt in commercial mint fields caused by *Verticillium dahliae*, describe the relationship between the drivers, and predict wilt. The combination of field experiments and advanced statistical tools like multiple linear regression, generalized additive model, random forest, and artificial neural network aided in concluding that all models selected *Pratylenchus* spp. as the most important predictor of mint wilt, and, interestingly, *V. dahliae* (thought to be the main cause of mint wilt) was the fourth most important predictor of mint wilt. Altogether, models explained that quantitative relationships between the two pathogens, mint cultivars, and cultivar age are required to explain wilt symptoms. Evidence like this shows how underestimated the contributions of PPNs are in disease complexes, and how important it is to integrate different tools of data analysis to maximize the understanding of drivers of plant disease.

Another limitation is the elucidation of mechanisms behind the interactions of the disease complexes. PPN interactions with other plant pathogenic microbes are not as studied and discussed as other types of relationships like symbiont bacteria-entomopathogenic nematodes (Zhang et al. 2020). It is hypothesized that PPNs can predispose the host plant by modifying host physical barriers, altering plant immune responses, increase of root exudation, generating changes in the microbial rhizosphere, and as direct vectors of another pathogen (Ravichandra, 2014). With genomic tools being improved and becoming more accessible every day, it would be interesting to study the gene expression of pathogens when interacting, and the effect that the pathogens combined have on the host gene expression related to plant response, physiology, and fitness (Rocha and Schwan, 2023). Results from such research can help to identify targets for the development of new chemistries with modes of action that inhibit such targets as well as improve plant breeding of resistant varieties (Eves-van den Akker, 2021).

1.2 POTATO PRODUCTION AND POTATO EARLY DIE DISEASE COMPLEX

Potato characteristics, production, and plant disease challenges

Roughly 7,000-13,000 years ago, the domestication of potatoes started in the high altiplano region of Peru, near Lake Titicaca. Potatoes are propagated by planting whole or cut tubers (e.g., seed pieces) instead of true seeds. As a result of this propagation method, potato crops often emerge slower, but development tends to be faster than other food crops, given the energy sources stored in the seed piece. The first evidence of growth is the formation of sprouts, structures that consist of stem tissue and meristem. Followed by sprouting, the root system develops in the first 12 inches of soil, in a shallow and sparse way with a small amount of root hairs. Later, stolons (underground shoots) are formed, from where potato tubers start to develop. However, the successful growth of potatoes is dependent on various factors like soil temperature, pH, moisture, nutrient availability, phytohormones, climate conditions, and successful pest and disease management (Thornton, 2020).

Today, potatoes are the world's most important vegetable crop with over one million acres in potato production. The United States potato sector contributes around $101 billion to the country's economy (Szymanski, 2023). Potato production is the second leading produce commodity in Michigan with 50,000 acres destined for potato production, generating $182.4 million in farm gate sales (Michigan Ag Facts and Figures, 2018). Michigan is the leading producer of potatoes for chip processing, however, other varieties such as table potatoes are

grown as well. Michigan has around 80 potato growers, where the majority of potato production is concentrated in Montcalm country, however, given that potatoes are more soil-particular than climate-particular, they are grown all over the state, where the soils are adequate.

One of the obstacles that face U.S. potato production is the presence of plant pathogens; there are around 32 diseases that negatively impact potato production (Stark, 2020). Some of the diseases that affect Michigan potato production are late blight, early blight, gray mold, potato scab, verticillium wilt, bacterial ring rot, silver scurf, stem cancer and black scurf, fusarium rot, white mold, pink rot, fusarium wilt, leak, bacterial soft rot, viruses like potato virus Y, and plant parasitic nematodes like root-knot and root-lesion. For the management of these pathogens, general disease management strategies are categorized by exclusion, eradication, protection, and use of resistant varieties. Moreover, the most effective management approaches use a combination of several methods, which is known as integrated pest management (IPM). Nonetheless, to achieve profitable yields, management decisions often result in intensive practices that utilize an abundance of chemical inputs for nutrient and pest management, which can negatively impact soil texture, biodiversity, organic matter, bulk density, and soil water holding capacity (Saini and Grant, 1980; Tsiafouli et al. 2015).

One serious disease that requires intensive pest management programs that include soil fumigation with broad-spectrum pesticides and regular pesticide applications, is Potato Early Die (PED) (Powelson and Rowe, 1993). The PED disease is caused by the synergistic interaction between the plant-parasitic nematode *Pratylenchus penetrans* and the wilt-inducing fungi *Verticillium dahliae* kleb. (Rowe et al. 1971) (Figure 2). This disease causes damage to the root system and reduces leaf area and therefore the plant's photosynthesis rate, thereby greatly impacting crop productivity and causing yield losses between 30% to 50% (Davis et al. 2001).

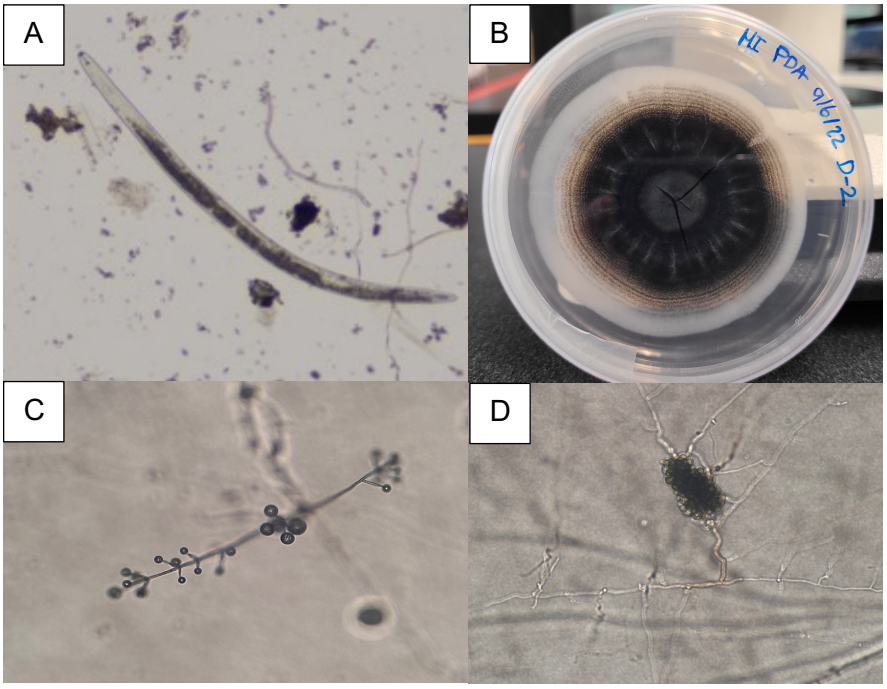

Figure 2. **A.** A juvenile of root-lesion nematode *Pratylenchus penetrans* extracted from potato roots. **B.** Colony morphology example of a Michigan *Verticillium dahliae* isolate, recovered from potato plants that showed symptoms of verticillium wilt. **C**. Verticil conidiophores characteristic of *Verticillium* spp. **D.** Microsclerotia of a Michigan *V. dahliae* isolate. All pictures taken by Luisa Parrado, 2022.

Root-lesion nematode *Pratylenchus penetrans*

Plant-parasitic nematodes are of great economic importance in the north-central region of the U.S. Cyst nematodes (e.g., *Heterodera glycines*, *H. schachtii*) are the most economically important, followed by the northern-root-knot nematode *Meloidogyne hapla*. Root lesion nematodes are the third most economically important nematode, with *P. penetrans* considered the most common of all in the mid-west U.S. (Westphal et al. 2018; Bird and Warner, 2018). *Pratylenchus penetrans* is an obligate migratory endoparasite that prefers temperate regions and sandy soils. It is widely distributed and is considered a regulated non-quarantine pest in the U.S. This nematode can infect over 400 plant species, such as fruit trees, orchards, cereals, field crops, vegetables, flowers, and weeds (Nemaplex, 2023).

Like all plant parasitic nematodes, *P. penetrans* has six life stages. It starts with the egg, followed by four juvenile stages, and the adult stage. Depending on environmental conditions and the host (e.g., susceptible, moderately susceptible, or moderately resistant), the life cycle can

be completed within 4-8 weeks. In the egg, the nematode molts from J1 to J2, thereafter, all motile stages can infect host plants. These nematodes prefer to invade their host by the root tips, root zone of elongation, or just anywhere on the entire root surface. As previously mentioned, they are migratory endoparasites and they migrate intracellularly within the roots. The roots from which they prefer to feed are in the root hairs or the cortical and stelar cells, which die after nematode feeding, causing extensive cell damage, root vascular disruption, and necrosis (Jones and Fosu-Nyarko, 2014). *Pratylenchus penetrans* reproduction is sexual and adult females can lay eggs singly inside the root or in soil, producing up to 3000 nematodes per gram of root (Fosu-Nyarko and Jones, 2016; Davis et al. 2000).

Pratylenchus spp. juveniles and adults can infect the host plant in response to gradients of compounds secreted by the roots (e.g., secretion of CO_2). Root infection is achieved by multiple mechanisms that include mechanical probing and enzyme secretion. PPNs, in general, may possess genes that encode for plant cell wall degrading enzymes (CAZymes) such as cellulases, xylanases, polygalacturonases, pectate lyases, and arabinases. Although most "parasitome" studies have been done on sedentary endoparasites, studies done on the root-lesion nematodes *P. coffeae* and *P. thornei*, indicate the presence of the same CAZymes classes (Haegeman et al. 2011). In *Pratylenchus* spp., is it proposed that these enable the nematode to migrate and feed from the root cells by softening the cell walls or cell wall dissolution (Jones and Fosu-Nyarko, 2014).

PPNs as well as other organisms that interact with plants, give themselves away by molecules the plants detect. For PPNs molecules could be found on their surfaces, released into the apoplast, or formed from cell wall degradation. These molecules are known as pathogen-associated molecular patterns (PAMPs). Once such molecules are detected by the plant, the PAMP-triggered immunity (PTI) is triggered, which includes the production of enzymes and compounds that are toxic to pathogens. For instance, it was found that on a resistant cultivar of banana, *P. coffeae* infection induces accumulations of peroxidases and polyphenol oxidases (Devi et al. 2007; Kumar et al. 2008). Another plant defense mechanism is the production of reactive oxygen species (ROS), yet it has been found that some PPNs can secrete antioxidant components that metabolize ROS (Zheng et al. 2015).

Most research about PPNs-plant interactions has been conducted on sedentary endoparasites, therefore there are knowledge gaps that need to be filled to understand the parasitism process of

migratory endoparasitic nematodes. Migratory endoparasites can avoid being affected by the host's immune responses just by moving before the host can even react, nonetheless, resistance of some host plants has been found to a specific species of *Pratylenchus*, while not to all; indicating that in migratory endoparasites like *Pratylenchus* spp., not all effectors are conserved. Studying the genes involved in *Pratylenchus* spp.-plant interaction could provide targets that can serve as leads for the development of new chemistries for *Pratylenchus* spp. control, but also to understand the role of *P. penetrans* in the PED disease complex.

Verticillium dahliae

The first species of *Verticillium* was first described in 1816, and ever since more than 150 species have been described, however, just a few are pathogenic to plants (Rasoul et al. 2004). *Verticillium* spp. belongs to the Ascomycota division and there are about 10 species that cause economic losses to agriculture. *Verticillium albo-atrum*, *V. alfalfa*e, *V. longisporum*, *V. nonalfalfae*, *V. tricorpus* and *V. zaregamsianum* are known to cause significant yield losses, while *V. nubilum* has minor pathogenicity only on tomatoes and potatoes (Inderbitzin and Subbarao, 2014). *Verticillium isaacii* and *V. klebahnii*, however, were isolated from lettuce and artichoke but their significance to agriculture is unknown (Inderbitzin and Subbarao, 2014). Amongst them all, *V. dahliae* has the most significant economic impact and is widely spread around the world, infecting over 200 plant species (Pegg and Brady, 2002). *Verticillium dahliae* economic impact depends on the crop. For instance, in highly infested fields, it can cause up to 50% yield losses on potatoes, however, yield losses are more common to be between 10-15% (Rowe et al., 1985). But, in lettuce, yield losses can be up to 100% (Attallah et al. 2011).
One characteristic feature of *V. dahliae* and other species of *Verticillium* is the verticil conidiophores (Inderbitzin et al. 2011). Resting structures such as resting mycelium, chlamydospores, and microsclerotia were traditionally used for *Verticillium* species identification. For instance, it was suggested that *Verticillium* spp. should be grouped by the ones that produce microsclerotia, and the ones that only produce dark-resting-mycelium (Barbara and Clewes, 2003). Correct identification of a strain is essential for disease management decisions, given that wrong identification could lead to unnecessary economic efforts in management. Current identification of *Verticillium* species is done by ITS amplification, however, given that the ITS region is similar for some groups of species of *Verticillium*, ITS amplification may lead to misidentification. This is specifically the case for *V. dahliae* and *V. longisporum* given that the

first is the parent of two of the three *V. longisporum* lineages (A1/D1 and A1/D3) (Inderbitzin et al. 2011). Consequently, Inderbitzin et al. 2013 successfully developed a multiplex PCR assay to overcome the many similarities these two species share, and now their protocol is used for diagnostic purposes of *V. dahliae*.

Given that *V. dahliae* has no known sexual reproductive cycle, there is evidence of different subspecific groups of *V. dahliae* that possess a multigene trait that allows them to form heterokaryons, which potentially allows them to exchange genetic information (Leslie, 1993). This subspecific group is known as Vegetative Compatibility Group (VCG). Fungal strains that anastomose and form heterokaryons with one another are assigned to a single VCG, moreover, strains that are incapable of anastomosing are referred to as vegetative incompatible (Joaquim and Rowe, 1990). Puhalla and Hummel, 1983, determined that there are 16 VCGs within *V. dahliae*.

Based on that, Joaquim, and Rowe, 1991, collected 187 *V. dahliae* isolates from potato plants from 22 potato fields in Ohio. They determined to which VCG group each *V. dahliae* isolates belongs, by the auxotrophic, nitrate-nonutilizing (*nit*) mutants' method and found that 128 out of the 187 strains belonged to VCG 4. Similarly, they obtained 47 *V. dahliae* isolates from 9 different U.S. states and found that 45 belonged to VCG 4. The isolates from VCG 4 were then subdivided into VCG 4A and VCG 4B based on the reaction when paired against tester strains of VCG 3; VCG 4A isolates were weakly compatible with the tester strain while VCG 4B isolates were incompatible. Later, they conducted pathogenicity trials in potatoes under greenhouse conditions with the strains from VCG 2 VCG 4A, and 4B. Their results allowed them to conclude that strains from VCG 4A are significantly more virulent than the others, indicating two distinct pathotypes (Joaquim and Rowe, 1991).

Verticillium dahliae it's a hemibiotroph fungi with a monocyclic disease cycle. The survival structure, microsclerotia, serves as the primary inoculum. Root exudates stimulate the germination of microsclerotia. The hyphae reach and penetrate the root epidermis, followed by the cortex, then the endodermis and lastly it colonizes the xylem. In the xylem, *V. dahliae* starts to produce conidia, which germinate and branch out, infecting the adjacent vascular cells. Through cell-wall degrading enzymes, *V. dahliae* obtains nutrients and continues its infection. The reproduction of the pathogen within the vascular tissue, plus the plant responses such as the ones related to containing the pathogen and preventing further spread, blocks the xylem, limiting

the flow of water and nutrients, which leads to leaf water deficits, resulting in reduced photosynthesis rate, leaf transpiration, and leaf longevity (Dhar et al. 2020; Deketelaere et al. 2017; Powelson and Rowe, 1993).

Verticillium dahliae secretome encodes for around 700 proteins that may play roles to facilitate infection and proliferation, which translate into the wilt symptoms. Such roles include production of cell-wall degrading enzymes that aid penetration of the epidermis, cortex, all the way to the xylem, proteins that aid the detox of reactive oxygen species released by the plant as a defense mechanism in the first phases of infection, effector proteins that manipulate the host immune responses like inactivating the production of salicylic acid and overall phytohormone imbalance, effector proteins that have an antagonistic effect on hosts microbiome which aids successful colonization of the niche, and proteins that cause cell death, resulting in tissue necrosis. Once in the xylem, *V. dahliae* produces more cell wall degrading enzymes to obtain nutrients from plant cells. Some leftover degradation products, plus the response of the plant host like the release of biomacromolecules to limit pathogen spread, cause the blockage of the xylem and, hence, wilt symptoms. Lastly, once it spreads up to the shoots and leaves, it secrets effectors that manipulate the plant production of Ethylene, which accelerates the disease symptoms and induces the production of microsclerotia (Zhang et al. 2022).

Given that *V. dahliae* has a wide host range, a very resistant survival structure that can survive for several years in the absence of a host, and the fact that once it infects the plant there are no curative measures to stop the disease, management is limited to 1. Significantly reducing soil primary inoculum which is only achieved by soil fumigation and 2. Prevention/avoidance methods. Such as, when possible, avoiding planting on highly infested fields, avoiding planting susceptible hosts, and consecutive cropping of hosts. There is an extensive amount of literature focused on management alternatives to either reduce soil primary inoculum or counteract the detrimental effects of Verticillium wilts on crop yields (Carrol et al. 2018). For instance, it has been found that broccoli can significantly reduce soil microsclerotia, wilt incidence, and severity and that such effects lasted long enough to prevent the buildup of soil inoculum for subsequent crops (Shetty et al., 1999). Another example is the use of organic amendments that either contain proteins or volatile fatty acids (VFA). Reduction of *V. dahliae* primary inoculum is explained by the release of nitrogen compounds such as NH_3 and NO_2^- which are highly toxic, however, their production is dependent on the acidity of the soil, and hence the success of organic soil

amendments is site-specific (Con et al. 2005). Biological control agents (BCA) are also an interesting alternative to manage *V. dahliae*, however, there are just a few products that have been developed with field-level efficacy, and just as with organic soil amendments, their efficacy is site-specific (Deketelaere et al. 2017).

Ultimately, up to date, soil fumigation and plant resistance remain the most effective strategies to control *V. dahliae*, however, plant resistance hasn't been a success in all crops. For instance, there are no *V. dahliae* resistant varieties for potatoes, only moderately resistant. As described above, not all *V. dahliae* isolates are highly virulent in potatoes, only VCG 4A. Therefore, knowledge of pre-existing genotypic diversity of *V. dahliae* in the field should be considered when making decisions on management strategies.

Mechanisms behind the interaction between *P. penetrans* and *V. dahliae*

For potatoes, the synergism between these two pathogens has been determined by the earlier onset of symptoms, higher disease incidence and severity, and lower yields, when compared to the effects caused by the pathogens alone (Francl and Wheeler, 1993). Specifically, VCGs 4A has a strong synergistic interaction with *P. penetrans*. Co-inoculations of *V. dahliae* VCG 4A + *P. penetrans* resulted in higher disease severity and lower tuber yields (Botseas and Rowe, 1994), however, their interaction nor their interaction as a complex with the host plant at a molecular level is not yet understood.

There are multiple hypotheses as to the mechanisms behind their interaction, one of them related to the feeding behavior of the nematode. *Pratylenchus penetrans* causes open necrotic wounds, also increases root exudation, and stimulates root branching, which is thought to promote *V. dahliae* microsclerotia germination and further plant infection (Riedel et al., 1985; Riedel and Rowe, 1985). In addition, in some cases, it has been observed that when *V. dahliae* is present, *P. penetrans* populations tend to increase (Bowers et al. 1996).

Both organisms have similar mechanisms to successfully infect and colonize their hosts. Therefore, studying how their gene expression changes when they encounter each other, could elucidate how these pathogens communicate. Furthermore, studying host gene expression to infections of the pathogens alone and combined could provide targets that can serve as leads for the development of resistant varieties. Understanding the biology of the PED complex and its interaction with its hosts is crucial for the development of new chemistries for successful PED disease complex management.

1.3 PED SYMPTOMS AND CURRENT MANAGEMENT ON POTATOES

Symptoms of *P. penetrans* damage can be observed as dark-brown lesions in the root cortex, and up close, black necrotic lesions can be observed in the root (Figure 3). High populations of *P. penetrans* can cause stunting which is visible before flowering as a lag in canopy closure and sometimes shallow lesions can occur on tubers.

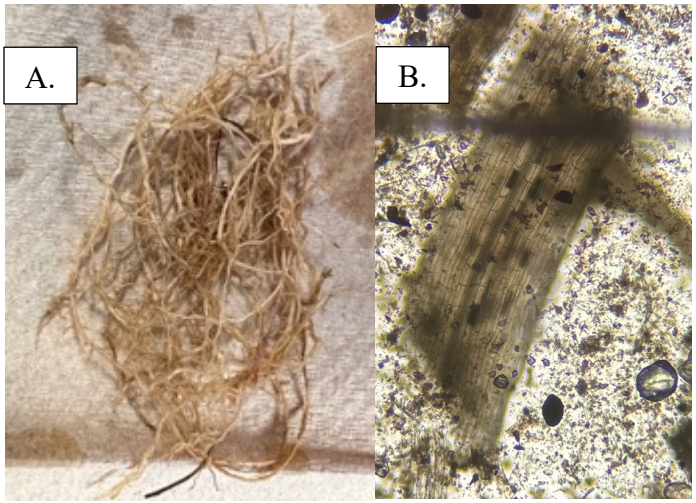

Figure 3. A. Reddish-brown necrotic lesions in the roots. **B.** Necrotic lesions in the root cells under the microscope. Pictures taken by Luisa Parrado, 2023.

In contrast, symptoms related to *V. dahliae* infection will normally start on the lower leaves, yellowing between leaf veins that turn brown, plus vascular discoloration of the stem at the base. Tubers and stems that are infected with *V. dahliae* show vascular discoloration (Figure 4).

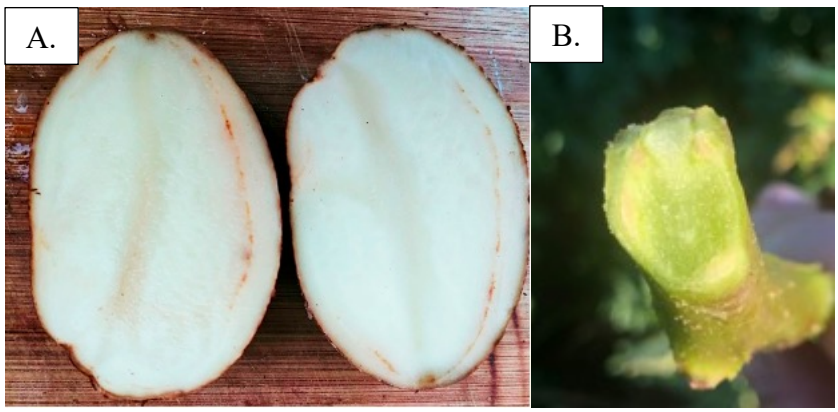

Figure 4. Vascular discoloration of **A.** potato tubers and **B.** potato stem, caused by V. dahliae infection. Pictures taken by Luisa Parrado, 2020.

In general, symptoms related to PED are stunted growth, uneven chlorosis, damage to the root system, premature senescence, and reduced yield (Rowe and Powelson, 2002). Soil fumigation with chemistries like metam sodium (Vapam®) is the industry standard for PED because it is effective against both pathogens. However, soil fumigation with metam sodium is highly regulated, and expensive and it requires the right temperature and soil moisture conditions to be effective. For better results, tillage is also recommended to expose roots before soil fumigation since *P. penetrans* overwinter in plant debris. After fumigation, soil sampling is encouraged to determine if additional control measurements are needed to lower nematode populations below threshold levels. The most used nematicide in potato production is oxamyl (Vydate®), however, some other non-fumigant nematicides and fungicides may be safer than soil fumigants or oxamyl (Table 1).

Table 1. Examples of some of the current non-fumigant products approved in the U.S. for Verticillium wilt and root-lesion nematode.

Pathogen	Chemical Group	Active Ingredient	Commercial name	FRAC /IRAC	Mode of Action
Broad spectrum	Methyl isothiocyanate generator	Metam sodium	Vapam®	N-UNX	Multi-site inhibitor
Verticillium spp/Plant-parasitic nematodes	Carboxamides	fluopyram	Velum® Prime	7	Fungal respiration complex II
Verticillium spp	Quinone outside inhibitors (QOL) Carboxamides	azoxystrobin and benzovindiflupyr	Elatus®	11 and 7	Fungal respiration complex III Fungal respiration complex II
Verticillium spp	Carboxamides	penfluten and prothioconazole	Emesto Silver®	7	Fungal respiration complex II
Verticillium spp	AP or anilo-pyrimidines	fluopyram and pyrimethanyl	Luna®	9	Methionine biosynthesis

Table 1. (cont'd)

Verticillium spp	Quinone outside inhibitors (QoI)	azoxystrobin	Quadris®	11	Fungal respiration complex III
Root-lesion nematode	Carbamate	oxamyl	Vydate® L	1A	Acetylcholine sterase inhibitors
Root-lesion nematode	Tetronic and Tetramic acid derivates	spirotetramat	Movento®	N-4	Lipid synthesis, growth regulation. Inhibitors of acetyl CoA carboxylase
Root-lesion nematode	Unknown	fluazaindolizine	Recklemel™	U-UN	Unkown

Potatoes were the most heavily fumigated crop with over 40 million pounds of fumigant applied reported by the EPA in 2005. However, more recent data about soil fumigation has not been disclosed. Instead, in 2022 USDA-NASS released a report about a survey conducted in 9 states, including Michigan, which accounts for 92% of the 901,000 acres planted with potatoes, and although the report did not disclose data regarding soil fumigation, 98% of planted acres were treated with fungicides such as chlorothalonil and azoxystrobin. Although effective in reducing pest pressure, soil fumigants and the application of dangerous and toxic chemistries can negatively impact soil microbial communities, contaminate groundwater systems, and increase soil erosion (Brussaard et al. 2007; Sinha et al. 1979; Toyota et al. 1999; Ibekwe et al. 2004). Thus, highlighting the need for safer management alternatives for detrimental diseases like PED.

1.4 DISSERTATION OBJECTIVES

The main goal of this dissertation is to investigate sustainable management strategies to manage *Pratylenchus penetrans* and *Verticillium dahliae* in a potato cropping system. To reach this goal, 4 objectives were established.

Objective 1: Determine the effectiveness of integrated management of Potato Early Die with manure-based amendments and biological control agents.

Two field trials were established to determine the efficacy of manure-based amendments and two biological control agents to control PED. This objective hypothesized that manure-based amendments differed on their pesticidal effect, and that combinations of manure-based

amendments and biological control agents could enhance the control of PED. All field research was conducted in commercial potato fields in Three Rivers, Michigan.

Objective 2: Determine the effectiveness of integrated management of Potato Early Die with non-fumigant nematicides, fungicides, and seed treatments.

One field trial was established to determine the efficacy of non-fumigant nematicides, fungicides, and seed treatments to control PED. The first hypothesis of this objective was that some combinations of non-fumigant nematicides and fungicides could enhance the control of PED. The second hypothesis was that seed treatments could protect the plant from the early growth stages, limiting early die infection and mitigating yield losses. All field research was conducted in commercial potato fields in Three Rivers, Michigan.

Objective 3: Identify commercially available biological control agents for Verticillium dahliae management.

This objective was accomplished through replicated laboratory assays and greenhouse trials. This objective hypothesized that at least one biological control agent would show significant antagonism against *Verticillium dahliae* in-vitro and in-planta. All lab assays were conducted in the Potato and Sugar Beet Pathology Laboratory at Michigan State University. A replicated greenhouse trial was established in the Applied Nematology Lab's Plant Greenhouse at Michigan State University in East Lansing, Michigan, to validate the results from the in-vitro screening.

Objective 4: Establish the effect of manure-based amendments on soil bacterial and fungal communities and Pratylenchus penetrans suppression under greenhouse conditions.

This objective was achieved through the conduction of two greenhouse trials. The hypothesis of this objective was that applications of manure-based amendments would have an influence on soil bacterial and fungal communities, and such response would correlate with the suppression of *Pratylenchus penetrans*. All greenhouse trials were conducted in the Applied Nematology Lab's Plant Greenhouse at Michigan State University in East Lansing, Michigan.

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CHAPTER 2: INTEGRATED MANAGEMENT OF POTATO EARLY DIE WITH MANURE-BASED AMENDMENTS AND BIOLOGICAL CONTROL AGENTS

Luisa M. Parrado, Emilie Cole, and Marisol Quintanilla

2.1 INTRODUCTION

Michigan is the leading producer of potato varieties for chip production. After all, Frito Lay, one of the largest chip producers in the world, sources around 40% of its potatoes from Michigan (Heberling, 2022). In addition, the Michigan potato industry also produces fresh varieties like the cv. Russet Norkotah. In 2022, 45,500 acres of potatoes were harvested. The yield per acre was 415 cwt (hundredweight; 20cwt=1ton), being the third largest yield on record, and in total, Michigan harvested 18.88 million cwt of potatoes. Fresh potato varieties like the Russet Norkotah are also grown in Michigan (Figure 5). This variety, in particular, has high yield potential because it produces a high percentage (>90%) of U.S. No. 1 tubers. However, it is susceptible to a variety of diseases such as foliar early blight, verticillium wilt, PED, black leg, PLVR, PVY, PVX, bacterial soft rot, late blight, Fusarium dry rot, Pythium leak, pink rot, and silver scurf, but moderately resistant to common scab (Stark, 2020). Overall, the Michigan potato industry contributes $1.24 billion to the state's economy; however, diseases that affect potato yields could affect profitability.

Figure 5. Grading potatoes cv. Russert Norkotah to determine yield (Pictures taken by Luisa Parrado, 2020).

One severe disease that requires intensive pest management programs that include soil fumigation with broad-spectrum pesticides and regular pesticide applications is Potato Early Die (PED) (Powelson and Rowe, 1993). The PED disease is caused by the synergistic interaction between the plant-parasitic nematode *Pratylenchus penetrans* and the wilt-inducing fungi *Verticillium dahliae* kleb. (Rowe et al. 1985). This disease causes damage to the root system and

leaf area, decreasing photosynthesis rate, leading the plant to senesce early, thereby causing yield losses between 30% and 50% (Davis et al. 2001). Hence, the Michigan Potato Industry Commission determined that one of their priorities is "*Integrated management of soil, seed, and foliar borne diseases to reduce vine and tuber rotting in potatoes*", in particular late blight and PED (Michigan Potato Industry Commission, 2022). Given the rising human health and environmental concerns around pesticide use in agriculture, such products may be banned in the future. Therefore, sustainable management alternatives for PED need to be investigated.

Over the years, the addition of organic amendments (e.g., compost) has been done to aid the promotion of soil quality parameters such as porosity, aggregate stability, water retention, and microbial diversity as well as improved nutrient availability through the addition of organic matter (Abawi and Widmer, 2000; Widmer et al. 2002). Nonetheless, organic soil amendments can be argued to be a great management alternative because it has been shown that they can decrease plant-parasitic nematode populations and other soil-borne pathogens in different cropping systems (Cole et al. 2020; Molina et al. 2014; Watson et al. 2017; Markakis et al. 2016; Tilston et al. 2002; Giotis et al. 2008; Noble and Coventry, 2010). For instance, potato fields treated with poultry manure one month before planting reduced scab and Verticillium wilt incidence to near 0 (Conn and Lazarovitz, 1998). Similarly, potato plants that were treated with composted cattle manure had 43% less *V. dahliae* incidence, compared to the control (Molina et al. 2014). In other pathosystems such as strawberries, the effectiveness of poultry manure in controlling *Fusarium* spp. and *Phytophthora* spp. was 92-100% and 67-89%, respectively (Zhang et al. 2021). As for the control of plant parasitic nematodes with soil organic amendments, poultry manure increased cucumber growth parameters and in addition, the PPN suppressive effect lasted for a longer period compared to oxamyl (Ali et al. 2022). In another study, applications of poultry manure aid in significantly reducing *Meloidogyne javanica* incidence and severity on cantaloupe as well as increasing the quality and quantity of yield (Karimipour Fard et al. 2019). On potatoes, Everts et al. 2007, showed that applications of raw or composted poultry manure can reduce *M. incognita* and *P. penetrans* populations but its effectiveness is dependent on proper incorporation into the soil, while in this same system, Cole et al. 2020 demonstrated one application of poultry manure or composted cattle and poultry manure to decrease Michigan *P. penetrans* populations and increase potato yields under field conditions.

Unfortunately, the pesticidal activity of organic soil amendments is inconsistent. For instance, a review of more than 1,000 studies showed that these products are suppressive in 45% of the cases, insignificant in 35%, and increase disease in 20% (Bonanomi et al. 2007). The literature argues that organic soil amendment effectiveness heavily depends on the nature of feedstock, composting process, compost additives, microbial communities, particle size, temperature, pH, C: N ratio, moisture, and oxygen (Martin and Ramsubhag, 2015). The inconsistency and unpredictability of such products compromise their practical use, emphasizing the need for more field studies that evaluate their variability (e.g., manure-based) in controlling detrimental plant diseases like PED.

Because of the inconsistency of sustainable management alternatives for PED control, growers often rely on chemical-based products such as Vydate® (oxamyl; Corteva Agriscience, Wilmington, DE). However, given its variable persistence length in the soil, consecutive applications are necessary to keep nematode populations under the damage threshold (Haydock et al. 2012). In contrast, it has been shown that one single application of poultry manure is highly effective at suppressing *P. penetrans* (Cole et al. 2020). Therefore, to reduce the need for constant applications of Vydate®, the response of *P. penetrans* to combined applications of poultry manure with one application of Vydate® should be evaluated to provide evidence of an effective management alternative with less pesticide input.

Another alternative to chemical-based pesticides for the management of soilborne pathogens is the use of biological control agents (BCAs). However, when comparing the effectiveness of chemical-based pesticides and BCAs, the latter often does not provide consistent control, present practical implementation issues, or there is not enough evidence of their field efficacy (Deketelaere et al. 2017; Ahmad et al. 2021). One possible approach to enhance the activity of BCAs is the addition of organic soil amendments which contain nutrients, specifically microelements, that can improve soil fertility (Noble and Conventry, 2005; Abd-Elgawad and Askary, 2018). One of the most studied microorganisms for PPN control is *Purpureocillium lilacinum* strain 251 (Syn. *Paecilomyces lilacinus*) (Luangsa-Ard et al. 2011), which is the active ingredient of the commercially available product MeloCon® by Certis USA, L.L.C (Figure 6). This fungus is effective against sedentary endoparasite nematodes like *Meloidogyne hapla*, *M. javanica*, *M. incognita*, *M. arenaria*, *M. enterolobii*, *Heterodera glycines*, *H. avenae*, *H. schachtii,* and *Globodera pallida*. Although significantly less evidence, *P. lilacinum* has also

been shown to be suppressive against migratory endoparasites like *Pratylenchus* spp. (Moreno-Gaviria et al. 2020; Constantin et al. 2022). Combined applications of *P. lilacinus* with green manures have shown significant control of *M. incognita* in pepper, while integrated applications of chicken manure and *P. lilacinus* did not show improved control of *M. incognita* in melon (Giri et al. 2020; Abdeldaym et al. 2014). This suggests that integrated management of PPNs with this strategy depends on the treatment combination, nematode, and crop, and it should be evaluated in a potato-PED system.

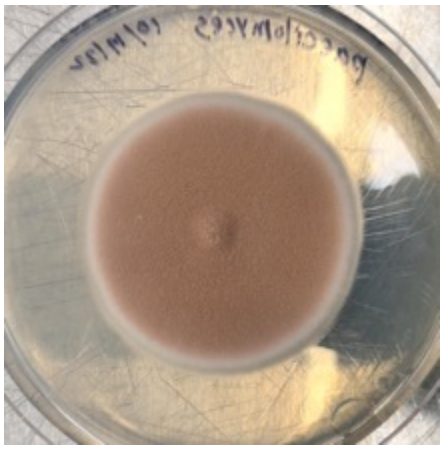

Figure 6. *Purpureocillium lilacinum* strain 251 in PDA isolated from MeloCon® formulation. (Picture taken by Luisa Parrado, 2022).

Paecilomyces lilacinum can be effective at infecting the different live stages of plant-parasitic nematodes, including eggs and juveniles through cell-wall degrading enzymes like proteases and chitinases that aid in degrading nematode eggshells (Cannayane and Sivakumar, 2001; Khan et al. 2006). Egg parasitation by this fungus occurs by the formation of appressoria, and once inside, the fungus spreads and forms conidiophores. Fungus infection of juveniles and cysts formed by *Meloidogyne* spp., *Heterodera* spp., and *Globodera* spp., can happen by hyphae entering through natural nematode openings. Still, it also secretes proteases, collagenases, and chitinases to penetrate the nematode cuticle and degrade cells (Kiewnick and Sikora, 2006). In addition, *P. licacinus* can also suppress plant-pathogenic fungi. For example, *P. lilacinum* is antagonistic to *Rhizoctonia solani*, *Sclerotina sclerotium*, *Fusarium oxysporum*, amongst others (Moreno-Gaviaria et al. 2020). Although most studies have been done in-vitro, the antagonistic effect of *P. lilacinum* is suggested to be mostly due to competition for space and nutrients, although there are other mechanisms such as mycoparasitism and antibiosis (Ahmad et al. 2021). Additionally, *P. lilacinum* has also shown evidence of promoting plant growth by increasing the

activity of polyphenols and antioxidants, which also resulted in the control of root rot fungi in okra (Baron et al. 2020; Bawa et al. 2020). There is evidence that some species such as *P. marquandii* and *P. variotii* are antagonistic to *V. dahliae*, however, not *P. lilacinum* (Moreno-Gaviria et al. 2020).

Some plant-growth-promoting bacteria (PGPB) can indirectly reduce plant pathogens infection by activating plant immune responses. One example of a PGPB is *Bacillus amyloliquefaciens* (Figure 7). This bacterium has been shown to reduce *V. dahliae* incidence in crops such as tomatoes, cotton, and olive (Pei et al. 2022; Liu et al. 2023). Literature suggests that the main mechanism by which this bacterium suppresses plant pathogens is by the secretion of metabolites such as surfactin and volatiles that induce plant systemic resistance. In contrast, the antimicrobial compounds secreted by this bacterium seemed to be of less importance (Chowdhury et al. 2015). In contrast, for PPNs suppression, perhaps antimicrobial compounds play a big role. Evidence that *B. amyloliquefaciens* can reduce *M. incognita* eggs, juveniles, and galls and increase tomato yield, was shown by Burkett-Cadena et al. 2008. Later in 2013, Liu et al. determined that this bacterium possesses a gene that encodes the production of a metabolite that has nematicidal activity considering that when they silenced this gene, the bacterium mutants did not show any nematicidal activity against *Caenorhabditis elegans*. *Bacillus amyloliquefaciens* strain D747 is the active ingredient of the commercially available product Double Nickel® by Certis USA, L.L.C, and because *P. lilacinum* and *B. amyloliquefaciens* have different antagonistic modes of action, it would be interesting to determine their combined effectiveness in controlling PED.

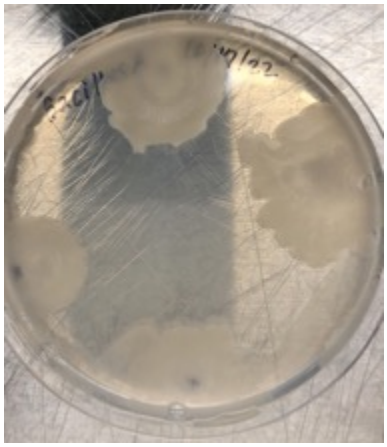

Figure 7. *Bacillus amyloliquefaciencs* strain D747 on PDA isolated from the formulation Double Nickel® (Picture taken by Luisa Parrado, 2022).

With the need for practical management alternatives for PED, the aims of this study were to 1. to determine the response of *P. penetrans*, *V. dahliae*, and potato yield to manure-based amendments that differ in feedstock, additives, and C: N ratios ("Trial 1"), and 2. to determine the response of *P. penetrans*, *V. dahliae*, and potato yield to combinations of manure-based amendments, biological control agents and Vydate® ("Trial 2").

2.2 METHODOLOGIES

Two separate field trials were conducted during the growing seasons of 2020 ("Trial 1") and 2021("Trial 2") at commercial potato fields that were naturally infested and had previous history of high incidence of PED. For both trials, manure-based amendments were sourced from Morgan Composting Inc., Sears, MI, and the composition, carbon to nitrogen ratio, nitrogen, phosphate, and potash composition can be found in Table 3 and Table 4.

Table 3. Description and nutrient composition of the manure-based amendments used in "Trial 1".

Manure-Based Amendment	Composition	Carbon:Nitrogen Ratio (C:N)	Nitrogen (TKN[1]) (kg/t)	Phosphate (P_2O_5) (kg/t)	Potash (K_2O) (kg/t)
Compost blend	Cattle and poultry manure Wood ash	8:1	21	15.3	15.4
Compost blend + Gypsum	Cattle and poultry manure Wood ash Gypsum	8:1	15.9	17.7	17.6
Raw poultry manure	Poultry manure	5:1	27.3	41.6	26.76
Composted poultry manure	Poultry manure	9.5:1	12.1	23.9	22.8
High carbon compost blend	Cattle manure Pine bark	35:1	3.7	2.72	3.7

Table 4. Description and nutrient composition of the manure-based amendments used in "Trial 2".

Manure-Based Amendment	Composition	Carbon:Nitrogen Ratio (C:N)	Nitrogen (TKN[1]) (kg/t)	Phosphate (P_2O_5) (kg/t)	Potash (K_2O) (kg/t)
Compost blend	Cattle and poultry manure Wood ash	9:1	18.3	33.5	25.8

Table 4. (cont'd)

Raw poultry manure	Poultry manure	5:1	34.2	28.7	24.6
High carbon compost blend	Cattle manure Pine bark	32:1	3.3	2.26	3.8

Variety of manure-based amendments field experiment "Trial 1"

Experimental Design

A field trial was conducted at a commercial potato farm in Three Rivers, Michigan (41.825701, -85.571021) from May to September 2020. The soil was a spinks loamy sand with 84.3% sand, 12.5% silt, 3.3% clay and 1.02% organic matter in the top 30 cm of soil (U.S. Department of Agriculture- Natural Resources Conservation Service, 2019), naturally infested with the root-lesion nematode, *Pratylenchus penetrans* (150 *P. penetrans*/100 cm^3 soil) and the soil-borne fungus *Verticillium dahliae* (5 Colony Forming Units (CFU)/g of soil).

The experimental design was a complete randomized block design with seven treatments and four replicates, for a total of 28 plots. Each plot was planted with potato (*Solanum tuberosum* cv. Norkotah Russet) due to its susceptibility to *Verticillium* spp. (Bae et al., 2007). Plots were 7.62 x 3.66 m (27.9 m^2), each with four rows with 86 cm spacing. In addition, a 1.5 m buffer was planted with cv. Dark Red Norland between plots, to prevent treatment overlap.

Poultry manure and compost A were selected based on previous results indicating that poultry manure and a compost blend (compost A) significantly reduce root-lesion nematode populations and increase yields (Cole et al. 2020). In addition, to determine the response of *P. penetrans* and *V. dahliae* to other manure-based amendments, compost A amended with gypsum, pine bark compost, and composted poultry manure were also included in this trial. Moreover, the experimental design also had a negative control (untreated) and a positive control (Vydate®) (Table 5). Forty-eight hours before planting, each manure-based amendment was applied and incorporated into the first 15 cm of soil at a rate equal to 3.08 tons/ha. At planting, Vydate® at a rate of 2.5 L/ha was applied in-furrow using a CO_2 backpack sprayer before row closure. We accounted for the extra nitrogen contributed by the amendments by applying extra urea to the plots that were not amended. Throughout the season, additional fungicides, insecticides, and herbicides were applied as necessary by the grower.

Table 5. Manure-based amendments that were selected for this field experiment that was conducted at a commercial potato production farm in Three Rivers, Michigan, with their respective composition, rate, application method, and application timing.

Treatment	Manufacturer	Active ingredient (a.i.)	Rate	Application method	Application timing
Untreated	-	-	-	-	-
Vydate®	Corteva Agriscience	Oxamyl (CAS number 23135-22-.0)	2.5 L/ha	In-furrow	At-planting
Compost A	Morgan Compost	60% chicken manure 20% dairy manure and 20% wood ash	3.08ton/ha	Incorporated 15cm deep	2 days before planting
Poultry manure	Morgan Compost	100% chicken manure	3.08ton/ha	Incorporated 15cm deep	2 days before planting
Compost A amended with Gypsum	Morgan Compost	60% chicken manure 20% dairy manure, 10% wood ash and 10% gypsum	3.08ton/ha	Incorporated 15cm deep	2 days before planting
Composted poultry manure	Morgan Compost	100% chicken manure	3.08ton/ha	Incorporated 15cm deep	2 days before planting
Compost HC	Morgan Compost	60% pine bark and 40% cattle manure	3.08ton/ha	Incorporated 15cm deep	2 days before planting

Combination of manure-based amendments trial and biological control agents field trial "Trial 2"

Experimental Design

A field trial was conducted at a commercial potato farm in Three Rivers, Michigan (41.92979, -85.56448) from May to September 2021. The soil was a spinks loamy sand with 84.3% sand, 12.5% silt, 3.3% clay and 1.02% organic matter in the top 30 cm of soil (U.S. Department of Agriculture- Natural Resources Conservation Service, 2020), naturally infested and with history of high incidence of PED. The experimental design was a complete randomized block design with 10 treatments and six replicates, for a total of 60 plots. Each plot was planted with potato cv. Norkotah Russet. Plots were 7.62 x 3.66 m (27.9 m^2), each with four rows with 86 cm spacing. In addition, a 1.5 m buffer was planted with cv. Dark Red Norland between plots, to prevent treatment overlap (Figure 8).

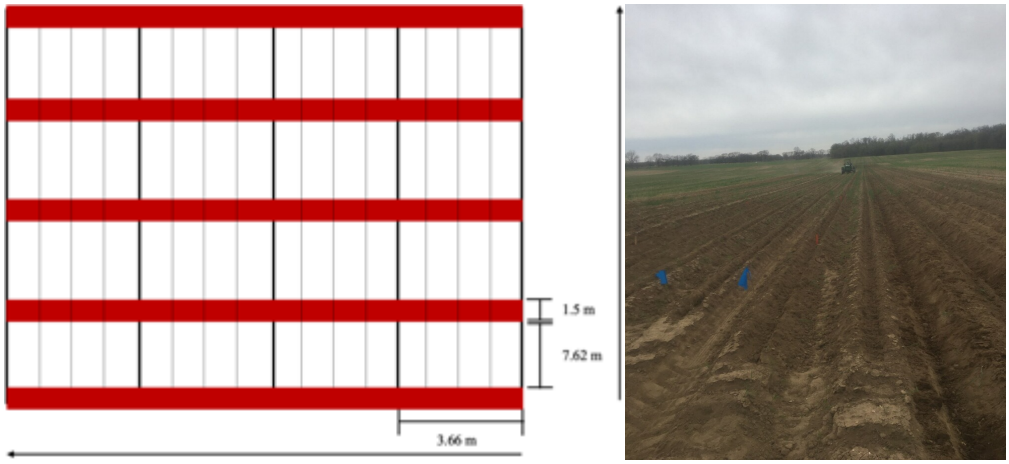

Figure 8. Visual representation of the potato field design that I used for all field experiments. Each rectangle represents a block (7.62 x 3.66 m). Each block had four rows with 86cm spacing in between. Each plot was planted with *S. tuberosum* cv. Norkotah Russet. To avoid treatment overlap, *S. tuberosum* cv. Dark Red Norland was planted between blocks on an area equal to 1.5 m, represented with the red rectangles.

Poultry manure, compost A, and pine bark-based compost were selected based on their potential to control *P. penetrans* populations, increase yields, and provide a high organic matter input. Combinations of the aforementioned amendments with *P. lilacinum*, the combination of *P. lilacinum* with *B. amyloliquefaciens*, and poultry manure and Vydate® were considered in this experiment. Moreover, we included a negative untreated control and two positive controls: the seed treatment Cruiser Maxx® (thiamethoxam and fludioxonil) and Vydate® alone (Table 6).

Table 6. Treatments to manage Potato Early Die with their respective active ingredients (AI), rate, application method, and application timing that were evaluated at a commercial potato production farm in Three Rivers, Michigan "Trial 2".

Treatment	Manufacturer	Active ingredient (a.i.)	Rate	Application method	Application timing
Untreated	-	-	-	-	-
Vydate®	Corteva Agriscience	Oxamyl (CAS number 23135-22-.0)	2.5 L/ha	In-furrow	At-planting
Cruiser Maxx® Potatoes	Syngenta US	Thiamethoxam (CAS No. 153719-23-4) fludioxonil (CAS No. 131341-86-1)	8ml/45kg of potatoes	Seed treatment	15 days before planting
Compost A	Morgan Compost	60% chicken manure 20% dairy manure and 20% wood ash	3.08ton/ha	Incorporated 15cm deep	2 days before planting
Poultry manure	Morgan Compost	100% chicken manure	3.08ton/ha	Incorporated 15cm deep	2 days before planting
Poultry manure + Vydate®	Morgan Compost	100% chicken manure	3.08 t/ha	Incorporated 15cm deep	2 days before planting
	Corteva Agriscience	Oxamyl (CAS number 23135-22-.0)	2.5 L/ha	In-furrow	At-planting
MeloCon® WG + poultry manure	Certis U.S.A. LLC	*P. lilacinum* Strain 251 (EPA registration No. 264-1175-70051	2.5 kg/ha	In-furrow	At-planting
	Morgan Compost, Sears, MI	100% poultry manure	3.08ton/ha	Incorporated 15cm deep	2 day before planting
MeloCon® WG + compost A	Certis U.S.A. LLC, Columbia, MD	*P. lilacinum* Strain 251 (EPA registration No. 264-1175-70051)	2.5 kg/ha	In-furrow	At-planting
	Morgan Compost, Sears, MI	60% chicken manure 20% dairy manure and 20% wood ash	3.08ton/ha	Incorporated 15cm deep	2 day before planting

Table 6. (cont'd)

MeloCon® WG + compost HC	Certis U.S.A. LLC, Columbia, MD	*P. lilacinum* Strain 251 (EPA registration No. 264-1175-70051)	2.5 kg/ha	In-furrow	At-planting
	Morgan Compost, Sears, MI	60% pine bark 40% cattle manure	3.08ton/ha	Incorporated 15cm deep	2 day before planting
Double Nickel 55® WDG + MeloCon® WG	Certis U.S.A. LLC, Columbia, MD	*B. amyloliquefaciens* (CAS No. 68038-60-8)	2.5 kg/ha	In-furrow	At-planting
		P. lilacinum Strain 251 (EPA registration No. 264-1175-70051)	2.13 L/ha		

Potato seeds corresponding to the seed-treated treatment were treated with Cruiser Maxx® at a rate of 8ml/45kg of potatoes, whereas the seeds corresponding to the other treatments were not treated with Cruiser Maxx®. Forty-eight hours before planting, manure-based amendments were applied and incorporated on the first 15cm of soil, at a rate equal to 3.08 tons/ha. At planting, Vydate® at a rate of 2.5 L/ha, MeloCon® WG at a rate of 2.5 kg/ha, and Double Nickel 55® WDG at a rate of 2.13 L/ha were applied in-furrow using a CO_2 backpack sprayer before row closure. We accounted for the extra nitrogen contributed by the amendments by applying extra urea to the plots that were not amended. Throughout the season, additional insecticides, and herbicides were applied as necessary by the grower.

Soil and root collection for nematode extraction and identification

Soil samples were taken before treatment application, 60 days after planting, and at harvest (100 days after planting) by randomly taking 10 soil cores from the center two rows in each plot at 15 cm depth. The soil was homogenized in a 1-gallon plastic bag and stored at 8 ºC until nematode extraction. Root-lesion nematode incidence in soil was quantified using the extraction method by Jenkins, 1964 based on elutriation and centrifugal flotation. Briefly, 100 cm^3 of soil was washed and passed through a stack of 250-µm and 25-µm sieves. Soil that was retained in the 25-µm sieve was placed into a centrifuge tube with water, followed by centrifugation at 4000 rpm for 5 min. After centrifugation, the supernatant was discarded, and the pellet of soil was left untouched. Then 40% sucrose was added, and tubes were centrifuged at 4000 rpm for 3 min. Lastly, the supernatant was passed through a 25-µm sieve to recover the nematodes which were then collected in a 10-ml glass tube for further nematode counting and identification. Morphological characteristics were observed using an inverted Nikon TMS microscope (Mai, 2018).

Root samples for *P. penetrans* extraction were collected 60 days and 90 days after planting, by randomly collecting 5 g of fine roots from ten plants from the middle two rows per plot. Root samples were placed in plastic bags and transported in a cooler to the laboratory. Once in the laboratory, roots were washed with cold water, left to dry for 2 min, and cut into 5mm pieces. Root pieces were placed in sterile plastic cups (Thermo Fisher Scientific, Hampton, NH) with 40ml of distillate water. The plastic cups were then placed in a shaker (G10 Gyratory Shaker, New Brunswick Scientific Co., Inc, New Brunswick, NJ) at 150 rpm for 72h. Subsequently, samples were then passed through a stack of 250-µm and 25-µm sieves. The nematodes on the

250-µm sieve were collected in a 10-ml glass tube for further *P. penetrans* nematode identification and quantification using an inverted Nikon TMS microscope (Figure 9).

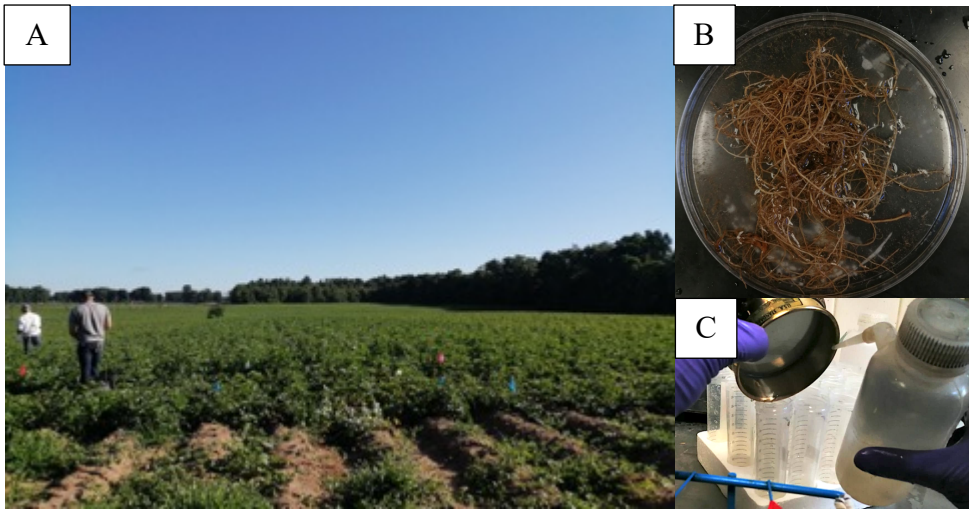

Figure 9. A. Field sampling. **B.** Roots collected from the field were washed with water for further extraction of *P. penetrans*. **C.** Extraction of *P. penetrans* from soil samples.

Stem Sampling for *Verticillium dahliae* Analysis

Potato stem samples for *V. dahliae* extraction were collected 60 days and 90 days after planting, by randomly collecting ten stems from ten plants from the middle two rows per plot. Samples were placed in paper bags and transported to the laboratory in a cooler. Samples were placed in paper bags and transported to the laboratory in a cooler. *Verticillium dahliae* incidence in stems was quantified by plating stem sections on Ethanol media (1 L distilled water 15g BD BactoTM, Thermo Fisher Scientific, Hampton, NH; 10ml of 200 proof ethanol; 0.4g streptomycin sulfate) (Nicot and Rouse 1987). From each stem, a 1 cm piece was cut, for a total of 10 pieces. The pieces were surface sterilized in 5% sodium hypochlorite solution (7.4% sodium hypochlorite concentrated Clorox® Disinfecting Bleach) for 5 min and left to dry. for 5 min and left to dry. Then, each stem piece was placed on Ethanol media and incubated at room temperature (26 ºC ± 5 ºC) for 15 days in darkness (Figure 10).

After 15 days of incubation, the number of stem portions with *Verticillium* germination characteristics such as verticillate conidiophores, white mycelium, and globose to elongate black microsclerotia arranged in a scattered pattern (Smith, 1965), were counted and the incidence of *V. dahliae* was established using the equation below, where Si corresponds to infected stems and Sn corresponds to non-infected stems:

$$\%\ of\ infection\ = (\frac{Si}{Sn}) \div 100)$$

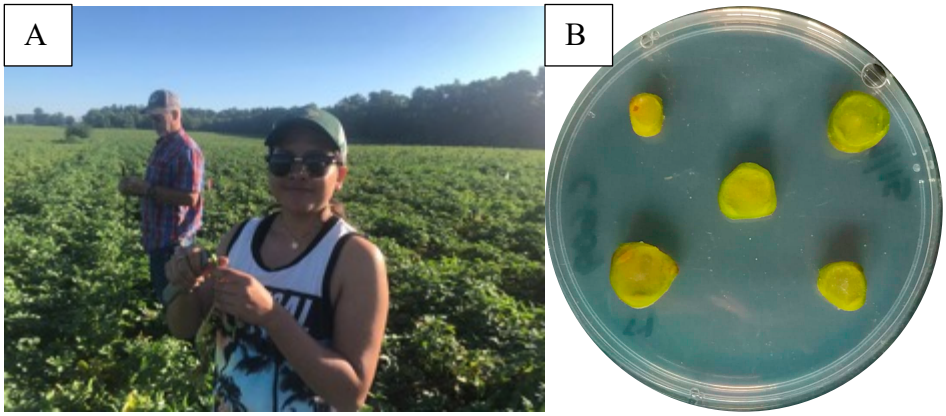

Figure 10. A. Potato stem collection at the field. **B.** Stem pieces placed on Ethanol agar.

Potato Yield and Quality

Potato yield was quantified by harvesting one row from each plot using a potato harvester 100 days after planting. Once harvested, the potatoes were brought to the Michigan State University Agronomy Farm (Lansing, MI) where they were washed, weighed, and graded. The total yield and size distribution were determined as oversized- ≥3 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). In addition, 10 tubers were randomly selected and set aside for further quality inspection. Potatoes were cut open and screened for brown center, internal brown spot, hollow heart, potato scab, and vascular discoloration (Figure 11).

Figure 11. A. Potato grading and yield quantification at the Michigan State University Agronomy Farm. **B.** Potato random pick-out for further inspection for internal defects. **C.** Vascular discoloration on potato tuber is a symptom caused by *V. dahliae* infection.

Statistical Analysis

Data collected from both field experiments were analyzed separately using RStudio version 2023 (R Core Team 2023). Response variables included *P. penetrans* counts in soil and roots, the number of stems infected with *V. dahliae*, the number of tubers with vascular discoloration, and potato yield. Treatment and sampling date were considered as the fixed effects, while replication was a random effect when included in the model.

For each data set, normality was determined with a normal quantile-quantile plot of residuals and a Shapiro-Wilk test (P-value>0.05). *Pratylenchus penetrans* abundance in soil and roots across time data were analyzed with a generalized linear mixed model (GLMM) with a Poisson distribution. Stem presence of *V. dahliae* across time and tuber presence of vascular discoloration data were analyzed with a GLMM model with a binomial distribution. Potato yield data were analyzed with a linear mixed-effects model (LMER) including replication as a random effect using the package 'lme4' (Bates et al. 2015). If treatment was determined to have a

significant effect, means separation was completed using Tukey's Honest Significant Difference (HSD) post-hoc tests (α=0.05), followed by pairwise comparisons among treatments using the packages 'emmeans', 'MASS', and 'mulcomp' (Lenth 2019; Venables and Ripley 2002; Hothorn et al. 2008). Statistically different means are represented by different letters in figures. Boxplots were developed using the package 'ggplot2' (Wickham, 2016) and letters were added manually onto graphs.

2.3 RESULTS

Root-Lesion Nematode Abundance in Soil when Treated with Different Manure-Base Amendments ("Trial 1")

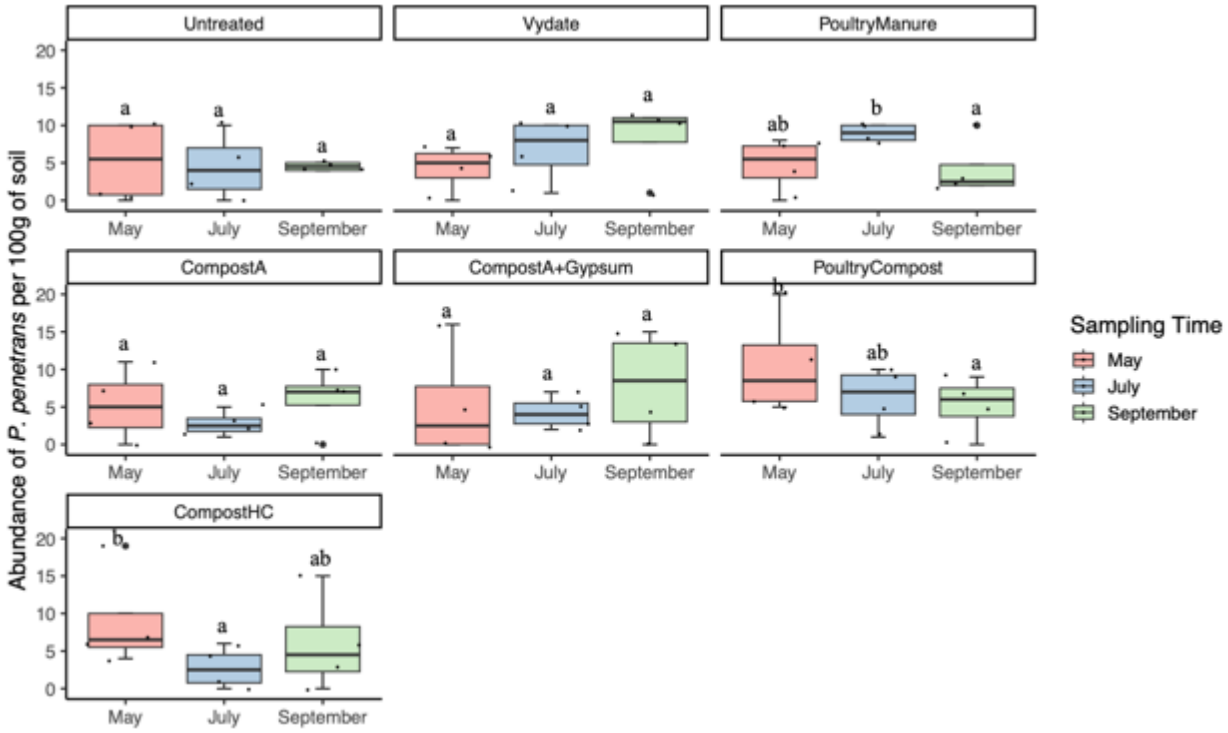

Figure 12. Standard boxplots to visualize *P. penetrans* abundance in 100g of soil determined for each of the treatments evaluated on "Trial 1", at each sampling time, which is denoted by the different colors (May, July, and September). The bold horizontal line within the box indicates the median and the error bars indicate the minimum and maximum data values. Jittered points visualize the data distribution of groups, and the bold points represent the outliers. The letters above boxplots indicate significant pairwise differences across time for each treatment (p-value<0.05).

Numerically, plots that were treated with poultry manure, poultry compost, and compost HC had a decrease of *P. penetrans* populations from May to September of 50%, 10%, and 33%,

respectively. In contrast, plots that were treated with compost A, compost A + gypsum, and Vydate® had an increase of *P. penetrans* populations from May to September of 14.2%, 52.3%, and 94.1%, respectively. Pairwise comparisons between sampling times indicated that in plots treated with compost A and compost A + gypsum, there was a slightly significant increase in nematode abundance from July to September (p-value=0.0814 and p-value=0.0883, respectively), while for Vydate® there was a slightly significant increase in nematode abundance from May to September (p-value=0.0675). For compost HC there was a significant decrease in nematode abundance in soil from May to July (p-value=0.0017), but populations slightly recovered and increased from July to September (p-value=0.0814). In contrast, nematode abundance in soil significantly decreased from May to September with both poultry manure and poultry compost (p-value=0.0257 and p-value=0.0575, respectively) (Figure 12). In September, the lowest average population was found in plots treated with 4 *P. penetrans*/100 g of soil.

Root-Lesion Nematode Abundance in Soil Response to the Combined Applications of BCAs and Manure-Base Amendments ("Trial 2")

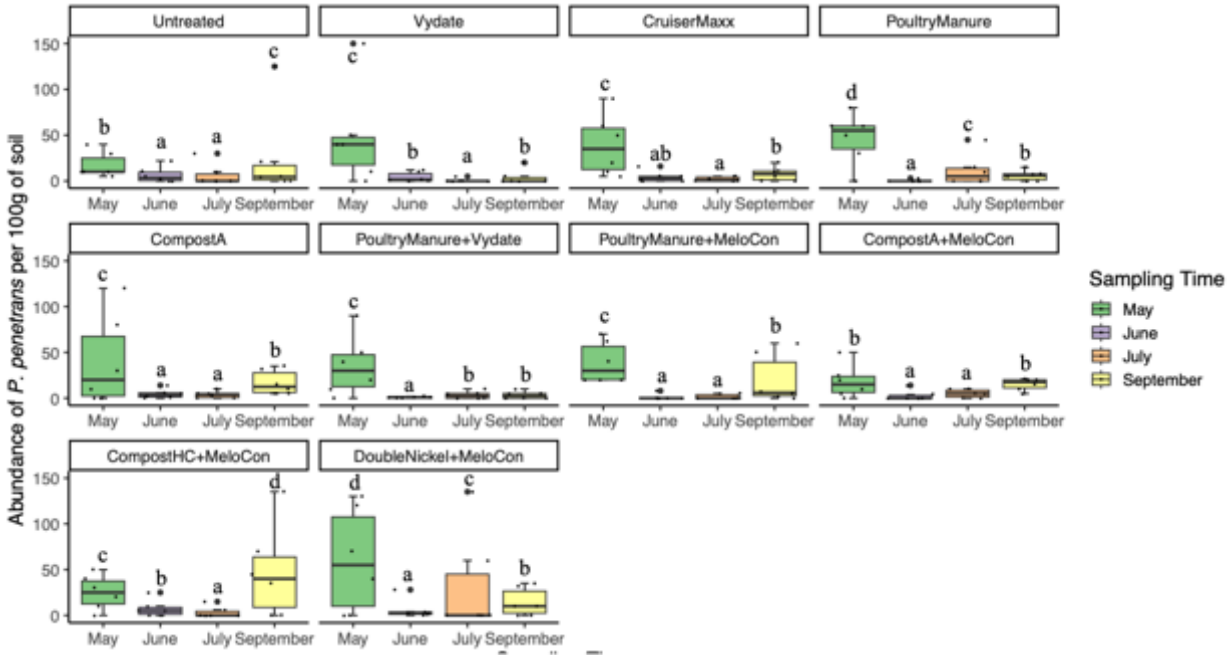

Figure 13. Standard boxplots to visualize *P. penetrans* abundance in 100g of soil determined for each of the treatments evaluated on "Trial 2", at each sampling time, which is denoted by the different colors (May, June, July, and September). The bold horizontal line within the box indicates the median and the error bars indicate the minimum and maximum data values. Jittered

Figure 13. (cont'd)

points visualize the data distribution of groups, and the bold points represent the outliers The letters above boxplots indicate significant pairwise differences across time for each treatment (p-value<0.05).

Overall, sampling time had a significant effect on *P. penetrans* populations in soil from May to September (z. value = 21.441, p-value<0.0001). Pairwise comparisons of sampling times showed that for the untreated control and compost HC + MeloCon®, *P. penetrans* abundance significantly increased over time by 47.6% and 90%, respectively (p-value<0.0001). In contrast, for the treatments Vydate®, poultry manure + Vydate®, poultry manure, Cruiser Maxx®, Double Nickel® + MeloCon®, compost A, poultry manure + MeloCon® and compost A + MeloCon® there was a significant decrease of *P. penetrans* abundance in the soil of 91%, 90.4%, 87.5%, 80.4%, 75.8%, 57.5%, 46.5% and 17.2%, respectively (p-value<0.0001). By September, the highest average number of *P. penetrans* in soil was found in the untreated control plots (26 *P. penetrans*/ 100 g of soil), while the lowest number was found in plots treated with poultry manure + Vydate® (3 *P. penetrans*/ 100 g of soil) (Figure 13).

Root-Lesion Nematode Abundance in Potato Roots When Treated with Different Manure-Based Amendments ("Trial 1")

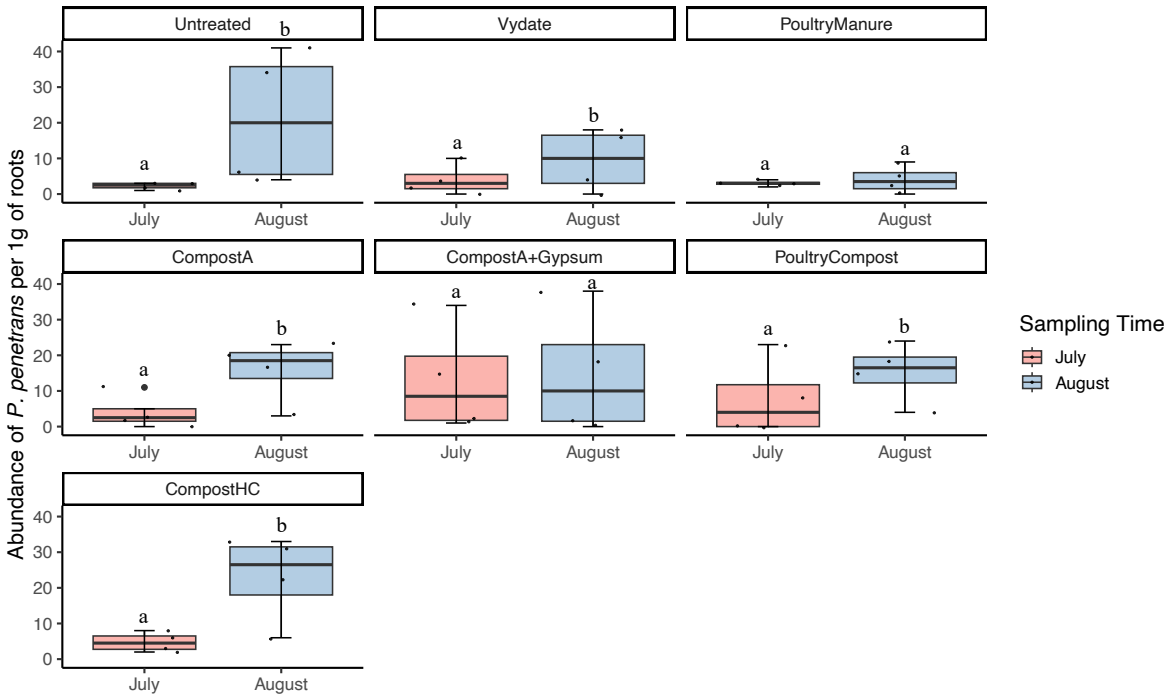

Figure 14. Standard boxplots to visualize *P. penetrans* abundance in 1g of roots determined for each of the treatments evaluated on "Trial 1", at each sampling time, which is denoted by the different colors (July and August). The bold horizontal line within the box indicates the median and the error bars indicate the minimum and maximum data values. Jittered points visualize the data distribution of groups, and the bold points represent the outliers. The letters above boxplots indicate significant pairwise differences across time for each treatment (p-value<0.05). *Pratylenchus penetrans* populations in roots were significantly impacted by sampling time (z. value = 9.329, p-value<0.0001). In most treatments, *P. penetrans* abundance in roots significantly increased over time. Pairwise comparisons among sampling times showed that in the untreated control, nematode abundance in roots in August was 9.4 times higher than in July, with an average of 21 P. *penetrans*/g of fresh root (p-value<0.0001). Similarly, in treatments Vydate®, compost A, poultry compost, and compost HC *P. penetrans* abundance in roots increased significantly over time by between 4-5 times from July to August (p-value<0.0001). In contrast, nematode abundance in the roots of plants treated with poultry manure and compost A + gypsum did not significantly increase over time (p-value=0.4513 and p-value=0.5675, respectively). The lowest increase in nematode abundance was on plants treated with poultry

manure with an average of 3 *P. penetrans*/g of fresh root in July and 4 *P. penetrans*/g of fresh root in August (Figure 14).

Root-Lesion Nematode Abundance in Potato Roots When Treated with Combined Applications of BCAs and Manure-Base Amendments ("Trial 2")

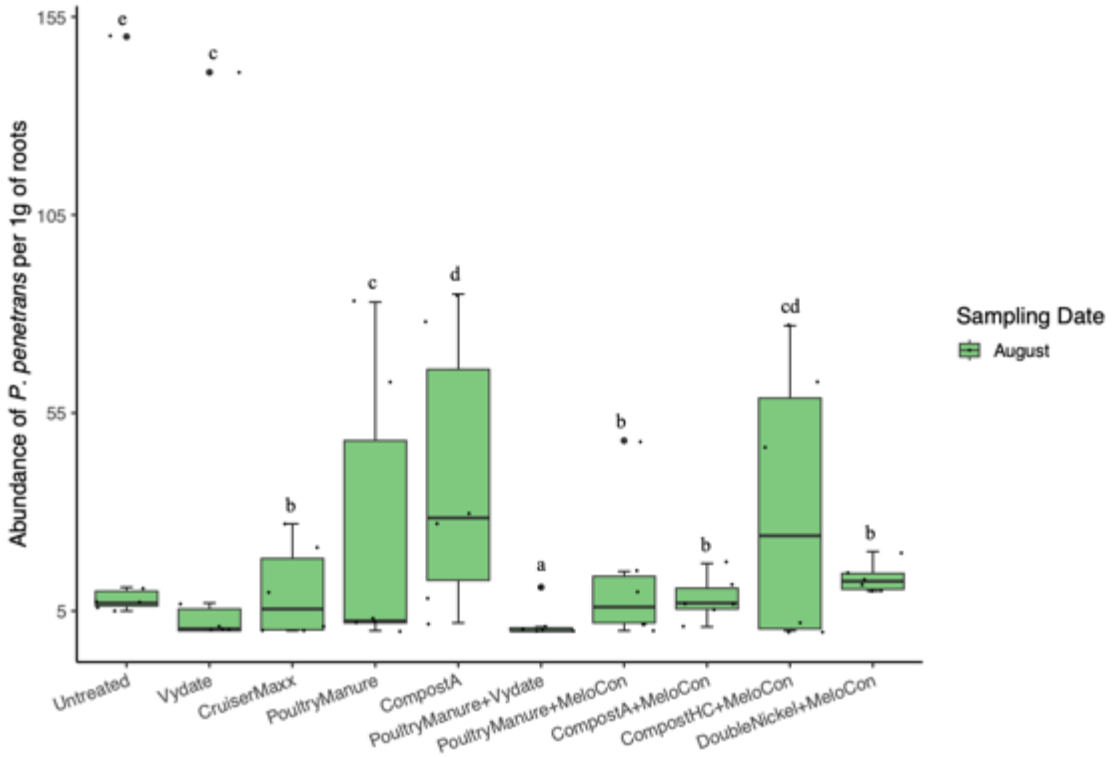

Figure 15. Standard boxplots to visualize *P. penetrans* abundance in 1g of roots determined for each of the treatments evaluated on "Trial 2", at the one sampling time (August). The bold horizontal line within the box indicates the median and the error bars indicate the minimum and maximum data values. Jittered points visualize the data distribution of groups, and the bold points represent the outliers. The letters above boxplots indicate significant pairwise differences in each treatment, compared to the untreated control (p-value<0.05)[1].

The abundance of *P. penetrans* in roots differed significantly among treatments (p-value<0.0001). In August, the highest average number of *P. penetrans* was found in roots from the untreated control plots (93 *P. penetrans*/ g of fresh root), while the lowest was found with

[1] For "Trial 2" root samples were collected in July, however, the protocol was not followed correctly, and the extraction did not work, so no nematodes were extracted, and the samples were discarded. Nevertheless, the protocol was followed correctly for the nematode extraction from the root samples collected in August, but no analysis among dates was able to be done; therefore, the data from August is the only one shown in this section.

poultry manure + Vydate® (2 *P. penetrans*/ g of fresh root) (pairwise comparison: p-value<0.0001). Interestingly, the average number of *P. penetrans* in roots from plots treated with poultry manure + Vydate® was 91.2% lower than the control, while poultry manure and Vydate® alone were 66.3% and 72.6% lower than the control, respectively. Pairwise comparisons indicated that the combined application of poultry manure and Vydate® resulted in significantly less abundance of nematodes in roots than applied alone (p-value<0.0001). Similarly, compared to the control, the combined applications of poultry manure or compost A with MeloCon® resulted in 73.3% and 89.4% fewer nematodes in the roots, respectively, while the application of poultry manure or compost A alone resulted in 66.3% and 53.8% fewer nematodes in the roots, respectively. As for the combined application of Double Nickel® and MeloCon®, it resulted in 53.8% fewer nematodes than the control (Figure 15).

Verticillium dahliae Potato Stem Infection When Treated with Different Manure-Based Amendments ("Trial 1")

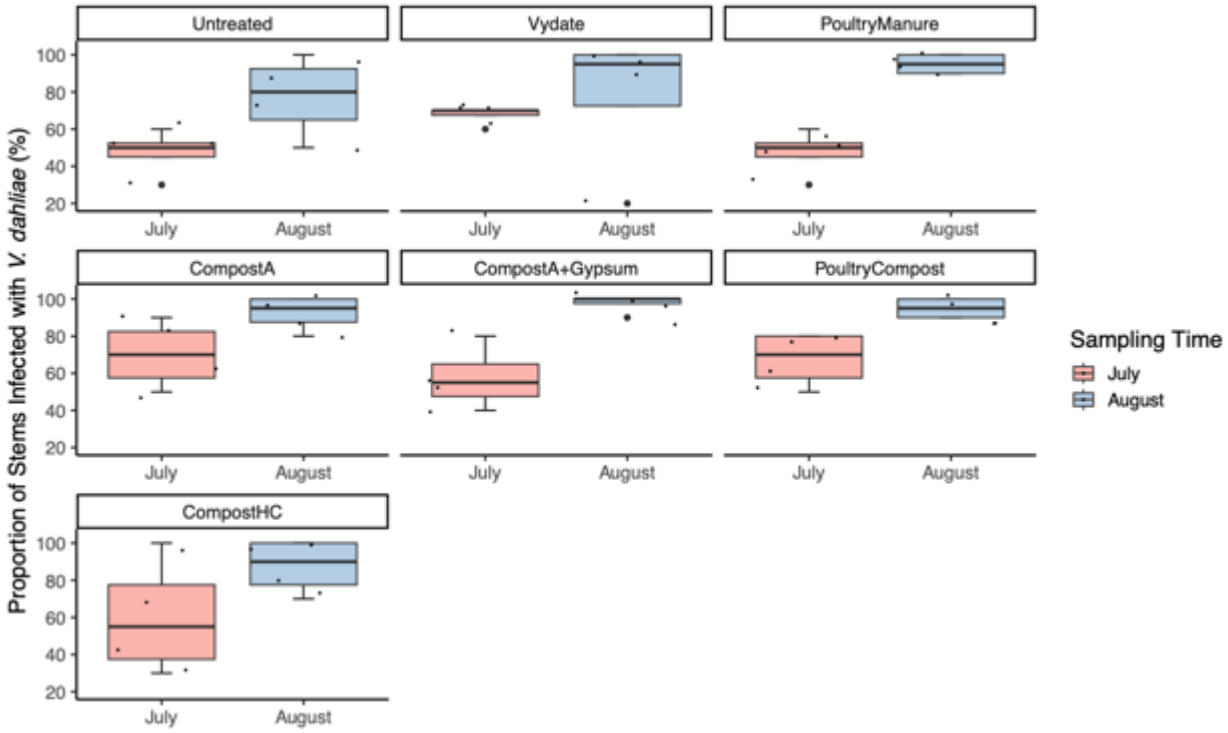

Figure 16. Standard boxplots to visualize the proportion (%) of potato stems infected with *V. dahliae* determined for each of the treatments evaluated on "Trial 1", at each sampling time, which is denoted by the different colors (July and August). The bold horizontal line within the box indicates the median and the error bars indicate the minimum and maximum data values. Jittered points visualize the data distribution of groups, and the bold points represent the outliers.

Figure 16. (cont'd)

The letters above boxplots indicate significant pairwise differences across time for each treatment (p-value<0.05).

Overall, *V. dahliae* potato stem infection significantly increased over time from July to August (z. value = 14.367, p-value<0.0001). In comparison to July infection, pathogen infection was significantly higher in August for all the treatments, except for Vydate®, in which the disease was only 1.1 times higher in August (pairwise comparison: p-value = 0.3186). In contrast, pathogen stem infection in plots treated with poultry manure had the highest increase of infection in August, being 2 times higher (pairwise comparison: p-value = 0.0001) (Figure 16).

Verticillium dahliae Potato Stem Infection When Treated with Combined Applications of BCAs and Manure-Base Amendments ("Trial 2")

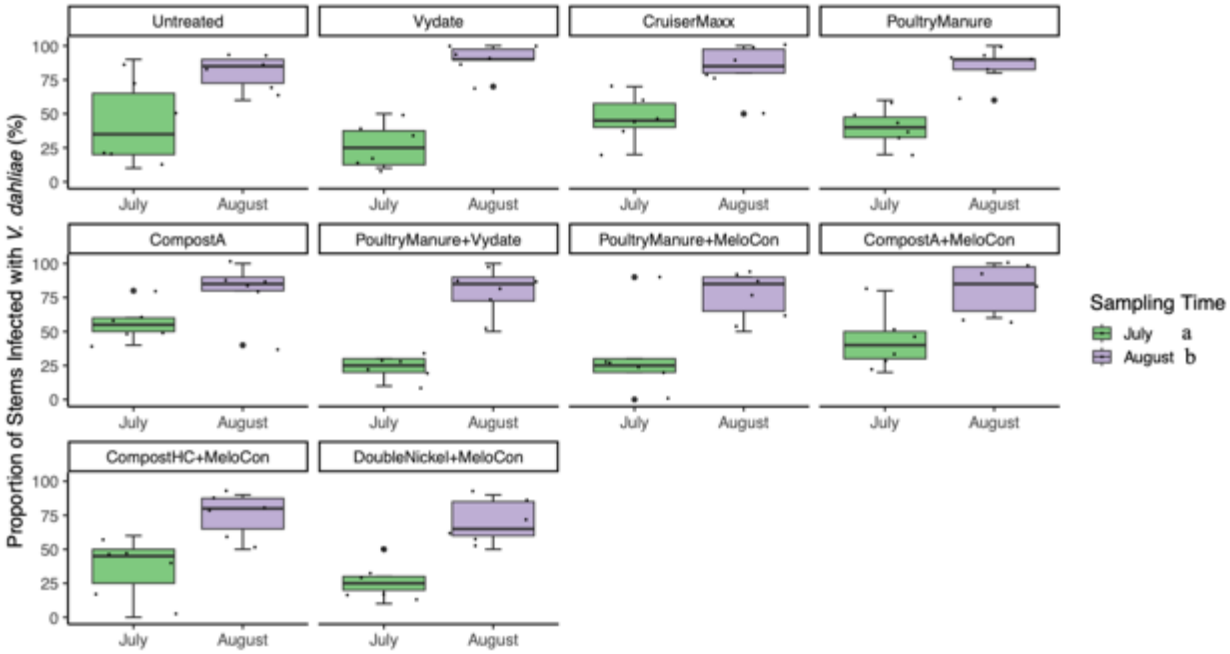

Figure 17. Standard boxplots to visualize the proportion (%) of potato stems infected with *V. dahliae* determined for each of the treatments evaluated on "Trial 2", at each sampling time, which is denoted by the different colors (July and August). The bold horizontal line within the box indicates the median and the error bars indicate the minimum and maximum data values. Jittered points visualize the data distribution of groups, and the bold points represent the outliers. The letters above boxplots indicate significant pairwise differences across time for each treatment (p-value<0.05).

Verticillium dahliae potato stem infection significantly increased over time from July to August, regardless of treatment (z. value = 14.367, p-value<0.0001). The increase in pathogen infection from July to August varied in certain treatments. For example, the highest increase of *V. dahliae* stem infection was found in plots treated with poultry manure + Vydate® and the control Vydate®, with 3 times more infection in August than in July. In contrast, the lowest disease increase was on plots treated with compost A, with just 1.4 times more infection in August than in July (Figure 17).

Potato Yield When Treated with Different Manure-Based Amendments ("Trial 1")

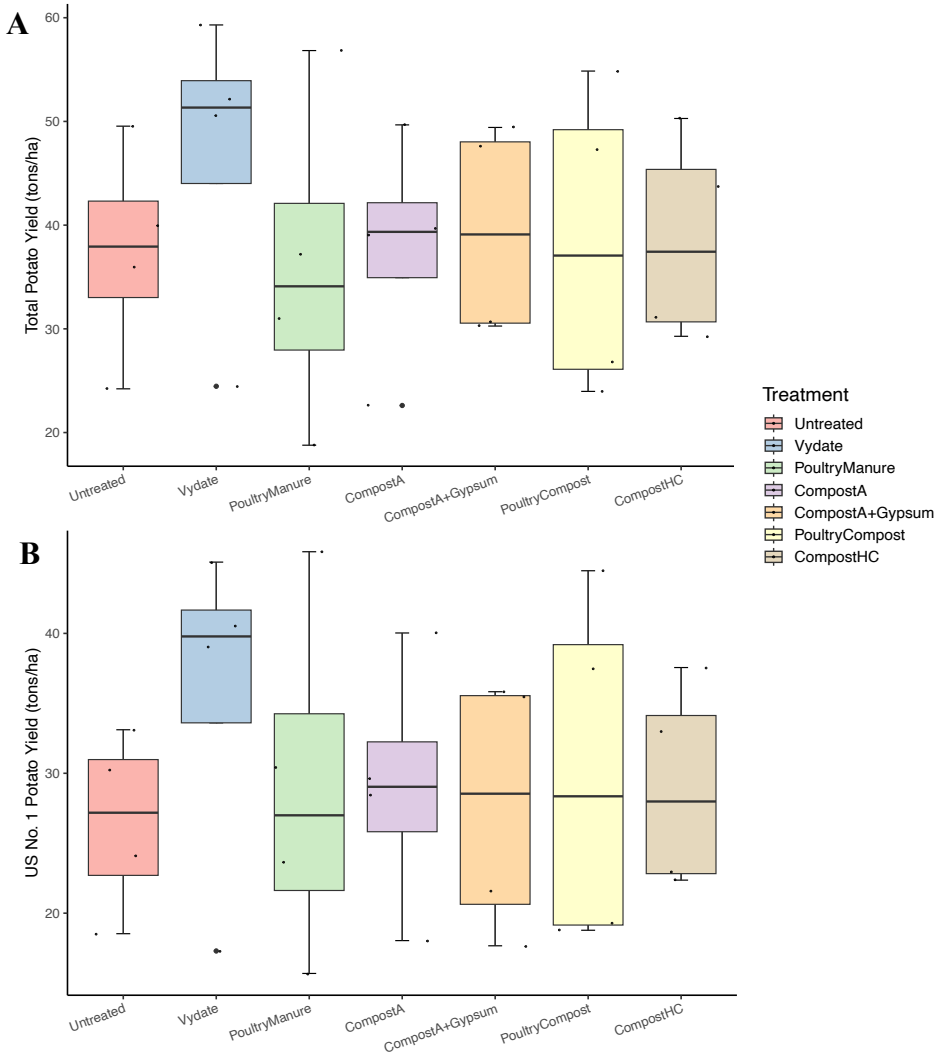

Figure 18. Standard boxplots to visualize the potato yield determined for each of the treatments evaluated on "Trial 1", denoted by the different colors. **A.** Total potato yield (all sizes) in tons per hectare. **B.** Total potato yield of tubers US. No. 1 in tons per hectare. The bold horizontal line within the box indicates the median and the error bars indicate the minimum and maximum data

Figure 18. (cont'd)

values. Jittered points visualize the data distribution of groups, and the bold points represent the outliers. The letters above boxplots indicate significant pairwise differences for each treatment compared to the untreated control (p-value<0.05).

Compared to the null model, no statistically significant overall treatment effect was found on total yield (DF = 6, F-value$_{(6,18.156)}$ = 1.145, *P-value* = 0.3778), nor US. No. 1 (DF = 6, F-value$_{(6,18.165)}$ = 1.1017, *P-value* = 0.3988). Numerically, Vydate® had the total yield (average = 46.6 tons/ha), and US No. 1 (average = 35.49 tons/ha). In comparison, the untreated control had the lowest yield on average, with 37 tons/ha of total yield, and 26.5 tons/ha of US No. 1 potatoes (Figure 18).

Potato Yield When Treated with Combined Applications of BCAs and Manure-Base Amendments ("Trial 2")

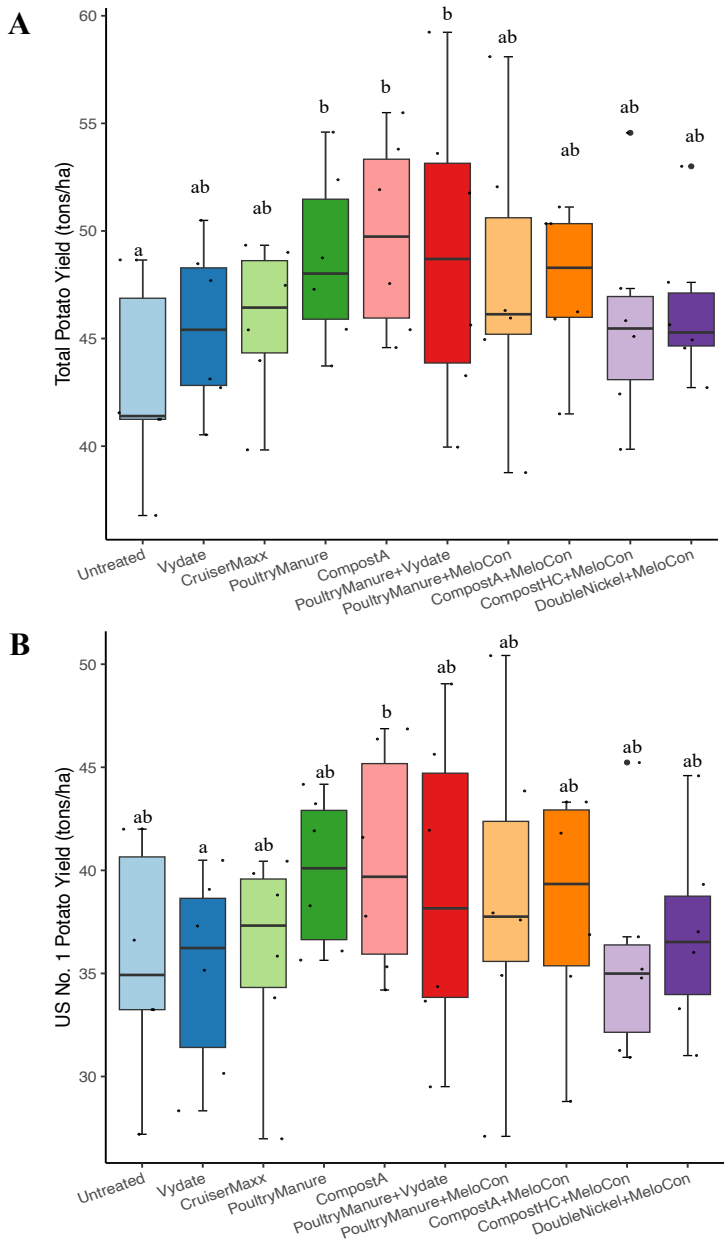

Figure 19. Standard boxplots to visualize the potato yield determined for each of the treatments evaluated on "Trial 1", denoted by the different colors. **A.** Total potato yield (all sizes) in tons per hectare. **B.** Total potato yield of tubers US. No. 1 in tons per hectare. The bold horizontal line within the box indicates the median and the error bars indicate the minimum and maximum data values. Jittered points visualize the data distribution of groups, and the bold points represent the

Figure 19. (cont'd)

outliers. The letters above boxplots indicate significant pairwise differences for each treatment compared to the untreated control (p-value<0.05).

Compared to the null model, treatment had an overall significant effect on total yield (DF = 9, F-value$_{(9,42)}$ = 1.4336, *P-value* = 0.03778), but not on US No. 1 yield (DF = 9, F-value$_{(9,42)}$ = 1.14, *P-value* = 0.3557). The lowest total yield was found on the untreated control plots (43 tons/ha), while the highest was found on plots treated with compost A alone (49.8 tons/ha), increasing total yield by 15.7% (pairwise comparison: p-value=0.0006). In contrast, Vydate® alone resulted in the second lowest total yield with an average of 45.5 tons/ha, while combined applications of poultry manure and Vydate® resulted in the second highest yield with an average of 49 tons/ha (7.7% more than Vydate® alone) (Figure 19A). Numerically, the lowest average marketable yield was found on plots treated with Vydate® with an average of 35 tons/ha, while the highest was found on compost A with an average of 40.4 tons/ha (pairwise comparison: p-value=0.0421(Figure 19B).

<u>Potato Internal Vascular Discoloration When Treated with Different Manure-Based Amendments ("Trial 1")</u>

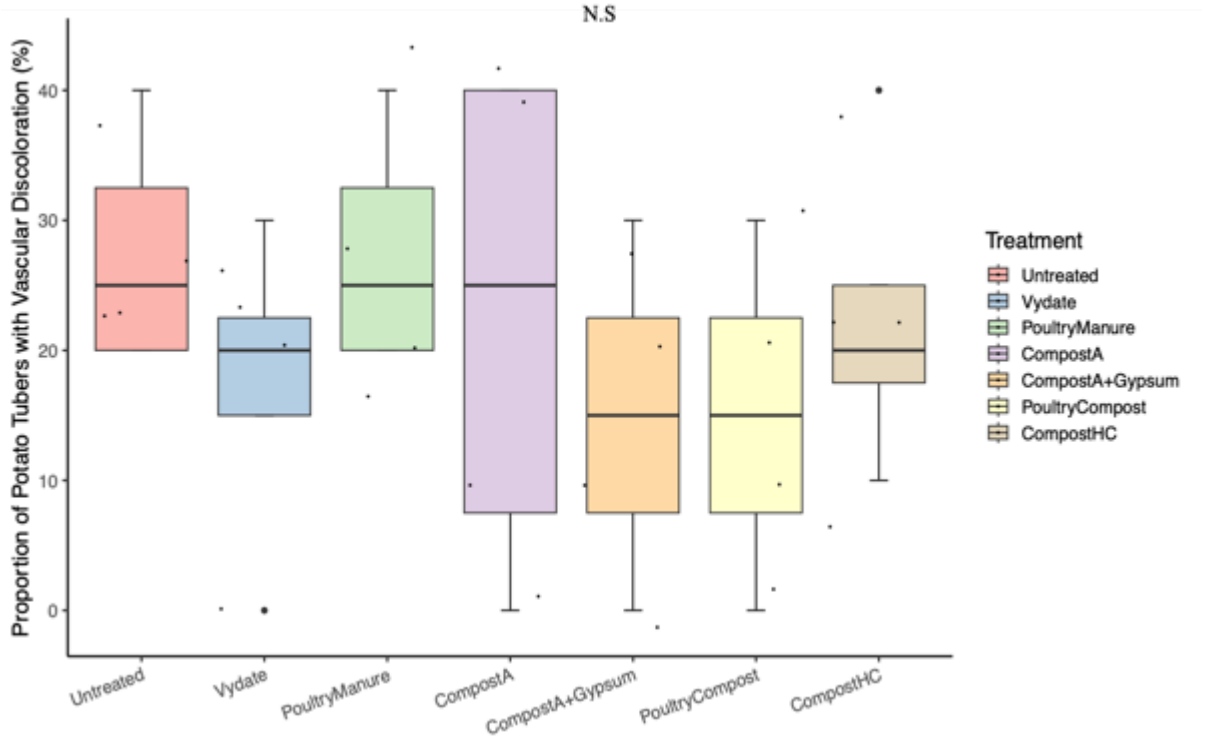

Figure 20. Standard boxplots to visualize the proportion of potato tubers exhibiting symptoms of vascular discoloration caused by *V. dahliae* for "Trial 1". The bold horizontal line within the box

Figure 20. (cont'd)

indicates the median and the error bars indicate the minimum and maximum data values. Jittered points visualize the data distribution of groups, and the bold points represent the outliers. The letters above boxplots indicate significant pairwise differences for each treatment compared to the untreated control(p-value<0.05).

There was no statistically significant effect of treatment on tuber vascular discoloration caused by *V. dahliae* (p-value>0.05). However, the average proportion of tubers with vascular discoloration was higher in the untreated control (27.5% (n=10)), compared to poultry compost (15% (n=10)) (Figure 20).

Potato Internal Vascular Discoloration When Treated with Combined Applications of BCAs and Manure-Base Amendments ("Trial 2")

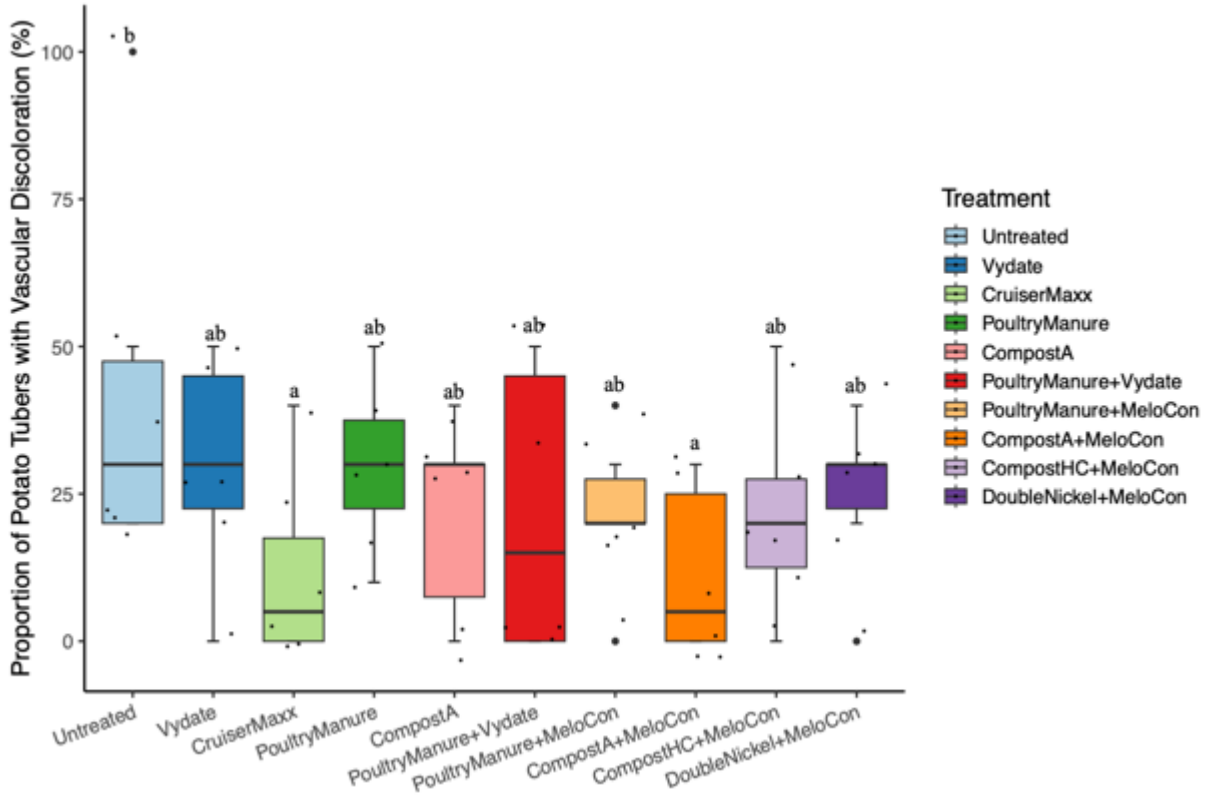

Figure 21. Standard boxplots to visualize the proportion (%) of potato tubers exhibiting symptoms of vascular discoloration caused by *V. dahliae* for "Trial 2". The bold horizontal line within the box indicates the median and the error bars indicate the minimum and maximum data values. Jittered points visualize the data distribution of groups, and bold points represent outliers.

Figure 21. (cont'd)

The letters above boxplots indicate significant pairwise differences for each treatment compared to the untreated control (p-value<0.05).

Lastly, tuber vascular discoloration was the highest on the tubers evaluated from the untreated control where an average of 41.7% of the evaluated tubers showed symptoms. In contrast, of the tubers evaluated from the compost A + MeloCon® and Cruiser Maxx®, only 11.7% had symptoms and pairwise comparisons showed that these treatments were significantly different from the untreated control (p-value=0.0158). As with the other treatments, between 21.6% and 30% of tubers showed vascular discoloration symptoms (Figure 21).

2.4 DISCUSSION

Literature suggests that control of pathogens with organic soil amendments is dependent on its characteristics, crop system, and ultimately on the changes they cause to the soil, resulting in an inconsistent and unpredictable effectivity to control plant pathogens (Oka, 2010; Rosskopf et al. 2020). In my first trial, I found that raw and composted poultry manure significantly lowered *P. penetrans* in soil over a season, with raw poultry manure also significantly maintaining nematode abundance low within the roots. Everts et al. 2005 reported that raw and composted poultry manure lowers root-lesion nematode numbers, yet the effect did not extend to later seasons. In another study, in infested carrot fields, raw poultry manure notably decreased *Meloidogyne* spp. populations, root galling, and increased yields (Kankam et al. 2014). My findings suggest that raw poultry manures' ability to reduce nematodes and limit reproduction within the root merits further study along with its potential long-term effects.

The other amendments compost A and compost A with gypsum were evaluated to determine if gypsum had any beneficial effect on pathogen control or yield. For instance, it was shown that gypsum-amended avocado soil can reduce sporangial production of *Phytophthora cinnamomi* (Messenger et al. 2000). The results from my trial showed that with compost A *P. penetrans* populations increased in soil, while with compost A with gypsum abundance in roots remained unchanged. However, the lack of research about gypsum-amended soil on root-lesion nematode reproduction within plant roots warrants further investigation. In addition, the literature suggests that applications of raw cattle manure can reduce plant parasitic nematodes more than composted (Nahar et al. 2006), which supports what was observed in my trial with composted cattle manure and raw poultry manure.

In the second field trial, the combination of poultry manure with Vydate® significantly reduced *P. penetrans* soil populations in soil and root infestation. In contrast to our findings, Osman et al. 2018, reported that poultry manure and oxamyl (the active ingredient of Vydate®) were less effective against *M. incognita* in eggplant when combined than when applied separately, potentially due to organic matter absorbing the nematicide's toxic components. As for the activity of oxamyl, Wright et al. 1980 found that oxamyl moves to cucumber plant areas under nematode attack, preventing root invasion, and concentrates at the root surface after foliar application. My findings suggest that Vydate®'s systemic action, combined with raw poultry manure's nematicidal effects, could more effectively limit nematode reproduction in roots, however, the nematicidal effects of poultry manure need further exploration.

Additionally, I hypothesized that adding manure-based amendments with MeloCon® would improve *P. penetrans* management. Poultry manure or Compost A with MeloCon® decreased *P. penetrans* abundance in soil by 46.5% and 17.2%, respectively, with an even greater reduction in roots (89.4% and 73.3%, respectively). In contrast, poultry manure and compost A alone resulted in 66.3% and 53.8% fewer nematodes in the roots. Likewise, Bawa et al. 2020, found that the enrichment of neem cake and farmyard manure with *P. lilacinum* was effective at reducing *M. incognita* damage to bell peppers. In another study, the combined application of cattle manure with *P. lilacinum* reduced root galling by *Meloidogyne* spp. and reproduction in tomatoes (Siddiqui and Futai, 2009). In my study, Compost HC with MeloCon® increased *P. penetrans* by 90%, potentially because of the nutrient imbalance and complex carbon content in pine bark, which could limit *P. lilacinum* growth (Gao et al. 2007; Valentin et al. 2010; Sun and Liu ,2006). My research provides compelling evidence that combined applications of manure-based amendments with *P. lilacinum* can lead to greater control of *P. penetrans* in potatoes, though amendment choice is critical.

Another one of my hypotheses was that combined applications of *P. lilacinum* and *B. amyloliquefaciens* could lead to greater nematode control given their different antagonistic modes of action. *Purpureocillium lilacinum* possesses hydrolytic enzymes that are required to degrade nematode-egg shell components (Kahn et al. 2004), while *B. amyloliquefaciens* induces plant systemic resistance (Chowdhury et al. 2015). Our study found that using both agents reduced *P. penetrans* in soil by 75.8% and root infestations by 53.8%. Similarly, de Paula et al., 2024 reported that co-inoculating cucumber roots with these agents significantly reduced *M.*

enterolobii eggs and galling, unlike separate inoculations. However, because we did not evaluate applications of the biological control agents alone, we cannot confirm that the combined application effect is greater.

Overall, treatments did not decrease *V. dahliae* potato stem infection throughout the two sampling times, instead, there was an increase. Cocozza et al. 2021 found that livestock manures-compost can be effective at suppressing *V. dahliae* but is dependent on low C: N ratio and low quantity of unsaturated fatty acids. In addition, ammonia and nitrous acid released from nitrogenous amendments were found to cause the highest mortality of *V. dahliae* microsclerotia (Tenuta and Lazarovits, 2001). In my study, although raw poultry manure had the lowest C:N ratio and the highest content of nitrogen and ammonium, it may have not provided the right nitrogen forms, or the soil conditions did not allow the transformation of nitrogen compounds to *V. dahliae* toxic forms.

As for potato yield, compost A increased total and marketable yield in both studies. This agrees with some literature where in a 7-year study conducted by Kimpinski et al. 2003, the addition of a compost blend that included cattle manure, increased potato yields by 27%. In another study, potato yields increased significantly after applications of raw or composted cattle manure, without affecting tuber quality (Moore et al. 2011). Although compost A did not significantly control PED, its yield impact suggests it may enhance soil nutrient content, warranting further investigation. As for tuber vascular discoloration, the combined application of compost A and MeloCon® resulted in the lowest proportion of tubers with this disease. It was shown in a recent study that 11 weeks after inoculation *V. dahliae* was detected in the stolon and the newly produced tubers (Zhang et al. 2023). In our study, the no reduction of *V. dahliae* stem infection, but less tuber vascular discoloration may suggest a possible limitation of *V. dahliae* transfer to progeny tubers but would require further study.

2.5 CONCLUSION

These results suggest that there are alternative methods to chemical-based products for *P. penetrans* control. Raw poultry manure at a rate of 3.08 tons/ha or combining it with one application of Vydate® at a rate of 2.5 L/ha, are the most effective methods. Another option is to use manure-based amendments with MeloCon®, but the efficacy varies by amendment. In addition, combining MeloCon® and Double Nickel® moderately controls *P. penetrans* under field conditions, though individual effects and their biocontrol mechanisms need more research.

Soil amended with compost A resulted in higher yields, but it did not control *P. penetrans* significantly. Therefore, the selection of manure-based amendments should consider their potential for reducing *P. penetrans* or enhancing yields. Disease complexes like PED are understudied and hence challenging to manage, but the evidence provided by these studies about management alternatives for *P. penetrans* is essential due to its important role in the PED disease complex.

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CHAPTER 3: MANAGEMENT OF POTATO EARLY DIE WITH NON-FUMIGANT ALTERNATIVES: NEMATICIDES, FUNGICIDES, AND SEED TREATMENTS

Luisa M. Parrado, Emilie Cole, and Marisol Quintanilla

3.1 INTRODUCTION

Metam sodium is the most used soil fumigant in agriculture and is registered on food and feed crops. This pesticide is a non-selective fumigant that is effective against fungi, nematodes, insects, and weeds. Metam sodium is of liquid form but is converted into methyl isothiocyanate (MITC), carbon disulfide, and hydrogen sulfide; yet MITC is the one with the pesticidal activity. Metam sodium can be applied by shank injection, rotary tiller, center pivot, sprinkler, drip, flood basin, furrow, and border. For metam sodium to be effective, several environmental parameters must be considered such as the wind conditions must be at a minimum of 2mph and 5 mph, the soil must be in good tilth and free of large clods as well as no crop residue present on the soil surface, the soil temperature must be maximum 90˚F and the soil moisture of the top 6" must be between 60% and 80%. Metam sodium conversion into MITC happens between 3 to 4 hours and its biodegradation increases as soil temperature and moisture increase, therefore such conditions are crucial parameters to consider when applying metam sodium, not only to ensure the effectiveness of the product but also to prevent unwanted exposure to the chemical (U.S. EPA, 2013).

Soil fumigation is a lengthy process that requires highly trained pesticide applicators and specialized materials and equipment. In addition, the amount of metam sodium needed per acre ranges between 40 to 60 gallons, costing $300 per acre, and an input of around 170 lb. of active ingredient applied per acre (Miller, J. 2019). Consequently, soil fumigation is not a sustainable long-term pest management approach, and growers need management alternatives. The most effective management approach is one that involves a combination of multiple methods, also known as Integrated Pest Management (IPM). For instance, currently, the top practices in potato pest management are treating seeds after purchase, cleaning materials and equipment to avoid the spread of inoculum, implementing at minimum a 3-year crop rotation, scouting for diseases, and using pesticides with different modes of action (NASS, 2022). Similar practices are used to prevent PED such as planting resistant varieties, implementing a 5-year crop rotation, and maintaining a balance of soil nutrients and moisture, but soil fumigation is the only method that can reduce PED soil inoculum before planting (Stark, 2020). Nevertheless, there are a handful of

non-fumigant fungicides and nematicides that if proven to be effective in reducing PED inoculum and detrimental impacts to potato yields, then soil fumigation could be replaced by these safer non-fumigant alternatives.

Some of the chemistries that may aid *Verticillium* control include penfluflen and prothioconazole, azoxystrobin, and fluopyram and pyrimethanil. Penflufen and prothioconazole are formulated into a product with the commercial name Emesto Silver®, which can be used as a potato seed treatment. Penfuflen (FRAC code 7) is a systemic, xylem-mobile fungicide that belongs to the pyrazole-4-carboxamides, and it targets mitochondrial respiration by blocking the succinate-dehydrogenase complex, while prothioconazole (FRAC code 3) is also a systemic fungicide that belongs to the triazolinthiones group and targets the biosynthesis of important components of fungal cell walls. Similarly, fluopyram and pyrimethanil are formulated as a product with the commercial name Luna® Tranquility. Fluopyram (FRAC code 7) is a systemic fungicide that has protectant and curative properties. It belongs to the chemical group pyridinyl-ethyl-benzamides, and it targets mitochondrial respiration by blocking the succinate-dehydrogenase complex, while pyrimethanil (FRAC code 9) is a broad-spectrum fungicide that belongs to the group anilino-pyrimidines, and it inhibits the secretions of fungal enzymes related to pathogenicity. Azoxystrobin (FRAC code 11) is a broad-spectrum systemic fungicide in the group methoxy acrylates which targets mitochondrial respiration by blocking electron transfer between cytochromes b and c1, ultimately preventing the generation of ATP and it is registered under the commercial name Quadris®.

Other fungicides that may be effective against *V. dahliae* are fludioxonil and flutolanil. Fludioxonil is a non-systemic, broad-spectrum fungicide and it has been demonstrated that this compound activates the HOG pathway by suppressing the activity of III HHK. These proteins are sensors to stress molecules like ROS, nitric oxide (NO), and aldehydic stressors, so it is believed that fludioxonil induces the production of these stress molecules in the pathogen (Brandhorts and Klein, 2019). For *Sclerotinia sclerotium*, it was found that fludioxonil treatment of oilseed rape was more protective rather than curative, whether it was applied on leaves or stems (Duan et al. 2013). In another study, it was found that combined applications of abamectin and fludioxonil were effective in reducing populations of *Meloidogyne incognita* and *Fusarium oxysporum*, and also, increased cucumber yields (Shi et al., 2019). In potatoes, fludioxonil is formulated as a seed treatment for tuber black scurf caused by *Rhizoctonia solani* but can also be

combined with other chemistries for the control of potato storage diseases like Fusarium dry rot and silver scurf (Budde-Rodriguez et al. 2022). As for flutolanil, this fungicide is part of the succinate dehydrogenase inhibitor group, and its antifungal effect is based on the disruption of the Krebs cycle and mitochondrial electron transportation chain (Zhao et al. 2019). In potatoes, combined applications of *Trichoderma harzianum* with flutolanil as a seed treatment provided the best protection against *R. solani* during the growing season (Wilson et al. 2008). However, no extensive research has been done on these fungicides for potatoes and PED.

Conversely, the most used chemistry for plant-parasitic nematode control is oxamyl (IRAC code N-1A). Oxamyl belongs to the carbamates chemical group, it acts as a neurotoxicant that inhibits acetylcholinesterase, and it is the active ingredient of a nematicide product commercially known as Vydate® L. This chemical acts as a systemic nematicide that requires contact with the nematode to be effective, and although very effective, this nematicide is of restricted use and overexposure of humans and animals can even cause death (Costa et la. 2008). In contrast, other compounds with nematicidal activity that are safer and perhaps just as effective, are spirotetramat, fluensulfone, fluopyram, and fluazaindolizine (Deseager et al. 2020) Spirotetramat (IRAC code N-4) is the active ingredient of Movento®, which is a systemic nematicide that can translocate within the xylem and the phloem, and to be toxic to nematodes it must be transformed in the plant to its spirotetramat-enol form (Vang et al. 2016). This compound inhibits the acetyl-CoA carboxylase activity, lipids synthesis, and fatty acid composition, and disrupts cuticle synthesis (Gutbrod et al., 2018). As fluensulfone (IRAC code N-UN), it was the first reduced-risk nematicidal compound registered in 2014 and it's the active ingredient of a product known as Nimitz®. This compound belongs to the group 1,3-thiazoles and it targets lipids and cell viability (Kearn et al., 2014; Kearn et al., 2017). Similarly, fluazaindolizine (IRAC code N-UN) is the newest nematicidal compound registered, being the active ingredient of Salibro® Recklemel®. Fluazaindolizine belongs to the chemical group imidazopyridines, however, its mode of action remains undiscovered. Lastly, fluopyram is the active ingredient of Velum® Prime, as it is registered for nematode control. It has been shown that just as in fungi, fluopyram is an inhibitor of the succinate dehydrogenase, and it can act as a nemastitic and nematicide for motile stages, but it is a poor ovicide (Wram and Zasada, 2020).

Although soil fumigation is the industry standard to reduce *V. dahliae* and *P. penetrans* soil inoculum, it is a practice that is currently highly restricted (U.S. EPA, 2024). The existence of

safer and more selective alternatives can potentially be adopted in pest management programs to replace soil fumigation, however, how effective they can be in reducing PED inoculum and damage, and in increasing crop yield under field conditions needs to be evaluated. Therefore, the main goal of this chapter was to determine the effect of various combinations of seed treatments, nematicides, fungicides, and seed treatments alone on PED, yield, and yield quality.

3.2 METHODOLOGIES

One field trial was conducted during the potato growing season of 2021. This trial was designed based on multiple combined applications of seed treatments, non-fumigant nematicides, and fungicides, or seed treatments alone. The field trial was established during the growing season of 2021 at a commercial potato field located in South-West Michigan (41.825701, -85.571021). The soil was a spinks loamy sand with 84.3% sand, 12.5% silt, 3.3% clay, and 1.02% organic matter in the top 30 cm of soil (U.S. Department of Agriculture- Natural Resources Conservation Service, 2020), naturally infested and with history of high incidence of PED.

The experimental design was a complete randomized block design. Fifteen treatments in total were included in the design, each with four replicates for a total of 60 plots (Table 8). Each plot was planted with potato cv. Norkotah Russet due to its susceptibility to *Verticillium* spp. (Atallah et al., 2007). Plots were 7.62 x 3.66 m (27.9 m^2), each with four rows with 86 cm spacing. In addition, a 1.5 m buffer was planted with potato cv. Dark Red Norland between plots, to prevent treatment overlap (Figure 22).

Depending on the treatment, fifteen days before planting the corresponding potato seed pieces were treated with either penflufen and prothioconazole (Emesto® Silver, 9.2 ml / 45 kg of potatoes), thiamethoxam and fludioxonil (Cruiser Maxx®, 8ml/45kg of potatoes), or the seed treatments “Potato IF”, “Potato IFN”, “Potato IFN2” and “Spirato 480 FS”, following the label and experimental protocols of each product provided by the respective company. The seed pieces corresponding to the other treatments were not treated with any seed treatment. At planting, the corresponding treatments were applied in-furrow using a backpack sprayer, and the follow-up applications were done by soil drenching as described in Table 7. All treatments were applied following the label and experimental protocols of each product provided by the respective company.

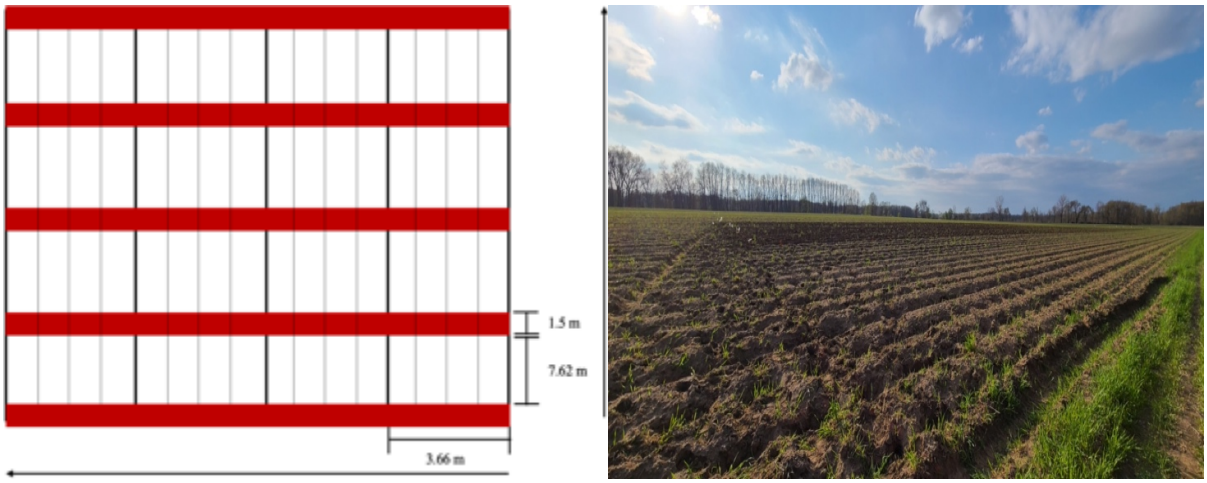

Figure 22. Visual representation of the potato field design that I used for all field experiments. Each rectangle represents a block (7.62 x 3.66 m). Each block had four rows with 86cm spacing in between. Each plot was planted with *Solanum tuberosum* cv. Norkotah Russet. To avoid treatment overlap, *Solanum tuberosum* cv. Dark Red Norland was planted between blocks on an area equal to 1.5 m, represented with the red rectangles.

Table 7. Generic name, active ingredient, chemical group, mode of action, FRAC/IRAC code, rate, and method and time of application for treatments evaluated for Potato Early Die control during the potato growing season of 2021 in a commercial potato field located at 41.825701, -85.571021.

Treatment	Active Ingredient	Chemical Group	Mode of Action	FRAC\IRAC code	Rate	Application Time
Untreated	-	-	-	-	-	-
Emesto® Silver 118 FS	Penflufen	Sterol biosynthesis in membranes	Demethylation inhibitors	3	9.2 mL / 45 kg of potato seeds	Seed treatment
	Prothioconazole	Respiration	Complex II: succinate-dehydrogenase	7		
Minuet™	Bacillus subtilis strain QST713	Biologicals	Multiple modes of action	BM02	871.9 mL	In-furrow at-planting
Emesto® Silver 118 FS	Penflufen	Sterol biosynthesis in membranes	Demethylation inhibitors	3	9.2 mL / 45 kg of potato seeds	Seed treatment
	Prothioconazole	Respiration	Complex II: succinate-dehydrogenase	7		
Minuet™	Bacillus subtilis strain QST713	Biologicals	Multiple modes of action	BM02	871.9 mL	In-furrow at planting
Velum® Prime 500 FC	Fluopyram	Respiration	Complex II: succinate-dehydrogenase	7	474.24 mL	In furrow at-planting

Table 7. (cont'd)

Luna® Tranquility 500 SC	Fluopyram	Respiration	Complex II: succinate-dehydrogenase	7	820 mL	Drench 28 days after row closure
	Pyrimethanil	Amino acid and protein synthesis	Anilio-pyrimidines	9		
Movento® HL 480 SC	Spirotetramat	Tetronic and Tretamic acid derivates	Inhibitors of acetyl CoA carboxylase	23	182.78 mL	Drench 28 days after row closure
Emesto® Silver 118 FS	Penflufen	Sterol biosynthesis in membranes	Demethylation inhibitors	3	9.2 mL / 45 kg of potato seeds	Seed treatment
	Prothioconazole	Respiration	Complex II: succinate-dehydrogenase	7		
Quadris® 240 SC	Azoxystrobin	Respiration	Quinone outside inhibitors	11	637.26 mL	In-furrow at planting
Velum® Prime 500 FC	Fluopyram	Respiration	Complex II: succinate-dehydrogenase	7	474.24 mL	In furrow at-planting
Velum® Prime 500 FC	Fluopyram	Respiration	Complex II: succinate-dehydrogenase	7	474.24 mL	Drench 42 days after planting
Vydate® C-LV 452SL	Oxamyl	Carbamates	Acetylcholinesterase inhibitors	N1-A	4.9 L	In-furrow at planting
Velum® Prime 500 FC	Fluopyram	Respiration	Complex II: succinate-dehydrogenase	7	474.24 mL	Drench Plants 25.4 cm tall

Table 7. (cont'd)

Movento® HL 480 SC	Spirotetramat	Tetronic and Tretamic acid derivates	Inhibitors of acetyl CoA carboxylase	23	182.78 mL	Drench at row closure
Movento® HL 480 SC	Spirotetramat	Tetronic and Tretamic acid derivates	Inhibitors of acetyl CoA carboxylase	23	182.78 mL	Drench 14 days after row closure
Vydate® C-LV 452SL	Oxamyl	Carbamates	Acetylcholinesterase inhibitors	N1-A	2.5 L	Drench 28 days after row closure
Velum® Prime 500 FC	Fluopyram	Respiration	Complex II: succinate-dehydrogenase	7	474.24 mL	In-furrow at planting
Velum® Prime 500 FC	Fluopyram	Respiration	Complex II: succinate-dehydrogenase	7	474.24 mL	Drench Plants 25.4 cm tall
Movento® HL 480 SC	Spirotetramat	Tetronic and Tretamic acid derivates	Inhibitors of acetyl CoA carboxylase	23	182.78 mL	Drench at row closure
Movento® HL 480 SC	Spirotetramat	Tetronic and Tretamic acid derivates	Inhibitors of acetyl CoA carboxylase	23	182.78 mL	Drench 14 days after row closure
Vydate® C-LV 452SL	Oxamyl	Carbamates	Acetylcholinesterase inhibitors	N1-A	2.4 L	Drench 28 days after row closure
Vydate® C-LV 452SL	Oxamyl	Carbamates	Acetylcholinesterase inhibitors	N1-A	4.9 L	In-furrow at planting

Table 7. (cont'd)

Velum® Prime 500 FC	Fluopyram	Respiration	Complex II: succinate-dehydrogenase	7	474.24 mL	Drench when plants are 25.4 cm tall
Velum® Prime 500 FC	Fluopyram	Respiration	Complex II: succinate-dehydrogenase	7	474.24 mL	Drench at row closure
Movento® HL 480 SC	Spirotetramat	Tetronic and Tretamic acid derivates	Inhibitors of acetyl CoA carboxylase	23	182.78 mL	Drench 14 days after row closure
Movento® HL 480 SC	Spirotetramat	Tetronic and Tretamic acid derivates	Inhibitors of acetyl CoA carboxylase	23	182.78 mL	Drench 28 days after row closure
Vydate® C-LV 452SL	Oxamyl	Carbamates	Acetylcholinesterase inhibitors	N1-A	4.9 L	In-furrow at planting
					2.4 L	Drench when plants are 25.4 cm tall.
					2.4 L	Drench at row closure
					2.4 L	Drench at 14 days after row closure
					2.4 L	Drench at 28 days after row closure

Table 7. (cont'd)

Emesto® Silver 118 FS	Penflufen	Sterol biosynthesis in membranes	Demethylation inhibitors	3	9.2 mL / 45 kg of potato seeds	Seed treatment
	Prothioconazole	Respiration	Complex II: succinate-dehydrogenase	7		
Serenade® ASO 1SC	Bacillus subtilis strain QST713	Biologicals	Multiple modes of action	BM02	2.3 L	In-furrow at planting
Emesto® Silver 118 FS	Penflufen	Sterol biosynthesis in membranes	Demethylation inhibitors	3	9.2 mL / 45 kg of potato seeds	Seed treatment
	Prothioconazole	Respiration	Complex II: succinate-dehydrogenase	7		
Serenade® ASO 1SC	Bacillus subtilis strain QST713	Biologicals with multiple modes of action	Multiple effects	BM02	2.3 L	In-furrow at planting
Velum® Prime 500 FC	Fluopyram	Respiration	Complex II: succinate-dehydrogenase	7	474.24 mL	In-furrow at planting
Movento® HL 480 SC	Spirotetramat	Tetronic and Tretamic acid derivates	Inhibitors of acetyl CoA carboxylase	23	182.78 mL	Drench 14 days after row closure
Emesto® Silver 118 FS	Penflufen	Sterol biosynthesis in membranes	Demethylation inhibitors	3	9.2 mL / 45 kg of potato seeds	Seed treatment
	Prothioconazole	Respiration	Complex II: succinate-dehydrogenase	7		
Quadris® 240 SC	Azoxystrobin	Respiration	Quinone outside inhibitors	11	703.9 mL	In-furrow at planting

Table 7. (cont'd)

Velum® Prime 500 FC	Fluopyram	Respiration	Complex II: succinate-dehydrogenase	7	474.24 mL	In-furrow at planting
Vydate® C-LV 452SL	Oxamyl	Carbamates	Acetylcholinesterase inhibitors	N1-A	2.4 L	Drench 42 days after planting
Spirato 480FS	-	-	-	-	88.7mL / 45 kg of potato seed	Seed Treatment
Potato IF	-	-	-	-	88.7mL / 45 kg of potato seed	Seed Treatment
	-	-	-			
Potato IFN	-	-	-	-	88.7mL / 45 kg of potato seed	Seed Treatment
	-	-	-			
	-	-	-	-		
Potato IFN2	-	-	-	-	88.7mL / 45 kg of potato seed	Seed Treatment
	-	-	-			
	-	-	-	-		

Soil and root collection for nematode extraction and identification

Soil samples were taken before treatment application, 60 days after planting, and at harvest (100 days after planting) by randomly taking 10 soil cores from the center two rows in each plot at 15 cm depth. The soil was homogenized in a 1-gallon plastic bag and stored at 8 ºC until nematode extraction. Root-lesion nematode incidence in soil was quantified using the extraction method by Jenkins, 1964 based on elutriation and centrifugal flotation. Briefly, 100 cm^3 of soil was washed and passed through a stack of 250-µm and 25-µm sieves. Soil that was retained in the 25-µm sieve was placed into a centrifuge tube with water, followed by centrifugation at 4000 rpm for 5 min. After centrifugation, the supernatant was discarded, and the pellet of soil was left untouched. Then 40% sucrose was added, and tubes were centrifuged at 4000 rpm for 3 min. Lastly, the supernatant was passed through a 25-µm sieve to recover the nematodes which were then collected in a 10-ml glass tube for further nematode counting and identification. Morphological characteristics were observed using an inverted Nikon TMS microscope (Mai, 2018).

Root samples for *P. penetrans* extraction were collected 60 days and 90 days after planting, by randomly collecting 5 g of fine roots from ten plants from the middle two rows per plot. Root samples were placed in plastic bags and transported in a cooler to the laboratory. Once in the laboratory, roots were washed with cold water, left to dry for 2 min, and cut into 5mm pieces. Root pieces were placed in sterile plastic cups (Thermo Fisher Scientific, Hampton, NH) with 40ml of distillate water. The plastic cups were then placed in a shaker (G10 Gyratory Shaker, New Brunswick Scientific Co., Inc, New Brunswick, NJ) at 150 rpm for 72h. Subsequently, samples were then passed through a stack of 250-µm and 25-µm sieves. The nematodes on the 250-µm sieve were collected in a 10-ml glass tube for further *P. penetrans* nematode identification and quantification using an inverted Nikon TMS microscope (Figure 23).

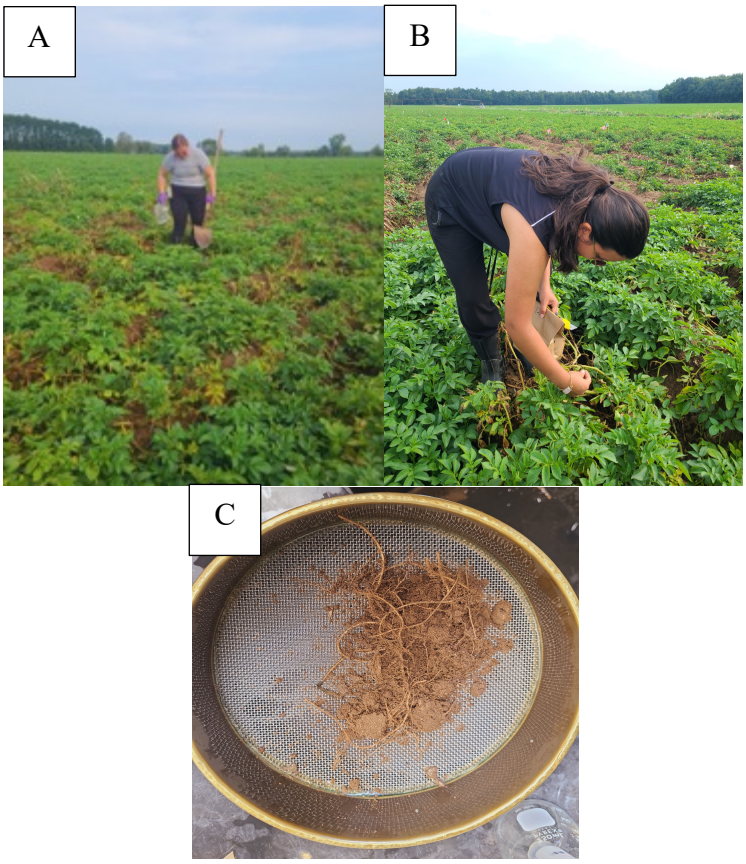

Figure 23. Field sampling of **A.** Potato roots for *P. penetrans* extraction and **B.** Potato stems for *V. dahliae* infection quantification. **C.** roots collected from the field are washed to get rid of soil debris.

Stem Sampling for *Verticillium dahliae* Analysis

Potato stem samples for *V. dahliae* extraction were collected 60 days and 90 days after planting, by randomly collecting ten stems from ten plants from the middle two rows per plot. Samples were placed in paper bags and transported to the laboratory in a cooler. Samples were placed in paper bags and transported to the laboratory in a cooler. *Verticillium dahliae* incidence in stems was quantified by plating stem sections on Ethanol media (1 L destilled water; 15g BD BactoTM, Thermo Fisher Scientific, Hampton, NH; 10ml of 200 proof ethanol; 0.4g streptomycin sulfate) (Nicot and Rouse 1987). From each stem, a 1 cm piece was cut, for a total of 10 pieces. The pieces were surface sterilized in 5% sodium hypochlorite solution (7.4% sodium hypochlorite concentrated Clorox® Disinfecting Bleach) for 5 min and left to dry. for 5 min and left to dry. Then, each stem piece was placed on Ethanol media and incubated at room temperature (26 ºC ± 5 ºC) for 15 days in darkness (Figure 24).

After 15 days of incubation, the number of stem portions with *Verticillium* germination characteristics such as verticillate conidiophores, white mycelium, and globose to elongate black microsclerotia arranged in a scattered pattern (Smith, 1965), were counted and the incidence of *V. dahliae* was established using the equation below, where Si corresponds to infected stems and Sn corresponds to non-infected stems:

$$\%\ of\ infection\ = (\frac{Si}{Sn}) \div 100)$$

Figure 24. Potato stems were randomly collected from the field.

Potato yield and quality

Potato yield was quantified by harvesting one row from each plot using a potato harvester 100 days after planting. Once harvested, the potatoes were brought to the Michigan State University Agronomy Farm (Lansing, MI) where they were washed, weighed, and graded. The total yield and size distribution were determined as oversized- ≥3 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). In addition, 10 tubers were randomly selected and set aside for further quality inspection. Potatoes were cut open and screened for brown center, internal brown spot, hollow heart, potato scab, and vascular discoloration (Figure 25).

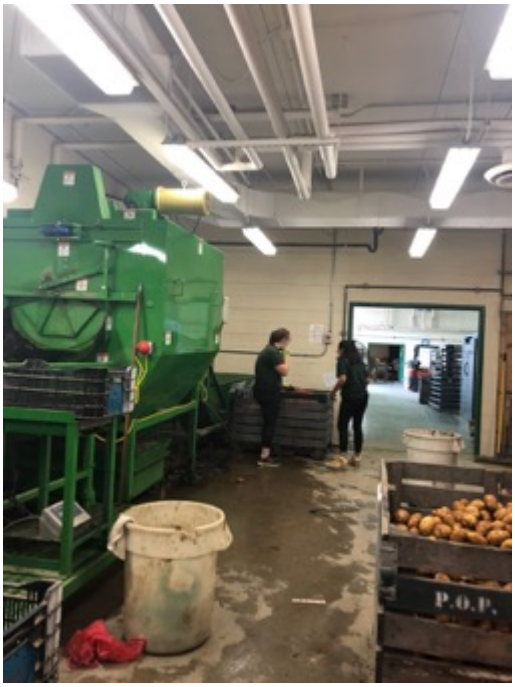

Figure 25. Potato grading and yield quantification at the Michigan State University Agronomy Farm.

Statistical Analysis

Data collected from both field experiments were analyzed separately using RStudio version 2023 (R Core Team 2023). Response variables included *P. penetrans* counts in soil and roots, the number of stems infected with *V. dahliae*, number of tubers with vascular discoloration, and potato yield. Treatment and sampling date were considered as the fixed effects, while replication was a random effect when included in the model.

For each data set, normality was determined with a normal quantile-quantile plot of residuals and a Shapiro-Wilk test (P-value>0.05). *Pratylenchus penetrans* abundance in soil and roots across time data were analyzed with a generalized linear mixed model (GLMM) with a Poisson distribution. Stem presence of *V. dahliae* across time and tuber presence of vascular discoloration data were analyzed with a GLMM model with a binomial distribution. Potato yield data were analyzed with a linear mixed-effects model (LMER) including replication as a random effect using the package 'lme4' (Bates et al. 2014). If treatment was determined to have a significant effect, means separation was completed using Tukey's Honest Significant Difference (HSD) post-hoc tests (α=0.05), followed by pairwise comparisons among treatments using the packages 'emmeans', 'MASS', and 'mulcomp' (Lenth 2019; Venables and Ripley, 2002; Hothorn

et al. 2008). Statistically different means are represented by different letters in figures. Boxplots were developed using the package 'ggplot2' (Wickham, 2016) and letters were added manually onto graphs.

3.3 RESULTS

Response of root-lesion nematode abundance in soil to the different chemical-based treatments evaluated across time

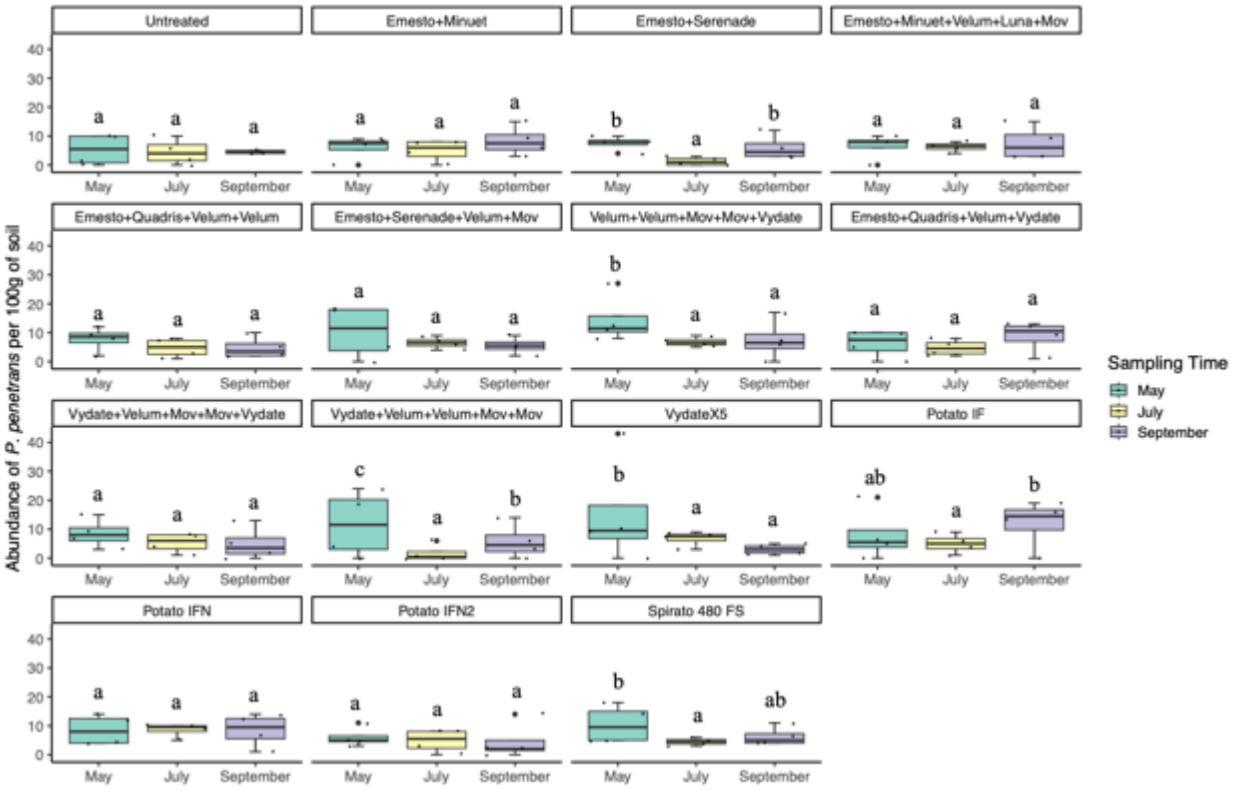

Figure 26. Standard boxplots to visualize *P. penetrans* abundance in 100g of soil determined for each of the treatments evaluated, at each sampling time, which is denoted by the different colors. The bold horizontal line within the box indicates the median and the error bars indicate the minimum and maximum data values. Jittered points visualize the data distribution of groups, and the bold points represent the outliers. The letters above boxplots indicate significant pairwise differences across time for each treatment (p-value<0.05).

Pratylenchus penetrans abundance in soil was significantly affected from May to July (z. ratio = -7.514, p-value = <0.0001), July to September (z. ratio = -3.400, p-value = 0.0019), and May to September (z. ratio = 4.401, p-value = <0.0001). From May to July, there was a significant decrease in *P. penetrans* in the soil in plots treated with “Emesto + Serenade” (pairwise

comparison: p-value = 0.0006), however, there was a significant increase of *P. penetrans* in soil from July to September (pairwise comparison: p-value = 0.0041). Similarly, *P. penetrans* populations significantly increased from July to September (pairwise comparison: p-value = 0.0029) in plots treated with "Potato IF", while for plots treated with "Spirato 480 FS", *P. penetrans* significantly decreased from May to July (pairwise comparison: p-value = 0.0074) and did not recover by September.

As for "Velum+Velum+Mov+Mov+Vydate", *P. penetrans* populations significantly decreased from May to September (pairwise comparison: p-value = 0.0095). Similarly, for the treatment "Vydate+Velum+Velum+Mov+Mov" *P. penetrans* populations significantly decreased from May to September (pairwise comparison: p-value = 0.0138), and as for "VydateX5", *P. penetrans* populations had a significant decline across time from May to September (pairwise comparison: p-value = <0.0001) (Figure 26).

Reproduction of root-lesion nematode in potato roots when treated with different chemical-based treatments

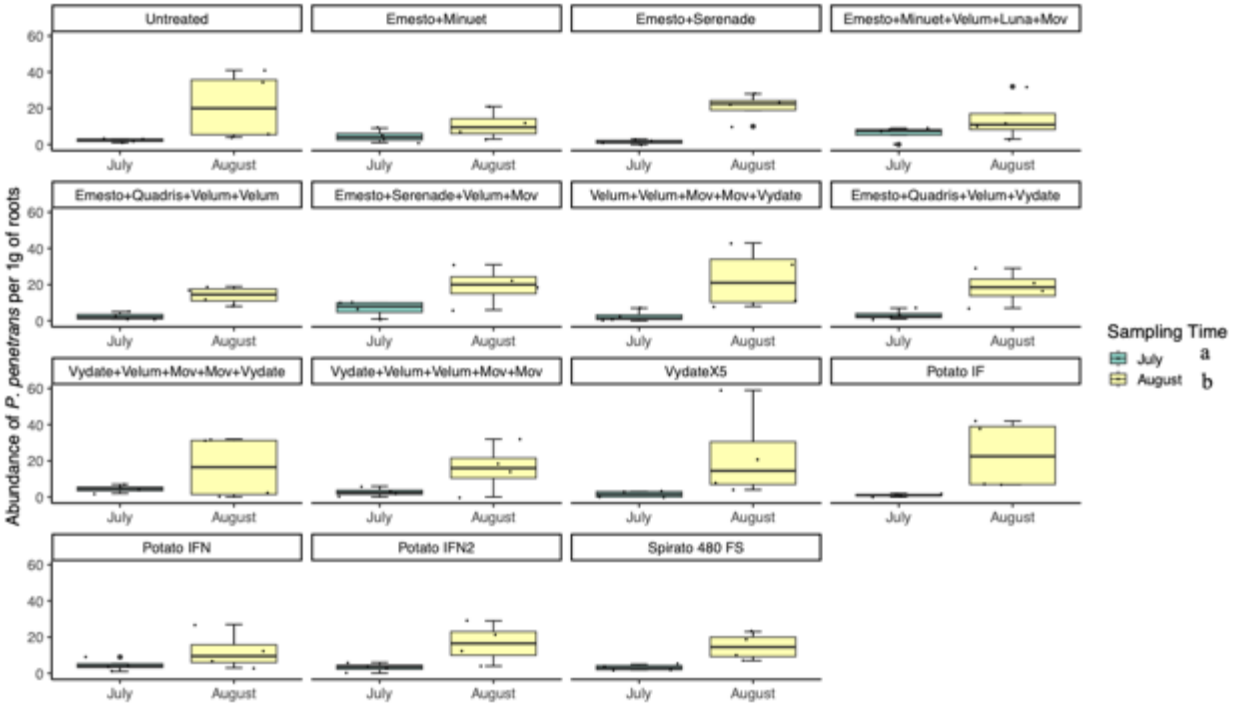

Figure 27. Standard boxplots to visualize *P. penetrans* abundance in 1g of roots determined for each of the treatments evaluated, at the respective sampling time, which is denoted by the different colors (July and August). The bold horizontal line within the box indicates the median and the error bars indicate the minimum and maximum data values. Jittered points visualize the

Figure 27. (cont'd)

data distribution of groups, while bold points indicate outliers. The letters above boxplots indicate significant pairwise differences across time for each treatment (p-value<0.05). *Pratylenchus penetrans* populations in roots significantly increased from July to September for all treatments (pairwise comparison: p-value = <0.0001). In August, the highest number of *P. penetrans* was found in roots collected from plots treated with "Potato IF", "VydateX5", "Velum + Velum + Mov + Mov + Vydate", "Untreated" control, and "Emesto + Serenade", with an average of 23.5, 23, 23.25, 21.25 and 20.75 *P. penetrans*/ g of root, respectively. In addition, for "Potato IF" there were 23 times more nematodes in August than in July. In comparison, the lowest number of *P. penetrans* was found in roots collected from plots treated with "Emesto + Minuet", with an average of 10.75 *P. penetrans*/ g of root, which resulted in only 2 times more nematodes in August than in July (Figure 27).

Verticillium dahliae Potato Stem Infection When Treated with Different Chemical-Based Treatments

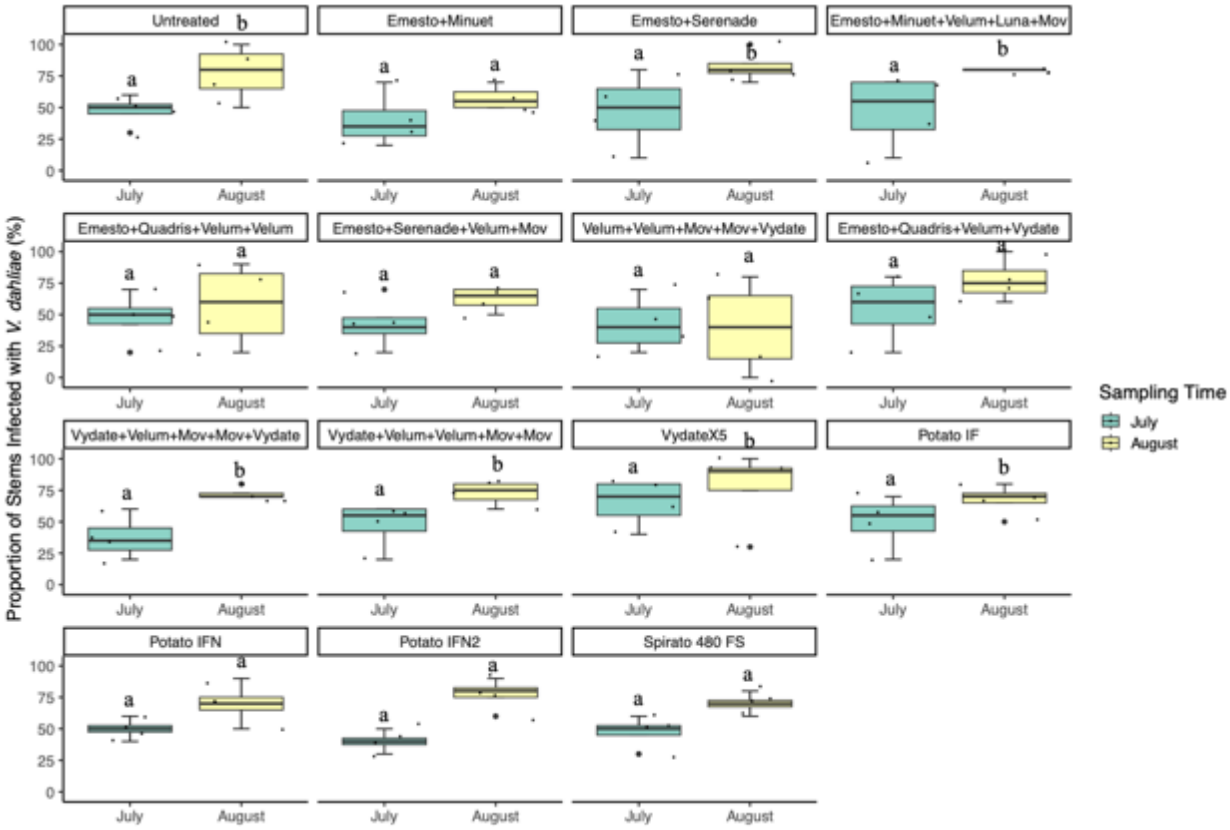

Figure 28. Standard boxplots to visualize the proportion (%) of potato stems (n=10) infected with *V. dahliae* determined for each of the treatments evaluated, at each sampling time, which is denoted by the different colors (July and August). The bold horizontal line within the box indicates the median and the whisker bars indicate the minimum and maximum data values. Jittered points visualize the data distribution of groups, and the bold points represent outliers. The letters above boxplots indicate significant pairwise differences across time for each treatment (p-value<0.05).

Verticillium dahliae stem infection from July to August was different for treatments (pairwise comparison: p-value = <0.0001). There was a significant increase of *V. dahliae* stem infection from July to August in the "Untreated" control (pairwise comparison: p-value = 0.0068), "Emesto+Minuet+Velum+Luna+Mov" (pairwise comparison: p-value = 0.0033), "Emesto+Quadris+Velum+Vydate" (pairwise comparison: p-value = 0.0361), "Emesto+Serenade" (pairwise comparison: p-value = 0.0016), "Vydate+Velum+Mov+Mov+Vydate" (pairwise comparison: p-value = 0.0021),

“Vydate+Velum+Velum+Mov+Mov” (pairwise comparison: p-value = 0.0244), “Potato IFN2” (pairwise comparison: p-value = 0.0010), and “Spirato 480 FS” (pairwise comparison: p-value = 0.0431). The highest increase of stem infection was found in “Vydate+Velum+Mov+Mov+Vydate” with 93% more infection in August than in July, while the “Untreated” control had a 63% increase in disease from July to August.

In contrast, “Emesto+Minuet”, “Emesto+Quadris+Velum+Velum”, “Emesto+Serenade+Velum+Mov”, “VydateX5”, “Velum+Velum+Mov+Mov+Vydate”, “Potato IF”, and “Potato IFN”, did not have a significant increase of stem infection from July to August (p-value>0.05). Although not significant, in “Velum+Velum+Mov+Mov+Vydate” there was a slight decrease in *V. dahliae* stem infection of only 6% (Figure 28).

Potato Yield When Treated with Different Chemical-Based Treatments.

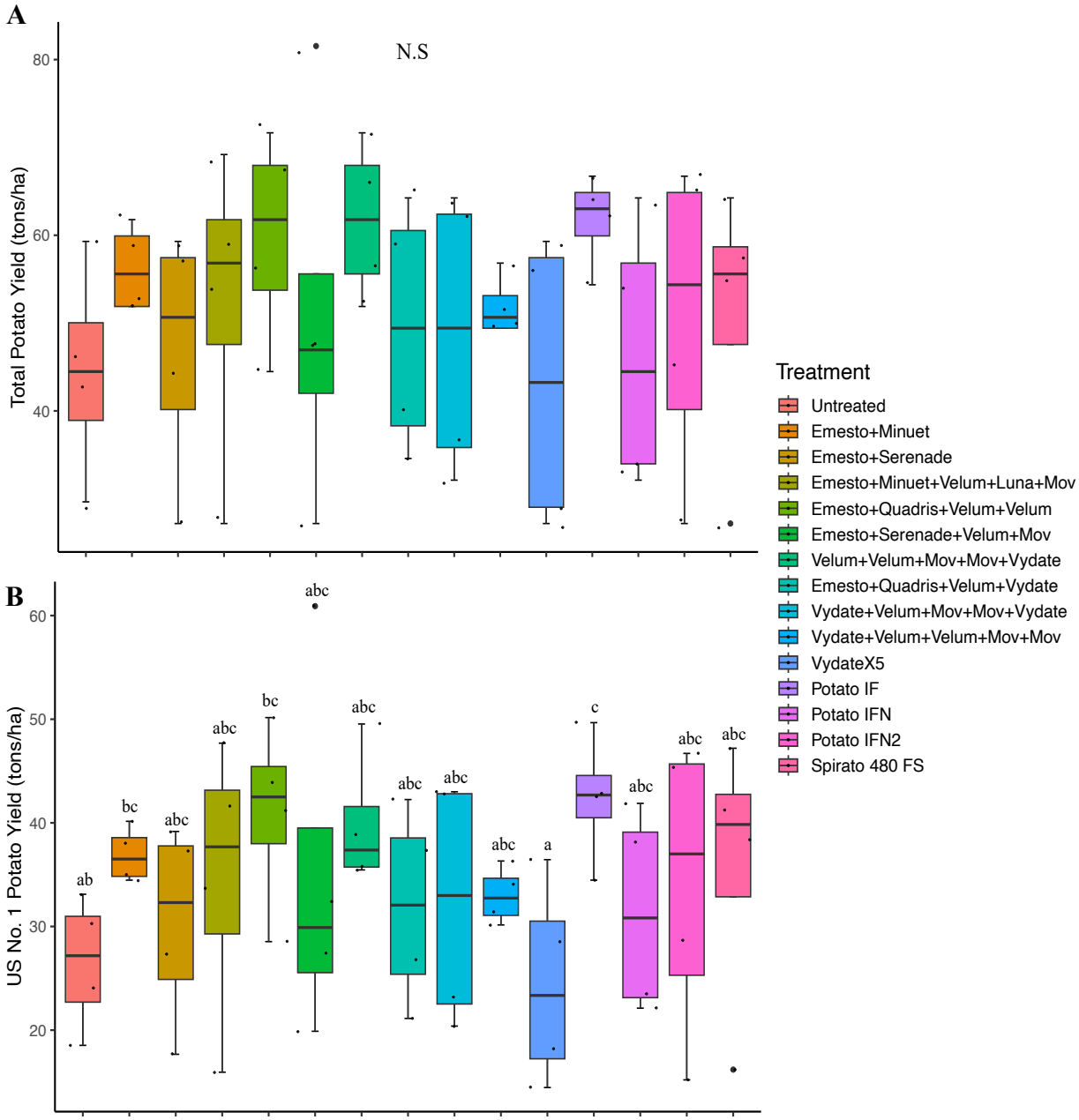

Figure 29. Standard boxplots to visualize the potato yield determined for each of the treatments evaluated. **A.** Total potato yield (all sizes) in tons per hectare. **B.** Total potato yield of tubers US. No. 1 in tons per hectare. The bold horizontal line within the box indicates the median and the error bars indicate the minimum and maximum data values. Jittered points visualize the data distribution of groups, and bold points represent outliers. The letters above boxplots indicate significant pairwise differences for each treatment, compared to the "Untreated" control (p-value<0.05).

Compared to the null model, overall, treatment did not have a significant effect on the production of tubers size A1 (DF = 14, F-value$_{(14,42)}$ = 0.9584, P-value = 0.5091), yield per plot (DF = 14, F-value$_{(14,42)}$ = 0.838, P-value = 0.6261), total yield per hectare (DF = 14, F-value$_{(14,42)}$ = 0.8382, P-value = 0.6259), and total yield of US No. 1 (DF= 14, F-value$_{(14,42)}$ = 1.12, P-value = 0.3697). Nevertheless, "VydateX5" showed the lowest total yield (43.2 tons/ha), and US No. 1 yield (24.4 tons/ha), followed by the "Untreated" control with a total yield of 44.4 tons/ha, and US No. 1 yield of 26.5 tons/ha. In contrast, "Potato IF" showed the highest total yield of 61.7 tons/ha (pairwise comparison: p-value = 0.0636), and US No. 1 yield of 42.3 tons/ha (pairwise comparison: p-value = 0.0396). That said, "Potato IF" produced 38.9% more total potato yield, and 60% more US No. 1 yield, compared to the "Untreated" control. In addition, "Potato IF" produced 42.8% more total potato yield, and 73% more US No. 1 yield, compared to "VydateX5" (Figure 29).

Potato Internal Defects When Treated with Different Chemical-Based Treatments

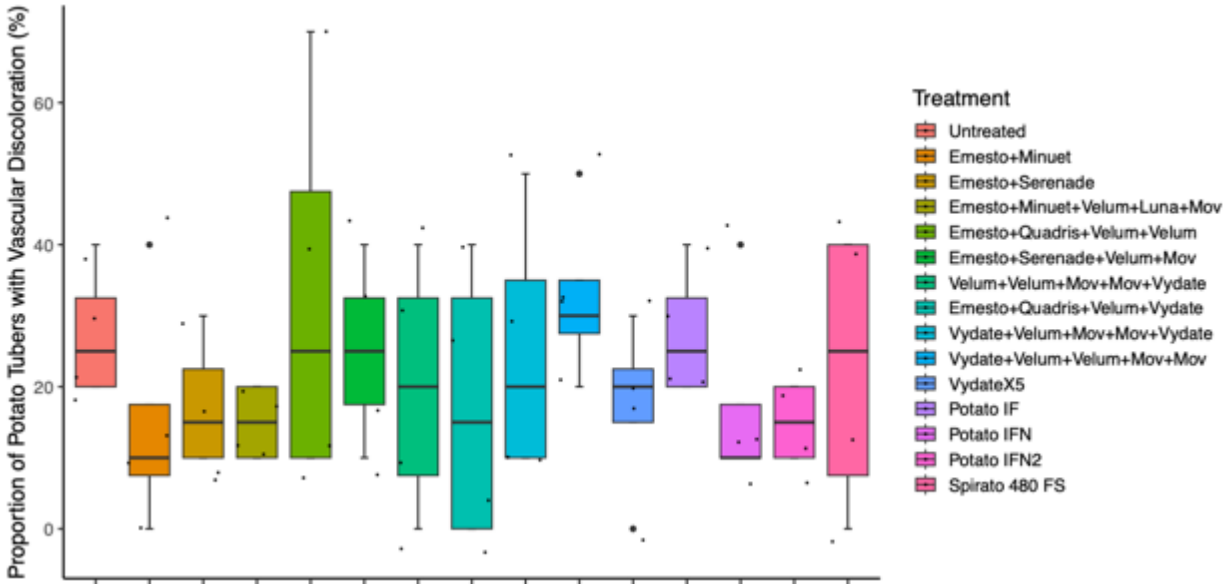

Figure 30. Standard boxplots to visualize the proportion (%) of potato tubers (n= 10) exhibiting symptoms of vascular discoloration caused by *V. dahliae*. The bold horizontal line within the box indicates the median and the error bars indicate the minimum and maximum data values. Jittered points visualize the data distribution of groups, and bold points represent outliers The letters above boxplots indicate significant pairwise differences across time for each treatment (p-value<0.05).

Potato tuber vascular discoloration caused by *V. dahliae* did not differ among treatments (p-value>0.05). Potato vascular discoloration was the highest in "Emesto+Quadris+Velum+Velum"

and “Vydate+Velum+Velum+Mov+Mov”, all with an average of 32.5% of evaluated tubers showing vascular discoloration. The lowest vascular discoloration was found in tubers from “Potato IFN2”, “Emesto+Minuet+Velum+Luna+Mov”, and “Emesto+Minuet” all with an average of 15% of evaluated tubers showing vascular discoloration (Figure 30).

3.4 DISCUSSION

In the past, control of plant-parasitic nematodes as well as soil-borne pathogens like *V. dahliae*, has been done with broad-spectrum products such as fumigants or organophosphates and carbamates. Although very effective at controlling pathogens, awareness was raised about their detrimental effects on the environment as well as the dangerous threat they pose to human health. Once these pesticides were banned, new, and reduced-risk products were needed. This resulted in products that growers use today which are considered non-fumigants. Some of these modern products are oxamyl, fluopyram, fluensulfone, fluazaindolizine, spirotetramat, and trioxazafen, which vary in mode of action, soil half-life, solubility, and human risk (Desaeger et al. 2020). The majority of treatments evaluated in this chapter include the compounds mentioned above in different combinations, rates, and time of application to determine which is the most effective at reducing the Potato Early Die disease complex inoculum and/or yield parameters under a commercial field setting.

Pratylenchus penetrans populations in soil throughout the growing season varied for treatments and the highest decrease was found in plots treated with 5 applications of Vydate® (80%). However, the other treatment combinations that included Vydate® had less impact on the *P. penetrans* soil population causing a decrease ranging from 41.1% to 51%, with one of the treatment combinations (Emesto+Quadris+Velum+Vydate) causing an increase of 40% in *P. penetrans* soil populations. A published study showed that fluensulfone caused the highest suppression of *M. javanica* compared to treatment combinations that included oxamyl (Desaeger and Watson, 2019). This may suggest that Vydate® is more effective when applied consecutively during the growing season.

The treatment combinations that included Vydate and that also resulted in reduced *P. penetrans* populations in soil were “Velum+Velum+Mov+Mov+Vydate”, “Vydate+Velum+Mov+Mov+Vydate” and “Vydate+Velum+Velum+Mov+Mov”. These treatments were a combination of the active ingredients oxamyl, fluopyram, and spirotetramat. Fluopyram® belongs to the class benzamides and its mode of action for both fungi and

nematodes is by inhibiting the enzyme succinate dehydrogenase (Heiken, 2017). Studies have shown that this chemical compound is very effective at controlling *Meloidogyne* species, however, it is not the case for other plant parasitic nematodes like *P. penetrans*. For instance, Watson and Desaeger, 2019, showed that fluopyram did not significantly reduce *P. penetrans* in soil and roots in strawberries. The lower *P. penetrans* reduction in soil observed for these treatment combinations evaluated in my experiment could then be attributed to the lack of effectivity of Velum® (fluopyram), however, further evaluation of this chemistry alone should be tested to confirm how effective fluopyram is at causing *P. penetrans* mortality in potatoes.

As for spirotetramat (Movento®), this is a systemic compound that is toxic to nematodes in its hydrolyzed form (Vang et al., 2016). Recently it was found that in *Caenorhabditis elegans* and *Hetrodera schachtii*, exposure to the enol form of spirotetramat leads to disruption of the nematode life cycle by inhibiting the acetyl-CoA-carboxylase, affecting lipid and fatty acid storage and composition (Gutbrod et al. 2020). Studies have shown that the efficacy of spirotetramat varies among plant parasitic nematodes. For instance, the egg-hatching of *Rotylenchus reniformis* was not affected by this compound (Waisen et al. 2019). In other studies, spirotetramat did not affect *P. penetrans* or *P. neglectus* (Zasada et al. 2010; Smiley et al. 2012). All together with the results from my experiment, may suggest that combinations of oxamyl, fluopyram, and spirotetramat are not effective in achieving the highest mortality of *P. penetrans* in soil, but instead, 5 applications of Vydate® (oxamyl) are enough.

As for the abundance of *P. penetrans* in roots, none of the treatments reduced nematode populations, however, overall *P. penetrans* populations remained low. For instance, the highest number of nematodes per gram of root was found in plots treated with 5 applications of Vydate® with an average of 23 *P. penetrans*. The economic threshold of *P. penetrans* depends on many variables including crop type, location, environment, and the potential for disease complexes (Castillo and Vovlas, 2007). However, in the presence of *V. dahliae*, the economic threshold of *P. penetrans* ranges between 10-20 nematodes per 100 g of soil (Powelson and Rowe, 1993). Hence, although by August the nematodes produced per gram of root do not seem a large amount, is enough to trigger the disease complex with *V. dahliae*. In fact, *V. dahliae* potato stem infection significantly increased over time, except for the treatment "Velum+Velum+Mov+Mov+Vydate", however, the decrease was only of 6% and was not statistically significant. The highest increase of *V. dahliae* infection was in plants treated with the

combination “Vydate+Velum+Mov+Mov+Vydate” (93%). This treatment in particular had two applications of Vydate® and two of Movento®, both with nematicide activity, while only one application of Velum® which has both fungicidal and nematicidal activity. In contrast, the treatment combination “Velum+Velum+Mov+Mov+Vydate” had two applications of Velum® early in the season, and in August the proportion of *V. dahliae* infected stems was 40%. These results suggest that perhaps more than one application of Velum® is necessary to provide some degree of control of *V. dahliae* but would require further exploration.

Despite the high control of *P. penetrans* provided by Vydate®, potato yield was the lowest for this treatment, followed by the control. In contrast, the seed treatment “Potato IF” provided the highest yields. The active ingredients of this seed treatments have been found to be effective fungicides against some potato diseases like silver scurf, leaf spot diseases, and potato scab, but no clear effect on potato yield was reported (Errampalli et al. 2001; Budde-Rodriguez et al. 2022; Al-Mughrabi et al. 2015). In addition, it was shown that combined applications of one of its active ingredients with *Trichoderma harzianum* decreased the incidence of black scurf and also increased potato marketable yield from 35% to 60%, but no effect of the chemical alone was reported (Wilson et al. 2008). The results of my experiment suggest that even though this seed treatment did not show a significant effect on *P. penetrans* or *V. dahliae*, this seed treatment may be having an effect against other potato pathogens that may have an impact on yield, in addition to potentially having a plant growth regulator effect, which may have resulted in high yields.

3.5 CONCLUSION

Soil fumigation with broad-spectrum pesticides like metam sodium is the current potato industry standard to control PED inoculum in soil. In addition, throughout the season consecutive applications of non-fumigant nematicides and fungicides are done to maintain populations of these pathogens low. Overall, there is little published evidence of the effectiveness of these non-fumigant products in controlling PED, therefore it is necessary to conduct field experiments to confirm the effect of these products on PED and potato yields. In the current field experiment, multiple combinations of non-fumigant nematicides and fungicides and seed treatments alone were considered for PED control. The results determined that as for *P. penetrans* control, 5 applications of Vydate® are enough to achieve the best control of this nematode in soil. In contrast, the seed treatment “Potato IF” resulted in an increase of more than 50% of marketable yield in comparison to the control. This result provides evidence that this is a product that by

some unknown underlying mechanism suppresses yield limiting pathogens, ensuring high yields. Nevertheless, it would be necessary to conduct more field trials to determine the consistency of this product in increasing potato yield. As for *V. dahliae*, none of the treatments significantly reduced disease severity. However, it is important to note that the treatment combination "Velum+Velum+Mov+Mov+Vydate" resulted in a slight decrease of 6% in disease severity, while in the other treatments disease severity significantly increased over time. This result suggests that it would be interesting to conduct more field trials to continue exploring the effectiveness of these chemicals separately and in different combinations and rates to determine the best alternative to reduce *V. dahliae* disease severity.

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CHAPTER 4: COMMERCIALLY AVAILABLE BIOLOGICAL CONTROL AGENTS FOR MICHIGAN *VERTICILLIUM DAHLIAE* MANAGEMENT

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4.1 INTRODUCTION

Verticillium dahliae is one of the most important species from the Verticillium genus given it is widespread across different regions and climates and has over 200 plant hosts which include economically important crops such as cotton, olive, lettuce, and potatoes (Baht and Subbarao, 1999; Ayele et al. 2020). In potatoes, it can cause up to 50% yield losses given its synergistic interaction with the root-lesion nematode *Pratylenchus penetrans* (Wheeler et al. 1992). Management of *V. dahliae* is challenging due to the melanized resting structure and primary inoculum, the microsclerotia, which allows it to persist in the soil for up to 14 years (Rauyaree et al. 2005). Currently, there is limited availability of resistant host germplasm therefore planting resistant potato varieties is not always feasible (Song et al. 2020). Additionally, the use of post-planting synthetic fungicides is not always effective because once *V. dahliae* infection happens, it locates in the vascular system of the host, becoming inaccessible, and in addition there is risk of resistance (Johnson and Dung, 2010). Moreover, controlling *V. dahliae* primary inoculum is only effective through pre-planting soil fumigation with broad spectrum pesticides (Kowalska, 2021).

The severity of the disease caused by *V. dahliae* is proportional to the quantity of initial inoculum, its efficacy in causing disease, and the length of time it interacts with its host (Van der Plank, 1963). Therefore, management strategies should effectively reduce initial inoculum and/or prevent the introduction and augmentation of inoculum in areas where *V. dahliae* population is below the economic threshold (Rowe and Powelson, 1993). Currently, soil fumigation is the only management strategy that effectively and consistently reduces *V. dahliae* soil inoculum; however, it should be considered that rising environmental and human safety concerns will lead this practice to future phase-out production and consumption (Kaikai et al. 2023). Therefore, implementing an integrated pest management (IPM) program is the best approach to managing plant pathogens like *V. dahliae* (Montes-Osuna and Mercado-Blanco, 2020).

An IPM program could include crop rotation, cover crops, green manures, organic soil amendments, biofumigation, and biological control agents (BCAs). It has been shown that some manure-based amendments are effective at reducing *V. dahliae* inoculum, however, such

effectiveness is correlated to the quantity of ammonia and nitrous acid within these amendments (Tenuta and Lazarovits, 2002). In addition, cover crops, green manures, and crop rotation are of limited use in Verticillium wilts control given its wide host range and its long persistence in the soil, even without a host. For instance, the effectiveness of broccoli, barley, mustards, canola corn, oat, sudangrass, and winterpea in reducing *V. dahliae* inoculum has been studied but the results have been variable (Johnson and Dung, 2010). An example of effective green manure for the reduction of *V. dahliae* inoculum is incorporating broccoli residue, which has been shown to reduce *V. dahliae* microsclerotia and wilt in cauliflower (Subbarao et al. 1999). However, green manures and cover crops are not widely integrated into potato production. Similarly, crop rotation can be highly effective for some species of *Verticillium* but not that effective for *V. dahliae*. In potato production usually 3 to 4-year rotations with non-susceptible crops are necessary, however, *V. dahliae* can also infect roots of weeds and non-hosts maintaining inoculum levels sufficient to cause disease in susceptible hosts (Joaquim et al. 1988). For instance, of often-found weed species in potato fields, a potato pathotype of *V. dahliae* can significantly increase inoculum in weed black nightshade (Frederick et al. 2017). Crop rotation with sudangrass, green peas, and corn has been shown to reduce propagule production in potato stems and increase yields (Easton et al. 1992). Therefore, successful crop rotation to avoid build-up of primary inoculum should also address the management of weeds such as the black nightshade in potato fields.

Another management alternative is the use of biological control agents. It is essential that effective *Verticillium* spp. biological control agents can either interfere with its survival structures, share the same niche, induce plant immune response, or promote plant growth. There are some bacteria and fungi that through the mechanisms mentioned above, can be effective at reducing the detrimental effects of Verticillium wilts. Some of the most studied genera of bacteria are *Bacillus*, *Streptomyces*, *Paenibacillus*, *Pseudomonas*, *Enterobacter*, *Acetobacter*, and *Serratia*, with the most studied genera being *Bacillus*. Within this genera, *B. amyloliquefaciens* and *B. subtilis* are the most effective species (Deketelaere et al. 2017; Li et al. 2014; Fira et al. 2018). Different strains of these species have been tested on cotton, eggplant, potato, oilseed rape, maple, and strawberry and some have shown *Verticillium* spp. antibiosis, competition, mycoparasitism, reduction of microsclerotia germination, and induction of plant resistance (Deketelaere et al. 2017). Likewise, bacteria like *Streptomyces* spp. and *Pseudomonas*

spp. have been shown to suppress *V. dahliae* disease in potatoes (Wiggins and Kinkel, 2005; Uppal et al. 2007 and 2008; El Hadrami et al. 2011). These bacteria own relevant characteristics like the production of antibiotics (e.g., amphotericin and nystatin), the production of chitinases that allow penetration of hyphae, and the induction of plant systemic resistance (Deketelaere et al. 2017).

As for fungi, species that belong to the genera Ascomycota such as *Trichoderma*, *Fusarium*, and *Verticillium* are the most extensively studied. For instance, *Trichoderma* spp. can inhibit the mycelial growth of *Verticillium* spp. by antibiosis, reduction of microsclerotia germination, and mycoparasitism (Fotoohiyan, 2017; Carrero-Carron et al. 2018; Varo et al. 2016). As for Fusarium, non-pathogenic *Fusarium oxysporum* strains can produce volatile organic compounds (VOCs) which can inhibit cotton *V. dahliae* mycelial growth, delay conidial germination, suppress germ-tube elongation, and cause hyphae collapse (Zhang et al. 2015). Similarly, there are non-pathogenic *Verticillium* species that have shown antagonism against pathogenic strains in cotton, tomato, potato, lettuce, and cauliflower, however, the mechanisms are not well understood (Deketelaere et al. 2017). In addition, there are also species of *Gliocladium* that have shown the potential to decrease *V. dahliae* severity in tomatoes and oilseed rape through similar mechanisms as the *Trichoderma* species such as antibiosis, mycoparasitism and reduction of microsclerotia germination (Jabnoun-Khiareddine et al. 2009).

In Chapters 2 and 3 I evaluated the pesticidal effect of different manure-based amendments and chemical-based fungicides on *P. penetrans* and *V. dahliae* under field conditions and one of the conclusions was that none of the evaluated treatments were effective in reducing *V. dahliae* incidence and severity. Therefore, in this chapter, I will focus on biological control agents as an alternative to control *V. dahliae*. There is a vast number of studies on potential BCAs for *Verticillium* spp. control that allows us to conclude that they can have multiple modes of action that interfere with the *Verticillium* spp. disease cycle at different stages, however, it can take up to 6 years for the product to be commercially available (Raymaekers et al. 2020; Köhl et al. 2020; Harman et al. 2010). Nevertheless, there are a handful of biological-based products labeled for *V. dahliae* control in potatoes in the U.S (Table 8), therefore the main goal of this chapter is to determine the efficacy of these BCAs against Michigan *V. dahliae* strains *in-vitro* and *in-planta*.

Table 8. Biological-based products have been massively produced and commercialized in the U.S. for *Verticillium dahliae* control.

Generic Name	Manufacturer	Active Ingredient	Chemical Group	Mode of Action	FRAC code
Double Nickel® LC	Certis U.S.A. L.L.C.	*Bacillus amyloliquefaciens* str. D747	Biologicals with multiple modes of action	Competition, mycoparasitism, antibiosis, membrane disruption and induced plant resistance	BM 02
Minuet™	Bayer Crop Science U.S.A.	*Bacillus subtilis* str. QST 713			
Actinovate® SP	Novonesis	*Streptomyces lydicus* str. WYEC 108			
Tenet® WP	Isagro U.S.A.	*Trichoderma asperellum* str. ICC 012			
		Trichoderma gamsii str. ICC 080			
Lalstop G46 WG	Lallemand Plant Care	*Clonostachys rosea* str.J1446			

4.2. METHODOLOGIES

Biological control agents and *Verticillium dahliae* isolates

Bacillus amyloliquefaciens strain D747 (Double Nickel® LC), *B. subtilis* strain QST 713 (Minuet™), *S. lydicus* strain WYEC 108 (Actinovate® SP), *T. asperellum* strain ICC 012, *T. gamsii* strain ICC 080 (Tenet® WP), and *C. rosea* strain J1446 (Lalstop G46 WG) were isolated from their respective commercial formulations by diluting 0.2 g of product on 1mL of sterile distilled water. After thoroughly mixing the solution, a 15µL aliquot was taken with a micropipette and placed in the middle of a Petri dish (90 mm by 15 mm) with potato dextrose agar (PDA, Difco, Scientific Laboratory Supplies). The plates were sealed with ParaFilm® M Lab Film (Bemis Company Inc.) and incubated at 25˚C in the dark for 7 days. After the

incubation period, purification of the biocontrol agent cultures was performed by streak transfer into Water agar (1 L distilled water and 15g of agar) to obtain monoconidial or single colony isolates for the fungi (single spore streak transfer) and bacteria (single colony streak transfer), respectively. From the purified colonies, mycelial tissue or bacteria cells were obtained why adding 1 mL of sterile distilled water and gently scraping the surface of the microorganism colony with the help of a sterile 90º glass cell spreader. The mixture of microbial tissue and water was transferred to the pre-labeled sterile tubes for further DNA extraction.

DNA extraction was performed for one representative colony of each microorganism using the QIAGEN DNeasy® Plant Mini Kit (QIAGEN, Germany). Tissue was mechanically lysed using Lysing Matrix A with 6.35-mm ceramic spheres (MP Biomedicals, Solon, OH) and the TissueLyserII (QIAGEN, Germany) for 3 minutes twice at 30 Hz. DNA was amplified via polymerase chain reaction (PCR) using the QIAGEN PCR kit, targeting the 16SrRNA region for bacteria (11F: 5'-AGAGTTTGATCCTGGCTCAG-3' and (11512R: 5'-ACGGTTACCTTGTTACGACTT-3') (Weisburg et al. 1991), and targeting the ITS region for fungi (ITS-1: 5'-TCCGTAGGTGAACCTGCGG-3' and ITS-4: 5'-TCCTCCGCTTA TTGATATGC-3') (Gardes and Bruns 1993). Thermal cycler conditions for the amplification of the 16sRNA region consisted of an initial denaturation cycle at 95 °C for 120s, then 30 cycles of denaturation at 95 °C for 30s, annealing at 57 °C for 30s, and extension at 72 °C for 45s, and followed by a final extension at 72 °C for 5 minutes. For amplification of the ITS region thermal cycler conditions consisted of an initial cycle denaturation at 95 °C for 120s, then 35 cycles of denaturation at 95 °C for 120s, annealing at 55 °C for 60s, and extension at 72 °C for 60s, and lastly, a final extension at 72 °C for 10 minutes (Schalchter et al. 2024).

Amplified DNA was then purified using the Qiaquick PCR Purification Kit (QIAGEN, Germany) for a target final DNA content of 10-40 ng. To verify the amplification of the correct region (approximate 680-bp product), agarose gel electrophoresis was performed using 1.5-2.0% Tris/borate/EDTA acetate agarose gel. Sanger sequencing was performed by the Michigan State University Research Technology Support Facility Genomics Core (East Lansing, MI). Species were confirmed by sequence alignment using blastn in NCBI (>90% identities) (Figure 31).

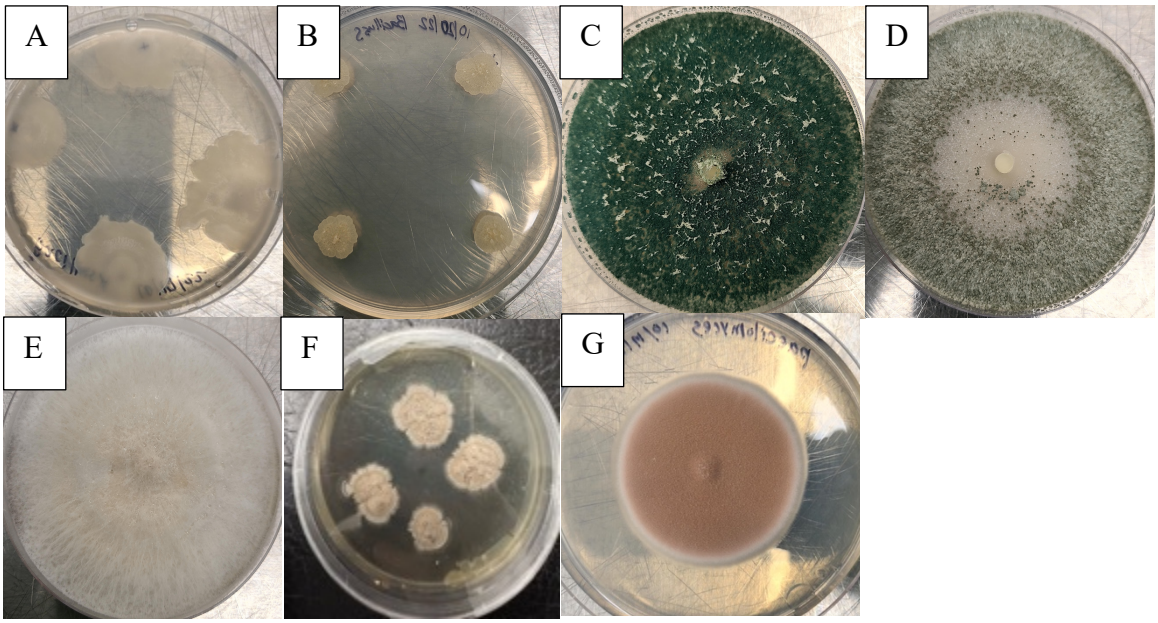

Figure 31. Microbial antagonists' colony morphology isolated from their respective formulations after 7 days of incubation in PDA media. **A.** *B. amyloliquefaciens* strain D747 (Double Nickel® LC; Certis U.S.A. L.L.C.), **B.** *B. subtilis* strain QST 713 (Minuet™; Bayer Crop Science U.S.A.), **C.** *T. asperellum* strain ICC 012 (Tenet® WP), **D.** *T. gamsii* strain ICC 080 (Tenet® WP; Isagro U.S.A.), **E.** *C. rosea* strain J1446 (Lalstop G46 WG; Lallemand Plant Care), **F.** *S. lydicus* strain WYEC 108 (Actinovate® SP; Novonesis), **G.** *P. lilacinum* strain 251 (MeloCon® WG; Certis U.S.A. L.L.C.).

Two different isolates of *V. dahliae* were obtained from infected field potato stems collected from farms located in southwest and northwest Michigan. In the laboratory, the stems were thoroughly washed and then dried with paper towels. From each stem, four 1 cm pieces were cut, and surface sterilized in 5% sodium hypochlorite solution (7.4% sodium hypochlorite concentrated Clorox® Disinfecting Bleach) for 5 min and left to dry. Then, each stem piece was placed on Water agar (1 L distilled water and 15g of agar) and incubated at room temperature (26 ºC ± 5 ºC) for 15 days in darkness. After the incubation period, fungal colonies with morphological characteristics such as verticillate conidiophores, white mycelium, and globose-to-elongate black microsclerotia arranged in a scattered pattern were selected for further culturing (Inderbitzin et al. 2011) (Figure 32).

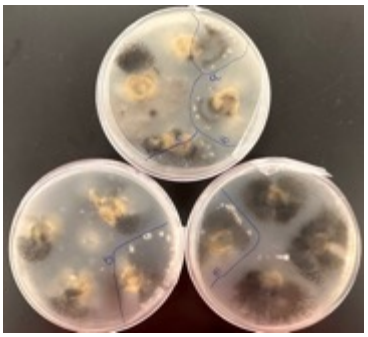

Figure 32. Fungal colonies isolated from infected potato stems were collected on commercial potato fields in southwest and northwest Michigan.

To obtain single colony cultures, single microsclerotia were picked from the fungal colonies that resembled those of *Verticillium* spp. and placed in Water agar (1 L distilled water and 15g of agar). The plates were sealed with ParaFilm® M Lab Film (Bemis Company Inc.) and incubated at room temperature (26 ºC ± 5 ºC) for 7 days in darkness. After incubation, single microsclerotia were picked from the fungal colonies and placed in potato dextrose agar (PDA, Difco, Scientific Laboratory Supplies) and plates were incubated at room temperature (26 ºC ± 5 ºC) for 7 days in darkness. After the incubation period, one plate of each colony was submitted to the Michigan State University Plant and Pest Diagnostics (East Lansing, MI 48824) for further DNA extraction and sequencing to determine which colonies were *V. dahliae* by performing a TaqMan real-time PCR assay based on the ribosomal DNA intergenic spacer of *Verticillium* according to Bilodeau et al. 2012. From the confirmed colonies, I selected two Michigan *V. dahliae* isolates, Vd1 and Vd2, for the antagonism and *in vivo* assays. Additionally, I also included a previously described *V. dahliae* isolate from Wisconsin as a reference, which was isolated from infected stem tissue of symptomatic Russet Burbank potato from the Hancock Research Station in central Wisconsin in 1980 and has maintained virulence in culture until today (Figure 33).

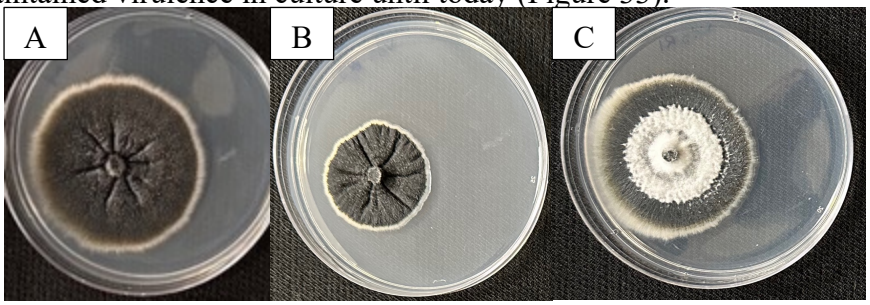

Figure 33. *Verticillium dahliae* isolates selected for the antagonism and *in vivo* assays. **A.** Michigan *V. dahliae* Isolate 1, **B.** Michigan *V. dahliae* Isolate 2, and **C.** Wisconsin *V. dahliae* Isolate.

Antagonism assays

Dual cultures between *B. amyloliquefaciens* strain D747, *B. subtilis* strain QST 713, *S. lydicus* strain WYEC 108, *T. asperellum* strain ICC 012, *T. gamsii* strain ICC 080, and *C. rosea* strain J1446 (Figure 35) and Michigan *V. dahliae* isolate 1, 2, and Wisconsin isolate were carried out according to (Carrero-Carrón et al., 2016), with some modifications. Michigan *V. dahliae* isolate 1, 2, and Wisconsin isolate and the antagonistic fungi and bacteria were cultured on PDA at room temperature (26 ºC ± 5 ºC) for 15 and 7 days, respectively, in darkness, to produce inoculum for the assays. After the incubation period, a 5-mm agar plug was taken from the edge of the *V. dahliae* colonies and placed in the middle of the Petri dish with PDA, for the bacteria-*V. dahliae* confrontation assays, or 3cm away from the edge of the Petri dish with PDA for the fungi-*V. dahliae* confrontation assays (Figure 34). The plates were sealed with parafilm paper and incubated at room temperature (26 ºC ± 5 ºC) for 4 days in darkness.

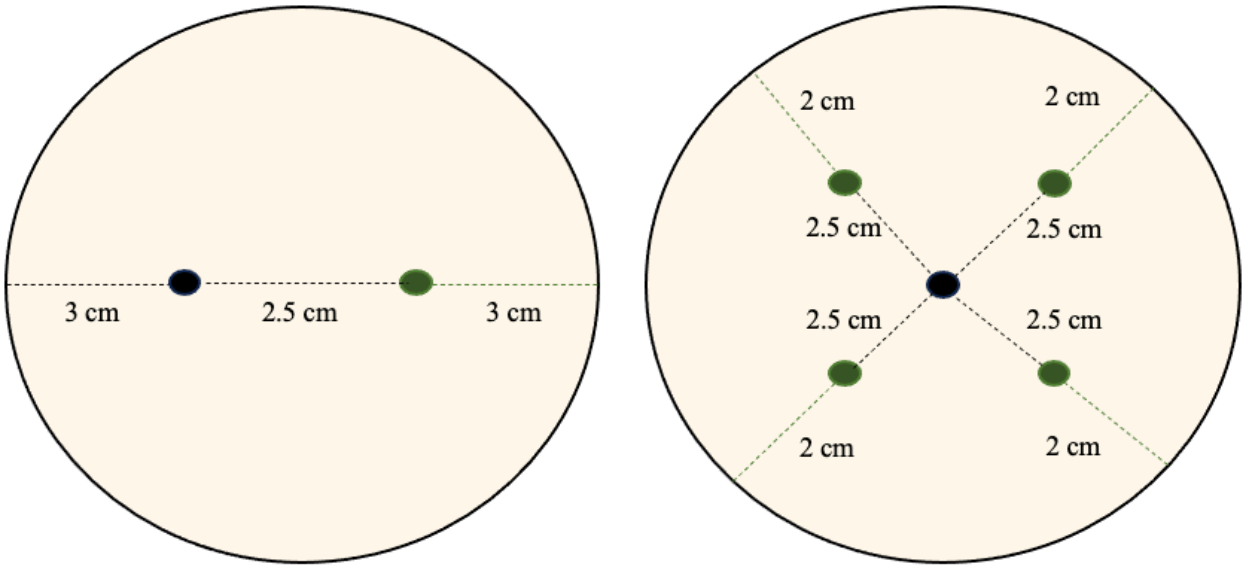

Figure 34. Spatial arrangement on Petri dish (90 mm by 15 mm) of **A.** Fungi-*V. dahliae* confrontation assays and **B.** Bacterial-*V. dahliae* confrontation assays. The black circles represent the *V. dahliae* isolate 5-mm plug, while the green circle represents either the fungal 5-mm plug **(A)** or the inoculation point for bacteria **(B)**. The black dashed lines represent the distance of the *V. dahliae* plug from the edge of the Petri dish and the fungal plug **(A)**, or the distance of the *V. dahliae* plug from the bacterial inoculation points **(B)**. The green dashed lines represent the distance of the fungal plug from the edge of the Petri dish **(A)**, or the distance of the bacterial inoculation points from the edge of the Petri dish **(B)**.

After four days of incubation, a 5-mm plug of the respective fungal antagonist was taken and placed 3 cm away from the edge, as represented in Figure 38. As for bacteria, with the use of a sterile micropipette tip, four bacterial point inoculations were made 2 cm away from the edge of the Petri dish as shown in Figure 34. After inoculation, the plates were sealed with ParaFilm® M Lab Film (Bemis Company Inc.) and incubated at room temperature (26 ºC ± 5 ºC) for 15 days in darkness. Each *V. dahliae* isolate had a total of six confrontation assays and eight controls. The controls consisted of the *V. dahliae* isolate alone, each antagonist alone, and the *V. dahliae* isolate growing on PDA media amended with azoxystrobin (FRAC group 11) and benzovindiflupyr (FRAC group 7) (Elatus®, Syngenta USA) and PDA media mended with azoxystrobin (FRAC group 11) (Quadris®, Syngenta USA), as the chemical fungicide controls. Each treatment had 4 replicates and the experiments were repeated twice. Every two days, colony growth was measured as shown in Figure 35. The four measurements were then averaged to obtain the colony radial growth. The mycelial growth inhibition percentage (MGI%) was calculated as follows (Poveda et al. 2022):

$$MGI\% = \left[\left(\frac{MGc - MGt}{MGc}\right)\right] \times 100$$

Where "MGt" corresponds to the mycelial growth of *V. dahliae* in confrontation with the antagonist and "MGc" corresponds to the mycelia growth of *V. dahliae* alone.

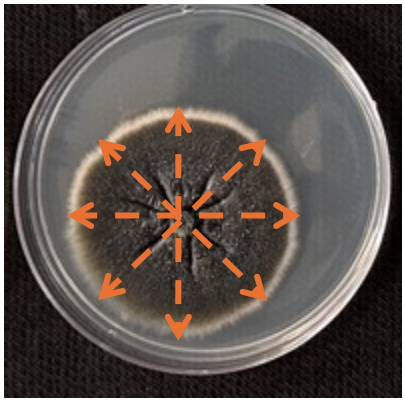

Figure 35. Measuring method of colony radial growth. Given that in some instances the colonies do not grow in an even circular growth, four direction measurements were taken represented with the orange dashed lines. The four measurements were then averaged to obtain the colony radial growth.

In vivo assays on potato cv. Russet Norkotah

Two repeated greenhouse experiments were established at the Michigan State University Plant Pathology Greenhouse complex (East Lansing, MI) to determine the *in vivo* efficacy of the biological control agents that reduced the Michigan *V. dahliae* isolates Vd1 and Vd2 colony growth by more than 60% in the *in vitro* experiments. For this, certified disease-free potato cv. Russet Norkotah tubers were obtained from Walther Farms (Three Rivers, MI) given its susceptibility to *V. dahliae* (Pasche et al. 2013). Tubers were cut into 56g seed pieces and one seed per pot was planted into a mix of 2:1 sand and soilless growing media in 2 L pots. Plants were fertilized at planting and every two weeks thereafter (15 ml/7.6 L, Peters' Professional 20-10-20 N-P-K, ICL Specialty Fertilizers, Dublin, OH), and kept at 16h:8h light: dark photoperiod at 26℃ for 2 weeks.

Simultaneously, colonies of both *V. dahliae* isolates were started in the laboratory for future conidia extraction. After the 2-week growth period, conidia of the Michigan *V. dahliae* isolates were collected by adding 5mL of sterile distilled water on top of 15 day-PDA cultures, scraping the surface, and filtering the conidial solution through a 100µm metal mesh (Figure 36). The inoculums were carefully transported to the greenhouse where plants were uprooted, the roots were washed with water and then inoculated by root dipping for 20 minutes into a conidial suspension of $1x10^6$ mL^{-1} according to Bubici et al. 2019.

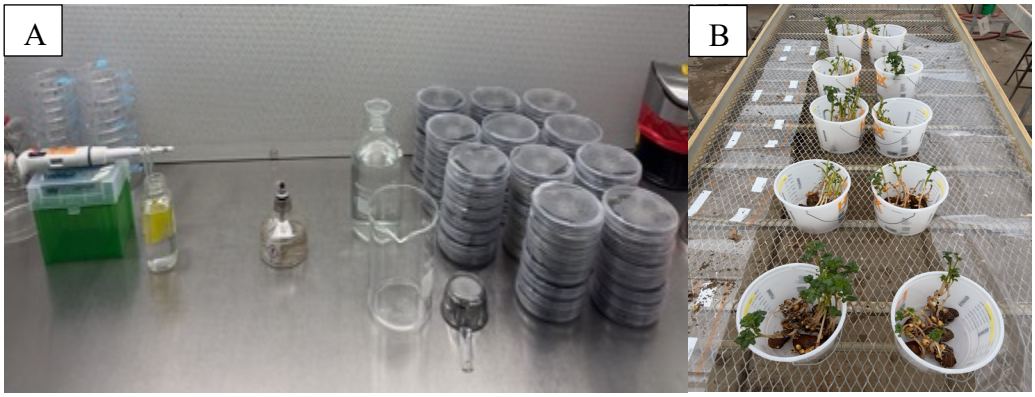

Figure 36. A. Extraction of *V. dahliae* conidia from 15 day-PDA cultures. **B.** Potato root dipping in the respective *V. dahliae* inoculum for 20 minutes.

The experimental design for these experiments was a randomized block design with three treatments and three controls. One experiment was designed to evaluate the effectiveness of the treatments *B. amyloliquefaciens* strain D747 (Double Nickel® LC; Certis USA LLC), *S. lydicus*

strain WYEC 108 (Actinovate® SP; Novonesis) and *T. asperellum* strain ICC 012 and *T. gamsii* strain ICC 080 (Tenet® WP; Isagro USA) on Michigan *V. dahliae* Isolate 1, while the second experiment was to evaluate the effectiveness of the same treatments but on Michigan *V. dahliae* Isolate 2 (Table 9). Controls for each experiment consisted of non- *V. dahliae* inoculated plants/non-treated, *V. dahliae* inoculated plants/non-treated which instead were treated with water, and *V. dahliae* inoculated plants treated with azoxystrobin (FRAC group 11) and benzovindiflupyr (FRAC group 7) (Elatus®, Syngenta USA), as the chemical fungicide control.

Table 9. Biological control agents' formulations were included in the greenhouse experiment to determine their efficacy in controlling the Michigan *V. dahliae* Isolates 1 and 2, plus the chemical-based fungicide control.

Generic Name	Active Ingredient	Rate (grams/gallon of water)	Application Method and Time
Double Nickel® LC	*Bacillus amyloliquefaciens* str. D747	2.27	Drench every two weeks
Actinovate® SP	*Streptomyces lydicus* str. WYEC 108	1.42	Drench every two weeks
Tenet® WP	*Trichoderma asperellum* str. ICC 012	1	Drench every two weeks
	Trichoderma gamsii str. ICC 080		
Elatus®	azoxystrobin and benzovindiflupyr	0.7	Drench every two weeks

Each treatment had five replicates, for a total of 30 pots per *V. dahliae* isolate experiment. The first application of Double Nickel® LC, Actinovate® SP, and Tenet® WP was done four days after *V. dahliae* inoculation, and a second one was done two weeks after. Rates for each of the

products were selected according to the label, and product applications were done by drenching each pot with 200 mL of solution. Plants were kept at a 16h:8h light: dark photoperiod at 26℃ for 30 days after *V. dahliae* inoculation and the experiments were repeated twice.

Plant height measurements were taken every week post-inoculation. In addition, disease severity was rated every week starting from one week after inoculation, following the Verticillium wilt scale established by Alkher et al. 2009. At the end of the experiment, plants were transported to the Applied Nematology Lab to measure plant biomass according to Bélanger et al. 2001, and record tuber weight and number (Figure 37).

Figure 37. A. Plants were transported to the Applied Nematology Lab and taken out of the pot for further evaluation. **B.** The aerial sections of the plant were cut and measured. **C.** The aerial section of the plant after oven-dried to determine plant biomass. **D.** Potato tubers harvested.

Statistical analysis

Data collected from the *in-vitro* and greenhouse experiments were analyzed using R version 2023.01 (R Core Team, 2023). For the *in-vitro* experiments, a linear mixed-effect model (LMER) was the best fit for the data using the package 'lme4' (Bates et al., 2014). In this model, the response variable was colony growth inhibition percentage, while experiment, treatment, and isolate were considered fixed effects. In addition, replicate and experiment, isolate and replicate, and treatment, replicate and experiment were considered nested random effects. Then, an ANOVA of the model was conducted. If treatment was found to be significant, Tukey's Honest Significant Difference (HSD) post-hoc tests (α=0.05) with a Bonferroni adjustment were conducted for means separation for each of the isolates, followed by pairwise comparison and compact letter of display of pairwise comparisons (CLD) using the packages 'emmeans', 'MASS', and 'mulcomp' (Lenth 2019; Venables and Ripley 2002; Hothorn et al. 2008).

For the greenhouse trials, experiments were analyzed separately (Vd1 and Vd2). Plant height measurement data was analyzed by first conducting an ANOVA to determine the effect of treatment, days after inoculation (DAI), and replicate, followed by a General Linear Model (GLM) with treatment and DAI as fixed effects, and height as the response variable. For the total number of tubers, a GLM was the best fit with treatment and replicates as fixed effects and the tuber number as the response variable, followed by an ANOVA to determine if the fixed effects had a significant effect on the response variable. As for tuber weight, a linear mixed-effects model (LMER) was the best fit, including tuber weight as the response variable, treatment as the fixed effect, and replication as a random effect, using the package 'lme4'(Bates et al., 2014), followed by an ANOVA to determine if treatment had a significant effect. A Tukey's Honest Significant Difference (HSD) post-hoc test (α=0.05) with a Bonferroni adjustment was conducted for means separation. Contrast analyses with pairwise comparisons were conducted to determine significant differences between treatments and the "Inoculated/Non-treated" control. Lastly, the compact letter of display of pairwise comparisons (CLD) was done using the packages 'emmeans', 'MASS', and 'mulcomp' (Lenth 2019; Venables and Ripley 2002; Hothorn et al. 2008).

To calculate de area under the disease progress curve (AUDPC), the function 'audpc' from the package 'agricolae' was used (de Mendiburu and Taseen, 2020). Then, a linear mixed-effects model (LMER) was found to be the best fit for the generated AUDPC data using the package 'lme4' (Bates et al., 2014). The AUDPC values were considered as the response variable, while treatment was the fixed effect, and repetition and experiment were the nested random effects. Then the significance of treatment was determined by conducting an ANOVA, and if significant, a Tukey's Honest Significant Difference (HSD) post-hoc test (α=0.05) with a Bonferroni adjustment was conducted for means separation. Contrast analyses with pairwise comparisons were conducted to determine significant differences between treatments and the "Inoculated/Non-treated" control. Lastly, the compact letter of display of pairwise comparisons (CLD) was done using the packages 'emmeans', 'MASS', and 'mulcomp' (Lenth 2019; Venables and Ripley 2002; Hothorn et al. 2008).

All graphs, except for the disease severity progress curves, were made with Microsoft® Excel (Version 18.81), and CLD letters were added onto graphs manually. As for the disease severity

progress curves, they were made in R version 2023.01 (R Core Team, 2023) using the package 'ggplot2' (Wickham 2016).

4.3 RESULTS

Mycelial growth inhibition percentage of *Verticillium dahliae* isolates 1, 2, and 3 when confronted with the different biological control agents

For all three *V. dahliae* isolates, treatment had a significant effect on the percentage of colony growth inhibition (Michigan *V. dahliae* isolate 1: DF = 9, F-value $_{(9, 27)}$ = 329.3395, P-value = <0.0001; Michigan *V. dahliae* isolate 2: DF = 9, F-value $_{(9, 27.8321)}$ = 203.740, P-value = <0.0001; Wisconsin *V. dahliae* isolate: DF = 9, F-value $_{(9, 27.6963)}$ = 79.8015, P-value = <0.0001). In addition, these effects were significantly different among isolates (DF= 2, F-value $_{(2, 6.376)}$ = 7.99, P-value = 0.01828), and there was a significant interaction between treatment and isolate (DF = 18, F-value $_{(18, 114.033)}$ = 18.4864, P-value = <0.0001) (Table 10).

For Michigan *V. dahliae* Isolate 1, pairwise comparisons showed that all treatments were significantly different from the control (p-value<0.0001) (Figure 38A). The highest colony growth inhibition was observed in the PDA media amended with azoxystrobin and benzovindiflupyr (100% inhibition), in which after 15 days of incubation, *V. dahliae* was mostly growing on the PDA plug. Colony growth inhibition was also high in the dual cultures with *T. gamsii* and *T. asperellum* with an average percentage of colony growth inhibition of 80.5% and 72.3%, respectively. It was interesting to observe that at 5 days after inoculation with either of these *Trichoderma* species, *V. dahliae* was outgrown when the *Trichoderma* species covered the whole area of the Petri dish (Figure 38B). *Streptomyces lydicus* also showed a high inhibition of Michigan-*V. dahliae* Isolate 1 (63.6%) and this was particularly interesting given how slow *S. lydicus* grew in comparison to other antagonists like the *Trichoderma* species (Figure 38A). On the contrary, the lowest Michigan *V. dahliae* Isolate 1 colony growth inhibition was observed in the dual cultures with *C. rosea* (12.3%), followed by azoxystrobin (26.8%), and *P. lilacinum* (34.1%).

For Michigan *V. dahliae* Isolate 2, all treatments except for *C. rosea* were significantly different than the control (p-value=0.8503) (Figure 38A). The highest percentage of colony growth inhibition was observed in dual cultures with *T. asperellum*, *S. lydicus*, *T. gamsii,* and azoxystrobin and benzovindiflupyr with an average of 79.0%, 71.8%, 70.1% and 65.9%, respectively. For this isolate, it was also interesting to see how the *Trichoderma* species outgrew

V. dahliae by covering the whole area of the petri dish by the 5th day after inoculation (Figure 38B). As for *S. lydicus*, it was also interesting to see that despite the slow growth of this actinobacteria it highly reduced *V. dahliae* colony growth. Interestingly, pairwise comparisons showed no difference between *S. lydicus* - *T. asperellum* (p-value = 0.31469), *S. lydicus* - *T. gamsii* (p-value = 0.9998), *T. asperellum* - *T.gamsii* (p-value = 0.1010). Different from Michigan *V. dahliae* Isolate 1, when Isolate 2 was grown on PDA amended with azoxystrobin and benzovindiflupyr, this isolate was able to grow out of the PDA plug and into the amended media but not as much as it did on PDA amended with just azoxystrobin, where the colony growth inhibition was just of 15.2%. Similarly, to Isolate 1, the lowest colony growth inhibition of Isolate 2 was observed in dual cultures with *C. rosea* (4.5%) (Figure 38A).

As for the Wisconsin *V. dahliae* Isolate, all the treatments were significantly different from the control except azoxystrobin (p-value = 1.0000) (Figure 38A). The highest percentage of colony growth inhibition was observed in dual cultures with *T. gamsii*, followed by *T. asperellum*, azoxystrobin and benzovindiflupyr, and *S. lydicus* with an average of 79.9%, 72.1%, 75.7% and 70.8%, respectively. Similar to the Michigan isolates, the Wisconsin isolate was outgrown by the *Trichoderma* species when these grew throughout the area of the Petri dish by the 5th day after inoculation (Figure 38B). As for *S. lydicus*, despite the slow growth of this actinobacteria it was interesting to see how much it was able to inhibit *V. dahliae* growth. Pairwise comparison showed no significant difference between *S. lydicus* - *T. asperellum* (p-value = 1.0000), *S. lydicus* - *T. gamsii* (p-value = 0.6881), *T. asperellum* - *T.gamsii* (p-value = 0.8406). Similar to Michigan Isolate 2, on the PDA amended with azoxystrobin and benzovindiflupyr, the Wisconsin isolate was able to grow out of the PDA plug and into the amended media. In contrast, there was an increase in growth of the Wisconsin isolate when grown in PDA amended with azoxystrobin, being 0.4% bigger than the control (Figure 38A).

Table 10. Inhibition of the 3 different *V. dahliae* isolates colony growth on potato dextrose agar (PDA) after 15 days in dual culture with the different biological control agents, and in fungicide-amended PDA as the chemical reference. The *V. dahliae* isolates were grown for 4 days before plating the respective biological control agents. Results are expressed as the mean of the replicated experiment with the corresponding standard error of the mean (SEM). Means followed by the same letter within a column are not significantly different according to Tukey's honestly significant difference test (P-value ≤ 0.05).

Treatment	Michigan-*V. dahliae* Isolate 1	Michigan-*V. dahliae* Isolate 2	Wisconsin-*V. dahliae* Isolate
	Percentage of colony growth inhibition (Mean ± SEM)	Percentage of colony growth inhibition (Mean ± SEM)	Percentage of colony growth inhibition (Mean ± SEM)
Control	0 a	0 a	0 a
Bacillus amyloliquefaciens str. D747	48.4 ± 1.9 d	51.4 ± 2.5 c	42.8 ± 0.2 c
Bacillus subtilis str. QST 713	52.4 ± 1.6 d	49.9 ± 2.6 c	36.7 ± 2.7 c
Streptomyces lydicus str. WYEC 108	63.6 ± 1.0 e	71.8 ± 1.5 de	70.8 ± 2.0 d
Trichoderma asperellum str. ICC 012	72.3 ± 1.8 ef	79.0 ± 1.8 e	72.1 ± 2.3 d
Trichoderma gamsii str. ICC 080	80.5 ± 0.8 f	70.1 ± 1.3 de	79.9 ± 2.1 d
Clonostachys rosea str.J1446	12.3 ± 2.8 b	4.5 ± 5.6 a	18.8 ± 3.9 b
Purpureocillium lilacinum PL225	34.1 ± 4.4 c	42.4 ± 3.0 c	31.8 ± 4.6 bc
Azoxystrobin and benzovindiflupyr	100 ± 0 g	65.9 ± 1.5 d	75.7 ± 1.2 d
Azoxystrobin	26.8 ± 2.6 c	15.2 ± 4.1 b	-0.4 ± 3.0 a
P-values	*<0.0001*	*<0.0001*	*<0.0001*

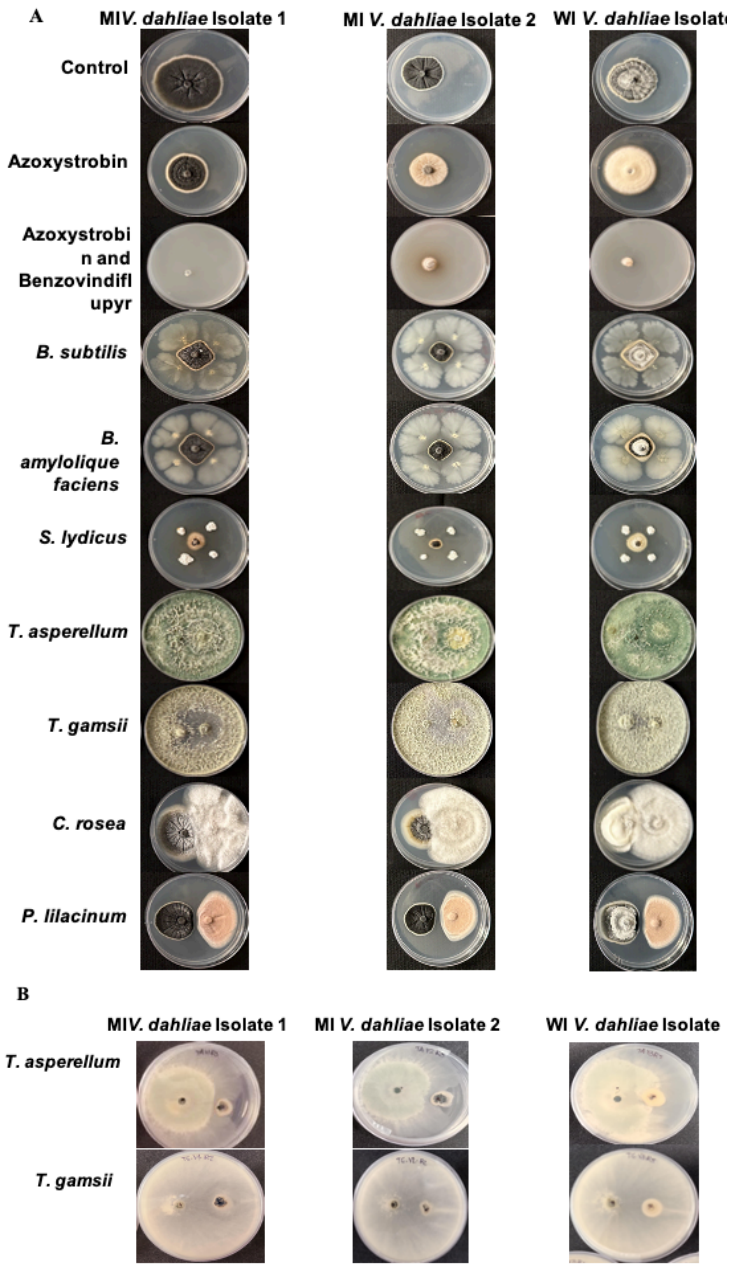

Figure 38. A. Photographs of the dual culture *in-vitro* study between Michigan *V. dahliae* isolate 1, Michigan *V. dahliae* isolate 2, Wisconsin *V. dahliae* isolate, and *B. subtilis, B. amyloliquefaciens, S. lydicus, T. asperellum, T. gamsii, C. rosea and P. lilacinum*. In addition, the controls of each *V. dahliae* isolate grown alone, and the positive chemical fungicide controls azoxystrobin, and azoxystrobin and benzovindiflupyr. Photographs show confrontation assays at 15 days after incubation period. **B.** Inhibition by *T. asperellum* and *T. gamsii* at day 5 of confrontation assay against Michigan *V. dahliae* isolate 1, Michigan *V. dahliae* isolate 2, Wisconsin *V. dahliae* isolate.

Effect of the different biological control agents on Michigan *Verticillium dahliae* isolates 1 and 2 severity in potato cv. Russet Norkotah, plant height, and tuber yield

Michigan *Verticillium dahliae* Isolate 1

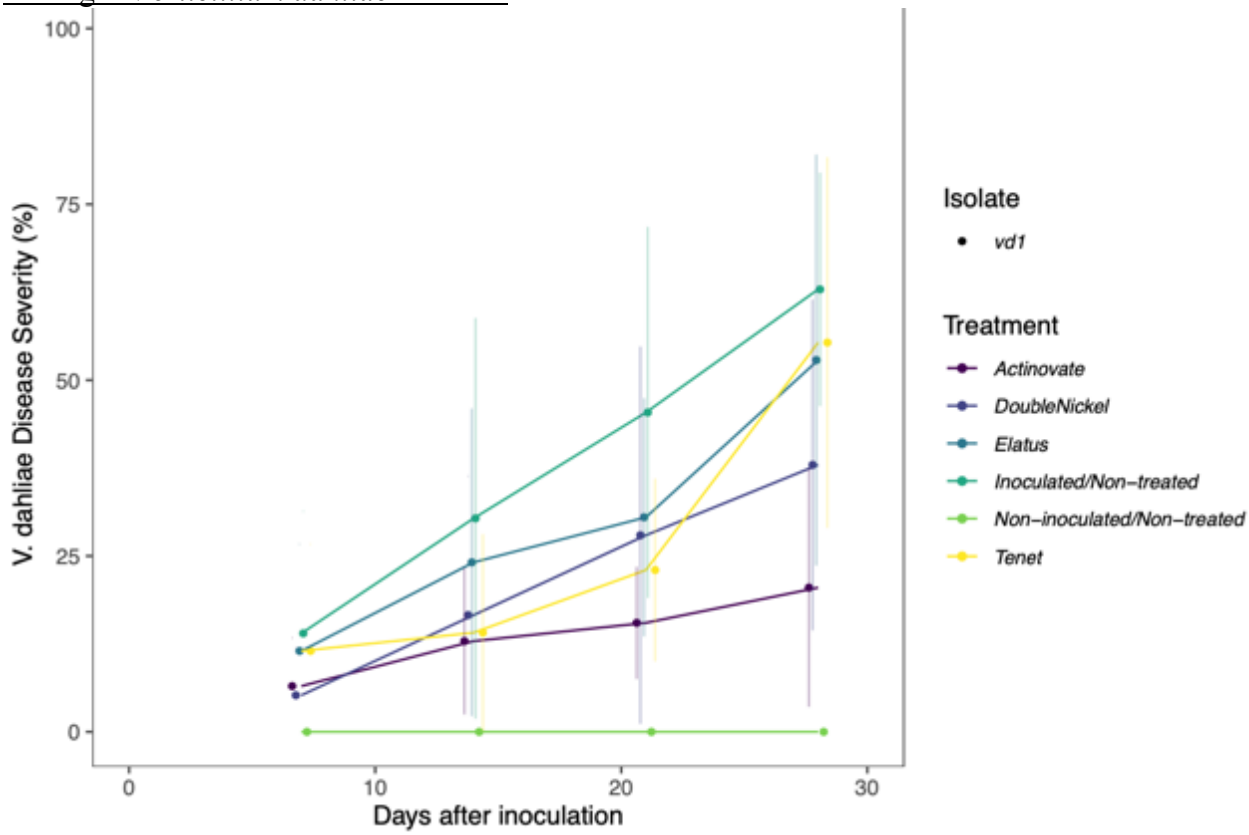

Figure 39. Michigan-*V. dahliae* Isolate 1 disease severity progress across time. The *y-axis* represents the point in time at which disease severity was recorded in days after inoculation (DAI), while the *x-axis* represents the percentage of disease severity for the respective sampling point. The different line colors represent each of the evaluated treatments and controls described in the legend of the figure (Tenet® (a.i. *T. asperellum* str. ICC 012 and *T. gamsii* str. ICC 080), Actinovate® (a.i. *S. lydicus* str. WYEC 108), Double Nickel® (a.i. *B. amyloliquefaciens* str. D747) and Elatus® (a.i. azoxystrobin and benzovindiflupyr). Results are expressed as the mean of the two experiments conducted with the respective standard deviation.

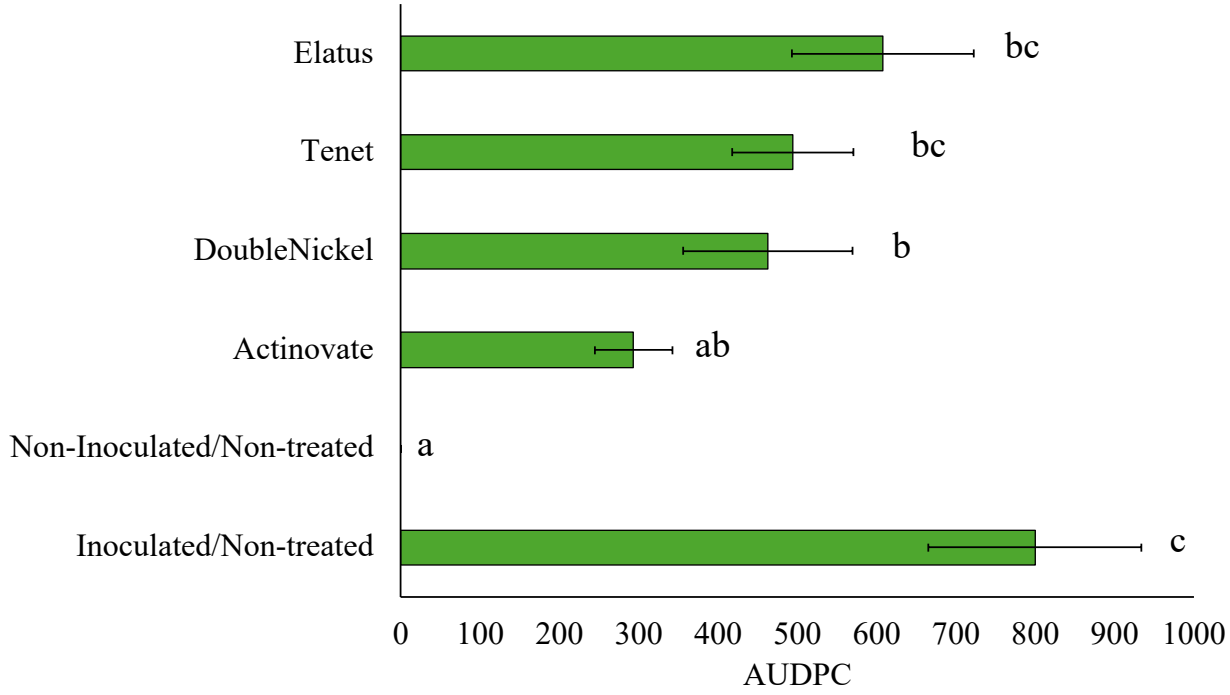

Figure 40. Michigan-*V. dahliae* Isolate 1 area under the disease progress curve (AUDPC) for each of the treatments evaluated in the greenhouse (Tenet® (a.i. *T. asperellum* str. ICC 012 and *T. gamsii* str. ICC 080), Actinovate® (a.i. *S. lydicus* str. WYEC 108), Double Nickel® (a.i. *B. amyloliquefaciens* str. D747) and Elatus® (a.i. azoxystrobin and benzovindiflupyr). AUDPC values are expressed as the mean of the two experiments with the respective standard error of the mean (SEM). The letters above each bar indicate significant pairwise differences in treatments compared to the "Inoculated/Non-treated" control (P-value<0.05).

The onset of symptom development was observed after 7 days post-inoculation. At this time point, the highest disease severity was observed in the "Inoculated/Non-treated" control with an average of 14%. In contrast, the lowest disease severity was observed in the "DoubleNickel" treatment with an average of 5.2%. As time progressed, disease severity increased significantly for all treatments (p-value<0.001). At 28 days post-inoculation, the highest disease severity was observed in the "Inoculated/Non-treated" control with an average of 62.9%, while the lowest disease severity was observed in the "Actinovate" treatment, with an average of 20.5% (Figure 39).

The AUDPC analysis showed that treatment had a significant effect on disease severity (DF = 5, F-value $_{(5, 50)}$ = 14.94, P-value<0.0001). Disease severity in plants treated with "Actinovate", "DoubleNickel" and "Tenet" were significantly lower than in the "Inoculated/Non-treated"

control (pairwise comparisons: p-value = 0.0002, p-value = 0.0274, and p-value = 0.0580, respectively). The lowest AUDPC value was observed on plants treated with "Actinovate" with an average value of 293.3 while the highest value was observed in the "Inoculated/Non-treated" (799.4) (Figure 40 and 41).

Figure 41. Photographs of the effect of the different treatments on Michigan *V. dahliae* isolate 1 after 40 days of growth in the greenhouse. **A.** non-inoculated/non-treated potato plants. **B.** inoculated/non-treated potato plants. **C.** *V. dahliae*-infected potato plants treated with Tenet® (*T. asperellum* str. ICC 012 and *T. gamsii* str. ICC 080). **D.** *V. dahliae*-infected potato plants treated with Actinovate® (*S. lydicus* str. WYEC 108). **E.** *V. dahliae*-infected potato plants treated with Double Nickel® (*B. amyloliquefaciens* str. D747). **F.** *V. dahliae*-infected potato plants treated with Elatus® (azoxystrobin and benzovindiflupyr).

Overall, treatment did not have a significant effect on plant height (DF = 5, F-value $_{(5,58)}$ = 1.511, P-value = 0.187). At 28 days after inoculation, plant height for all treatments ranged between 24.71 cm to 27.65 cm, with the highest plants being the ones treated with "Actinovate" (Figure 42).

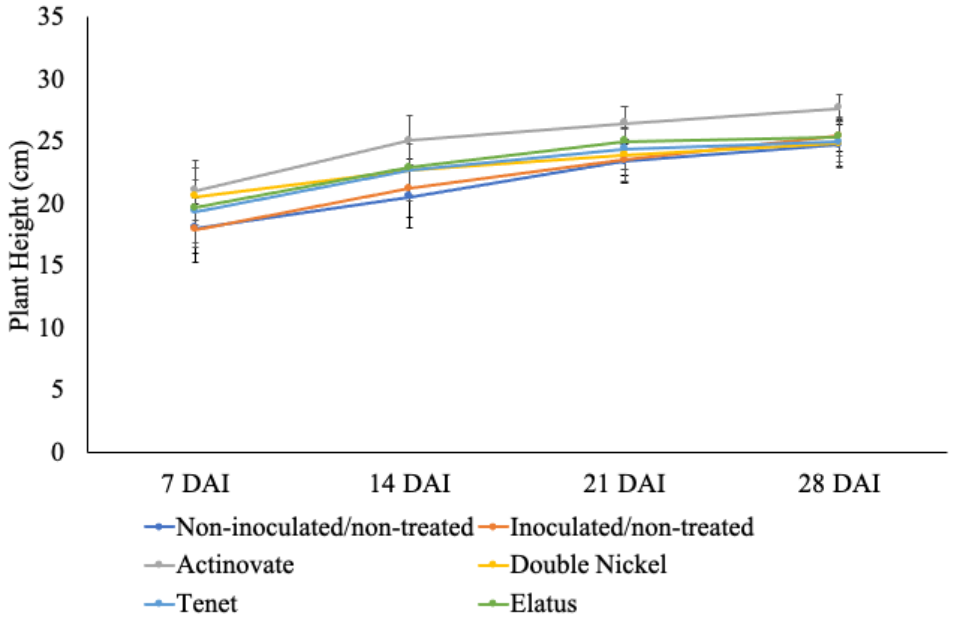

Figure 42. Plant height measurements taken every 7 days after inoculation with Michigan-V. dahliae Isolate 1 for the duration of the experiment (4 weeks) for each treatment (Tenet® (a.i. *T. asperellum* str. ICC 012 and *T. gamsii* str. ICC 080), Actinovate® (a.i. *S. lydicus* str. WYEC 108), Double Nickel® (a.i. *B. amyloliquefaciens* str. D747) and Elatus® (a.i. azoxystrobin and benzovindiflupyr). Results are expressed as the mean of 2 experiments with the respective standard error of the mean (SEM). The asterisk (*) above the DAI time represents significant pairwise differences of treatments compared to the "Inoculated/Non-treated" control (p-value<0.05).

The number of tubers produced by plants was significantly affected by treatment (DF= 5, F-value $_{(5, 29.6167)}$ = 13.7571, P- value < 0.0001) (Figure 43A). The highest average number of tubers produced was found in the "Non-inoculated/Non-treated" control (6 tubers). In contrast, the lowest number of tubers was found in the "Inoculated/Non-treated" control with an average of 2 tubers (pairwise comparison: p-value = 0.0004). Similarly, there were significantly more tubers in plants treated with "Actinovate", "DoubleNickel" and "Tenet" with an average of 4.7, 5.5, and 5.3 tubers, respectively (pairwise comparison: p-value = 0.012, p-value = 0.0012, p-value = 0.0017). However, total tuber weight was not significantly affected by treatment (DF = 5, F-value $_{(5, 54)}$ = 1.3496, P-value = 0.2579) (Figure 43B).

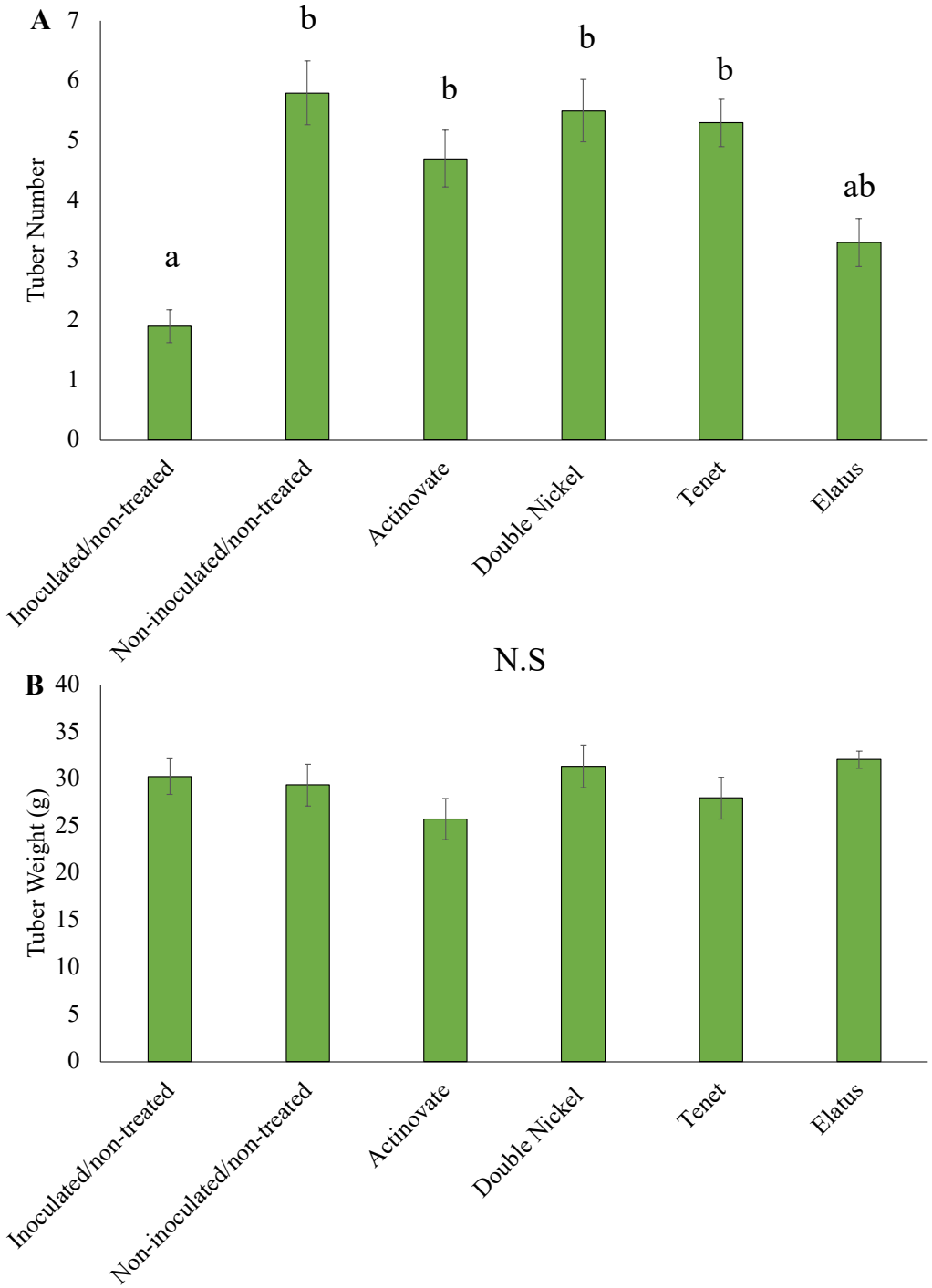

Figure 43. A. Average tuber number and **B.** Average tuber weight of total tubers 4 weeks after Michigan-*V. dahliae* Isolate 1 inoculation for each treatment (Tenet® (a.i. *T. asperellum* str. ICC 012 and *T. gamsii* str. ICC 080), Actinovate® (a.i. *S. lydicus* str. WYEC 108), Double Nickel® (a.i. *B. amyloliquefaciens* str. D747) and Elatus® (a.i. azoxystrobin and benzovindiflupyr). Results are expressed as the mean of 2 experiments with the respective standard error of the

Figure 43. (cont'd)

mean (SEM). The letters above each bar indicate significant pairwise differences of treatments compared to the "Inoculated/Non-treated" control and N.S. indicated "Not Significant"(p-value<0.05).

Verticillium dahliae Isolate 2

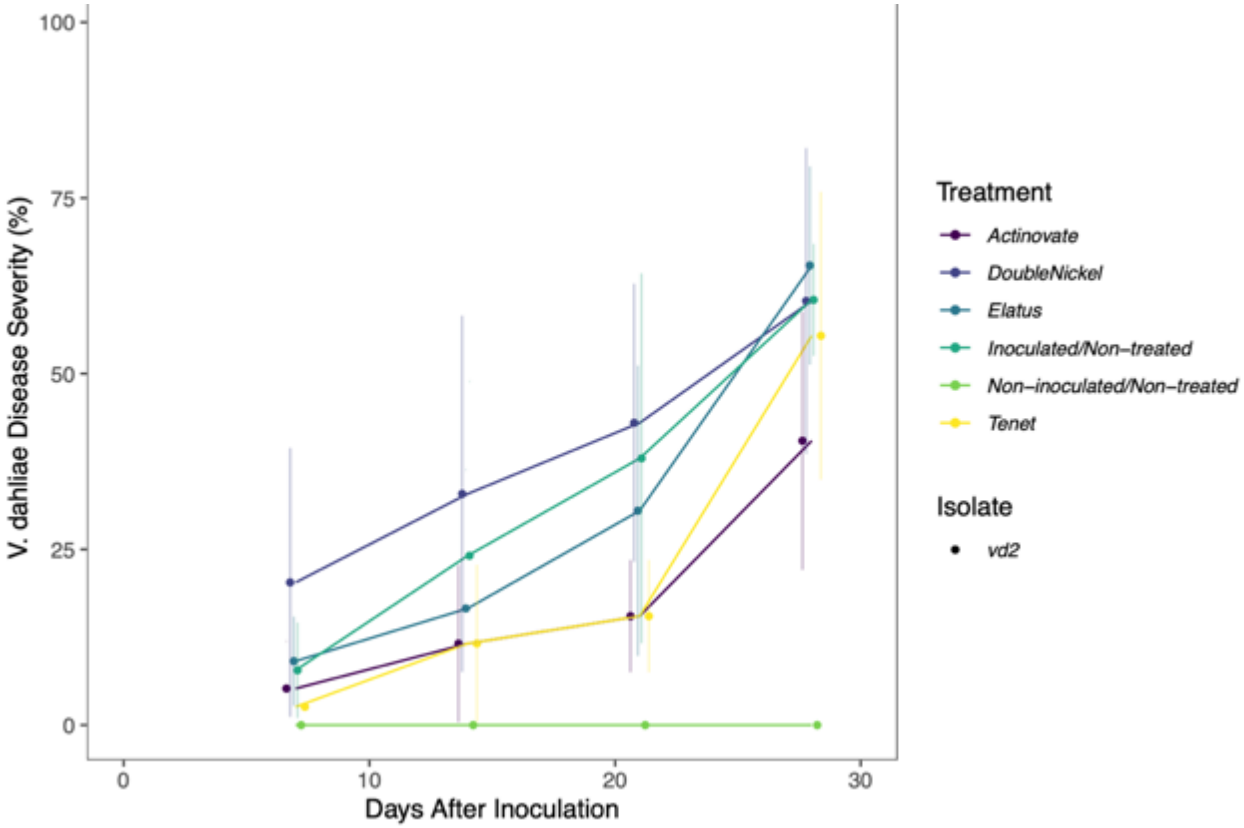

Figure 44. Michigan-*V. dahliae* Isolate 2 disease severity progress across time. The *y-axis* represents the point in time at which disease severity was recorded in days after inoculation (DAP), while the *x-axis* represents the percentage of disease severity for the respective sampling point. The different line colors represent each of the evaluated treatments and controls described in the legend of the figure (Tenet® (a.i. *T. asperellum* str. ICC 012 and *T. gamsii* str. ICC 080), Actinovate® (a.i. *S. lydicus* str. WYEC 108), Double Nickel® (a.i. *B. amyloliquefaciens* str. D747) and Elatus® (a.i. azoxystrobin and benzovindiflupyr). Results are expressed as the mean of the two experiments with the respective standard deviation.

The onset of disease symptoms was observed 7 days after inoculation. Interestingly, at this time point, the highest disease severity was observed in plants treated with "DoubleNickel" with an average of 20.3%. In contrast, the lowest disease severity was observed in plants treated with

"Tenet", with an average of 2.6%. As time passed, disease severity increased significantly (p-value<0.001). At 28 days after inoculation, the highest disease severity was observed in plants treated with "Elatus", "DoubleNickel" and the "Inoculated/Non-treated" control with an average of 65.4%, 60.4%, and 60.5%, respectively. At this time point, the lowest disease severity was observed in plants treated with "Actinovate" and "Tenet" with an average of 40.45% and 55.4%, respectively (Figure 44).

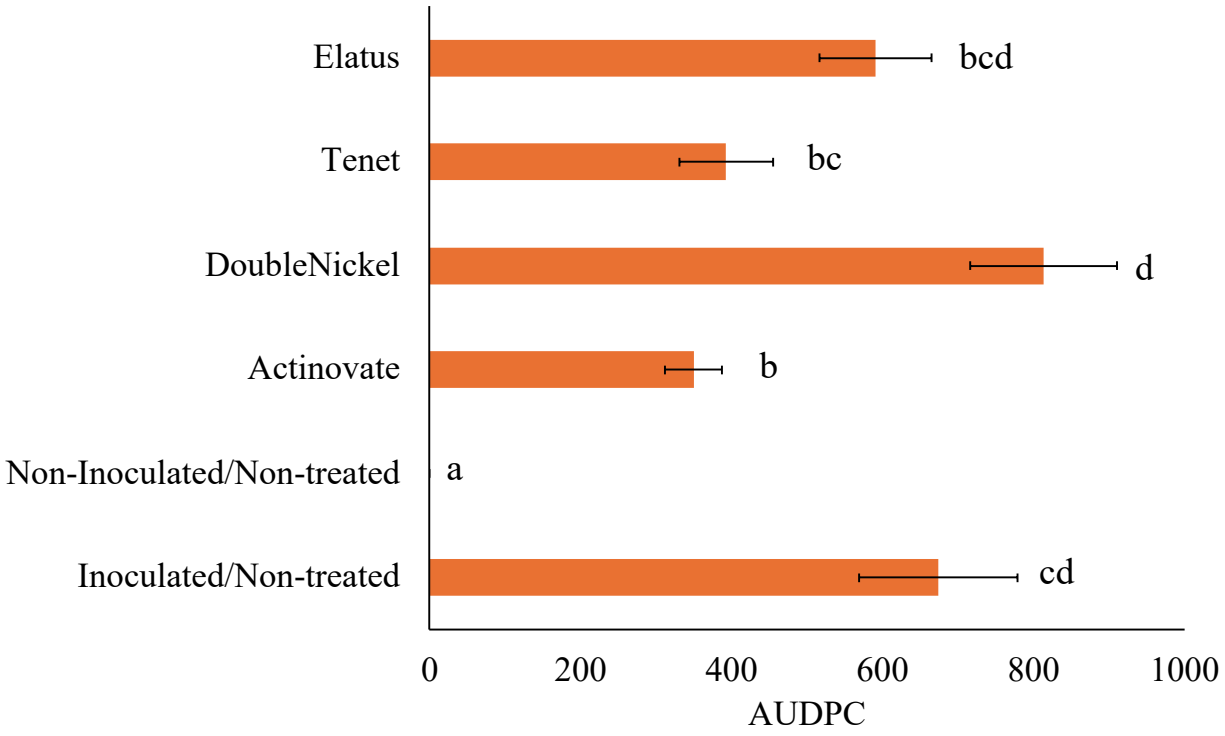

Figure 45. Michigan-*V. dahliae* Isolate 2 area under the disease progress curve (AUDPC) for each of the treatments evaluated in the greenhouse (Tenet® (a.i. *T. asperellum* str. ICC 012 and *T. gamsii* str. ICC 080), Actinovate® (a.i. *S. lydicus* str. WYEC 108), Double Nickel® (a.i. *B. amyloliquefaciens* str. D747) and Elatus® (a.i. azoxystrobin and benzovindiflupyr). AUDPC values are expressed as the mean of the two experiments with the respective standard error of the mean (SEM). The letters above each bar indicate significant pairwise differences of treatments compared to the "Inoculated/Non-treated" control (p-value<0.05).

The AUDPC analysis showed that the highest value was observed in plants treated with "DoubleNickel" (813.57), while the lowest was observed in plants treated with "Actinovate" (349.47). In the same way, contrast analysis showed that "Actinovate" and "Inoculated/Non-

treated" were significantly different (pairwise comparisons: p-value = 0.0139), and also "Tenet" and "Inoculated/Non-treated" (pairwise comparisons: p-value = 0.0476) (Figure 45 and 46).

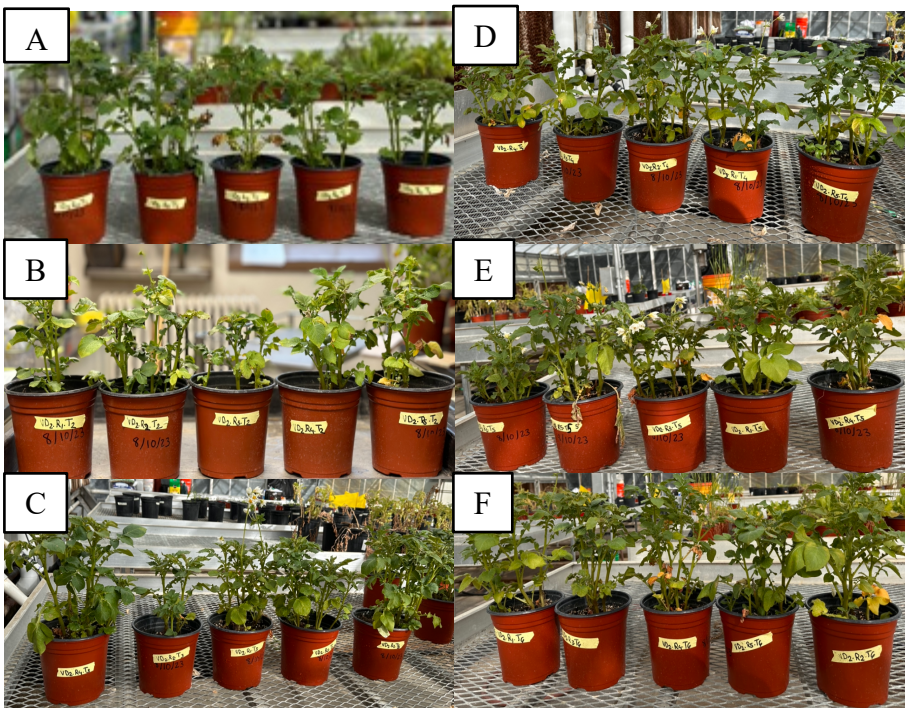

Figure 46. Photographs of the effect of the different treatments on Michigan *V. dahliae* isolate 2 after 40 days of growth in the greenhouse. **A.** non-inoculated/non-treated potato plants. **B.** inoculated/non-treated potato plants. **C.** *V. dahliae*-infected potato plants treated with Tenet (*T. asperellum* str. ICC 012 and *T. gamsii* str. ICC 080). **D.** *V. dahliae*-infected potato plants treated with Actinovate (*S. lydicus* str. WYEC 108). **E.** *V. dahliae*-infected potato plants treated with Double Nickel (*B. amyloliquefaciens* str. D747). **F.** *V. dahliae*-infected potato plants treated with Elatus (azoxystrobin and benzovindiflupyr).

Treatment had a slightly significant effect on height (DF = 5, F-value $_{(5,\ 103.5)}$ = 2.121, P-value = 0.0638). At the end of the experiment, despite the symptoms of chlorosis and wilt, the tallest plants were the ones treated with "DoubleNickel" and "Elatus" in comparison to the "Inoculated/Non-treated" control (pairwise comparisons: p-value = 0.0416 and p-value = 0.0117, respectively), with an average height of 28.2 cm and 28.9 cm, respectively, while the control was 20.2 cm (Figure 47).

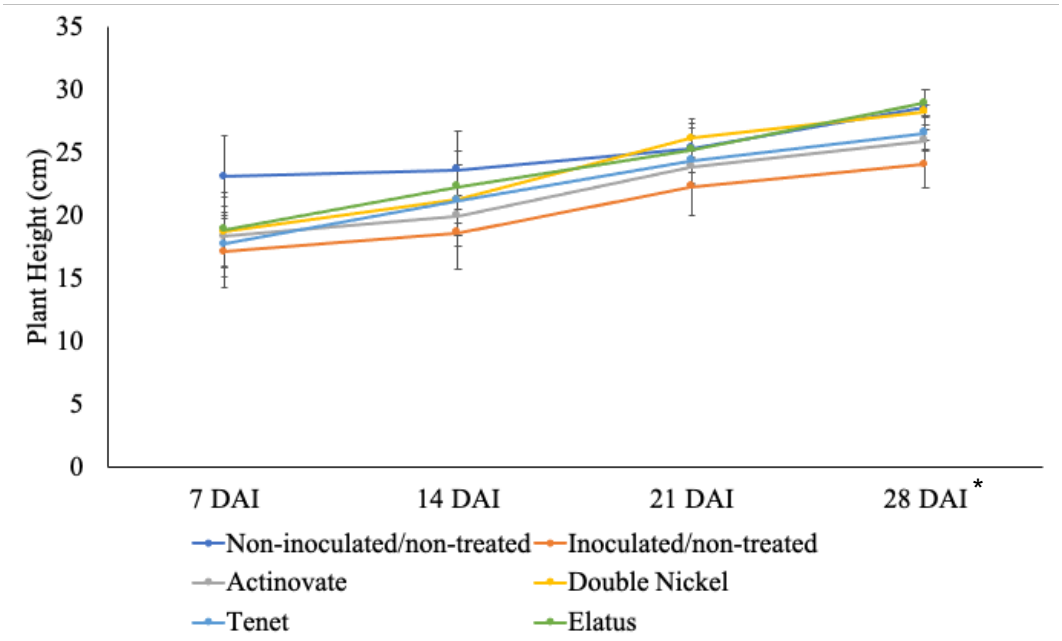

Figure 47. Plant height measurements taken every 7 days after inoculation with Michigan-V. dahliae Isolate 2 for the duration of the experiment (4 weeks) for each of the treatments (Tenet® (a.i. *T. asperellum* str. ICC 012 and *T. gamsii* str. ICC 080), Actinovate® (a.i. *S. lydicus* str. WYEC 108), Double Nickel® (a.i. *B. amyloliquefaciens* str. D747) and Elatus® (a.i. azoxystrobin and benzovindiflupyr). Results are expressed as the mean of 2 experiments with the respective standard error of the mean (SEM). The asterisk (*) above the DAI time represents significant pairwise differences of treatments compared to the "Inoculated/Non-treated" control (p-value<0.05).

The number of tubers produced by plants was significantly affected by treatment (DF= 5, F-value $_{(5, 23.7)}$ = 6.7445, P-value<0.0001) (Figure 48A). The highest number of tubers was observed in plants treated with "Actinovate" with an average of 6.5 tubers. In contrast, the lowest number of tubers was observed in the "Inoculated/Non-treated" control with an average of 2.3 tubers. Contrast analysis showed that the treatments "Actinovate", "DoubleNickel", "Tenet" and "Elatus" produced significantly more tubers than the "Inoculated/Non-treated" control (pairwise comparison: p-value = 0.0003, p-value = 0.0440, p-value = 0.0358, and p-value = 0.0029, respectively). Treatment also had a significant effect on tuber weight (DF = 5, F-value $_{(5, 50)}$ = 7.0532, P-value<0.0001) (Figure 48B). The highest weight of total tubers was found in the "Non-inoculated/Non-treated" control with an average weight of 35 g. In contrast, the lowest

weight was found for tubers from the "Inoculated/Non-treated" control with an average weight of 14.6 g. Contrast analysis showed that the treatments "Actinovate", "DoubleNickel", "Tenet" and "Elatus" had significantly heavier tubers than the "Inoculated/Non-treated" control (pairwise comparison: p-value = 0.0036, p-value = 0.0214, p-value = 0.0033, and p-value = 0.0090, respectively).

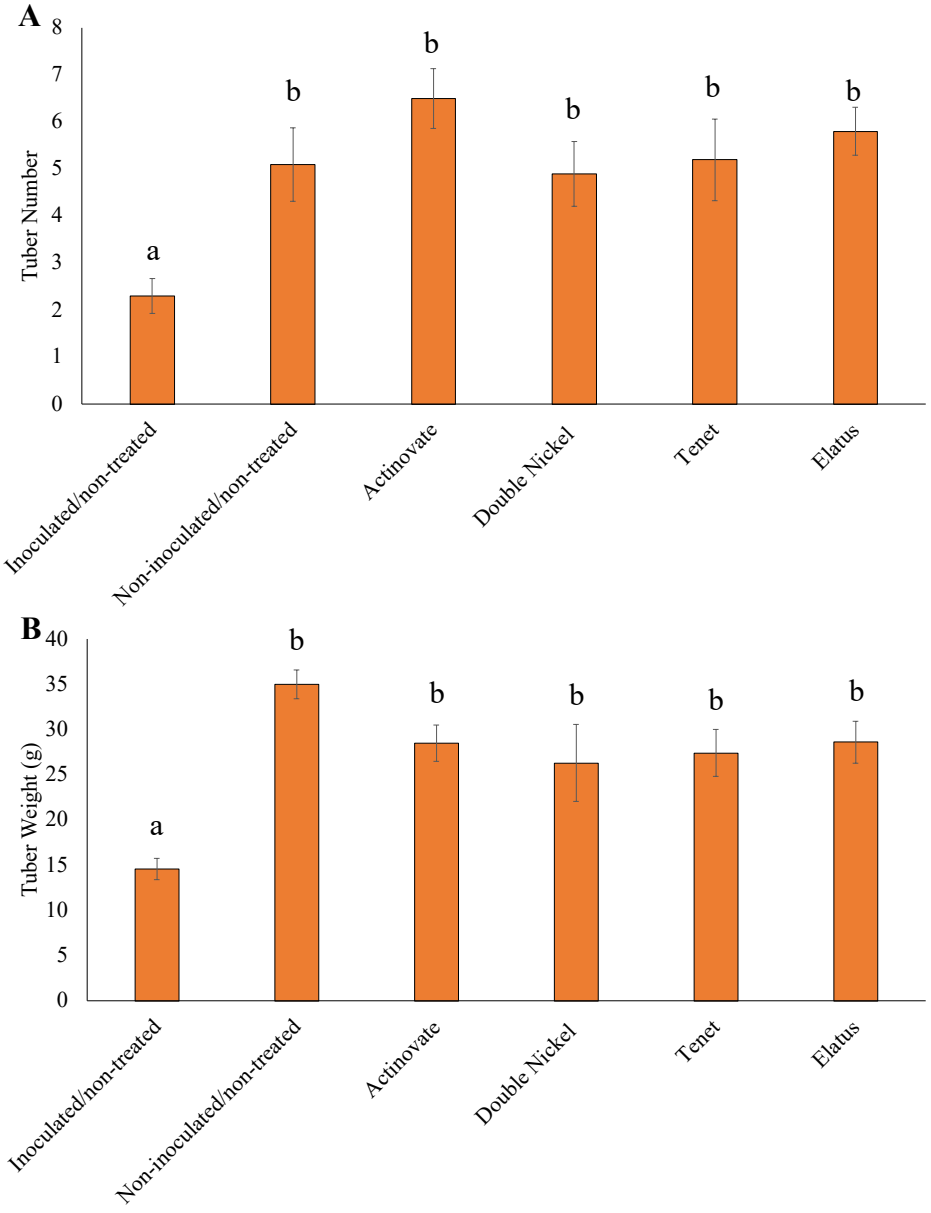

Figure 48. Average ± SEM of **A.** Tuber number and **B.** Tuber weight of total tubers 4 weeks after Michigan-*V. dahliae* Isolate 2 inoculation for each treatment (Tenet® (a.i. *T. asperellum* str. ICC 012 and *T. gamsii* str. ICC 080), Actinovate® (a.i. *S. lydicus* str. WYEC 108), Double

Figure 48. (cont'd)

Nickel® (a.i. *B. amyloliquefaciens* str. D747) and Elatus® (a.i. azoxystrobin and benzovindiflupyr). The letters above each bar indicate significant pairwise differences of treatments compared to the "Inoculated/Non-treated" control (p-value<0.05).

4.4 DISCUSSION

Verticillium dahliae management heavily relies on soil fumigation with the pesticide metam sodium (MS). When applied to soil with moisture, MS decomposes to the active compounds methyl-isothiocyanate (MITC), however, consecutive fumigation with MS can lead to an accelerated degradation of MITC, which results in lower effectiveness in controlling soil-borne pathogens (Di Primo et al. 2003). Currently, the use of metam sodium is highly regulated, and due to rising environmental and human health concerns around pesticide use, it is a matter of time before metam sodium is banned just as it happened with other soil fumigants like methyl-bromide. Thus, it is essential to invest efforts into determining the effectiveness of management alternatives for *V. dahliae*. In this chapter, I focused on biological control agents (BCAs) as a potential alternative to manage *V. dahliae*. First, presumptively *V. dahliae*-infected potato stem samples were collected from commercial potato fields for further purification of different isolates of this pathogen. From the isolates that were confirmed to be *V. dahliae*, 2 were selected to conduct the studies. For the *in-vitro* experiments, a highly aggressive and well-studied *V. dahliae* isolate from Wisconsin was also included to contrast with our Michigan isolates. The BCAs included in this experiment were selected based on being commercially available and having listed on the label that they are effective against *Verticillium* spp. in potatoes. For the greenhouse experiments, only the products that showed >50% colony growth inhibition were included to be evaluated against Michigan Isolate 1 and 2.

From my in-vitro studies, the BCAs had a variable effect for each of the three isolates evaluated. However, for the three *V. dahliae* isolates, *Trichoderma gamsii* strain ICC 080, *T. asperellum* strain ICC 012, and *Streptomyces lydicus* strain WYEC 108 showed the highest percentage of *V. dahliae* colony growth inhibition (>60%). As for the greenhouse assays, the disease severity observed in the "Infected/Non-treated" control was not significantly different between the Michigan isolates (Michigan Isolate 1: 62.9% and Michigan Isolate 2: 60.5%), however, the disease control provided by the evaluated treatments was different between the isolates.

From the *in-vitro* experiments, it was interesting to observe that the *Trichoderma* species outgrew the *V. dahliae* isolates by the 5th day of dual culturing. Carrero-Carrón et al. 2016 had a similar observation. The authors found that three different strains of *T. asperellum* overgrew the colony of different olive pathotypes of *V. dahliae* and that the diffusible compounds of low molecular weight (<14 kDa) secreted by only two of the *T. asperellum* isolates showed more antifungal activity against *V. dahliae*. Mycoparasitism and antibiosis are the main mechanisms by which *Trichoderma* spp. suppress plant pathogens (Druzhinina et al. 2011). Literature suggests that the secretion of hydrolytic enzymes facilitates the disruption of host structures (Schirmböck et al. 1994), hence the results from my study may suggest that the *Trichoderma* species were successful at reducing *V. dahliae* colony growth due to hydrolytic enzyme activity induced by contact with *V. dahliae*, however, such extracellular compounds should be characterized in future experiments to elucidate potential mechanisms of antagonism and differences between the *Trichoderma* species. In my experiment, although not significantly different, the percentage of *V. dahliae* colony growth inhibition between *T. gamsii* and *T. asperellum* was different among *V. dahliae* isolates. For Michigan *V. dahliae* isolate 1 and Wisconsin *V. dahliae* isolate, *T. gamsii* showed the highest inhibition (80.5% and 79.97%, respectively), while for Michigan *V. dahliae* isolate 2, *T. asperellum* showed the highest inhibition (79%). This could indicate that the effectiveness of *Trichoderma* is dependent on the species/strain and also the *V. dahliae* isolate. For instance, in a recent study, 39 *Trichoderma* strains' antagonistic activity against *V. dahliae* was evaluated and the colony growth inhibition ranged between 32%-90%, being the highest (>90%) for just 6 of the *Trichoderma* strains. In addition, the authors also found that the effect of these strains varied between the two *V. dahliae* isolates evaluated (Carrasco et al. 2023). As for my greenhouse experiments, for both Michigan Isolate 1 and 2, the active ingredients of Tenet® (a mixture of *T. gamsii* and *T. asperellum*) resulted in similar disease severity values at the end of the experiment. Michigan Isolate 1 inoculated potato plants that were treated with Tenet® had a disease severity of 55.3%, while for Michigan Isolate 2 plants treated with Tenet® had a disease severity of 55.4%. Carrero-Carrón et al. 2016 determined the effectiveness of two strains of *T. asperellum* on *V. dahliae* in olives and found that prior root colonization by *T. asperellum* potentially significantly reduces the onset of Verticillium wilt in olives and they go on to discuss that prior root colonization is crucial for *Trichoderma* antagonism, plant growth promotion, and induced systemic resistance (Shoresh et

al. 2010). In my experiment, Tenet® was applied by soil drench 4 days post-*V. dahliae* root dipping inoculation, which may suggest that if Tenet® had been applied before *V. dahliae* inoculation, less disease severity would have been observed, however, different application timings and methods should be evaluated in future experiments.

Similarly, from the *in-vitro* experiments, a significantly high percentage of colony growth inhibition was observed in dual cultures with *S. lydicus*. Despite the lower growth rate of *S. lydicus*, *V. dahliae* colony growth inhibition was >60% for all three isolates. A study conducted by Yuan and Crawford, 1995, found that the colony growth of strains of *Pythium ultimum*, *Aphanomyces euteiches*, *Fusarium oxysporum*, *F. solani,* and *Rhizoctonia solani* was significantly restricted after 5 days in dual culture with *S. lydicus*. The same authors evaluated the morphology of the pathogen's hyphae that were facing the colony of *S. lydicus and* found no abnormal morphology or lysis of hyphal tips. The authors then indicate that the mechanism by which *S. lydicus* limits pathogen colony growth may be due to the secretion of diffusible antifungal compounds. The antagonistic activity of *Streptomyces* spp. has been found to be due to the activity of chitinases, glucanases, antibiotics, and volatile compounds (Pacios-Michelena et al. 2021). In the confrontation assays, *S. lydicus* colonies did not come in contact with *V. dahliae* colonies which may suggest that the high colony growth inhibition may have been due to the release of antibiotics or volatile compounds; it would be significant to characterize such compounds in future experiments to understand the mechanisms of *S. lydicus* antagonism. Additionally, in our study we did not find a significant effect of *V. dahliae* isolate on *S. lydicus* inhibitory potential, however, a study that screened the activity of *S. lydicus* against 148 *Pythium* isolates found that although *S. lydicus* inhibited all pathogen isolates colony growth, the percentage growth inhibition significantly varied amongst them (Weiland, 2014). In my study, only 3 *V. dahliae* isolates were considered, and although *S. lydicus* was effective at limiting growth for all three isolates, it would be essential to include more *V. dahliae* isolates in future antagonism screenings because of the complexity of *V. dahliae* populations (different races, defoliating/non-defoliating, and vegetative compatibility groups) (Chen et al. 2021). As for the performance of *S. lydicus* (Actinovate®) under greenhouse conditions, it was one of the products that provided the best control of *V. dahliae*, although the disease severity at the end of the experiment was different for both Michigan isolates. For Michigan Isolate 1, the disease severity was 20.5% while for Isolate 2 disease severity was 40.4%, but for both, it was the treatment with

the lowest disease severity. This could indicate that the degree of effectiveness could be dependent on the *V. dahliae* isolate, therefore more isolates should be included in any future experiments to determine how widely effective *S. lydicus* can be. One explanation by which *S. lydicus*-treated plants had the lowest disease severity could be because this bacterium is considered a Plant Growth Promoting Rhizobacteria (PGPR) which can induce local and systemic immunity responses in plants (Pacios-Michelena et al. 2021) Additionally, *Streptomyces* spp. can also promote plant growth through the release of indole acetic acid (IAA) and siderophores (Gopalakrishnan et al. 2013). The many different mechanisms of antagonism displayed by *Streptomyces* spp. (antibiotics, volatile organic compounds, lytic enzymes, induction of plant resistance, plant growth promotion, and colonization of roots) positions *S. lydicus* as a promising biocontrol alternative for the control of *V. dahliae*. Nevertheless, our results were based on artificial inoculations of *V. dahliae* onto a mixture of sand and plant-growing media; therefore, it would be interesting to evaluate the effectiveness of Actinovate® under field conditions that consider a variety of natural biotic and abiotic factors.

For the chemical-based controls included in the *in-vitro* experiments (PDA amended with azoxystrobin and benzovindiflupyr or azoxystrobin only) the percentage of colony growth inhibition was different for the three *V. dahliae* isolates. Azoxystrobin and benzovindiflupyr are the active ingredients of a commercially available fungicide under the name Elatus®. The concentrations of these active ingredients are 0.3lb and 0.15lb, respectively, per 1lb of product. Elatus® is marketed for the control of *Rhizoctonia* in potatoes, however, it is also stated that it has been shown to suppress *Verticillium* levels when applied in-furrow at-planting as a management tool in combination with other tools like the use of resistant potato varieties, proper irrigation and fertilization, crop rotation and fumigation (2ee label recommendation). To date, no peer-reviewed publication was found that evaluated the potential of these two active ingredients on Michigan *V. dahliae* isolates from potatoes. Therefore, it was interesting to find that the percentage of colony growth inhibition for Michigan isolate 1 was 100%, while for Michigan isolate 2 and Wisconsin isolate was 65.9% and 75.7%, respectively. As for the greenhouse experiments, at the end of the experiment plants treated with Elatus® had a disease severity of 52.5% and 65.4% for Michigan Isolate 1 and 2, respectively. For Michigan Isolate 2, disease severity was slightly higher than in the “Inoculated/Non-treated” control.

In contrast, another commercially available fungicide, Quadris® was included in my *in-vitro* assays as another control. The active ingredient in this fungicide is azoxystrobin only, at a concentration of 2.08lb per 1 gallon of product. Even though the label of this fungicide does not state that the product is effective against *Verticillium* spp., it was included to contrast with Elatus®. The results on PDA amended with azoxystrobin only found that the percentage of colony growth inhibition was low, compared to some of the BCAs and Elatus®. For Michigan isolate 1 the colony growth inhibition was 26.8%, while for Michigan isolate 2 and Wisconsin isolate, it was 15.2% and -0.4% (an increase in colony growth compared to the *V. dahliae* isolate grown alone), respectively. Therefore, Quadris® was not considered to be evaluated in the greenhouse experiments. In a published paper by Bubici et al. 2019, they found that *in-vitro* the EC_{50} of azoxystrobin against *V. dahliae* was 311.8 µg/mL, which was the highest compared to the other fungicides included in the assay. Regardless, because azoxystrobin acts as a systemic fungicide, further *in-planta* evaluations showed that four soil applications of azoxystrobin reduced verticillium wilt severity index by up to 90% and *V. dahliae* colonization by 40%, and they suggest that azoxystrobin may have a low direct toxic effect on *V. dahliae* but highly reduce disease severity and pathogen colonization by altering plant responses to pathogen infection. This agrees with the results I observed in the *in-vitro* assays, however, because of the low colony growth inhibition observed, Quadris® was not included in the greenhouse trials. Another study determined the percentage of colony growth inhibition of 4 *V. dahliae* isolates from avocado in PDA amended with azoxystrobin and they found that for all *V. dahliae* isolates, azoxystrobin inhibited fungi mycelial growth by 95% (Ramirez-Gil and Morales-Osorio, 2021). This may suggest that perhaps the effectiveness of azoxystrobin is dependent on the *V. dahliae* isolate, however, more *in-vitro* studies should be carried out that include more *V. dahliae* isolates. Although it may be possible that the results of azoxystrobin *in-planta* could be the opposite to what we observed in the *in-vitro* experiments, the use of azoxystrobin may present a big limitation. Azoxystrobin is part of the QoI FRAC code 11 fungicide group, having a single-site mode of action. These fungicides inhibit mitochondrial respiration by binding to the Qo site of the cytochrome enzyme complex *bc1*, limiting electron transfer (Barlett et al. 2002). One limitation of using single-site inhibitors like azoxystrobin is the high risk of resistance. For instance, Ma et al. 2003 found that *Alternaria alternata, A. tenuissima* and *A. arborescens* isolates from pistachio orchards under consecutive applications of azoxystrobin for 3-4 years

were found resistant to this fungicide due to a single mutation in the cytochrome *b* gene. Thus, one possible explanation for the low *V. dahliae* colony growth inhibition observed in our *in-vitro* experiments could be that there is a possibility these isolates have mutated and become resistant to such type of fungicides with that mode of action.

On the other hand, benzovindiflupyr is a pyrazole-carboxamide and part of the succinate-dehydrogenase inhibitors (SDHI) fungicides FRAC group 7 which have a multi-site mode of action but can have medium-high risk of resistance. For instance, in a recent study, benzovindiflupyr-resistant isolates of *Sclerotium rolfsii* showed cross-resistance to other SDHI inhibitors fungicides and were found to transmit resistance genes to sensitive isolates (Ciu et al. 2024). In another recent publication, the EC_{50} of benzovindiflupyr was found to be 1.08 µg/mL against *V. dahliae* and the same authors found that Maine *V. dahliae* populations can be resistant to benzovindiflupyr but no cross-resistance with other fungicides like azoxystrobin was found (Li et al., 2023). Hence, to reduce the risk of developing resistance by avoiding constant exposure to benzovindiflupyr, combined applications of azoxystrobin and benzovindiflupyr may limit the risk and also successfully control *V. dahliae*. We demonstrated that Elatus® (a.i. azoxystrobin and benzovindiflupyr) was one of the most effective treatments to limit *V. dahliae* colony growth *in-vitro*, however, Elatus® treated plants had a high disease severity. Some studies have shown that benzovindiflupyr can have both protectant and curative effects. For instance, it was found that the application of this fungicide one day after inoculation of strawberries with *Colletotrichum gloesporioides* was effective in reducing plant mortality and disease development (Oliveira et al., 2020). Similarly, applications of benzovindiflupyr before or after inoculation of *Corunespora cassiicola* in cucumber showed preventive and curative efficacies of 87% and 77%, respectively (Zhu et al. 2021). Due to this fungicide was applied 4 days after *V. dahliae* inoculation, the results observed in our greenhouse trials could indicate that Elatus® may not have a curative effect but rather a protectant effect for *V. dahliae*. Nevertheless, for future experiments it would be significant to compare applications of Elatus® before and after *V. dahliae* inoculations. Given the results from our greenhouse experiments Elatus® alone may not be the most effective management tool for the two Michigan *V. dahliae* isolates but integrated management of *V. dahliae* with biological control agents like *S. lydicus*, fungicides like Elatus®, resistant potato varieties, proper irrigation and fertilization, and crop rotation could potentially enhance control of Michigan isolates and limit the risk of fungicide resistance.

4.5 CONCLUSION

In conclusion, the active ingredients of Tenet® (*T. asperellum* and *T. gamsii*) are highly antagonistic to the three evaluated *V. dahliae* isolates *in-vitro*. Overall, both species were able to overgrow *V. dahliae* and significantly limit its growth. However, when evaluated under greenhouse conditions, Tenet® applications to plants infected with either Michigan *V. dahliae* Isolates 1 or 2 did not show the same degree of effectivity and the disease severity at the end of the experiment was 12% and 8.4% less than the "Inoculated/Non-treated" control, respectively. As for the chemical-based fungicides, PDA media amended with Elatus® resulted in one of the highest colony growth inhibitions for the *V. dahliae* isolates, however, this was not observed under greenhouse conditions. Treatment with Elatus® resulted in 17% less disease severity caused by Michigan Isolate 1, while 8% more disease severity caused by Michigan Isolate 2. In contrast, despite the slower growth rate of the active ingredient of Actinovate® (*S. lydicus*), this actinobacteria was highly antagonistic against the three *V. dahliae* isolates *in-vitro*. This effect translated to the greenhouse experiments where applications of Actinovate® to plants infected with Michigan Isolate 1 resulted in 67.4% less disease severity than the "Inoculated/Non-treated" control, but for Michigan Isolate 2, disease severity was 33.2% less than the control. Still, Actinovate® was the treatment that provided the best control of both *V. dahliae* isolates.

The results obtained by these experiments are based on only 2 Michigan *V. dahliae* isolates. However, because of the complexity of *V. dahliae* populations, it would be interesting to survey the Michigan potato fields *V. dahliae* populations to determine the distribution of such populations categorized by the VCGs they belong to given that not all VCGs are pathogenic to potatoes, neither form a synergistic interaction with the root-lesion nematode *P. penetrans*. This will provide a better guide for growers when making decisions regarding management measures and also provide a better reference about the risk of interaction with *P. penetrans*. Likewise, more *V. dahliae* isolates should be included in any future screening of potential treatments to determine how widely effective such treatments can be. These results were based on artificial inoculations of *V. dahliae* onto a mixture of sand and plant-growing media; therefore, it would be interesting to evaluate the effectiveness of Actinovate® under field conditions that consider a variety of natural biotic and abiotic factors.

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CHAPTER 5: MANURE-BASED AMENDMENTS INFLUENCE ON POTATO SOIL BACTERIAL AND FUNGAL COMMUNITIES AND ITS IMPACT ON *PRATYLENCHUS PENETRANS* POPULATIONS

Luisa M. Parrado and Marisol Quintanilla

5.1 INTRODUCTION

The results found in Chapter 2 of this dissertation showed that poultry manure and a compost blend made of poultry and cattle composted manure amended with wood ash are effective at keeping *P. penetrans* populations low. In general, the mechanism(s) underlying the pesticidal effect of organic soil amendments on plant parasitic nematodes is understudied, but the proposed mechanisms are hypothesized to be one or more of the following: 1. release of pre-existing nematicidal compounds, 2. generation of nematicidal compounds during degradation such as ammonia and fatty acids, 3. enhancement and/or induction of antagonistic microorganisms, 4. increase of plant tolerance and resistance, and 5. changes in soil physiology that are unsuitable for nematode survival (Oka, 2010).

Further research done to study the fungal soil-borne suppressive effect of organic soil amendments has led to the conclusion that chemical parameters such as pH and nutrient content have significant but not exclusive effects on the development of disease severity. After sterilization, the suppressive effect of the amendments decreased; highlighting that the microorganisms present in such amendments also play a significant role in the suppression of disease severity along with abiotic factors such as nitrogenous compounds (Tilston et al. 2002; Oka and Yermiyahu, 2002). The mechanisms by which these microbes contribute to disease control could be: 1. successful parasitism of pathogen, 2. antibiotic production, 3. successful competition for nutrients and/or space, 4. plant-induced systemic resistance, and 5. improved plant nutrition and vigor (Hoitink and Boehm, 1999). Mature composts can contain mesophilic microorganisms that possess antagonistic features such as the synthesis of appreciable quantities of antibiotics (Makan et al., 2013). Studies have been conducted to isolate microorganisms within certain composts, and *Bacillus* spp., *Enterobacter* spp., *Pseudomonas* spp., *Streptomyces* spp., as well as *Penicillium* spp., *Aspergillus* spp., and *Trichoderma* spp., have been identified with known biocontrol activity (Suarez et al., 2007; Chung and Hoitink, 1990; Gorodecki and Hadar, 1990; Phae et al. 1990; Hadar and Papadopoulou, 2012; Neher et al. 2022). These biocontrol agents can decrease pathogen populations through the excretion of lytic enzymes

(Ruanpanun et al. 2010), endoparasitism, production of compounds that inhibit pathogen growth (Ruanpanum et al. 2010; Watson et al. 2017), colonization of root-system, or competition for nutrients and space (Lorito et al. 1993; Erhart et al. 1999; Hoitink and Boehm, 1999; McKellar and Nelson, 2003; Kavroulakis et al. 2005; Borrero et al. 2006; Perez-Piqueres et al. 2006). Similarly, some microbial communities can secrete molecules that promote plant growth and/or enhance the plant defense system like DAPG (2,4-diacetylphloroglucinol), PRN (pyrrolnitrin), AAA (indole acetic acid), and siderophores (Noble and Coventry, 2005; de Souza et al. 2003; Garbeva et al. 2004; Latz et al. 2012; Mazzola, 2004; Thakur and Vyas, 1983; Gupta et al. 2002). According to this information, there are likely potential biocontrol agents within composts and manures which could be enhanced via environmental enrichment (Postma et al. 2003; Zhang et al. 1998; Trillas et al. 2006; Siddiqui et al. 2008; Kavroulakis et al. 2010). However, a review of more than 1,000 studies showed organic soil amendments are suppressive in 45% of the cases, insignificant in 35%, and increase disease in 20% (Bonanomi et al. 2007). Nevertheless, it has been shown that organic soil amendments can change the microbial community composition of the soil and as a result, enhance the competition and/or antagonism of plant pathogens (Hoitink and Boehm, 1999; Steinberg et al., 2004). For potatoes, it has been found that many members of Proteobacteria phylum are found in the plant rhizosphere with beneficial activity (Weinert et al. 2011). Likewise, other microorganisms such as the Streptomycetaceae, Micromonosporaceae, and Pseudomonadaceae families which have been widely studied for their biocontrol effects (Haas and Defago, 2005; Weller, 2007; Raaijmakers et al. 2009; Berg and Smalla, 2009; Weinert et al., 2010). Additionally, they play a key role in preventing tuber bacteria rots (Koiv et al. 2015), avoiding quality loss due to sprouting (Slininger et al, 2004), saccharification (de Souza et al. 2010), water loss (Aulakh, 2013) or spoilage (Liebe et al. 2016). Consequently, there is an increasing interest in using organic soil amendments for disease management to potentially augment microbial communities with beneficial activity that may have a direct or indirect effect on plant pathogens (Reuveni et al.2002; Garbeva et al. 2004).

The addition of organic soil amendments to the soil can favor the development of suppressive soil microbiota and therefore contribute to the development of suppressive soils (Baker and Cook, 1974; Hornby, 1983). However, the effectiveness of organic soil amendments is dependent on their characteristics, the soil characteristics, the crop, the pathogen, and the environmental conditions, hence its effectiveness cannot be generalized nor the microbial

composition and effect on natural soil microbiome. Previous field trials conducted in 2020 and 2021 showed that poultry manure and a compost blend (compost A or LAB=Layer Ash Blend) prevented a significant increase in *P. penetrans* populations. Considering that one of the mechanisms by which organic soil amendments suppress plant-parasitic nematodes is by influencing the soil microbiome, the main goal of this chapter is to determine the response of *P. penetrans* to poultry manure and compost A (LAB=Layer Ash Blend) autoclaved vs. not-autoclaved and the changes in the natural potato soil microbiome.

5.2 METHODOLOGIES

Experimental design and treatments

Two greenhouse trials were established at the Michigan State University Plant Pathology Greenhouse complex (East Lansing, MI). The first experiment was established in the fall of 2022 using soil collected from a commercial potato field located near Mendon Township, MI (42.027054, -85.516433). The soil textural series was an Osthemo sandy loam with 1.02% organic matter, 7.9% clary, 72.7% sand, and 19.4% silt. In addition, the pH of the soil was 6.1, with a bulk density of 1.53 g/cm^3 and an available water capacity of 0.13 cm/cm (United States Department of Agriculture, Web Soil Survey, 2023). Potato cv. Lamoka was planted at the time of soil collection and the preceding crop history was a 5-year crop rotation of corn-alfalfa-corn-alfalfa-potato. The field did not have any history of cover crops in the past 15 years; however, hog manure had been applied in the spring of 2009 and dairy manure had been applied in the springs of 2015 and 2021.

The second experiment was established in the summer of 2023 using soil collected from a different commercial potato field located near Lockport Township, MI (41.899160, -85.601069). The soil textural series was a Spink loamy sand with 1.02% organic matter, 3.3% clary, 84.3% sand, and 12.5% silt. In addition, the pH of the soil was 6.0, with a bulk density of 1.68 g/cm^3 and an available water capacity of 0.11 cm/cm (United States Department of Agriculture, Web Soil Survey, 2024). The potato cv. Russet Norkotah was planted at the time of soil collection, and the crop history was a 4-year rotation of seed corn-potato-seed corn-soybean-potato. Every year the field is planted with rye or triticale as cover crops and the soil has never been fumigated nor treated with manures.

Table 11. Description and nutrient composition of the manure-based amendments used in the 2022 greenhouse experiment.

Manure-Based Amendment	Composition	Carbon:Nitrogen Ratio (C:N)	Nitrogen (TKN[1]) (kg/t)	Phosphate (P_2O_5) (kg/t)	Potash (K_2O) (kg/t)
Compost blend	Cattle and poultry manure Wood ash	8.7:1	12.3	18.1	26.2
Raw poultry manure	Poultry manure	5:1	38.1	24.6	18.6

Table 12. Description and nutrient composition of the manure-based amendments used in the 2023 greenhouse experiment.

Manure-Based Amendment	Composition	Carbon:Nitrogen Ratio (C:N)	Nitrogen (TKN[1]) (kg/t)	Phosphate (P_2O_5) (kg/t)	Potash (K_2O) (kg/t)
Compost blend	Cattle and poultry manure Wood ash	9.2:1	22.9	14.1	14
Raw poultry manure	Poultry manure	5:1	33.9	25.5	21.4

The experimental design for both greenhouse experiments was a randomized complete block design with four manure-based amendment treatments, an untreated control, and a positive control using fluopyram (Velum® Prime; FRAC code 7); each treatment had five replicates, for a total of 30 experimental units. The treatments were selected based on results obtained from previous field trials. One was composted poultry and cattle manure amended with wood ash (compost A; LAB=Layer Ash Blend), and the second one was raw poultry manure (Morgan Composting Inc. Sears, MI) and their characteristics can be found in Tables 11 and 12. Literature suggests that autoclaved compost does not have the same effectiveness at reducing plant pathogens incidence as non-autoclaved, therefore, I also included autoclaved compost A (LAB=Layer Ash Blend) and autoclaved raw poultry manure. To autoclave these products, 200g of each manure-based amendment was autoclaved at 121˚C for 60 minutes (Tilston et al. 2002).

Both greenhouse experiments were set up in 1.5-gallon round black plastic pots with drainage holes (21.5 cm in diameter by 21.5 cm deep). Two days before scheduled planting, each pot was filled with a total of 5.5 kg of soil, and manure-based amendment treatments were incorporated with the soil by hand at a rate equal to 3.08 tons/ha. Potato cv. Russet Norkotah seed was obtained from Walther Farms (Three Rivers, MI) and cut into 56.7 g seed pieces; one seed piece was planted in each pot (Figure 49).

Figure 49. Potato cv. Russet Norkotah certified disease-free tuber were cut into 56.7 g seed pieces.

Initial soil samples were taken before treatment application to determine the abundance of different plant-parasitic nematodes and free-living nematodes, however, artificial inoculation of *P. penetrans* was made to ensure the threshold at which symptoms are displayed in potatoes (Orlando et al. 2019). *Pratylenchus penetrans* inoculum was obtained from the Michigan State University Applied Nematology Lab *in-vitro* cultures maintained on carrot disks. Two weeks after planting, each pot was artificially inoculated with *P. penetrans* at a rate of 3 nematodes/g of soil, for a total of 16,500 *P. penetrans* mixed stages (juveniles, adults, and eggs) per pot (Figure 50).

Figure 50. *Pratylenchus penetrans* inoculum was prepared in the Applied Nematology Lab and then taken to the greenhouse to inoculate the pots.

One week after inoculation, the respective pots were treated with fluopyram (Velum® Prime; FRAC code 7) by soil drenching at a rate of 496.47 ml/ha, while the untreated pots were treated with an equal amount of water. Pots were watered daily and the greenhouse environmental conditions for both experiments were 16h:8h light: dark photoperiod at 26°C. The potato variety used in these experiments is medium maturing (95 to 110 days after planting), therefore the experiment was kept for a total of 90 days, which would also allow at least two *P. penetrans* reproduction cycles (Orlando et al. 2019) (Figure 51).

Figure 51. Potato plants growing in the greenhouse.

Soil and Root Sampling for *P. penetrans* quantification

Soil samples were taken before treatment application, 45 days after planting, and 90 days after planting. Using a disinfected measuring scoop, 100g of soil was taken per pot, placed in ¼ gallon

Ziploc® bags, and transported to the laboratory in a cooler (Figure 52). Then they were placed in a cold storage room at 8˚C until nematode extraction. Root-lesion nematode incidence in soil was quantified using the extraction method by Jenkins, 1964 based on elutriation and centrifugal flotation. Briefly, 100 cm^3 of soil was washed and passed through a stack of 250-µm and 25-µm sieves. Soil that was retained in the 25-µm sieve was placed into a centrifuge tube with water, followed by centrifugation at 4000 rpm for 5 min. After centrifugation, the supernatant was discarded, and the pellet of soil was left untouched. Then 40% sucrose was added, and tubes were centrifuged at 4000 rpm for 3 min. Lastly, the supernatant was passed through a 25-µm sieve to recover the nematodes which were then collected in a 10-ml glass tube for further nematode counting and identification. Morphological characteristics were observed using an inverted Nikon TMS microscope (Mai, 2018).

Figure 52. Soil samples were carefully taken using a disinfected plastic scoop.

Root samples were collected 45 days after planting, and 90 days after planting. Two grams of roots were taken per pot, placed in ¼ gallon Ziploc® bags, and transported to the laboratory in a cooler (Figure 53). Once in the laboratory, roots were washed with cold water, left to dry for 2 min, and cut into 5mm pieces. Root pieces were placed in sterile plastic cups (Thermo Fisher Scientific, Hampton, NH) with 40ml of distillate water. The plastic cups were then placed in a shaker (G10 Gyratory Shaker, New Brunswick Scientific Co., Inc, New Brunswick, NJ) at 150 rpm for 72h. Subsequently, samples were then passed through a stack of 250-µm and 25-µm sieves. The nematodes on the 25-µm sieve were collected in a 10-ml glass tube for further *P. penetrans* nematode quantification using an inverted Nikon TMS microscope.

Figure 53. Root samples were carefully taken from each pot.

Soil Sampling for Microbial Analysis

Soil samples were taken before treatment application, 45 days after planting, and 90 days after planting. Using sterile 25 mL falcon tubes with a scoop attached to the lid, 10g of soil adjacent to the roots was taken and placed in the tube (Figure 54). Once all the samples were taken, they were shipped overnight to Biome Makers Inc. (202 Cousteau PI, Suite 100. Davis, CA. 95618) for DNA extraction, sequencing process, bacterial and fungal library preparation, and sequencing data processing. According to the protocol provided by Biome Makers Inc., DNA extraction was done using a DNeasy Powerlyzer Powersoil Kit using QIAcube Connect. PCR assays for the 16S rRNA gene V4 region and the ITS gene by amplification of the ITS1 region were done using WineSeq® custom primers according to the Patent WO2017096385 (Becares and Fernandez, 2017). Quality control of the amplification product was checked by gel electrophoresis, the 16S rRNA and ITS libraries were pooled and sequenced on an Illumina MiSeq instrument using a 2x200bp MiSeq Reagent Kit v3 kit. Biome Makers Inc. completed the bioinformatic analysis by through sequence quality filtering, followed by chimera and singletons removal, and clustering against their curated databases from the latest versions of SILVA 138.1 for 16S sequences and UNITE 8.3 for ITS sequences at a 97% identity alignment and taxonomy was predicted to the most probable species or family level.

Figure 54. Soil sampling for DNA extraction and sequencing. 10g of soil adjacent to the roots was taken and placed in sterile tubes.

Plant Fitness Measurements

Plant height measurements were taken every two weeks using a measuring stick, and ninety days after planting, shoot fresh weight, and the number and weight of tubers were recorded.

Statistical Analysis

P. penetrans, plant height, and tuber number and weight data analyses

Data collected from both greenhouse experiments were analyzed separately using RStudio version 2023 (R Core Team 2023). Response variables included *P. penetrans* counts in soil and roots, the number of progeny tubers, and total tuber weight. Treatment and sampling date were considered as the fixed effects, while replication was a random effect when included in the model. For each data set, normality was determined with a normal quantile-quantile plot of residuals and a Shapiro-Wilk test (P-value>0.05). *Pratylenchus penetrans* abundance in soil and roots across time data were analyzed with a generalized linear mixed model (GLMM) with a Poisson distribution. Progeny tuber number data were analyzed with a GLMM model with a binomial distribution, while total tuber weight data were analyzed with a linear mixed-effects model (LMER) including replication as a random effect with the package 'lme4' (Bates et al. 2015). Plant height measurement data was analyzed by first conducting an ANOVA to determine the effect of treatment, days after inoculation (DAI), and replicate, followed by a General Linear Model (GLM) with treatment and DAI as fixed effects, and height as the response variable. If treatment was determined to have a significant effect, means separation was completed using Tukey's Honest Significant Difference (HSD) post-hoc tests (α=0.05), followed by pairwise comparisons among treatments using the packages 'emmeans', 'MASS', and 'mulcomp' (Lenth

2019; Venables and Ripley 2002; Hothorn et al. 2008). Statistically different means are represented by different letters in figures. Bar plots were developed using Microsoft® Excel (Version 16.81) and the CLD results were added manually onto graphs.

Microbial Data Statistical Analysis

Data were processed, analyzed, and visualized using the R statistical software v. 4.3.1 (R Core Team, 2023). Gaussian multilevel mixed models were used to test the fixed effects of treatments, time (before treatment application (T0), 45 days after treatment application (T1), and 90 days after treatment application (T2)) and their two-way interactions, and random effects of blocks and plots within blocks on the relative abundances of the bacterial and fungal phyla using the 'lme4' package (Bates et al., 2015). Post hoc tests were conducted using the 'emmeans' package (Lenth et al., 2018). On day 45 and day 90 after the treatments were applied, Pearson's moment correlations were calculated between relative abundances of bacteria/fungal phylum and the nematode counts.

Diversity indices (Shannon diversity index, Pielou's evenness, and Chao1 richness) were calculated from the bacterial and fungal relative abundance data using the 'vegan' package (Oksanen et al. 2018). Each diversity indices measures were fitted separately to test the effect of treatments and time. Specifically, Gaussian mixed models were used with the fixed effects of treatment, time (before treatment application (T0), 45 days after treatment application (T1), and 90 days after treatment application (T2)), and the 2-way interaction, and random effects of blocks and plots within blocks on Shannon diversity index and Pielou's evenness. A generalized linear mixed model with a Poisson distribution to test for Chao1 richness was used.

To evaluate the shifts in beta-diversity of the bacterial and fungal community as affected by the treatments, time (before treatment application (T0), 45 days after treatment application (T1), and 90 days after treatment application (T2)), and their interactions, permutational multivariate analysis of variance (perMANOVA) were performed using the function *adonis2* in the 'vegan' package. Pairwise comparisons between treatments and time were performed using the function *pairwise.adonis2*.

Principal components analysis was performed on the Hellinger transformed relative abundance data after Bray-Curtis distances were calculated across samples. PCAs were conducted on data from each year separately. Heat maps were generated using the z-scaled relative abundance of

the bacterial and fungal phyla for each treatment and sampling time for each year. All visualizations were performed using the 'ggplot2' package (Wickham, 2009).

5.3 RESULTS

Pratylenchus penetrans abundance in soil when treated with manure-based amendments

In "Trial 1" (Figure 55), *P. penetrans* abundance in soil varied significantly among treatments ($X^2_{(5, 143)} = 6617.6$, P-value<0.001) and among days after planting (DAP) ($X^2_{(1, 88)} = 5299.0$, P-value<0.001). Similarly, there was an interaction between treatment and DAP ($X^2_{(5, 143)} = 132.9$, P-value<0.001). At 45 DAP, all treatments were significantly different from the "Untreated" control (pairwise comparison: p-value<0.05). As for the manure-based amendments, *P. penetrans* abundance in "CompostA" and "CompostA-Autoclaved" and "PoultryManure" and "PoultryManure-Autoclaved" was not significantly different. The lowest number of *P. penetrans* was found in "PoultryManure" with an average of 45 *P. penetrans*/100 g of soil, while for "PoultryManure-Autoclaved" the average was of 70.2 *P. penetrans*/100 g of soil.

At 90 DAP the treatment that had the highest number of *P. penetrans* in soil was "Velum" with an average of 986 *P. penetrans*/100 g of soil, while the "Untreated" control had an average number of 445 *P. penetrans*/100 g of soil. *Pratylenchus penetrans* abundance in soil significantly differed in all treatments compared to the "Untreated" control (pairwise comparison: p-value <0.0001). The contrast analysis of "Autoclaved" vs. "Not Autoclaved", was not significant between "autoclaved vs. not autoclaved. The lowest number of *P. penetrans* was found in "Poultry Manure" with an average of 135 *P. penetrans*/100 g of soil, while in "PoultryManure-Autoclaved" the average number was 210.6 *P. penetrans*/100 g of soil.

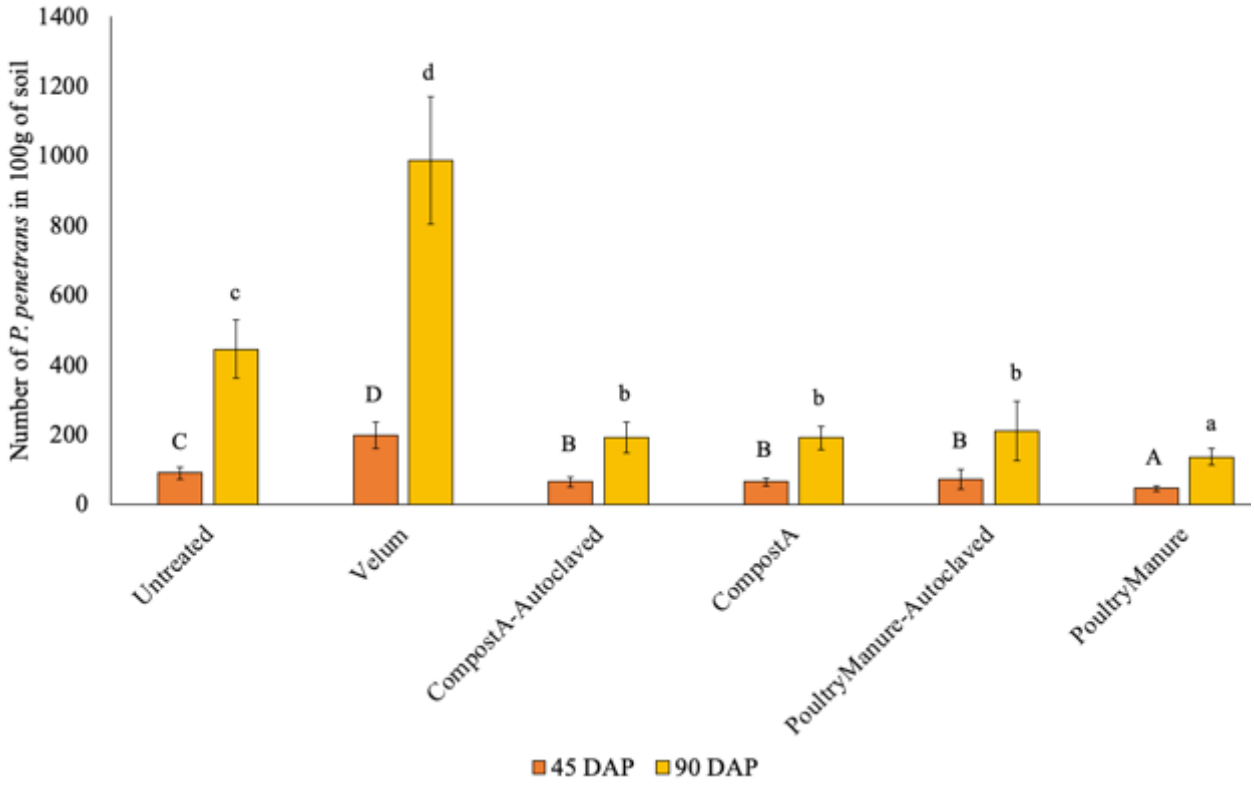

Figure 55. Average ± SEM number of *P. penetrans* abundance in 100g of soil for each treatment (control, Velum (a.i. fluopyram), Compost A (composted poultry and cattle manure amended with wood ash), and raw poultry manure) evaluated in the greenhouse "Trial 1". Samples were taken 45 days after planting and then 90 days after planting. The letters above each bar indicate significant pairwise differences among treatments. For 45 DAP data, significant differences are indicated in upper case letters while for 90 DAP data significant differences are indicated in lower case letters (DAP = days after planting) (p-value<0.05).

In "Trial 2" (Figure 56), *P. penetrans* abundance also differed significantly among treatments ($X^2_{(5, 143)} = 7935.4$, P-value<0.001), and among DAP times ($X^2_{(1,88)} = 5188.7$, P-value<0.001). At 45 DAP, *P. penetrans* abundance differed significantly in most treatments from the "Untreated" control except for "CompostA-Autoclaved" (pairwise comparisons: p-value = 0.08) and, "PoultryManure-Autoclaved" (pairwise comparisons: p-value = 0.99). At 45 DAP, "PoultryManure-Autoclaved" had an average of 95 *P. penetrans*/100 g of soil, and "Compost A-Autoclaved" had an average of 82.2 *P. penetrans*/100 g of soil, while the "Untreated" control had an average of 98.2 *P. penetrans*/100 g of soil. Nevertheless, the highest number of *P. penetrans* was found in "Velum" with an average of 232.8 *P. penetrans*/100 g of soil. As for the contrast between "Autoclaved" and "Not Autoclaved", there was no significant difference

between “CompostA” and “CompostA-Autoclaved” and “PoultryManure” and “PoultryManure-Autoclaved”. The lowest number of *P. penetrans* was found in “PoultryManure” with 54.2 *P. penetrans*/100 g of soil, followed by “CompostA” with an average of 70.6 *P. penetrans*/100 g of soil.

At 90 DAP, all treatments were significantly different from the control (pairwise comparisons: p-value<0.0001). The highest number of *P. penetrans* was found in “Velum” with an average of 1103 *P. penetrans*/100 g of soil, while the “Untreated” control had an average of 517.2 *P. penetrans*/100 g of soil. As for the manure-based amendments treatments, there was no significant difference between “CompostA” and “CompostA-Autoclaved” and “PoultryManure” and “PoultryManure-Autoclaved”. The lowest number of *P. penetrans* was found in “PoultryManure” with an average of 147.8 *P. penetrans*/100 g of soil, while “PoultryManure-Autoclaved” had an average of 197.6 *P. penetrans*/100 g of soil.

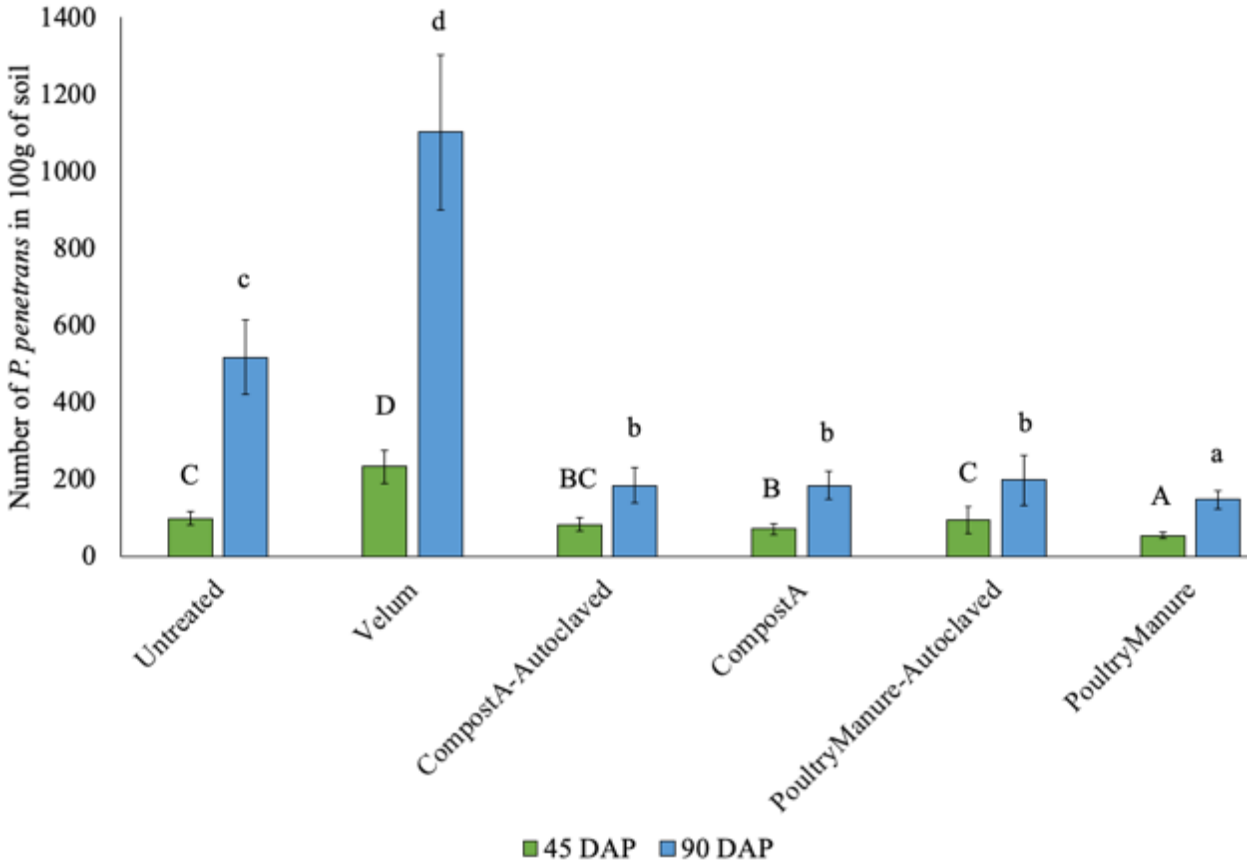

Figure 56. Average ± SEM number of *P. penetrans* abundance in 100g of soil for each treatment (control, Velum (a.i. fluopyram), Compost A (composted poultry and cattle manure amended with wood ash), and raw poultry manure) evaluated in the greenhouse “Trial 2”. Samples were

Figure 56. (cont'd)

taken 45 days after planting and then 90 days after planting. The letters above each bar indicate significant pairwise differences among treatments. For 45 DAP data, significant differences are indicated in upper case letters while for 90 DAP data significant differences are indicated in lower case letters (DAP = days after planting) (p-value<0.05).

Pratylenchus penetrans abundance in roots when treated with manure-based amendments

In "Trial 1" (Figure 57), overall, *P. penetrans* abundance in 1g of root was significantly different among treatments ($X^2_{(5, 143)} = 13298.2$, P-value<0.0001) as well as among DAP ($X^2_{(1, 88)} = 19046.7$, P-value<0.001). As for the contrast between "Autoclaved" vs. "Not Autoclaved", at 45 DAP abundance of *P. penetrans* in roots from treatments "CompostA" and "CompostA-Autoclaved" was significantly different (pairwise comparison: p-value<0.0001), while it was not between "PoultryManure" and "PoultryManure-Autoclaved" (pairwise comparison: p-value = 0.1727). The average number of *P. penetrans* in "CompostA" was 75.7 *P. penetrans*/ 1 g of root, while in "CompostA-Autoclaved" the average was 150.2 *P. penetrans*/ 1 g of root.

Even though there was an overall significant increase of *P. penetrans* numbers in 1g of root (p-value<0.0001), the highest number of *P. penetrans* at 90 DAP was found in the "Untreated" control and "Velum" with an average of 1786.2 and 1346.9 *P. penetrans*/ 1 g of root. In contrast, the lowest number of *P. penetrans* was found in "PoultryManure" with an average of 223 *P. penetrans*/ 1 g of root, being 87.51% fewer *P. penetrans* than in the "Untreated" control. However, *P. penetrans* abundance was highest in "CompostA" and "CompostA-Autoclaved" with an average of 995 and 1076 *P. penetrans*/ 1 g of root, while for "PoultryManure" and "PoultryManure-Autoclaved" the averages were 223 and 305 *P. penetrans*/ 1 g of root.

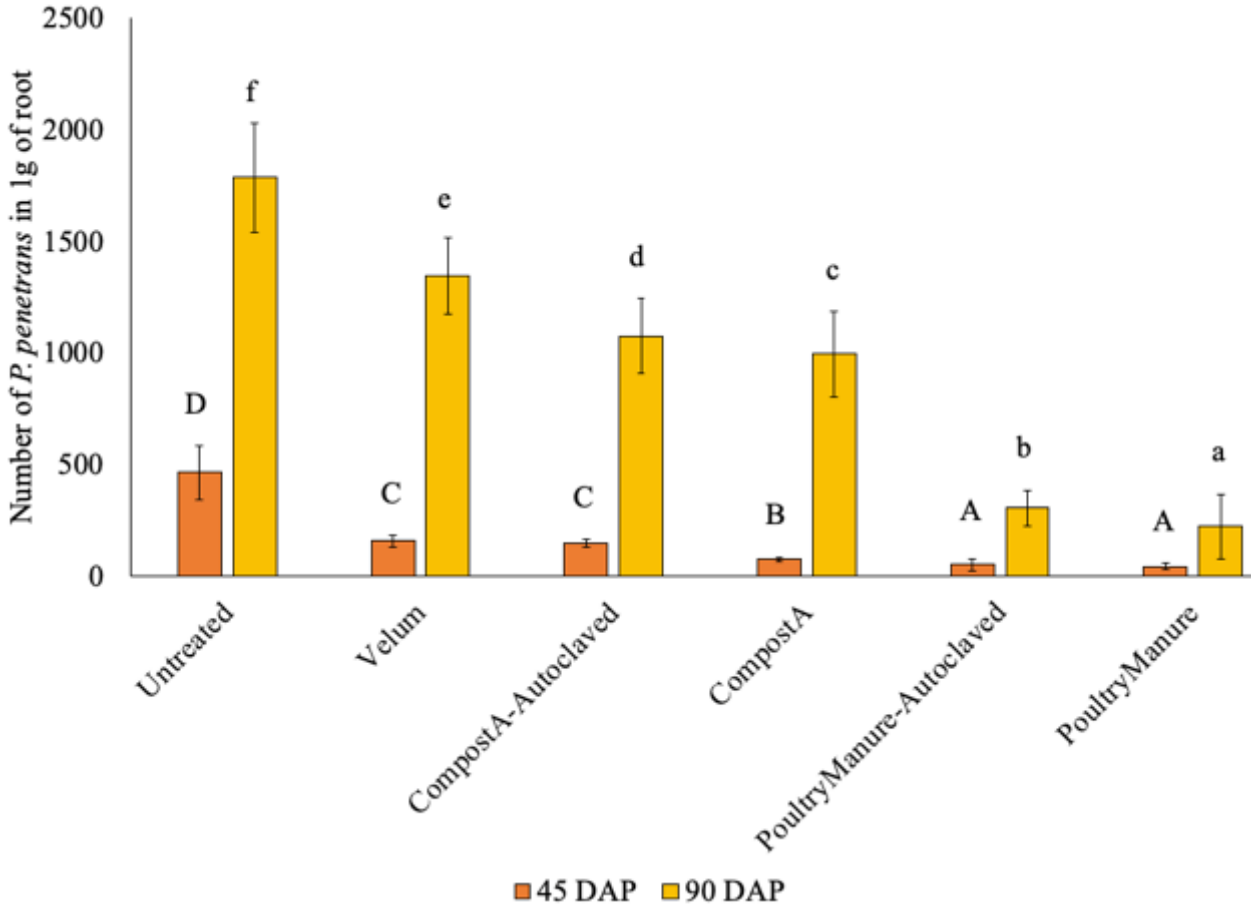

Figure 57. Average ± SEM number of *P. penetrans* abundance in 1g of root for each treatment (control, Velum (a.i. fluopyram), Compost A (composted poultry and cattle manure amended with wood ash), and raw poultry manure) evaluated in the greenhouse "Trial 1". Samples were taken 45 days after planting and then 90 days after planting. The letters above each bar indicate significant pairwise differences among treatments. For 45 DAP data, significant differences are indicated in upper case letters while for 90 DAP data significant differences are indicated in lower case letters (DAP = days after planting) (p-value<0.05).

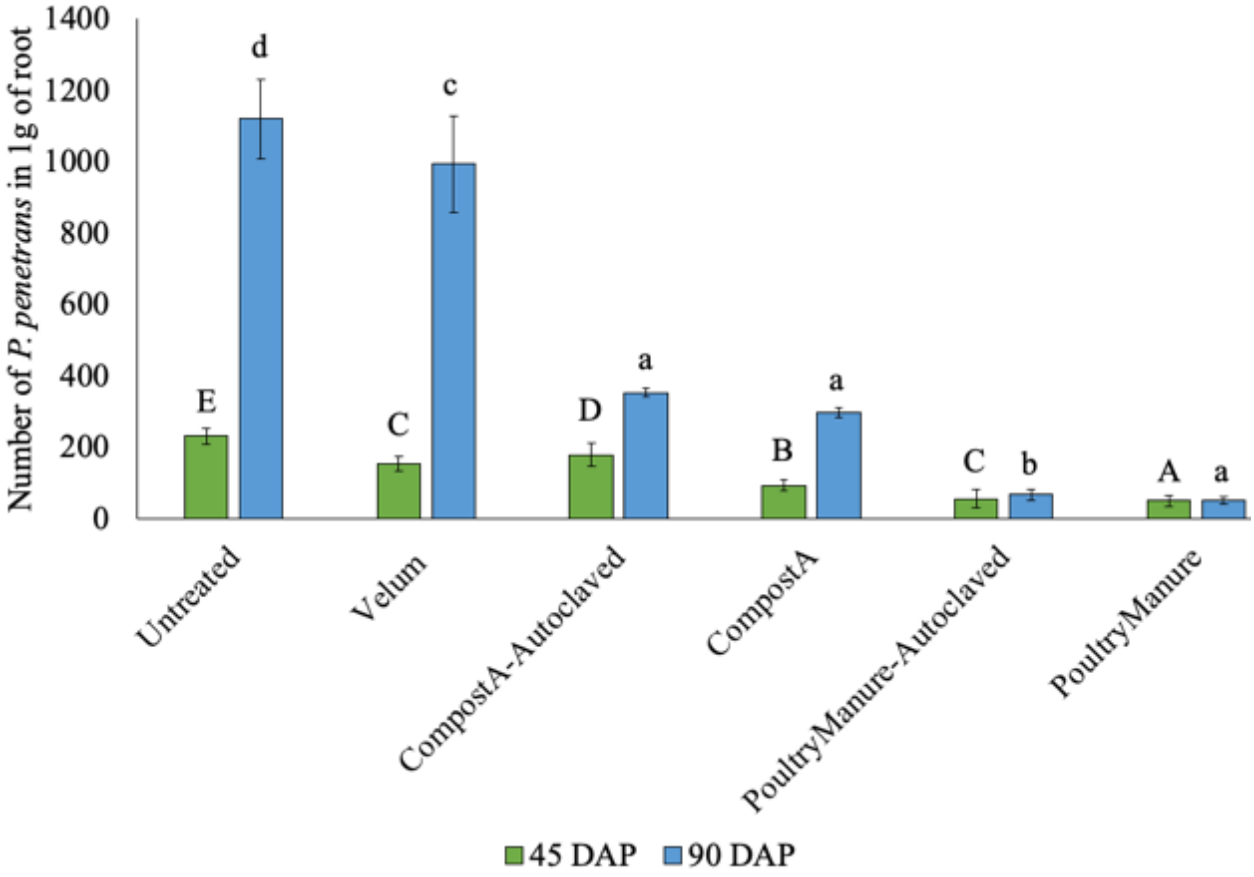

Figure 58. Average ± SEM number of *P. penetrans* abundance in 1g of root for each treatment (control, Velum (a.i. fluopyram), Compost A (composted poultry and cattle manure amended with wood ash), and raw poultry manure) evaluated in the greenhouse "Trial 2". Samples were taken 45 days after planting and then 90 days after planting. The letters above each bar indicate significant pairwise differences among treatments. For 45 DAP data, significant differences are indicated in upper case letters while for 90 DAP data significant differences are indicated in lower case letters (DAP = days after planting) (p-value<0.05).

In "Trial 2" (Figure 58), overall *P. penetrans* abundance in 1g of root was significantly different among treatments ($X^2_{(5, 143)} = 15104.4$, P-value<0.0001) as well as among DAP ($X^2_{(1, 88)} = 4147.9$, P-value<0.001). Overall, there was a significant increase of *P. penetrans* abundance in roots from 45 DAP to 90 DAP (pairwise comparison: p-value<0.0001), however, no significant increase of *P. penetrans* was found in "PoultryManure" (pairwise comparison: p-value = 0.8243).

At 45 DAP, the highest number of *P. penetrans* was found in the "Untreated" control with an average of 232.2 *P. penetrans*/ 1 g of root, while the lowest was found in "PoultryManure" with

an average of 50.2 *P. penetrans*/ 1 g of root, followed by "PoultryManure-Autoclaved" with an average of 56.1 *P. penetrans*/ 1 g of root. At 90 DAP, the highest number of *P. penetrans* was found in the "Untreated" with an average of 1121 *P. penetrans*/ 1 g of root, while the lowest was found in "PoultryManure" with an average of 51.2 *P. penetrans*/ 1 g of root, being 95.4% fewer *P. penetrans* than in the "Untreated" control. *Pratylenchus penetrans* abundance in roots was significantly different between the manure-based amendments that were "Autoclaved" vs. "Not Autoclaved". For instance, at 45 DAP *P. penetrans* abundance was significantly different between "CompostA" and "CompostA-Autoclaved" with an average of 93.8 and 178.2 *P. penetrans*/ 1 g of root, respectively (pairwise comparison: p-value<0.0001). However, these treatments were not significantly different at 90 DAP (pairwise comparison: p-value = 0.8505). On the contrary, the abundance of *P. penetrans* in "PoultryManure" and "PoultryManure-Autoclaved" was not significantly different.

Potato plant height when treated with manure-based amendments

In "Trial 1" (Figure 59), plant height was significantly affected by treatment ($X^2_{(5, 143)} = 96.45$, P-value<0.0001) and DAP ($X^2_{(5, 143)} = 404.89$, P-value<0.0001). At 30 DAP there was a significant difference between "PoultryManure-Autoclaved" and "Velum" (pairwise comparison: p-value = 0.0131). At this measuring time point, plants treated with "PoultryManure-Autoclaved" were on average 31.6 cm while the "Velum" treated ones were 20.4 cm. At 60 DAP, plants treated with manure-based amendments were significantly taller than the "Untreated" (pairwise comparison: p-value<0.05). The average height of plants treated with manure-based amendments ranged between 40.2 and 41.2 cm, while the "Untreated" was 30.2 cm. After 75 DAP plant started to senesce as observed in Figure 59.

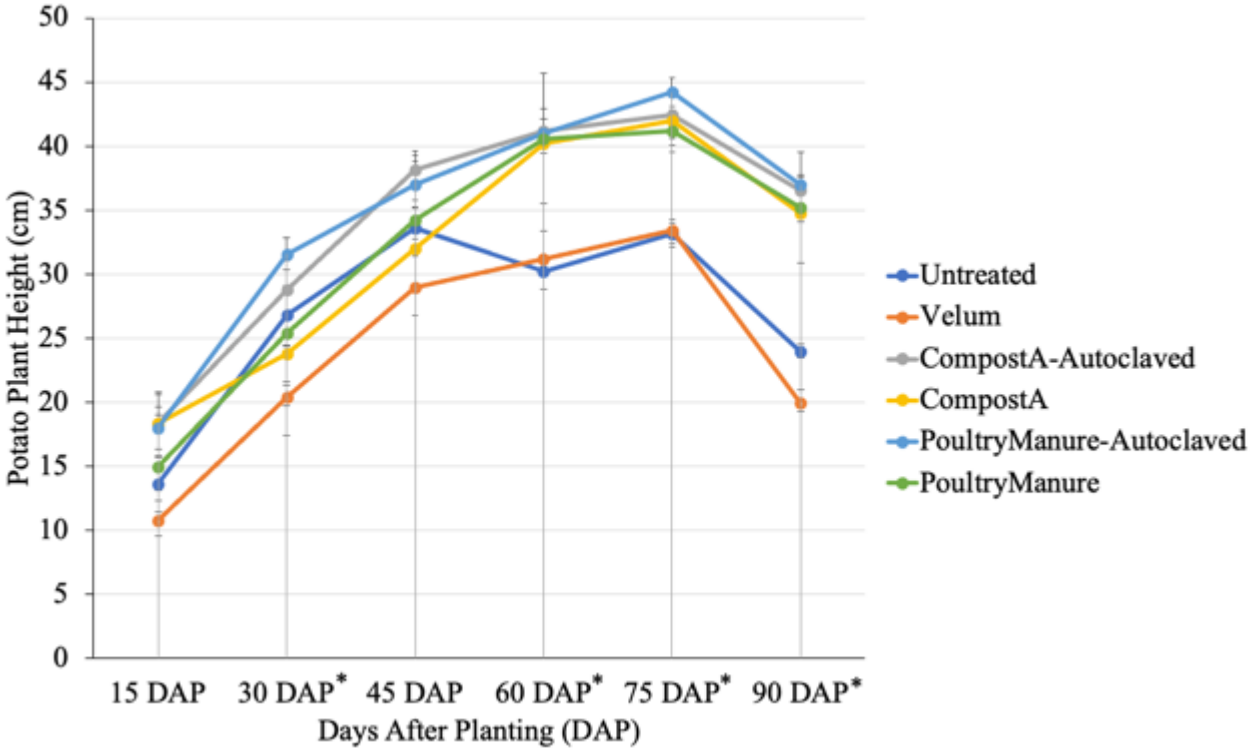

Figure 59. Average ± SEM height of potato plants corresponding to each treatment (control, Velum (a.i. fluopyram), Compost A (composted poultry and cattle manure amended with wood ash, and raw poultry manure) evaluated in the greenhouse "Trial 1" every 15 days for the duration of the experiment (90 days) (DAP = days after planting). The asterisks (*) above each measuring time point, indicate significant pairwise differences of treatments compared to the "Untreated" control (p-value<0.05).

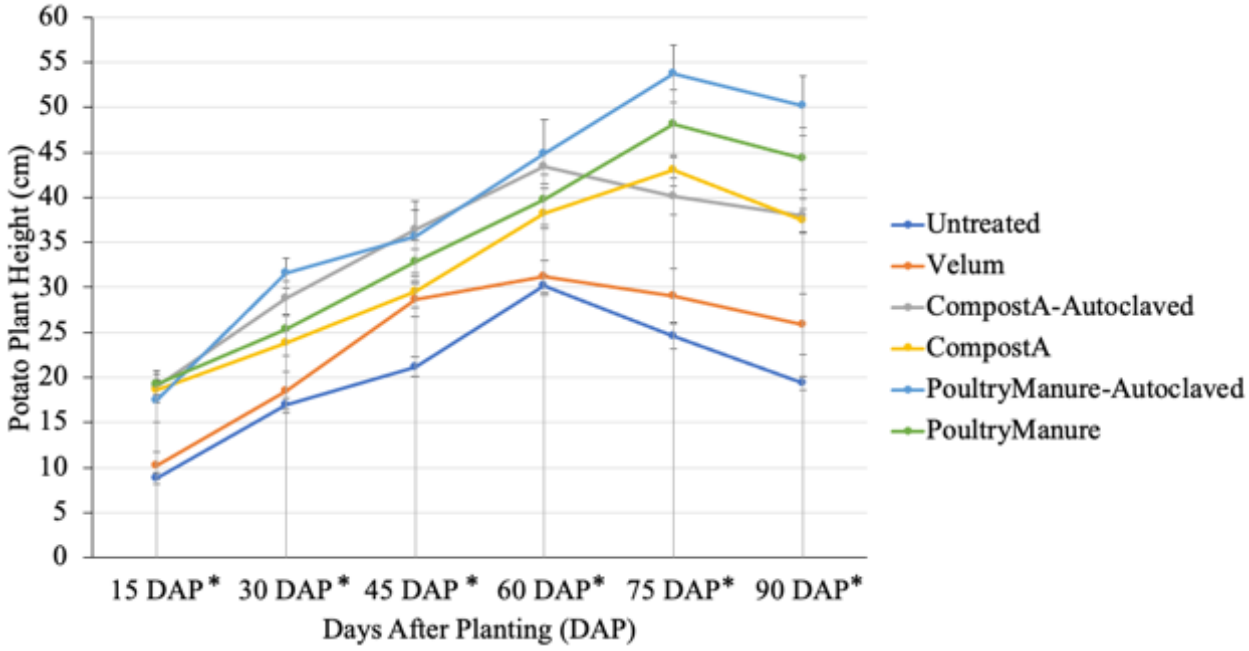

Figure 60. Average ± SEM height of potato plants corresponding to each treatment (control, Velum (a.i. fluopyram), Compost A (composted poultry and cattle manure amended with wood ash), and raw poultry manure) evaluated in the greenhouse "Trial 2" every 15 days for the duration of the experiment (90 days) (DAP = days after planting). The asterisks (*) above each measuring time point, indicate significant pairwise differences of treatments compared to the "Untreated" control (p-value<0.05).

In "Trial 2" (Figure 60), plant height was significantly affected by treatment ($X^2_{(5, 143)} = 567.46$, P-value<0.0001) and DAP ($X^2_{(5, 143)} = 637.77$, P-value<0.0001). At 15 DAP, plants treated with manure-based amendments were significantly taller than the "Untreated" (pairwise comparison: p-value<0.05). The tallest plants were the ones treated with "PoultryManure" with an average height of 19.2 cm, while the shortest plants were the "Untreated" with an average height of 8.8 cm. Similarly, at 30, 45, 60, 75, and 90 DAP, plants treated with manure-based amendments continued to be taller than the "Untreated" (pairwise comparison: p-value<0.05). At 75 DAP, the tallest plants were the ones treated with "PoultryManure-Autoclaved" with an average height of 53.7 cm, while the "Untreated" plants had an average height of 24.6 cm. After 75 DAP, plants started to senesce as observed in Figure 60.

Potato tuber number and weight when treated with manure-based amendments

In "Trial 1" (Figure 61), treatment had a significant effect on the number of tubers produced ($X^2_{(5, 143)} = 17.14$, P-value = 0.004) (Figure 61A). Compared to the control, "PoultryManure"

treated plants produced significantly more tubers. On average, treated plants with "PoultryManure" produced 23 tubers, while the "Control" plants produced 13 tubers. Therefore, "PoultryManure" produced 43.5% more tubers than the "Untreated". In addition, there was no significant difference between "Autoclaved" vs. "Not Autoclaved" manure-based amendments. As for the average weight of the total number of tubers, treatment overall did not have a significant effect (DF = 5, F-value $_{(5, 24)}$ = 1.74, p-value = 0.16). Numerically, "PoultryManure-Autoclaved" treated plants had the heaviest tubers with a total average of 207 g, while the "Untreated" plants produced tubers with a total average of 134.4 g (Figure61B).

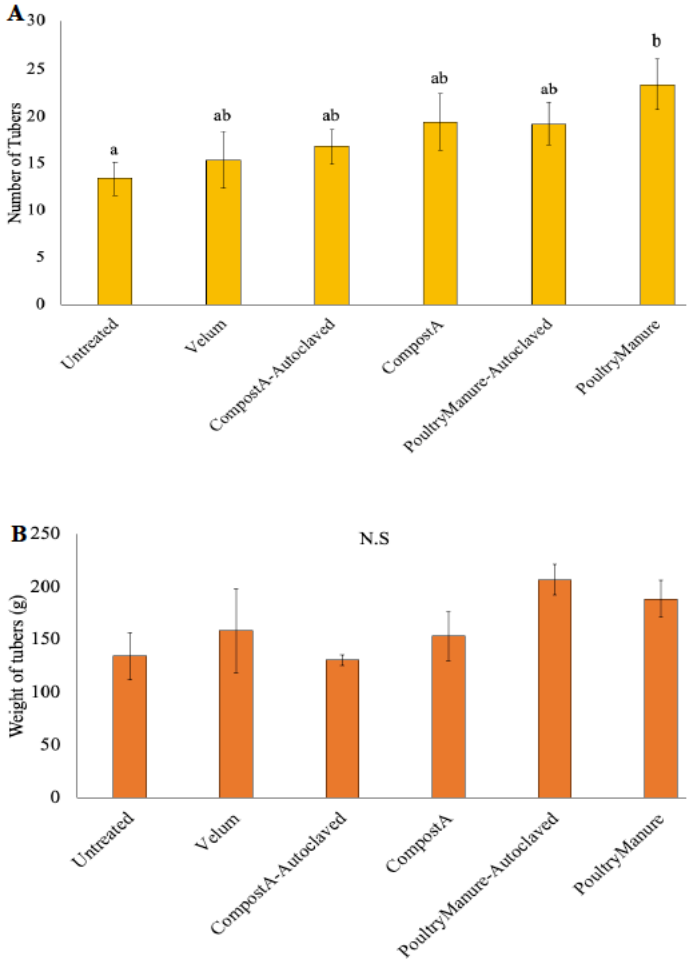

Figure 61. Average ± SEM of **A.** Number of potato tubers produced after 90 days for each treatment, **B.** Weight of the total number of tubers produced after 90 days for each treatment (control, Velum (a.i. fluopyram), Compost A (composted poultry and cattle manure amended with wood ash), and raw poultry manure) evaluated in the greenhouse "Trial 1". The letters

Figure 61. (cont'd)

above each bar indicate significant pairwise differences in treatments compared to the "Untreated" control. N.S. indicates no significant differences (p-value<0.05).

In "Trial 2" (Figure 62), the number of tubers produced by plants was significantly impacted by treatment ($X^2_{(5, 143)} = 15.06$, P-value = 0.01). Compared to the "Untreated", plants treated with "PoultryManure" yield significantly more tubers with an average of 7 tubers, while the "Untreated" plants yield an average of 3 tubers (pairwise comparison: p-value = 0.03) (Figure 66A). As for the weight of the total number of tubers, treatment had a significant effect (DF = 5, F-value$_{(5, 24)}$ = 5.66, p-value = 0.001). Plants treated with "PoultryManure" had the heaviest tubers with an average weight of 171.8 g for the total number of tubers, while the tubers from the "Untreated" plants weighed an average of 78.4 g (Figure 62B).

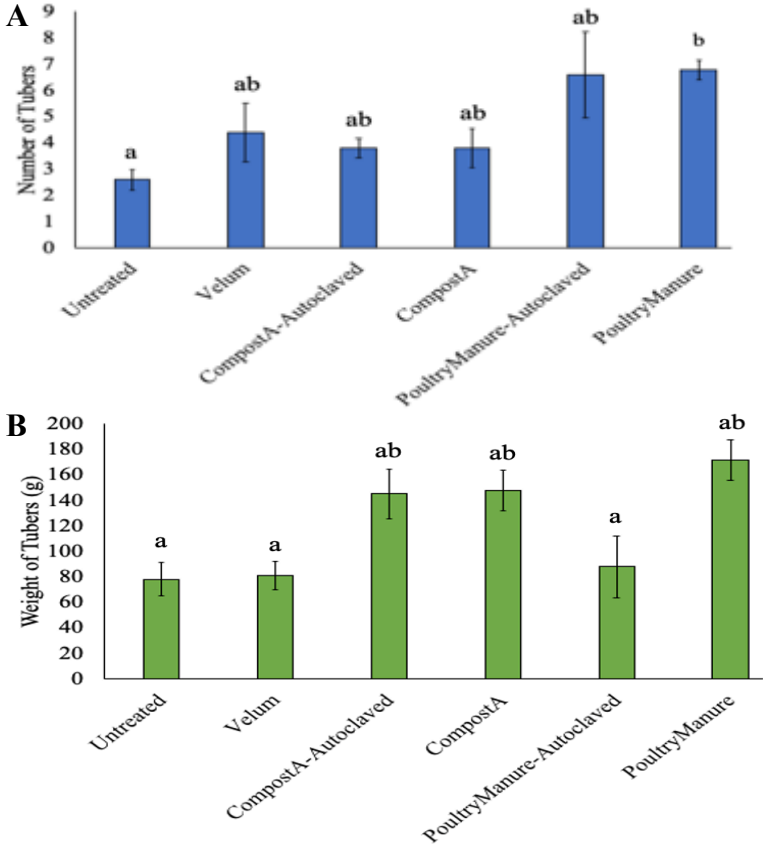

Figure 62. Average ± SEM of **A.** Number of potato tubers produced after 90 days for each treatment (control, Velum (a.i. fluopyram), Compost A (composted poultry and cattle manure amended with wood ash), and raw poultry manure), **B.** Weight of the total number of tubers produced after 90 days for each treatment that was evaluated in the greenhouse "Trial 2". The

Figure 62. (cont'd)

letters above each bar indicate significant pairwise differences of treatments compared to the "Untreated" control (p-value<0.05).

Relative Abundance of Bacterial Communities of Soil Over Time when Treated with Manure-based Amendments Autoclaved Vs. Non-Autoclaved

Table 13. Mean ± SEM of relative abundances (%) of most abundant bacterial phyla in soils for each of the evaluated treatments (LAB = Compost A, LABs = Compost A-Autoclaved, PM = Poultry Manure, PMs = Poultry Manure-Autoclaved and Vel = Velum) at before treatment application (T0), 45 days after treatment application (T1), and 90 days after treatment application (T2) for the greenhouse experiments conducted in 2022 and 2023. ANOVAs were performed on relative abundance for each treatment separately across time. Only means with significant differences are indicated. Means with the same letter in the same row are not significantly different.

Experiment	Phylum	Treatment																	
		Control			LAB			LABs			PM			PMs			Vel		
		T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2
2022	Actinobacteriota	24.6±1.7 a	26.7±1.7 a	26.3±1.7 a	24.7±1.7 a	25.9±1.7 a	26.0±1.7 a	23.0±1.7 a	23.7±1.7 a	25.3±1.7 a	22.6±1.7 a	24.3±1.7 a	23.8±1.7 a	23.4±1.7 a	20.6±1.7 a	18.4±1.7 a	23.7±1.7 a	24.2±1.7 a	27.6±1.7 a
	Proteobacteria	31.3±2.6 a	31.3±2.6 a	39.7±2.6 a	30.8±2.6 a	35.3±2.6 a	36.8±2.6 a	34.8±2.6 a	33.0±2.6 a	36.1±2.6 a	32.3±2.6 a	35.6±2.6 a	36.6±2.6 a	32.0±2.6 a	36.1±2.6 a	36.5±2.6 a	31.5±2.6 a	30.4±2.6 a	40.2±2.6 b
2023	Actinobacteriota	28.3±2.1 a	29.9±2.1 a	23.6±2.1 a	29.8±2.1 a	27.9±2.1 a	23.8±2.1 a	26.5±2.1 a	22.7±2.1 a	25.0±2.1 a	27.1±2.1 a	29.7±2.1 a	20.3±2.1 b	27.2±2.1 a	20.3±2.1 b	23.1±2.1 ab	27.2±2.1 a	25.6±2.1 a	22.8±2.1 a
	Proteobacteria	39.9±2.0 a	47.3±2.0 b	31.2±2.0 c	40.5±2.0 a	43.4±2.0 a	37.8±2.0 a	41.9±2.0 a	44.5±2.0 a	41.3±2.0 a	39.4±2.0 a	38.7±2.0 ab	32.4±2.0 b	40.1±2.0 a	29.7±2.0 b	28.6±2.0 b	39.9±2.0 a	33.4±2.0 a	34.5±2.0 a
	Firmicutes	7.5±2.5 a	6.1±2.5 a	11.8±2.5 a	6.6±2.5 a	6.8±2.5 a	13.7±2.5 a	5.8±2.5 a	6.7±2.5 ab	14.4±2.5 b	6.3±2.5 a	17.8±2.5 b	30.5±2.5 c	7.3±2.5 a	24.9±2.5 b	30.2±2.5 b	6.8±2.5 a	7.2±2.5 a	10.6±2.5 a

Table 14. Mean ± SEM of relative abundances (%) of the least abundant bacterial phyla in soils for each of the evaluated treatments (LAB = Compost A, LABs = Compost A-Autoclaved, PM = Poultry Manure, PMs = Poultry Manure-Autoclaved and Vel = Velum) at before treatment application (T0), 45 days after treatment application (T1), and 90 days after treatment application (T2) for the greenhouse experiments conducted in 2022 and 2023. ANOVAs were performed on relative abundance for each treatment separately across time. Only means with significant differences are indicated. Means with the same letter in the same row are not significantly different.

Experiment	Phylum	Treatment																	
		Control			LAB			LABs			PM			PMs			Vel		
		T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2
2022	Acidobacteriota	6.2±0.5 a	3.6±0.5 b	4.2±0.5 b	6.4±0.5 a	4.1±0.5 b	4.6±0.5 b	6.0±0.5 a	3.2±0.5 b	3.8±0.5 b	6.7±0.5 a	3.0±0.5 b	2.9±0.5 b	5.9±0.5 a	2.8±0.5 b	4.9±0.5 a	6.2±0.5 a	4.7±0.5 b	3.7±0.5 b
	Bacteroidota	3.8±2.0 a	9.8±2.0 a	4.3±2.0 a	2.5±2.0 a	6.4±2.0 a	2.6±2.0 a	2.6±2.0 a	4.2±2.0 a	2.9±2.0 a	2.1±2.0 a	7.7±2.0 a	4.2±2.0 a	3.2±2.0 a	11.8±2.0 b	3.6±2.0 a	2.9±2.0 a	6.7±2.0 a	8.3±2.0 a
	Myxococcota	3.3±0.3 a	1.6±0.3 b	1.8±0.3 b	3.2±0.3 a	1.5±0.3 b	1.6±0.3 b	3.2±0.3 a	1.4±0.3 b	1.5±0.3 b	3.1±0.3 a	1.1±0.3 b	1.4±0.3 b	3.2±0.3 a	1.3±0.3 b	1.9±0.3 b	3.5±0.3 a	1.9±0.3 b	1.2±0.3 b
	Planctomycetota	3.6±0.3 a	1.9±0.3 b	1.8±0.3 b	3.4±0.3 a	2.5±0.3 a	2.6±0.3 a	3.1±0.3 a	3.1±0.3 a	2.4±0.3 a	3.6±0.3 a	2.1±0.3 b	2.4±0.3 b	3.8±0.3 a	3.3±0.3 a	2.1±0.3 b	3.5±0.3 a	2.4±0.3 b	1.9±0.3 b
	Verrucomicrobiota	3.9±0.3 a	2.6±0.3 b	1.7±0.3 b	3.7±0.3 a	2.4±0.3 b	2.4±0.3 b	3.3±0.3 a	2.2±0.3 b	2.0±0.3 b	4.1±0.3 a	1.5±0.3 b	2.1±0.3 b	3.9±0.3 a	1.8±0.3 b	1.5±0.3 b	7.0±0.3 a	3.0±0.3 a	1.9±0.3 b
	Armatimonadota	0.06±0.008 a	0.02±0.008 b	0.01±0.008 b	0.09±0.008 a	0.02±0.008 b	0.009±0.008 b	0.04±0.008 a	0.007±0.008 b	0.009±0.008 b	0.06±0.008 a	0.003±0.008 b	0±0.008 b	0.04±0.008 a	0.003±0.008 b	0.007±0.008 b	0.04±0.008 a	0.04±0.008 a	0.02±0.008 a
	Bdellovibrionota	0.2±0.08 a	0.1±0.08 a	0.3±0.08 a	0.2±0.08 a	0.1±0.08 a	0.3±0.08 a	0.2±0.08 a	0.2±0.08 a	0.3±0.08 a	0.2±0.08 a	0.3±0.08 ab	0.40.08 b	0.3±0.08 a	0.6±0.08 b	0.5±0.08 ab	0.2±0.08 a	0.2±0.08 a	0.5±0.08 b
	Desulfobacterota	0.5±0.06 a	0.06±0.06 b	0.1±0.06 b	0.5±0.06 a	0.07±0.06 b	0.05±0.06 b	0.4±0.06 a	0.02±0.06 b	0.04±0.06 b	0.4±0.06 a	0.01±0.06 b	0.002±0.06 b	0.3±0.06 b	0.02±0.06 b	0.01±0.06 b	0.4±0.06 a	0.1±0.06 b	0.07±0.06 b
	Gemmatimonadota	1.0±0.2 a	0.5±0.2 b	0.7±0.2 ab	1.0±0.2 a	0.7±0.2 a	1.0±0.2 a	1.0±0.2 a	0.5±0.2 a	0.7±0.2 a	1.1±0.2 a	0.8±0.2 a	0.7±0.2 a	1.0±0.2 ab	0.6±0.2 a	1.2±0.2 b	1.0±0.2 a	0.7±0.2 a	1.0±0.2 a
	Nitrospirota	1.5±0.1 a	0.6±0.1 b	1.1±0.1 ab	1.7±0.1 a	0.9±0.1 b	1.2±0.1 ab	1.5±0.1 a	0.7±0.1 b	0.9±0.1 b	1.6±0.1 a	0.4±0.1 b	0.4±0.1 b	1.7±0.1 a	0.2±0.1 b	0.4±0.1 b	1.7±0.1 a	0.8±0.1 b	0.7±0.1 b
	Spirochaetota	0.01±0.002 a	0±0.002 b	0±0.002 b	0±0.002 a	0.001±0.002 a	0±0.002 a	0.02±0.002 a	0±0.002 b	0±0.002 b	0±0.002 a	0±0.002 a	0±0.002 a	0±0.002 a	0±0.002 a	0±0.002 a	0.005±0.002 a	0±0.002 a	0±0.002 a
	Sumerlaeota	0.02±0.02 a	0.01±0.02 a	0.01±0.02 a	0.006±0.02 a	0.05±0.02 ab	0.7±0.02 b	0.01±0.02 a	0.05±0.02 a	0.03±0.02 a	0.01±0.02 a	0.1±0.02 b	0.05±0.02 ab	0.01±0.0 2 a	0.11±0.02 b	0.1±0.02 b	0.01±0.02 a	0.02±0.02 ab	0.007±0.02 b
2023	Acidobacteriota	5.9±0.6 a	3.8±0.6 b	6.2±0.6 a	6.7±0.6 a	3.4±0.6 b	4.7±0.6 b	5.4±0.6 a	3.1±0.6 b	3.1±0.6 b	5.9±0.6 a	2.9±0.6 b	3.2±0.6 b	6.2±0.6 a	3.2±0.6 b	3.4±0.6 b	6.4±0.6 a	6.3±0.6 a	5.8±0.6 a
	Bacteroidota	7.4±1.3 a	5.3±1.3 a	3.6±1.3 a	7.5±1.3 ab	10.7±1.3 a	3.4±1.3 b	10.4±1.3 a	11.8±1.3 a	4.6±1.3 b	10.4±1.3 a	5.3±1.3 b	3.6±1.3 b	8.0±1.3 a	8.4±1.3 a	2.8±1.3 b	8.7±1.3 a	5.1±1.3 ab	3.7±1.3 b
	Myxococcota	2.2±0.4 a	1.0±0.4 a	4.9±0.4 b	2.3±0.4 a	0.8±0.4 b	5.0±0.4 c	2.2±0.4 a	1.8±0.4 a	3.9±0.4 b	2.8±0.4 a	1.2±0.4 b	3.7±0.4 a	2.8±0.4 ab	2.3±0.4 a	3.8±0.4 b	2.1±0.4 a	3.6±0.4 b	5.6±0.4 c
	Nitrospirota	1.0±0.1 a	0.6±0.1 b	1.1±0.1 a	1.0±0.1 a	0.4±0.1 b	0.9±0.1 a	0.9±0.1 a	0.3±0.1 b	0.7±0.1 ab	0.7±0.1 a	0.3±0.1 b	0.5±0.1 ab	0.9±0.1 a	0.5±0.1 b	0.8±0.1 ab	0.9±0.1 a	1.2±0.1 b	1.3±0.1 b
	Verrucomicrobiota	1.3±0.01 a	0.8±0.01 a	2.6±0.01 b	0.8±0.01 ab	0.6±0.01 a	1.5±0.01 b	1.2±0.01 ab	1.8±0.01 a	0.9±0.01 b	1.3±0.01 a	0.7±0.01 a	0.9±0.01 a	1.1±0.01 a	2.2±0.01 b	1.0±0.01 a	1.6±0.01 a	2.8±0.01 b	2.2±0.01 ab
	Abditibacteriota	0.02±0.01 a	0.01±0.01 a	0.01±0.01 a	0.03±0.01 a	0.01±0.01 a	0.003±0.01 a	0.05±0.01 a	0.06±0.01 a	0.002±0.01 b	0.05±0.01 a	0.04±0.01 a	0.03±0.01 a	0.05±0.01 a	0.05±0.01 a	0.005±0.01 b	0.04±0.01 a	0.03±0.01 a	0.001±0.01 a
	Armatimonadota	0.005±0.005 a	0±0.005 a	0.03±0.005 b	0.004±0.005 a	0.005±0.005 a	0.004±0.005 a	0±0.005 a	0.02±0.005 b	0±0.005 a	0.003±0.005 a	0.005±0.005 a	0±0.005 a	0.007±0.005 ab	0.02±0.005 a	0±0.005 b	0.02±0.005 a	0.06±0.005 b	0.03±0.005 a
	Chloroflexi	0.07±0.02 ab	0.04±0.02 a	0.14±0.02 b	0.03±0.02 a	0.03±0.02 a	0.04±0.02 a	0.07±0.02 a	0.1±0.02 a	0.06±0.02 a	0.1±0.02 a	0.02±0.02 a	0.06±0.02 a	0.11±0.02 a	0.09±0.02 ab	0.03±0.02 b	0.13±0.02 a	0.3±0.02 b	0.1±0.02 a
	Cyanobacteria	0±0.03 a	0.007±0.03 a	0.05±0.03 a	0.006±0.03 a	0.004±0.03 a	0.08±0.03 a	0.03±0.03 a	0.02±0.03 a	0.03±0.03 a	0±0.03 a	0.003±0.03 a	0.07±0.03 a	0.002±0.03 a	0.14±0.03 b	0.11±0.03 b	0.006±0.03 a	0.18±0.03 b	0.05±0.03 a
	Desulfobacterota	0.4±0.07 a	0.8±0.07 b	0.3±0.07 ab	0.3±0.07 a	0.06±0.07 a	0.3±0.07 a	0.2±0.07 a	0.04±0.07 a	0.1±0.07 a	0.2±0.07 a	0.04±0.07 a	0.3±0.07 a	0.3±0.07 a	0.06±0.07 a	0.06±0.07 a	0.2±0.07 a	0.2±0.07 a	0.3±0.07 a
	Gemmatimonadota	0.7±0.1 a	0.7±0.1 a	1.3±0.1 b	0.9±0.1 a	0.8±0.1 a	0.9±0.1 a	0.7±0.1 a	0.9±0.1 a	0.9±0.1 a	0.7±0.1 ab	0.6±0.1 a	1.0±0.1 b	0.8±0.1 a	0.9±0.1 a	0.9±0.1 a	0.8±0.1 a	1.6±0.1 b	1.3±0.1 c
	Spirochaetota	0±0.006 a	0±0.006 a	0.04±0.006 b	0±0.006 a	0±0.006 a	0.4±0.006 b	0±0.006 a	0±0.006 a	0.01±0.006 a	0±0.006 a	0±0.006 a	0.01±0.006 a	0±0.006 a	0.01±0.006 a	0.006±0.006 a	0±0.006 a	0.006±0.006 a	0.04±0.006 b
	Sumerlaeota	0.005±0.01 a	0.01±0.01 a	0.04±0.01 a	0±0.01 a	0.03±0.01 a	0.01±0.01 a	0.007±0.01 a	0.009±0.01 b	0.02±0.01 a	0.009±0.01 a	0.04±0.01 a	0.02±0.01 a	0.01±0.01 a	0.12±0.01 b	0.01±0.01 a	0±0.01 a	0.06±0.01 b	0.02±0.01 a

For the greenhouse experiment conducted in 2022, treatment only had a significant effect on Actinobacteriota (DF = 5, F-value $_{(5,20)}$ = 3.83, p-value = 0.01), Armatimonadota (DF = 5, F-value $_{(5,20)}$ = 58.65, p-value<0.0001), Bdellovibrionota (DF = 5, F-value $_{(5,20)}$ = 3.93, p-value = 0.01), Nitrospirota (DF = 5, F-value $_{(5,20)}$ = 4.48, p-value = 0.006), Spirochaetota (DF = 5, F-value $_{(5,20)}$ = 3.82, p-value = 0.01), and Sumerlaeota (DF = 5, F-value $_{(5,20)}$ = 4.20, p-value = 0.008). Across all treatments and sampling times, the most abundant bacteria were the Proteobacterium phylum with a relative abundance between 30% and 40%, followed by the Actinobacteria with a relative abundance between 18% to 27% (Table 13), while the other Phyla were less abundant (Table 14).

As for the most abundant bacteria phyla, the Actinobacteria numerically increased over time in all treatments, except for in PMs-treated soils, in which it decreased from 23.4% at T0 to 18.4% at T2. Similarly, the relative abundance of the phylum Proteobacteria numerically increased over time in all treatments, but only in Velum-treated soils the relative abundance of this phylum significantly increased from 31.5% at T0 to 40.2% at T2. The relative abundance of the least abundant bacteria phyla Myxococcota, Desulfobacterota, and Verrucomicrobiota decreased significantly over time in all the treatments, in contrast, the relative abundance of the other phyla varied among treatments. For instance, Acidobateriota significantly decreased over time in all treatments, except for in soils treated with PMs in which it significantly decreased from T0 to T1, but populations recovered from T1 to T2. In PMs-treated soils, the phylum Bacteroidota significantly increased from T0 to T1 but then significantly decreased from T1 to T2. The phylum Bdellovibrionota significantly increased over time in soils treated with PM or PMs, and also in Velum-treated soils. The only phylum that significantly increased over time with the addition of compost, specifically LAB, PM, and PMs was the Sumerlaeota.

For the greenhouse experiment conducted in 2023, treatment had a significant effect on Abditibacteriota (DF = 5, F-value $_{(5,20)}$ = 2.61, p-value = 0.05), Acidobacteriota (DF = 5, F-value $_{(5,20)}$ = 6.61, p-value = 0.0008), Armatimonadota (DF = 5, F-value $_{(5,20)}$ = 12.37, p-value<0.0001), Bacteroidota (DF = 5, F-value $_{(5,20)}$ = 2.70, p-value = 0.05), Chloroflexi (DF = 5, F-value $_{(5,20)}$ = 9.01, p-value = 0.0001), Cyanobacteria (DF = 5, F-value $_{(5,20)}$ = 2.54, p-value = 0.061), Desulfovacterota (DF = 5, F-value $_{(5,20)}$ = 2.78, p-value = 0.04), Firmicutes (DF = 5, F-value $_{(5,20)}$ = 15.05, p-value<0.0001), Gemmatimonadota (DF = 5, F-value $_{(5,20)}$ = 6.12, p-value = 0.001), Myxococcota (DF = 5, F-value $_{(5,20)}$ = 3.30, p-value = 0.02), Proteobacteria (DF = 5, F-value $_{(5,20)}$

= 8.35, p-value = 0.0002), Spirochaetota (DF = 5, F-value $_{(5,20)}$ = 2.66, p-value = 0.05), Sumerlaeota (DF = 5, F-value $_{(5,20)}$ = 2.49, p-value = 0.06), and Verrucomicrobiota (DF = 5, F-value $_{(5,20)}$ = 9.47, p-value<0.0001). Overall, the most abundant bacteria phyla were the Proteobacteria with a relative abundance ranging from 30% to 47%, followed by the phylum Actinobacteria with a relative abundance ranging between 20-30%, and the Firmicutes, which significantly increased in abundance only in PM and PMs-treated soils by 79.9% and 75.8%, respectively. (Table 15). The rest of the phyla had a low relative abundance (Table 16).

The relative abundance of bacteria phyla varied depending on the treatment and sampling time. For instance, the relative abundance of Abditibacteriota significantly decreased over time for all treatments, decreasing statistically significantly in LABs and PMs-treated soils from T0 to T2. As for Acidobacteriota, its relative abundance significantly decreased over time in the compost-treated soils, while it significantly increased over time in the control soils. Similarly, Actinobateriota decreased in all the treatments, with the most significant decrease in PM and PMs-treated soils. As for Armatimonadota, its relative abundance significantly fluctuated over time in the control, LABs, PMs, and Velum-treated. Likewise, the relative abundance of Bacteroidota significantly fluctuated in the LAB and LABs by significantly increasing from T0 to T1, while decreasing from T1 to T0, while in PM, PMs, and Velum-treated soils, the relative abundance of this phylum significantly decreased over time. Interestingly, Chloroflexi significantly decreased in PMs and Velum-treated soils while significantly increased in the control soils. The phylum Cyanobacteriota significantly increased over time in the PMs-treated soils only. Another bacterial phylum that fluctuated over time in all treatments was the Desulfobacterota, however, it only significantly increased from T0 to T1 in the control soils but from T1 to T2, its relative abundance decreased. As for the Gemmatimonadota, this phylum only increased significantly on the control, PM, and Velum-treated soils. The phylum Myxococcota relative abundance only increased significantly in the control and Velum-treated soils, while fluctuating over time in the compost-treated soils. Nitrospirota relative abundance also fluctuated over time among treatments, except for in Velum-treated soils, where they significantly increased over time. As for Spirochaetoa, its relative abundance only significantly increased from T0 to T1 in the control and LAB-treated soils. The phylum Sumerlaeota significantly increased from T0 to T1 in the PMs and Velum-treated soils, but from T1 to T2 its relative abundance decreased. Lastly, Verrucomicrobiota significantly increased in the control and LAB-

treated soils, while in LABs and PMs-treated soils, the relative abundance of this phylum increased from T0 to T1, but then significantly decreased at T2.

Relative Abundance of Fungal Communities of Soil Over Time when Treated with Manure-Based Amendments Autoclaved Vs. Non-Autoclaved

Table 15. Mean ± SEM of relative abundances (%) of the most abundant fungal phyla in soils for each of the evaluated treatments (LAB = Compost A, LABs = Compost A-Autoclaved, PM = Poultry Manure, PMs = Poultry Manure-Autoclaved and Vel = Velum) at before treatment application (T0), 45 days after treatment application (T1), and 90 days after treatment application (T2) for the greenhouse experiments conducted in 2022 and 2023. ANOVAs were performed on relative abundance for each treatment separately across time. Only means with significant differences are indicated. Means with the same letter in the same row are not significantly different.

Experiment	Phylum	Treatment																	
		Control			LAB			LABs			PM			PMs			Vel		
		T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2
2022	Basidiomycota	11.0±4.8 a	38.0±4.8 b	21.6±4.8 a	14.9±4.8 a	23.3±4.8 a	15.4±4.8 a	13.3±4.8 a	33.9±4.8 b	20.3±4.8 ab	11.2±4.8 a	18.4±4.8 a	14.5±4.8 a	14.1±4.8 ab	26.0±4.8 a	8.3±4.8 b	15.8±4.8 a	13.2±4.8 a	14.9±4.8 a
	Mortierellomycota	38.3±4.7 a	25.8±4.7 a	33.7±4.7 a	43.7±4.7 a	29.8±4.7 a	39.7±4.7 a	33.9±4.7 a	24.5±4.7 a	35.6±4.7 a	33.5±4.7 a	34.3±4.7 a	33.7±4.7 a	38.7±4.7 a	15.5±4.7 b	14.2±4.7 b	42.1±4.7 a	39.3±4.7 a	41.1±4.7 a
2023	Ascomycota	52.9±5.8 a	42.6±5.8 a	36.3±5.8 a	45.2±5.8 a	33.2±5.8 a	37.6±5.8 a	51.1±5.8 a	46.0±5.8 a	44.3±5.8 a	46.2±5.8 a	42.4±5.8 a	43.7±5.8 a	45.6±5.8 a	39.9±5.8 a	38.2±5.8 a	50.2±5.8 a	41.3±5.8 a	34.1±5.8 a
	Mortierellomycota	24.3±3.2 a	22.6±3.2 a	16.8±3.2 a	24.4±3.2 ab	30.5±3.2 a	13.8±3.2 b	25.8±3.2 a	16.1±3.2 a	20.1±3.2 a	26.2±3.2 a	24.3±3.2 a	17.1±3.2 a	30.0±3.2 ab	39.0±3.2 a	25.3±3.2 b	23.9±3.2 a	19.7±3.2 ab	11.4±3.2 b
	Oomycota	14.2±5.4 a	27.3±5.4 ab	34.9±5.4 b	24.8±5.4 a	27.4±5.4 a	40.5±5.4 a	14.7±5.4 a	28.4±5.4 a	26.5±5.4 a	16.3±5.4 a	20.4±5.4 a	22.7±5.4 a	17.5±5.4 a	4.8±5.4 a	18.6±5.4 a	18.2±5.4 a	30.7±5.4 ab	47.8±5.4 b

Table 16. Mean ± SEM of relative abundances (%) of the least abundant fungal phyla in soils for each of the evaluated treatments (LAB = Compost A, LABs = Compost A-Autoclaved, PM = Poultry Manure, PMs = Poultry Manure-Autoclaved and Vel = Velum) at before treatment application (T0), 45 days after treatment application (T1), and 90 days after treatment application (T2) for the greenhouse experiments conducted in 2022 and 2023. ANOVAs were performed on relative abundance for each treatment separately across time. Only means with significant differences are indicated. Means with the same letter in the same row are not significantly different.

Experiment	Phylum	Treatment																	
		Control			LAB			LABs			PM			PMs			Vel		
		T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2
2022	Aphelidiomycota	0±0.02 a	0.008±0.02 a	0.06±0.02 a	0.009±0.02 a	0.112±0.02 b	0.06±0.02 ab	0.004±0.02 a	0.03±0.02 a	0.02±0.02 a	0.01±0.02 a	0.02±0.02 a	0±0.02 a	0.03±0.02 a	0.02±0.02 a	0.002±0.02 a	0.009±0.02 a	0.09±0.02 b	0.04±0.02 ab
	Mucoromycota	1.0±3.0 a	1.2±3.0 a	0.5±3.0 a	2.1±3.0 a	8.4±3.0 a	6.8±3.0 a	1.6±3.0 a	1.9±3.0 a	2.6±3.0 a	5.2±3.0 a	6.2±3.0 a	2.6±3.0 a	1.3±3.0 a	17.1±3.0 b	19.1±3.0 b	2.2±3.0 a	1.0±3.0 a	0.2±3.0 a
	Oomycota	0±0.02 a	0.002±0.02 a	0.03±0.02 ab	0±0.02 a	0±0.02 a	0.09±0.02 b	0±0.02 a	0.001±0.02 a	0.04±0.02 a	0.007±0.02 a	0.008±0.02 a	0.05±0.02 a	0.007±0.02 a	0.004±0.02 a	0.14±0.02 b	0.001±0.02 a	0±0.02 a	0.03±0.02 a
2023	Entorrhizomycota	0±0.005 a	0±0.005 a	0±0.005 a	0.007±0.005 ab	0±0.005 a	0.02±0.005 b	0±0.005 a	0±0.005 a	0.007±0.005 a	0±0.005 a	0±0.005 a	0.01±0.005 a	0±0.005 a	0±0.005 a	0.009±0.005 a	0±0.005 a	0±0.005 a	0.007±0.005 a
	Glomeromycota	0.02±0.004 a	0.01±0.004 a	0.07±0.004 a	0.004±0.004 a	0±0.004 a	0.132±0.004 b	0.02±0.004 a	0±0.004 a	0.03±0.004 a	0.001±0.004 a	0±0.004 a	0.04±0.004 a	0.002±0.004 a	0±0.004 a	0.118±0.004 a	0±0.004 a	0.02±0.004 a	0.1±0.004 a
	Mucoromycota	1.2±2.1 a	0.8±2.1 a	0.4±2.1 a	0.4±2.1 a	1.4±2.1 a	0.6±2.1 a	0.6±2.1 a	2.3±2.1 a	1.6±2.1 a	0.6±2.1 a	7.2±2.1 b	11.4±2.1 b	0.3±2.1 a	8.6±2.1 b	7.8±2.1 b	0.9±2.1 a	0.8±2.1 a	0.3±2.1 a
	Olpidiomycota	0.02±0.007 a	0.005±0.007 a	0.002±0.007 a	0.06±0.007 a	0.004±0.007 b	0±0.007 b	0.1±0.007 a	0±0.007 b	0±0.007 b	0.04±0.007 a	0±0.007 b	0±0.007 b	0.05±0.007 a	0.002±0.007 b	0±0.007 b	0.1±0.007 a	0±0.007 b	0±0.007 b

For the greenhouse experiment conducted in 2022, treatments only affected the phyla Mortierellomycota (DF = 5, F-value $_{(5,20)}$ = 4.82, p-value = 0.004), and Mucoromycota (DF = 5, F-value $_{(5,20)}$ = 6.66, p-value = 0.0008).The most abundant fungal phyla were the Mortierellomycota with a relative abundance between 24% to 44%, followed by the Basidiomycota with a relative abundance ranging from 8.3% to 38% (Table 15). While the rest of the phyla were less abundant (Table 16).

As for the most abundant phyla, the Mortierellomycota and the Basidiomycota, populations fluctuated across time and treatment. For instance, numerically, the relative abundance of the Mortierellomycota decreased from T0 to T1 but then increased from T1 to T2 in the control, LAB, and LABs-treated soils, while its abundance significantly decreased over time in the PMs-treated soils. As for the Basidiomycota, the relative abundance increased from T0 to T1, while it decreased from T1 to T2 in all treatments, except in Velum-treated soils. In both the control and LABs-treated soils, populations significantly increased from T0 to T1, then decreased. However, only in PMs-treated soils, the abundance of this phylum significantly decreased from T1 to T2. As for the Mucoromycota phylum, its relative abundance only significantly increased in the PMs-treated soils. As for the least abundant, the Oomycota phylum, increased significantly over time in the control, LAB, and PMs-treated soils.

For the greenhouse experiment conducted in 2023, the phyla that were affected by treatment and time were the Mortierellomycota (DF = 5, F-value $_{(5,20)}$ = 6.08, p-value = 0.001 and DF = 2, F-value $_{(2,48)}$ = 13.23, p-value<0.0001, respectively), Mucoromycota (DF = 5, F-value $_{(5,20)}$ = 3.11, p-value = 0.03 and DF = 2, F-value $_{(2,48)}$ = 5.19, p-value = 0.009, respectively), and Oomycota (DF = 5, F-value $_{(5,20)}$ = 5.00, p-value = 0.009 and DF = 2, F-value $_{(2,48)}$ = 10.66, p-value = 0.0001, respectively).

In 2023, the most abundant phyla were the Ascomycota with a relative abundance between 34% and 53%, followed by the Oomycota and Mortierellomycota phyla with a relative abundance ranging from 10% to 30% (Table 15). In contrast, the rest of the phyla were less abundant (Table 16). The lowest decrease of Ascomycota relative abundance was on PM-treated soils, where the decrease was 5.4%, while in the control or Velum-treated soils, the decrease was 32%. The Oomycota phylum relative abundance significantly increased in the control and Velum-treated soils, while the Mortierellomycota relative abundance significantly decreased in the Velum, LAB, and PMs-treated soils. The Mucoromycota significantly increased in abundance in the PM,

PMs, and Velum-treated soils, while numerically decreasing in the control soils and fluctuating in the LAB and LABs-treated soils.

α-Diversity indices and ß-Diversity of Bacterial Communities of Soil Over Time when Treated with Manure-based Amendments Autoclaved Vs. Non-Autoclaved

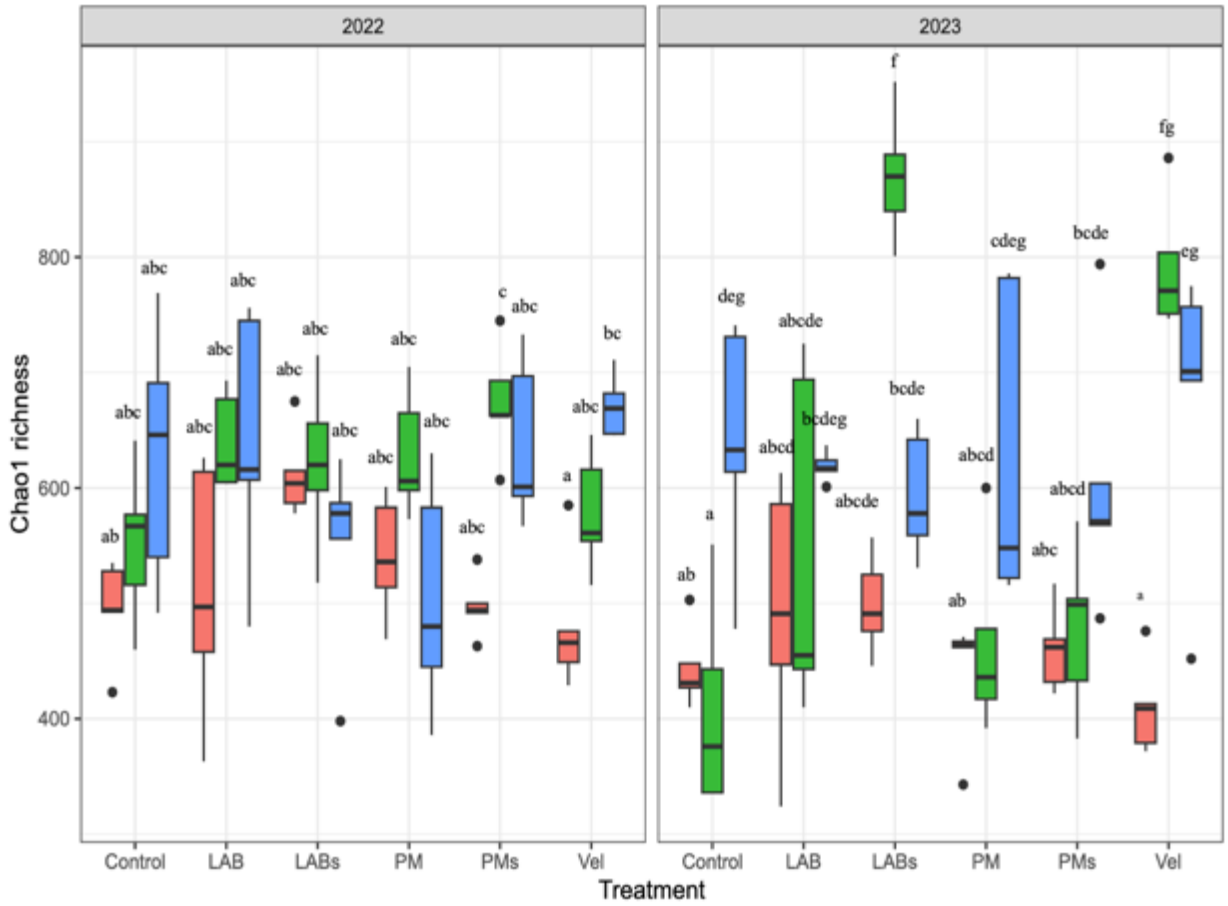

Figure 63. Standard boxplots to visualize the effect of treatments on Chao-1 richness for the experiments conducted in 2022 and 2023. Chao1 richness index were calculated before treatment application (T0), 45 days after treatment application (T1), and 90 days after treatment application (T2). The center line of boxplots shows the median, and the bottom and upper limits indicate the 25 and 75th percentiles, respectively. LAB = Compost A, LABs = Compost A-Autoclaved, PM = PoultryManure, PMs = PoultryManure-Autoclaved and Vel = Velum. Within each year, different letters above each boxplot indicate significant differences among the combinations of treatments and time.

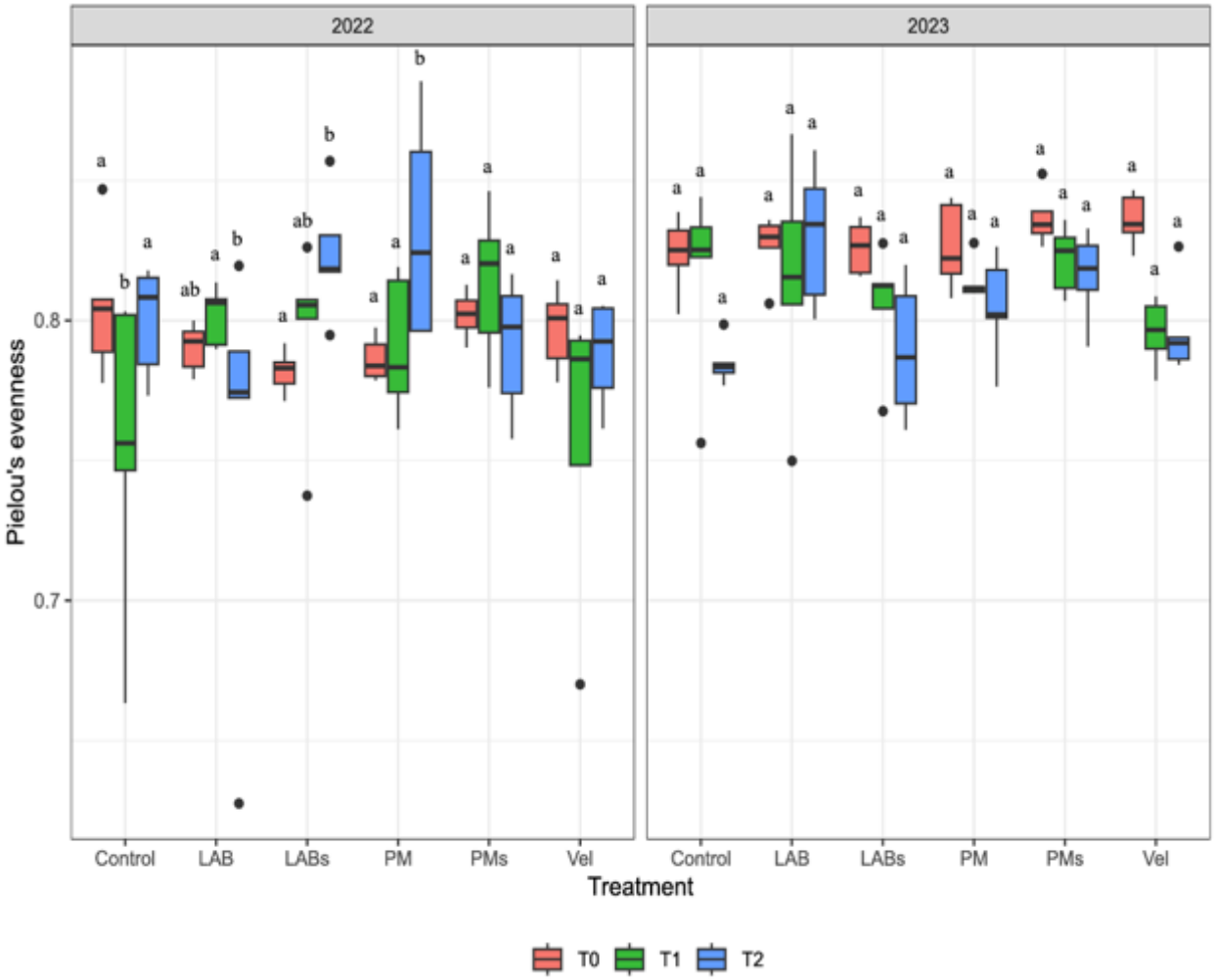

Figure 64. Standard boxplots to visualize the effect of treatments on bacteria Pielou's evenness index for the experiments conducted in 2022 and 2023. Pielou's evenness was calculated before treatment application (T0), 45 days after treatment application (T1), and 90 days after treatment application (T2). The center line of boxplots shows the median, and the bottom and upper limits indicate the 25 and 75th percentiles, respectively. LAB = Compost A, LABs = Compost A-Autoclaved, PM = PoultryManure, PMs = PoultryManure-Autoclaved and Vel = Velum. Within each year, different letters above each boxplot indicate significant differences among the combinations of treatments and time.

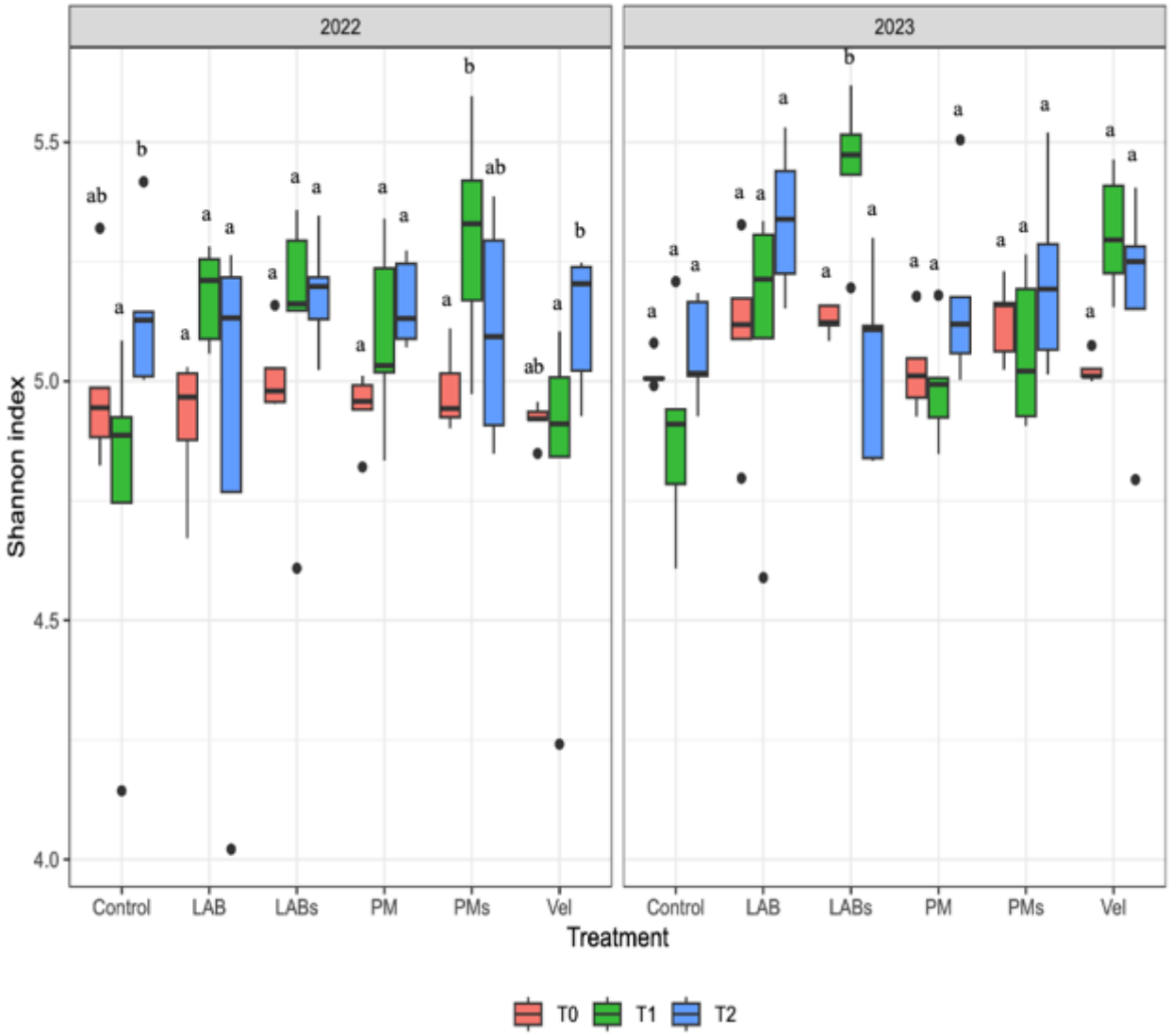

Figure 65. Standard boxplots to visualize the effect of treatments on bacteria Shannon diversity index for the experiments conducted in 2022 and 2023. Shannon diversity index was calculated before treatment application (T0), 45 days after treatment application (T1), and 90 days after treatment application (T2). The center line of boxplots shows the median, and the bottom and upper limits indicate the 25 and 75th percentiles, respectively. LAB = Compost A, LABs = Compost A-Autoclaved, PM = PoultryManure, PMs = PoultryManure-Autoclaved and Vel = Velum. Within each year, different letters above each boxplot indicate significant differences among the combinations of treatments and time.

For the experiments conducted in 2022 and 2023, Chao 1 richness of the bacterial community was significantly affected by treatment (DF = 5, F-value $_{(5,40)}$ = 7.201, p-value = 0.0001) and time (DF = 2, F-value $_{(2,96)}$ = 49.317, p-value<0.0001) (Figure 63). In the 2022 experiment, at T0,

Chao1 richness started ranging between 481 to 612. At T1, the highest Chao1 richness was found in PMs (674), followed by LAB (640), PM (629), LABs (621), Velum (579), and the control (552). From T0 to T1, the highest Chao1 richness increase was found in PMs-treated soils, in which the increase was 26.3%, followed by LAB (20%), Velum (16.9%), PM (13%), control (10.4%), and LABs-treated soils (1.4%). From T1 to T2, Chao1 richness increased only in the control soils and in the Velum-treated soils (12.1% and 13.7%, respectively). As for the compost-treated soils, Chao1 richness decreased at T2, and the highest decrease was found in PM-treated soils (19.7%). As for the 2023 experiment, at T0, Chao1 richness ranged between 410 and 499. At T1, Chao1 richness decreased 8.10% in the control soils, while it increased in all the treated soils. The highest increase in richness was found in Velum-treated soils (48.2%), followed by LABs-treated soils (42%). However, at T2, there was a decrease in richness for these two treatments (15% and 32%, respectively). At this same sampling time point, Chao1 richness increased for all the other treatments, including the control in which richness increased 36.2% from T1 to T2. The second highest increase was found in PM-treated soils (26.3%), followed by PMs (21%), and LAB (12%).

As for Pielou's evenness index, it did not significantly vary among treatments (DF = 5, F-value $_{(5,40)}$ = 1.977, p-value = 0.1031) but changed across time (DF = 2, F-value $_{(2,96)}$ = 3.952, p-value = 0.0224) (Figure 64). In 2022, the evenness of bacterial communities decreased from T0 to T1 in the control and Velum-treated soils (6.3% and 4.9%, respectively), while it increased in the compost-treated soils. However, from T1 to T2, evenness decreased only in LAB and PMs-treated soils (5.6% and 2.7%, respectively), while it increased in the other treatments. As for the 2023 experiment, bacterial communities' evenness decreased from T0 to T1 in all the treatments and kept decreasing from T1 to T2 in all treatments except in LAB, in which there was an increase of 1.8%.

Lastly, the Shannon diversity index, it varied among treatments (DF = 5, F-value $_{(5,40)}$ = 3.015, p-value = 0.0211) and time (DF = 2, F-value $_{(2,96)}$ = 5.508, p-value = 0.0054) (Figure 65). In 2022, from T0 to T1, diversity decreased in the control and Velum-treated soils (4.6% and 2%, respectively), and in the compost-treated soils, diversity increased. The highest increase was found in PM-treated soils (6%), followed by LAB-treated soils (5.1%). From T1 to T2, diversity decreased in the LAB-treated soils (5.8%) and in the PMs-treated soils (3.6%), while it increased in the control and Velum-treated soils (7.4% and 6%, respectively). In 2023, diversity decreased

from T0 to T1 in the control soils (2.6%), PM and PMs-treated soils (0.8% and 1.4%, respectively), while in LABs and Velum-treated soils there was a diversity increase (5.9% and 5.5%, respectively). In contrast, from T1 to T2 there was a decrease in diversity in LABs and Velum-treated soils (7.5% and 2.4%, respectively), while in the other treatments, there was an increase.

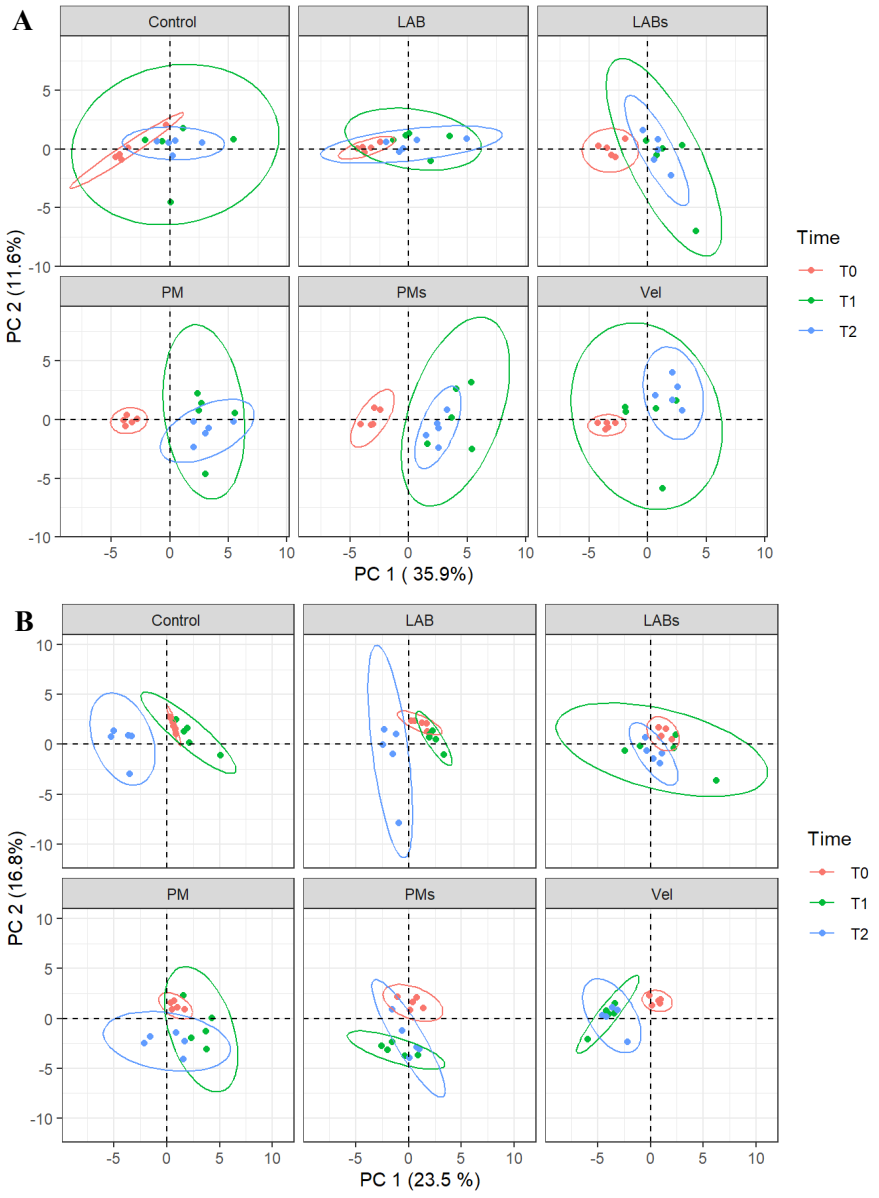

Figure 66. Principal component analysis of bacterial communities for **A.** 2022 greenhouse experiment and **B.** 2023 greenhouse experiment at the different sampling time points differentiated with the different colors (Red= before treatment application (T0), Green=45 days after treatment application (T1), and Blue=90 days after treatment application (T2)) using a

Figure 66. (cont'd)

Principal component Analysis plot (PCA) based on a Bray-Curtis dissimilarity on Hellinger transformed data for each of the treatments (LAB = Compost A, LABs = Compost A-Autoclaved, PM = PoultryManure, PMs = PoultryManure-Autoclaved and Vel = Velum). PERMANOVA analysis showed that the ß-diversity of bacterial communities in the 2022 greenhouse experiment was significantly different among treatments (P-value = 0.003) and significantly differed across time (P-value = 0.001) (Figure 66A). At all-time points, only the application of PM and PMs resulted in a significant/marginally significant impact on the ß-diversity of bacterial communities in comparison to the control (P-value = 0.078 and P-value = 0.021, respectively). Pairwise comparisons among treatments showed that the application of PMs also resulted in a significant/marginally significant impact on bacteria ß-diversity in comparison to the Velum-treated soil (P-value = 0.055) and LAB (P-value = 0.027). In contrast, there were no significant differences between bacteria ß-diversity of PM and PMs-treated soils (P-value = 0.628) nor LAB and LABs-treated soils (P-value = 0.415). However, there was a marginally significant difference between PM and LAB-treated soil bacteria ß-diversity (P-value = 0.089). Pairwise comparisons between sampling times showed that ß-diversity was significantly affected between T0 and T1 as well as between T0 and T2 (P-value = 0.001) while marginally affected between T1 and T2 (P-value = 0.076).

As for the ß-diversity of bacterial communities in the 2023 greenhouse experiment, it was significantly different among treatments (P-value = 0.001), and also significantly different across time (P-value = 0.001) (Figure 66B). Across all time points, applications of PM and PMs resulted in significantly different ß-diversity of bacterial communities compared to the control (P-value = 0.011, and P-value = 0.014, respectively), while applications of Vel resulted in a marginally significant effect in comparison to the control (P-value = 0.06). Application of Vel also resulted in significant differences when compared to PMs (P-value = 0.001), LABs (P-value = 0.001), PM (P-value = 0.001), and LAB (P-value = 0.001). Applications of PMs resulted in significant differences when compared to LABs (P-value = 0.002), and LAB (P-value = 0.002), while application of LABs resulted in a significant difference when compared to PM (P-value = 0.03). In addition, there was a marginally significant difference between PM and LAB (P-value = 0.057). In contrast, there were no significant differences between PM and PMs (P-value) = 0.185) nor LAB and LABs (P-value = 0.471). Pairwise comparison between sampling times

showed that ß-diversity was significantly affected between T0 and T1 as well as between T0 and T2 and T1 and T2 (P-value = 0.001).

α-Diversity indices and ß-Diversity of Fungal Communities of Soil Over Time when Treated with Manure-Based Amendments Autoclaved Vs. Non-Autoclaved

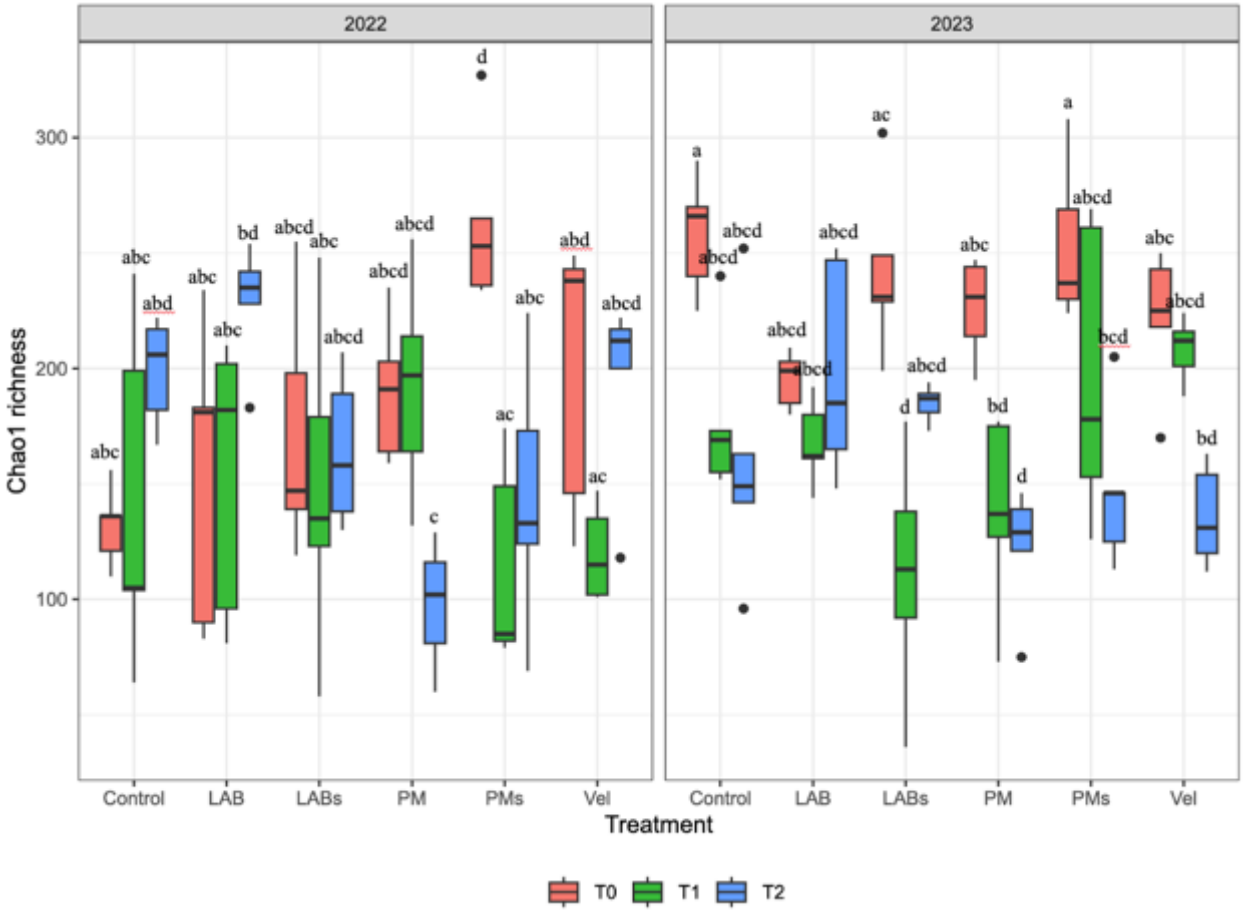

Figure 67. Standard boxplots to visualize the effect of treatments on fungal community Chao-1 richness for the experiments conducted in 2022 and 2023. Chao1 richness was calculated before treatment application (T0), 45 days after treatment application (T1), and 90 days after treatment application (T2). The center line of boxplots shows the median, and the bottom and upper limits indicate the 25 and 75th percentiles, respectively. LAB = Compost A, LABs = Compost A-Autoclaved, PM = PoultryManure, PMs = PoultryManure-Autoclaved and Vel = Velum. Within each year, different letters above each boxplot indicate significant differences among the combinations of treatments and time.

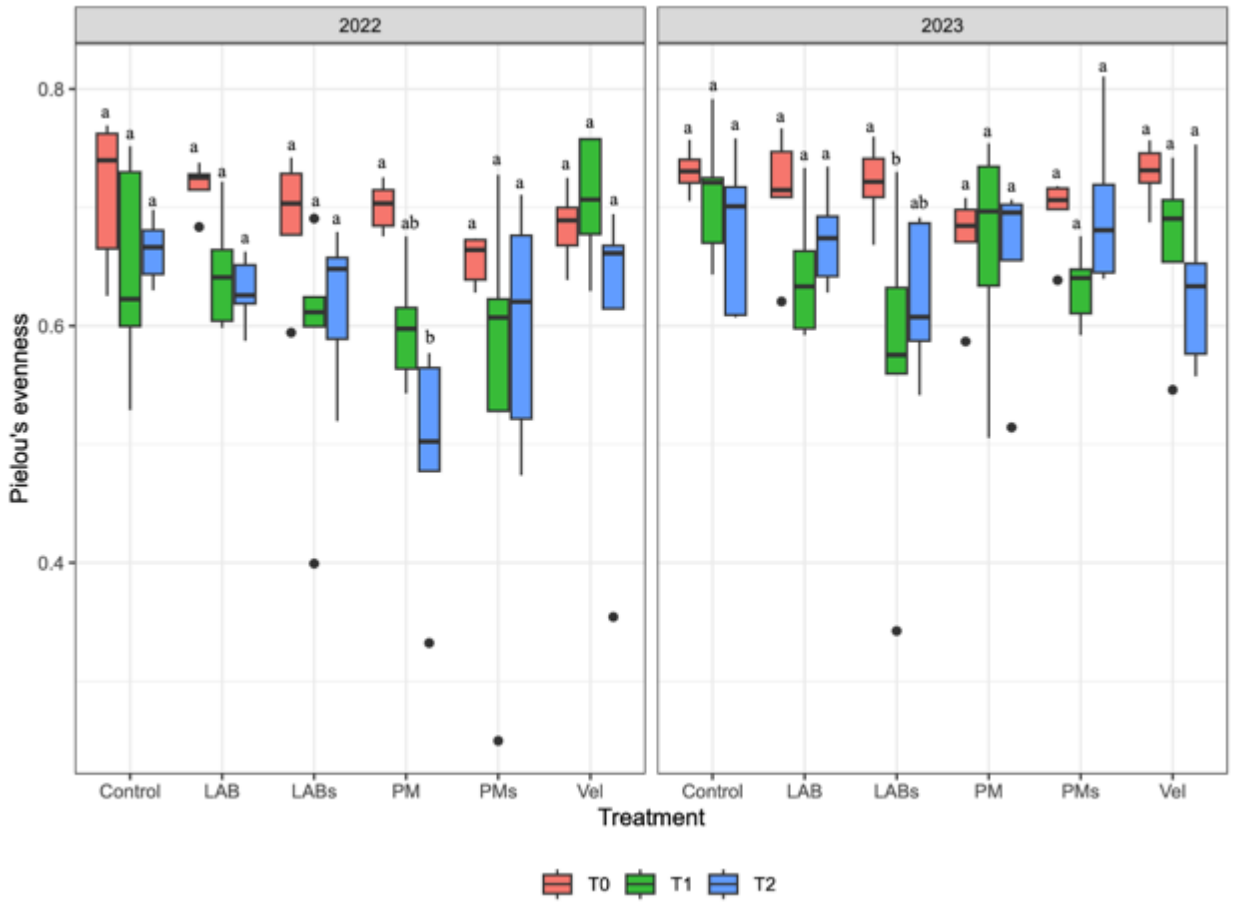

Figure 68. Standard boxplots to visualize the effect of treatments on fungal community Pielou's evenness for the experiments conducted in 2022 and 2023. Pielou's evenness was calculated before treatment application (T0), 45 days after treatment application (T1), and 90 days after treatment application (T2). The center line of boxplots shows the median, and the bottom and upper limits indicate the 25 and 75th percentiles, respectively. LAB = Compost A, LABs = Compost A-Autoclaved, PM = PoultryManure, PMs = PoultryManure-Autoclaved and Vel = Velum. Within each year, different letters above each boxplot indicate significant differences among the combinations of treatments and time.

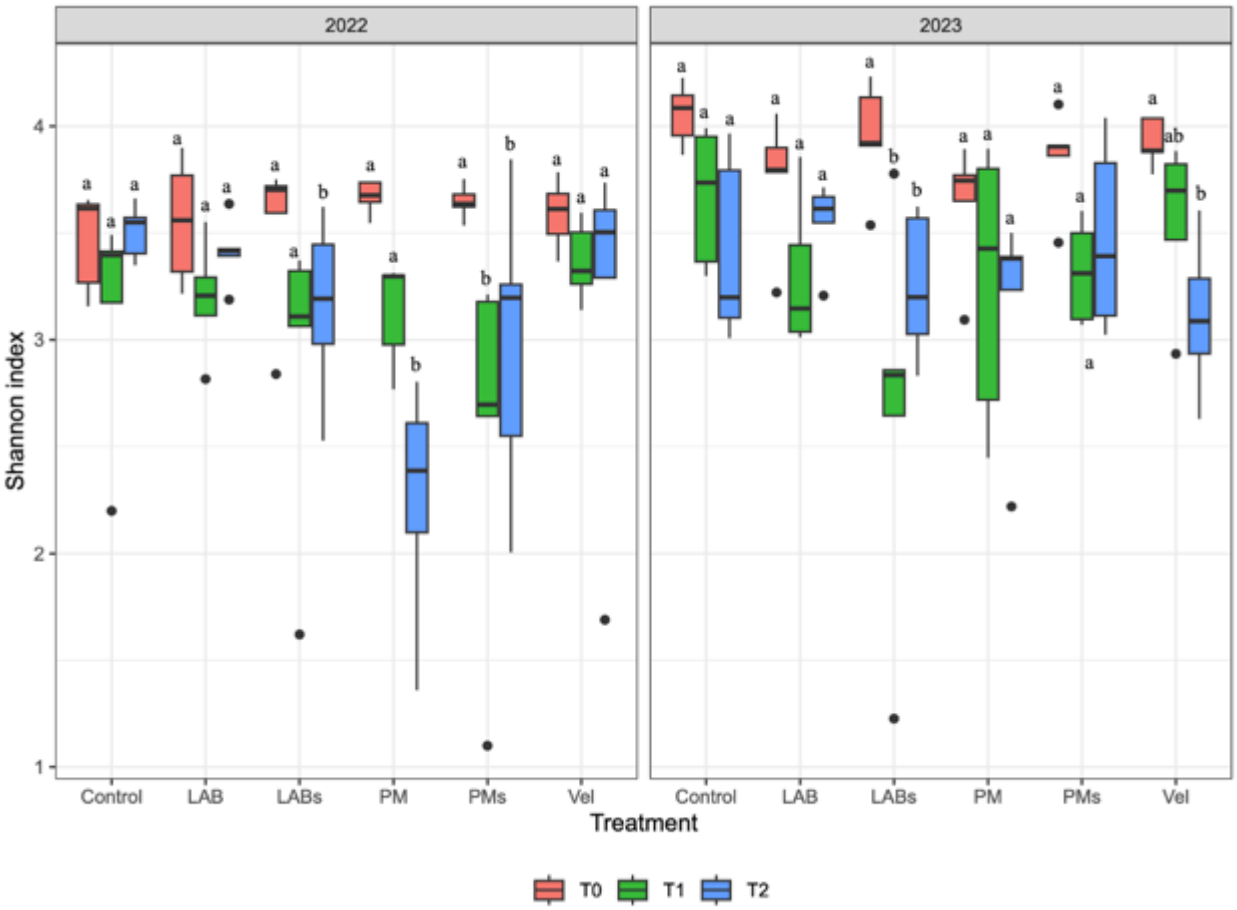

Figure 69. Standard boxplots to visualize the effect of treatments on fungal community Shannon index for the experiments conducted in 2022 and 2023. Shannon diversity index was calculated before treatment application (T0), 45 days after treatment application (T1), and 90 days after treatment application (T2). The center line of boxplots shows the median, and the bottom and upper limits indicate the 25 and 75th percentiles, respectively. LAB = Compost A, LABs = Compost A-Autoclaved, PM = PoultryManure, PMs = PoultryManure-Autoclaved and Vel = Velum. Within each year, different letters above each boxplot indicate significant differences among the combinations of treatments and time.

For the experiments conducted in 2022 and 2023, Chao 1 richness of the soil fungal community was not significantly different among treatments (DF = 5, F-value $_{(5,40)}$ = 1.45, p-value = 0.22) while it was among sampling times (DF = 2, F-value $_{(2,96)}$ = 26.99, p-value<0.0001) (Figure 67). In the greenhouse experiment conducted in 2022, Chao1 richness decreased from T0 to T1 in LABs, PMs, and Velum-treated soils by 13.4%, 57%, and 40%, respectively, while in the control, and PM-treated soils, richness increased by 7.57% and 1.14%, respectively. However,

from T1 to T2, richness increased in all treatments, except for in PM-treated soils, where richness decreased by 49.3%. In the 2023 experiment, Chao1 richness decreased in all treatments from T0 to T1. The biggest decrease in richness was found in LABs-treated soils and the control (54% and 31.1%, respectively). This same trend was followed from T1 to T2, during which richness decreased in all treatments but LAB-treated soils in which richness increased by 16%. The highest decrease in richness was found in LABs-treated soils, followed by Velum and PMs-treated soils (39.8%, 34.6%, and 22.5%, respectively).

As for Pielou's evenness index, it was significantly different among treatments (DF = 5, F-value $_{(5,40)}$ = 3.34, p-value = 0.01) and among sampling times (DF = 2, F-value $_{(2,96)}$ = 17.30, p-value = 0.02) (Figure 68). In the 2022 experiment, fungal communities' evenness decreased from T0 to T1 in all treatments but in Velum-treated soils, in which evenness increased by 3.1%. As for the other treatments, the highest decrease was found in PMs, PM, and LABs treated soils (17%, 15%, and 15%, respectively). From T1 to T2, evenness decreased in all treatments but LABs, where evenness increased by 5.5%, while the highest decrease was in Velum-treated soils (15.3%). In the 2023 experiment, evenness also decreased in all treatments from T0 to T1, especially in LABs-treated soils in which the decrease was 21%. However, from T1 to T2, evenness increased only in LAB, LABs, and PMs-treated soils (4.4%, 8.8%, and 9.4%, respectively.

Lastly, the Shannon diversity index was significantly different among treatments (DF = 5, F-value $_{(5,40)}$ = 3.20, p-value = 0.01) and time (DF = 2, F-value $_{(2,96)}$ = 29.76, p-value<0.0001) (Figure 69). In the 2022 greenhouse experiment, fungal diversity decreased in all treatments from T0 to T1. The highest decrease in diversity was found in PMs, LABs, and PM-treated soils in which the decrease was 30%, 17.6%, and 14.7%, respectively. From T1 to T2, diversity increased in the control, LAB, LABs, and PMs by 10.5%, 6.2%, 7.9%, and 13.5%, respectively. In contrast, diversity decreased in PM, and Velum-treated soils by 28.1% and 5.6%. A similar trend was observed in the 2023 greenhouse trial in which diversity decreased in all treatments from T0 to T1 and kept decreasing in all treatments but LAB, LABs, and PMs, where diversity increased from T1 to T2 by 7.04%, 17.8%, and 4.6%.

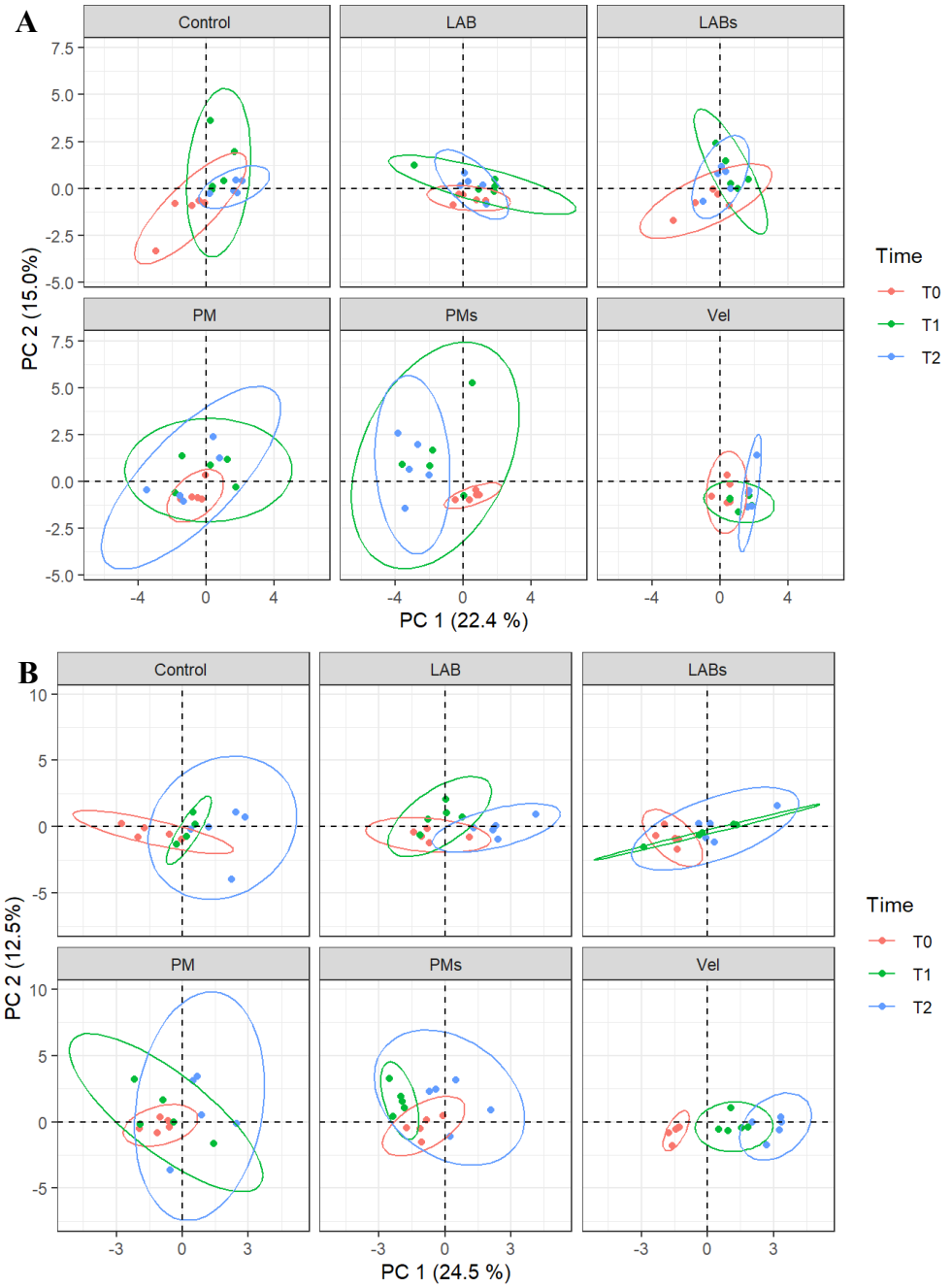

Figure 70. Principal component analysis of fungal communities for **A.** 2022 greenhouse experiment and **B.** 2023 greenhouse experiment at the different sampling time points differentiated with the different colors (Red= before treatment application (T0), Green=45 days after treatment application (T1), and Blue=90 days after treatment application (T2)) using a Principal component Analysis plot (PCA) based on a Bray-Curtis dissimilarity on Hellinger

Figure 70. (cont'd)

transformed data for each of the treatments (LAB = Compost A, LABs = Compost A-Autoclaved, PM = PoultryManure, PMs = PoultryManure-Autoclaved and Vel = Velum). PERMANOVA analysis showed that the ß-diversity of soil fungal communities in the 2022 greenhouse experiment significantly differed among treatments (P-value = 0.001) and time (P-value = 0.001) (Figure 70A). Across all sampling times, compared to the control, the application of Vel, PMs, PM, and LAB significantly affected soil fungal ß-diversity (P-value = 0.025, P-value = 0.002, P-value = 0.016 and P-value = 0.007, respectively), while application of LABs did not (P-value = 0.298). ß-diversity of soil fungal communities in Vel-treated soil significantly differed from PMs, LABs, PM, and LAB-treated soils (P-value = 0.001, P-value = 0.004, P-value = 0.021 and P-value = 0.01, respectively). The fungal ß-diversity of PMs-treated soils significantly differed from LABs and LAB-treated soils (P-value = 0.007 and P-value = 0.011, respectively), while it did not significantly differ from PM-treated soil (P-value = 0.139). In contrast, the fungal ß-diversity of LABs-treated soils was marginally different from LAB-treated soils (P-value = 0.063). As for the fungal ß-diversity of PM-treated soils and LAB-treated soils, it was not significantly different (P-value = 0.173). Pairwise comparisons between sampling times showed that soil fungal ß-diversity significantly changed between T0 and T1, T0 and T2, and T1 and T2 (P-value = 0.001, P-value = 0.039 and P-value = 0.02, respectively).

As for the 2023 greenhouse experiment, the ß-diversity of soil fungal communities significantly differed among treatments (P-value = 0.001) and time (P-value = 0.001) (Figure 70B). Across all sampling times, compared to the control, only the ß-diversity of PMs-treated soils was significantly different (P-value = 0.002). In contrast, the ß-diversity of soil fungal communities in Vel-treated soil significantly differed from PMs and PM-treated soils (P-value = 0.001, and P-value = 0.015, respectively). The fungal ß-diversity of PMs-treated soils significantly differed from LABs and LAB-treated soils (P-value = 0.003 and P-value = 0.001, respectively), while it did not significantly differ from PM-treated soils (P-value = 0.19). The fungal ß-diversity of LABs-treated soils was marginally different from LAB-treated soils (P-value = 0.086), as well as between PM-treated soils and LAB-treated soils (P-value = 0.06). Pairwise comparisons between sampling times showed that soil fungal ß-diversity significantly changed between T0 and T1, T0 and T2, and T1 and T2 (P-value = 0.014, P-value = 0.001 and P-value = 0.003, respectively).

Correlation Between the Relative Abundance of Bacterial and Fungal Phyla, and *P. penetrans* Abundance at the Different Sampling Times

The relative abundance of some bacteria phyla were significantly correlated with *P. penetrans* populations at 45 (T1) and 90 (T2) days after treatment application, however, correlations were different between the greenhouse experiment conducted in 2022 and 2023 (Table 17). Overall, there were a total of 30 positive correlations and 22 negative correlations and the only bacteria phylum that was only negatively correlated with *P. penetrans* abundance was the Firmicutes in the greenhouse experiment conducted in 2023.

Table 17. Pearson's correlation coefficients (=Coef.) between the relative abundance of bacterial phyla and *P. penetrans* abundance at 45 days after treatment application (T1), and 90 days after treatment application (T2) for each of the treatments (LAB = Compost A, LABs = Compost A-Autoclaved, PM = PoultryManure, PMs = PoultryManure-Autoclaved and Vel = Velum). (-) indicates no correlation found.

	Phylum	Treatment																							
		Control				LAB				LABs				PM				PMs				Vel			
		T1		T2		T1		T2		T1		T2		T1		T2		T1		T2		T1		T2	
		Coef. (r)	*p* value	Coef. (r)	*p* value	Coef. (r)	*p* value	Coef. (r)	*p* value	Coef. (r)	*p* value	Coef. (r)	*p* value	Coef. (r)	*p* value	Coef. (r)	*p* value	Coef. (r)	*p* value	Coef. (r)	*p* value	Coef. (r)	*p* value	Coef. (r)	*p* value
2022	Acidobacteriota	-	-	-	-	-	-	-	-	-	-	-	-	0.87	0.05	-	-	-	-	-	-	-0.84	0.06	0.88	0.04
	Actinobacteriota	-0.84	0.07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Armatimonadota	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.94	0.02	-	-	-	-	0.88	0.04
	Bacteroidota	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.92	0.02	-0.88	0.04	-	-
	Bdellovibrionota	0.81	0.09	-	-	-	-	-0.99	<0.001	-0.84	0.07	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Chloroflexi	-	-	-	-	-0.87	0.05	0.96	0.009	-	-	0.87	0.05	-	-	-	-	-	-	-	-	-	-	-0.85	0.06
	Cyanobacteria	-	-	-	-	-	-	-	-	-	-	-	-	0.85	0.07	-	-	0.88	0.05	-	-	-	-	-	-
	Desulfobacterota	-	-	0.82	0.08	-	-	0.82	0.08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Gemmatimonadota	0.96	0.007	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Myxococcota	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-0.99	<0.001	-	-	-	-
	Nitrospirota	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Proteobacteria	-0.87	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Spirochaetota	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Sumerlaeota	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Verrucomicrobiota	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-0.89	0.04	-	-	-	-	0.88	0.04	-	-
2023	Abditibacteriota	-	-	0.83	0.08	-	-	-	-	0.95	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Acidobacteriota	-0.90	0.03	-0.93	0.02	-	-	-	-	-	-	0.99	0.02	-	-	-	-	0.85	0.06	-	-	0.89	0.04	-	-
	Actinobacteriota	-0.81	0.07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.81	0.09	0.89	0.04
	Armatimonadota	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.81	0.09	-	-	-	-	-	-
	Bacteroidota	0.99	<0.001	-	-	-0.84	0.06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Chloroflexi	-	-	0.94	0.02	-	-	-	-	0.84	0.07	-	-	-	-	-	-	0.96	0.009	-	-	-0.97	0.004	-	-
	Cyanobacteria	0.86	0.05	0.85	0.06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-0.85	0.06	-	-
	Desulfobacterota	-	-	-0.94	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Firmicutes	-	-	-	-	-	-	-	-	-	-	-0.95	0.01	-	-	-	-	-	-	-	-	-	-	-	-
	Gemmatimonadota	-0.91	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.97	0.005	-	-	-	-	-	-
	Myxococcota	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-0.87	0.05	-	-	-	-
	Nitrospirota	-0.90	0.03	-0.91	0.03	-	-	-	-	-	-	-	-	-	-	-0.92	0.02	-	-	-	-	0.94	0.01	-	-
	Proteobacteria	0.87	0.05	-	-	-	-	-	-	-	-	-0.92	0.02	-	-	-	-	-0.98	0.002	-0.86	0.05	-	-	-	-
	Spirochaetota	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Sumerlaeota	-	-	0.97	0.004	-	-	-	-	0.93	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Verrucomicrobiota	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

As for the relative abundance of fungal phyla, fewer correlations were found with *P. penetrans* abundance, and the correlations found were different between the experiments conducted in 2022 and 2023 (Table 18). In total, there were 6 positive correlations and 6 negative correlations. Mucoromycota was the only phylum only positively correlated with *P. penetrans* abundance, while Oomycota phylum was the only phylum only negatively correlated with *P. penetrans* abundance.

Table 18. Pearson's correlation coefficients (=Coef.) between the relative abundance of fungal phyla and *P. penetrans* abundance at 45 days after treatment application (T1), and 90 days after treatment application (T2).

	Phylum	Treatment																							
		Control				LAB				LABs				PM				PMs				Vel			
		T1		T2		T1		T2		T1		T2		T1		T2		T1		T2		T1		T2	
		Coef. (r)	*p* value	Coef. (r)	*p* value	Coef. (r)	*p* value	Coef. (r)	*p* value	Coef. (r)	*p* value	Coef. (r)	*p* value	Coef. (r)	*p* value	Coef. (r)	*p* value	Coef. (r)	*p* value	Coef. (r)	*p* value	Coef. (r)	*p* value	Coef. (r)	*p* value
2022	Ascomycota	-	-	-	-	-	-	-	-	0.82	0.08	-	-	-	-	-	-	-	-	-	-	-	-	0.90	0.03
	Basidiomycota	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Glomeromycota	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mortierellomycota	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-0.90	0.03
	Mucoromycota	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Olpidiomycota	-	-	-	-	-	-	-0.91	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Oomycota	-	-	-	-	-	-	-0.82	0.08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2023	Ascomycota	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-0.92	0.02	-	-
	Basidiomycota	-	-	-	-	-0.90	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.83	0.08
	Glomeromycota	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mortierellomycota	-	-	-0.92	0.03	-	-	-0.90	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mucoromycota	-	-	-	-	-	-	0.96	0.006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Olpidiomycota	0.94	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Oomycota	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

5.4 DISCUSSION

Autoclaved vs. non-autoclaved manure-based amendments effect on nematode abundance and plant performance.

Literature suggests that the disease suppression activity of manures and composts could be attributed to the microbiome within these amendments and/or the effect of these amendments on the natural soil microbiome. For instance, in a study published by Kaplan et al. 1992, they showed that in soils amended with autoclaved poultry manure, there was more infection of tomatoes by *Meloidogyne arenaria* than in soils amended with not-autoclaved poultry manure and concluded that such result is correlated with the microbiome of the manure. Similarly, Ringer et al, 1997 showed that poultry manure was the most efficient amendment to reduce damping-off caused by *Pythium* spp. and that the loss of disease suppression by autoclaved manure was evidence that disease suppression is mediated by microorganisms as well as low levels of NO_2 and NO_3. Zhang et al. 1998, demonstrated that autoclaving a pine bark compost fortified with *Trichoderma hamatum* and *Flavobacterium balustinum* did not induce systemic acquired resistance (SAR) in cucumbers to *Colletotrichum orbiculare* while inoculating the autoclaved compost with non-autoclaved compost restored the SAR effect. The authors then conclude that the SAR activity of the compost is attributed to the microbiome.

Some studies show that there could be no correlation between the biological activity of organic soil amendments and disease suppression. For example, non-aerated compost teas inhibited *Alternaria solani*, *Botrytis cinerea,* and *Phytophthora infestans* in-vitro, while autoclaved teas did not inhibit pathogens growth. However, correlation analysis showed that neither microbial communities nor physic-chemical compositions of the teas were correlated with their relative efficacy (Koné et al. 2010). In my study, I found that the application of manure-based amendments poultry manure, and LAB (Compost A) autoclaved or not autoclaved resulted in fewer *P. penetrans* abundance in soil and roots throughout the 90-day experiment period compared to the controls. In addition, there were no significant differences in nematode abundance between autoclaved vs. not autoclaved manure-treated soils and overall, the lowest abundance of *P. penetrans* was found in PM and PMs-treated soils. There are other mechanisms by which organic soil amendments are suppressive to plant-parasitic nematodes which include the release of nematicidal compounds that exist in the amendment, the production of nematicidal compounds like fatty acids and ammonia during degradation in the soil, an increase in plant

tolerance and resistance and changes in the soil physico-chemical properties (Oka, 2010). In my study only the possible role of microbial activity was explored by also evaluating autoclaved manure-based amendments, however, some studies have provided evidence that suggests the disease suppression activity of such amendments, especially manure-based, is more due to abiotic factors. For example, cow manure-based compost was tested against *M. javanica* and chemical analysis of the compost and leachates of the treated soils showed that high electrical conductivity and high concentrations of nitrogen forms were the main cause of nematode suppression (Oka and Pivonia, 2003). Nitrogenous compounds, specifically NH_3, are thought to be very efficient at killing nematodes, and such form of nitrogen is usually found in low C/N ratio amendments like the raw poultry manure used in my experiment. However, the conversion to NH_3 is highly dependent on soil conditions, and oxidization of NH_3 to NO_2 and then to NO_3 can happen rapidly through the activity of nitrifying microorganisms (Oka et al. 2010). Animal manures like poultry manure can also be rich in short-chain fatty acids like acetic, propionic, butyric, isovaleric, and caproic, which can be toxic against plant-parasitic nematodes. For instance, valeric acid from liquid hog manure was shown to be the most toxic to *P. penetrans* (Mahran et al. 2008). Cole et al. 2020 showed that poultry manure and LAB had high concentrations of butyric and acetic acid, while only poultry manure had concentrations of valeric acid. This together with my greenhouse experiments suggests that the disease-suppressive activity of PM and LAB amendments used in this experiment may not be entirely due to microbial activity and that instead, the pesticidal activity of these amendments may heavily rely on the release of nematicidal compounds like short-chain fatty acids. Hence, to establish the correlation between organic acids and *P. penetrans* low abundance in amended soils, the concentration of organic acids with known nematicidal activity should be determined in the manure-based amendments and once incorporated into the soil throughout the growing season. Aside from the low abundance of *P. penetrans* in PM, PMs, LAB, and LABs-treated soils, the application of PM and LAB autoclaved or not autoclaved resulted in the tallest plants in comparison to the untreated and Velum control. In addition, applications of PM resulted in the highest number of progeny tubers, and in the greenhouse experiment conducted in 2023, this treatment also resulted in the heaviest tubers. In a study conducted by Siddiqui, 2004, among the organic fertilizers used to control *M. incognita* in tomato plants, poultry manure application resulted in the tallest plants when compared to the infected-untreated control, as well as less

galling and nematode reproduction. In another study, the addition of plant-based compost to soil planted with tomatoes significantly increased shoot weight and fruit yield (Tahiri et al. 2022). Compost tea-based treatments also showed to increase potato sprouting, plant growth, and yield as well as reduce *Rhizoctonia solani* infection (Gonzalez-Hernandez et al. 2022). In contrast, one study showed that a variety of composts, including poultry manure, affected N and K, but that despite these effects on nutrient availability, yield was not significantly affected, and concluded that there is no short-term nutrient benefit for potatoes (Wilson et al. 2019). However, they mentioned they applied their compost in the fall before planting potatoes in the spring, and considering my results, the application of manure-based amendments may be more efficient at increasing tuber yield if applied not too far before planting (*i.e.* in the spring). Nevertheless, the changes in soil physical and chemical parameters such as macro and micronutrients in soils treated with poultry manure and LAB should be determined to conclude if these changes are correlated with the higher plant height, as well as the higher number and weight of tubers and if these changes are also correlated with *P. penetrans* low abundance in amended soils.

Applications of manure-based amendments on bacterial and fungal diversity and correlations with *P. penetrans* abundance

The soil microbiome plays a key role in crop productivity and protection from plant pathogens and the addition of organic soil amendments is a promising alternative to shape these communities and stimulate their beneficial activity (de Corato, 2020). Most literature reports an increase in microbial diversity after the addition of different types of compost. For instance, a study conducted by Nair and Ngouajio, 2012 showed that soil respiration was high in soils treated with dairy manure, as well as higher microbial biomass in treatments that included manure. In addition, they also found a positive correlation between soil organic matter and microbial biomass and concluded that this is due to the addition of cover crop residue and manure. In another study, Gomez et al., 2006 reported higher microbial diversity in soils amended with vermicompost and poultry manure. Another study showed that the addition of different composts, including manure, increased microbial biomass and respiration, however, they did not find a difference in bacterial community structure among the amended soils (Ros et al. 2006). In a 25-year study that evaluated the addition of a compost mix based on poultry manure, Liu et al. 2022 found that the diversity of bacterial and fungal phyla was significantly affected by compost applications, particularly increasing beneficial microorganisms, and

decreasing plant pathogens. This same study found that electrical conductivity, organic matter, and phosphorus concentrations were directly correlated with the increase in microbial diversity. In another study, the evaluation of a diverse number of composts, including one based on poultry manure, on soil chemical properties and microbial communities over 11 months, showed that compost increases different fractions of organic carbon and soil pH. The authors then suggest that the changes in soil bacterial and fungal communities were probably due to these soil chemical changes. In addition, they also demonstrated the contribution of microbial communities originating from the compost to the soil and showed that some of these persisted in the soil for almost one year (Goyer et al. 2022). Thus, from my experiments, the increase in microbial diversity in the autoclaved amendments-treated soils could suggest that other parameters like the addition of organic matter and other macro and micronutrients to the soil could have influenced microbial diversity. Additionally, in my experiments, I did not consider the microbial profile of the manure-based amendments and, therefore, did not consider the introduction and persistence of such communities in the soil. Hence, for future experiments, it would be crucial to consider it to determine if there are beneficial microorganisms in the compost that persist in the soil in addition to conducting experiments for more than one growing season.

Heisey et al., 2022, conducted an experiment with a single application of a high N compost made from human feces and sugarcane waste on radishes to evaluate the changes in soil microbial communities and network for six growing cycles. They found that fungal and procaryotic communities were significantly different in treated soils than in the control or urea-treated soils. In addition, they also found that fungal communities responded faster than the procaryotic communities, with the latter significantly changing after the third growing cycle. In addition, they also found that compost affected fungal communities more than procaryotic ones. In contrast, in my experiment, I found that bacterial communities were more diverse than fungal ones. At the same time, bacteria community diversity changed at 45 days after treatment application, while fungal communities changed significantly at 90 days in the amended soils. Thus, although my results only covered one growing cycle, it may suggest that the changes in microbial communities caused by the addition of amendments are dependent on the amendment and soil used, considering too that the bacterial and fungal phyla were different in 2022 and 2023 and the soil used was sourced from different locations as well as the manure-based amendments batches were different.

In my experiment, it was interesting to see that for bacteria, the most abundant taxa were the Actinobacteria and Proteobacteria, which was consistent in both experiments. However, the low abundant (<10%) ones were different in both years. At the same time, for bacteria, there were a high number of correlations with *P. penetrans* abundance, and the majority of these correlations were with the least abundant phyla. For example, the phyla that were only positively correlated with *P. penetrans* numbers were the Armatimonadota, Summerlaeota, Abditibacteriota, and Enthotenelaeota (relative abundance of <1%). In contrast, the phyla that were only negatively correlated with *P. penetrans* were the Firmicutes, which started in low abundance but increased significantly over time with the addition of amendments. As for fungi, there were fewer correlations, with most phyla showing both positive and negative correlations. In a paper published by Chen et al. 2020, applications of compost changed the microbial profile. In addition, through Random Forest regression modeling to identify the most important phyla to predict ecosystem functionality the authors found that the top three most important phyla were the Cyanobacteria, Armatimonadetes, and Fibrobacteres, which played the most ecologically important roles and also were the least abundant phyla (<3%). In contrast, the most abundant phyla such as the Proteobacteria played less significant roles. Hence, the correlation results from my experiments may suggest that low-abundant microbial taxa could provide unique functional traits relevant to *P. penetrans* survival, infection, and reproduction, but would need further investigation. In addition, the Firmicutes phyla relative abundance increased significantly over time only in the year 2023 in some of the amended soils. A published study by Zhou et al. 2019, showed that Firmicutes were the most abundant bacterial phyla in root-knot nematode-free soils. The authors discuss that a relevant genus from this phylum, *Bacillus* spp., is frequently associated with biological control activity against a variety of plant pathogens including nematodes like root-knot. Therefore, the results from my experiment could indicate that members of the Firmicutes phylum with biological control activity against *P. penetrans* were enhanced by the addition of manure-based amendments, but because these results were observed only in 2023, more experiments should be conducted to confirm their persistence in addition to identify the species of *Bacillus*.

5.5 CONCLUSION

Organic soil amendments are a promising management alternative for soil-borne pathogens like the root-lesion nematode *P. penetrans*. In Chapter 2 of this dissertation, results from field trials

showed that poultry manure and LAB (Compost A) are effective at keeping *P. penetrans* abundance low in soils and roots. One of the proposed mechanisms by which organic soil amendments like manure-based ones reduce plant-parasitic nematode populations is by the introduction or enhancement of beneficial microorganisms to the soil. The results from my greenhouse experiments led to the conclusion that poultry manure and LAB whether autoclaved or not, showed significantly less *P. penetrans* numbers in soil and roots compared to the untreated and Velum controls. The maximum level of control was provided by PM and PMs applications, which simultaneously also yielded the highest number and heaviest tubers. To study further the role of the soil microbiome on *P. penetrans* abundance, I determined the changes that the addition of poultry manure and LAB autoclaved and not-autoclaved caused to soil microbial communities, and identified microbial phyla that correlate with *P. penetrans* abundance. The results from this chapter led to the conclusion that the mechanism by which poultry manure and LAB maintain *P. penetrans* populations low is not entirely due to beneficial microorganisms. Hence, other proposed mechanisms such as the release or production of nematicidal compounds like nitrogenous forms or short-chain fatty acids, and changes to soil chemical properties should be explored in future experiments. Additionally, future experiments should be conducted to determine the persistence of changes in the soil's biotic and abiotic parameters throughout more than one growing season. The results from such experiments could provide evidence of manure-based amendments as management alternatives that aid in recovering and improving soil health, which can lead to less pesticide and synthetic fertilizer inputs.

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CHAPTER 6: IMPLICATIONS OF RESEARCH ON SUSTAINABLE MANAGEMENT STRATEGIES FOR POTATO EARLY DIE (PED)

The current dissertation provides evidence of management alternatives for Potato Early Die that are as effective as chemical-based tools, advocating for a shift towards sustainable agriculture and providing a roadmap to future experiments for achieving it. Disease complexes like PED are often understudied and hence challenging to manage, but this dissertation presents management alternatives for both *P. penetrans* and *V. dahliae*. The most effective management alternatives for *P. penetrans* control are raw poultry manure, both standalone and in combination with a singular application of Vydate® or MeloCon® (*Purpureocilium lilacinum*), and Compost A, a composite of composted raw poultry and cattle manure with wood ash, in combination with a singular application of MeloCon®. For *V. dahliae*, applications of Actinovate® (*Streptomyces lydicus*) showed the best control of disease symptoms under controlled greenhouse conditions while the treatment combination Velum®+Velum®+Movento®+Movento®+Vydate® showed a slight decrease of *V. dahliae* stem infection of 6% under field conditions, compared the untreated control where disease increased by 63%.

Disease complexes often require an integrated management approach to ensure maximum control of primary inoculum. Therefore, the findings from this research fortify the need for an Integrated Pest Management framework of biological, cultural, and chemical methodologies to minimize PED risk and optimize management efficacy. One example of an IPM framework that could be adopted by the potato production industry based on results from this research could be soil incorporation of raw poultry manure before planting, early season application of Vydate®, and applications of biological control agents like *P. lilacinum* and *S. lydicus* coupled with crop rotation with non-hosts, and meticulous weed, irrigation, and nutrient management. In addition, it was also found that the Compost A application resulted in higher yields. Reduced yield losses translate into higher profits for farmers and can have a positive ripple effect on the regional economy, especially considering the significant contribution of the food and agriculture industry to Michigan's economy.

Pest management practices that promote soil microbe activity are essential for soil natural pathogen suppression. Although *P. penetrans* abundance in manure-treated soils was significantly lower than the control in both autoclaved or non-autoclaved manure-treated soils, the relative abundance of soil bacterial and fungal phyla and the α- and ß-diversity indices of

bacterial and fungal species changed in response to both autoclaved and non-autoclaved manure amendments. In particular, the relative abundance of the Firmicutes bacteria phyla significantly increased with the addition of manure-based amendments and negatively correlated with *P. penetrans* abundance. A representative genus of this phylum is *Bacillus* which is often associated with biological control activity and some species are commercially available for use in agriculture. These findings highlight the importance of promoting microorganisms that aid in natural soil pathogen suppression and underscores the potential of organic waste like manure as a resource for agricultural pest management which may lead to more efficient resource utilization. Overall, these research findings are of global relevance and the insights gained are applicable worldwide, particularly in other regions where potato is a staple crop and PED is a concern. This research is also a foundation of evidence for the composting industry to design and improve their products as pest control tools which could have long-term benefits for the industry's sustainability. The results from this dissertation can also serve as points to promote the adoption of practices that are sustainable, economically viable and that address consumer health concerns around chemical-based pesticides and the market demand for “pesticide-free” food. Lastly, the results from this dissertation have added to the understanding of the impact of sustainable control alternatives for soil-borne diseases and provided ideas for grant proposals to gain funding for future experiments that will continue to benefit stakeholders such as the potato industry.

APPENDIX

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 in fluid-preserved specimens.

Voucher Number: 2024-01

Author and Title of Dissertation:

Author: Luisa M. Parrado

Title: Investigating Sustainable Strategies to Manage the Root-Lesion Nematode *Pratylenchus penetrans* and the Wilt-Inducing Fungus *Verticillium dahliae* in Potato Production

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*	Juvenile	1	Photograph