

EVALUATING DISTRIBUTION, PATHOLOGY, AND MANAGEMENT OF
ECONOMICALLY SIGNIFICANT PLANT-PARASITIC NEMATODE SPECIES IN
MICHIGAN CARROT AND WHEAT PRODUCTION

By

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ABSTRACT

Carrot (*Daucus carota* L.) and winter wheat (*Triticum aestivum*) are two important crop systems in Michigan agriculture. Bountiful yields are challenged by a myriad of bacterial, fungal, and viral diseases, herbivorous insects, nematodes, and animals. Particularly, plant-parasitic nematodes threaten yields as damage results in nonspecific symptoms, which can prohibit proper diagnosis. Root lesion nematodes (*Pratylenchus* spp.) are widespread in North America and are known to infect over 350 crop hosts. Because of their broad host range, typical recommendations for management like crop rotation are obsolete. Additionally, the distribution and abundance of *Pratylenchus* at the species level is unknown, further exacerbating issues with detection and developing tolerance levels. A series of objectives was established to help fill knowledge gaps and pave a pathway to effective management. During harvest, soil and root surveys were conducted for carrots (early October) and winter wheat (late June) to determine the top plant-parasitic nematode genera. In tandem, individual adult *Pratylenchus* females were collected for molecular identification from both carrot and wheat fields (N=100). Results indicated that *Pratylenchus* spp. were the most abundant and widespread plant-parasitic nematodes in both crops, occurring in 100% of carrot fields and 90% of winter wheat fields. *Pratylenchus penetrans*, *P. crenatus*, and *P. neglectus* were the most identified *Pratylenchus* species, emphasizing the need to investigate relationships with these hosts.

Next, under a series of greenhouse trials, the top three *Pratylenchus* species were inoculated to young carrot and wheat seedlings above the threshold level. Strikingly, *P. penetrans* and *P. crenatus* damaged nematode to carrots, eliciting root weight reduction by 36.94% and 21.76%, compared to non-inoculated control plants. *P. neglectus* did not reduce root weight or taproot length. Contrastingly, *P. neglectus* was the most damaging nematode in wheat

and reduced root weight by 24.07% compared to control plants, while *P. crenatus* did not reduce winter wheat plant height or root weights.

Finally, the grower standard, oxamyl, is a nematicide that may be subject to increased environmental bans. To investigate alternative strategies, such as alternative chemically based nematicides, biologically based nematicides, manure-based compost soil amendments, and cover crop incorporation for managing plant-parasitic nematode populations, we evaluated these strategies under field and greenhouse conditions. Under field conditions, *Pratylenchus* nematode harvest concentrations applied with biological or chemical nematicides were not significantly different from untreated control plots. In a two-year parsnip field trial, Nimitz and Vydate reduced root-knot nematodes and resulted in higher yields than control plots. Finally, the oilseed radish (cv. Control) was a nonhost to both *Pratylenchus penetrans* and *Meloidogyne hapla*, and it may be a suitable dead-end cover crop if field studies support our findings. None of the products effectively treated *Pratylenchus*-infested carrot fields, highlighting the complexities of this pest system. However, in parsnips, *M. hapla* populations were sufficiently managed with Nimitz, making this treatment a potential alternative to Vydate. Our studies provide evidence that *Pratylenchus* spp. are severe pests of carrot and winter wheat plants in Michigan and that species-specific identification of these species is essential in determining when and how to manage them.

This dissertation is dedicated to all four of my grandparents, Margaret Darling, Kenneth Darling, Rollin Garrett, and Linda Garrett, who are no longer with us.

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CHAPTER 1: CHALLENGES WITH *PRATYLENCHUS* SPP. NEMATODES IN CARROT SYSTEMS: SPECIES IDENTIFICATION AND MANAGEMENT

1.1 INTRODUCTION

Plant-parasitic nematodes (PPNs) invade plant cells and divert nutrients away from root cytoplasm, often resulting in tissue necrosis, plant stunting, and consequent yield losses (Mejias et al., 2019; Singh et al., 2013). These silent yield killers produce nonspecific above-ground symptoms, like wilting or patchiness, that often leave farmers unaware of issues unless samples are tested via submission to plant and pest diagnostic clinics. Feeding by PPNs stresses the plant, inducing it to activate immune responses and divert energy to fight infection or sustain the parasite itself (Khan & Khan, 2021). In root vegetables, three PPN taxa are predominantly responsible for yield losses: *Meloidogyne* spp. (root-knot nematodes), *Heterodera* and *Globodera* spp. (cyst nematodes), and *Pratylenchus* spp. (root lesion nematodes) (Bernard et al., 2017).

Globally, *Pratylenchus* spp. are within the top three PPNs that threaten agriculture, infecting over 350 plant species worldwide (Castillo & Vovlas, 2007; J. T. Jones et al., 2013). *Pratylenchus* nematodes are migratory endoparasites, entering plants by puncturing cell walls while utilizing a toolkit of digestive enzymes to tunnel through roots during feeding, leaving a path of necrotic tissue behind (Zunke, 1990a, 1990b). High concentrations of these pests will destroy entire root regions, leaving them susceptible to co-infection by other plant pathogens. Additionally, unlike other top PPNs like *Meloidogyne* spp. and *Heterodera* spp. that produce distinct galling and root disfiguration, *Pratylenchus* spp. below-ground symptoms are visibly indistinguishable from other pathogens (Castillo & Vovlas, 2007; J. T. Jones et al., 2013). For this reason, *Pratylenchus* spp. nematode damage often eludes proper diagnosis, exacerbating yield losses (Hallmann et al., 2007).

Researchers and farmers are challenged to develop management strategies for

Pratylenchus spp. that are effective, economically feasible, and non-toxic to the consumer or cause adverse environmental impacts (Atolani & Fabiyi, 2020). Methyl bromide and related broad-spectrum fumigant nematicides became unavailable in 2000 and 2005 in the United States and Europe, respectively (Johnson et al., 2012). Concerns of drawbacks with applicator safety, consumer health, and impact on environmental resources and non-target organisms led to bans, leaving farmers without protection against one of the most significant pathogens impacting global crop production (Noling & Becker, 1994; Zasada et al., 2010). In addition, common recommendations in vegetable IPM practices, such as crop rotation, resistant varieties, and chemical applications, are challenged by *Pratylenchus* spp. broad host range, lack of available resistant lines, multiple generations within a season, and difficulties with diagnosis. Synthesizing current knowledge on *Pratylenchus* species identification, reproduction, pathogenicity, damage, and available management strategies will uncover existing knowledge gaps, challenges, and implications in root vegetables.

1.1.1 *Pratylenchus* spp. identification

Like other PPN genera, *Pratylenchus* nematodes can be distinguished using a standard genera key (Mai & Mullin, 1996). These organisms are defined by their vermiform body shape, bold flat lip, stubby stylet, and overlapping esophagus (Castillo & Vovlas, 2007). However, obtaining a species-level diagnosis requires using molecular and morphological identification. Unfortunately, due to the high skillset necessary to identify individual nematodes to species, getting accurate identification is costly and time-consuming (Orlando et al., 2020). In addition, several critical morphological characteristics can be inconsistent or overlapping between *Pratylenchus* species (Table 1.1), presenting further challenges in obtaining confident species identification. Consequently, recent work has focused on incorporating molecular tools to further

our knowledge of nematode identification and related host interactions (Bogale et al., 2020; Orlando et al., 2020).

The development of these molecular tools for *Pratylenchus* species-level identification has been addressed for more than 30 years. First, standard barcoding procedures of using universal primers in the ITS region followed by sequencing were proposed for confident identification. Due to the time-consuming nature of this procedure paired with physical sample collection, quick-tool finger-printing protocols were proposed, starting with the use of PCR-restriction fragment length polymorphism (RFLP) patterns within the ITS region for *Pratylenchus* spp. identification (Orui, 1996). However, PCR-RFLP requires that PCR amplification and subsequent enzymatic digestion produce a distinctive species specific pattern. While this tool was seemingly effective for a small group of species, nematologists decided to move away from this strategy due to the volume of described species (>100) and refocus on species-species primer development (Orui & Mizukubo, 1999). Similarly, amplified fragment length polymorphism (AFLP) and random amplification of polymorphic DNA (RAPD), Sequence Characterized Amplified Region (SCAR), and Simple Sequence or Variable Number Tandem Repeats have also been investigated but have significant limitations with identifying more than a few species (Bogale et al., 2021).

From the 2000s onwards, proposed protocols for *Pratylenchus* spp. have heavily focused on the development of sequence-based methods of species-specific regions of rDNA, which encompasses conserved coding of 5.8S, 18S, and 28S regions, along with more variable regions of ITS and ETS (Bogale et al., 2020). Primarily, barcoding methods for *Pratylenchus* have focused on the COX1 gene region, although the D2-D3 expansion site of 28S is a viable region for species delimitation, too (Subbotin et al., 2008; Ozbayrak et al., 2018; Bogale et al., 2020). Most recently, machine learning and artificial intelligence strategies have been introduced for

reading samples, though it has been confined to egg and juvenile stages of *Heterodera* so far (Bogale et al., 2020). Furthermore, the creation of AI software that could identify and count *Pratylenchus* spp. would significantly limit human error and lead to less effort spent on monotonous, time-consuming tasks like sample reading, further propelling research on these organisms.

Table 1.1. *Pratylenchus* spp. morphometrics associated with North American carrot systems†.

Species	Lip annuli	V% range*	Stylet length	Body Length	Tail shape	Males?
<i>P. penetrans</i>	3	67 – 85.9	14.5 – 16.8	381 - 666	Round, smooth tip, or Crenate, annulated tip	Yes, 50%
<i>P. crenatus</i>	3	72 - 93	12 - 16.5	452 – 614	Round, smooth tip, or Crenate, annulated tip	No
<i>P. thornei</i>	3	71 - 82	14 - 17	427 - 688	Round, smooth tip Broad, truncate tip	No
<i>P. neglectus</i>	2	71 - 85	13 – 18	425 - 581	Round, smooth tip	Rarely, up to 7.5%

*V% signifies vulval percentage, which is the measurement between the tip of the head to the vulva, divided by the total body length and multiplied by 100.

†Measurements obtained from: Tarte & Mai, 1976; J. L. Townshend et al., 1978; Wu et al., 2002; İmren, 2007; D. Y. Chen et al., 2009; Mokrini et al., 2016; Junior et al., 2016; Janssen et al., 2017; Divsalar et al., 2018; Al-Khafaji et al., 2019; Divsalar et al., 2019; Bogale et al., 2021; Yüksel et al., 2023.

1.1.2 *Pratylenchus* life cycle and pathogenicity

The life cycle of *Pratylenchus* spp. begins with the egg, where the embryogenic and preliminary juvenile stages (J1 and J2) are developed. Following J2 development and hatching, all life stages can enter and feed on host roots. The remaining life stages before adulthood (J3 and J4) and during adulthood are vermiform (Pudasaini et al., 2008). Some *Pratylenchus* spp. (e.g., *P. penetrans*, *P. vulnus*) are sexually dimorphic with an even ratio of the two sexes. In contrast, others are exclusively female-dominated (*P. crenatus*) or majority female-dominated, where males are rare (*P. neglectus*, *P. thornei*) (Castillo & Vovlas, 2007). If the species is dimorphic, females and males become sexually distinguishable during adulthood by developing either a vulva

or spicule, respectively. *Pratylenchus* spp. reproduce sexually or asexually with parthenogenesis (Castillo & Vovlas, 2007; Zunke, 1990b). While all *Pratylenchus* spp. follow this development, the timing of the life cycle of *Pratylenchus* spp. greatly ranges from 21 to 65 days, dependent on species identity and several abiotic and biotic environmental conditions (Figure 1.1) (Castillo & Vovlas, 2007; Chitambar & Raski, 1985; Mizukubo & Adachi, 1997). *Pratylenchus penetrans* reproduce optimally at pH 5.2-6.4 and hatch in temperatures between 15-30°C, but most optimally at 20°C (Pudasaini et al., 2007; Willis, 1972).

Pratylenchus spp. nematodes infect plant hosts using mechanosensory and chemosensory organs to sense and locate ideal host roots (Basyoni & Rizk, 2016). Once a root is located, individuals use their stylet (mouthpart) to probe it for an ideal penetration site. The nematode stylet then pierces through the cell wall, injecting a slew of digestive enzymes to silence the plant's immune system and degrade the cell cytoplasm for feeding (Zunke, 1990b, 1990b). This behavior results in localized necrosis of root tissue, leaving visible brown lesions on the root surface. As the nematodes feed, they tunnel throughout the root, leaving an open pathway for other opportunistic plant pathogens. Feeding by *Pratylenchus* spp. on lateral root hairs can also limit nutrient uptake, stunting overall plant growth. Feeding can damage all portions of the root, but the zone of elongation, root tips, and root surface are most associated with yield and quality losses (M. Jones & Fosu-Nyarko, 2014).

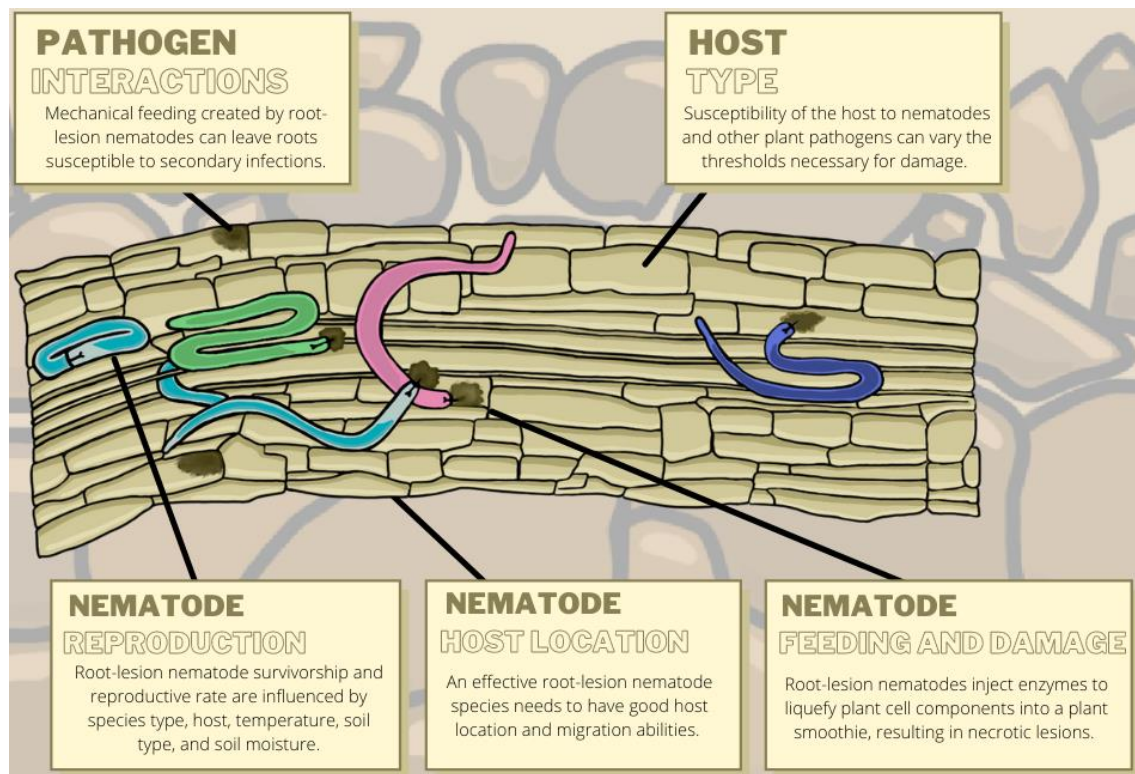


Figure 1.1. An illustration identifying the key components that influence *Pratylenchus* spp. ability to produce damage to host roots.

1.1.3 *Pratylenchus* spp. in worldwide carrot production

In carrots, damage to cells in the zone of elongation or apical meristem of the taproot can prompt the stressed plant to produce alternate taproots (forking) or to shorten the taproot (stubbiness). These root distortions, forking and stubbiness, are primarily associated with *Pratylenchus* spp. feeding; however, mechanical damage and other plant pathogens that hinder these root regions can result in similar symptoms (Hay & Pethybridge, 2005). Symptoms caused by *Pratylenchus* spp. are less evident and catastrophic than carrot cyst and root-knot nematodes, so the severity of this pest has historically been downplayed (Hallmann et al., 2007). This can further challenge proper diagnosis without confirmation of soil and root samples. Quality losses by *Pratylenchus* spp. are more severe than yield losses in carrots, but both should be considered in evaluating PPN damage severity (Teklu et al., 2016). Aggressive feeding early in the season can produce an unthrifty taproot, increasing the risk of root splitting, stunting, brown lesions, and

even plant death (Castillo & Vovlas, 2007). Distorted taproots are often rejected in fresh markets and processing carrot production, resulting in further economic losses (Sumonsiri & Barringer, 2014).

Prior distribution studies in essential regions for carrot production suggest *Pratylenchus* spp. occupy 84-90% of carrot fields (Hay & Pethybridge, 2005; Tangvik, 2017). Field surveys of carrot systems in Norway, Tasmania, and Germany have reported that *Pratylenchus crenatus* is the most prominent species in carrot fields grown in cool, temperate regions (Hay & Pethybridge, 2005; Hallmann et al., 2007; Tangvik, 2017). These regions all occupy moist, cool, temperate climates (Sayre et al., 2020), suggesting that conditions would be within optimal range for northern *Pratylenchus* species. Despite most existing literature describing *P. penetrans*-carrot relationships, these studies on distribution cohesively reported low or no *P. penetrans* prevalence within carrot systems. When *P. penetrans* were reported in fields, it was almost exclusively coexistent with other species (Hay & Pethybridge, 2005; Hallmann et al., 2007). Strikingly, of the total field samples (N=269) assessed within these three national surveys, 34% contained *P. crenatus*, 20.8% contained *P. neglectus*, 11.2% had *P. thornei*, and 4.8% of fields contained *P. penetrans*. This is a significant contrast to the available literature within temperate regions that focus on *P. penetrans* on carrots; however, field surveys in other cool, temperate areas of carrot production, like Canada and the northern United States, should be completed to understand the worldwide distribution of northern *Pratylenchus* species further. For example, *P. penetrans* and *P. crenatus* distribution ranges largely overlap in North America (Townshend et al., 1978).

Both *P. penetrans* and *P. crenatus* are associated with severely damaging carrot plants. Feeding above action thresholds by these species within the first thirty days of growth often results in increased seedling death and malformation of the taproot, primarily by forking and

stunting (Coosemans, 1975; Vrain & Belair, 1981; George et al., 2016). In severe infestations, populations can cause visible patchiness within carrot fields by killing and stunting young plants. However, there have been conflicting tolerance levels provided for these species, with some studies documenting severe damage ranging from 10-180 individuals of *P. penetrans* (Oostenbrink, 1972; Coosemans, 1975; Vrain & Belair, 1981; Seinhorst, 1998; Teklu et al., 2016) and 36-78 individuals of *P. crenatus* (Hay & Pethybridge, 2005; Seinhorst, 1998; Weischer & Brown, 2000). Two studies concluded that *P. crenatus* infestations resulted in forking and stunting of the taproot (P. A. A. Loof, 1991; J. W. Potter & Olthof, 1993), while a field study found increased populations corresponded with lower yield weight but not increased carrot forking or stunting (Hay & Pethybridge, 2005). One study even reported that in one field site, 71-163 root lesion nematodes/100 cm³ soil (species unknown) resulted in no carrot yield or quality loss (Holmstrom et al., 2008). Another study observed that 75% of carrots became forked after exposure to as few as ten individuals/100cm³ soil (Holmstrom et al., 2008). Finally, others report concentrations of 100/100cm³ soil result in reduced yield weight, but forking only occurred at higher concentrations of 200 and 400/100cm³ soil (Vrain & Belair, 1981). Due to conflicting information on carrot tolerance, there are currently no clear levels at which damage occurs, and tolerance levels are highly variable for the same species. *P. crenatus* can be easily confused with *P. penetrans* (Loof, 1960), and due to a history of discrepancies in the criterion for *Pratylenchus* spp. morphological identification and overlapping regional distribution, studies that do not use a combination of morphological and molecular strategies for identification should be used with caution.

1.1.4 Role of winter wheat in Midwestern carrot production

In Midwestern carrot production, winter wheat or barley is planted with carrots in non-tilled rows to serve as a windbreak and is promptly killed with herbicide once carrot seedlings reach the two- or three-leaf stage (Brainard & Noyes, 2012). Additionally, several high-production farms in Michigan include winter wheat within their crop rotation (*personal communication, Ralph and Justin Oomen, carrot farmers*). Two species of *Pratylenchus* (*P. neglectus* and *P. thornei*) have high reproductive rates on winter wheat, resulting in 12-70% yield losses in the Pacific Northwest (Washington, Oregon, Idaho) and Montana (Smiley & Machado, 2009; May et al., 2016). *P. neglectus* and *P. thornei* are considered a severe threat to Pacific Northwest winter wheat farms, even developing several resistant lines to prevent economic losses (Smiley et al., 2014; Briar et al., 2018). Studies in the PNW suggest that winter wheat is a better host to *Pratylenchus* spp, than barley (Smiley et al., 2014).

Since winter wheat is considered an excellent host to *Pratylenchus* spp. and relevant in Michigan carrot production, it would be worthwhile to investigate how crop rotations with winter wheat compare to those without. After eggs hatch, young juveniles typically exit the roots in search of a new host (Briar et al., 2018), which suggests that interplanting or the presence of weed hosts early in the season may augment population growth, infection, and corresponding losses. However, this interaction has not been investigated. Additionally, distribution surveys like the ones conducted in the PNW and surrounding states have not been conducted in Michigan (Smiley et al., 2004; Briar et al., 2018).

1.1.5 Management strategies for *Pratylenchus* in carrot and related vegetable systems

1.1.5.1 Chemically based nematicides for management in root vegetables

Pratylenchus spp. management is challenging, which can be exacerbated by the organisms' wide host range and migratory behavior (Castillo & Volvas, 2007). Migratory PPNs like *Pratylenchus* spp. are less susceptible to nematicidal compounds than *Meloidogyne* spp. (Oka, 2013). For example, after 24 hr of exposure, the nematicide fluazaindolizine did not affect *P. penetrans* or *P. thornei* but did significantly reduce *M. hapla*, *M. incognita*, and *M. chitwoodi* populations (Wram and Zasada, 2020). Another study investigating the efficacy of monoterpene compounds and the nematicide oxamyl for control of *P. penetrans* found that effects were less potent than on the free-living nematode, *C. elegans* (Tsao & Yu, 2011). In addition, nicotine had a nematicidal impact on *M. hapla* and *M. incognita* but did not cause mortality in a population of *P. penetrans* (Yu & Potter, 2008).

In root vegetable production systems, like carrots, parsnips, potatoes, and beets, the product oxamyl is typically used to control PPNs (Bird & Warner, 2018). Most published chemical trials focus on *P. penetrans* management in potato production. For example, microplot trials in potatoes (cv. Russet Burbank) found that all rates of an in-furrow, pre-plant treatment of oxamyl decreased nematode populations and increased yields by 30.7-73.1% (Olthof et al., 1985). Efficacy of dimethyl disulfide, oxamyl, fluensulfone, fluopyram, and fluzaindolizine for management of *Meloidogyne* spp. are comparatively well studied in carrots and related vegetable systems (Gugino et al., 2006; Curto et al., 2014; Morris et al., 2016; Becker et al., 2019). However, similar published studies investigating the efficacy of nematicides on *Pratylenchus* spp. in carrot systems are lacking.

1.1.5.2 Biological control agents and soil amendments for *Pratylenchus* spp. management

Several prominent antagonistic bacterial and fungal taxa are associated with altering *Pratylenchus* spp: pathogenicity and survivorship. The most studied bacterium for nematode management worldwide is *Pasteuria*, which is an obligate parasite that adheres itself to the nematode cuticle and then colonizes the host's body, rendering it unable to reproduce successfully (Chen & Dickson, 1998; Elhady et al., 2017). *Pasteuria* spp. (*Pa. thornei* and *Pa. penetrans*) are useful biocontrol agents for *Pratylenchus zaeae* and *P. scribneri* (Oostendorp et al., 1991; Stirling et al., 2017; Confort & Inomoto, 2023). However, *Pa. penetrans* spore attachment and corresponding efficacy vary by species and populations of *Pratylenchus* (Oliveira et al., 2015).

Burkholderia is another bacterial taxon capable of infecting all life stages of *Pratylenchus*, limiting survivorship (Elhady et al., 2017). In a study investigating the bacterial and fungal communities associated with the cuticles of *Pratylenchus* spp., *Burkholderia* was the most abundant bacterial genus (Omarjee et al., 2008; Elhady et al., 2017). In addition to reducing *Pratylenchus* spp., *Burkholderia* spp. also reduced the growth of the plant pathogen *Fusarium oxysporum*, suggesting that this taxon exhibits both nematocidal and fungicidal qualities (Duong et al., 2021). Majestene® (Pro Farm Group, Davis, CA) is a bionematicide comprising heat-killed *Burkholderia* spp. and spent fermentation media. This bioagent is available to commercial carrot growers; however, effectiveness against *Pratylenchus* spp. within carrot production is unpublished.

Purpureocillium lilacinum (formerly *Paecilomyces lilacinum*) is a nematophagous fungus that possesses insecticidal, nematocidal, and antimicrobial qualities (Hotaka et al., 2015; W. Chen & Hu, 2022). *Pu. lilacinum* infects nematode eggs by colonizing the surface cuticle with its hyphae, which secrete a toolkit of enzymes that degrades chitin and protein structures essential for

nematode survivorship (W. Chen & Hu, 2022). During the colonization process, *Pu. lilacinum* infection produces lipopeptides called leucinostatins, which are associated with mortality of nematode, insect, mite, and other plant pathogens (Wang et al., 2016; Jiao et al., 2019; Girardi et al., 2022). This fungal bioagent is available as an organically registered product, MeloCon® WG (Certis Biologicals, Columbia, MD), for use in carrots.

The addition of animal-manure-based composts, compost teas, and plant-based green manures in crop production can significantly modify the microbial composition of the rhizosphere and plant health (Oka, 2010; Milkereit et al., 2021; Ali et al., 2022). Preplant application of poultry, hog, and cattle-based composted manure reduces *Pratylenchus* spp. abundance in fruit, vegetable, and field crops (Forge et al., 2016; Watson et al., 2017; Izuogu & Usman, 2019; El-Ashry, 2021). The addition of pelletized poultry manure increased bacteria and beneficial nematodes associated with N cycling and C-based compound breakdown, allowing plants to uptake nutrients and increase plant growth more readily (Milkereit et al., 2021). Additionally, the production of volatile fatty acids (VFAs), such as *n*-butyric acid, during the composting and fermentation process of animal-based manures has been discussed as the mechanism of *Pratylenchus* spp. mortality (Mahran et al., 2008; Cole et al., 2020). It is important to note that, to the contrary, the addition of raw manures increased *P. penetrans* densities (Kimpinski et al., 2003; T. Forge et al., 2005), suggesting that the fermentation/compost process is significant for the mortality mechanism. The mechanism that reduces *Pratylenchus* spp. populations are currently debated but hypothesized to be multifaceted and indirect through enhancing physical changes in soil, augmenting antagonistic bacterial and fungal communities, releasing nematicidal compounds and phytochemicals, and increasing overall plant tolerance to PPNs (Oka, 2010; Rouse-Miller et al., 2020). Furthermore, as previously mentioned, this concept has been

demonstrated in other crops, but the addition of composted manures for managing *Pratylenchus* spp. in carrots has not been explored.

1.1.5.3 Cover crop incorporation for *Pratylenchus* spp. management

In carrots, limiting variability in soil moisture and nutrient supply during growth stages can positively impact root development, so management practices that aim to reduce this variability can impact subsequent yield (Brainard & Noyes, 2012). The incorporation of cover crops is one of the most widely used agroecological practices in vegetable crops worldwide (Silva & Moore, 2017). In addition to stabilizing nutrient and water availability, cover crop incorporation offers mechanical and physical benefits to soil, such as increased drainage, increased aeration, decreased compaction, and reduced water and wind erosion (Scholberg et al., 2010). Additionally, cover crops can positively influence antagonistic bacterial and fungal communities (Patkowska et al., 2016), along with free-living and PPN communities in carrot fields (Grabau et al., 2017).

Cover crops augment PPN communities by serving as non-hosts or trap crops. Non-hosts interrupt a generational cycle by preventing reproduction and forcing nematodes to rely on a diapause period or desiccate without a host. This can be implemented for PPNs that cannot survive prolonged periods of diapause, i.e., oat for managing *Meloidogyne hapla* in vegetable systems (Vestergård, 2019). Trap-cropping strategies are split up into two methods: “open-end” and “dead-end.” The “open-end” strategy involves a good host that allows nematodes to infect and develop host sites within the host root but requires farmers to promptly kill the crop before nematode generation occurs (Waisen et al., 2019). This “open-end” strategy can effectively manage sedentary endoparasitic nematodes, like *Heterodera*, *Globodera*, and *Meloidogyne* spp. because after their initial infective stage, all subsequent life stages are confined to feeding sites

(Mhatre et al., 2022; Samara, 2022). However, because environmental factors can significantly influence nematodes' life cycle and regeneration periods, timing when the crop is killed is essential for efficacy (Waisen et al., 2019). Additionally, the “open-end” trap crop strategy is unlikely to be effective for managing migratory PPNs like *Pratylenchus* spp., as nematodes can freely migrate in and out of roots as needed.

Contrastingly, “dead-end” trap crop strategies may be more effective for *Pratylenchus* spp. management. The “dead-end” trap crop strategy involves the plant first attracting PPNs and producing secondary metabolites for plant defense that inhibit feeding, survival, and reproduction. Plants in the Brassicaceae family have been investigated for their suitability as “dead-end” hosts and green manures because of their allelopathic effects on PPNs. Compounds and secondary metabolites (i.e., isothiocyanates, glucosinolates, etc.) produced by the plant shoots or roots cause nematode mortality (M. J. Potter et al., 1998). If biofumigation is the goal, above-ground growth is either winter-killed or incorporated into the field as green manure. Several cultivars of oilseed radish (*Raphanus sativus*) have been investigated for *Pratylenchus* spp. management in rotational/subsequent crop studies (Grabau et al., 2017; Schmidt et al., 2017), but studies investigating these crops' host status and reproductive capability are lacking. Incorporating oilseed radish as green manure decreased populations of *P. neglectus* in potatoes by 60% (Al-Rehiyani & Hafez, 1998), providing evidence that oilseed radishes may be effective cover crops for nematode management. Another Brassica, oil rapeseed (*Brassica napus*), has been proposed as a potential trap crop. However, several *Pratylenchus* spp. can feed and reproduce successfully on roots and lead to significant damage (Webb, 1990; Kumari, 2012). Incorporating oilseed radishes as cover crops is an effective option for PPN management; however, existing studies note that the nematicidal effect of glucosinolates varies between nematode species and host cultivars (M. J.

Potter et al., 1998). This emphasizes that uniform investigations are necessary to understand host status and reproduction within specific cropping systems.

1.2 OBJECTIVES

In prior reviews and extension bulletins summarizing plant pathogens of carrots, *Pratylenchus* spp. are routinely omitted (Davis, 2004; Kelley & Phatak, 2009; Que et al., 2019). This creates the illusion that *Pratylenchus* nematodes do not threaten carrot production despite their broad distribution and evidence of associated economic losses. While other PPN pests like *Meloidogyne* spp. and *Heterodera carotae* certainly deserve attention for their threat to carrot production, *Pratylenchus* spp. infestations should warrant action and concern, too. There is evidence of *Pratylenchus* spp. infection limiting corresponding yield and quality in carrots (Hay & Pethybridge, 2002; Pudasaini et al., 2007; Seinhorst, 1998; Vrain & Belair, 1981), though investigations comparing species under uniform conditions are lacking. What became increasingly striking is that challenges with confident species identification and tolerance to nematicides blur the lines between if, when, and how growers should manage *Pratylenchus* spp. in carrots.

The four *Pratylenchus* species discussed in this introduction (*P. penetrans*, *P. crenatus*, *P. neglectus*, and *P. thornei*) have not been evaluated under uniform conditions on carrots or winter wheat, so future studies should aim to quantify the disease impact of these four species on carrot systems utilizing a holistic approach, with an emphasis on monitoring parameters of quality and pathogen coinfection under uniform environmental conditions. In Michigan, farmers report up to 50% yield losses from *Pratylenchus* nematodes (Bird & Warner, 2018). Recent small-scale surveys anticipate they are widespread in the region (Grabau et al., 2017; Habteweld et al., 2020) and 100% of MI carrot farmers agreed investing and evaluating nematode management strategies

is essential (Darling et al., 2020), indicating this would be a good region for investigating knowledge gaps discussed within this introduction. Determining if and to what extent damage occurs for these species is critical to carrot growers worldwide, as past soil surveys reveal these nematodes routinely occupy >90% of carrot fields. Based on my comprehensive review of the available literature on *Pratylenchus* spp. distribution within temperate carrot fields and available reproductive data, I suggest that carrot is a great host for *Pratylenchus crenatus* and *P. penetrans*. Still, studies are necessary to understand when they should be managed. Consumers believe carrots with visible brown lesions are old, rotting, or disease-ridden, while those with an even medium saturation are fresher, tastier, healthier, and sweeter (Schifferstein et al., 2019). These associations emphasize that quality loss can reach beyond just weight loss. Despite *Pratylenchus* spp. limiting yield by up to 50% (Bird & Warner, 2018), effective management strategies are lacking. This is especially important to uncover because nematicides and management strategies are expensive inputs for specialty crop growers. Unnecessary applications or a lack of application, when necessary, have the potential for severe economic repercussions. Additionally, there is mounting evidence that biocontrol agents, soil amendments, and “dead-end” trap cropping could be effective options for *Pratylenchus* spp. management, but similar studies should investigate applications within carrots.

To help fill some of the knowledge gaps outlined within this introduction, my dissertation’s primary objectives are to 1) determine the abundance and distribution of plant-parasitic nematodes in Michigan carrot and wheat fields, with a focus on identifying *Pratylenchus* to species; 2) investigate to what extent *Pratylenchus* species elicit damage to carrot and wheat plants; 3) evaluate the effect of chemical, biological, and cover crop strategies for nematode management of *Pratylenchus* spp. in carrot systems.

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CHAPTER 2: PLANT-PARASITIC NEMATODE COMMUNITIES IN MICHIGAN CARROT AND WHEAT FIELDS

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2.1 INTRODUCTION

Michigan is an important state for carrot (*Daucus carota* L.) production, ranking 4th in the United States and yielding over \$14.5 million annually (USDA-NASS 2019). Carrots are subject to disease from several fungal, bacterial, and viral infections and damage from insect, nematode, mite, and animal pests (Viric Gasparic et al. 2022; Donne et al. 2020). Plant-parasitic nematode infestations and corresponding damage occur below ground, so issues can be difficult to identify until the end of the season after yield and quality losses have already occurred.

Plant-parasitic nematode genera historically most associated with yield and/or quality

losses to carrots are *Meloidogyne* spp., *Heterodera carotae*, and *Pratylenchus* spp. (Berney and Bird 1992; Greco et al. 1998). *Meloidogyne hapla* is a sedentary endoparasitic nematode that forms galls on secondary root systems and thereby can reduce yields in unmanaged fields by 26-77% (Gugino et al. 2006). Comparatively, the carrot cyst nematode, *Heterodera carotae*, is another sedentary endoparasitic nematode that can cause yield losses as high as 12-20% (Greco et al. 1993; Greco et al. 1998). These two significant pests cause visual distortion to root systems, which results in unmarketable yield losses from quality issues. As previously discussed, *M. hapla* and *H. carotae* have listed tolerance or risk densities, whereas *Pratylenchus* spp. lacks these guidelines and management recommendations.

Within the last 50 years, three surveys have been conducted addressing some aspects of Michigan carrots' plant-parasitic nematode communities. The first survey was conducted in 1986-1988, which focused solely on the frequency of occurrence of *Heterodera carotae* and *M. hapla* (Berney and Bird 1992). Since then, however, nematode communities in Michigan fields have been explored in a limited capacity. In 2017, an in-depth survey of two fields focused on cover crop rotations for *P. penetrans* management (Grabau et al. 2017). Another survey was recently conducted in 2020 but aimed to determine the role of nematodes as bioindicators for soil health, focusing on four fields (Habteweld et al. 2020). Both recent surveys conducted in 2017 and 2020 uncovered that *Pratylenchus* spp. were the most prevalent plant-parasitic nematode in western counties, suggesting these nematodes may pose a risk to processing carrot production.

Winter wheat is commonly included in carrot crop rotations in the northern United States, most important for carrot production: Washington, Wisconsin, and Michigan (*personal communication*, Michigan Vegetable Council). Additionally, carrot farmers intercrop winter wheat and other cereal grains with fragile seedlings to protect them from wind and water erosion

(Brainard et al. 2015). This is relevant because wheat is considered an excellent host for *Pratylenchus* species and may contribute to a seasonal build-up. For example, *Pratylenchus neglectus* is abundant and negatively correlated with winter wheat yield in the Pacific Northwest (Smiley & Machado 2009), yet limited information exists on how plant-parasitic nematodes occupy and impact yield in Michigan winter wheat fields. Winter wheat can be subject to extensive yield losses of up to 85% by *Pratylenchus thornei*, 8-36% by *P. neglectus*, or 10-19% by *P. penetrans* (Smiley et al. 2010; Yu et al. 2012), which highlights that species may be considered more or less damaging within crop systems.

The objectives of this chapter are to 1) determine the plant-parasitic and free-living nematode abundance, frequency of occurrence, and distribution in Michigan carrot and winter wheat fields, 2) determine if crop history and soil type influence nematode taxa densities, 3) investigate if higher *Pratylenchus* densities correlated with reduced growth parameters, and 4) identify *Pratylenchus* nematodes to species in both Michigan carrot and winter wheat fields. Based on prior surveys of Michigan carrot fields and previously discussed wheat studies from the Pacific Northwest, I hypothesize that *Pratylenchus* will be the most abundant plant-parasitic nematodes in Michigan carrot and wheat fields.

2.2 METHODS

2.2.1 Michigan carrot survey design and sample collection

In October 2021, 25 carrot fields were visited and surveyed for plant-parasitic nematode abundance and distribution, spanning 1,117 acres (Table 2.1). Field sites were included based on farmer interest, aided by communication with the Michigan Carrot Committee and Michigan State University Extension. Before samples were collected, farmers were asked for crop rotation history from the prior two years, 2019 and 2020, and carrot type (processing or fresh market) to include

in data analysis. Additionally, GPS Coordinates were used to map acreage sampled and soil type on the USDA Web Soil Survey (Soil Survey Staff, 2022).

At each field, ten soil and root samples were collected in a W-shape throughout the field. At each of the ten sampling points within a field, a standard soil core (25.4 by 2.54 cm) was used to collect soil surrounding a random, one-meter section of the middle rows of carrots. This sample was then individually labeled and stored. Following soil collection, one taproot was collected from the middle of the one-meter sampled section and stored in a gallon-sized plastic bag for further processing. Next, a Garmin eTrex 10 worldwide handheld device was used to collect GPS coordinates for each sample area within a field. Following transportation to the MSU Applied Nematology laboratory, soil samples were stored at 4°C until processing. More information on field information can be found in Tables 2.1 and 2.2.

Table 2.1. Field location, carrot type, crop history and soil type of fields sampled for a Michigan carrot soil and root survey, conducted in October 2021 (N=25).

Field Code	GPS Coordinates	Type*	Crop History (2020-2019)	Soil Type
JO.04	43°50.769' N, 86°21.258' W	PR	Wheat-Greenbean	Sand
JO.05	43°50.451' N, 86°21.564' W	PR	Wheat-Greenbean	Sand
JO.06	43°50.208' N, 86°21.130' W	PR	Corn-Corn	Sand
JO.08	43°44.189' N, 85°39.073' W	PR	Corn-Soybean	Sand
JS.01	43°50.107' N, 86°14.992' W	PR	Wheat-Soybean	Sand
RO.01	43°44.902' N, 86°15.399' W	PR	Broccoli-Snapbean	Sand
RO.02	43°44.908' N, 86°15.255' W	PR	Broccoli-Snapbean	Sand
RO.03	43°46.735' N, 86°15.443' W	PR	Wheat-Zucchini	Sand
RO.04	43°53.857' N, 86°17.579' W	PR	Corn-Corn	Sand
RM.01	43°43.800' N, 86°22.652' W	PR	Pumpkin-Sugarbeet	Sand
RM.02	43°43.641' N, 86°10.260' W	PR	Wheat-Zucchini	Loamy Sand
JO.01	44°16.080' N, 85°34.804' W	PR	Wheat-Greenbean	Loamy Sand
JO.02	44°14.972' N, 85°34.846' W	PR	Wheat-Greenbean	Loamy Sand
JO.03	44°15.162' N, 85°35.001' W	PR	Wheat-Greenbean	Loamy Sand
JO.07	43°50.712' N, 86°20.401' W	PR	Wheat-Greenbean	Loamy Sand
VF.01	43°03.470' N, 83°03.450' W	FR	Carrot-Corn	Muck
VF.02	43°03.405' N, 83°03.329' W	FR	Soybean-Carrot	Muck
VF.03	43°03.569' N, 83°03.507' W	FR	Carrot-Corn	Muck
VF.04	43°08.298' N, 83°00.525' W	FR	Corn-Corn	Muck
VF.05	43°07.203' N, 82°59.448' W	FR	Soybean-Corn	Muck
VF.06	43°06.099' N, 82°57.300' W	FR	Carrot-Corn	Muck
VF.07	43°03.995' N, 83°03.914' W	FR	Carrot-Corn	Muck
VF.08	43°07.066' N, 83°02.958' W	FR	Soybean-Carrot	Muck
VF.09	43°15.105' N, 82°57.822' W	FR	Soybean-Carrot	Muck
VF.10	43°15.321' N, 82°57.693' W	FR	Carrot-Carrot	Muck

*PR and FR: Processing (PR) carrots vs. Fresh Market (FR) carrots.

2.2.2 Michigan wheat survey design and sample collection

In late June 2022, 30 soft red winter wheat (*Triticum aestivum*) fields were surveyed for plant-parasitic nematode abundance and distribution in Michigan. Fields were selected based on farmer interest, aided by communication with the Michigan Wheat Program. Before samples were collected, I collected the past two years of crop history from all surveyed fields. Soil and root collection methodology was consistent between carrot and wheat fields; however, the number of samples per field was reduced from ten to five due to the laborious requirements of processing samples. Finally, roots were collected and stored in a plastic bag until processing. Additionally, plant height was recorded. Figure 2.1 visualizes the statewide location of sample sites for crops.

Table 2.2. Field location, crop history and soil type of fields sampled for a soil and root wheat survey, conducted in July 2022 (N=30).

Field Code	GPS Coordinates	Crop History (2021-2020)	Soil Type
SH1	43°38.847' N, 86°16.649' W	Soybean-Corn	Sand
PF16	43°38.256' N, 86°20.060' W	Soybean-Corn	Sand
RS1	43°39.696' N, 86°17.905' W	Soybean-Corn	Sand
SB1	43°39.676' N, 86°17.872' W	Soybean-Corn	Sand
SB2	43°39.634' N, 86°17.878' W	Soybean-Corn	Sand
RA1	43°38.491' N, 86°16.703' W	Soybean-Corn	Loamy Sand
SH2	43°38.755' N, 86°16.087' W	Soybean-Corn	Loamy Sand
JM9	43°38.146' N, 86°12.222' W	Soybean-Corn	Loamy Sand
MF	41°53.107' N, 83°33.190' W	Soybean-Corn	Loamy Sand
LW	41°52.276' N, 83°34.325' W	Soybean-Corn	Loamy Sand
TR	42°20.930' N, 84°41.699' W	Soybean-Corn	Sandy Loam
FR	42°21.354' N, 84°41.324' W	Soybean-Corn	Sandy Loam
TNR	42°21.342' N, 84°43.775' W	Soybean-Corn	Sandy Loam
MS	42°23.018' N, 84°42.025' W	Soybean-Corn	Sandy Loam
HR	42°19.087' N, 84°41.397' W	Soybean-Corn	Sandy Loam
FSA	43°39.110' N, 83°10.438' W	Bean-Soybean	Loam
FSB	43°39.512' N, 83°11.028' W	Bean-Soybean	Loam
FSC	43°38.892' N, 83°11.805' W	Bean-Soybean	Loam
FSD	43°40.444' N, 83°11.007' W	Bean-Soybean	Loam
FSE	43°40.388' N, 83°10.970' W	Bean-Corn	Loam
FSF	43°40.479' N, 83°10.130' W	Bean-Corn	Loam
FSG	43°36.535' N, 83°12.627' W	Bean-Soybean	Loam
TL	41°52.909' N, 83°32.986' W	Soybean-Corn	Loam
1DB1	43°14.625' N, 82°50.738' W	Soybean-Sugarbeet	Loam
2DB2	43°14.721' N, 82°51.924' W	Soybean-Sugarbeet	Clay Loam
23DB3	43°15.482' N, 82°51.482' W	Soybean-Sugarbeet	Clay Loam
7DB4	43°13.780' N, 82°52.353' W	Soybean-Sugarbeet	Clay Loam
18DB5	43°13.797' N, 82°52.088' W	Soybean-Sugarbeet	Clay Loam
33DB6	43°13.326' N, 82°52.910' W	Sugarbeet-Oat	Clay Loam
LU	41°52.782' N, 83°33.369' W	Soybean-Corn	Clay Loam

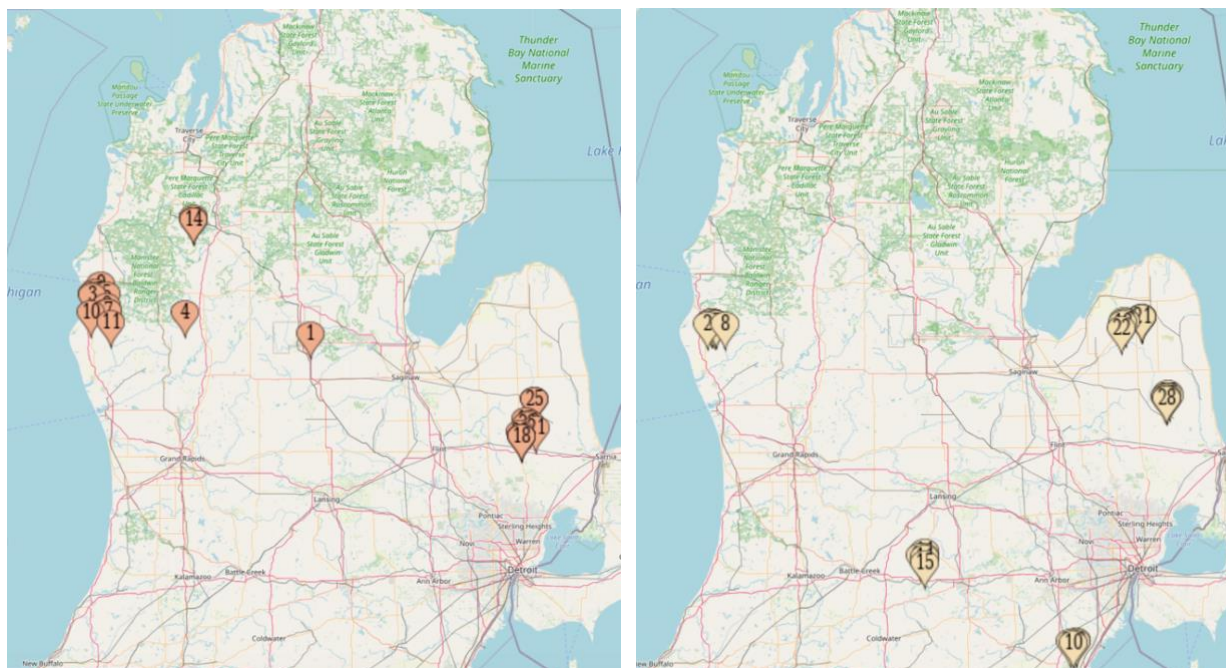


Figure 2.1. Distribution of fields surveyed in Michigan A) carrot fields and B) wheat fields.

2.2.3 Nematode extraction from root and soil samples

To extract nematodes from collected soil, samples from both surveys were processed using the sucrose centrifugal-floatation procedure (Jenkins, 1964). First, the soil cores were homogenized within each bag to ensure even distribution, and a 100 cm³ subsample was collected. Each subsample was mixed with 400mL of water and poured on a pair of 250-µm over 25-µm sieves. This step was repeated three times to collect nematodes on the bottom 25-µm sieve. Next, the accumulations on the finer sieve were collected into a 100 mL centrifuge tube. The samples were then spun in rounds of eight at 4,000 rpm for 5 min. and the water was poured off to reveal a soil pellet. A 70% sucrose solution was added to each tube and centrifuged again at 4,000 rpm for three min., suspending the nematodes at the top of the solution. Finally, the sugar solution was poured onto a 7.62 cm, 25-µm sieve, and all nematodes were collected into a 10 mL glass test tube. Samples were processed and counted within five days of collection to prevent desiccation.

The root incubation method was used to elucidate existing endoparasitic nematodes from

roots. This has been modified slightly over the years (Moore et al. 1992; Behn, n.d.). First, root systems were lightly washed of residual soil debris and pat dry in replicate sets from each field. Following cleaning, one gram of each root system was weighed and cut into 1-2 cm sections. Each root system was labeled and placed in a 250 mL flask. Next, 20 mL of 0.01% NaClO⁻ solution was added to each flask. Flasks were placed onto a G10 Gyrotory Shaker (New Brunswick Scientific Co., Inc.) at 125 rpm for 24 hours. Each sample was then poured onto 250- μ m over a 25- μ m sieve. Contents from the 25- μ m sieve were collected in a 10mL glass tube for nematode enumeration. However, wheat root samples contained sufficiently more residual root tissue and debris, so further processing was required to ensure clean samples. For wheat samples, the accumulates from the bottom 25- μ m sieve was collected into a 100mL centrifuge tube. Samples were then processed using the centrifugal-sucrose floatation procedure. Once the final step occurred and samples were collected into 10mL glass tubes, the remaining step was to identify, enumerate, and record nematode communities within each sample. Because nematodes can quickly die off and degrade in root systems, the shaking process was initiated the same day as root collection, and the root samples were processed and counted within three days of collection.

2.2.4 Nematode identification, classification, and enumeration

Each sample was read using an inverted Nikon TMS microscope at 200x and 1000x magnification. Nematodes were grouped by trophic grouping into one of five categories: bacterial feeders, fungal feeders, herbivores, predators, and omnivores, based on mouthpart (Yeates 1971; Wardle & Yeates 1993). Furthermore, plant-parasitic nematodes (herbivores) were identified to genus via defining morphological characters (Mai & Mullin 1996). One adult female *Pratylenchus* nematode was randomly selected from five samples per field. A total of 20 fields (ten carrot, ten wheat) containing *Pratylenchus* nematodes were randomly selected to be

molecularly identified at the species level.

Selected individuals were photographed at 1000x, and body length, stylet length, and the vulval percentage were collected. Next, samples were stored in a 0.2mL PCR tube, coded, and stored under deep storage conditions (-80°C) until molecular identification (N=100). First, DNA was extracted by adding 1µl of Tween 20 (3.5%) and 10µl NaOH (0.05 M) and incubated at 95°C for 15 min. Next, a PCR mastermix was created, composed of the AllTaq mastermix (QIAGEN), the D2A (5'-ACAAGTACCGTGAGGGAAAGT-3')/D3B (5'-TGCGAAGGAACCAGCTACTA-3') primers (Subbotin et al. 2006) and nuclease-free water. Samples were then placed in a thermal cycler with an initial denaturing cycle (93°C) of 3 min, followed by 40 cycles of 15s denaturing cycle (93°C), 40s annealing cycle (58°C), 70s extension cycle (68°C), with a final extension of 5 min (68°C). Samples were mixed with purple dye with 3% gel red, loaded into 1.1% agar gel, and ran at 128 V for 20-30 minutes until bands ran roughly 80% down the column. Gel was then photographed, and bands were excised under UV light. The gel was dissolved, and samples were purified for submission using the Zymoclean Gel DNA Recovery Kit (Irvine, CA). Finally, samples were loaded into a 96-well plate with 0.2µl forward primer (D2A) and submitted to the Research Technology Support Facility Genomics Core at Michigan State University for Sanger sequencing. Sequences were received and next, every sample was trimmed using 4Peaks software and matched against the National Center for Biotechnology Information's BLAST database to yield the highest species identity.

2.2.5 Abundance, incidence, and field occurrence of plant-parasitic nematodes

Several calculations were performed for each plant-parasitic nematode genus to estimate the extent of distribution in Michigan fields. First, abundance was determined by averaging the total samples containing ≥ 1 individual. Next, the frequency of occurrence was calculated (number

of total samples containing ≥ 1 individual/total number of samples *100). Finally, the percentage of positive fields (number of fields containing ≥ 1 individual/total number of fields) was determined.

2.2.6 Survey data analysis

Exploratory data analysis (EDA) was performed on carrot soil survey data to investigate the effects of soil type and crop history on eight nematode taxa densities (*Pratylenchus*, *Meloidogyne*, *Heterodera*, *Paratylenchus*, bacterial feeders, fungal feeders, omnivores, and predators) at harvest. For the wheat survey, another EDA was performed to investigate the effects of soil type and crop history on eight nematode taxa densities (*Pratylenchus*, *Helicotylenchus*, *Tylenchorhynchus*, *Heterodera*, bacterial feeders, fungal feeders, omnivores, and predators) again at harvest. For all nematode count data, zero-inflated generalized linear mixed models (GLMM) were created with nematode counts as the response variable, two fixed effects (crop rotation history, combined soil type), and one random effect (field nested into grower). Because the surveyed fresh market fields were only planted in muck soils, soil and carrot types were combined into one fixed effect for the carrot dataset. The count models were best fit with negative binomial distribution and a log link function, using the package ‘glmmTMB’, and checked for overdispersion (Brooks et al. 2017). Next, GLMs were best fit for yield parameter response variables with normal or negative binomial distribution using the same fixed and random effects. For all models, if F-values were significant for treatment type, Tukey’s Honest Significant Difference (HSD) post-hoc tests ($\alpha=0.05$) were conducted for means separation, using the “emmeans” and “multcompView” packages in R (Lenth 2020; Graves et al. 2023). Graphs were constructed using ggplots in the ‘tidyverse’ package, and compact letter display (CLD) for pairwise comparisons were manually annotated onto bar and boxplots (Wickham et al. 2019).

Finally, if figures contained multiple graphs, they were combined using the ‘cowplot’ package (Wilke 2020).

2.3 RESULTS

2.3.1 Abundance and incidence of plant-parasitic nematodes

In Michigan carrot fields, we used morphological identification under an inverted-light microscope to determine the presence of nine plant-parasitic nematode genera in soil:

Pratylenchus, *Meloidogyne*, *Heterodera*, *Helicotylenchus*, *Paratylenchus*, *Tylenchorhynchus*, *Xiphinema*, *Trichodorus*, and *Criconema*; while in roots, we detected four nematode genera:

Pratylenchus, *Meloidogyne*, *Heterodera*, and *Paratylenchus*. In wheat soil samples, we detected eleven genera: *Pratylenchus*, *Meloidogyne*, *Heterodera*, *Helicotylenchus*, *Paratylenchus*,

Tylenchorhynchus, *Scutellonema*, *Hoplolaimus*, *Xiphinema*, *Trichodorus*, *Criconema* and

Longidorus; while in wheat roots, we detected four genera: *Pratylenchus*, *Heterodera*,

Helicotylenchus, and *Tylenchorhynchus*.

Table 2.3. Densities (Average \pm SEM) and frequency of plant-parasitic nematodes detected in Michigan carrot field soil and roots.

Genus	Soil (/100cm ³)			Roots (/1g)		
	Average \pm SEM	Samples ≥ 1 individual	Fields ≥ 1 individual	Average \pm SEM	Samples ≥ 1 individual	Fields ≥ 1 individual
<i>Pratylenchus</i>	113.0 \pm 13.2	63.2%	96%	126.4 \pm 13.5	84.0%	100%
<i>Meloidogyne</i>	56.1 \pm 7.7	34.0%	56%	46.2 \pm 5.7	39.6%	60%
<i>Heterodera</i>	35.7 \pm 5.7	36.8%	40%	24.1 \pm 2.5	35.6%	40%
<i>Paratylenchus</i>	111.6 \pm 11.8	28.8%	32%	65.8 \pm 6.5	32.4%	40%
<i>Helicotylenchus</i>	73.0 \pm 14.6	25.2%	44%	0.0 \pm 0.0	0%	0%
<i>Tylenchorhynchus</i>	3.2 \pm 0.5	6.4%	28%	0.0 \pm 0.0	0%	0%
<i>Xiphinema</i>	9.9 \pm 2.0	4.4%	16%	0.0 \pm 0.0	0%	0%
<i>Trichodorus</i>	32.3 \pm 20.4	1.6%	4%	0.0 \pm 0.0	0%	0%
<i>Criconema</i>	2.0 \pm 0.0	0.4%	4%	0.0 \pm 0.0	0%	0%

Table 2.4. Densities (Average \pm SEM) and frequency of plant-parasitic nematodes detected in Michigan wheat field soil and roots.

Genus	Soil (/100cm ³)			Roots (/1g)		
	Average \pm SEM	Samples ≥ 1 individual	Fields ≥ 1 individual	Average \pm SEM	Samples ≥ 1 individual	Fields ≥ 1 individual
<i>Pratylenchus</i>	40.4 \pm 8.6	61.5%	90.0%	16.1 \pm 2.4	47.3%	73.3%
<i>Helicotylenchus</i>	41.6 \pm 6.0	62.2%	86.7%	6.9 \pm 1.6	30.4%	56.7%
<i>Tylenchorhynchus</i>	25.0 \pm 4.0	52.0%	76.7%	7.2 \pm 1.9	13.5%	50.0%
<i>Heterodera</i>	16.6 \pm 3.4	33.8%	63.3%	18.2 \pm 8.5	14.2%	30.0%
<i>Paratylenchus</i>	5.7 \pm 0.1	13.5%	30.0%	0.0 \pm 0.0	0%	0%
<i>Longidorus</i>	1.1 \pm 0.2	9.5%	30.0%	0.0 \pm 0.0	0%	0%
<i>Xiphinema</i>	7.5 \pm 3.9	8.1%	23.3%	0.0 \pm 0.0	0%	0%
<i>Hoplolaimus</i>	1.0 \pm 0.0	2.0%	10.0%	0.0 \pm 0.0	0%	0%
<i>Criconema</i>	1.0 \pm 0.1	1.4%	6.7%	0.0 \pm 0.0	0%	0%
<i>Scutellonema</i>	1.0 \pm 0.0	1.4%	6.7%	0.0 \pm 0.0	0%	0%
<i>Trichodorus</i>	1.0 \pm 0.0	0.7%	3.3%	0.0 \pm 0.0	0%	0%

2.3.2 Molecular identification of *Pratylenchus* spp. in carrot and wheat fields

Of the ten fields randomly selected for *Pratylenchus* spp. molecular identification, we identified three species in Michigan carrot fields: *Pratylenchus penetrans*, *P. crenatus*, and *P. neglectus* (Figure 2.2). Additionally, we determined one specimen with a 98.1% identity to *P. penetrans*, which may be a different species based on morphometrics and DNA sequence identity. Strikingly, 66% of molecularly identified samples were *P. penetrans*, 22% *P. crenatus*, 10% *P. neglectus*, and 2% *P. penetrans* sp.?. Additionally, 20% of carrot fields contained a mixture of two species: *P. penetrans* and *P. crenatus*. Further, we selected three nematodes from the top five most concentrated fields of *Meloidogyne* and *Heterodera* and molecularly identified those individuals. *Meloidogyne hapla*, *Heterodera carotae*, *Heterodera glycines*, *Tylenchorhynchus maximus*, *Paratylenchus* sp. D and *Xiphinema rivesi* were also molecularly identified.

Four species were detected in wheat fields: *P. neglectus*, *P. penetrans*, *P. crenatus*, and *P. scribneri*. Again, *Pratylenchus penetrans* was the most abundant species, detected in 42% of specimens and in 60% of wheat fields. However, *P. crenatus* closely followed with 40% of specimens identified in 70% of fields. Lastly, *P. neglectus* and *P. scribneri* were identified as 16% and 2% of specimens, respectively. Half of winter wheat fields contained a mixture of at least two

of the four species.

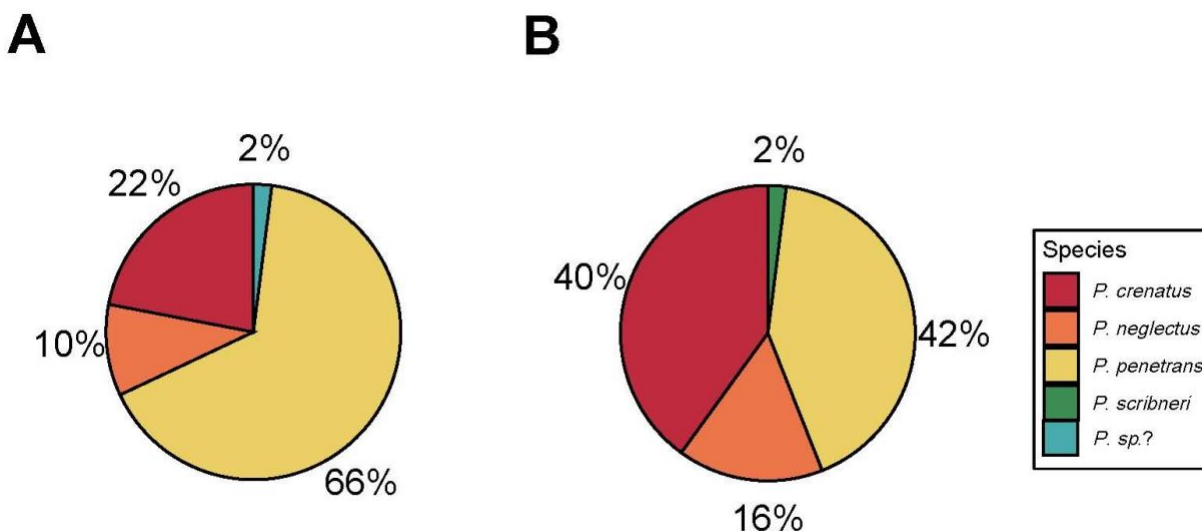


Figure 2.2. *Pratylenchus* spp. identified using molecular identification and morphological measurements from (A) carrot fields and (B) winter wheat fields containing positive samples (N=50 each).

2.3.3 Impact of soil type and crop history on *Pratylenchus* spp. densities in carrot and wheat fields

In winter wheat, two soil types (sand and sandy loam) had higher densities of *Pratylenchus* spp. than fields with clay loam ($F_{(4, \infty)}=2.75$; P -values<0.05; Figure 2.3A). In contrast, the prior two years of crop history for winter wheat fields did not significantly impact *Pratylenchus* densities ($F_{(4, \infty)}=0.74$; P -value>0.1; Figure 2.4A).

In carrot fields, soil type significantly impacted *Pratylenchus* spp. soil densities ($F_{(2, \infty)}=5.42$; P -value<0.005). Processing carrots in loamy sand fields contained significantly higher densities of *Pratylenchus* spp. than fresh market carrots grown in muck fields (P -value<0.05; Figure 2.3B). Additionally, crop history from the prior two years (2020-2019) significantly influenced *Pratylenchus* spp. soil densities at carrot harvest ($F_{(11, \infty)}=4.00$; P -value<0.0001; Figure 2.4B). Three of the 12 possible rotations (Table 2.2) included wheat the year prior (wheat-zucchini, wheat-soybean, and wheat-green bean). Carrots rotated with winter

wheat in 2020 had significantly higher *Pratylenchus* spp. than other crop rotations without wheat ($F_{(6,243)}=16.91$; $P\text{-value}<0.0001$). Additionally, carrot-carrot rotations had significantly higher densities of *Pratylenchus* spp. than corn-soybean, soybean-carrot, and carrot-corn rotations ($P\text{-values}<0.05$; Figure 2.4A).

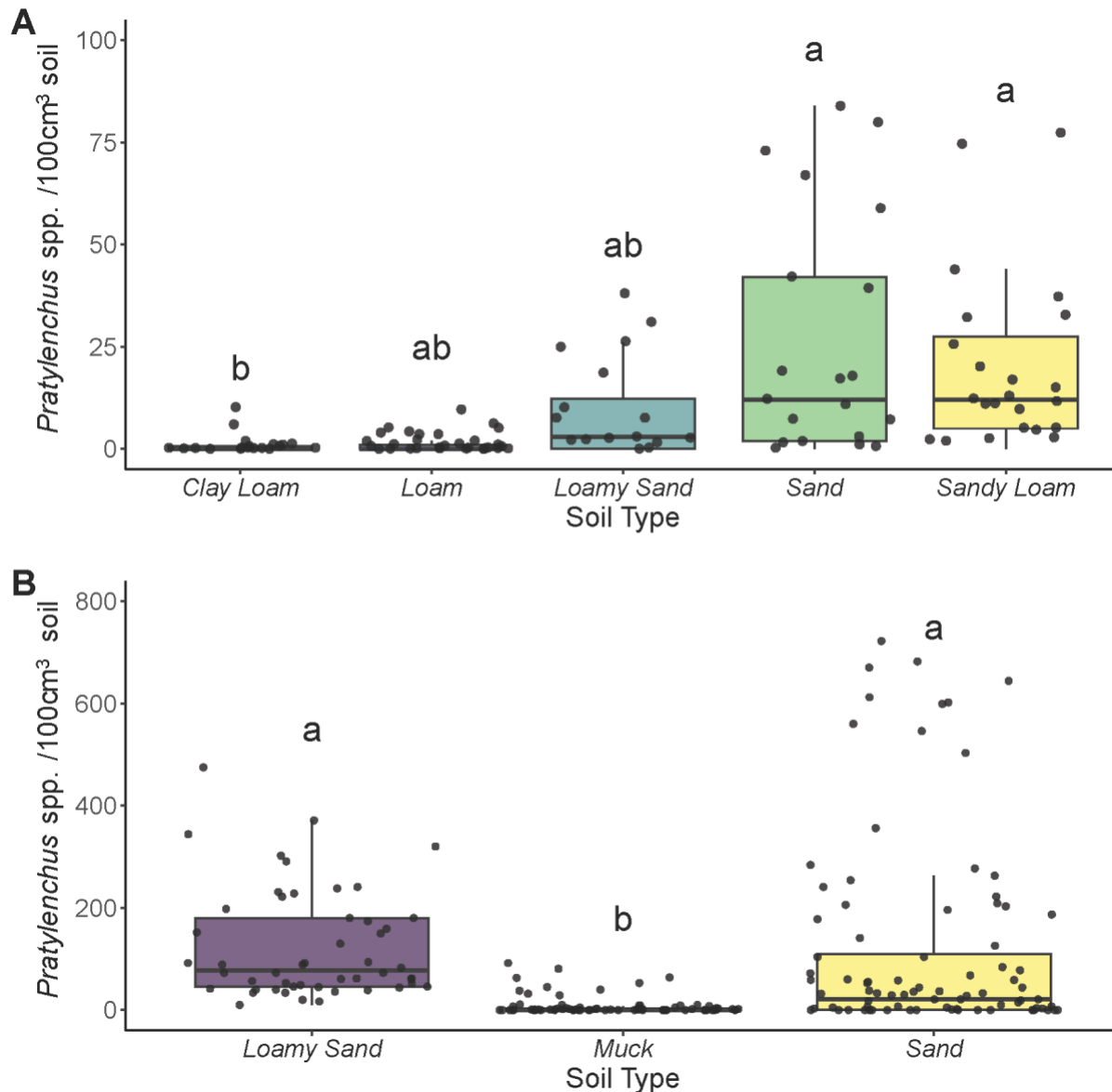


Figure 2.3. *Pratylenchus* spp. densities in Michigan (A) winter wheat and (B) carrot field soil separated by soil type using standard boxplots, where the bold horizontal line within the box indicates the median, the box itself signifies the interquartile range of data points, and the whiskers indicate the minimum and maximum data values. Jittered points visualize data distribution of groups. Unique letters above boxplots indicate that soil type had significant pairwise differences for that crop ($P\text{-value}<0.1$).

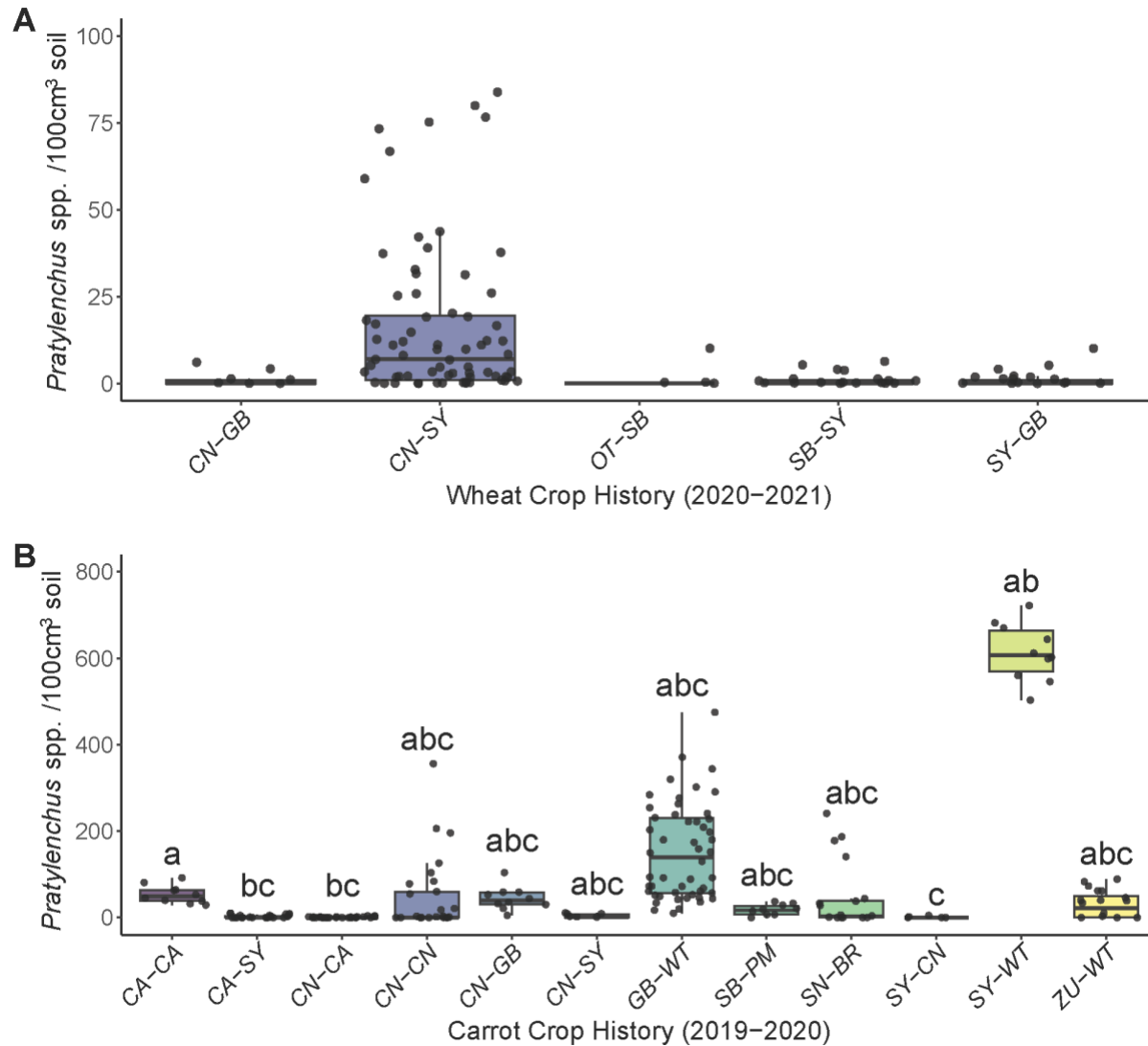


Figure 2.4. *Pratylenchus* spp. densities in Michigan (A) wheat and (B) carrot fields, separated by the past two years of crop history using standard boxplots, where the bold horizontal line within the box indicates the median, the box itself signifies the interquartile range of data points, and the whiskers indicate the minimum and maximum data values. Jittered points visualize data distribution of groups. Crops name abbreviations: BR-Broccoli, CA-Carrot, CN-Corn, GB-Greenbean, OT-Oat, PM-Pumpkin, SB-Sugarbeet, SN-Snapbean, SY-Soybean, WT-Wheat, and ZU-Zucchini. Unique letters above boxplots indicate that soil type had significant pairwise differences for that crop (P -value<0.1).

2.3.4 Influence of soil type and crop history on other plant-parasitic nematodes

In carrot, *Meloidogyne*, *Heterodera*, and *Paratylenchus* spp. were not impacted by soil type or crop history ($F_{(2, \infty)} < 1.0$ and $F_{(11, \infty)} < 1.0$, P -values>0.5). In wheat, *Helicotylenchus*

nematodes were more abundant in sandy loam soils than sand ($F_{(4,\infty)}=3.08$; $P\text{-value}<0.05$).

However, other abundant plant-parasitic nematodes (*Tylenchorhynchus*, *Heterodera*) in Michigan winter wheat were not influenced by soil type or crop history ($F_{(4,\infty)}<1.00$ and $F_{(4,\infty)}<1.80$, $P\text{-values}>0.1$).

2.3.5 Impact of soil type and crop history on beneficial and root-affiliated nematodes

Fungal-feeding and omnivorous nematodes were significantly impacted by the prior two years of crop history in carrots ($F_{(11,\infty)}=4.52$ and $F_{(11,\infty)}=2.51$; $P\text{-values}<0.005$; Figure 2.5) but were not affected by soil type ($F_{(2,\infty)}=1.75$ and $F_{(2,\infty)}=1.55$; $P\text{-value}>0.1$). Bacterial-feeding nematodes were not affected by crop history or soil type ($F_{(2,\infty)}=1.14$ and $F_{(11,\infty)}=0.13$; $P\text{-values}>0.1$), and neither were predatory nematodes ($F_{(2,\infty)}=3.51$ and $F_{(11,\infty)}=0.69$; $P\text{-values}>0.1$).

In contrast, fungal-feeding nematodes were more abundant in winter wheat fields planted in sandier soil types (sand, loamy sand, sandy loam) than clay loam soils ($F_{(4,\infty)}=3.06$; $P\text{-values}<0.05$; Figure 2.6) but were not impacted by crop history ($F_{(4,\infty)}=2.22$; $P\text{-value}>0.1$). Predatory nematode densities were higher in sandy loam than in clay loam winter wheat fields ($F_{(4,\infty)}=2.3$; $P\text{-value}<0.05$). Omnivorous nematode densities in wheat were not impacted by crop history or soil type ($F_{(4,\infty)}=1.06$ and $F_{(4,\infty)}=1.36$; $P\text{-value}>0.1$). Bacterial-feeding nematodes were also not impacted by crop history or soil type ($F_{(4,138)}=0.69$ and $F_{(4,138)}=0.83$; $P\text{-values}>0.1$).

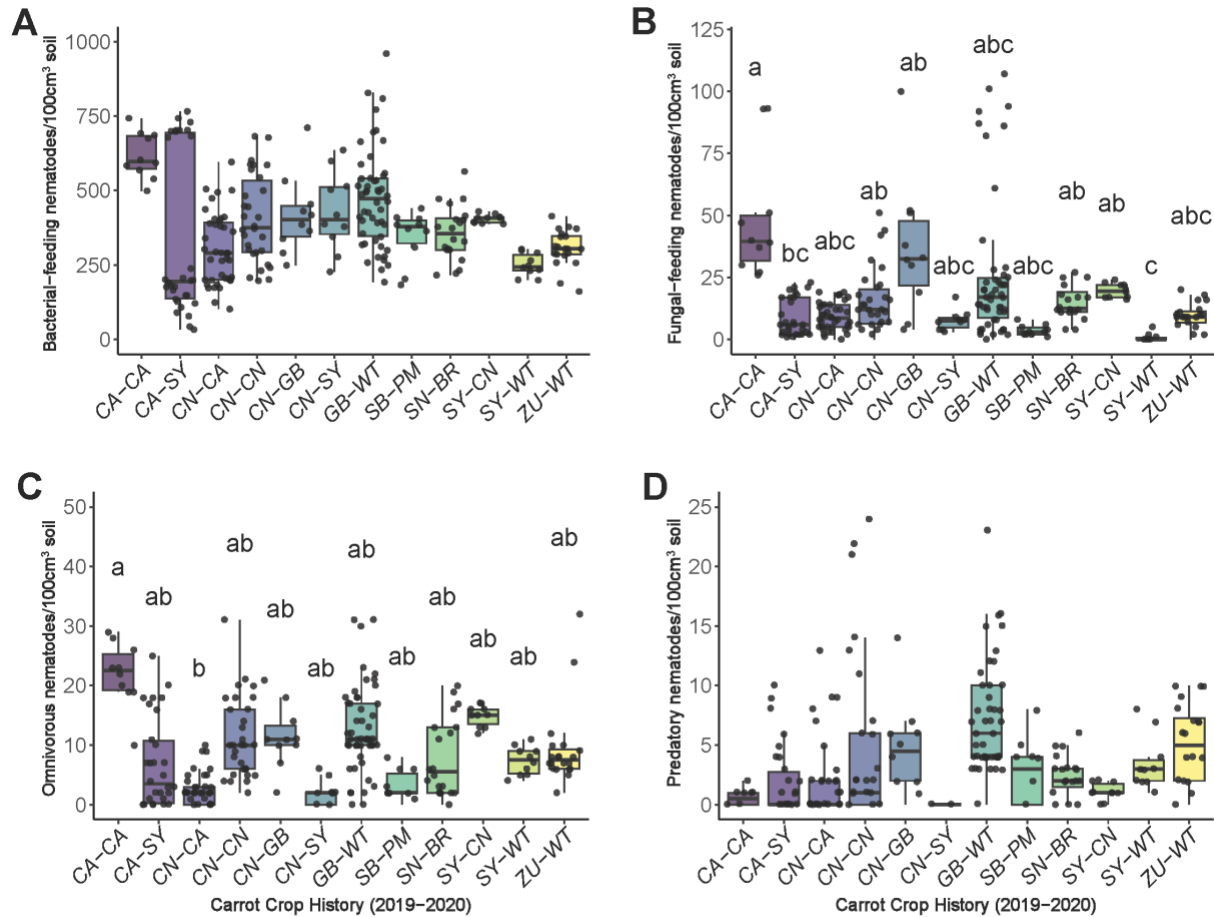


Figure 2.5. Impact of crop history on beneficial and root-affiliated nematodes recovered from carrot fields: (A) The number of bacterial-feeding nematodes, (B) fungal-feeding nematodes, (C) omnivorous nematodes, and (D) predatory nematodes. Crops name abbreviations: BR-Broccoli, CA-Carrot, CN-Corn, GB-Greenbean, PM-Pumpkin, SB-Sugarbeet, SN-Snapbean, SY-Soybean, WT-Wheat, and ZU-Zucchini. Unique letters indicate groups were significantly different (P -value<0.1); graphs lacking letters indicate that no groups were significantly different from others.

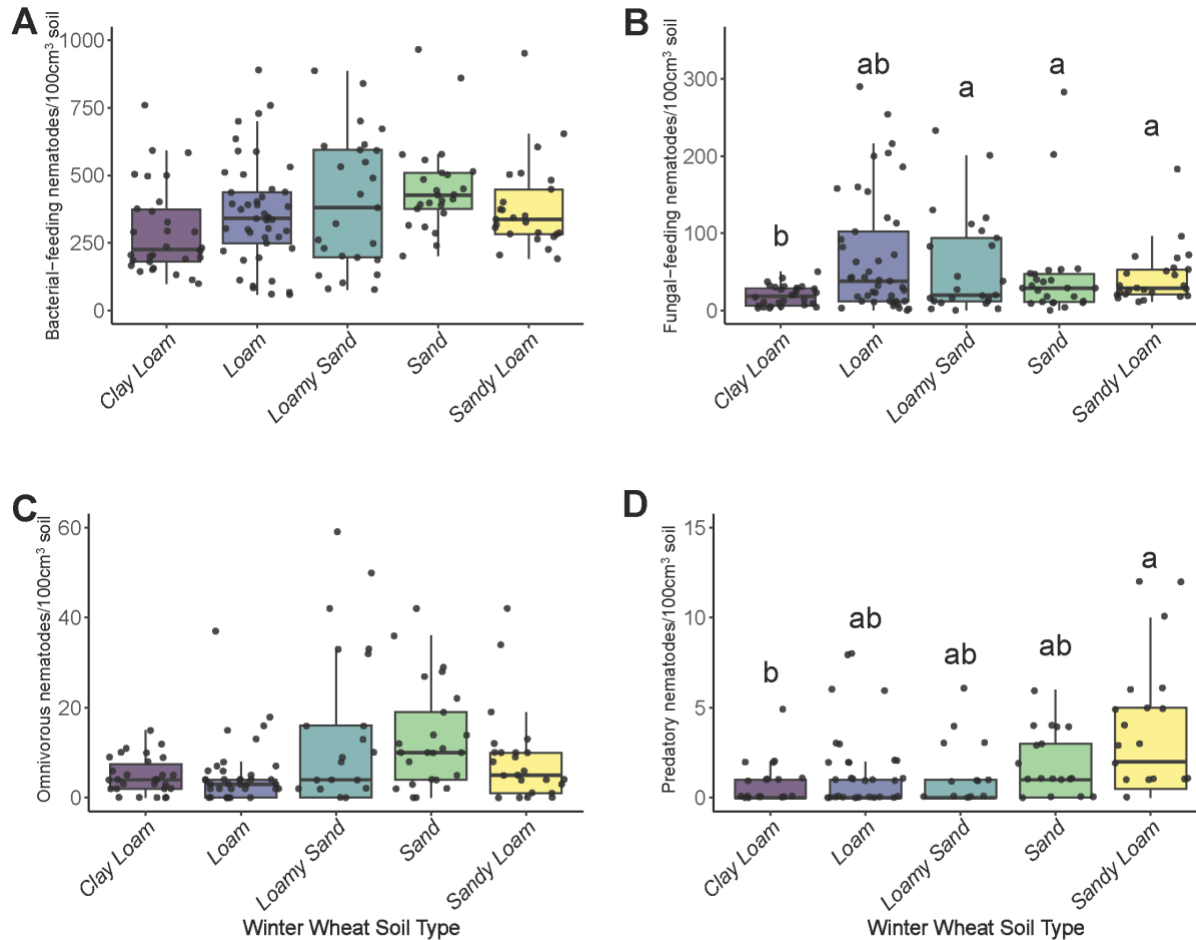


Figure 2.6. Impact of soil type on the number of beneficial and root-affiliated nematodes recovered from winter wheat fields: (A) bacterial-feeding nematodes, (B) fungal-feeding nematodes, (C) omnivorous nematodes, and (D) predatory nematodes. Unique letters indicate groups were significantly different (P -value <0.1); graphs lacking letters indicate that no groups were significantly different from others.

2.3.6 Impact of plant-parasitic nematode densities on yield and quality in carrot and winter wheat

Higher *Pratylenchus* spp. densities in soil at carrot harvest did not result in reduced carrot taproot weight or length ($F_{(1,172)}=0.44$ and $F_{(1,194)}=0.96$; P -values >0.1). However, higher *Meloidogyne* spp. resulted in reduced taproot length ($F_{(1,22)}=9.25$, P -value <0.05), but not weight ($F_{(1,26)}=1.91$, P -value >0.1). Similarly, higher *Heterodera* spp. resulted in reduced taproot length ($F_{(1,233)}=11.94$, P -value <0.005), but not weight ($F_{(1,233)}=0.18$, P -value >0.1). *Paratylenchus* spp. did not result in reduced taproot weight or length ($F_{(1,37)}=0.54$ and $F_{(1,55)}=1.5$, P -values >0.1).

In winter wheat, increased *Pratylenchus* spp. and *Tylenchorhynchus* spp. densities did not reduce plant height ($F_{(1,66)}=0.34$ and $F_{(1,137)}=1.25$; $P\text{-values}>0.1$). However, higher densities of *Helicotylenchus* spp. were recovered from lower plant heights ($F_{(1,133)}=5.05$; $P\text{-values}<0.1$).

2.4 DISCUSSION

We conducted statewide root and soil surveys to identify the abundance and identity of plant-parasitic nematodes in Michigan carrot and wheat production. We surveyed >1,000 acres of active production fields for each crop within two weeks of harvest. We documented at least ≥ 1 *Pratylenchus* spp. individual in 100% and 90% of surveyed Michigan carrot and wheat fields, respectively. This widespread distribution emphasizes the importance of developing effective management strategies for controlling this nematode pest. We did not find that higher numbers of *Pratylenchus* spp. caused reduced taproot weight or length; however, at-plant densities are more associated with resulting damage than harvest populations. Additionally, we identified 100 female *Pratylenchus* individuals to species using a combination of morphological and molecular methods from carrot and wheat fields. In both surveys, we identified five species: *P. penetrans* (54), *P. crenatus* (31), *P. neglectus* (13), *P. scribneri* (1), and *P. sp?* (1).

We found that *Pratylenchus*, *Meloidogyne*, *Heterodera*, and *Paratylenchus* were the most abundant plant-parasitic nematode genera in carrot fields. We detected *Paratylenchus* in 40% and 32% of carrot root and soil samples. Five individuals were selected and identified as *Paratylenchus* sp. D, a species unable to be delimited to a described species in recent literature (Singh et al. 2021). Additionally, *Meloidogyne hapla*, *Heterodera carotae*, *H. glycines*, *Tylenchorhynchus maximus*, and *Xiphinema riversi* were identified from carrot fields. We document the first record of *Pratylenchus neglectus*, *Xiphinema rivesi*, *Tylenchorhynchus maximus*, and *Paratylenchus* sp. D in carrot fields in the United States. *Pratylenchus neglectus*

was previously recovered from Australian carrot fields; however, damage or tolerance levels are unknown (Hay and Pethybridge, 2005).

Notably, the average statewide abundance of *Pratylenchus penetrans* nematode in carrots superseded the previously estimated tolerance level of 50 individuals/100cm³ soil (Teklu et al. 2016) by 2.26x (soil). Ten of the 25 carrot fields (40%) breached the tolerance level of ≥ 50 individuals. Additionally, because the tolerance limit of both *Meloidogyne hapla* and *Heterodera carotae* is ≥ 1 individual/100cm³, 60% and 40% of fields were above tolerance, respectively. In total, 88% of surveyed carrot fields were above the threshold for at least a tolerance level for *Pratylenchus*, *Meloidogyne*, and/or *Heterodera*, providing updated and relevant evidence that these three plant-parasitic nematodes pose a threat to the Michigan carrot industry. A recent Michigan corn survey also identified that 26.8% of corn fields contained the corn needle nematode, *Longidorus breviannulatus* (Thapa et al. 2023). Similarly, we determined that 30% of surveyed Michigan winter wheat fields had *Longidorus breviannulatus*, and every sample with ≥ 1 nematode was collected from a field with corn planted the two years prior.

The past two years of crop history and soil type impacted *Pratylenchus* spp. densities in carrot fields, but only soil type influenced *Pratylenchus* spp. densities in winter wheat. However, increased *Pratylenchus* spp. did not result in reduced carrot taproot weight, length, or winter wheat height. However, we identified different species of *Pratylenchus* spp., and it is unclear when damage occurs for each species, as outlined in the introduction. Interestingly, the only plant-parasitic nematode we found in higher abundance in winter wheat fields with lower plant heights was *Helicotylenchus* spp., which are widespread but not typically considered severe pests of field crops unless in extreme densities (Tylka et al., 2018). The impacts of *Helicotylenchus* on winter wheat should be further explored.

One of the most significant findings of the wheat survey was the recovery of *Heterodera glycines* juveniles (J2s) from winter wheat roots. Upon morphological observation, I initially suspected these nematodes would be identified as *H. avenae* due to their relationship with cereal production in other regions (Smiley et al., 2005). However, unlike *Heterodera carotae* collected from carrot roots, no further developmental life stages of *Heterodera* spp. were observed in winter wheat roots. Five cyst juveniles (J2 stages) were collected for molecular confirmation from root tissue from 2 wheat fields: 3 (43°39.470' N, 83°11.028' W) and 2 (42°21.342' N, 84°43.749' W). All five samples matched 100% identity to the soybean cyst nematode, *H. glycines*. Because these nematodes were recovered from roots but lacked developmental stages, I hypothesize that winter wheat operates as a dead-end trap crop for *H. glycines*. Soybean crop rotations, including winter wheat, yielded 10% higher than rotations without (Janovicek et al., 2021). Additionally, established soybean fields with winter wheat the prior year possessed lower *H. glycines* populations (*personal communication*, Dr. George Bird, Michigan State University). In a recent multi-year field study, winter wheat strips reduced *H. glycines* egg densities by 31.8% compared to fallow strips in Illinois, suggesting that winter wheat suppresses populations beyond normal desiccation (Rocha et al. 2021). The results of our survey and recent literature indicate that winter wheat should be investigated for its potential as a cover crop for nematode management in midwestern soybean production.

There were several limitations of our protocols within this survey. The root extraction protocol was selected based on laboratory availability and the method's fast processing time. Other methods frequently used in nematode extraction, like the Seinhorst mistifier and Baermann funnel, require expensive equipment and/or large areas of space for their use across several days (Griesbach, 1999). These crops have different growing seasons (carrot- April to October, winter

wheat – November to July) that only briefly overlap at various points. For this reason, we were tasked with deciding to keep the growth stage or time of year uniform. We opted to take soil and root samples near the end of the season for each respective crop to ensure that the nematodes recovered were dominantly reproducing and feeding on each crop. Because nematodes are affected by soil moisture, temperature, and seasonality, I suggest that these surveys should not be statistically compared but can serve as evidence of statewide incidence at their respective harvest periods. As mentioned in the data analysis method section, these surveys were analyzed separately. Secondly, collecting nematode individuals directly from roots would have been ideal for molecular identification. However, given the timely cost of transportation, sampling, processing, extraction, and counting, collecting measurements or storing nematodes from root samples was not feasible – so specimens were selected from soil samples. Because *Pratylenchus* spp. travel in and out of roots during the season, our results are valid and provide valuable information for future research. Despite these challenges, our survey provides helpful information on the abundance, identity, and distribution of plant-parasitic nematodes for nematologists, extension agents, and diagnostic staff.

2.5 CONCLUSION

Conducting these surveys provided information on the distribution and abundance of nine plant-parasitic nematodes inhabiting carrot and eleven genera infesting wheat fields in Michigan. Additionally, we successfully identified the dominant species infesting fields: *Pratylenchus penetrans*, *P. crenatus*, *P. neglectus*, *P. scribneri*, and an unknown *Pratylenchus* species. Our survey concurs with prior literature suggesting *Pratylenchus* spp. are widespread and abundant within carrot systems; we documented that they are widespread at densities above tolerance limits in Michigan. However, *Pratylenchus* spp. did not approach tolerance levels in winter wheat.

Notably, *Pratylenchus thornei*, which is associated with *P. neglectus* in causing winter wheat yield losses in other northern temperate regions, was not detected in our survey. Future studies are necessary to further understand species' interactions with carrot and winter wheat plants.

Additionally, preliminary observations indicate that winter wheat can potentially serve as a dead-end trap crop for *H. glycines*.

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CHAPTER 3: ROOT LESION NEMATODES, *PRATYLENCHUS* SPP., ELICIT DIFFERENT REPRODUCTION AND DAMAGE TO CARROT AND WINTER WHEAT

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3.1 INTRODUCTION

Root lesion nematodes, *Pratylenchus* spp., have a broad host range of over 350 crops, including fruit, vegetables, small cereal grains, legumes, specialty crops, and more (Castillo & Vovlas, 2007). *Pratylenchus* spp. damage root systems by sensing, probing, penetrating, feeding, and tunneling through root cells (Zunke, 1990). All *Pratylenchus* spp. life stages, except for the egg, feed on root tissue. This feeding leads to localized root necrosis, which in severe cases results in root death or stunted growth.

Acquiring sufficient *Pratylenchus* cultures necessary for the volume of inoculation can be challenging. One standard method to successfully culture *Pratylenchus* spp. is via sterile carrot discs (Verdejo-Lucas & Pinochet, 1992; Castillo et al., 1995; Coyne et al., 2014; Özdemir,

2021). However, issues with contamination and labor requirements for colony upkeep can present challenges. Another standard method is rearing on wheat roots in media (Castillo & Vovlas, 2007), but this method is usually only sufficient for 1-2 life cycles.

Research on differences between *Pratylenchus* spp. damage in carrots has been limited, as detailed in Chapter 1. Studies showed that carrots and wheat are both excellent hosts for *Pratylenchus* spp. (Potter & Olthof, 1993; Smiley et al., 2010). Yet, there is a lack of literature describing the comparison of multiple *Pratylenchus* species reproduction and corresponding damage in carrot and wheat systems. In the previous chapter, we identified that the top three *Pratylenchus* spp. encountered in Michigan carrot and winter wheat fields are *P. penetrans*, *P. crenatus*, and *P. neglectus*. However, to date, reproductive factors, tolerance levels, and corresponding damage for each species within each crop are conflicting, limited, or absent. For this reason, the objectives of this chapter are 1) to investigate reproductive factors of *P. penetrans*, *P. crenatus*, and *P. neglectus* in carrot disc cultures; 2) to quantify reproduction and damage *P. penetrans*, *P. crenatus*, and *P. neglectus* to carrot; and 3) to quantify *P. penetrans*, *P. crenatus*, and *P. neglectus* to winter wheat. We hypothesize that different *Pratylenchus* species will have different reproductive success and elicit varying severity of damage unique to each crop system.

3.2 METHODS

3.2.1 *Pratylenchus* species confirmation

Pratylenchus penetrans (43°50.107' N, 86°14.992' W) and *P. crenatus* (44°15.162' N, 85°35.001' W) isolates originated from single, gravid females obtained from Michigan carrot soil. *Pratylenchus neglectus* isolates originated from Montana and were obtained from a winter wheat field (*personal communication*, Erika Consoli). Before these experiments, all three *Pratylenchus* isolates were reared in carrot disc cultures. Species identification was confirmed using

morphological characters and 28S region molecular identification with the primers D2A/D3B (Subbotin et al. 2006).

3.2.2 Carrot disk trials

A laboratory experiment was conducted to determine the reproductive rates of three northern root lesion nematode species. First, conventionally grown processing carrots were purchased from a local grocery store (Meijer, Michigan). Each carrot was transferred to the laboratory and washed thoroughly with Dawn dish soap. After washing any residual soil off the carrots, the surface layers were peeled twice using a standard vegetable peeler, and the top sections were disposed of (2-3 cm). The carrots were then transferred to a sterilized fume hood. Under the hood, the carrots were sliced into 2 cm sections. Following slicing, carrot discs were dipped in 90% ethanol solution, flash-flamed for 10 s, washed with distilled water, and transferred to individual Petri dishes (100 mm x 60 mm). We exposed the carrot discs to UV light for 45 s; the carrot discs were carefully flipped using sterile forceps to expose both sides. We developed and adjusted this process from a prior method (Coyne et al. 2014). Following this procedure, the carrot discs were inoculated with 100 μ l of 100 mixed-stage nematodes of *Pratylenchus penetrans*, *P. neglectus*, *P. crenatus*, or nothing. Each species had ten replicates. Controls with no nematodes were included to monitor visual differences between infected and uninfected carrot discs. We inoculated the discs in five equidistant droplets of 20 μ l along the root cortex:core. These cultures were maintained in the dark at 25°C for the duration of the experiment. Forty days later, each disc from the first five replicates was cut into 5 mm x 5 mm grid pieces, and 20 ml of distilled water was added to the dish. Thirty hours later, the solution was poured onto a 25 μ m sieve to catch nematodes, washed thoroughly with distilled water, and collected into standard glass test tubes (10 ml). Another 40 days later, the remaining five

replicates were deconstructed and counted for each species and water control. This experiment was conducted in Fall 2022 and repeated in Spring 2023. Final populations from both periods were collected to calculate reproductive factors (RF), calculated by dividing the total extracted nematodes (all life stages from soil and roots per pot) by the initial inoculum for each pot and averaged by species for each crop.

3.2.3 Establishment of greenhouse trials

A series of greenhouse trials were conducted to determine to what extent three *Pratylenchus* species, *P. penetrans*, *P. crenatus*, and *P. neglectus*, elicit damage to carrots. Before planting, 200 carrot seeds (cv. Cupar) were germinated in a Petri dish with distilled water (5ml). Seven days later, pots (1.5L) were filled with a 70:30 ratio of play sand: steam sterilized field soil and each pot was seeded with six germinated carrot seeds. Because mechanical barriers in the soil can promote taproot forking or splitting, large rocks or gravel were removed from the soil mix before seeding. Five days later, the seedlings were thinned down to three plants per pot and inoculated directly to the plant roots with distilled water (2ml) containing either 750 mixed life stages of *P. neglectus*, *P. penetrans*, *P. crenatus*, or no nematodes. Pots inoculated with no nematodes served as controls; each treatment had six replicates. The pots were monitored over 12 weeks and fertilized weekly with 20N-20P-20K to promote normal growth and development.

To determine to what extent three *Pratylenchus* species, *P. penetrans*, *P. crenatus*, and *P. neglectus*, elicit damage to winter wheat, a series of greenhouse trials were conducted similar to the carrot greenhouse trial previously described. Before planting, 100 winter wheat seeds (cv. Jupiter) were germinated in a Petri Dish with distilled water (5ml). Three days later, pots (1.5L) were filled with a 70:30 ratio of play sand: steam sterilized field soil and each pot was seeded

with two germinated wheat seeds. Five days later, nematodes were inoculated directly to the plant roots with distilled water (2ml) containing either 3,000 mixed life stages of *P. neglectus*, *P. penetrans*, *P. crenatus*, or no nematodes. Pots inoculated with no nematodes served as controls; each treatment had six replicates. The pots were monitored over 12 weeks and fertilized weekly with 20-20-20 to promote normal growth and development.

The initial inoculum was based on prior documented tolerance levels at 50 individuals per 100 cm³ for carrots (Teklu et al., 2016) and 200 individuals per 100 cm³ for wheat (Thompson et al., 2008). After 12 weeks, each trial was deconstructed by carefully collecting 1 g roots, 100 cm³ soil, and root weight (g). Additionally, root length (cm) or plant height (cm) were collected for carrots and wheat to monitor the impact on yield and vigor.

3.2.4 Data analysis

The goal of these experiments was to determine if *P. penetrans*, *P. crenatus*, and *P. neglectus* had significant impacts on different growth parameters for carrot (root weight, root length) and winter wheat (plant height, root weight), compared with non-inoculated control plants. To evaluate this, the effect of species type was assessed on the growth parameter response variables using linear mixed-effect models (lmm), with species as the fixed effect and replicate and trial as random effects, using the “dplyr” and “multComp” packages in R (Hothorn et al., 2016; Wickham et al., 2019; R Core Team, 2023). If F-values were significant, Tukey’s HSD post hoc tests ($\alpha=0.05$) were conducted for means separation, using the “emmeans” and “MASS” packages in R (Lenth, 2018; Ripley et al., 2013). Graphs were constructed using the ‘ggplot2’ package and combined when necessary, using ‘cowplot’ (Wickham, 2011; Wilke et al., 2019).

3.3 RESULTS

3.3.1 Carrot disc trials

For both experiment repetitions, RF of *Pratylenchus penetrans* carrot disks were the lowest of the three species at 40 days and the highest at 80 days ($F_{(2,21)}=3.702$ and $F_{(2,21)}=19.8$, P -values < 0.05 ; Table 3.1). Additionally, the RFs of *P. crenatus* were significantly higher ($F_{(2,21)}=19.8$, P -value < 0.0001) than *P. neglectus* for both repetitions at 80 days. Additionally, RF values were higher in the first trial than in the repeated trial ($F_{(1,19)}=5.17$, P -value < 0.0001), so trial was included in the analysis as a random effect.

Table 3.1. Average (\pm SEM) Reproductive Factors (RF) and life stage densities of *P. penetrans*, *P. crenatus*, and *P. neglectus* on carrot discs at 40 and 80 days.

Species	Females	Males	Juveniles	Eggs	RF
Forty Days					
<i>P. penetrans</i>	9.3 \pm 1.6	6.3 \pm 1.1	27.8 \pm 6.5	16.4 \pm 2.3	6.0 \pm 1.1 b
<i>P. crenatus</i>	50.3 \pm 10.1	0.0 \pm 0.0	72.1 \pm 20.4	104.9 \pm 34.6	22.7 \pm 6.4 a
<i>P. neglectus</i>	43.1 \pm 7.6	0.1 \pm 0.1	48.1 \pm 10.2	65.4 \pm 22.4	15.7 \pm 4.0 ab
Eighty Days					
<i>P. penetrans</i>	3707.5 \pm 122.7	2472.5 \pm 296.8	2616.3 \pm 80.4	6191.3 \pm 261.1	1498.8 \pm 39.5 a
<i>P. crenatus</i>	5633.8 \pm 252.7	0.0 \pm 0.0	4986.3 \pm 388.0	2495.0 \pm 320.3	1311.5 \pm 83.6 b
<i>P. neglectus</i>	4827.5 \pm 303.2	2.1 \pm 0.8	3567.5 \pm 353.4	895.0 \pm 32.2	929.2 \pm 65.1 c

3.3.2 Reproductive ability of *Pratylenchus* spp. in carrot and wheat greenhouse plants

For both carrot and winter wheat, RFs > 1.0 for *Pratylenchus crenatus*, *P. neglectus*, and *P. penetrans*, indicating moderate host status or greater (Oostenbrink, 1972). The reproductive factors of all three species were statistically different from each other in carrot ($F_{(2,33)}=27.46$, P -value < 0.05 ; Figure 3.1A) and in winter wheat ($F_{(2,27)}=40.54$, P -value < 0.05 ; Figure 3.1B). In carrot, *Pratylenchus neglectus* and *P. crenatus* had the highest average RFs of 153.72 ± 3.90 and 136.69 ± 6.28 (mean \pm se), higher than *P. penetrans* ($F_{(2,33)}=27.46$, P -value < 0.05). *Pratylenchus neglectus* had the highest reproduction in winter wheat, with an average RF of 4.55

± 0.38 (mean \pm se), and *P. crenatus* had the lowest average reproduction at 1.19 ± 0.16 ($F_{(2,27)} = 40.54$, P -values < 0.05 ; Figure 3.1B).

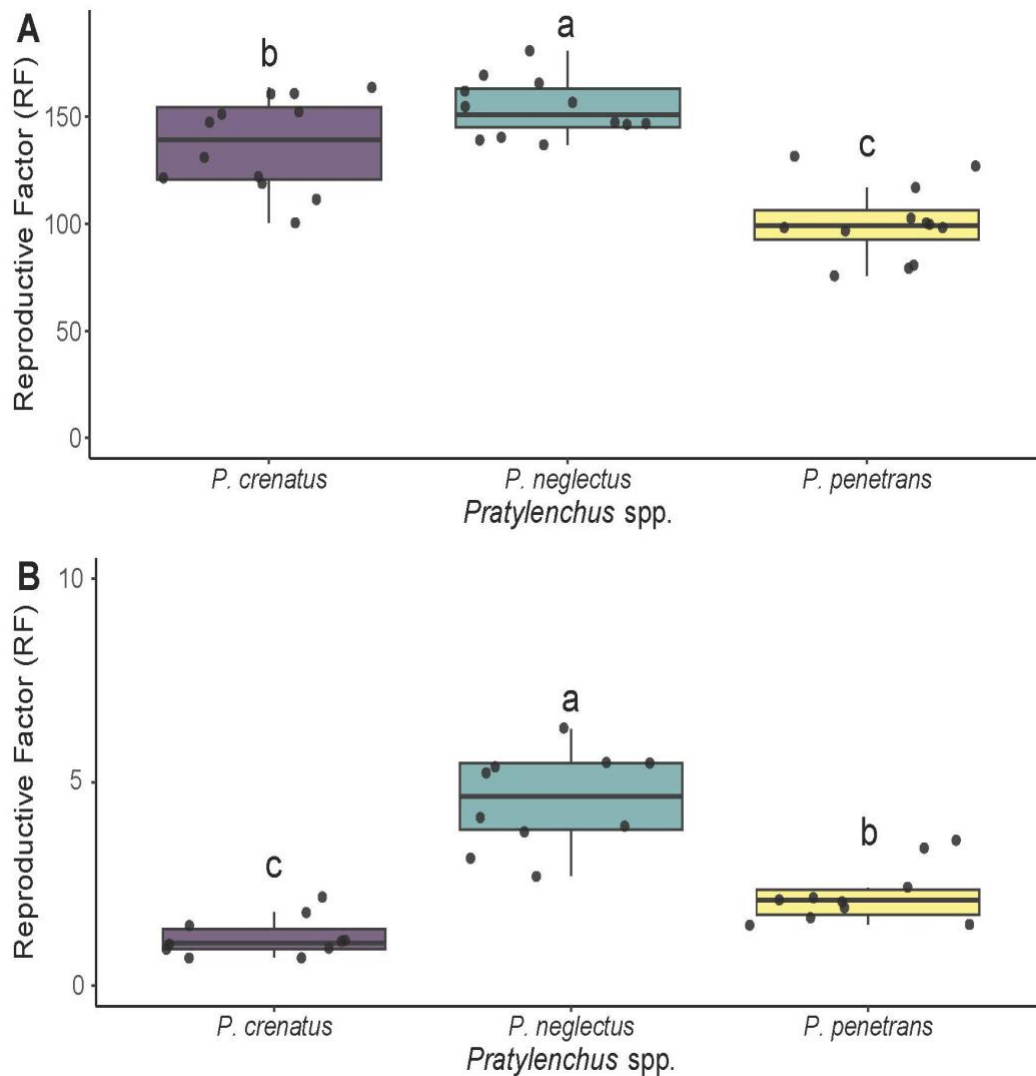


Figure 3.1. Reproductive Factors (RF) of *Pratylenchus crenatus*, *P. neglectus*, and *P. penetrans* isolates in (A) carrot, cv. Cupar, and (B) winter wheat, cv. Jupiter, in separate greenhouse trials. Jittered dots represent the distribution of individual data points within groups. A unique letter above a group indicates that the group is statistically significant (P -value < 0.05).

3.3.3 Impact of *Pratylenchus* spp. on carrot harvest parameters

Taproot weights of carrot plants infected with *Pratylenchus penetrans* and *P. crenatus* resulted in a 36.94% and 21.76% weight reduction, respectively, in comparison with non-

inoculated control plants ($F_{(3,44)} = 44.52$, $P\text{-value} < 0.05$; Figure 3.2 A). Additionally, carrot plants infected with *Pratylenchus penetrans* and *P. crenatus* were 33.48% and 7.05% shorter in length, respectively ($F_{(3,44)} = 83.47$, $P\text{-value} < 0.05$; Figure 3.2 B). *Pratylenchus neglectus* did not significantly reduce taproot weight or length compared to control plants ($P\text{-value} = 0.2297$ and $P\text{-value} = 0.9969$). Uniform photographs of each carrot root were taken to illustrate root distortions caused by different *Pratylenchus* species (Figure 3.3).

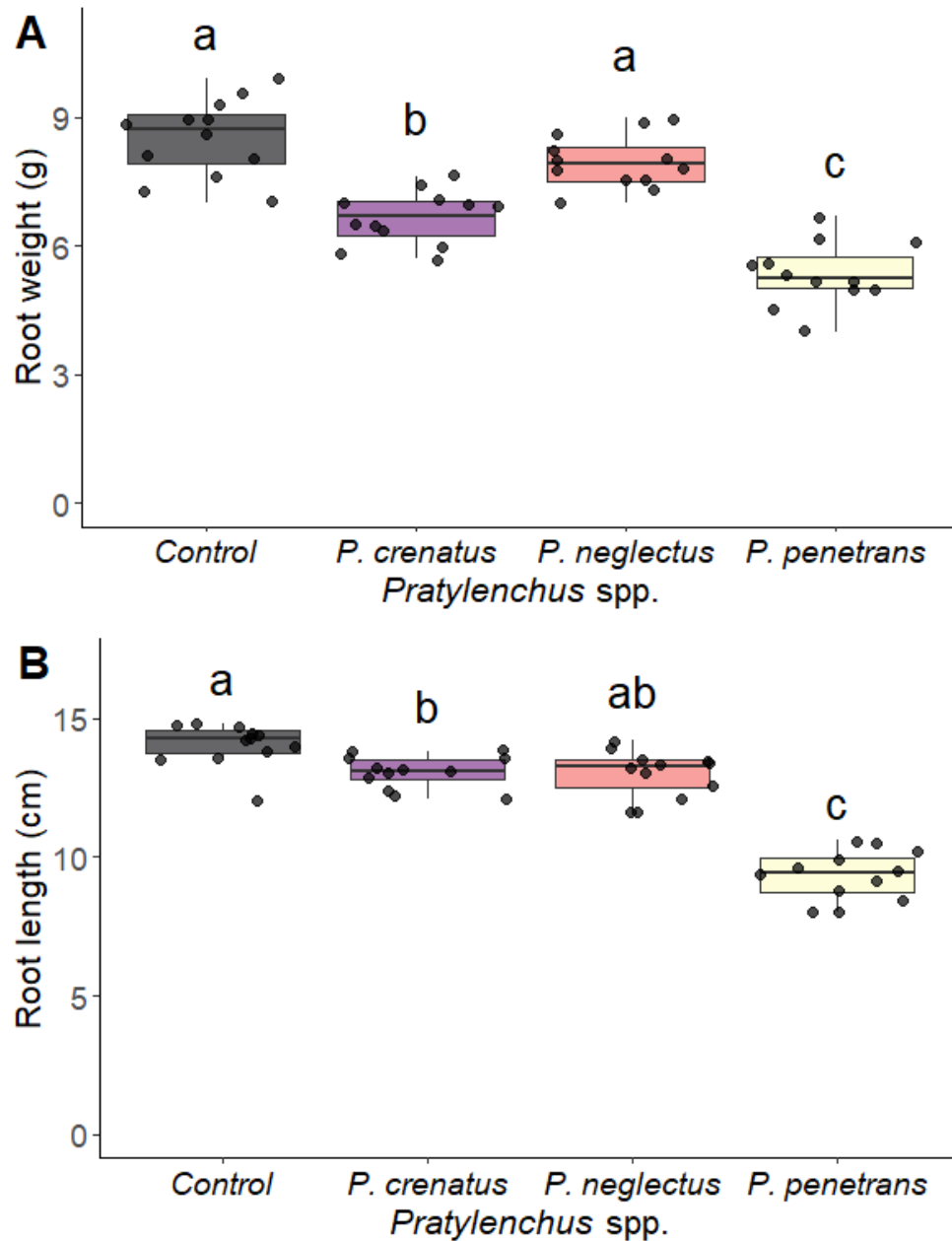


Figure 3.2. Carrot taproot weights (A) and lengths (B) of carrots exposed to equal populations of *Pratylenchus penetrans*, *P. crenatus*, and *P. neglectus*, in comparison with controls with no nematodes (Control). Jittered dots represent the distribution of individual data points within groups. A unique letter above a group indicates that the group is statistically significant (P -value < 0.05).

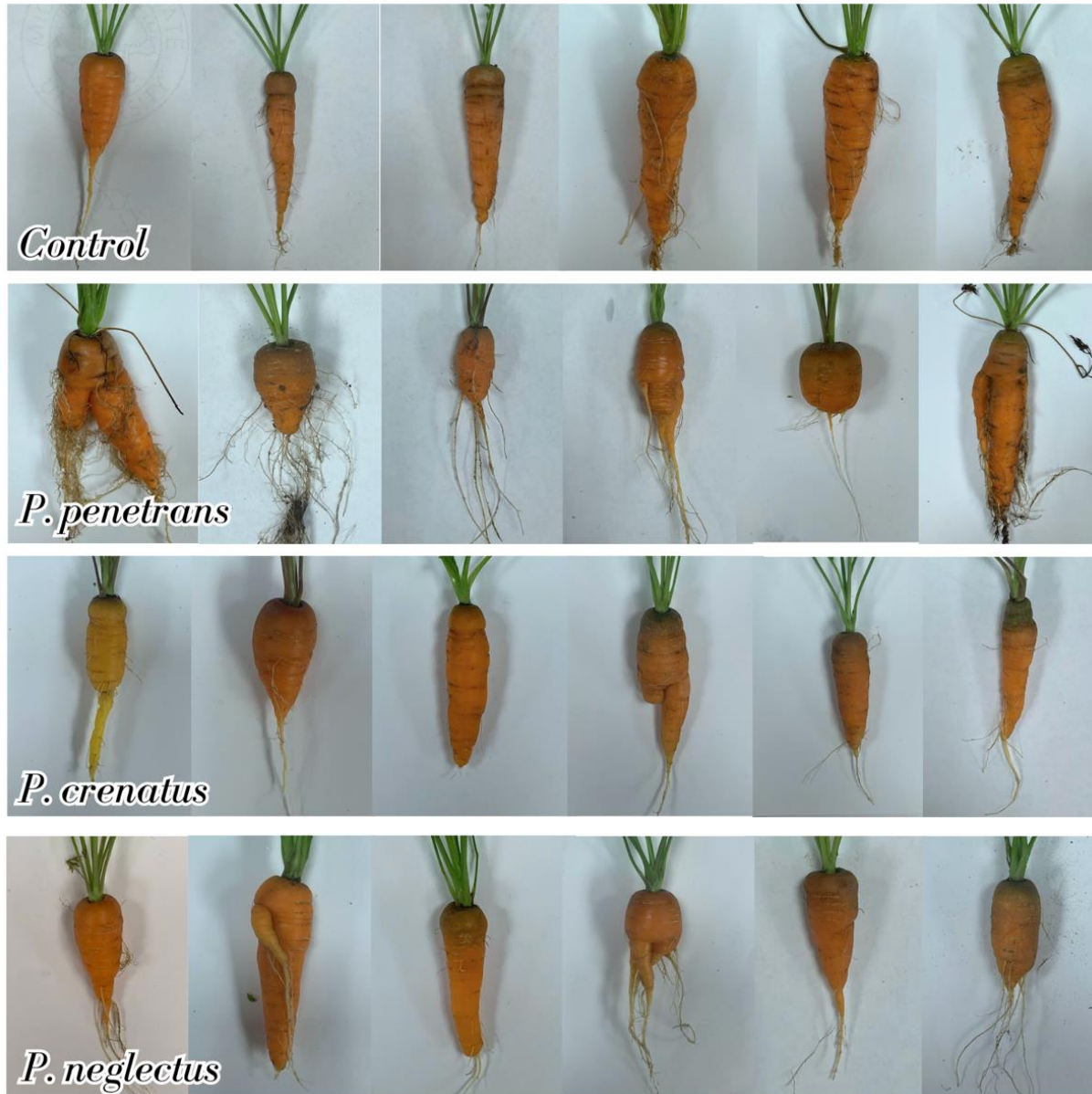


Figure 3.3. Impact of 50 individuals/100cm³ soil of *Pratylenchus penetrans*, *P. crenatus*, or *P. neglectus* on carrot taproot under uniform greenhouse conditions in carrot (cv. Cupar), compared to untreated control roots.

3.3.4 Impact of *Pratylenchus* spp. on winter wheat growth parameters

In winter wheat, *Pratylenchus neglectus*- and *P. penetrans*-infected plants reduced root weight (g) by 24.07% and 23.50%, respectively, compared to non-inoculated control plants (Figure 3.4A; $F_{(3,36)} = 11.59$, $P\text{-values} < 0.01$). However, only *Pratylenchus neglectus* significantly reduced winter wheat plant height (cm) by 11.25% (Figure 3.4B; $F_{(3,36)} = 5.59$, $P\text{-value} < 0.01$).

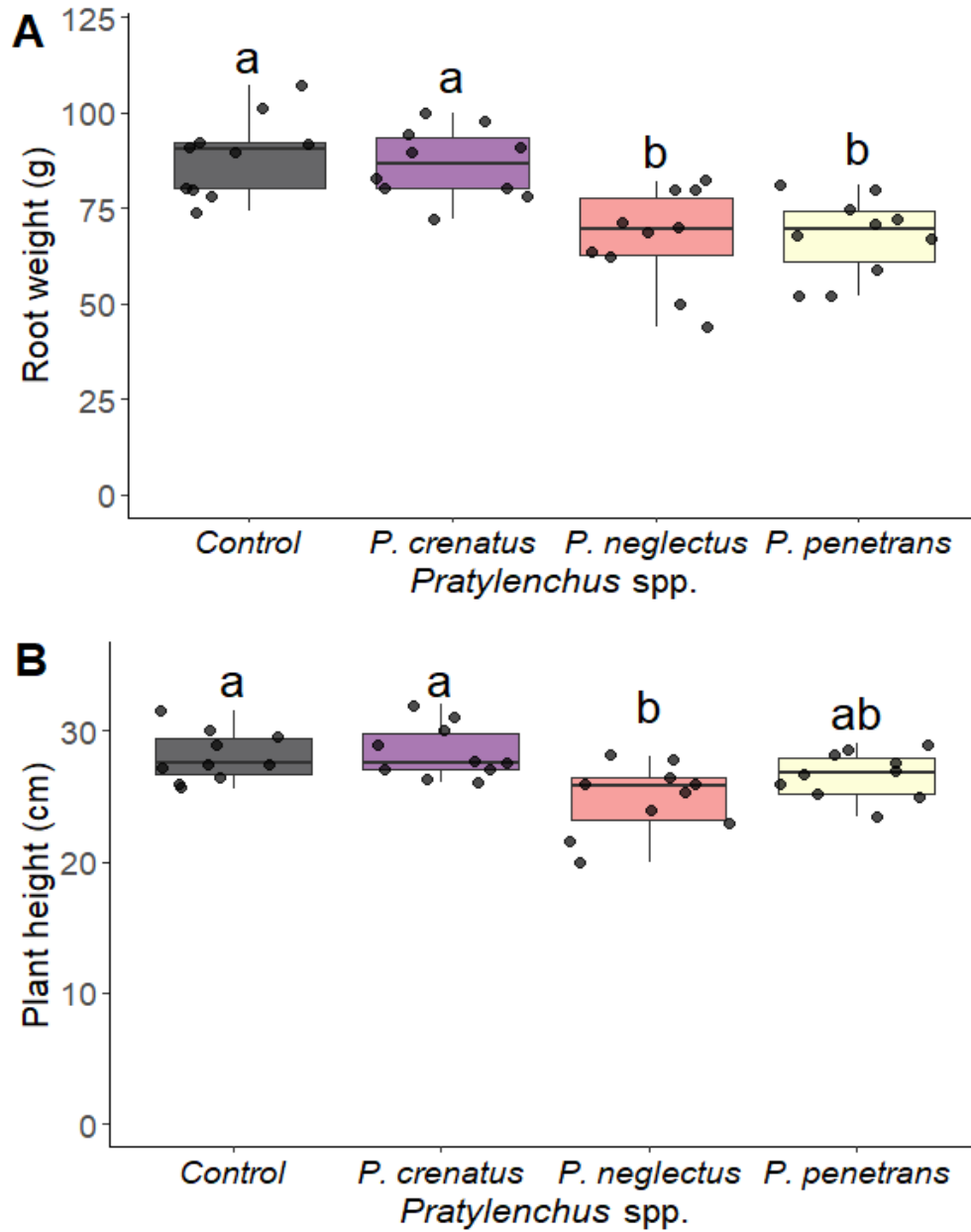


Figure 3.4. Root weights (A) and above-ground plant height (B) of wheat exposed to equal populations of *Pratylenchus penetrans*, *P. crenatus*, and *P. neglectus*, compared with controls with no nematodes. Letters indicate that groups differed significantly, while graphs lacking letters indicate no significant differences (P -value >0.01).

3.4 DISCUSSION

P. penetrans, *P. crenatus*, and *P. neglectus* were recovered from Michigan carrot and wheat fields during a 2021/22 field survey, indicating these species potentially threaten carrot and winter wheat production. However, no existing studies have compared these species under uniform inoculum densities and conditions for these hosts. To provide enhanced knowledge on species pathogenicity and reproductive capability on carrot and wheat crops, three trials were established to fulfill the objectives of this chapter. Each trial was repeated twice.

Carrot disc experiments showed significant differences in reproductive factors between species. Interestingly, *P. penetrans* maintained the lowest RF after 40 days but reversed after 80 days, consistent between trial repetitions. Because *P. penetrans* is a dimorphic species that relies on sexual reproduction, the inability to find a mating partner may disrupt population growth in low densities at early periods. This may help explain why *P. penetrans* at high densities can match or exceed the reproductive rate of the asexual species, *P. neglectus* and *P. crenatus*. Inoculation with 100 mixed life-stage individuals resulted in RFs superseding 900 at 80 days post-inoculation for all species, indicating that carrot disk culturing is an excellent way to multiply nematodes if time and proper care is taken to maintain them, which agrees with other reproductive studies (Verdejo-Lucas & Pinochet, 1992; Castillo et al., 1995; Waele & Elsen, 2002).

When species were evaluated under greenhouse conditions, *P. neglectus* maintained the highest average RF of the three species in both carrot and wheat trials at harvest (12 weeks/84 DAI). Despite this, *P. neglectus* significantly reduced winter wheat root weight and plant height but not carrot root weight or root length. Additionally, *P. crenatus* had an average RF<2.00 in wheat, indicating that it is not a suitable host (Oostenbrink, 1972). *Pratylenchus neglectus* and *P.*

penetrans RFs surpassed this level for carrot and wheat, making these crops suitable/excellent hosts for the two species.

In the early stages of carrot growth, seedlings are very susceptible to damage. Disruption of taproot development can lead to severe repercussions, including forking, stunting, and plant death (Coosemans, 1975; Hay & Pethybridge, 2005; Teklu et al., 2016). The clustered feeding and mating behavior of *Pratylenchus penetrans* in the zone of elongation (Zunke, 1990) may explain the reduction of weight and length of harvested taproots. In contrast, *Pratylenchus neglectus* enters thicker roots but first engages in ectoparasitic feeding while roots are finer (Anderson, 1976), meaning life stages of *P. neglectus* do not enter the root system and feed on external root cells early in root development. This behavior may provide insight into why feeding by *P. neglectus* did not result in taproot stunting or weight reduction in carrots.

Lastly, species identification is critical for pest diagnosis for other plant-parasitic genera like *Heterodera* and *Meloidogyne* spp. However, studies that compare *Pratylenchus* spp. are lacking within similar systems. This is likely due to a combination of factors including difficulties with culturing methods, confident identification, and extraction protocols of migratory nematodes (Orlando et al., 2020). However, these challenges are necessary to overcome for *Pratylenchus* spp. that threaten crop yields. The experiments conducted in this chapter add to mounting evidence that species identity is critical in understanding how these organisms elicit damage. Until then, tolerance levels or risk ratings may be misleading if they do not account for species differences.

3.5 CONCLUSION

We found that *P. neglectus* did not reduce carrot root weight or length, and *P. crenatus* did not reduce plant height or root weight in winter wheat. Developing risk levels or tolerance for carrots and winter wheat based on species may be more accurate; however, given that many abiotic and biotic factors influence nematode reproduction and pathogenesis, more research should be done to further investigate the complexities of migratory plant-parasitic nematodes like *Pratylenchus* species within these crop systems.

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CHAPTER 4: EVALUATING ALTERNATIVE MANAGEMENT STRATEGIES FOR *PRATYLENCHUS* SPP. AND *MELOIDOGYNE* HAPLA IN SPECIALTY CROPPING SYSTEMS

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4.1 INTRODUCTION

Specialty crops comprise 27% of total U.S. crop production in sales, despite only accounting for 7.7% of total U.S. farmland acres (Johnson 2017; USDA-NASS 2022). Public funding of research geared towards increasing yield and the success of specialty crop production has historically been underfunded (Alston & Pardey 2008). Specialty crops are subject to increased risk because of their higher production value, and pest/pathogen pressure can result in devastating losses to the farmer. The plant family *Apiaceae* contains several agronomically

significant specialty crops, most of which are either vegetables, f.e., celery (*Apium graveolens* L.), carrot (*Daucus carota* L.), parsnip (*Pastinaca sativa* L.) or culinary herbs and spices, f.e., parsley (*Petroselinum crispum* L.), dill (*Anethum graveolens* L.), fennel (*Foeniculum vulgare* L.), anise (*Pimpinella anisum* L.). Notably, Michigan is one of the highest-producing states for celery and carrot, ranking 2nd, 4th, respectively in the US (USDA-NASS 2019). However, this family is susceptible to damage by various pests and pathogens, including insects, bacterial, fungal, and viral diseases, and plant-parasitic nematodes.

Root vegetables like carrots and parsnips can suffer exceptional quality and/or yield losses from plant-parasitic nematodes (Brzeski 1970; Kimpinski & Sanderson 2004). Additionally, issues may go unnoticed until harvest due to nonspecific symptoms like wilting and stunting, which are also caused by many pathogens. Two prominent herbivorous nematodes that can impact carrot and parsnip yield are root-knot nematodes (*Meloidogyne* spp.) and root lesion nematodes (*Pratylenchus* spp.) (Gugino et al., 2006). In northern U.S. regions like Michigan, *Meloidogyne hapla* and *Pratylenchus penetrans* are responsible for damaging root vegetables (Bird & Warner 2018; Darling et al. 2020). *Meloidogyne* spp. are sedentary endoparasites, eliciting damage by juveniles entering root systems, creating host cells, and maturing within the root, leading to the formation of galls (Cailluad et al., 2008). *Pratylenchus* spp. are migratory endoparasites that disrupt healthy root production via tunneling through root cells, leaving a trail of necrotic tissue behind throughout their lifecycle (Mokrini et al. 2019). Common symptoms of *M. hapla* infestations in carrots and parsnips result in reduced yields and the presence of galls on lateral roots, while *P. penetrans* elicit damage by tunneling through the apical meristem which can result in the forking of the main taproot or stunting (Potter & Olthof 1993; Becker et al. 2019). In our recent soil and root survey, *Pratylenchus* spp. and *Meloidogyne*

hapla were the top-most encountered plant-parasitic nematodes in Michigan carrot fields.

Nematicides, alongside other classes of pesticides, have recently become subject to increased environmental bans and limitations. For example, the United States enacted an almost complete ban of the fumigant methyl bromide in 2005 (Zasada et al., 2010; Johnson et al., 2012). While beneficial to natural resources and other systems, these restrictions can leave growers with few effective management options (Desaeger et al., 2020). A desirable nematicide or management strategy significantly reduces the target plant-parasitic nematode pest, resulting in a corresponding yield bump or reduction of quality loss, which outweighs the application cost. Alternative nematode management strategies for fumigants include breeding resistant crop lines, less toxic chemical and biological control products, soil amendments, and the use of non-host cover crops (Lopes et al. 2019). Rotating resistant crop varieties is effective in field crops like soybean, but there are no existing lines for specialty crop root vegetables like carrots and parsnip (Thapa et al. 2022).

Using chemical and biological control products can result in effective management of nematodes. Biological control methods can also be effectively utilized to suppress nematodes via the application of an antagonistic strain of bacteria or fungi. The benefits of using a biologically based product can minimize the risk to the applicator and surrounding resources. Two biologically based nematicides, MeloCon (Certis; *Purpureocillium lilacinum*) and Majestene (Marrone; heat-killed *Burkholderia* spp.), are registered for use on organic crops. The active ingredient of MeloCon, *Purpureocillium lilacinum*, is a soil-borne egg-parasitic fungus, originally isolated from tropical regions (Domsch et al. 1980). Over the years, organic amendments (*e.g.*, compost and manure-based amendments) have been used in agricultural systems to promote soil quality parameters such as porosity, aggregate stability, water retention,

microbial diversity, and nutrient availability (Abawi & Widmer 2000; Widmer et al. 2002; Oka 2010). However, in the last few decades, compost and manures have been screened and shown to be promising as management approaches for plant-plant parasitic nematodes (Noble & Roberts 2004). Additionally, as discussed in the introductory chapter, incorporating non-host cover crops is another potential option for limiting plant-parasitic nematode populations in root vegetable production. Cover cropping in vegetable systems also benefits the cropping system by increasing nitrogen supply to subsequent crops, suppressing noxious weeds, increasing nutrient cycling, and improving soil physical properties (Adetunji et al., 2020; Blesh et al., 2019). Cover crops like oat (*Avena sativa* L.), radish (*Raphanus sativus* L.), and rape seed (*Brassica napus* L.) influence the nematode community in carrot production in the long run which can be beneficial to soil health (Grabau et al., 2017). Additionally, many grasses (e.g., oat, barley) are non-hosts of *Meloidogyne* spp. and can be incorporated easily (Opperman et al., 1988). Some cover crops act as trap crops which stimulate nematode hatch, however, do not provide a host for reproduction (Scholte, 2000; Smith et al., 2004).

To understand potential management alternatives to fumigation, we developed experiments with the following objectives (i) to evaluate chemically-, biologically-based products, and soil amendment (compost) effectiveness at reducing *Pratylenchus* nematodes in carrot production, (ii) to evaluate chemically- and biologically-based products effectiveness at reducing *Meloidogyne* nematodes in parsnip production, and (iii) to evaluate the host status of oilseed radish and rapeseed varieties for *P. penetrans* and *M. hapla*, and their use as cover crops within these systems.

4.2 METHODS

4.2.1 Carrot field site establishment

Nematicide trials were established on carrot (cv. Cupar) in northwest Michigan for four years (2017-2020). Treatment rates and application style were applied as recommended by the manufacturer (Table 4.1). Each year, a field was selected that had a history of high infestations of root lesion nematodes, *Pratylenchus spp.*, to evaluate the efficacies of non-fumigant nematicides and soil amendments. Each plot consisted of one-planter pass (3 rows, 1.62 m x 6.10 m), and the plots were arranged in a randomized complete block design with 5 replicates. Compost applications were applied to the soil just prior to planting and manually incorporated into the soil surface. Soil samples were collected three times throughout the season: prior to crop planting (April), midseason (July), and during harvest (October). During the first year, initial samples were collected in a zig-zag pattern across the experimental area prior to crop establishment to represent the field average. For the remaining three years, initial samples were collected from each plot to account for clustering variability that is common in nematode-infested fields. Midseason and harvest soil cores were collected using a zig-zag pattern within each plot. Soil cores from the same plot were homogenized into one sample, and a subsample (100cm³) of each bag was processed to extract nematodes via the sugar centrifugal flotation method (Jenkins 1964). Samples were identified and counted using an inverted Nikon TMS microscope at 200x and 1000x magnification and plant-parasitic nematodes were identified to genus via morphological characters. Non-plant parasitic nematode counts were also collected to observe impacts on non-target nematodes. Nematode taxa were grouped according to trophic level (Yeates et al. 1971). Carrots (1 m) were pulled per plot during harvest to determine treatment impact on yield. Carrots were weighed and graded according to processing standards

(USDA 1960). Any unmarketable carrots associated with *Pratylenchus* spp. nematode damage (stubbiness and forking of the taproot) was recorded.

Table 4.1. Products applied during the field nematicide trials.

Product	Company	Active Ingredient	Rate	Application	Years – Carrots (of 4)	Years – Parsnips (of 2)
Control	NA	Untreated	NA	NA	4	2
Velum Prime	Bayer CropScience	Fluopyram	0.48 L/ha	In-furrow	4	2
Nimitz	ADAMA	Fluensulfone	2.50 L/ha	At seeding	3	2
Majestene 305	Marrone Biosciences	Heat-killed <i>Burkholderia</i> spp.	18.71 L/ha	In-furrow	4	2
MeloCon	Certis USA	<i>Purpureocillium lilacinum</i>	5.60 kg/ha	In-furrow	4	0
Vydate	Corteva Agriscience	Oxamyl	9.35 L/ha	In-furrow	4	2
Compost	Dairy Doo	Layer Ash Blend	2.80 tons/ha	Spreader (Pre-plant)	3	2

4.2.2 Parsnip field site establishment

For two years, a seasonal nematicide trial was established on parsnip (cv. Panorama) in southwest Michigan. In both years, we selected a field with a history of high infestations of the northern root-knot nematode, *Meloidogyne hapla*. Treatments and applications (Table 4.1) were applied as recommended by the manufacturer. Plots consisted of one-planter pass (4 rows, 3.5 m x 15.2 m) and were arranged in a randomized complete block design with 5 replicates. Oxamyl was applied by the grower in the surrounding field and not the specified plots for this trial, but replicated plots were selected to sample for comparison. Soil samples were collected, processed, and quantified three times throughout the season as described in the previous section. Due to increased demand for parsnips, harvest occurred earlier than usual, late July in Y1 (2019) and mid-August in Y2 (2020). Parsnips were measured and evaluated for galling by *M. hapla*, excess of lateral roots (hairy), shortened taproots (stubby), forking of the taproot (forked), and insect

damage (fly-damaged) by the carrot rust fly (*Psila rosae*).

4.2.3 *Pratylenchus penetrans* source colony

Nematode cultures were maintained *in vitro* on carrot discs with slight modifications (Coyne et al. 2014). Carrots were purchased from local grocery stores and hand-selected for diameters (>5 cm) and inspected for any visual deformities (cracks, rot). Healthy carrots were washed with dish soap and scrubbed of any residual soil. The remaining steps were conducted under a laminar-flow hood. Carrots were peeled with a standard metal peeler three times and submerged in ethanol, followed by distilled water. This process was repeated twice, and all tools were flame sterilized prior to use. After this process, carrots were sliced into sections (1 cm) and placed in a standard plastic 100 x 15 mm Petri dish. A single mature female *P. penetrans* nematode was collected from fresh field soil, sanitized in an antimicrobial bath of Streptomycin Sulfate (Sigma-Alrich, St. Louis, MO.), then thoroughly rinsed with distilled water. Quickly after, each nematode was added to a sterile disc and the dish was sealed tightly with parafilm (Bemis™). Discs showing any contamination (bacterial ooze, fungal bodies, etc.) were promptly disposed of and only healthy discs were used for colony splitting, continuation, and trials. Nematodes were extracted by carefully dicing carrots, submerging pieces in distilled water for 24 hrs and solution was poured over a 25-μm sieve to collect nematodes.

4.2.4 *Meloidogyne hapla* source colony

Northern root-knot nematode (*M. hapla*) eggs were extracted from infected parsnips using a diluted NaOCl shaking method (Hussey & Barker 1973). Eggs were then collected from roots, rinsed thoroughly, and inoculated to a susceptible tomato variety (cv. Rutgers). Colonies were maintained under greenhouse conditions (16:8 hr light/dark) and extracted when they wilted or had stunted growth.

4.2.5 Establishment of cover crop trials

Experiments were established to evaluate the host status of candidate cover crops for nematode control. The selected cover crops for root-lesion nematodes and root-knot nematodes are represented in Table 4.2 and 4.3, respectively. Oilseed radish varieties were selected based on communication and interest of local Michigan vegetable growers and Michigan State University vegetable extension educators. As previously described, *P. penetrans* were reared on carrot disks and *M. hapla* nematodes were reared on tomatoes, so we kept these crops as corresponding positive controls (suitable hosts) for their respective trials.

For the evaluation of *P. penetrans*, three seeds of each crop (Table 4.2) were planted in cone-tainers (100 cm³) in an RL200 tray (Ray Leach), with a soil mixture of 70:30 play sand to sterilized field sandy soil (100 cm³). Cone-tainers were arranged in a complete random block design with 3-4 replicates. Seven days post-planting, each cone was thinned to one seedling and then inoculated with *P. penetrans* nematodes (1,000) of mixed life stages at the base of the plant. Carrots were also included to represent a suitable host of *P. penetrans* for comparison. Eight weeks following inoculation, the trial was concluded. Soil (100 cm³) and root samples (1 g) were collected for extraction and enumeration for each container. The trial was then repeated following the same protocols. Reproductive factors (RF) were calculated for each of the three trials by dividing the final extracted populations (P_f) by the initial inoculum (P_i). RF of each crop was then categorized into four groupings: Nonhost (RF = 0.00-0.09), Poor Host (RF = 0.10-0.99), Moderate Host (RF = 1.00-2.00), and Suitable Host (RF > 2.00) (Oostenbrink, 1972). The experiment was repeated to ensure consistency of host status.

For the evaluation of the host status of various cover crops to the root-knot nematode, a trial was established as previously described apart from inoculating with 1,000 *M. hapla* eggs

onto cover crop options (Table 4.3). Tomato (cv. Rutgers) was used as a control host for *M. hapla* in this trial (Stephan 1983), and the methods for this trial were similar to the methods described above. Only nematode densities and calculated reproductive factors were collected for the cover crop trials at trial conclusion. Both trials were repeated.

Table 4.2. Oil seed radish cultivars evaluated within greenhouse trials for reducing the root lesion nematode, *P. penetrans*.

Cover crop (Trade name)	Company
Control	Allied Seeds
Concorde	Allied Seeds
Defender	Petersen Seed
Eco-Till	Allied Seeds
Enricher	Advance
Image	La Crosse Seed
NitroRadish	Byron Seeds
Respect	Star of the West
Select	La Crosse Seed
Tillage	La Crosse Seed

Table 4.3. Cover crop seeds evaluated for efficacy at reducing the northern root knot nematode, *Meloidogyne hapla*.

Cover crop (Trade name)	Species	Company
Control	Oil Seed Radish	Allied Seeds
Concorde	Oil Seed Radish	Allied Seeds
Eco-Till	Oil Seed Radish	Petersen
Select	Oil Seed Radish	La Crosse Seed
NitroRadish	Oil Seed Radish	Byron Seeds
Dwarf Essex	Rapeseed	Advance Cover Crops
Pratex	Oat	Allied Seeds

4.2.6 Data analysis

Data collected from all trials were analyzed using R version 4.3.1 (R Core Team, 2023). For the field trials, response variables included yield parameters (total yield, marketable yield, stubbed yield, forked yield, hairy yield) and nematode counts (mid-season and harvest *Pratylenchus* (carrot) or *Meloidogyne* (parsnip) counts per 100cm³ soil, and free-living nematode

counts per 100cm³ soil). The treatment type was the fixed effect, whereas year and replication were considered random effects. For nematode count data, generalized linear mixed models (GLMM) were best fit with negative binomial distribution and a log link function, using the package ‘glmmTMB’, and checked for overdispersion (Brooks et al. 2017). Additional GLMMs were best fit for yield parameter response variables with normal distribution. If F-values were significant for treatment type, Tukey’s Honest Significant Difference (HSD) post-hoc tests ($\alpha=0.1$) were conducted for means separation, using the “emmeans” and “multcompView” packages in R (Lenth 2020; Graves et al. 2023). Graphs were constructed using ggplots in the ‘tidyverse’ package, and compact letter display (CLD) for pairwise comparisons were manually annotated onto bar and boxplots (Wickham et al. 2019). Finally, if figures contained multiple graphs, they were combined using the ‘cowplot’ package (Wilke 2020).

For the cover crop greenhouse trials, RF values were calculated from soil and root densities at trial conclusion. The RF values were then scaled to standardize so every datapoint had a mean value of zero. Normality was confirmed with a normal quantile-quantile plot of residuals and a Shapiro-Wilk test ($P\text{-value}>0.05$). Next, a linear mixed model (LMM) was constructed with RF as the response variable, crop type as the fixed effect, and trial and replicate as random effects. If F-values were significant, Tukey’s honest significant difference post-hoc tests were conducted ($\alpha=0.05$) following model analysis for means separation, using the “emmeans” and “MASS” packages in R (Lenth, 2020; Venables & Ripley, 2002).

4.3 RESULTS

4.3.1 Carrot field trials

4.3.1.1 Reduction of *Pratylenchus* spp. by treatment

At midseason, *Pratylenchus* spp. densities within plots that were treated with Velum

Prime were 45.27% lower than untreated control plots (Figure 4.1; $F_{(6,\infty)}=3.78$, $P\text{-value}<0.01$). However, densities had recovered at harvest and were not different from controls. Plots treated with Nimitz were lower than untreated plots at harvest ($P\text{-value}<0.1$), but not midseason ($P\text{-value}>0.1$).

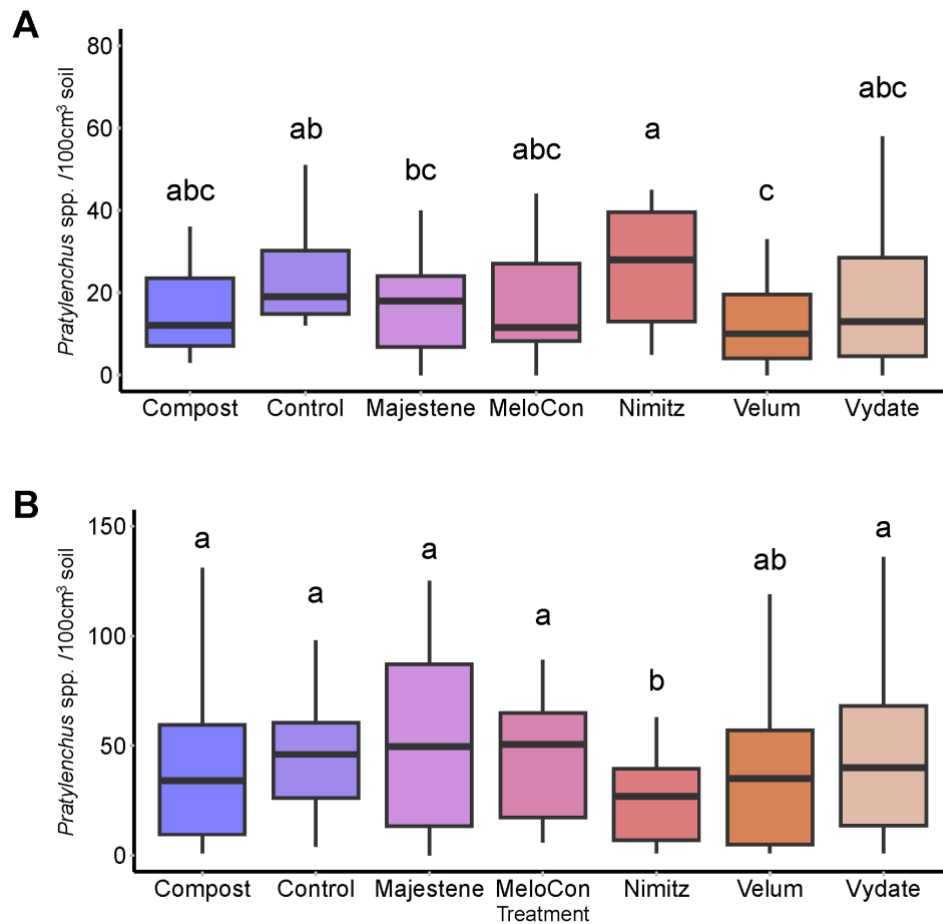


Figure 4.1. *Pratylenchus* spp., root lesion nematode, densities at midseason (A) and harvest (B) by control method. Unique letters above a group indicates statistical difference ($P\text{-value} < 0.1$).

4.3.1.2 Impact of treatments on free-living nematodes

At midseason, densities of free-living nematodes were not impacted by treatment application (Figure 4.2; $F_{(6,120)}=0.88$, $P\text{-value}>0.1$); however, at harvest, densities of plots treated with Nimitz were 47.05% lower than untreated control plots ($F_{(6,\infty)}=2.54$, $P\text{-value}<0.1$).

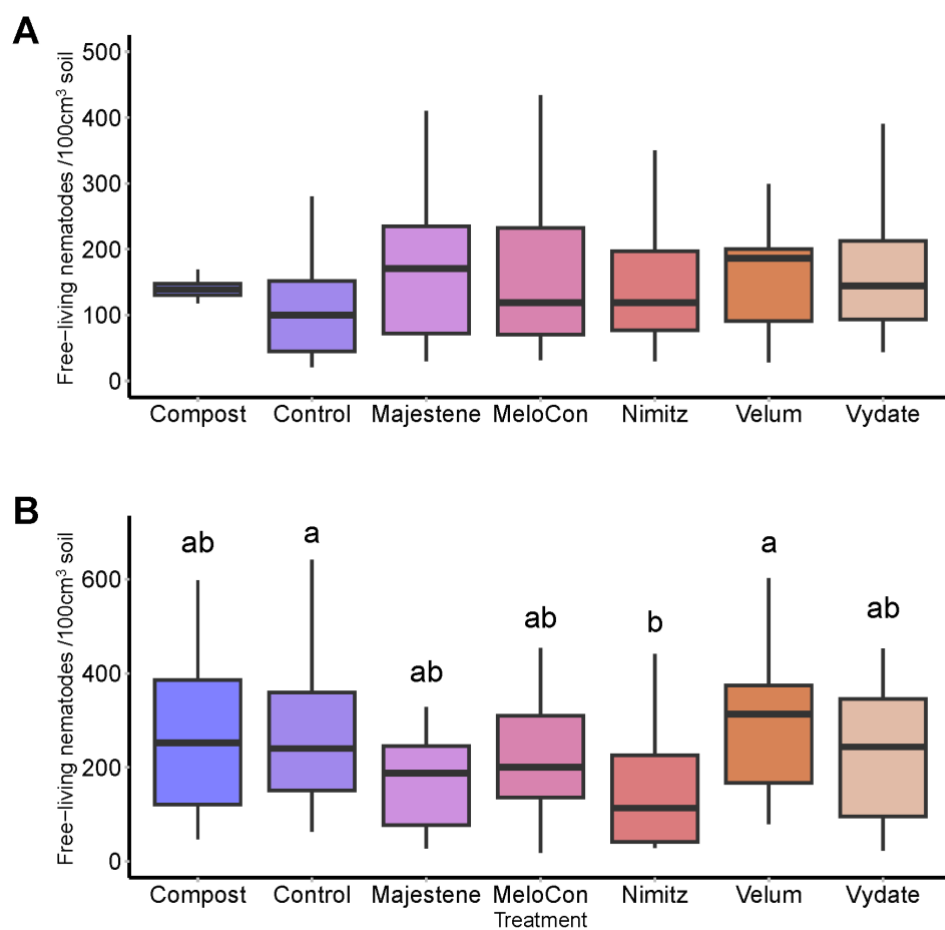


Figure 4.2. Free-living nematode densities at midseason (A) and harvest (B) by control method. Unique letters above a group indicates statistical difference (P -value < 0.1).

4.3.1.3 Impact on yield and presence of unmarketable characteristics

Total yield of carrots was not impacted by treatment type (Figure 4.3A; $F_{(6,\infty)}=1.86$, P -value >0.1). However, marketable yield was different between treatments (Figure 4.3B; $F_{(6,\infty)}=2.85$, P -value <0.01). Plots treated with Vydate produced 8.58% more marketable carrots than untreated control plots (P -value >0.1). Treatments did not impact stubby or forked carrot yield (Figure 4.3C, D; $F_{(6,\infty)}=0.48$ and $F_{(6,\infty)}=1.16$, P -values >0.1).

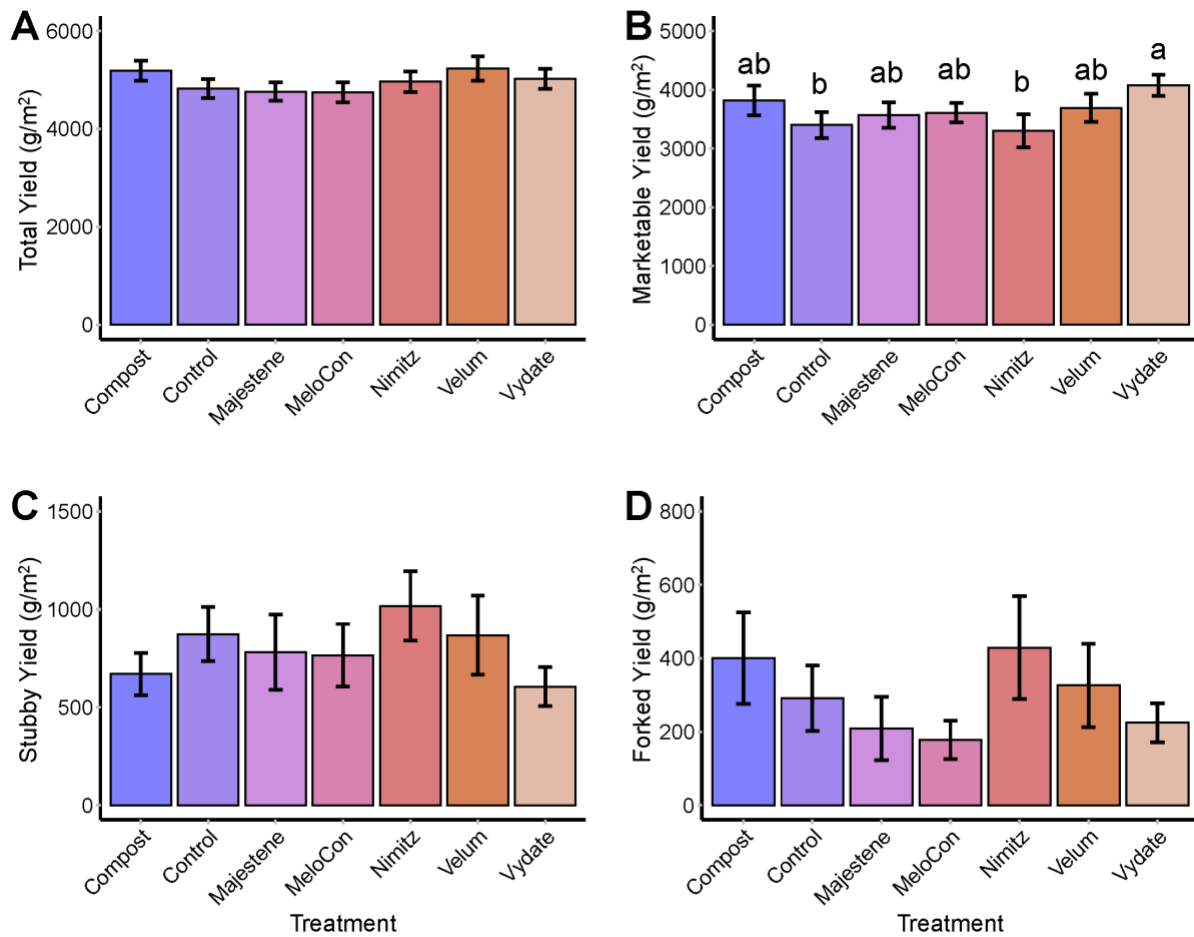


Figure 4.3. Average \pm SEM (A) total, (B) marketable, (C) stubby, (D) forked yield produced by each control method in the carrot nematicide trial. Unique letters above a group indicates statistical difference (P -value < 0.1).

4.3.2 Parsnip field trials

4.3.2.1 Reduction of *Meloidogyne* spp. by treatment

At midseason, Velum, Vydate, and Nimitz applications decreased *Meloidogyne hapla* densities by 45.37%, 69.44%, and 75.62%, compared with untreated control plots (Figure 4.4A; $F_{(5,\infty)}=9.19$, $P\text{-values}<0.0001$). At harvest, *M. hapla* densities in plots treated with Velum Prime rebounded and were not different from untreated plot (Figure 4.4B; $F_{(5,46)}=4.88$, $P\text{-value}>0.1$). Meanwhile, applications of Majestene, Nimitz, and Vydate reduced *M. hapla* harvest populations by 67.36%, 78.16%, and 78.24%, respectively, compared to untreated plots ($P\text{-values}<0.01$).

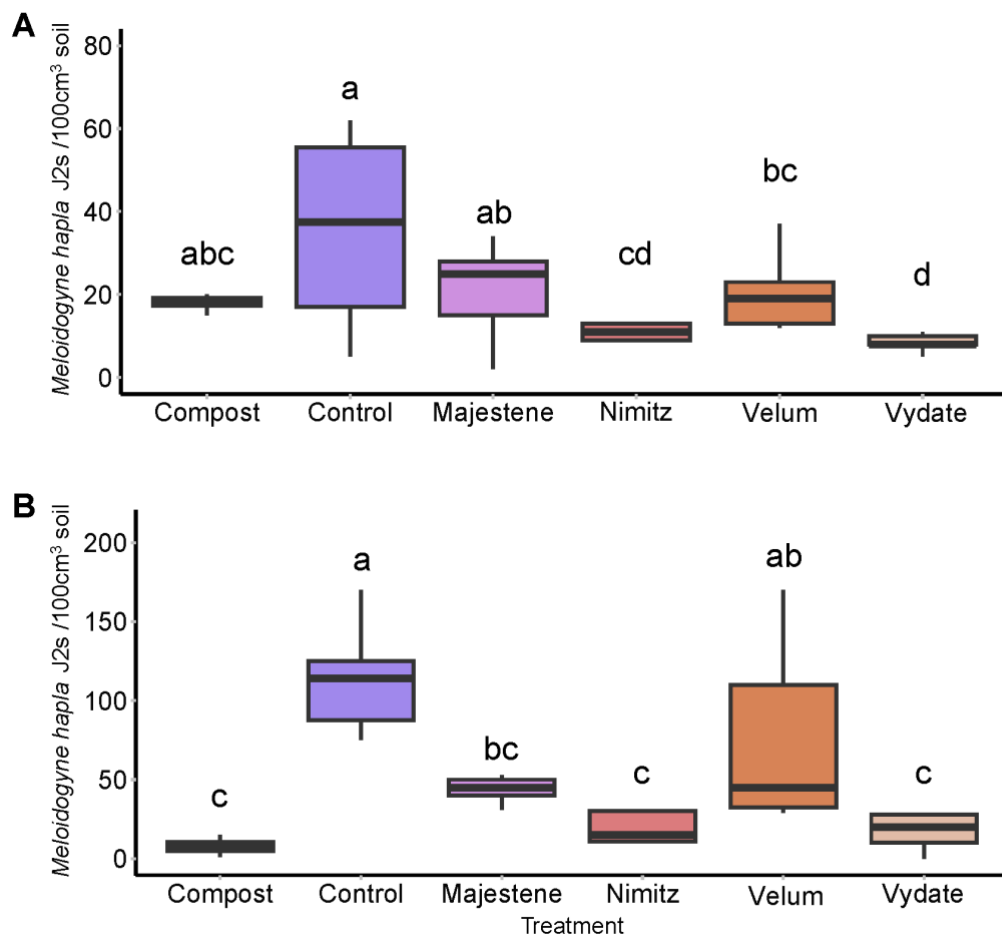


Figure 4.4. *Meloidogyne hapla*, root-knot nematodes, in soil at midseason (A) and harvest (B) by control method. Unique letters above a group indicates statistical difference ($P\text{-value} < 0.01$).

4.3.2.2 Impact of treatments on free-living nematodes

At midseason and harvest, free-living nematodes were impacted by treatment type (Figure 4.5; $F_{(5,48)}=4.19$ and $F_{(5,46)}=11.88$, P -values<0.01). Compost and Majestene applications increased the number of free-living nematodes by 92.41% and 34.18%, respectively, compared to Vydate-applied plots at midseason (P -values<0.01). Free-living nematode densities in Vydate plots were 58.15% lower than untreated plots at harvest ($F_{(5,46)}=11.88$, P -value<0.001).

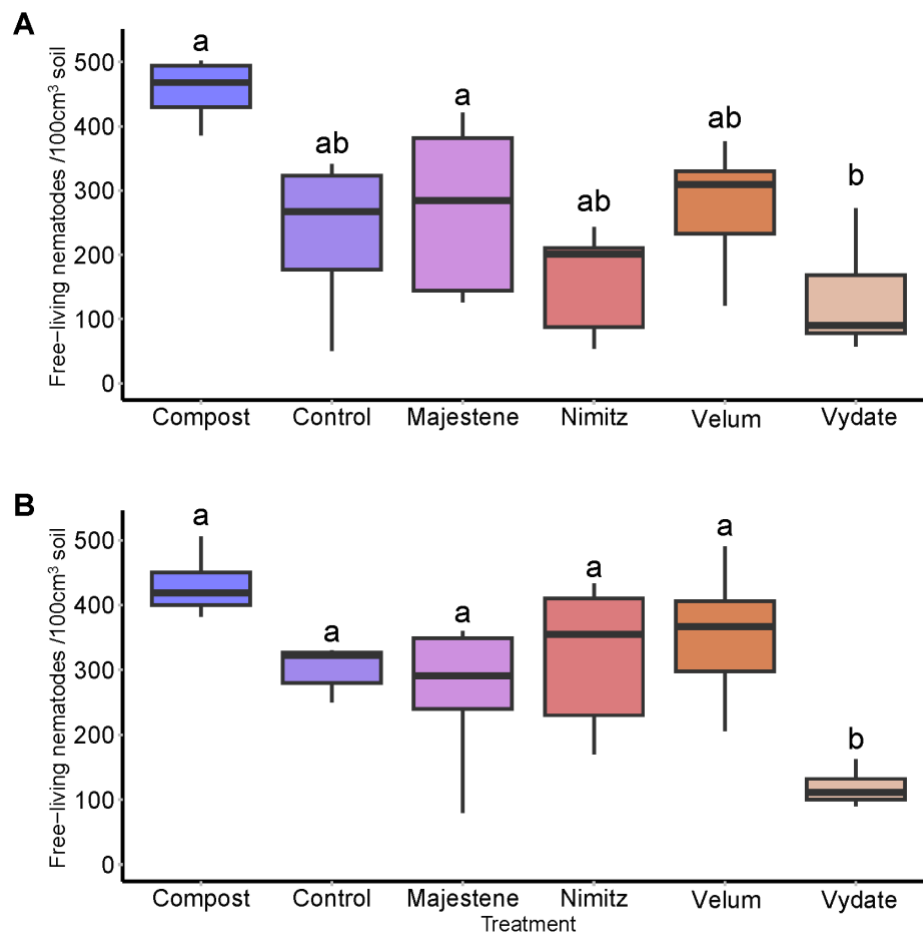


Figure 4.5. Free-living nematodes in soil at midseason (A) and harvest (B) by control method. Unique letters above a group indicates statistical difference (P -value < 0.01).

4.3.2.3 Impact on yield and presence of unmarketable characteristics

Compost-applied plots increased total yield by 99.81% compared with untreated plots (Figure 4.6A; $F_{(5,\infty)}=2.03$, $P\text{-value}<0.05$). However, when considering unmarketable characteristics, compost increased forking by 1,684.49% (Figure 4.6E; $F_{(5,\infty)}=3.80$, $P\text{-values}<0.01$). Vydate and Nimitz increased marketable parsnip yield by 60.53% and 43.68%, respectively, compared with untreated control plots (Figure 4.6B; $F_{(5,46)}=2.28$, $P\text{-values}<0.01$). Nimitz was also the only treatment that reduced hairy parsnip yield by 85.37%, compared to untreated plots (Figure 4.6C) $F_{(5,\infty)}=2.15$, $P\text{-value}<0.05$). Finally, treatments did not affect yield of stubby and fly-damaged parsnips (Figure D, F; $F_{(5,46)}=0.43$ and $F_{(5,\infty)}=0.16$, $P\text{-values}>0.1$).

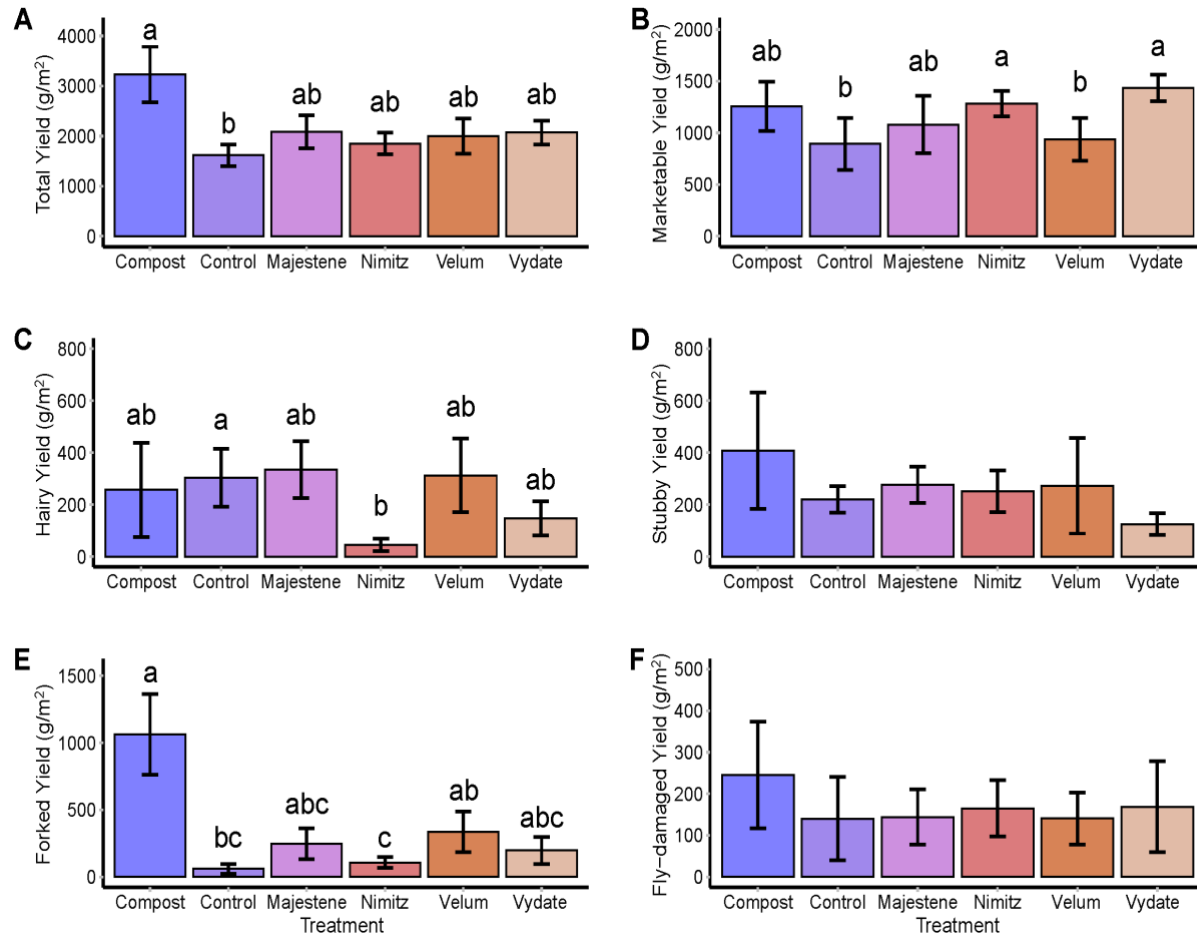


Figure 4.6. Parsnip total yield (average \pm SEM) (A) is separated by marketable(B), hairy (C), stubby (D), forked (E), and fly-damaged (F) characteristics by control method.

4.3.3 Cover crop trials

In greenhouse trials evaluating the host status of cover crops for *P. penetrans*, all oilseed radishes had RFs lower than the host carrot (Table 4.4; $F_{(11,78)} = 52.25$, $P\text{-value} < 0.05$). The oilseed radishes cvs. Control and Concorde both had an average reproductive factor of 0.05, indicating they are non-hosts to *P. penetrans*. Additionally, the remaining varieties of oilseed radish were poor hosts for *P. penetrans*.

In our greenhouse trials evaluating the host status of *M. hapla*, the susceptible tomato (cv. Rutgers) host had an RF value higher than all other crops tested (Table 4.5; $F_{(7,59)} = 17.30$, $P\text{-value} < 0.05$). Specifically, oat (cv. Pratex) and oilseed radishes (cvs. Control and Select) were non-hosts for *M. hapla*. The oilseed radish (cv. Control) was a non-host for both *P. penetrans* and *M. hapla*.

Table 4.4. Reproductive factors (RF; mean \pm SEM) of *Pratylenchus penetrans* on potential cover crop hosts between two trials. Pairwise differences are indicated by unique letters ($F_{(11,78)} = 52.25$, $P\text{-value} < 0.05$; $\alpha = 0.05$).

Cover crop	RF (mean \pm SEM)	Host Status
Carrot (cv. Cupar)	1.41 \pm 0.18 a	Moderate
Oilseed Radish (cv. Control)	0.05 \pm 0.01 f	Non
Oilseed Radish (cv. Concorde)	0.05 \pm 0.01 f	Non
Oilseed Radish (cv. Defender)	0.38 \pm 0.04 cde	Poor
Oilseed Radish (cv. Eco-Till)	0.54 \pm 0.09 c	Poor
Oilseed Radish (cv. Enricher)	0.46 \pm 0.06 cd	Poor
Oilseed Radish (cv. Image)	0.83 \pm 0.02 b	Poor
Oilseed Radish (cv. NitroRadish)	0.39 \pm 0.06 cd	Poor
Oilseed Radish (cv. Respect)	0.47 \pm 0.05 cd	Poor
Oilseed Radish (cv. Select)	0.23 \pm 0.03 def	Poor
Oilseed Radish (cv. Tillage)	0.84 \pm 0.08 b	Poor

Table 4.5. Reproductive factors (RF; mean \pm SEM) of *Meloidogyne hapla* on potential cover crop hosts between two trials. Pairwise differences are indicated by unique letters ($F_{(7,59)} = 17.30$, $P\text{-value} < 0.05$; $\alpha = 0.05$).

Cover crop	RF (mean \pm SEM)	Host Status
Tomato (cv. Rutgers)	4.55 \pm 0.72 a	Suitable
Oat (cv. Pratex)	0.00 \pm 0.01 b	Non
Oilseed Radish (cv. Control)	0.02 \pm 0.01 b	Non
Oilseed Radish (cv. Concorde)	1.1 \pm 0.75 b	Moderate
Oilseed Radish (cv. Eco-Till)	0.16 \pm 0.11 b	Poor
Oilseed Radish (cv. NitroRadish)	0.12 \pm 0.04 b	Poor
Oilseed Radish (cv. Select)	0.03 \pm 0.01 b	Non
Rapeseed (cv. Dwarf Essex)	0.49 \pm 0.30 b	Poor

4.3 DISCUSSION

Evaluating non-fumigant nematicides has recently become increasingly important as historically effective products are pulled from the shelves due to increased environmental usage restrictions and a shift in consumer preferences (Desaeger et al., 2020). This study is the first to investigate the effectiveness of several nematicides and a soil amendment for reducing *Pratylenchus* spp. and *M. hapla* populations within carrot and parsnip fields. In our field trial on *Pratylenchus* spp. in carrots, we found that an in-furrow application of Velum Prime reduced midseason densities, however, these populations had rebounded by harvest and did not result in increased total or marketable yield compared to untreated plots. Nimitz-applied plots had higher *Pratylenchus* densities than control plots at midseason, but lower densities at harvest, which did not correspond to a significant impact on yield. Vydate had no effect on *Pratylenchus* densities at midseason or harvest and reduced free-living, beneficial nematodes at harvest; yet in-furrow applications of Vydate at planting resulted in the highest marketable yield. This suggests that Vydate may have had an alternative benefit to carrot growth.

In our field trial on *M. hapla* in parsnips, we found that Nimitz and Vydate were both effective for reducing *M. hapla* populations and increasing marketable parsnip yield. Nimitz also decreased the presence of hairy parsnips, making it a viable product for reducing *M. hapla*

densities in parsnips. In a study comparing similar products in carrots, Nimitz was also the most effective treatment for reducing yield loss caused by the root-knot nematode (*M. incognita*) management (Becker et al. 2019). This suggests that Nimitz is a promising method to manage *Meloidogyne* spp. in taproot vegetable systems and results in nematode control and increased yield comparable to Vydate application. In contrast, plots treated with Velum Prime reduced *M. hapla* populations in comparison to control plots at midseason, but densities had rebounded by harvest and resulted in yields no different than untreated control plots. Interestingly, Velum Prime's active ingredient, fluopyram, is a contact kill nematicide and another study on carrot found that it provided good control within the first few weeks but, only the top 6-8 cm of carrots were free of *Meloidogyne* galling (hairiness) (Becker et al. 2019). We noticed the same trend in our field trials, where an in-furrow application of Velum Prime at planting did not decrease harvest densities of *Pratylenchus* spp. or *M. hapla*, nor did it increase carrot and parsnip yield. This indicates it may not be an effective tool for managing plant-parasitic nematodes of taproot vegetables.

The biological product Majestene did not increase the total or marketable yield of carrots or parsnips. Also, plots treated with Majestene did not have reduced plant-parasitic nematodes at midseason in either crop. Since biological ingredients are organisms, they require optimal conditions to persist in different locations dependent on soil moisture, soil microbiome, pH, and other factors, and not all bionematicides may be successful (Pires et al. 2022). We also found that an in-furrow application of MeloCon did not reduce *Pratylenchus* spp. densities in our carrot field trial at midseason or at harvest. Consequentially, our findings support past studies highlighting that use of these two biologically based products can be inconsistent between year and crop. For example, Majestene applications did not significantly reduce plant-parasitic

nematode populations in tomato (*Solanum lycopersicum* L.), tobacco (*Nicotiana tabacum* L.), and peach (*Prunus persica* L.), but did in cucurbits (*Cucurbitaceae*) and daylilies (*Hemerocallis*) (Desaeger & Watson, 2019; Khanal & Desaeger, 2020; Alam et al., 2022; Khanal et al., 2022; Howland et al., 2022). As discussed, differences in abiotic and biotic factors between different fields and crop types may explain this variability.

Additionally, the effects of compost application were inconsistent, but overall they seemed ineffective. In parsnips, compost significantly reduced *M. hapla* densities at harvest and increased total yield. However, when the total yield was separated into marketable, forked, stubby, hairy, and fly-damaged parsnips, we found that this yield increase was due to a 1684.49% increase in forked parsnips and no change in marketable yield. Several authors have documented the reduction of plant-parasitic nematodes on crops treated with animal-based manure or composts (Renčo and Kováčik, 2012; Cole et al., 2020). One of the main ingredients of the layer ash-based compost is cattle manure. The increase in free-living nematodes could be related to improved soil structure, augmentation of beneficial microorganisms, and supply of nutrients (Siddiqui and Futai, 2008; Nahar et al., 2016). The effect of compost and manures on plant-parasitic nematodes abundance is dependent on the type of feedstock used and the rate applied, therefore, the effectiveness of such a management approach can be variable (Kimpinski et al., 2003). In carrots and parsnips, the addition of compost had no significant impact on marketable yield in comparison with untreated controls.

There were several challenges with the establishment of the field trials. Soil moisture and annual precipitation can influence nematode communities in soil and can impact nematodes in diverse ways (Nielsen & Ball, 2015; Ankrom et al., 2020). In northwest Michigan, the carrot growing seasons (Apr.-Oct.) of 2017-2020 received approximately 58.2, 73.8, 79.9, and 62.3 cm

of precipitation, respectively (US-NWS, 2023). Interestingly, the year with the highest precipitation (year 3, 2019), was when we detected the lowest *Pratylenchus* pressure.

Lastly, several varieties of oilseed radishes were evaluated for their host status and suitability as cover crops under greenhouse conditions. All the oilseed radishes that were evaluated had significantly lower *P. penetrans* populations than carrots ($P\text{-value} < 0.05$). However, only the radishes cvs. Control and Concorde were categorized as non-hosts when using the Oostenbrink (1972) host status scale. Among the tested crops in our experiments, oat (cv. Pratex) was the only definitive nonhost for the root knot nematode, *M. hapla*. This was expected, as oats and other grasses are not typically hosts to many *Meloidogyne* species (Belair 1992; Viaene & Abawi, 1998). Additionally, we found the oilseed radish (cv. Control) was a non-host to both lesion nematode, *P. penetrans*, and the northern root knot nematode, *M. hapla* (Table 4.4 and 4.5). This oilseed radish variety may be a suitable option for vegetables with co-infestations of these nematodes. Prior to our research, few studies evaluated oilseed radish varieties offered to Michigan growers for both *P. penetrans* and *M. hapla* management. A better understanding of oilseed radish variety-specific host/non-host relation with two economically significant nematodes of root vegetables is necessary to make informed decisions regarding cover crop incorporation for nematode management.

4.5 CONCLUSION

As nematicides become banned, many growers are left with limited choices for nematode control. Many field crops like soybean (*Glycine max* L.), cotton (*Gossypium*), corn (*Zea mays* L), and potato (*Solanum tuberosum* L.) may be (comparatively) better evaluated on relevant plant-parasitic nematodes for their effectiveness within the system. However, specialty crop growers that plant root vegetables, like carrots, parsnips and many other high value, lower acreage crops,

are also subject to these product limitations without current recommendations for replacement strategies. Problems can also be exasperated by a lack of published field studies testing effectiveness within the crop systems. Carrots and parsnips are higher value crops planted on fewer acres, so even small yield losses can often be economically devastating for the farmer.

In our carrot trials, we did not find that any treatment both significantly reduced *Pratylenchus* spp. at harvest and increased marketable yield. However, one application of Vydate in-furrow at plant resulted in a marketable yield increase of 8.8%, despite not reducing *Pratylenchus* spp. densities. In contrast, Vydate and Nimitz applications at-plant can reduce *M. hapla* under field conditions with one in-furrow application at seeding, with corresponding substantial marketable yield increases of 60.53% and 43.68%. These nematode reductions also resulted in a corresponding marketable yield bump of plots treated with these nematicides, and Nimitz resulted in an additional reduction of unmarketable characteristics (hairiness).

We also found that the host status of oilseed radishes tested were poor hosts (cvs. Defender, Eco-Till, Enricher, Image, NitroRadish, Respect, Select, and Tillage) or non-hosts (cvs. Control and Concorde) for the root lesion nematode, *P. penetrans*. For this reason, these oilseed radishes would be a suitable cover crop to plant if the goal is to limit *P. penetrans* reproduction. Similarly, we found that several of the oilseed radishes (cvs. Eco-Till and NitroRadish) and the oil rapeseed (cv. Dwarf Essex) were poor hosts; while oat (cv. Pratex) and two oilseed radishes (cv. Control and Select) were non-hosts to *M. hapla*. We also determined that the oilseed radish (cv. Concorde) was a moderate host for *M. hapla* and thus would not be a suitable choice for a cover crop establishment aiming to prevent reproduction. Greenhouse Planting these crops in a multi-season field trial to determine impact to soil health, free-living nematode communities, and crop yield would be an important next step for gauging impact.

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CHAPTER 5: CONCLUSION

Pratylenchus spp. are damaging to a wide variety of over 350 crops, and lack of non-host commodities make the pests particularly difficult to manage (Castillo and Volvas, 2007). Plant-parasitic nematode infestations commonly result in “silent” damage because symptoms are nonspecific, resulting in stunting, lower weight, and wilting. However, other significant plant-parasitic nematodes like *Meloidogyne* and *Heterodera*, usually result in the presence of galled or severely misshapen roots, whereas infestations by *Pratylenchus* spp., below-ground symptoms are nonspecific as well. Often in heavily infested winter wheat fields necrotic root sections are present, but this could be due to co-infestation with other pathogens as well. Relationships with secondary soil pathogens can form devastating disease complexes, like *Verticillium dahliae* in potatoes, resulting in further damage (Riedel et al., 1985).

Despite modern molecular tools, only a handful of statewide soil surveys on plant-parasitic nematode communities have included species-level identification of *Pratylenchus* nematodes. One example of the importance of species identity includes *P. thornei* and *P. neglectus* in the Pacific Northwest. For example, *Pratylenchus thornei* is generally considered to be more aggressive and can reduce yields by 85%, while *P. neglectus* is associated with 8-35% of yield losses (Taylor et al., 1999; Thompson et al., 2008; Smiley et al., 2013). Additionally, for diseases like Potato Early Die, some species like *P. penetrans* have the capability to facilitate disease complexes between other pathogens. These two examples in *Pratylenchus* emphasize why proper species identification is important and can be crop specific.

The main goal of this dissertation was to aid in overcoming significant knowledge gaps or inconsistencies about *Pratylenchus* spp. in Michigan carrot and wheat production. To tackle this goal, we used a combination approach which included evaluating distribution and identity of

the top three *Pratylenchus* spp. in fields (*P. penetrans*, *P. crenatus*, and *P. neglectus*), how they reproduce and elicit damage to each cropping system under greenhouse conditions, and how/if current management strategies are effective and can consistently control *Pratylenchus* spp.

In Chapter 1, I overviewed the current available literature and major knowledge gaps about *Pratylenchus* spp., and the challenges associated with maintaining adequate inoculum, inconsistent tolerance ratings, and proper identification. Some of these can be explained by environmental factors like regional differences, soil type, pH, or water availability. However, it is important to note that these differences usually do not inhibit the success of trials for other significant species, like *Meloidogyne hapla* and *Heterodera carotae*, which are well documented on carrot. We also expand on how future strategies should incorporate molecular tools to both enhance our current understanding of nematode pathogenicity and increase our toolkit of management strategies.

In our first research chapter (Chapter 2), I determined the distribution and abundance of relevant plant-parasitic nematodes in Michigan carrot and wheat fields. Like other northern, temperate regions worldwide (Townshend et al., 1987), we uncovered that *P. penetrans*, *P. crenatus*, and *P. neglectus* are the top three *Pratylenchus* spp. occupying Michigan carrot and wheat fields. With this survey, we also document the first report of four species in carrot fields in the United States: *Pratylenchus neglectus*, *Xiphinema rivesi*, *Tylenchorhynchus maximus*, and *Paratylenchus* sp. D. Additionally, *P. thornei* was not recovered in any of our samples, indicating that either it is not abundant in Michigan carrot and/or wheat fields, or that its distribution does not extend to counties we sampled. This is a particularly important finding due to the severity of damage associated with winter wheat and *P. thornei*, and if detected in Michigan, it would be an important finding for Michigan wheat producers.

During the wheat survey, I observed *Heterodera glycines* (soybean cyst nematode) juveniles (J2 life stage) inside of winter wheat roots and confirmed this with molecular identification of five individual samples from two fields. Recent literature suggests that winter wheat before soybeans increases yield up to 10% and also decreases *H. glycines* abundance (Janovicek et al. 2021; Rocha et al., 2022). Studies also found that nominal factors of suppressive soil like enriched microbial communities and corresponding antagonistic microorganisms and compounds were highest in wheat-soybean cropping rotations (Sun et al., 2022; Rocha et al., 2023). My observation paired with this recent literature suggests that winter wheat could function as a dead-end trap crop for *H. glycines*. I hypothesize that winter wheat recruits these antagonistic microbes to kill cyst juveniles once infected, lowering subsequent populations. If repeated studies can support this finding, it would be monumental to winter wheat acreage and corresponding soybean yield increases. These findings and observations also emphasize that surveys play an important role in nematology research, deserve attention, and can even extend beyond the crop of interest.

In Chapter 3, I conducted a series of greenhouse trials to determine the extent of reproduction and damage if the top three *Pratylenchus* species were inoculated to young carrot and wheat seedlings under uniform conditions. Strikingly, *P. penetrans* and *P. crenatus* damaged carrots severely, eliciting root weight reduction by 36.94% and 21.76% compared to non-inoculated control plants. *P. neglectus* did not reduce root weight or taproot length, suggesting it may not be a significant pest to carrots. Contrastingly, *P. neglectus* was the most damaging nematode in wheat and reduced root weight by 24.07% compared to control plants, while *P. crenatus* did not reduce winter wheat plant height or root weights.

Lastly, in a 4-year field trial in carrots, we determined that none of the alternative

management strategies were effective for *Pratylenchus* spp. management in carrots. Vydate did increase marketable yield by 8.8%, but it did not impact *Pratylenchus* spp. densities at midseason or harvest. We also found that both Nimitz and Vydate applications decreased *M. hapla* populations and resulted in increased marketable yield, adding to evidence that these products are effective for root-knot nematode infestations in root vegetables. Finally, we determined that the oilseed radish (cv. Control) could be an effective dead-end host crop for *P. penetrans* and *M. hapla*, as it was a nonhost for both of these organisms. Even though we did not find an effective management strategy for *P. penetrans* in carrots, I strongly support the publication of this data, as negative results help researchers build a better toolkit for farmers.

The results of each chapter within this dissertation weave together to support our initial hypothesis: *Pratylenchus* species identification, with a combination of morphological and molecular methods, is critical in further understanding the extent of damage produced by *Pratylenchus* spp. Future work should be conducted on the behavioral and pathogenic differences between species. Specifically, *P. penetrans* had a lower reproductive factor than *P. crenatus* and *P. neglectus* in carrot greenhouse trials yet resulted in a higher production of taproot weight loss compared to other species. I hypothesize this could be the result of several factors 1) secretory enzymes of *P. penetrans* could cause greater necrosis in cell tissue, 2) *P. penetrans* could overcome plant defenses to a greater extent, 3) the existence of males could result in greater damage via clustering for mating, or 4) behavioral differences in probing, penetration, tunneling, feeding, or secondary pathogens by species could further elicit damage. It is also interesting to note that while *P. penetrans* was damaging to winter wheat, *P. neglectus* elicited the most damage. This indicates that each species may have a unique relationship with its host, which may differ by crop type.

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