EXPLORING COVER CROPS FOR MANAGING PLANT-PARASITIC NEMATODES

HETERODERA GLYCINES AND *PRATYLENCHUS PENETRANS*

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Three studies were conducted to assess the effects of cover crops on plant-parasitic nematodes (PPNs) of potato and soybean. The first study investigated the hosting and population reduction abilities of 25 cover crops to *Pratylenchus penetrans* and found alfalfa (Bullseye) to reduce the initial population densities consistently. The second study tested the population reduction abilities of ten cover crops to two soybean cyst nematode (SCN; *Heterodera glycines*) populations in the microplot. Sunnhemp (cultivar not specified) was the most effective to reduce both SCN populations. The third study evaluated ten cover crops in a growth chamber for their impacts on hatching and root penetration of SCN and their potential as trap crops. Faba bean (Petite) showed the greatest potential to act as a trap crop for SCN, based on its effect on hatching and root penetration by SCN. These results help select suitable cover crops to manage PPNs in infested fields.

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DEDICATION

This work is dedicated to my parents Kul Prasad Neupane and Ganga Neupane. I also like to dedicate this work to my brothers, Krishna and Narayan, and sisters, Kamala and Anita.

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LIST OF ABBREVIATIONS

ASA.....American Soybean Association.

CRD .....Completely Randomized Design.

DNA.....Deoxyribonucleic Acid.

FAO.....Food and Agriculture Organization.

HSD.....Honestly Significant Difference.

MT.....Metric Ton.

ND.....North Dakota.

NDSU.....North Dakota State University.

PCR .....Polymerase Chain Reaction.

PPN .....Plant-parasitic Nematode.

RCBD.....Randomized Complete Block Design.

RF.....Reproductive Factor.

RLN.....Root-lesion Nematode.

RPM .....Rotation Per Minute.

SBCN .....Sugarbeet Cyst Nematode.

SCN.....Soybean Cyst Nematode.

US .....United States.

USA.....United States of America.

USDA-NASS .....United States Department of Agriculture- National Agricultural Statistics service.

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CHAPTER 1: INTRODUCTION

The economically important plant-parasitic nematodes can cause significant yield losses of major crops. The annual crop yield losses caused by plant-parasitic nematodes have been estimated to be USD 13 billion in the United States and USD 173 billion globally (Elling 2013). Plant-parasitic nematodes generally affect the roots of major crops and disrupt the nutrient and water uptake, which ultimately impact the overall performance reducing quality and yields. They produce symptoms like yellowing, stunting, and patchy growth of plants, which could be confused with symptoms produced by other soil-borne pathogens and abiotic factors.

Soybean and potato are among the important crops in our region. North Dakota is a major soybean producer with a production of 4.75 million metric tons in 2019 (USDA-NASS 2020a). Similarly, North Dakota and Minnesota together produced 2.1 million metric tons of potatoes (USDA-NASS 2020b). There are several biotic and abiotic constraints in soybean and potato production. Among biotic factors, plant-parasitic nematodes are the major yield reducing factors in both crops. Soybean cyst nematode (SCN; *Heterodera glycines*) is the most important pathogen of soybean that causes yield loss of more than USD 1.5 billion annually in the U.S (Bandara et al. 2020). Similarly, root-lesion nematodes (*Pratylenchus* spp.) are economically important pathogens of potatoes. Among them, *P. penetrans* is the most destructive root-lesion nematode, and its damage ranges from 30% to 70% in the form of yield losses (Holgado et al. 2009; Philis 1996).

Several different strategies are available for managing plant-parasitic nematodes from infested fields. Host resistance and crop rotation are considered the most effective strategies to manage SCN (Niblack 2005). However, the diversification of the SCN virulent population due to continuous use of the single source of resistance (PI 88788) is causing reduced resistance levels

in the currently available resistant cultivars (Chowdhury et al. 2021; Colgrove and Niblack 2008). Similarly, there is a lack of recent research on the host resistance of potatoes against *P. penetrans* (Orlando et al. 2020). Crop rotation is very challenging to use against *P. penetrans* due to its broad host range (Davis and MacGuidwin 2005). The use of chemical nematicide is restricted due to its potentially detrimental effect on the environment and non-target organisms (Haydock et al. 2006).

Cover crops belonging to several plant families have been evaluated for managing plant-parasitic nematodes. They have shown potential suppression and control of nematodes, including SCN (Acharya et al. 2021; Chen et al. 2008; de Nascimento et al. 2016; Harbach et al. 2021; Riga et al. 2001) and *P. penetrans* (Belair et al. 2005; Everts et al. 2006; Hooks et al. 2010; Kimpinski et al. 2000). However, there is limited information on the hosting and population reduction abilities of cover crops on these nematodes from our region. Additionally, there is no information on the mechanism involved in suppressing nematodes by effective cover crops. Hence, this study was conducted with the following objectives.

- To determine the hosting and population reduction abilities to *Pratylenchus penetrans* under greenhouse conditions.
- To study the distribution of *P. penetrans* in roots and soil habitats after three months of growth in the greenhouse.
- To evaluate cover crops for their impacts on the reduction and suppression of two SCN populations from North Dakota fields under outdoor microplot conditions.
- To study cover crops for their effects on the hatching of SCN eggs and to compare the penetration of roots by second-stage juveniles (J2s) among tested cover crops.

CHAPTER 2: LITERATURE REVIEW

Potato and its Production in the U.S.

Potato (*Solanum tuberosum* L.) is an herbaceous crop that belongs to the Solanaceae family. Potatoes were domesticated around 8,000 years ago in the region of modern-day Peru and Bolivia (Spooner et al. 2005). Around the sixteenth century, it was introduced to Europe and spread to the rest of the world from Europe (Hawkes 1992). In the U.S., the potato was introduced from England through Bermuda in 1621. However, it became a field crop after 1750 (Sauer 2017). The potato was responsible for the population growth and urbanization of the Old World between 1700 and 1900 (Nunn and Qian 2011). Potatoes are considered cool-season crops but are grown under temperate, subtropical, and tropical conditions in over 130 countries. Carbohydrates, vitamin C, potassium, phosphorus, and magnesium are some of the key nutrients available in potatoes (Beals 2019).

Potato is the fourth most important crop after corn, wheat, and rice worldwide. The U.S. ranks fifth in production worldwide with an annual production of 23 million metric tons after China, India, Ukraine, and Russia (FAO 2020). In the U.S., potato is grown in around one million acres of land in 30 states. Idaho is the biggest producer of potatoes, followed by Washington, Wisconsin, Oregon, North Dakota, Michigan, Colorado, California, Minnesota, and Maine. North Dakota and Minnesota produced 2.1 million metric tons in 2019 (USDA-NASS 2020b).

Constraints in Potato Production in the U.S.

Potato crops can be adversely affected by different biotic and abiotic stresses. Abiotic stresses include salinity, water (drought and flooding), and heat that directly affect the potato plant growth and yield (Kumar et al. 2021, Chourasia et al. 2021). Similarly, soil abiotic factors

affect the incidence and severity of several soil-borne diseases of potatoes. Worldwide, there are about 40 soil-borne diseases of potatoes caused by soil inhibiting fungi, bacteria, and nematodes (Fiers et al. 2011). These soil-borne diseases may affect crop development, tuber qualities, or both. Some of the major diseases of potatoes for North Dakota and Minnesota are black dot (*Colletotrichum coccodes*), verticillium wilt (*Verticillium dahliae*), pink rot (*Phytophthora erythroseptica*), and leak tuber rot (*Pythium ultimum*) (Gudmestad et al. 2007). Plant-parasitic nematodes are one of the most detrimental yield-reducing biotic factors of potatoes. They also affect the physical and chemical properties of tubers and their qualities (Lima et al. 2018). There are many plant-parasitic nematodes associated with potatoes, some of them cause significant damage to potatoes, and some of them are of minor or local importance. Plant-parasitic nematodes of high importance are potato cyst nematodes (*Globodera* spp.), root-knot nematodes (*Meloidogyne* spp.), false root-knot nematode (*Nacobbus aberrans*), root-lesion nematodes (*Pratylenchus* spp.), root rot nematode (*Ditylenchus destructor*), stem and bulb nematode (*D. dipsaci*), and stubby root nematodes (*Trichodorus* spp. and *Paratrichodorus* spp.) (Lima et al. 2018).

Root-lesion Nematode (*Pratylenchus* spp.)

Root-lesion Nematode and its Importance

Root-lesion nematodes (*Pratylenchus* spp.) are major plant parasites of potato in temperate, subtropical, and tropical regions (Castillo and Vovlas 2007). There are at least 15 species of *Pratylenchus,* including *P. andinus, P. brachyurus, P. crenatus, P. penetrans, P. scribneri, P. thornei, P. vulnus, P. neglectus, and P. zeae*, that infect the potato (Lima et al. 2018). They are migratory endo-parasitic nematodes with a broad host range (more than 400 species of crop plant) (Davis and MacGuidwin 2005). *Pratylenchus penetrans* is the most

important root-lesion nematode for potatoes (Lima et al. 2018; Waeyenberge et al. 2009). *Pratylenchus penetrans* is associated with more than 350 plant hosts (Belair et al. 2007) and is considered the most damaging to potatoes in Europe, North America, and Australia (Orlando et al. 2020). They can parasitize several plants, including vegetables, cereals, fruits, grasses, and ornamental crops (Castillo and Vovlas 2007).

Life Cycle of Root-lesion Nematode

The life stages of root-lesion nematodes comprise eggs, juveniles, and adults. Eggs are laid in the host roots or the soil, singly or in groups, and the first molt occurs within the egg to change first-stage juvenile to second-stage juvenile (Pudasaini et al. 2008). The eggs are deposited in the soil upon degradation of roots. The second-stage juvenile (J2) remains inside the eggs until hatching. Subsequent molting occurs to change J2 into J3, J3 into J4, and then to adults, becoming either male or female (Castilo and Vovlas 2007). Males are generally absent or rare in some species (*P. crenatus, P. neglectus, and P. thornei*) but are common in other species, including *P. penetrans* and *P. coffeae*. Species without males reproduce by parthenogenesis, and reproduction is amphimictic in species with males (Duncan and Moens 2013). The length of the life cycle of root-lesion nematodes is greatly affected by different factors, including temperature. *Pratylenchus penetrans* completed its life cycle in 54-65 days in red clover (Turner and Chapman 1972), while it took 22-46 days in Ladino white clover (*Trifolium repens* L.) under the temperature range of 17 to 30^{o}C (Mizukubo and Adachi 1997). In carrot (*Daucus carota* L.) callus at 24^{0}C, it completed a life cycle in 34-35 days (Wu et al. 2002).

Symptoms and Economic Importance of Root-lesion Nematode

Root-lesion nematodes are migratory endoparasites, but they can also feed on the root surface as ectoparasites (Duncan and Moens 2013). *Pratylenchus* spp. can feed on roots, stolons,

underground stems, and tubers. All stages of the nematode can infect the crops by penetrating the zone of elongation and feeding on parenchyma cells of roots. Feeding of nematodes results in brown lesions at the points of penetration and leads to necrosis. These lesions affect the root growth resulting in stunting and yellowing of crops (Castillo and Vovlas 2007). *Pratylenchus penetrans* can infect the tubers and produces scab or wart-like appearances (Holgado et al. 2009). The yield losses from *P. penetrans* in potatoes range from 30% to 70% (Holgado et al. 2009; Lazarovits et a. 1992; Philis 1996). Furthermore, associations of *P. penetrans* with other pathogens increase the severity of damage to potato tubers and the overall growth of the plant (Rotenberg et al. 2004; Holgado et al. 2009; Upadhaya et al. 2020). Different potato cultivars and environmental factors, such as temperature, moisture, and soil texture, determine the economic damage thresholds of *P. penetrans* (Castillo and Vovlas 2007). An economic threshold of 100 nematodes per 250 g of soil was reported to cause 50% yield loss in cultivar Saturna from Norway (Holgado et al. 2009). Only 1-2 *P. penetrans*/cm^3 of soil have been shown to cause damage to potato (Castillo and Vovlas 2007), whereas Martin et al. (1982) reported 0.76 nematode/cm^3 would be enough to cause damage on potato growth in dry years. Furthermore, Bernard and Laughlin (1976) showed the yield loss of 23% to 30% on potato cultivar Superior by 0.38 *P. penetrans*/cm^3, but cultivar Russet Burbank was unaffected by nematode density of 0.38-2 per g of soil.

Management of Root-lesion Nematode (*Pratylenchus penetrans*)

The effective management methods generally help reduce the initial nematode population density and restrict further reproduction during the growing season (Perry and Moens 2013). Host plant resistance is the best approach for disease control, as it is an economically viable and environmentally sound approach. Many studies had identified several *P. penetrans* resistant

potato cultivars in the past (Brodie and Plaisted 1993; Davis et al. 1992; Kimpinski and McRae 1988). However, many of those resistant cultivars have been discontinued and research for finding resistance in current potato cultivars is very few (Orlando et al. 2020).

The use of chemical compounds such as soil fumigants and non-fumigants is still the best approach to manage *P. penetrans* from soil (Davis and MacGuidwin 2005). But global restrictions have been put on the use of those compounds due to their potentially detrimental impact on the environment, human health, and other non-target organisms (Haydock et al. 2006). Some nematicides have shown promising results in reducing the nematode population from soil without phytotoxicity to the main crop (Zasada et al. 2010). Similarly, some biological control agents have been tested against root-lesion nematodes and some studies have shown their effectiveness. Certain trapping fungi, such as *Hirsutella rhossiliensis*, have shown the potential to control *P. penetrans* in potatoes (Timper and Brodie 1993, 1994). Bacteria species, such as *Microbacterium esteraromaticum* and *Kocuria varians*, isolated from two marigold species (*Tagetes* spp.), were able to cause highest mortality in a study conducted by Sturz and Kimpinski (2004). However, their commercialization is limited for nematode management (Palomares-Rius et al. 2014; Stirling 2014). Cultural strategies, such as organic amendments by poultry manure (Conn and Larovitis 1999) and soil solarization (Lazarovits et al. 1991; Pinkerton et al. 2000), can be utilized to manage *P. penetrans*. Crop rotation could effectively manage plant-parasitic nematodes, but it is challenging to use for managing root-lesion nematodes due to their wide host range (Orlando et al. 2020).

Several studies have shown the efficient management of *P. penetrans* population density from the infested field by integrating cover crops in the cropping system. Belair et al. (2005) reported a significant reduction of *P. penetrans* in a potato field when it was rotated with pearl

millet (*Pennisetum glaucum* L.) and found an increase in potato yield compared with the control crop after one-year rotation. Sorghum × sudangrass (*Sorghum bicolor* (L.) × *S. arundinaceum* (Desv.) Stapf var. *sudanense* (Stapf) Hitchc.) was effective in suppressing *P. penetrans* in a three-year rotational sequence (Everts et al. 2006). Similarly, different species of marigold (*Tagetes* spp.) have been established as effective cover crops for managing *P. penetrans* (Reynolds et al. 2000; Alexander and Waldenmaier 2002; Evenhuis et al. 2004; Hooks et al. 2010). Tobacco (*Nicotiana tabacum* L.) yield was increased by an average of 197 kg/ha after rotation with two species of marigold, in addition to a reduction of *P. penetrans* below the economic threshold level (Reynolds et al. 2000). A significant increase in potato tuber yield was observed in a field trial in Canada due to crop rotation with marigold cultivars (Kimpinski et al. 2000). Moreover, rotation of potato with marigold (*T. erecta*) resulted in an average 93% reduction in *P. penetrans* compared with the initial nematode population during a three-year study (Alexander and Waldenmaier 2002).

In addition to previous approaches, the exclusion is critical approach for nematode management. Sanitation of farm equipment and use of planting materials from nematode-free fields is significant to prevent the infestation of healthy fields. As *P. penetrans* can reside under the potato skin, tuber should be checked for the presence of nematodes before using them as planting materials (Figueiredo et al. 2021). Also, accurate identification of nematode species and densities are vital for selecting the best approach or combination for their management (Castillo and Vovlas 2007).

Soybean and its Production in the U.S.

Soybean [*Glycine max* L. (Merr.)] is an important leguminous crop worldwide that is believed to be originated from northeastern and central China. Domestication of soybean was

started in China around 1100 BC (Carter et al. 2004; Hymowitz 1970; Hymowitz and Newell 1981; Wilson 2008). However, it was first introduced to the United States in 1765 to the British colony of Georgia by a seaman, Samuel Bowen (Hymowitz and Harlan 1983; NCSOY 2019). An American chemist, George Washington Carver, identified soybean to have high protein and oil content in 1904 and realized the beneficial effects of soybean for good soil health (Hyomowitz and Shurtleff 2005; NCSOY 2019). Soybean has been grown for several purposes, including oil, meal, animal feed, and biodiesel production. It is a commercial crop with high protein and seed soil content (Singh and Shivakumar 2010).

The world's largest source of animal feed protein is processed soybean, and it is also the second largest source of vegetable oil (USDA-NASS 2020a). North and South America produce more than 86% of the world's soybean (ASA 2020). In 2019, Brazil was the largest producer with a 37% contribution to the world's production. Brazil was followed by the U.S. with 28%, Argentina with 16%, China with 5%, Paraguay and India each with 3%, Canada with 2%, and the rest of the world with 6% world's soybean production (ASA 2020). North Dakota is the ninth largest producer of soybean in the U.S. with 4.75 million metric tons in 2019 (USDA-NASS 2020a).

Constraints in Soybean Production in the U.S.

Several biotic and abiotic factors can adversely affect soybean growth affecting the yield and seed quality. Water stress, nutrient availability, salt toxicity, and low temperature are included under abiotic factors, whereas weeds, soil-borne and foliar pathogens, and insects are the biotic factors (Hartman et al. 2011). Among the biotic factors limiting soybean production, soybean cyst nematodes (SCN), sudden death syndrome, seedling diseases, charcoal rot, *Septoria* brown spot, *Phytophthora* root and stem rot, *Sclerotinia* stem rot, brown stem rot, *Fusarium* wilt,

root rot, and pod and stem blight are the most destructive diseases in the northern United States and Ontario, Canada from 2010 to 2014 (Allen et al. 2017). Among these diseases, SCN continues to remain the most destructive disease of soybean in the U.S. over the last two decades (Allen et al. 2017; Koenning and Wrather 2010).

Soybean Cyst Nematodes (SCN; *Heterodera glycines*)

History and Distribution

Soybean cyst nematode, *Heterodera glycines* Ichinohe, is an obligatory endo-parasitic nematode that forms a permanent feeding site inside the roots of its host (Niblack et al. 2006). SCN was first discovered in northeast China in 1899 (Li et al. 2011) and after that, its discovery continued throughout the world. In the U.S., it was first discovered in North Carolina in 1954 (Winstead et al. 1955), and it continues to spread in almost all states where soybean is grown (Tylka and Marett 2021). Imported soil from China and Japan during the 20th century for *Rhizobium* studies was the source of SCN in the U.S. (Noel 1986). Soybean cyst nematode was first detected in North Dakota in 2003 in Richland County (Bradley et al. 2004), and by 2015, at least 19 counties had been positive for SCN infestation (Yan et al. 2015). The spread of SCN to other counties of North Dakota is following the trend of increase in an acreage of soybean production (Tylka and Marret 2017, 2021).

Life Cycle of SCN

Eggs, juveniles, and adult males or females (cysts) are the three major life stages of SCN. A cyst contains several eggs that are survival units of SCN. The multiplication of a single cell embryo occurs inside an egg to differentiate into a first stage juvenile (J1). The second stage juvenile (J2) emerges from the egg after the molting of J1 inside the egg (Niblack et al. 2005). The J2 is the infective stage of this nematode, and the hatching of J2 is a complex process

affected by multiple biotic and abiotic factors (Niblack et al. 2005; Tefft et al. 1982; Turner and Subbotin 2013). The chemical gradient created by root exudates of host plants guides the movement of J2 towards the roots, and J2 penetrates the root tips with the stylet. J2 releases different enzymes, including cellulases, that degrade the cell wall and dissolve the boundaries between the cells (Papademetriou and Bone 1983; Smant et al. 1998). Then the J2 moves intracellularly to find a permanent feeding site, called syncytium, near the vascular tissues (Johnson et al. 1993). Chemical secretion by J2 allows the incorporation of adjacent cells to the feeding cell through cell wall dissolution, ultimately forming a nutrient sink. The nutrient sink continuously feeds the developing nematodes for their growth and reproduction (Kandoth et al. 2011; Levin et al. 2020).

Once the juvenile starts feeding, it becomes sedentary and starts swelling up. Then it molts three times to become an adult female or male. The third stage juvenile male (J3) molts into an adult male 10 to 15 days after infection and regains mobility before leaving the host root. However, the female continues to feed and swell up (Lauritis et al. 1983). The pressure exerted by the growing female body ruptures the epidermis, and the posterior end of the nematode emerges out of the root (Raski 1950). The pheromones secreted by the female then attract the male for mating to fertilize the eggs. Hundreds of eggs are produced by a single SCN female. However, some are deposited outside of the body in a gelatinous matrix (Niblack et al. 2006; Sipes et al. 1992). The death of the SCN female occurs after the fertilization of eggs, and the female starts turning into a cyst. The cyst is a protective structure for eggs against desiccation and other microbial predators in the soil (Niblack et al. 2006). Soybean cyst nematode can remain viable in natural conditions for more than a decade based on their life stages (Davis and

Tylka 2021; Duan et al. 2009). Generally, SCN completes its life cycle within three to four weeks, regulated by temperature and moisture conditions in soil (Niblack et al. 2006).

Symptoms and Economic Importance of SCN

The above-ground symptoms produced on soybean by SCN are often confused with symptoms caused by bacterial and fungal diseases and abiotic stresses, such as drought, nutrient deficiency, and herbicide injuries (Mueller et al. 2016). Moreover, up to 30% yield loss in soybean can be caused by SCN, without producing any visible symptoms on plants in the field (Wang et al. 2003; Niblack 2005). Generally, stunting and yellowing of host plants are seen in the field with a high population density of SCN in the soil. Additionally, white lemon shaped SCN females can be found on the surface of roots when plants are uprooted and examined (Davis and Tylka 2021).

Soybean cyst nematode is the major pest of soybean worldwide and it causes soybean yield loss of more than 1.5 billion dollars annually in the U.S. (Allen et al. 2017; Bandara et el. 2020; Koenning and Wrather 2010). Additionally, since dry bean (*Phaseolus vulgaris* L.) is also a good host of SCN (Poromarto et al. 2011), the overall economic losses could be more due to infestation of SCN.

SCN Management

There are several strategies to manage SCN from infested fields, including host resistance, chemical control, crop rotation, biological control, and others. Host resistance and crop rotations are commonly used strategies because of their effectiveness and minimal detrimental effects to the environment and non-target organisms (Niblack 2005; Oyekanmi and Fawole 2010).

Several studies have revealed resistance sources against SCN in different soybean accessions. Three recessive genes *rhg1*, *rhg2*, and *rhg3* (Caldwell 1960) and two dominant genes *Rhg4* and *Rhg5* (Matson and William 1965; Rao-Arelli 1994), have been reported to confer resistance against SCN. Among them, *rhg1* and *Rhg4* have been found to provide most of the resistance in resistant-soybean cultivars (Concibido et al. 2004). Moreover, the *rhg1-b* allele of the *rhg1* gene, derived from the plant introduction line PI88788, provides resistance to about 90% of the resistant cultivars in the U.S. (Concibido et al. 2004; Tylka and Mullaney 2018). Additionally, the copy number variation of this *rhg1* gene significantly affects the resistance level in the plants (Cook et al. 2012). On the other hand, continuous use of this single resistance source has caused rapid diversifications of the SCN virulence population (Chowdhury et al. 2021; Yan and Baidoo 2018). The virulence of diversified SCN populations may result in reduced level of a resistance in the resistant cultivars creating a threat to current management strategies (Colgrove and Niblack 2008).

Another strategy to manage SCN is the use of chemical compounds. However, this strategy is not economical and eco-friendly, because of its high cost and negative impact on the environment and non-target organisms (Oka 2010). Seed treatments with nematicidal chemical or biologically active ingredients have also been tested against SCN. However, their effects and performance have not been consistent in the field conditions (Rossman et al. 2018).

Biological control is an economically rational approach for the management of SCN in the field. A fungal species *Hirsutella rhossiliensis* has been found to infect SCN juveniles and eggs, thus reducing infection to host roots (Chen 2007; Zhang et al. 2006). The soybean seedlings infected with a bacterium, *Sinorhizobium fredii* strain Sneb183, were able to show systemic resistance to SCN, and population reduction of the nematode was observed in the field

(Tian et al. 2014). Other bacteria species such as *Pasteuria nishizawae*, *Bacillus amyloliquefaciens,* and *Bacillus firmus* have shown a suppressive effect on the SCN population in fields (PMRA 2011, PMRA 2015, PMRA 2016). However, their commercial utilization for nematode management is very limited.

The host range of SCN is narrow compared with other plant-parasitic nematodes such as root-lesion nematodes. Apart from soybean, SCN infects a few other leguminous crops and some weeds species (Creech et al. 2007; Poromarto et al. 2011; Poromarto et al. 2015). Because of this narrow host range, crop rotation with non-host crops, such as corn (*Zea mays* L.) and wheat (*Triticum aestivum* L.), can be effective for the SCN management from infested fields (Chen et al. 2001; Warnke et al. 2008). Similarly, crop rotation between resistant soybean cultivars with different resistant sources is helpful to avoid building of SCN in the field.

The utilization of cover crops is another approach for managing SCN, as many studies have pointed out its potential use. Cover crops reduce the leaching of the nutrients, soil erosion and increase soil organic matter (Clark 2007). Furthermore, they can suppress weeds, pests, and pathogens, including plant-parasitic nematodes (Snapp et al. 2005; Abawi and Widmer 2000). Non-host or poor host cover crops may reduce the nematode population from infested soil by producing chemical compounds from their roots and residues. The chemical compound may directly be detrimental to the nematodes, or they may affect the nematode biology by enhancing the egg hatching and allowing juveniles to penetrate the root systems. Hatched juveniles would die in the absence of suitable hosts, and the penetrated juveniles would not be able to complete their life cycle inside the roots of non-host cover crops (Hooks et al. 2010; Kushida et al. 2003; Riga et al. 2001; Tylka 2014). In some cases, cover crops may provide a suitable niche for

organisms antagonistic to the plant-parasitic nematodes to suppress the population density in the soil (Wang et al. 2004).

Many published studies have reported effective reductions of SCN population from the infested soil using different cover crops. *Crambe abyssinica* Hochst cv FMS Brilhante significantly reduced the SCN from infested field during its growing season and caused a reduction in SCN reproduction when it was incorporated into the soil during the soybean growing season (de Nascimento et al. 2016). Annual ryegrass (*Lolium multiflorum* L.) was the most effective to reduce the SCN population by enhancing the hatching of eggs in the absence of a host and depleting the lipid reserves of juveniles (Riga et al. 2001). Harbach et al. (2021) reported crimson clover (*Trifolium incarnatum* L.) induced the greatest hatching of SCN eggs and penetration of its roots by the hatched juveniles among tested cover crops in controlled conditions. The SCN juveniles could not complete the life cycle inside crimson clover, which shows its potential as a trap crop for managing SCN. Sunnhemp (*Crotalaria juncea* L.) and red clover (*Trifolium pratense* L.) were the most effective to stimulate hatching of SCN, thereby reducing the nematode population from infested soil (Chen et al. 2008). They also found a reduction in egg density and juveniles' infectivity after incorporating plant residues into the soil. Acharya et al. (2021) evaluated diverse cover crops for their effect on two SCN populations in a microplot experiment. They found daikon radish (*Raphanus sativus* L.), annual ryegrass, winter rye (*Secale cereale* L.), red clover, and sweetclover (*Melilotus officinalis* L.) to be consistently effective in reducing the significant nematode populations than fallow treatment.

In addition to the earlier strategies, sanitation is significant to prevent the spread of SCN to healthy fields. Sanitation of farm machinery, harvesting crops without contamination, and cleaning root crops grown in the infested soil are important activities to prevent the spread of

SCN (Giesler and Wilson 2011). Moreover, management of soil water content, weeds, pest, and overall proper agronomic practices are vital for enhancing plant growth and minimizing the yield losses by SCN (Chen et al. 2021).

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CHAPTER 3: HOST STATUS AND POPULATION REDUCTION ABILITY OF COVER CROPS TO *PRATYLENCHUS PENETRANS* ASSOCIATED WITH POTATO[1]

Abstract

Non-host or poor host cover crops can provide an alternative method of nematode management. A total of 25 cover crop species/cultivars, along with susceptible potato (*Solanum tuberosum* L.) (Red Norland), a rotational crop wheat (*Triticum aestivum* L.) (Glenn), and an unplanted control, were evaluated in a greenhouse experiment for their hosting and population reduction abilities to *Pratylenchus penetrans*. Three trials were conducted in a completely randomized design using nematode-infested soil and terminated three months after planting. Nematodes were extracted from the roots and soil of each crop to determine their final population densities, reductions, and distributions in the soil and root habitats. Reproductive factor (Rf, ratio of final nematode population density to initial nematode population density) was used to determine the host status of crops. Faba bean (*Vicia faba* Roth) (Petite) produced the greatest nematode population density in all trials, whereas only alfalfa (*Medicago sativa* L.) (Bullseye) constantly reduced the initial nematode population, indicating a poor host range. Annual ryegrass (*Lolium multiflorum* L.), winter rye (*Secale cereale* L.) (ND Dylan), and white proso millet (*Panicum miliaceum* L.) also demonstrated poor hosting abilities in most trials. Five cover crops maintained the initial population throughout the experiment with Rfs less than 2, and the remaining crops were suitable hosts for *P. penetrans*. Analysis of nematode distribution

[1] The material in this chapter was co-authored by Kamal Neupane (Department of Plant Pathology, North Dakota State University) and Guiping Yan (Department of Plant Pathology, North Dakota State University). Kamal Neupane had primary responsibility for increasing nematode population and performing the experiment in the greenhouse. Kamal Neupane was the primary developer of the conclusions presented here. Guiping Yan served as the principal investigator and corresponding author and checked the statistical analysis conducted by Kamal Neupane. This paper was submitted to journal, Plant Disease.

indicated that the majority of the crops had less than or equal to 30% of the final population residing in the roots after three months of growth. This research will help potato growers select effective cover crops and avoid susceptible hosts to manage *P. penetrans* in the field to minimize potato yield losses caused by nematode infestation.

Keywords: Potato, cover crops, root-lesion nematode. *Pratylenchus penetrans*, reproductive factor, host range

Introduction

The United States is ranked fifth globally in production of potato (*Solanum tuberosum* L.), the most important nongrain food worldwide (FAO 2020). About 40 soil-borne diseases, including fungi, bacteria, viruses, and nematodes, negatively impact the production of potatoes (Fiers et al. 2012). *Globodera* spp., *Pratylenchus* spp., *Ditylenchus* spp., *Meloidogyne* spp., *Trichodorus*, and *Paratrichodorus* spp. are some of the economically important nematode species affecting potato production (Lima et al. 2018). In temperate regions, root-lesion nematodes (RLN, *Pratylenchus* spp.) are the most common nematode pests of potato (Florini and Loria 1990; Yan et al. 2016).

Root-lesion nematodes are migratory endo-parasitic nematode pests of diverse crop species and their economic damage on crops ranks third behind sedentary endo-parasitic root-knot and cyst nematodes, respectively (Troccoli et al. 2021). The worldwide economic impact on several crops is mainly due to the broad host range (more than 400 species of crop plants) and their distribution in almost every temperate and tropical environment (Castillo and Vovlas 2007). Among many species of RLN, *P. penetrans* (Cobb) is the most damaging species in potato (Lima et al. 2018), and the economic losses from *P. penetrans* range from approximately 30% to 70% (Holgado et al. 2009; Lazarovits et al. 1991). Additionally, the synergetic interaction of this

nematode species with the fungus *Verticillium dahliae* exacerbates the damage from potato early dying (PED) disease (Rotenberg et al. 2004). Also, the coexistence of *P. penetrans* with other secondary pathogens suggests possible associations to form different disease complexes in potato fields (Holgado et al. 2009; Upadhaya et al. 2020). The economic damage threshold of *P. penetrans* on potatoes varies depending upon the cultivars and environmental factors like soil texture, moisture, and temperature (Castillo and Vovlas 2007; Orlando et al. 2020). Holgado et al. (2009) reported an economic threshold of 100 nematodes/250 g of soil caused 50% of damage in cultivar Saturna in Norway.

Several strategies for managing *P. penetrans* may facilitate the reduction of the initial nematode population and diminish the nematode reproduction during the growing season. The use of soil fumigants and non-fumigants still is the main approach for *P. penetrans* management from the infested field (Orlando et al. 2020). However, fumigant's potential impacts on human health, the environment, and other non-target organisms cause global restrictions on their use (Haydock et al. 2006). Several studies were conducted in the past to assess different potato cultivars for resistance to *P. penetrans* (Brodie and Plaisted 1993; Davis et al. 1992; Kimpinski and McRae 1988) but many resistant cultivars identified from those studies have been discontinued and recent research is lacking (Orlando et al. 2020). Similarly, many potentially effective biological control agents like 'trapping fungi' (Timper and Brodie 1993) and bacteria (Sturz and Kimpinski 2004) have been studied against *P. penetrans*. However, their commercial utilization is limited in agriculture for nematode management (Poveda et al. 2020; Stirling 2014). Managing RLN by biological control agents is difficult as nematodes mainly live inside the roots (Stirling 2014). Crop rotation also is an environment-friendly strategy to manage nematodes, but it is very challenging to employ for RLN because of their wide host range (Orlando et al. 2020).

The integration of cover crops in the cropping system can be an alternative means of *P. penetrans* management, reducing the nematode population through different mechanisms. Many researchers in the past have reported the potential use of several species of cover crops to suppress plant-parasitic nematodes from the infested soil. Several *Brassica* spp. including brown mustard (*Brassica juncea* L.), rapeseed (*B. napus* L.), and oilseed radish (*Raphanus sativus* L.), were tested against the pale potato cyst nematode (*Globodera pallida*) in vitro and in the soil in Europe and were found to cause over 95% mortality of encysted eggs of *G. pallida* (Lord et al. 2011). Similarly, wild and cultivated *Brassica* spp. resulted in 56%-95% mortality of exposed *P. neglectus* under laboratory conditions (Potter et al. 1998). Different species of *Crotalaria* spp. have been extensively studied for their effect on nematode suppression and found to effectively reduce different plant-parasitic nematodes, including soybean cyst nematodes (SCN: *Heterodera glycines* Ichinohe), sugarbeet cyst nematode (SBCN; *Heterodera schachtii*), and *Globodera* spp., from different crop hosts (Sedaghatjoo et al. 2017; Wang et al. 2002). Acharya et al. (2020) evaluated the host range of 35 cover crops species/cultivars from four plant families to SCN and found 28 cover crop species/cultivars to be non-hosts, crops which can be utilized in SCN management without concern of nematode population increase.

Bélair et al. (2005) evaluated the impact of rotation of forage and grain pearl millet (*Pennisetum glaucum*) on *P. penetrans* population in Quebec, Canada. They found a significant reduction of nematode population after a 1-year forage pearl millet rotation and a 10% increase in potato yield compared with the control crop oat (*Avena sativa* L.). Similarly, in a microplot experiment conducted in Maryland, *P. penetrans* was suppressed by sorghum × sudangrass (*Sorghum bicolor* (L.) × *S. arundinaceum* (Desv.) Stapf var. *sudanense* (Stapf) Hitchc.) in a 3-year rotational sequence comprising potato, cucumber (*Cucumis sativus* L.), and soybean

cultivars. In the same experiment, sorghum × sudangrass and castor bean (*Ricinus communis* L.) both reduced the root-knot nematode (*Meloidogyne incognita*) from the infested soil as compared with fallow treatment (Everts et al. 2006). Some cover crops provide an alternative means of nematode management by stimulating the nematode eggs to hatch but not allowing them to reproduce within the plants. Reproduction of *P. penetrans* was effectively suppressed by French marigold (*Tagetes patula nana* cultivar Sparky) in a field trial in Belgium (Pudasaini et al. 2006). In another field study conducted in the Netherlands, *T. patula* was found to be more effective with a longer-lasting effect than a chemical soil fumigant in reducing *P. penetrans* populations (Evenhuis et al. 2004). Kimpinski et al. (2000) reported significantly higher potato tuber yield in addition to the population reduction of *P. penetrans* following marigolds planting.

Cover crops are mainly utilized for their role in soil erosion, soil health and land productivity with added benefits of pests and weeds management (Fageria et al. 2005; Weerasekara et al. 2017). As farmers are utilizing cover crops on more and more land acreage, the hosting ability of those crops to pests and pathogens needs to be evaluated to prevent them from harboring and providing a suitable environment for the reproduction of pests and pathogens. Despite the knowledge of the broad host range of *P. penetrans*, many cover crops in the northern Great Plains have not been evaluated for their hosting and population reduction ability for this RLN species. The objectives of this research were to screen 25 cover crop species and cultivars to 1) determine their hosting and population reduction ability to the root-lesion nematode, *P. penetrans*, under greenhouse conditions, and 2) to study the distribution of this nematode species in roots and soil habitats after three months of growth in the greenhouse.

Materials and Methods

Cover Crops

Cover crops species included in the experiments belong to four families, Brassicaceae, Fabaceae, Linaceae, and Poaceae. These cover crops have been commonly used or have the potential to be utilized as cover crops in the northern Great Plains (Table 3.1). A *P. penetrans*-susceptible potato (Red Norland) was used as a positive control and an unplanted treatment with infested soil (fallow) was used as a negative control. A common rotational crop wheat (Glenn) was used for comparison in this study. The cover crops seeds were acquired from the Forage and Biomass Crop Production Program (North Dakota State University, Fargo, ND, USA), Allied Seed (Nampa, ID, USA), and Great Northern AG (Plaza, ND, USA).

Nematode Species Identification and Population Maintenance

Root-lesion nematode population was initially collected from a nematode-infested potato field located in Becker County, Minnesota. Species-specific polymerase chain reaction (PCR) was used to identify and confirm the root-lesion nematode to species level. Nematodes were extracted from the collected sample and picked with a dental pick based on the morphological characteristics (Castillo and Vovlas 2007). The proteinase K method was then used to extract DNA individually from a single nematode (Huang and Yan 2017). The forward primer D2A (5′-ACAAGTACCGTGAGGGAAAGTTG-3′) and reverse primer D3B (5′-TCGGA AGGAACCAGCTACTA-3′) were used to amplify the D2-D3 expansion region of the 28S rRNA gene to validate the presence of DNA in the sample (Subbotin et al. 2008). Species-specific PCR was performed using a *P. penetrans*-specific forward primer PP5F (5-ACATGGTCGACACGGTGATA-3′) and reverse primer PP5R (5´-TGTTGCGCAAATCCTGTTTA-3′) that produces an amplification fragments size of 520 bp

Table 3.1. List of cover crops and controls tested for their hosting ability to root-lesion nematode, *P. penetrans* under controlled greenhouse conditions.

Crop (Cultivar or Cultivar Not Stated = CNS)	Scientific Name	Family	No. of Plants Per Pot
Alfalfa (Bullseye)	*Medicago sativa* L.	Fabaceae	4
Annual ryegrass (CNS)	*Lolium multiflorum* L.	Poaceae	2
Carinata (CNS)	*Brassica carinata* L.	Brassicaceae	1
Crambe (Belann)	*Crambe abyssinica*	Brassicaceae	1
Crimson clover (Dixie)	*Trifolium incarnatum* L.	Fabaceae	3
Daikon radish (Eco-till)	*Raphanus sativus* L.	Brassicaceae	1
Ethiopian cabbage (CNS)	*Brassica carinata* L.	Brassicaceae	1
Faba bean (Petite)	*Vicia faba* Roth	Fabaceae	2
Flax (Carter)	*Linum usitatissimum* L.	Linaceae	1
Forage oat (CNS)	*Avena sativa* L.	Poaceae	2
Forage Pea (Arvika)	*Pisum sativum*	Fabaceae	2
Foxtail millet (Siberian)	*Setaria italica* (L.) P. Beauvois	Poaceae	2
Japanese millet (CNS)	*Echinochloa esculenta* L.	Poaceae	2
Brown mustard Mighty Mustard™ (Kodiak)	*Brassica juncea* L.	Brassicaceae	1
Oilseed radish (Concorde)	*Raphanus sativus* L.	Brassicaceae	1
Oilseed radish (Control)	*Raphanus sativus* L.	Brassicaceae	1
Oilseed radish (Image)	*Raphanus sativus* L.	Brassicaceae	1
Potato (Red Norland)	*Solanum tuberosum*	Solanaceae	1
Sunnhemp (CNS)	*Crotolara juncea* L.	Fabaceae	1
Turnip (Pointer)	*Brassica rapa subsp. rapa* L.	Brassicaceae	1
Turnip (Purple Top)	*Brassica rapa subsp. rapa* L.	Brassicaceae	1
Wheat (Glenn)	*Triticum aestivum* L.	Poaceae	2
White mustard (Master)	*Sinapis alba* L.	Brassicaceae	1
White proso millet (CNS)	*Panicum miliaceum L*	Poaceae	2
Winter camelina (Bison)	*Camelina sativa* (L.) Crantz	Brassicaceae	2
Winter camelina (Joelle)	*Camelina sativa* (L.) Crantz	Brassicaceae	2
Winter rye (ND Dylan)	*Secale cereale* L.	Poaceae	2
Unplanted infested soil	-	-	-

(Mekete et al. 2011). Briefly, PCR mixture [8.62 µl double-distilled H_2O, 3.2 µl 5xGreen GoTaq Flexi buffer, 0.96 µl $MgCl_2$ (25 mM), 0.32 µl dNTP (10 mM), 0.64 µl of each primer (10 µM), 0.12 µl of GoTaq Flexi DNA polymerase (Promega Corp., Madison, WI, USA)] was prepared

and 1.5 µl template DNA was added to the PCR mixture. The amplification conditions for the primer set were initial denaturation at 94°C for 3 min followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 1.5 min, extension at 72°C for 1.5 min, and a final extension for 10 min at 72°C. A 2% agarose gel stained with ethidium bromide was prepared and 8 µl of the PCR products were added to the wells of agarose gel after DNA amplification. Gel electrophoresis was conducted for 45 min at 85 V and the banding pattern of the PCR products was visualized and photographed using AlphaImager Gel Documentation System (Proteinsimple Inc., Santa Clara, CA, USA).

A nematode population was reared and increased on a potato (cultivar Red Norland) susceptible to *P. penetrans* using pasteurized soil obtained from a potato field in Sargent County, ND (91% sand, 7% silt, 2% clay, pH 5.9). Nematodes were reared in the greenhouse located at the North Dakota Agriculture Experimental Station, Fargo, ND. Potato plants were grown in a greenhouse room with 16-hof daylight at an average temperature of 22°C for 12 weeks. Further rearing of the nematode was continued by planting potatoes in already infested soil until enough population density for the experiment was obtained. Soil from all pots was mixed thoroughly and average nematode population density was determined by extracting nematodes from three 200 g of soil subsamples using the sugar centrifugal flotation technique (Jenkins 1964). Briefly, 200 g of soil sample was placed in a 3-L jug filled with water and stirred vigorously to mix them thoroughly. Then, one-third of the solution was poured onto a stack of sieves (250 µm and 20 µm mesh size) to separate plant debris from nematodes along with soil particles. Finer soil particles and nematodes were caught in a 20-µm sieve, which was then collected in centrifuge tubes. After centrifuging the soil suspensions at 4,000 rpm speed for 10 min, the supernatant was discarded and the remaining soil samples were then suspended in 1.3 M sugar (American Crystal Sugar

Company, Moorhead, MN, USA) solution to separate nematodes from heavier soil particles. The suspension was stirred thoroughly and centrifuged at 4,000 rpm for 30 s. The supernatant was poured through a 20-µm sieve to collect root-lesion nematodes. The nematodes were thoroughly rinsed with tap water to wash the sugar from their body. The nematodes were collected in a 50-ml capacity storage tube (Thermo Fisher Scientific Inc. Waltham, MA, USA) for identification and quantification. Then, the infested soil was mixed with pasteurized soil from the same Sargent County, ND sample to obtain the required amount of soil for the greenhouse experiment. Three subsamples were again taken after mixing to determine the average initial nematode population of the nematode. Mixed soil was kept in a cold room at 4^{o}C to avoid changes in the nematode population until planting.

Greenhouse Experiments

Two greenhouse trials were conducted to evaluate the hosting and population reduction ability of the 25 cover crops species and cultivars to *P. penetrans*. An additional trial was conducted to further validate the response of the cover crop entries demonstrating distinct responses for hosting range against *P. penetrans* in the first two trials. The initial nematode population densities were 1,590/kg of soil and 1,670/kg of soil for the first and second trials, respectively. Trial 1 and 2 were set up in February and September of 2020, respectively. A third trial was set up in May of 2021 for some crop entries with distinct reactions to the nematode in the first two trials. However, the third trial did not include cover crops with distinct but suitable host ranking (good and excellent) in the previous two assessments. Faba bean (Petite) and alfalfa (Bullseye), which were excellent and poor hosts, respectively, were included in the third trial for comparison and further confirmation. The initial population density for the third trial was 2,125/kg of soil. Slow-release fertilizer (14-14-14 NPK, The Scotts Company, Marysville, OH,

USA) was mixed with soil at a rate of 5 g per kg of soil before planting. The experiment was arranged in a completely randomized design (CRD) with one kg of soil in each pot. All treatments were replicated four times in the first and second trials, while five replicates were used in the third trial.

All crops were directly seeded into the soil in plastic pots (15 cm in top diameter and 15 cm in height, Dillen Products, Middlefield, Ohio, USA) at variable depth depending upon their seed size. Potato was pre-sprouted before planting. For sprouting of potato, tubers were spread on moist paper towels placed in plastic trays (Van Ness Cat Pan Litters, Van Ness, Clifton, NJ, USA) and kept at room temperature of 22^{o}C for 15 days. Pre-sprouting of potato tubers helps grow plants quickly and makes roots available for nematode infection after planting. Sprouted tubers were cut into 2 to 3 pieces with at least one sprout per piece. Tubers were cut into pieces about a week before planting to provide adequate time for healing of the cut section. Different seeding rates were applied for crops during planting due to their different germination rate but only one sprouted tuber was used for potato in a pot. After emergence, seedlings were thinned out to an appropriate plant density for each treatment (Table 3.1) (Midwest Cover Crop Council 2014). All trials were conducted in the greenhouse at the North Dakota Agriculture Experiment Station, Fargo, ND with 16-h of daylight at an average temperature of 22^{o}C for 12 weeks. Adequate water was provided to the plants throughout the trials. Upon termination of trials, plant tops were removed, roots were separated from soil, and all the samples were stored in a cold room at 4^{o}C with respective label tags in separate individual plastic bags until they were processed for nematode extraction.

Soil and Root Samples Processing and Nematodes Quantifying

Soil and root samples from each pot were processed separately for nematode extraction. A 200-g subsample of soil was taken from each pot for nematode extraction using the sugar centrifugal flotation technique (Jenkin 1964) as described above. To extract the nematodes from roots, first, the soil particles around the roots were removed by gently rinsing the roots with tap water. The roots were soaked with a paper towel to remove all the moisture around them and were cut into 1-cm pieces before taking the fresh weight. Nematodes from roots were extracted by incubating chopped root pieces for 48 h in tap water (Whitehead and Hemming 1965). Extracted nematodes from soil and roots were collected separately in 50-ml suspension tubes (Thermo Fisher Scientific Inc. Waltham, MA). *Pratylenchus penetrans* was identified and counted using an inverted light microscope at 100X magnification (Zeiss Axiovert 25, Carl Zeiss Microscopy, NY, USA). Nematode populations extracted from 200 g of soil were converted to the total number of nematodes in 1 kg of soil for each individual pot. Total nematodes in 1 kg of soil were added to the nematode numbers obtained from the roots of a respective plant to get the final nematode population for each pot. The final nematode population densities were averaged across replicates for each trial.

Reproductive Factor and Host Range

Reproductive factor (Rf) was calculated for each experimental unit by dividing the final nematode population density on the tested crop by the initial nematode population density. The average Rf of nematodes for treatment was calculated as an average of Rf from four replicates for trials 1 and 2, and five replicates for trial 3. To determine the hosting ability of cover crops, five groups, including N = non-host ($Rf < 0.15$), P = poor host ($0.15 \leq Rf < 1.0$), M = maintenance host ($1.0 \leq Rf < 2.0$), G = good host ($2.0 \leq Rf < 4.0$), and E = excellent host ($Rf \geq$

4.0), were designated based on the average Rf as described in previous studies (Mbiro and Wesemael 2016; Schomaker et al. 2013). Hosting ability ranking was assigned to each crop in each of the trials.

Data Analysis

SAS software (SAS 9.4; SAS Institute Inc., Cary, NC) was used to analyze the final population densities of *P. penetrans,* population reduction of *P. penetrans* by cover crops, *P. penetrans* present in the soil and roots, and number of *P. penetrans* per g of fresh roots of cover crops. The PROC GLIMMIX with Tukey's honestly significant difference (HSD) mean separation was used to perform the analysis of variance (ANOVA) at a significance level of 5% to determine significant differences in the values of the above-mentioned parameters. Similarly, *P. penetrans* present in the soil and roots were compared using the pairwise t-test ($P \leq 0.05$).

The population reduction of *P. penetrans* was expressed as an average percentage reduction in nematode population densities from replicates (n = 4 for trials 1 and 2, n = 5 for trial 3) compared with the initial population densities. Population reduction was calculated using the formula; (initial population density on the tested crop - final population density on the tested crop)/initial population density on the tested crop x 100. The number of *P. penetrans* per g of fresh roots of cover crops was expressed as an average from all the trials for each crop and calculated by dividing the nematode number in the roots by the corresponding fresh root weight.

Results

Confirmation of the Nematode Species as *P. penetrans*

The root-lesion nematodes collected from the naturally infested field were identified to genus level based on their morphological features. Flat sclerotized lip region, short and stout stomato-stylet with basal knobs, ventral overlapping of the pharynx with intestine, vulva

positioned at 70-85% of the body length from the anterior end of an adult, and rounded tail with smooth tip were the characteristic features used to identify them to genus level and separate them from other vermiform nematodes (Castillo and Vovlas 2007). The species-specific primer set PP5F/PP5R was able to amplify a single amplicon of approximately 520 bp that matched the original description of the fragment size produced by the same primer set for *P. penetrans* (Mekete et al. 2011). This primer set did not produce any amplification with the control DNA samples of *Pratylenchus scribneri* and a new root-lesion nematode species (not named yet) from North Dakota. These results confirmed the root-lesion nematode species as *P. penetrans*. All vermiform life stages of the root-lesion nematode including juveniles, females, and males were counted to determine population densities.

Final Population Densities of *P. penetrans*

The mean final population density of root-lesion nematode showed significant differences among the cover crops tested in all three trials (Table 3.2). Alfalfa (Bullseye) and white proso millet (CNS), had lower mean final population densities than initial nematode density in trial 1 (Table 3.2). However, none of the cover crops had a lower mean final population density than the negative control, unplanted infested soil, in trial 1. Eight cover crops, crimson clover (Dixie), forage oat (CNS), flax (Carter), winter camelina (Bison), foxtail millet (Siberian), annual ryegrass (CNS), white proso millet (CNS), and alfalfa (Bullseye) had significantly lower mean final population density than potato (Red Norland), that was used as the positive control. The mean final population densities in faba bean (Petite), crambe (Belann), and brown mustard Mighty Mustard™ (Kodiak) were significantly higher than potato (Red Norland) (Table 3.2).

Table 3.2. Mean final population density of root lesion nematode, *Pratylenchus penetrans*, for different cover crops and controls, and their host rankings based on reproductive factor (Rf), in three greenhouse trials.

Cover crop (cultivar or cultivar not stated = CNS)	Mean final population density[x]			Rf[y]			Host ranking[z]		
	Trials[w]			Trials			Trials		
	1	2	3	1	2	3	1	2	3
Alfalfa (Bullseye)	999 m-n	788 l	344 g	0.63	0.47	0.16	P	P	P
Annual ryegrass (CNS)	1,819 k-m	690 l	646 f-g	1.14	0.41	0.30	M	P	P
Carinata (CNS)	5,799 c-g	5,686 b-e	-	3.65	3.41	-	G	G	-
Crambe (Belann)	13,237 b	7,746 b-c	-	8.39	4.64	-	E	E	-
Crimson clover (Dixie)	2,313 i-l	3,031 d-i	-	1.45	1.81	-	M	M	-
Daikon radish (Eco-till)	7,055 b-e	3,340 d-i	-	4.38	2.00	-	E	G	-
Ethiopian cabbage (CNS)	8,274 b-d	4,970 c-f	-	5.05	2.98	-	E	G	-
Faba bean (Petite)	26,325 a	24,360 a	28,468 a	16.56	14.59	13.40	E	E	E
Flax (Carter)	2,145 k-l	2,108 g-k	-	1.35	1.26	-	M	M	-
Forage oat (CNS)	2,182 j-l	3,936 c-h	7,752 b-c	1.37	2.36	3.65	M	G	G
Forage pea (Arvika)	9,016 b-d	6,300 b-d	-	5.61	3.78	-	E	G	-
Foxtail millet (Siberian)	2,078 k-l	2,414 f-k	-	1.31	1.45	-	M	M	-
Japanese millet (CNS)	4,303 e-i	3,850 c-h	-	2.71	2.31	-	G	G	-
Brown mustard Mighty Mustard™ (Kodiak)	9,474 b-c	3,448 c-i	-	5.96	2.07	-	E	G	-
Oilseed radish (Concorde)	4,141 e-j	1,554 i-l	4,611 c-d	2.61	0.93	2.17	G	P	G
Oilseed radish (Control)	4,190 e-j	3,095 d-i	4,284 c-e	2.79	1.85	2.02	G	M	G
Oilseed radish (Image)	3,309 f-k	2,521 e-j	6,206 b-c	2.08	1.51	2.92	G	M	G
Potato (Red Norland)	4,720 d-h	5,555 c-e	7,821 b-c	2.97	3.33	3.68	G	G	G
Sunnhemp (CNS)	3,083 g-k	12,906 a-b	19,040 a-b	1.94	7.73	8.96	M	E	E
Turnip (Pointer)	5,535 c-g	1,840 h-k	4,526 c-d	3.48	1.10	2.13	G	M	G
Turnip (Purple Top)	6,340 c-f	4,518 c-g	-	3.99	2.70	-	G	G	-
Wheat (Glenn)	1,937 k-l	1,900 h-k	-	1.22	1.14	-	M	M	-
White mustard (Master)	6,600 c-e	3,591 c-h	-	4.29	2.15	-	E	G	-
White proso millet (CNS)	1,374 l-n	3,469 c-i	1,832 d-f	0.86	2.08	0.86	P	G	P
Winter camelina (Bison)	2,140 k-l	1,979 h-k	-	1.35	1.19	-	M	M	-
Winter camelina (Joelle)	2,873 h-k	2,823 d-i	-	1.81	1.69	-	M	M	-
Winter rye (ND Dylan)	2,525 h-l	1,134 j-l	1,809 d-f	1.59	0.68	0.85	M	P	P
Unplanted infested soil	885 n	1,100 k-l	1,330 e-f	0.56	0.66	0.63	-	-	-
P > *F*	< 0.0001	< 0.0001	< 0.0001						

[w] Trial 1 was initiated in February 2020 with initial nematode density of 1,590 *P. penetrans*/1 kg of soil, trial 2 was initiated in September 2020 with the initial nematode density of 1,670 *P. penetrans*/1 kg soil and trial 3 was initiated in May 2021 with the initial nematode density of 2,125 *P. penetrans*/1 kg soil.

[x] Mean final population density is the mean final population of replicates (n = 4 for trials 1 and 2, n = 5 for trial 3) for each treatment. Mean final population with same letters are not significantly different from each other in each column.

[y] Rf (reproductive factor) is the mean reproductive factor of replicates (n = 4 for trials 1 and 2, n = 5 for trial 3) for each treatment and was calculated by dividing the final population density of nematode by the initial population density of nematode.

[z] Host ranking was the categorization into five classes based on reproductive factors of treatments: N = non-host (Rf < 0.15), P = poor host (Rf = 0.15 to 1.0), M = maintenance host (Rf = 1.0 to 2.0), G = good host (Rf = 2.0 to 4.0), and E=excellent host (Rf ≥ 4) (Mbiro and Wesemael et al. 2016; Schomaker et al. 2013).

In trial 2, the mean final population densities in alfalfa (Bullseye), annual ryegrass (CNS), oilseed radish (Concorde), and winter rye (ND Dylan) were lower as compared with the initial population density. Alfalfa (Bullseye) and annual ryegrass (CNS) had lower mean final population densities but not significantly different than unplanted infested soil (Table 3.2). Faba bean (Petite) and sunnhemp (CNS) had significantly higher mean final population densities than potato (Red Norland). However, the mean final population densities in eight cover crops including, foxtail millet (Siberian), flax (Carter), winter camelina (Bison), turnip (Pointer), oilseed radish (Concorde), winter rye (ND Dylan), alfalfa (Bullseye), and annual ryegrass (CNS) were significantly lower than the positive control in trial 2 (Table 3.2).

Similarly, alfalfa (Bullseye), annual ryegrass (CNS), white proso millet (CNS), and winter rye (ND Dylan) had lower mean final population density than the initial population density in trial 3, suggesting population reduction of the nematode as compared with the initial population. Furthermore, alfalfa (Bullseye) and annual ryegrass (CNS) had lower mean final population densities as compared with unplanted infested soil where alfalfa exhibited a significant difference with unplanted infested soil (Table 3.2). White proso millet (CNS), winter rye (ND Dylan), annual ryegrass (CNS), and alfalfa (Bullseye) produced significantly lower mean final populations than potato (Red Norland) whereas only faba bean (Petite) had a significantly greater mean final population density as compared with potato (Red Norland) (Table 3.2).

Faba bean (Petite) produced the greatest mean final population densities among the cover crops in all three trials. The lowest mean final population densities in trials 1 and 3 were found in alfalfa (Bullseye), whereas annual ryegrass (CNS) had the lowest mean final population density in trial 2 (Table 3.2). The mean final population densities ranged from 999 (alfalfa cv. Bullseye)

to 26,325 (faba bean cv. Petite) nematodes per kg of soil and roots in trial 1. In trial 2, the mean final population densities ranged from 690 (annual ryegrass) to 24,360 (faba bean cv. Petite) nematodes per kg of soil and roots. Similarly, the mean final population densities ranged from 344 (alfalfa cv. Bullseye) to 28,468 (faba bean cv. Petite) in trial 3 (Table 3.2).

Reproductive Factors (Rfs) of *P. penetrans* and Host Ranking of Cover Crops

Alfalfa (Bullseye) consistently had the Rf values less than one in all three trials, indicating its poor hosting ability for *P. penetrans* (Table 3.2). All other cover crops belonging to the Fabaceae family had Rf values more than one in at least one of the trials. Faba bean (Petite) had the highest value of Rf among all cover crops in all three trials (Rf = 16.56 for trial 1, Rf = 14.59 for trial 2, and Rf = 13.40 for trial 3), exhibiting its excellent hosting ability to the nematode. Maintenance hosting ability was shown by crimson clover (Dixie) consistently, with Rf values less than two, while forage pea (Arvika) was found to be a suitable host in both trials (Rf = 5.61 for trial 1 and Rf = 3.78 for trial 2). Sunnhemp, inconsistently, showed maintenance hosting ability in trial 1 (Rf = 1.94), but it served as an excellent host later in two trials (Rf = 7.73 for trial 2 and Rf = 8.96 for trial 3) (Table 3.2).

All the cover crops from the Brassicaceae family tested in this experiment had Rf values more than one in at least one of the trials (Table 3.2). Winter camelina (Bison and Joelle) consistently maintained the nematodes with Rf values less than 2 in all trials, whereas carinata (CNS), crambe (Belann), daikon radish (Eco-till), Ethiopian cabbage (CNS), brown mustard Mighty Mustard™ (Kodiak), turnip (Purple Top), and white mustard (Master) served as suitable hosts throughout the experiment. Three cultivars of oilseed radish and turnip (Pointer) increased the initial population of the nematode by more than two folds in trial 1, but they demonstrated

variable reactions to the nematode in trial 2. All of them were further tested in trial 3 and confirmed to be good hosts (Rf > 2.00) for *P. penetrans* (Table 3.2).

Two cover crops from the Poaceae family, foxtail millet (Siberian) (Rf = 1.31 for trial 1 and Rf = 1.45 for trial 2) and Japanese millet (CNS) (Rf = 2.71 for trial 1 and Rf = 2.31 for trial 2) showed maintenance and good hosting ability, respectively, throughout the experiment while other cover crops demonstrated varied host ranges in first two trials. Annual ryegrass (CNS) and winter rye (ND Dylan) both showed maintenance host range in trial 1, but they were confirmed to be poor hosts from trials 2 and 3 with Rf values less than one. Similarly, the host ranges of forage oat (CNS) and white proso millet (CNS) for *P. penetrans* were established to be good and poor, respectively. Flax (Carter), which was the only crop from the Linaceae family, showed the maintenance host range throughout the experiment (Table 3.2).

Wheat (Glenn) showed the maintenance hosting range for the nematode in two trials. In the susceptible potato (Red Norland), *Pratylenchus penetrans* reproduced very well (Rfs = 2.97 for trial 1, 3.33 for trial 2, and 3.68 for trial 3), suggesting a conducive environment in the greenhouse for the nematode reproduction. From the negative control, 56%, 66%, and 63% of the initial nematode population density were recovered at the end of trial 1, trial 2, and trial 3, respectively (Table 3.2).

The COV test for homogeneity performed using PROC GLIMMIX suggested the Rfs from three trials were homogeneous. Therefore, the Rfs for all cover crops in the three trials were combined, averaged, and analyzed to determine the overall hosting ability of those cover crops to *P. penetrans*. Alfalfa (Bullseye) and annual ryegrass (CNS) were poor hosts for the nematode according to the combined results. Eight cover crops, winter camelina (Bison), crimson clover (Dixie), flax (Carter), foxtail millet (Siberian), oilseed radish (Concorde), white proso millet

(CNS), winter camelina (Joelle), and winter rye (ND Dylan) showed maintenance hosting ability. White proso millet (CNS) and winter rye (ND Dylan) were ranked as maintenance hosts for *P. penetrans* based on the combined Rf values. However, they had a poor host range in the majority of the trials. Additionally, oilseed radish (Concorde) despite serving as a good host in the first and third trials, showed the maintenance host range based on the combined results. Nine cover crops, carinata (CNS), daikon radish (Eco-till), forage oat (CNS), Japanese millet (CNS), two cultivars of oilseed radish (Control and Image), two cultivars of turnip (Pointer and Purple Top), and white mustard (Master) were good hosts. Similarly, six cover crops, including crambe (Belann), Ethiopian cabbage (CNS), faba bean (Petite), forage pea (Arvika), brown mustard Mighty Mustard™ (Kodiak), and sunnhemp (CNS), were excellent hosts for *P. penetrans*.

Population Reductions of *P. penetrans* by Cover Crops

Twenty cover crops evaluated in this study had a greater mean final population of *P. penetrans* than the initial population in all trials (Table 3.2). However, five cover crops, including alfalfa (Bullseye), annual ryegrass (CNS), oilseed radish (Concorde), white proso millet (CNS), and winter rye (ND Dylan), reduced the initial nematode density from the infested soil in at least one trial during the experiments (Table 3.3). Alfalfa (Bullseye) and white proso millet (CNS) reduced the nematode population by 37.18% and 13.60% from the initial population, respectively, in trial 1. In trial 2, annual ryegrass (CNS) had the greatest population reduction percentage (PRP), followed by alfalfa (Bullseye), winter rye (ND Dylan), and oilseed radish (Concorde). In trial 3, alfalfa (Bullseye) had the greatest nematode population reduction, followed by annual ryegrass (CNS), winter rye (ND Dylan), and white proso millet (CNS). However, none of these cover crops were significantly different from negative control for PRP.

Unplanted infested soil had PRP of 44.34%, 34.13%, and 37.41% for trials 1, 2, and 3, respectively (Table 3.3).

Table 3.3. Population reduction percentage (PRP) of *P. penetrans* by cover crops and control treatments in greenhouse experiments[v].

	Population reduction percentage (PRP) [w]		
Cover crops/cultivars	Trial 1	Trial 2	Trial 3
Alfalfa (Bullseye)	37.18 ab	52.84 a	83.81 a
Annual ryegrass (CNS)	-14.39 bc	58.68 a	69.6 a
Oilseed radish (Concorde)	-160.41 d	6.96 a	-116.99 b
White proso millet (CNS)	13.6 ab	-107.71 b	13.79 ab
Winter rye (ND Dylan)	-58.8 c	32.11 a	14.87 ab
Unplanted infested soil	44.34 a	34.13 a	37.41 a
P > *F*	< 0.0001	< 0.0001	0.0032

[v] Population reduction percentage (PRP) for treatments with population reduction in at least one of the trials. Other crops and controls did not reduce the initial nematode population during the experiments.
[w] Population reduction percentage (PRP) is average of % reduction in nematode populations from replicates (n = 4 for trials 1 and 2, n = 5 for trial 3) compared to the initial nematode population. Nematode population reduction (%) = (the initial population density on the tested crop – the final population density on the tested crop)/initial population density on the tested crop x 100. CNS = cultivar not stated. Negative population reduction % indicates population increase in treatment during that trial. Population reduction (%) with same letter are not statistically different in each column.

Nematode Population Distribution in Soil and Roots of Cover Crops

The nematode populations extracted from soil and roots were statistically compared, and the percentage of nematodes in roots compared with the total population for each cover crop was determined. For the majority of the cover crops in trial 1 and trial 2, the mean population of nematodes in roots was significantly different ($P \leq 0.05$) from the mean population of nematodes in soil (Table 3.4). Similarly, the mean nematode population in soil was greater than the mean nematode population in roots for the majority of the cover crops throughout the experiment. However, the recovery of nematodes from soil or roots was inconsistent for some cover crops among trials. For example, the excellent host crop, sunnhemp (CNS), had a significantly higher

number of nematodes in the roots than the soil in the second trial, but nematodes numbers in soil and roots statistically similar in trials 1 and 3. Two poor host cover crops, alfalfa (Bullseye) and white proso millet (CNS), also showed variable distribution of nematodes in the soil and roots. Potato (Red Norland) consistently had a significantly higher root-lesion nematode population in the soil than the roots in all three trials. However, wheat (Glenn) had statistically similar nematode population in roots and soil in both trials (Table 3.4).

When the distribution of nematodes in soil and roots were combined for the whole experiment and compared, the majority of the cover crops had less than 30% of the total nematodes in the roots after three months of plant growth (Fig. 3.1). However, more than 50% of the total nematode population was extracted from roots of five cover crops including, alfalfa (Bullseye), crimson clover (Dixie), forage oat (CNS), sunnhemp (CNS), and white proso millet (CNS). The proportion of nematodes in roots ranged from 5.75% (white mustard cv. Master) to 59.35% (sunnhemp). Faba bean with the greatest mean final population had more than 60% of nematodes present in the soil at the end of the experiment (Fig. 3.1).

The average number of nematodes per g of fresh root weight was calculated for each cover crop and plant control (potato cv. Red Norland and wheat cv. Glenn) based on the mean number of nematodes in roots and the fresh weight of the roots. The average numbers of nematodes per g of fresh roots were homogeneous among the trials, therefore, the data were combined and analyzed. Significant differences ($P \leq 0.001$) were observed between the average number of nematodes per g of fresh roots for cover crop treatments (Fig. 3.2). Faba bean (Petite) had the highest number of nematodes (1,615) in 1 g of fresh roots followed by forage pea cv. Arvika (1,597 nematodes/g of fresh roots), and both were significantly higher than the susceptible check potato cv Red Norland (285 nematodes/g of fresh roots). Six cover crops, two

cultivars of oilseed radish (Image and Control), Japanese millet (CNS), alfalfa (Bullseye), winter rye (ND Dylan), and annual ryegrass (CNS) had statistically fewer number of nematodes per g of fresh roots compared with potato (Red Norland). Annual ryegrass (CNS) had the lowest number of mean nematodes (15) per g of roots among all the tested cover crops. Wheat (Glenn) had 211 nematodes in 1 g of roots which was not significantly different from mean nematodes per g of roots in potato (Red Norland) (Fig. 3.2).

Table 3.4. Distribution of *Pratylenchus penetrans* in soil and root habitats of cover crops and controls after three months of growth in three greenhouse trials.

	Mean final population					
	Trial 1		Trial 2		Trial 3	
Cover crop/cultivar	Soil[y]	Root[z]	Soil	Root	Soil	Root
Alfalfa (Bullseye)	574	425	138	650	280	64
Annual ryegrass (CNS[x])	1,300	519	375	315	580	66
Carinata (CNS)	4,700	1,099*	4,906	780*	-	-
Crambe (Belann)	11,288	1,949*	5,975	1,771*	-	-
Crimson clover (Dixie)	950	1,363	1,331	1,700	-	-
Daikon radish (Eco-till)	6,215	840*	2,900	440*	-	-
Ethiopian cabbage (CNS)	6,600	1,674*	4,500	470*	-	-
Faba bean (Petite)	14,975	11,350*	15,419	8,941*	17,470	10,998*
Flax (Carter)	1,250	895	1,688	420*	-	-
Forage oat (CNS)	813	1,370	1,056	2,880*	4,365	3,387
Forage pea (Arvika)	6,600	2,416*	4,700	1,600*	-	-
Foxtail millet (Siberian)	1,415	663	1,594	820	-	-
Japanese millet (CNS)	2,600	1,703	1,750	2,100	-	-
Brown mustard Mighty Mustard™ (Kodiak)	8,476	997*	3,063	385*	-	-
Oilseed radish (Concorde)	2,875	1,266*	900	654	4,345	266*
Oilseed radish (Control)	3,200	990*	2,350	745*	4,100	184*
Oilseed radish (Image)	2,500	809*	1,881	640*	5,900	306*
Potato (Red Norland)	4,425	295*	3,775	1,780*	5,695	2,126*
Sunnhemp (CNS)	1,106	1,976	3,444	9,463*	9,920	9,120
Turnip (Pointer)	4,575	960*	1,425	415	4,290	236*
Turnip (Purple Top)	5,400	940*	4,094	424*	-	-
Wheat (Glenn)	1,431	506	1,225	675	-	-
White mustard (Master)	6,125	475*	3,481	110*	-	-
White proso millet (CNS)	784	590	1,306	2,163	1,096	736
Winter camelina (Bison)	1,575	565	1,644	335*	-	-
Winter camelina (Joelle)	2,139	734*	2,313	510*	-	-
Winter rye (ND Dylan)	2,175	350*	719	415	1,450	359

[x] CNS = Cultivar not stated

[y] Nematode population (soil) is the mean population of replicates (n = 4 for trials 1 and 2, n = 5 for trial 3) in 1 kg of soil for each treatment from the respective trial. Nematodes from soil were extracted using sugar centrifugal flotation method (Jenkins 1964).

[z] Nematode population (root) is the mean population of replicates (n = 4 for trials 1 and 2, n = 5 for trial 3) in all available roots for each treatment from the respective trial. Nematodes from roots were extracted using Whitehead tray extraction method (Whitehead and Hemming 1965). Asterisk (*) represents significant difference ($P \leq 0.05$) between the *P. penetrans* populations in soil and roots among treatments for each trial.

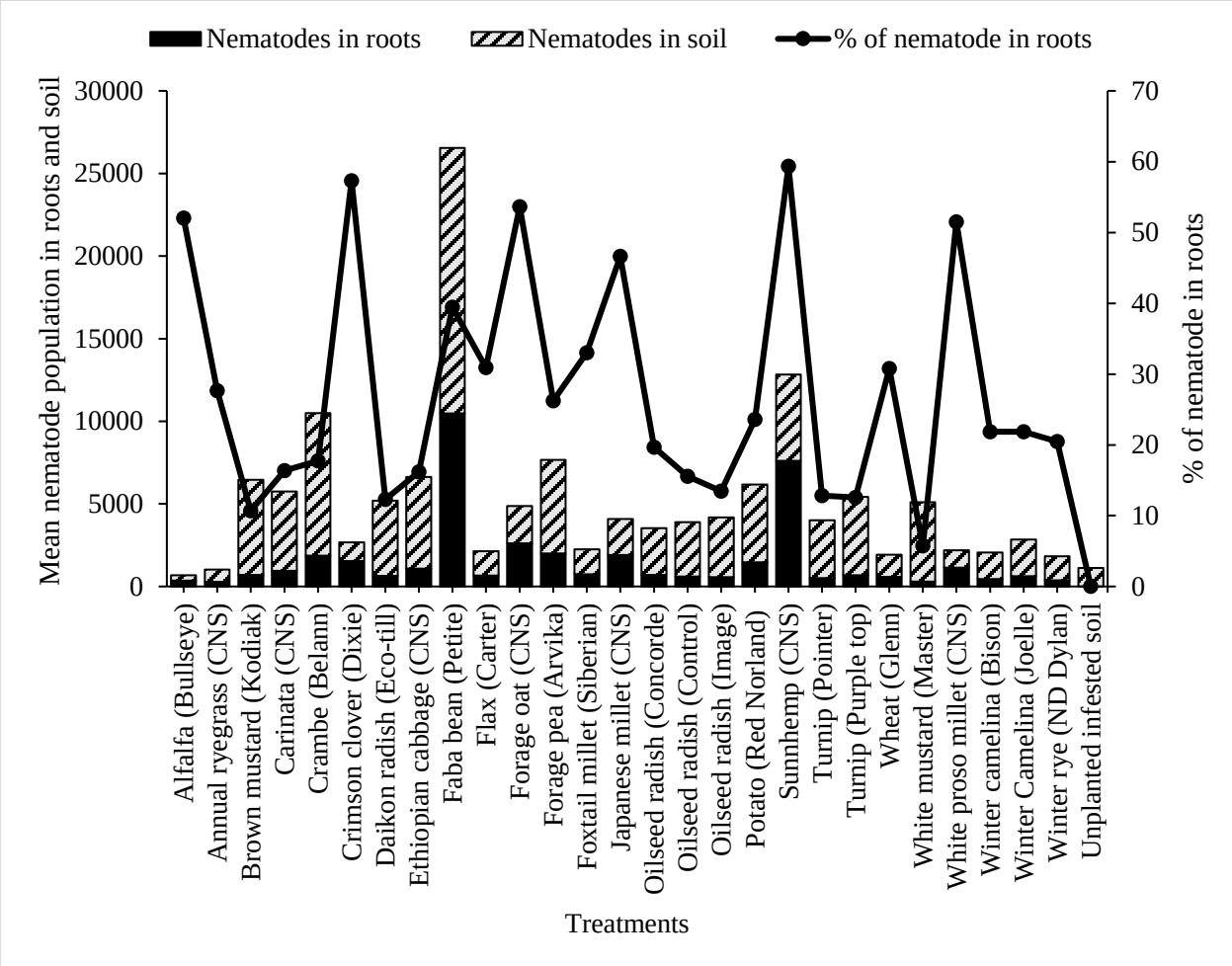

Fig. 3.1. Mean proportion of *Pratylenchus penetrans* nematodes in roots vs soil after three months of growth in each cover crop and control treatment. Mean percentage of nematodes in roots is the mean of percentage of nematodes in roots of all replicates from all the trials for each treatment and calculated as mean nematode population in roots/(mean nematode population in roots + mean nematode population in soil) × 100. Primary Y-axis (on the right) is for mean population of nematodes in roots and in soil, and secondary Y-axis (on the left) is dedicated to mean percentage of nematode in roots.

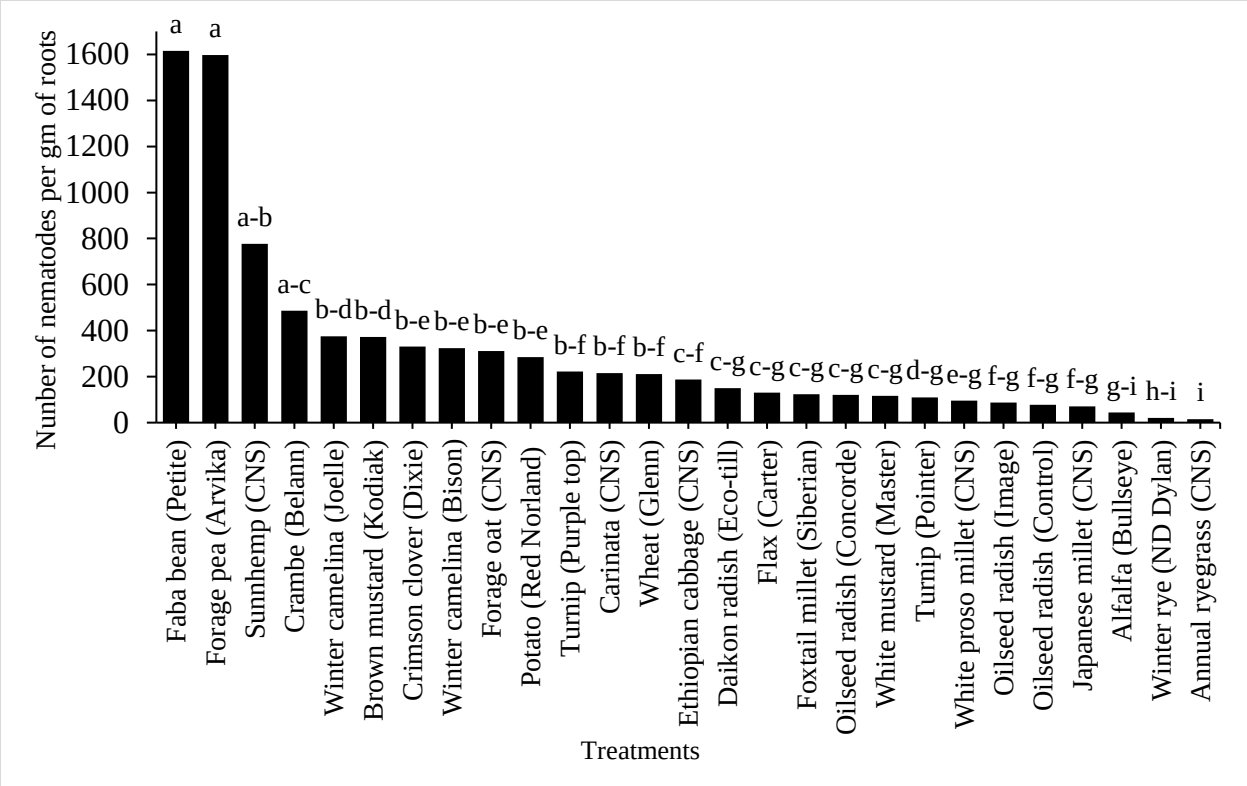

Fig. 3.2. Number of *Pratylenchus penetrans* per gm of fresh roots in each crop entry tested. Nematode per gm of roots is the mean of nematode per gm of fresh roots combined from all the trials for each treatment and measured by dividing the nematode number in roots by fresh root weight. Number of nematodes per gm of fresh roots with same alphabet are not significantly different ($P \leq 0.05$) from each other.

Discussion

This study presents the host suitability of 25 different cover crop species/cultivars for root-lesion nematode, *Pratylenchus penetrans*, based on the reproductive factor (Rf) from the greenhouse experiment and provides information on population reductions of *P. penetrans* by cover crops. Additionally, the distributions of nematode population in soil and root after three months of cover crops growth were evaluated in this study.

Previously, many studies have evaluated different species/cultivars of cover crops and revealed a wide range of host ranges for *P. penetrans* (Florini and Loria 1990; Forge et al. 2000; Grabau et al. 2017; Rudolph et al. 2017; Thies et al. 1995; Vrain et al. 1996). Among 25 cover

crop species/cultivars evaluated in this study, 11 cover crops showed the same host range level in all trials. Six of the cover crops tested had similar hosting abilities, all supporting the reproduction of nematodes with Rfs more than two. However, nine of them had distinct reactions to *P. penetrans* in two trials and were further evaluated to confirm their host ranges. To our knowledge, none of the cultivars of cover crops studied in this research were tested before for hosting ability to *P. penetrans*. Furthermore, crimson clover (Dixie), carinata (CNS), Ethiopian cabbage (CNS), forage pea (Arvika), turnip (Pointer and Purple Top), white proso millet (CNS), and winter camelina (Joelle) had never been evaluated for the reproduction of *P. penetrans* before.

Cover crops species of the Fabaceae family had varied responses to *P. penetrans* in our trial. Alfalfa (Bullseye) was the only leguminous crop that consistently reduced the initial nematode populations from the soil throughout the experiments showing poor hosting ability. Similar to our finding, alfalfa cv. MNGRN-16 was reported to be resistant against this RLN species (Thies et al. 1995), but cv. Baker was found to be a suitable host in another study (Nelson et al. 1985). The highest number of this RLN was observed per total root mass in alfalfa cv. Saranac (Miller 1978), and cv. Alpha was a good host in a study conducted in Belgium (Mbiro and Wesemael 2016). Thies et al. (1995) also found two legumes, sweetclover (*Melilotus officinalis var. alba* L.) and crown vetch (*Coronilla varia* L.), to be the least suitable host for *P. penetrans* despite the nematode reproduced on all tested legumes in greenhouse and growth chamber. All the other cover crops of the Fabaceae family in our study supported the reproduction of nematodes with reproductive factors greater than one. In a study by Forge et al. (2000), all the leguminous cover crops, including faba bean (Banner), supported the reproduction

of *P. penetrans* very well. Similarly, we found faba bean (Petite) as an excellent host in our experiment with the highest reproductive factor among the tested crops.

A total of 13 cover crop species/cultivars of the Brassicaceae family were tested in this study and none of them were able to reduce the initial nematode population from the soil, except oilseed radish (Concorde) in the second trial. Winter camelina (Bison and Joelle) consistently maintained the nematode with Rfs less than two in two trials. Winter camelina has a great potential to be utilized as a winter annual for relay cropping or interseeding in our regions to provide soil cover and reduce nutrient leaching from soil (Berti et al. 2017). Smiley et al. (2014) tested three camelina cultivars from the Pacific Northwest region for two root-lesion nematode species, *P. neglectus* and *P. thornei*. They found that all three cultivars (Yellowstone, Blaine Creek, and Calena) supported the reproduction of *P. neglectus* but showed poor host ranges for *P. thornei*. The same study revealed that brown mustard cv. Pacific Gold and white mustard cv. Ida Gold were excellent hosts for *P. neglectus* but poor hosts for another RLN, *P. thornei*. Similarly, those mustard cultivars (Pacific Gold and Ida Gold) maintained the initial population density of *P. penetrans* in a greenhouse study in Washington (Rudolph et al. 2017). Bélair et al. (2002) also reported excellent reproductions of *P. penetrans* on brown and white mustard in a greenhouse experiment in Quebec, Canada. In our study, *P. penetrans* reproduced very well in a brown mustard (Kodiak) and a white mustard (Master). Crambe (Belann) was found to support excellent reproduction of nematode in the present study, but Kok and Coenen (1995) reported maintenance host range of crambe in a greenhouse study.

We evaluated three cultivars of oilseed radish (Concorde, Control, and Image) and a cultivar (Eco-till) of daikon radish for the host suitability to *P. penetrans*. All cultivars showed good host suitability for nematodes except for Concorde that showed a small percentage

reduction (7%) of the initial nematode density in one of the three trials. Grabau et al. (2017) reported the population density increased in radish (Defender) in the Michigan carrot production system. Miller (1978) also described the susceptibility of radish to *P. penetrans* with severe necrosis of roots. Mbiro and Wesemael (2016), however, found radish line RsV79/80 to be a poor host with reduction in the initial nematode density. Crops belonging to the Brassicaceae family contain glucosinolates that convert to isothiocyanates in the soil (Devi 2018). The volatile compounds are known to be suppressive to the plant-parasitic nematodes including *Globodera pallida* (Lord et al 2011; Ngala et al. 2015), *Meloidogyne incognita* (Oliveira et al. 2011), and *Pratylenchus neglectus* (Potter et al. 1998). The release of those compounds and their activity depend on many factors including crop species, plant growth stage at the time of incorporation in soil, plant parts, degree of maceration of plant parts, soil temperature, soil depth, and soil water content (Devi 2018; Potter et al. 1998). The determination of hosting abilities of Brassica cover crops is very important as their nematicidal effects may be overcome by nematode reproduction in roots when they are incorporated as green manure (Grabau et al. 2017; McLeod and Steel 1999).

Previously, many species of cover crops belonging to the Poaceae family have been evaluated for hosting ability to *P. penetrans* from different geographical regions. Forage oat showed a good host range for the nematode in the present study. Similar host ranges for oat cultivars have been reported in past studies (Bélair et al. 2002; Florini and Loria 1990; Rudolph et al. 2017; Thies et al. 1995). However, Oat cv. Saia suppressed the nematode reproduction in research conducted in Oregon, USA (Forge et al. 2000) and British Columbia, Canada (Vrain et al. 1996). Forge et al. (2000) also found the suppressive effect of winter rye cv. Wheeler to *P. penetrans* and revealed to establish well in winter season with suppression of weeds that are host

to the nematode. Florini and Loria (1990) found increased mortality of *P. penetrans* on winter rye cultivars under field conditions despite their good hosting abilities in pot studies. We found winter rye cv. ND Dylan had poor hosting ability in this study, supporting previous studies showing suppressive effects of winter rye to the nematode. Winter rye consistently survives the winters in our region and provides the best soil cover in the spring. Winter rye also is effective in suppressing weeds because of competition for resources and their strong allelopathic effect on weeds (Wick et al. 2018). Some studies, however, have reported substantial reproduction of *P. penetrans* on winter rye (Bélair et al. 2002; Thies et al. 1995).

Another winter cover crop, annual ryegrass, was found to reduce the initial nematode population in two of our trials, showing poor host ranges. Mbiro and Wesemael (2016) also reported a reduction of the initial population of this RLN on annual ryegrass cv. Meltador in a greenhouse study. Foxtail millet (Golden German) and Japanese millet were excellent hosts of *P. penetrans* after 11 weeks of growth in the greenhouse (Bélair et al. 2002). Similar to that study, Japanese millet (CNS) supported the reproduction of this nematode very well in our study. However, foxtail millet (Siberian) only maintained the initial nematode without a substantial increase in population. Another millet crop, white proso millet reduced the initial nematode population in two of the trials in our study but had shown good host range in one of the trials, which suggests that it may have the potential to favor the nematode reproduction under certain conditions. The only crop from the family Linaceae, flax (Carter), showed a maintenance host range for *P. penetrans* in our study. McKeown and Potter (2001) reported flax (Norlea) to be a host of *P. penetrans* in the field trials but they did not categorize the specific host category. However, flax cv. Pembina was found to reduce the initial population of *P. neglectus* and *P. thornei* from the soil in a greenhouse assay (Smiley et al. 2014).

Considerable variations in responses of different cover crop species and cultivars were observed in the present study. Some variations in host ranking of cover crops between the trials of this study could have resulted due to minor discrepancies in the environmental conditions of the greenhouse, such as slight changes in room temperature, soil moisture in pots, and the time of the year the crops were evaluated on. While comparing the results with results from past studies, we also noticed distinct reactions of different cultivars of the same crop to *P. penetrans*. Townshend (1989) also found varying susceptibility among cultivars when the reproduction of four species of *Pratylenchus* was evaluated in two cultivars of oat. The variations in reactions of different cultivars of the same crop to this nematode could be the result of differences in the genetic makeup of the cultivars. Resistance in alfalfa against *P. penetrans* and their molecular interactions has been evaluated in some studies, but the complete mechanism is still unclear (Vieira et al. 2019). Thus, to understand the host-pathogen interaction and to gain knowledge on the genetics of host resistance in cover crops, further research needs to be performed. Similarly, it is crucial to evaluate all available cultivars of cover crops for host suitability for plant-parasitic nematodes before utilizing them in the fields.

The study of the population distribution of *P. penetrans* in soil and roots of cover crops revealed that significant differences prevailed between the population in soil and roots of most cover crops. After three months of growth, most of the cover crops had a higher population of nematode resided in the soil habitat than root habitat. Moreover, nematode population distribution varied between the trials for the same crop. For example, sunnhemp had more nematodes in roots in the first and second trials, but the nematode population in the soil habitat was greater than in the root habitat in the third trial. These discrepancies in nematode distribution could be the result of different factors, including the growth period of plants, individual plant

vigor, development of root systems, availability of feeder roots, and greenhouse conditions. The visual observation during the harvest of the plants suggests that crops with greater vigor, such as sunnhemp that had the green growing plants in the vegetative stage, had higher nematodes in roots. That, along with past studies, indicates that the quantification of nematodes both from the soil and roots is crucial for determining the total nematode population from host plants (Chowdhury et al. 2022; MacGuidwin and Bender 2012).

This study found some cover crops with the ability to reduce nematode population from the infected soil, and it is important because of the broad host range of *P. penetrans*. Based on the result presented in our study, faba bean (Petite) was consistently the most suitable host, which increased the initial population up to 16 times of the initial population. It was much more susceptible than potato (Red Norland), which was consistently a good host and had the mean final population about three times the initial population. In our study, alfalfa (Bullseye) remained a poor host for *P. penetrans*, with nematodes reduction up to 84% compared with the initial population. Cover crops with poor hosting ability could potentially be utilized for managing this RLN in the infested fields. Additionally, the cover crops that maintained the nematode population in our trial without a substantial increase in the initial population may be evaluated under the field conditions to determine their performance against *P. penetrans*. This research will help potato growers select effective cover crops and avoid susceptible hosts to integrate them into the current cropping system to manage *P. penetrans* from infested field.

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CHAPTER 4: EVALUATION OF COVER CROPS FOR REDUCING *HETERODERA GLYCINES* POPULATIONS IN MICROPLOT EXPERIMENTS[2]

Abstract

Soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe) is a major yield-reducing pathogen of soybean worldwide. Microplot experiments were conducted to evaluate ten cover crops for their effects on two SCN populations (SCN103 and SCN2W) collected from North Dakota soybean fields in 2019 and 2020. Experiments were conducted in a randomized complete block design using naturally infested field soil. A susceptible soybean [*Glycine max* (L.) Merr.] (cv. Barnes) and unplanted natural soil (fallow) were used as controls. Plants were grown in outdoor conditions for 75 days before soil samples were collected. SCN eggs and juveniles were extracted from the soil samples to determine final population, population reduction and suppression. Soybean (Barnes) had significantly greater final population densities than all the cover crops and fallow. All tested cover crops and fallow reduced the initial densities of both SCN populations. All the cover crops, except chickling vetch (*Lathyrus sativus* L.) (Pulse), had lower final population densities than fallow and suppressed the SCN populations throughout the experiments. Sunnhemp (*Crotalaria juncea* L.) (cultivar not stated; CNS), oilseed radish (*Raphanus sativus* L.) (Concorde and Control), and daikon radish (Eco-Till) significantly reduced the SCN103 population compared with fallow. Sunnhemp (CNS), alfalfa (Bullseye), and daikon radish (Eco-Till) had significant population reductions of SCN2W than fallow.

[2] The material in this chapter was co-authored by Kamal Neupane (Department of Plant Pathology, North Dakota State University), Addison Plaisance (Department of Plant Pathology, North Dakota State University), and Guiping Yan (Department of Plant Pathology, North Dakota State University). Kamal Neupane had primary responsibility for collecting soil samples from the fields and performing microplot experiment. Kamal Neupane was the primary developer of the conclusions presented here. Addison Plaisance helped with soil sampling and served as proofreader. Guiping Yan served as the principal investigator and corresponding author and checked the statistical analysis conducted by Kamal Neupane. This paper was submitted to journal, Nematology.

Sunnhemp (CNS) was found to have the greatest reductions in SCN populations ranging from 55% to 86% compared with the initial densities. This study demonstrated cover crops species/cultivars with the ability to reduce SCN populations in outdoor conditions, and the findings suggest that they could be utilized in infested fields to manage SCN.
Keywords: Soybean cyst nematode (SCN), soybean, outdoor conditions, infested soil, management, final population, population reduction, and suppression

Introduction

Soybean [*Glycine max* L. (Merr.)] is an important leguminous crop with high protein and seed oil content (Singh and Shivakumar 2010). The processed form of soybean is the largest source of animal feed protein and the second-largest source of vegetable oil worldwide (USDA-NASS 2020). The United States (U.S.) is the second-biggest soybean producer, with a value of USD 31.2 billion in 2019 (ASA 2020). North Dakota ranks fourth in the U.S. for the total soybean planted area, with over five million acres planted in 2019 (USDA-NASS 2020).

Soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe) is an obligate sedentary endo-parasitic nematode that continues to be the prominent yield-reducing biotic factor for soybean production throughout the world (Hartman et al. 2011; Koenning and Wrather 2010; Niblack et al. 2006). The economic loss caused by SCN is estimated to be more than USD 1.5 billion annually in the U.S. (Allen et al. 2017; Bandara et al. 2020; Bradley et al. 2021). Despite the massive economic impact, farmers often do not notice the losses and 30% or greater yield reduction by SCN can occur without any obvious above-ground symptoms (Niblack 2005; Wang et al. 2003). Since SCN was first detected in North Carolina in 1954 (Winstead et al. 1955), it has spread to nearly every soybean-producing state in the U.S. (Tylka and Marett 2017, 2021). In

North Dakota, SCN was first detected in Richland County in 2003 (Bradley et al. 2004), and at least 19 counties were positive for SCN infestation by 2015 (Yan et al. 2015).

Among multiple strategies for SCN management, the use of resistant cultivars and crop rotation are considered the best approach (Mueller et al. 2016; Niblack et al. 2006). However, rapid diversification of SCN virulence due to the continuous use of the major resistance source, PI 88788, poses a great threat to current SCN management strategies (Chowdhury et al. 2021; Niblack et al. 2008; McCarville et al. 2017; Yan and Baidoo 2018). Newly evolved virulent populations of SCN can cause reduced effectiveness of resistant cultivars (Colgrove and Niblack 2008), prompting the development of alternative management strategies of SCN (McCarvill et al. 2017).

Cover crops provide an alternative approach as several experiments have shown the potential of cover crops in managing plant-parasitic nematodes. Cover crops help prevent soil erosion, maintain soil health, and suppress weeds, pests, and pathogens (Snapp et al. 2005; Weerasekara et al. 2017). Managing plant-parasitic nematodes by cover crops depends on the interaction between cover crops and nematodes species (Abawi and Widmer 2000; Wang et al.2002a). Cover crops from the Brassicaceae family, including brown mustard (*B. juncea*), rapeseed (*B. napus*), and oilseed radish (*Raphanus sativus* L.) were found to kill over 95% of encysted eggs of the pale cyst nematode (*Globodera pallida*) in vitro and soil (Lord et al. 2011). Similarly, oilseed radish (*R. sativus*) and white mustard (*Sinapis alba* L.) significantly reduced the sugarbeet cyst nematode (*Heterodera schachtii*) in two locations of Iran when planted before sugarbeet (*Beta vulgaris* L.) (Hemayati et al. 2017). The root-knot nematode (*Meloidogyne incognita*) infestation and root galling were significantly reduced in tomato planted after French (*Tagetes patula* cultivars Single Gold and Tangerine) and African marigold (*T. erecta* cv. Flor de

Muerto) compared with tomato (*Solanum lycopersicum* L.) planted after fallow (Ploeg and Maris 1999).

In 2016, Nascimento *et al.* conducted a field study to evaluate crambe (*Crambe abyssinica* Hochst, cultivar FMS Brilhante) for its biofumigant effect on SCN and found a significant reduction of SCN population during its growing season. They also observed a significantly reduced number of adult SCN females in soybean roots and cysts in the soil during the soybean growing period after incorporating *C. abyssinica* in the SCN infested soil. A group of non-host plants was tested in vitro and under greenhouse conditions to evaluate their plant residues and root exudates on SCN hatching and juvenile survivability. Annual ryegrass (*Lolium multiflorum* L.) was found the most effective for SCN egg hatching in the absence of hosts and population reduction by depleting lipid contents of the juveniles (Riga et al. 2001). Several cover crop species were evaluated for their effects on SCN biology by Harbach et al. (2021) using a mechanistic approach. The root exudates (REs) and soil leachates (SLs) of crimson clover (*Trifolium incarnatum* L.) induced the greatest hatching of SCN and allowed the penetration of roots by a significant number of juveniles, showing potential as a trap crop. They also found more juveniles attracted towards REs and SLs of annual ryegrass (*L. multiflorum*) and winter rye (*Secale cereale* L.) without significant penetration. Similarly, Chen et al. (2008) evaluated non-host cover crops for their effects on SCN hatch, viability, and development. They found that sunnhemp (*Crotalaria juncea* L.) and red clover (*Trifolium pratense* L.) were the most effective to manage SCN by stimulating the hatching of juveniles. They also reported the reduced egg density and infectivity of juveniles when plant residues were added to the soil.

Acharya et al. (2019) assessed the reduction of a SCN population on three cover crops, winter camelina (*Camelina sativa* cv. Joelle), crambe (*C. abyssinica* cv. BelAnn), and brown

mustard (*B. juncea* cv. Kodiak), from North Dakota, and reported an average of 51% and 48% reduction of the initial population by brown mustard and winter camelina, respectively, in greenhouse experiments. Similarly, ten different cover crops from the same region were evaluated in an outdoor microplot experiment. Among them, daikon radish (*R. sativus*), annual ryegrass (*L. multiflorum*), winter rye (*Secale cereale* cv. ND Dylan), red clover (*Trifolium pratense* cv. Allington), and sweetclover (*Melilotus officinalis*) significantly reduced two SCN populations as compared with unplanted natural soil in two-year experiments (Acharya et al. 2021). However, the information regarding SCN reduction and suppression by other cover crop species/cultivars in North Dakota is still limited as more cover crops are being utilized or introduced in the region. Hence, the objective of this research was to evaluate these cover crops for their effects on the reduction and suppression of two SCN populations from North Dakota fields under outdoor microplot conditions.

Materials and Methods

Cover Crops

A total of ten cover crop species/cultivars were evaluated in this study that belong to three plant families, including Brassicaceae, Fabaceae, and Linaceae. Selected cover crops have been commonly used in the northern Great Plains, or they have the potential to be utilized in the region (Table 4.1). A SCN-susceptible soybean cultivar, Barnes, and an unplanted treatment with infested soil (fallow) were included as controls. The seeds were acquired from Forage and Biomass Crop Production Program (North Dakota State University, Fargo, ND, U.S.), Allied Seed (Nampa, ID, U.S.), and Great Northern AG (Plaza, ND, U.S.).

Table 4.1. List of cover crops and controls tested for their effects on SCN under microplot conditions.

Crops (Cultivar or Cultivar Not Stated = CNS)	Scientific Name	Family	Plants Per Pot
Alfalfa (Bullseye)	*Medicago sativa* L.	Fabaceae	8
Chickling vetch (Pulse)	*Lathyrus cicera* L.	Fabaceae	3
Daikon radish (Eco-Till)	*Raphanus sativus* L.	Brassicaceae	5
Faba bean (Petite)	*Vicia faba* Roth	Fabaceae	3
Flax (Carter)	*Linum usitatissimum* L.	Linaceae	3
Oilseed radish (Concorde)	*Raphanus sativus* L.	Brassicaceae	5
Oilseed radish (Control)	*Raphanus sativus* L.	Brassicaceae	5
Oilseed radish (Image)	*Raphanus sativus* L.	Brassicaceae	5
Soybean (Barnes)	*Glycine max* L.	Fabaceae	2
Sunnhemp (CNS)	*Crotalaria juncea* L.	Fabaceae	3
White mustard (Master)	*Sinapis alba* L.	Brassicaceae	5
Unplanted natural soil (fallow)			

Soybean Cyst Nematode Populations

Two SCN populations, HG type 0 and HG type 7, were used in this study to evaluate the effect of cover crops on their reductions. HG typing of these SCN populations was previously performed and confirmed by Acharya et al. (2021). These two populations are the most common SCN populations (36% frequency of HG type 0 and 27% frequency of HG type 7) in North Dakota (Chowdhury et al. 2021). Soil naturally infested with HG type 0 population was collected from a field SCN103 (sandy loam; Richland County, ND, U.S.), whereas soil naturally infested with HG type 7 was collected from a field SCN2W (loam; Cass County, ND, U.S.). Names of the fields will be interchangeably used as nematode populations hereafter. The soil samples collected from each field were mixed thoroughly to distribute the nematode population evenly in the soil mixture before planting. Three subsamples of 200 g from each soil were taken for nematode extraction to determine the initial SCN population densities.

A combination of standard methods described by Krushberg et al. (1994) and Jenkins (1964) was used to extract SCN cysts and hatched juveniles from the soil samples. Three sieves with mesh sizes of 710 µm, 250 µm, and 20 µm were arranged from top to bottom. The soil sample was placed in a 4-L pitcher filled with three-quarters water and stirred vigorously before pouring the suspension into the sieve arrangement. The 710-µm sieve separated the plant debris from the soil. Soybean cyst nematode cysts were collected in a 250-µm mesh size sieve, and hatched juveniles in the sample were collected in 20-µm along with the soil. The juveniles were recovered using the sugar centrifugal floatation method (Jenkins 1964). The soil suspension was centrifuged for ten minutes at 4,000 rpm speed to separate lighter particles from nematodes and other heavier particles. The supernatant was discarded, and nematodes were separated from soil particles by suspending in 1.3 M sugar (American Crystal Sugar Company, Moorhead, MN, U.S.) solution. The suspension was mixed thoroughly with a metallic stirrer and centrifuged at 4,000 rpm for 30 s. The juveniles were collected on sieve of 20-µm mesh size and rinsed with tap water to wash sugar solution from the nematode bodies. The nematodes were collected in a 50-ml capacity suspension tube for identification and quantification.

A rubber stopper attached to a motorized drill press (MasterForce Drill Press, Menards, Fargo, ND, U.S.) was used to crush the extracted cysts to release the SCN eggs and juveniles (Faghihi and Ferris 2000). The extracted eggs and juveniles were identified and quantified under the light microscope at 100X magnification (Zeiss Axiovert 25; Carl Zeiss Microscopy, NY, U.S.). The eggs and juveniles obtained from soil and cyst crushing were combined for respective subsamples to obtain the initial population density. The initial SCN populations (eggs and juveniles) per 200 g of soil were 4,524 and 5,974 for SCN103 and SCN2W, respectively, for the experiments conducted in 2019. For the trials conducted in 2020, the initial SCN population

(eggs and juveniles) per 200 g of soil for each treatment was 2,670 for both soil SCN103 and SCN2W, at the time of planting.

Microplot Experiments

Two experiments were conducted in 2019 and 2020, with freshly collected SCN-infested field soil each year, in a research field site on the campus of North Dakota State University. A field area (dimension 4.57 m × 18.18 m) was selected for microplot, and large plastic pots (diameter of 22.86 cm and depth of 20.30 cm, High Performance 200, Haviland, OH, U.S.) were used for cover crop planting. Plastic pots were inserted in holes dug in the field, and approximately 8 cm of the pots were kept above the soil surface to prevent the contamination of soil in the pot from blowing dust and from surrounding pots. Additionally, the remaining areas of the plot were covered with weed barrier (5OZ Pro garden weed barrier landscape fabric, ECO gardener, Houston, TX, U.S.) to prevent the contamination of the pots from surrounding soil and the weed growth. The microplot was surrounded by wired fence to prevent the entry of animals.

Each plastic pots contained about 5 kg of infested soil from each field, and cover crops were planted during the first week of August in both years. They were kept in a greenhouse for 2 weeks for seedlings establishment before transferring them to external environmental conditions. Standard seeding rates and spacing were applied for maintaining the number of plants per pot (Table 1) (Midwest Cover Crops Council 2014). The plants were regularly watered to provide the required moisture for the first two weeks and were fertilized twice with water-soluble 20-20-20 fertilizer (SCHULTZ All Purpose Plant Food 20-20-20, Knox Fertilizer Company, Inc. IN, U.S.) to aid in the growth of the plants. The experiments were terminated 75 days after the plants were transferred to microplot, and soil samples were collected from the root zone of each pot. Soil samples were collected randomly from the unplanted fallow treatment. Collected soil

samples were then mixed thoroughly, and a 200-g subsample was used to determine the final nematode population density (eggs and juveniles) at harvest. A combination of standard methods described by Krushberg et al. (1994) and Jenkins (1964) was used to extract SCN cysts and hatched juveniles from the soil samples as described above. The SCN eggs and juveniles were then quantified under the light microscope at 100X magnification (Zeiss Axiovert 25; Carl Zeiss Microscopy, NY, U.S.), and the final nematode population density was determined.

Data Analysis

The SAS software (SAS 9.4; SAS Institute Inc., Cary, NC) was used to analyze the obtained data from the trials. All the obtained data were analyzed separately for two trials from different years and for two SCN populations because the data were not homogenous, and the initial population densities in the two trials for each SCN population were different. The analysis of variance and mean separations were performed using the PROC GLIMMIX with Tukey's honestly significant difference (HSD), at 5% significance level. Means separation was applied to mean final population densities (FPD), nematode suppression percentages, and population reduction percentages.

The reproductive factor (Rf) was calculated as the final population density on tested crop/the initial population density in the tested crop for each treatment. Population reduction (%) for each treatment (entry) was calculated by using the formula: population reduction (%) = [(initial SCN population density on tested crop – mean FPD on the tested crop)/initial SCN population density on the tested crop] × 100. Nematode suppression (%) was calculated to evaluate the additional nematode mortality by cover crops other than natural mortality in unplanted natural soil (fallow), and it was calculated by using the formula; nematode suppression

(%) = [(mean FPD on unplanted natural soil – mean FPD on tested crop)/mean FPD on the unplanted natural soil] × 100.

Results

Final SCN Population Densities and Reproductive Factors

In 2019, there were significant differences among the treatments, including soybean (Barnes) and unplanted natural soil, for the mean final population density of SCN103 (Table 4.2). The SCN susceptible soybean cultivar, Barnes, produced a significantly ($P \leq 0.05$) higher final population density and higher Rf (FPD = 13,906; Rf = 3.07) than all other treatments in this trial. Similarly, the mean FPD in all cover crops was significantly lower than the unplanted natural soil (2,207; 0.49). The highest and the lowest final population densities and Rfs were found on chickling vetch cv. Pulse (1,498; 0.33) and sunnhemp (799; 0.18), respectively among the tested cover crops in the first trial (Table 4.2). The SCN2W population had significant differences among cover crops and controls for mean FPD in 2019 (Table 4.2). Soybean (Barnes) had the highest mean FPD and Rf (21,160; 3.54), and FPD in soybean (Barnes) was significantly ($P \leq 0.05$) greater than the rest of the entries. All the tested cover crops, except chickling vetch (Pulse), had significantly lower mean FPD than unplanted natural soil (1,723; 0.29). Among the cover crops, chickling vetch (Pulse) had the highest final nematode population (1,659; 0.28) and sunnhemp (CNS) had the lowest final nematode population (816; 0.14) at harvest (Table 2).

In 2020, all tested cover crops and fallow resulted in significantly ($P \leq 0.05$) lower mean FPD of SCN103 than soybean (6,565; 2.46) (Table 4.3). Similarly, four cover crop entries, including sunnhemp (CNS), oilseed radish (Concorde), oilseed radish (Control), and daikon radish (Eco-Till), had significantly lower mean FPD than unplanted natural soil (1,716; 0.64). Similar to the previous year, Sunnhemp (CNS) had the lowest FPD and Rf (1,189; 0.45),

whereas oilseed radish (Image) had the highest FPD and Rf (1,664; 0.63) among the cover crops (Table 4.3). Soybean (Barnes) produced a significantly higher mean FPD (32,380) of SCN2W than the rest of the treatments in 2020 (Table 4.3). In this trial, chickling vetch (Pulse) had a greater SCN2W population and Rf (2,094; 0.79) than fallow treatment (1,555; 0.58). All other cover crops had lower mean FPD than fallow, but the mean FPD on five cover crops, including sunnhemp (CNS), alfalfa (Bullseye), daikon radish (Eco-Till), oilseed radish (Concorde), and flax (Carter), were significantly lower. Sunnhemp continued to result in the lowest final population (618; 0.23) of SCN.

SCN Population Suppression Compared with Fallow

All the tested cover crops suppressed the SCN103 populations in infested soil compared with fallow in 2019 (Table 4.2). However, soybean (Barnes) did not suppress the nematode as it increased the densities of both SCN populations. Sunnhemp (CNS) caused the greatest nematode suppression (62.49%), while suppression by chickling vetch (Pulse) was the lowest (30.86%) for SCN103 among the cover crops. In 2019, all the cover crops suppressed the SCN2W population compared to unplanted natural soil. Sunnhemp (CNS) induced significantly greater suppression (51.64%) than other cover crops. The nematode population was suppressed by 2.03% in the pots containing chickling vetch (Pulse) compared with the fallow treatment (Table 4.2).

Table 4.2. Final SCN population densities, reproductive factors (Rfs), and population suppression (%) of SCN103 and SCN2W by cover crops in comparison with unplanted natural soil from microplot experiment 2019[v].

Treatments	SCN103			SCN2W		
	Final Population[x]	Rf[y]	Suppression (%)[z]	Final Population	Rf	Suppression (%)
Soybean (Barnes)	13,906 a	3.07	-559.21	21,160 a	3.54	-1126.00
Unplanted natural soil	2,207 b	0.49	0.00	1,723 b	0.29	0.00
Chickling vetch (Pulse)	1,498 c	0.33	30.86 b	1,659 bc	0.28	2.03 e
Flax (Carter)	1,463 c	0.32	31.32 b	1,575 cd	0.27	8.27 cde
White mustard (Master)	1,418 c	0.31	34.50 b	1,589 cd	0.27	7.35 de
Oilseed radish (Image)	1,268 c	0.28	40.43 b	1,457 ef	0.24	14.44 bcd
Oilseed radish (Concorde)	1,247 c	0.28	41.11 b	1,537 de	0.26	9.36 bcde
Oilseed radish (Control)	1,159 c	0.26	45.82 ab	1,401 f	0.23	18.41 bc
Alfalfa (Bullseye)	1,119 c	0.25	47.28 ab	1,380 f	0.23	19.52 b
Faba bean (Petite)	1,099 cd	0.24	47.39 ab	1,557 d	0.26	8.56 cde
Daikon radish (Eco-Till)	1,084 cd	0.24	48.43 ab	1,445 f	0.24	15.94 bcd
Sunnhemp (CNS[w])	799 d	0.18	62.49 a	816 g	0.14	51.64 a
P > F	< .0001		< .0001	< .0001		< .0001

[v] The initial population densities of SCN (eggs and juveniles) per 200 g of soil for each treatment were 4,524 and 5,974 for SCN103 and SCN2W, respectively.
[w] CNS = cultivar not stated
[x] Mean final SCN population (eggs and juveniles) per 200 g of soil for each treatment, including unplanted natural soil (fallow), and soybean (Barnes) averaged from five replicates. Average final population with same letters are not significantly different from each other.
[y] Reproductive factor (Rf) was the mean Rf for each treatment averaged from five replicates and was calculated by dividing the final SCN population density by the initial SCN population density.
[z] Average population suppression (%) by each crop compared with unplanted natural soil (fallow) averaged for five replicates and was calculated as [(final SCN population on fallow – final SCN population on tested crop)/final SCN population on fallow] × 100. Negative (-) value of suppression (%) indicates a higher final nematode population in the treatment compared to unplanted natural soil. Average population suppressions with same letters are not significantly different from each other in each column. Treatments with negative suppression (%) were not included for statistical analysis as they did not suppress SCN compared to fallow.

In 2020, all tested cover crops, except chickling vetch (Pulse), suppressed both SCN populations compared with fallow (Table 4.3). Similar to the first trial, sunnhemp (CNS) produced the greatest suppression (30.46%) of SCN103, whereas suppression by oilseed radish (Image) was the lowest (1.88%) among the tested cover crops. The suppression of SCN2W was

greatest (60.03%) in plots containing sunnhemp (CNS), whereas white mustard (Master) resulted in the least nematode suppression (11.05%) in 2020 (Table 4.3). Chickling vetch (Pulse) could not suppress the SCN2W population, other than natural mortality, compared with the unplanted natural soil. Similarly, soybean (Barnes) did not suppress the SCN2W population as it supported nematode reproduction, and the FPD on soybean (Barnes) was greater than the FPD on fallow (Table 4.3).

Table 4.3. Final SCN population densities, reproductive factors (Rfs), and population suppression (%) of SCN103 and SCN2W by cover crops in comparison with unplanted natural soil from microplot experiment 2020[v].

Treatments	SCN103			SCN2W		
	Final Population[x]	RF[y]	Suppression (%)[z]	Final Population	RF	Suppression (%)
Soybean (Barnes)	6,565 a	2.46	-284.25	32,380 a	12.13	-1,991.00
Unplanted natural soil	1,716 b	0.64	0.00	1,555 bc	0.58	0.00
Oilseed radish (Image)	1,664 bc	0.63	1.88 e	1,296 bcd	0.49	16.25 de
Chickling vetch (Pulse)	1,631 bcd	0.61	4.24 de	2,094 b	0.79	-36.8
Faba bean (Petite)	1,505 bcd	0.57	11.96 cd	1,077 cde	0.41	30.01 bcd
White mustard (Master)	1,465 bcd	0.55	14.41 cd	1,376 bcd	0.52	11.05 e
Flax (Carter)	1,445 bcd	0.54	15.66 bc	1,037 de	0.39	32.97 bcd
Alfalfa (Bullseye)	1,421 bcde	0.53	16.65 abc	818 ef	0.31	47.13 ab
Daikon radish (Eco-Till)	1,342 cde	0.50	21.59 abc	937 ef	0.35	38.69 abc
Oilseed radish (Control)	1,257 de	0.47	26.49 abc	1,176 cde	0.44	24.06 cde
Oilseed radish (Concorde)	1,197 de	0.45	29.38 ab	977 de	0.37	37.35 abc
Sunnhemp (CNS[w])	1,189 e	0.45	30.46 a	618 f	0.23	60.03 a
P > F	< .0001		0.0027	< .0001		0.001

[v] The initial population densities of SCN (eggs and juveniles) per 200 g of soil for each treatment were 2,670 for both SCN103 and SCN2W.
[w] CNS = cultivar not stated
[x] Mean final SCN population (eggs and juveniles) per 200 g of soil for each treatment, including unplanted natural soil (fallow), and soybean (Barnes) averaged from five replicates. Average final population with same letters are not significantly different from each other.
[y] Reproductive factor (Rf) was the mean Rf for each treatment averaged from five replicates and was calculated by dividing the final SCN population density by the initial SCN population density
[z] Average population suppression (%) by each crop compared with unplanted natural soil (fallow) averaged for five replicates and was calculated as [(final SCN population on fallow – final SCN population on tested crop)/final SCN population on fallow] × 100. Negative (-) value of suppression (%) indicates a higher final nematode population in the treatment compared to unplanted natural soil. Average population suppressions with same letters are not significantly different from each other in each column. Treatments with negative suppression (%) were not included for statistical analysis as they did not suppress SCN compared to fallow.

SCN Population Reduction Compared with Initial Population Density

The percentages of SCN population reduction by cover crops and unplanted natural soil were significantly different ($P \leq 0.05$) among them for both SCN populations in both trials (Fig. 4.1 and Fig. 4.2). In 2019, all the tested cover crops reduced the initial nematode population of SCN103 from the microplot (Fig. 4.1). The SCN reduction percentages on those crops were significantly ($P \leq 0.05$) greater than the nematode reduction percentage in unplanted natural soil (51.17%). Sunnhemp (CNS) reduced the highest SCN population density (82.32%), followed by daikon radish cv. Eco-Till (76.02%) and faba bean cv. Petite (75.69%). The population reductions in remaining crops ranged from 66.84% in chickling vetch (Pulse) to 75.24% in alfalfa (Bullseye). For SCN2W, all the cover crops, except chickling vetch (Pulse), flax (Carter), white mustard (Master), faba bean (Petite), and oilseed radish (Concorde), had significantly higher percentages of SCN population reductions than fallow treatment (71.21%) in 2019 (Fig. 4.1). Sunnhemp (CNS) produced the highest reduction (86.27%), followed by alfalfa cv. Bullseye (76.9%), oilseed radish cv. Control (76.57%), daikon radish cv. Eco-Till (75.90%), and oilseed radish cv. Image (75.56%). Chickling vetch (Pulse) caused the lowest reduction (72.21%) of SCN among the cover crops (Fig. 4.1). However, soybean (Barnes) increased the initial population of SCN103 and SCN2W by 207% and 254%, respectively, in 2019.

In 2020, all cover crops reduced the initial SCN103 population from the infested soil, but only four cover crops, including sunnhemp (CNS), oilseed radish (Concorde), oilseed radish (Control), and daikon radish (Eco-Till), caused significantly ($P \leq 0.05$) higher reduction than fallow treatment (35.58%) (Fig. 4.2). Sunnhemp (CNS) resulted in the greatest reduction (55.36%) among tested crops, followed by 55.06% by oilseed radish (Concorde), 52.81% by oilseed radish (Control), and 49.63% by daikon radish (Eco-Till). The lowest SCN reduction

(37.53%) was in oilseed radish (Image). For SCN2W, all cover crops reduced the initial nematode population density from the microplot in 2020 (Fig. 4.2). However, only sunnhemp (CNS), alfalfa (Bullseye), daikon radish (Eco-Till), and oilseed radish (Concorde) reduced the population significantly more than the unplanted natural soil. The highest nematode reduction was demonstrated by sunnhemp (76.78%), followed by alfalfa cv. Bullseye (69.29%), daikon radish cv. Eco-Till (64.79%), and oilseed radish cv. Concorde (63.30%). The reduction of SCN2W by chickling vetch cv. Pulse (21.35%) was lower than fallow treatment (41.57%). In contrast, soybean (Barnes) increased the initial population of SCN103 and SCN2W by 146% and 1,113%, respectively, in 2020.

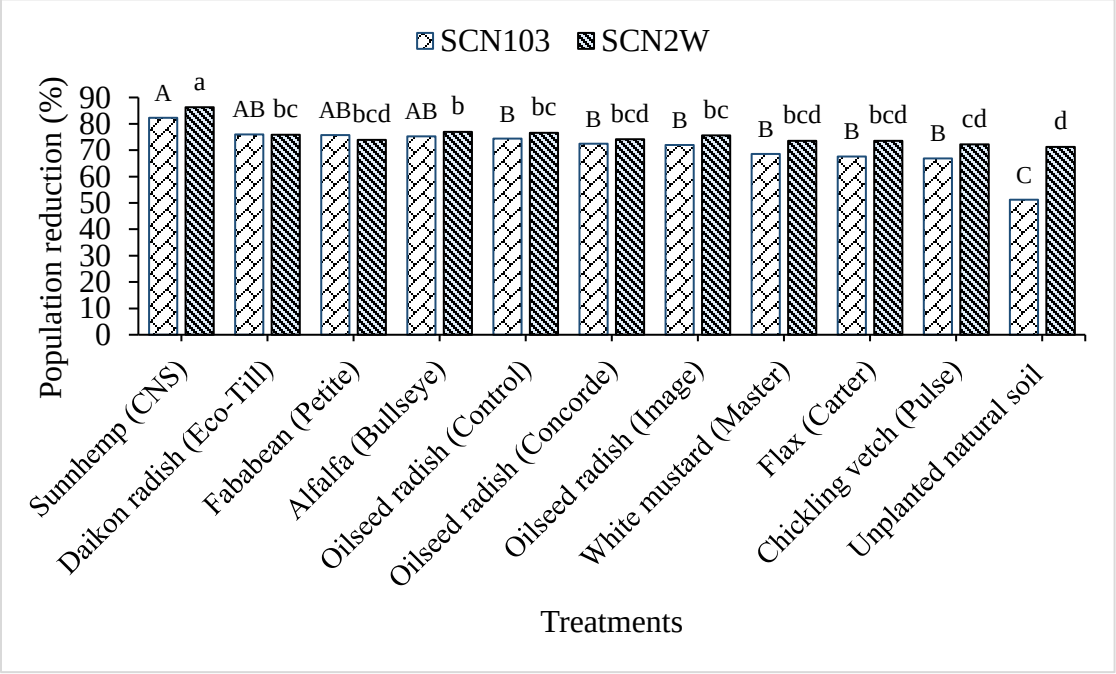

Fig. 4.1. Population reduction (%) of SCN103 and SCN2W by different cover crops and unplanted natural soil in microplot experiment conducted in 2019. CNS = cultivar not stated. The population reduction (%) was calculated by using the formula: [(initial SCN population density on the tested treatment – final SCN population density on the tested treatment)/initial SCN population density on the tested treatment] × 100. Population reduction (%) was the mean of population reductions (%) from five replicates for each SCN population. Treatments with same capital letters are not significantly different from each other for mean population reduction (%) for SCN103 ($P \leq 0.05$) and treatments with same lowercase letters are not significantly different from each other for mean population reduction (%) for SCN2W ($P \leq 0.05$).

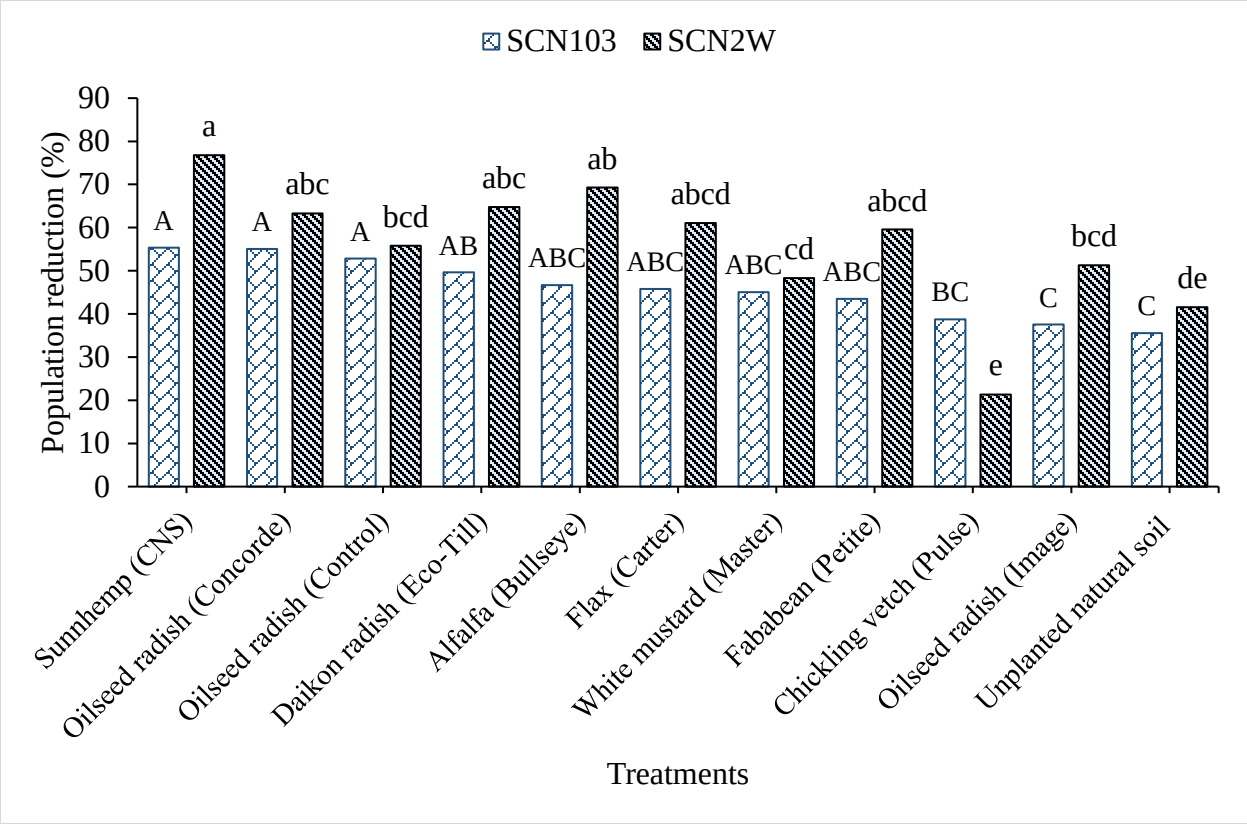

Fig. 4.2. Population reduction (%) of SCN103 and SCN2W by different cover crops and unplanted natural soil in microplot experiment conducted in 2020. CNS = cultivar not stated. The population reduction (%) was calculated by using the formula: [(initial SCN population density on the tested treatment – final SCN population density on the tested treatment)/initial SCN population density on the tested treatment] × 100. Population reduction (%) was the mean of population reductions (%) from five replicates for each SCN population. Treatments with same capital letters are not significantly different from each other for mean population reduction (%) for SCN103 ($P \leq 0.05$) and treatments with same lowercase letters are not significantly different from each other for mean population reduction (%) for SCN2W ($P \leq 0.05$).

Discussion

This study evaluated ten cover crops species/cultivars for their effects in reducing two SCN populations from naturally infested soil under outdoor microplot conditions. All the tested cover crops, except chickling vetch (Pulse), were evaluated in a greenhouse experiment and found to be non-hosts for two SCN populations (SCN103 and SCN2W) (Acharya et al. 2020). Susceptible soybean (Barnes) produced the significantly higher FPD of both SCN populations in both years than all the tested cover crops and unplanted natural infested soil (fallow). All the

cover crops reduced the initial densities of both nematode populations in both years. Similarly, almost all the cover crops suppressed both SCN populations compared with fallow, which suggested that cover crops caused the mortality of SCN other than natural reduction in fallow soil. However, chickling vetch (Pulse) did not suppress SCN2W in 2020 as it had a greater final population than unplanted natural soil. To our knowledge, none of the cultivars of cover crops, except oilseed radish cv. Image, studied in this research were evaluated before for SCN population reduction and suppression. Furthermore, the crops chickling vetch and flax had never been tested for their ability to reduce and suppress SCN from infested soil before.

In the present study, the responses of cover crops were determined by calculating the nematode population reduction compared with the initial population density and the population suppression compared with the final population density on the unplanted natural soil (fallow). Population reduction reveals the overall change in nematode population in the rhizosphere of a particular cover crop over the period of plant growth. It requires having information on the pre-plant and post-harvest nematode population densities in the soil to evaluate crops based on the population reduction percentage. Additionally, reproductive factors were calculated using pre-plant and post-harvest densities, which is generally used for the categorization of host ranges of crops to vermiform plant-parasitic nematodes, including root-lesion nematodes (Mbiro and Wesemael 2016; Schomaker et al. 2013; Smiley et al. 2014). Population suppression presents the change in nematode population densities other than from the natural mortality occurred in fallow soil. It requires comparing the post-harvest population densities on tested crops and fallow based on the population suppression percentage.

Sunnhemp (CNS) consistently caused the highest reduction of both SCN populations from the infested soil in both trials. Species of sunnhemp have been extensively evaluated for

their effect on reducing plant-parasitic nematodes from infested fields from different geographical regions (Kushida et al. 2003; Sedaghatjoo et al. 2017; Wang et al. 2002a, 2004). The population reduction of SCN from the infested soil was significantly higher in plots containing sunnhemp than unplanted natural soil in pot and field trials conducted in Japan (Kushida et al. 2003). Sedaghatjoo et al. (2017) found two cultivars of sunnhemp (Tillage Sunn and Tropic Sun) to be resistant against five species of cyst nematodes. Warnke et al. (2006) also reported the lowest SCN population in sunnhemp evaluated in greenhouse conditions. The effectiveness of sunnhemp against different plant-parasitic nematodes is attributed to several mechanisms. They can reduce the nematode density from the soil by acting as a non-host or poor host (Rodríguez-Kábana et al. 1992), by producing toxic or inhibitory chemicals (Jasy and Koshy 1994; Wang et al. 2001), acting as a trap crop (Kushida et al. 2003; Chen et al. 2008), and enhancing the antagonistic micro-organisms in the soil (Wang et al. 2002b, 2004). One or a combination of these mechanisms might have resulted in SCN reduction in our experiment. However, since sunnhemp is a warm-season annual (Schomberg et al. 2007), its practical utilization seems challenging in cold regions especially if it is fall-planted or planted after soybean harvest. Moreover, sunnhemp is a suitable host for many vermiform plant-parasitic nematodes, including root-lesion nematodes (*Pratylenchus* spp.) (Neupane and Yan, unpublished; Wang et al. 2002a)., which needs to be considered when they are used in the fields. Nonetheless, its evaluation under temperate growing conditions showed the increased richness of free-living nematodes in field conditions (Hinds et al. 2013).

Among other cover crops from the Fabaceae family, alfalfa (Bullseye) consistently showed significant reductions of both SCN populations compared with unplanted natural soil. Our observation is supported by previous studies (Kobayashi-Leonel et al. 2017; Riga et al.

2001; Warnke et al. 2006) that reported alfalfa as a non-host legume to SCN. Alfalfa cv. Apollo Supreme induced significantly higher hatching of SCN juveniles from encysted eggs than water and effectively reduced the neutral lipid content in the juveniles, which is responsible for reduced infectivity of many cysts and root-knot nematode species (Riga et al. 2001). However, chickling vetch (Pulse) caused the lowest reductions and suppressions of both SCN populations among the tested cover crops in most trials. The population reduction of SCN populations by chickling vetch was statistically similar to unplanted natural soil in both years, except for SCN103 in 2019. It even had a higher mean final population of SCN2W than the fallow treatment in 2020 showing a negative suppression value. Chickling vetch (Pulse) was found to be a poor host for SCN103 and SCN2W in greenhouse experiments (Yan, unpublished), which may explain the lowest reduction of nematode populations in the present experiments.

Five cover crops of the family Brassicaceae were tested in this research, including oilseed radish (Concorde, Control, and Image), daikon radish (Eco-Till), and white mustard (Master). Daikon radish (Eco-Till) and two oilseed radish cultivars (Concorde and Control) consistently and significantly reduced the population of SCN103 than fallow in 2019 and 2020. For SCN2W, only daikon radish (Eco-Till) resulted significantly greater nematode reductions than fallow in both trials. Similar to this finding, daikon radish (CNS) had consistently reduced the initial population of two SCN populations (SCN103 and SCN2W) in microplot experiments conducted by Acharya et al. (2021). Our experiments showed some variations in reduction and suppression percentages of SCN populations by different cultivars of oilseed radish. That emphasizes the significance of evaluating available cultivars of cover crops for nematode reduction and suppression before using them in the fields. Several other cover crops of the family Brassicaceae have been tested against different plant-parasitic nematodes, including SCN, and found effective

in reducing them in infested soil (Acharya et al. 2019, 2021; Harbach et al. 2021; Ngala et al. 2015). Biofumigation by brassica crops is considered the main mode of action for nematode suppression in the infested fields (do Nascimento et al. 2016; Dutta et al. 2019; Ngala et al. 2015), but the biofumigation effect was not evaluated in the present study.

We found consistent reproduction of both SCN populations on susceptible soybean (Barnes), despite a greater increase of SCN2W in the second trial. This increase could be due to better soybean growth with vigorous root growth providing more feeding sites to SCN in the second trial. Variation was observed in the SCN reductions in cover crops in trials for two years. Trials conducted in 2020 had relatively lower declines of both SCN populations on cover crops compared with the trial in 2019. In 2020, the plants experienced early frost during the second week of September that affected their growth, and some pots had reduced plant densities than the standard set at the beginning of the experiment, which may explain reduced effect of cover crops against SCN populations in the second trials. Similarly, we observed variations in the population reductions of SCN from unplanted natural soil, ranging from almost 36% to 71% for two years. Previous studies also have shown variations in the reduction of SCN populations in fallow (Acharya et al. 2021; Kushida et al. 2003). Several biotic and abiotic factors may affect the decline in SCN populations from unplanted soil, including the spontaneous hatching of infective juveniles, which itself is dependent on multiple factors such as temperature, soil moisture, plant root exudates, and soil pH (Davis and Tylka 2021; Duan et al. 2009; Niblack et al. 2006; Tefft et al. 1982; Thompson and Tylka 1997).

This research studied the reduction and suppression of two SCN populations (HG type 0 and HG type 7) by different cover crop species/cultivars in North Dakota. Based on the results presented, sunnhemp (CNS), oilseed radish (Concorde and Control), and daikon radish (Eco-Till)

showed significant reductions in SCN103 population compared with fallow. Three cover crops, sunnhemp (CNS), alfalfa (Bullseye), and daikon radish (Eco-till), demonstrated significant reductions in SCN2W population than fallow. Additionally, sunnhemp (CNS) consistently exhibited the greatest reduction and suppression of two SCN populations in all trials conducted. We observed variable effects of different oilseed radish cultivars on the same SCN population, which emphasizes evaluating all available cultivars of cover crops for finding effective cultivars against nematode. Moreover, further research would be beneficial to assess agronomic practices, including their integration into the current farming system (Berti et al. 2021; Chen et al. 2006). Some of the cover crops, such as sunnhemp, need further evaluation for their performance and growth, despite significant SCN population reduction in our trials. As sunnhemp is not a frost-tolerant crop, a slight drop in temperature below 0°C could kill it when planted after soybean or wheat harvest in the fall (Mansoer et al. 1997). Furthermore, studying the mechanisms behind their suppressive effects would help their selections and utilizations in different cropping systems for managing SCN.

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CHAPTER 5: EFFECTS OF COVER CROPS ON HATCHING AND ROOT PENETRATION OF SOYBEAN CYST NEMATODE (*HETERODERA GLYCINES*)

Abstract

The use of non-host cover crops can be a viable strategy for managing soybean cyst nematode (SCN). Cover crops may affect SCN biology and reduce populations by acting as trap crops which promote the hatching and/or penetration by second-stage juveniles (J2s) without supporting their development. Two growth chamber experiments were conducted to assess ten cover crops for their impacts on SCN hatching and penetration into the roots. For each experiment with four replicates, crops were planted in naturally infested soil in two separate sets to be harvested 15 and 30 days after planting (DAP). Soybean cysts nematode-susceptible soybean [*Glycine max* (L.) Merr.] (cv. Barnes) and unplanted natural soil (fallow) were used as controls. Faba bean (*Vicia faba* Roth) (cv. Petite), a non-host of SCN, had the highest hatching rate among the cover crops, which was statistically similar to soybean. Turnip (*Brassica rapa* L.) (cv. Purple Top) and red clover (*Trifolium pratense* L.) (cv. Allington) also showed significant hatching compared with hatching numbers in fallow. Root staining revealed that the greatest number of J2s penetrated the faba bean roots 15 DAP, followed by soybean and winter rye (*Secale cereale* L.) (cv. ND Dylan). While J2s penetrated all tested crops, they completed their development to become adult females only in soybean and turnip. The SCN development to adult females did not occur in faba bean, and the number of SCN inside the faba bean roots 30 DAP was significantly lower than 15 DAP and significantly lower than in soybean. These results suggest that the faba bean affects SCN biology and has the greatest potential to act as a trap crop for managing SCN.

Keywords: soybean cyst nematodes (SCN), *Heterodera glycines*, cover crops, trap crops, hatching, penetration, nematode development, management

Introduction

Soybean [*Glycine max* L. (Merr.)] is a globally important leguminous crop and a great source of plant protein and vegetable oil for human consumption. Also, it is the largest source of protein in animal feed in the world (USDA-NASS 2020). The United States (U.S.) ranks second in world soybean production with a production of 96.89 million MT (ASA 2020). North Dakota has the fourth largest total soybean acreage of U.S. states, with an annual production of about five million metric tons (USDA-NASS 2020). Plant-parasitic nematodes are one of the most detrimental biotic factors that affect plant growth ultimately reducing soybean yield (Koenning and Wrather 2010).

Soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe) is the biggest yield-limiting factor of soybean among different diseases in the world (Hartman et al. 2011; Koenning and Wrather 2010). This obligate sedentary endo-parasitic nematode causes annual economic loss of more than 1.5 billion dollars in the U.S. (Allen et al. 2017; Bandara et al. 2020; Bradley et al. 2021). Moreover, SCN can cause more than 30% yield loss without visible above-ground symptoms (Niblack 2005; Wang et al. 2003). This pathogen was first discovered in the U.S. in North Carolina in 1954 (Winstead et al. 1955), and it has now spread to almost every soybean-producing state (Tylka and Marett 2021). Similarly, SCN had spread to at least 19 counties of North Dakota by 2015 (Yan et al. 2015), and its spread has continued because of increased soybean production acreage in the state (Tylka and Marett 2017, 2021). The use of resistant cultivars along with crop rotation is the primary strategies for SCN management (Mueller et al. 2016; Niblack et al. 2006). However, continued use of a single source of resistance has resulted

in the increase of SCN virulence that challenges the current management strategies (Chowdhury et al. 2021; Niblack et al. 2008; Yan and Baidoo 2018). The resistance in current SCN-resistant cultivars could be overcome by new virulent SCN populations resulting in the need of alternative methods for better management of SCN (McCarville et al. 2017).

The use of cover crops by farmers in the U.S. has become increasingly popular (Conservation Technology Information Center 2017). Cover crops prevent soil erosion and positively affect soil health, land productivity, and pest management (Clark 2007; Weerasakera et al. 2017). Moreover, cover crops can be utilized for nematode management depending on nematode species and cover crops species/cultivars (Abawi and Widmer 2000; Wang et al. 2002). Cover crops can reduce nematodes population densities from the soil through one or more mechanisms, such as producing nematicidal/allelochemical compounds, providing suitable conditions for antagonistic organisms, and acting as trap crops (Niblack and Chen 2004; Tylka et al. 2014). A cover crop would be a successful trap crop if it allows a large number of second-stage juveniles (J2s) to penetrate the roots but does not permit the completion of the nematode life cycle. The nematode could fail to initiate feeding sites, or the feeding sites might get degenerated by resistant host response (Wang et al. 2003). Similarly, trap crops may enhance nematode hatching in the absence of a suitable host resulting in the starvation of J2s (Hooks et al. 2010; Riga et al. 2001). However, susceptible cover crops or cultivars could be utilized as trap crops if they are destroyed before the nematodes complete their development (LaMondia 1996; Whitehead 1977).

Several studies have reported the reductions of plant-parasitic nematode population densities from infested fields by trap crops. Oilseed radish (*Raphanus sativa* cultivars Adagio and Colonel) and white mustard (*Sinapis alba* cultivars Accent and Luna) can act as trap crops

for sugarbeet cyst nematode (*Heterodera schachtii*) and result in a significant reduction of nematode reproduction (Hemayati et al. 2017; Wright et al. 2018). Similarly, sticky nightshade (*Solanum sisymbriifolium* Accession PI 381291) has shown its efficiency as a trap crop against three species of potato cyst nematode, *Globodera* spp., in three locations of the U.S. It was also reported that the prolonged exposure of nematodes to a trap crop increased the percentage reduction of nematode populations (Dandurand et al. 2019). The trapping mechanisms of marigold species (*Tagetes* spp.) have been well established for root-knot nematodes (*Meloidogyne* spp.) and root-lesion nematode (*Pratylenchus penetrans*) (Hooks et al. 2010).

As previously mentioned, cover crops with the potential to act as trap crops can enhance the nematode hatching, but ultimately result in the starvation of hatched juveniles. Riga et al. (2001) tested the root exudates (REs) of several crops for their effects on the hatching of SCN eggs. They found annual ryegrass (*Lolium multiflorum* L.) and white clover (*Trifolium repens* L.) to induce greater hatching than susceptible soybean. Also, REs from those crops enhanced the depletion of the neutral lipid content of juveniles, leading to starvation and reduced infectivity. Similarly, Harbach et al. (2021) revealed crimson clover (*T. incarnatum* L.) as the best potential trap crop for SCN among the tested crops based on the number of J2s hatched in its REs and soil leachates (SLs) and the juveniles inside its roots. Two species of sunnhemp (*Crotalaria juncea* and *C. spectabilis*) were tested as trap crops against SCN by Kushida et al. (2003). They found significantly greater hatching of SCN eggs in sunnhemp REs and similar penetration of roots by J2s as in soybean. However, most of the SCN inside the sunnhemp roots did not complete their life cycle. Chen et al. (2008) also reported sunnhemp, along with red clover (*Trifolium pretense*), having a similar effect as susceptible soybean on SCN eggs and J2s. Sunnhemp and red clover

stimulated the hatching of eggs and allowed the J2 penetration, but they did not allow complete development of penetrated J2s into adults.

Some studies have evaluated cover crops from the northern Great Plains for their hosting and population reduction abilities against common SCN populations (Acharya et al. 2019, 2020, 2021). Similarly, we evaluated additional cover crop species and cultivars for their effects on population reductions of two SCN populations from North Dakota in outdoor microplot conditions (Neupane et al. unpublished data). All the cover crops tested were able to reduce the initial nematode population density, and they had significantly lower final population density than found with susceptible soybean (Barnes). However, the mechanisms behind those reductions in population densities of SCN are unclear. One or combination of two or more mechanisms, as described previously, could play roles in suppressing the SCN reproduction in naturally infested soil. The objectives of this study were to evaluate cover crops for their effects on the hatching of SCN eggs and to compare the penetrations of roots by second-stage juveniles (J2s) among the tested crop species and cultivars.

Materials and Methods

Selection of Cover Crop Species and Cultivars

Ten cover crops were evaluated for their effect on SCN eggs hatching and penetration of roots by SCN juveniles (Table 5.1). Cover crops tested in this study belong to three plant families, including Brassicaceae, Fabaceae, and Poaceae. Among these test crops, turnip (cv. Purple Top) was described as a poor host and the remaining test cover crops were described as non-hosts of SCN (Acharya et al. 2020). Soybean (cv. Barnes), susceptible to SCN, was included as a positive control, and an unplanted natural field soil (fallow) as a negative control. A common rotation crop in our region, corn (cv. DKC44-13), was included in the experiments for

comparison. Planting materials (seeds) were received from Forage and Biomass Crop Production Program (North Dakota State University, Fargo, ND, U.S.), Allied Seed (Nampa, ID, U.S.), and Great Northern AG (Plaza, ND, U.S.).

Table 5.1. List of cover crops and controls evaluated for SCN eggs hatching and penetration of their roots by second-stage juveniles under controlled growth chamber conditions and their host status to SCN.

Cover crops and controls	Scientific Name	Family	Host status[z]
Alfalfa (Bullseye)	*Medicago sativa* L.	Fabaceae	Non-host
Daikon radish (Eco-Till)	*Raphanus sativus* L.	Brassicaceae	Non-host
Faba bean (Petite)	*Vicia faba* Roth	Fabaceae	Non-host
Foxtail millet (Siberian)	*Setaria italica* subspp. *Rubofructa* (L.) P. Beauv.	Poaceae	Non-host
Oilseed radish (Concorde)	*Raphanus sativus* L.	Brassicaceae	Non-host
Oilseed radish (Control)	*Raphanus sativus* L.	Brassicaceae	Non-host
Red clover (Allington)	*Trifolium pratense* L.	Fabaceae	Non-host
Turnip (Purple Top)	*Brassica rapa* subsp. *rapa* L.	Brassicaceae	Poor host
White mustard (Master)	*Sinapis alba* L.	Brassicaceae	Non-host
Winter rye (ND Dylan)	*Secale cereale* L.	Poaceae	Non-host
Corn (DKC44-13)	*Zea mays* L.	Poaceae	Non-host
Soybean (Barnes)	*Glycine max* L.	Fabaceae	Host
Unplanted natural soil (fallow)			

[z] Host status of cover crops were determined by Acharya et al. (2020).

Soybean Cyst Nematode (*H. glycines*) Population

The SCN population used in this study was SCN103 (HG type 0). Soil naturally infested with SCN103 was collected from a soybean field in Richland County, North Dakota. The collected soil samples (sandy loam with 65% sand) were mixed comprehensively to distribute the nematode population evenly in the soil mixture. The mean initial population density was determined by extracting nematodes from three 200 g samples of mixed field soil using a combination of the nematode extraction methods described by Krushberg et al. (1994) and Jenkins (1964). The 200-g subsample was placed in a pitcher of 4-Lcapacity filled with three-

quarters water, subsequently vigorously stirred, and then poured into the sieve (mesh sizes of 710 µm, 250 µm, and 20 µm) arrangement placed from top to bottom. The top sieve caught the plant debris, while the middle sieve caught the SCN cysts. A sieve of mesh size 20 µm, placed at the bottom of the arrangement, trapped the SCN juveniles and soil. The sugar centrifugal flotation method (Jenkin 1964) was used to recover SCN juveniles from the soil. The nematodes and heavier soil particles were separated from the lighter particles by centrifuging the soil suspension at 4,000 rpm for 10 min. Nematodes were recovered from the remaining soil suspension by suspending them in 1.3 M sugar solution (American Crystal Sugar Company, Moorhead, MN, U.S.). The suspension was stirred and centrifuged for 30 s at 4,000 rpm, and the nematodes were collected from the supernatant in a 20-µm mesh sized sieve.

The eggs and J2s were released by crushing the extracted cysts using a rubber stopper attached to a motorized drill press (MasterForce Drill Press, Menards, Fargo, ND, U.S.) (Faghihi and Ferris 2000). The eggs and J2s were then quantified and added to juvenile numbers extracted from the sugar centrifugal flotation method (Jenkins 1964) to obtain the total initial population density. The initial nematode population densities for the first and second trials set up in a growth chamber were 2,300 and 2,200 per 200 g of soil, respectively. The crops were planted using cone containers (diameter - 3.8 cm, height - 21 cm; Stuewe and Sons, Inc., Tangent, OR, U.S.) that accommodated an average of 163.25 g and 171.13 g of soil in the first and second trial, respectively. Therefore, the initial population densities per experimental unit (container) in the first and second trials were 1,877 and 1,883, respectively.

Growth Chamber Experiments

The plants were grown in controlled environmental conditions (temperature of 27^{o}C and daylight period of 16 h) in a growth chamber (GR64, Conviron, Winnipeg, Manitoba, Canada)

for this study. The seeds of all the crops were directly planted into the soil in cone containers (diameter-3.8 cm, height-21 cm; Stuewe and Sons, Inc., Tangent, OR, U.S.), and the containers were placed in 14 × 7 -cell plastic racks. Cone containers were arranged in a completely randomized design, and each treatment had four replicates. Plants were thinned out to one plant per cone container 4 to 6 days after planting. However, the cone containers with foxtail millet and winter rye often had more than one plant remaining per experimental unit due to difficulties in removing their growing points from the soil. The cover crops and controls were planted in two sets for each of the two trials and then were harvested 15 and 30 days after planting (DAP). The first and second trials were set up in September of 2020 and December of 2021, respectively.

The plant set grown for 15 days was used to evaluate crop effect on SCN eggs hatching and penetration. Fifteen days were long enough for sufficient plant root development and root penetration by SCN J2s but not long enough for completion of SCN life cycle. The plant tops were discarded, and the remaining soil and root samples with the cone containers were stored at 4^{o}C until their processing. The soil and root samples were processed for nematode extraction and root staining, respectively. Soil sample and roots were gently placed in a 3-L jug containing water. The roots were recovered as intact as possible with minimum fragmentation by gently dislodging the soil particles in water. They were further washed to remove any remaining soil particles from the surface and were kept separately for the staining. A combination of methods described by Krushberg et al. (1994) and Jenkins (1964) was used to extract cysts and hatched juveniles from soil samples, as described before. The extracted cysts were crushed to release eggs and J2s using a rubber stopper attached to a motorized drill press (MasterForce Drill Press, Menards, Fargo, ND, U.S.) (Faghihi and Ferris 2000). The SCN juvenile numbers obtained from

the cysts crushing and from the soil sample using the sugar centrifugal flotation method were combined to obtain total J2s outside the root systems for the 15 DAP treatment.

Similarly, the plant set grown for 30 days was used to observe the development of SCN adults and the changes in numbers of SCN inside and outside of the plant roots. Thirty days were enough for SCN to complete the life cycle under suitable conditions (Niblack et al. 2006). The soil samples obtained from experimental units (cone containers) 30 DAP were processed using the same method for the plant set harvested 15 DAP. However, before crushing the cyst samples to release eggs and juveniles, root samples were observed and white females formed in the roots of crops were quantified. The white females were identified and counted using a dissecting microscope (SM 100 Series, Swift Optical Instrument, INC. TX, U.S.).

Root Staining Experiments

The roots from the harvested plants 15 DAP were stained using the method described by Thies et al. (2002) using red food coloring dye (McCormick & Co., Inc., Hunt Valley, MD, U.S.). Briefly, the roots were chopped into 1- to 2-cm pieces and agitated in 100 ml 1.5% NaOCl solution for 4 min. They were then rinsed with tap water for 30 s and placed in a beaker containing about 150 ml tap water for 15 min. After that, they were placed in 12.5% (v/v) food coloring dye solution (McCormick & Co., Inc., Hunt Valley, MD, U.S.), brought to boil for about 30 s, and left for cooling to room temperature. The dyed roots were rinsed with tap water, and 40 ml glycerol (VWR Chemicals, Solon, Ohio, U.S.) and a few drops of 5N HCl were added. This glycerol mix was then heated to about 40^{o}C and allowed to cool to room temperature and subsequently kept at 4^{o}C until roots were observed under a stereo microscope (ZEISS Stemi 508, Carl Zeiss Microscopy, White Plains, NY, U.S.). The total number of SCN of all life stages present inside the roots was added to the number of J2s outside the root system 15 DAP to

determine the total number of hatched J2s for each crop. For unplanted natural soil, only J2s obtained from soil samples were considered.

The roots obtained from plants 30 DAP were stained using the same methods used for the plants harvested 15 DAP. The numbers of SCN of all life stages present inside the roots of the crops, 15 and 30 DAP, were compared to evaluate the changes in the number of SCN that penetrated roots over the 15 days. The images of SCN inside the plant roots were taken using a microscope camera (ZEISS Axiocam 105 Color, Carl Zeiss Microscopy, White Plains, NY, U.S.) and analyzed with software ZEISS ZEN 3.0 (Blue Edition) (Carl Zeiss Microscopy, White Plains, NY, U.S.).

Data Analysis

Data obtained from the experiments were analyzed using the software SAS 9.4 (SAS Institute Inc., Cary, NC). Data were evaluated separately for the two trials as the data from two trials were not homogenous. An analysis of variance was performed using SAS PROC GLIMMIX, and the means of treatment effects were separated using Tukey's honestly significant difference (HSD), at a 5% significance level. The mean separation test was applied to the number of total hatched SCN, SCN J2s in the soil 15 and 30 DAP, and SCN (all life stages) in the roots 15 and 30 DAP. The mean number of hatched SCN was calculated using the formula; (mean SCN J2s in the soil 15 DAP + mean SCN in the roots 15 DAP – pre-plant SCN J2s). The numbers of SCN inside the roots 15 and 30 DAP were compared using a simple paired t-test ($P \leq 0.05$) to observe the changes in SCN inside the roots. The fallow treatment was excluded from analysis for the number of nematodes inside the roots 15 and 30 DAP as it did not have plants. The same paired comparison was conducted for the number of J2s in the soil 15 and 30 DAP. The changes in population densities from the beginning of the experiments to 15 DAP

and from 15 DAP to 30 DAP were also compared. The simple paired t-test was carried out between the initial nematode population density and the final population density (FPD) 15 DAP and between the FPD 15 DAP and FPD 30 DAP.

Results

Number of Hatched SCN in Soil and Roots

In the first trial, the mean number of hatched SCN ranged from 104 (white mustard) to 680 (soybean) (Fig. 5.1). The hatched SCN numbers in soybean, faba bean (621), turnip (329), and red clover (301) were significantly greater ($P < 0.05$) than hatching in unplanted infested soil (251). Faba bean had the highest hatching among the tested cover crops ($P \leq 0.05$). Hatching in corn, winter rye, and foxtail millet were not significantly different from unplanted soil control. However, other remaining cover crops had significantly fewer hatching numbers of SCN compared with unplanted infested soil in the first trial (Fig. 5.1).

Similarly, a significant difference ($P \leq 0.05$) was observed between the treatments for the mean hatched SCN in the second trial (Fig. 5.1). The number of hatched SCN ranged from 82 (white mustard) to 572 (soybean). Soybean, faba bean (523), turnip (259), daikon radish (252), corn (250), red clover (229), and alfalfa (190) had statistically greater hatched SCN than unplanted infested soil (152). In the second trial as well, the hatching was the greatest in faba bean among the cover crops, which was significantly greater than hatching on other cover crops. Winter rye and oilseed radish (cv. Concorde) were not significantly different from the unplanted infested soil control for hatched SCN. Foxtail millet, oilseed radish (cv. Control), and white mustard had significantly smaller numbers of hatched SCN than the unplanted infested soil (Fig. 5.1).

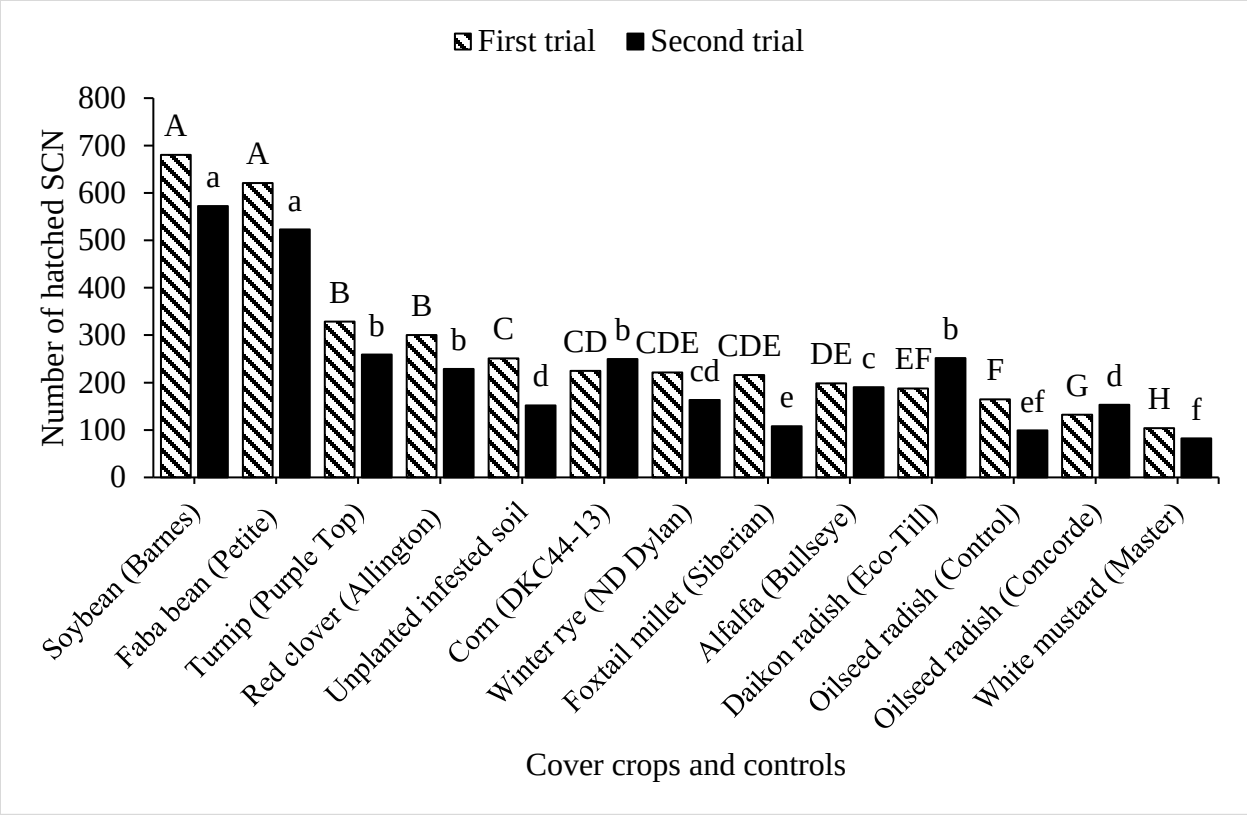


Fig. 5.1. Number of hatched SCN on cover crops and controls. Number of hatched SCN is the mean of total number of juveniles (J2s) in the soil and all life stages of SCN inside the roots 15 DAP minus the number of pre-plant juveniles (J2s). For unplanted infested soil, only number of J2s in the soil 15 DAP was considered as it did not have plants. Number of hatched SCN is the average of four replicates for each treatment in each trial. Number of hatched SCN with the same capital letter are not significantly different ($P > 0.05$) from each other for the first trial and number of hatched SCN with the same lowercase letter are not significantly different ($P > 0.05$) from each other for the second trial.

SCN Root Penetration and Hatched Juveniles in the Soil

A significant difference ($P \leq 0.05$) among the cover crops and controls occurred for the number of SCN inside the roots (Table 5.2). In the first trial, faba bean had the highest SCN (449) inside the roots, followed by soybean (414) at 15 DAP. The number of SCN inside soybean at 30 DAP (618) was significantly greater than that of faba bean (143) at 30 DAP. All the remaining crops had significantly lower SCN penetrated inside their roots than faba bean and soybean, both at 15 and 30 DAP. In the second trial, we observed a trend similar to the first trial for nematode number inside the roots of cover crops and controls, both at 15 and 30 DAP. We

observed reductions of SCN 30 DAP compared with SCN 15 DAP inside the roots for most crops in both trials. However, soybean, white mustard, alfalfa, turnip, and red clover had greater SCN inside the roots 30 DAP than that 15 DAP (Table 5.2).

Table 5.2. Number of total SCN penetrated inside the roots of cover crops and controls 15 and 30 days after planting (DAP) in two growth chamber trials.

Cover crops (cultivar) and controls	Number of SCN inside the roots of crops			
	First trial		Second trial	
	15 DAP[y]	30 DAP[z]	15 DAP	30 DAP
Faba bean (Petite)	449 a	143* b	302 a	116* b
Soybean (Barnes)	414 a	618* a	295 a	452* a
Winter rye (ND Dylan)	144 b	72* cd	61 b	25* d
Corn (DKC44-13)	112 c	12* fg	4 g	0 f
Daikon radish (Eco- Till)	86 d	53* d	37 c	26* d
Oilseed radish (Control)	78 d	6* g	28 cd	4* f
Oilseed radish (Concorde)	67 de	14* fg	13 ef	4* f
White mustard (Master)	52 e	69* cd	23 de	32 d
Foxtail millet (Siberian)	24 f	16 fg	31 cd	6* ef
Alfalfa (Bullseye)	8 g	36* e	6 fg	12* e
Turnip (Purple Top)	7 g	75* c	25 cd	51* c
Red clover (Allington)	3 g	21* f	8 fg	31* d
P > *F*	< 0.0001	< 0.0001	< 0.0001	< 0.0001

[y] Number of SCN inside the roots of cover crops and controls 15 DAP is the mean of total SCN of all life stages inside the roots of cover crops and controls across four replicates for each treatment. Number of SCN inside the roots of crops 15 DAP with same letter are not significantly different ($P > 0.05$) from each other in both trials.
[z] Number of SCN inside the roots of cover crops and controls 30 DAP is the mean of total SCN of all life stages inside the roots of cover crops and controls across four replicates for each treatment. Number of SCN inside the roots of crops 30 DAP with same letter are not significantly different ($P > 0.05$) from each other in both trials.
* Numbers of SCN inside the roots 30 DAP with asterisk (*) are significantly different ($P \leq 0.05$) from numbers of SCN inside the roots 15 DAP of respective treatment for both trials.

All crop species evaluated in this study allowed the penetration of roots by SCN juveniles. We observed minimal development of SCN reaching third- or fourth-stage juveniles in faba bean (Fig. 5.2A) but penetrated juveniles did not develop into adult females during the experiment (Fig. 5.2B). There were few adult males observed in the roots of faba bean, daikon

radish, and white mustard. However, susceptible soybean (Fig. 5.3A and 5.3B) and turnip allowed the full development of SCN to adult females.

Table 5.3. Number of second-stage juveniles (J2s) in the soil 15 and 30 days after planting (DAP) in two growth chamber trials.

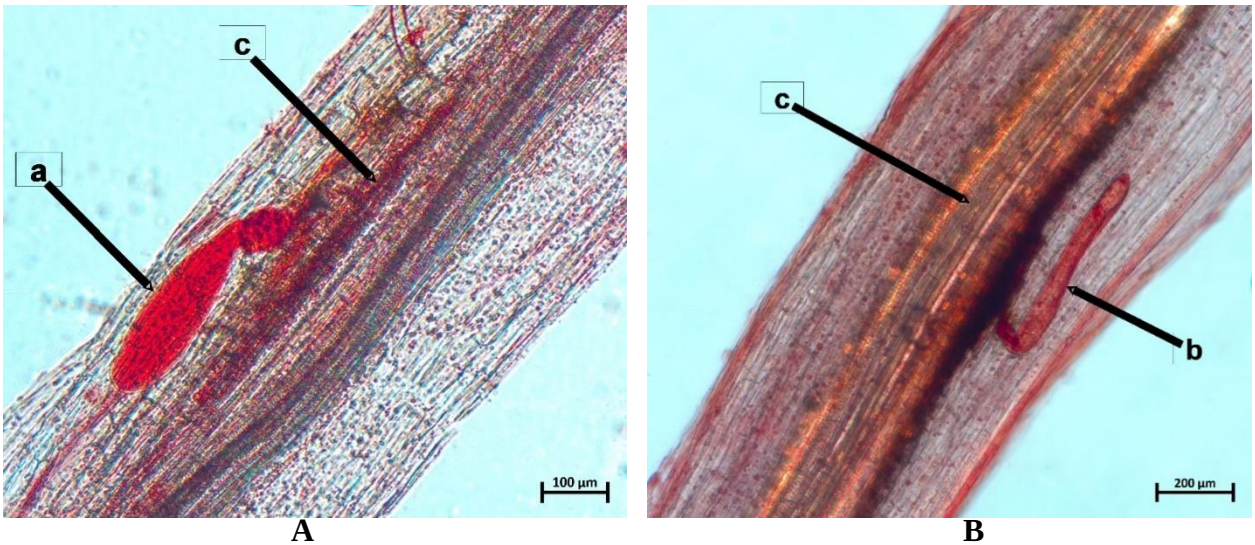
Cover crops (cultivars) and controls	Number of hatched J2s in the soil (outside the roots)			
	First trial		Second trial	
	15 DAP[y]	30 DAP[z]	15 DAP	30 DAP
Turnip (Purple Top)	431 a	240* d	363 ab	363 b
Red clover (Allington)	406 ab	175* e	350 bc	250* cd
Soybean (Barnes)	375 b	2,648* a	406 a	6,081* a
Unplanted infested soil	360 b	281* bc	281 de	275 c
Foxtail millet (Siberian)	301 c	300 b	206 gh	231 de
Alfalfa (Bullseye)	300 c	250* cd	313 cd	206* ef
Faba bean (Petite)	281 c	275 bcd	350 bc	231* de
Corn (DKC44-13)	223 d	150* ef	375 ab	131* g
Daikon radish (Eco- Till)	211 de	156* e	344 bc	181* f
Oilseed radish (Control)	196 def	150* ef	200 gh	231* de
Winter rye (ND Dylan)	186 efg	125* fg	231 fg	206 ef
Oilseed radish (Concorde)	175 fg	106* g	269 ef	281 c
White mustard (Master)	161 g	106* g	188 h	175 f
$P > F$	< 0.0001	< 0.0001	< 0.0001	< 0.0001

[y] Number of juveniles in the soil 15 DAP is the mean of the hatched juveniles in the soil 15 DAP obtained from sugar flotation centrifugal method and cyst crushing of four replicates for each treatment. Number of juveniles in soil 15 DAP with same letter are not significantly different ($P > 0.05$) from each other for each trial.

[z] Number of juveniles in the soil 30 DAP is the mean of the hatched juveniles in the soil 30 DAP obtained from sugar flotation centrifugal method and cyst crushing of four replicates for each treatment. Number of juveniles in soil 30 DAP with same letter are not significantly different ($P > 0.05$) from each other for each trial. Number of juveniles in the soil 30 DAP with the asterisk (*) are significantly different ($P \leq 0.05$) from the number of juveniles in the soil 15 DAP of respective treatment for each trial.

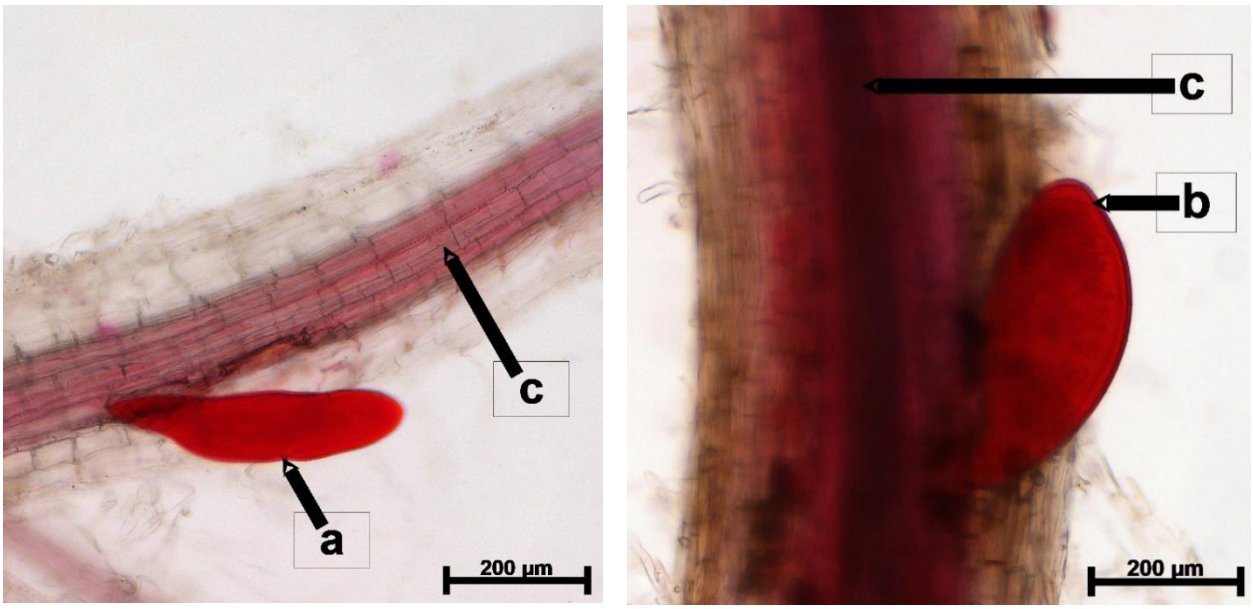
When the number of J2s in the soil were analyzed, we found considerable variations among treatments for both 15 and 30 DAP time points (Table 5.3). In the first trial, turnip had the highest J2s in soil (431) 15 DAP followed by red clover (406) and soybean (375). However, in the second trial, soybean had the highest J2s in the soil (406), followed by corn (275) and

turnip (363) at 15 DAP. Soybean had the greatest J2s (2,648) in soil 30 DAP followed by foxtail millet (300) and unplanted infested soil (281) in the first trial. In the second trial, the highest J2 numbers in soil were found in soybean (6,081) followed by turnip (363) and oilseed radish cv. Concorde (281). Most of the cover crops and controls had statistically similar or lower ($P \leq 0.05$) J2s in the soil 30 DAP than 15 DAP, except for soybean which had significantly higher numbers of J2s in the soil 30 DAP than 15 DAP.



A **B**

Fig. 5.2. SCN development in faba bean (Petite). **A.** A developing SCN juvenile inside a root 15 DAP. **B.** A degenerating SCN juvenile inside a root 30 DAP. **a** = a developing SCN juvenile, **b** = a degenerating SCN juvenile, **c** = vascular bundle.



A **B**

Fig. 5.3. SCN development in susceptible soybean (Barnes). **A.** A developing SCN juvenile inside a root 15 DAP. **B.** fully developed SCN white female still attached to the root 30 DAP. a = a developing SCN juvenile, **b** = a fully developed SCN white female, **c** = vascular bundle.

SCN Population Densities and their Changes 15 and 30 Days after Planting

The mean final population densities (FPD) of each treatment 15 and 30 DAP were compared with the initial population density and the FPD 15 DAP, respectively (Table 5.4). All the tested cover crops and controls had significantly lower ($P \leq 0.05$) FPD 15 DAP than the initial population densities in both trials. In the first trial, white mustard had the highest reduction (54%) with the lowest FPD (861) at 15 DAP. The unplanted infested soil had the highest FPD (1,635) and the lowest reduction (13%) of the SCN population compared with the initial population. On the other hand, oilseed radish (cv. Control) had the lowest FPD (963) 15 DAP in the second trial with the highest reduction (49%) of the initial population density. The least population reduction (17%) was observed in unplanted infested soil with the highest FPD (1,556) at 15 DAP.

Table 5.4. Final population density (FPD) 15 and 30 days after planting (DAP) and SCN population changes from pre-plant to 15 DAP and 15 DAP to 30 DAP in two growth chamber trials.

	First trial[v]				Second trial			
Cover crops (cultivars) and controls	FPD 15 DAP[w]	% Change (15DAP - 0DAP)[x]	FPD 30 DAP[y]	% Change (30DAP - 15DAP)[z]	FPD 15 DAP	% Change (15DAP - 0DAP)	FPD 30 DAP	% Change (30DAP - 15DAP)
Unplanted infested soil	1,635*	-13	1,456	-11	1,556*	-17	1,350	-13
Red clover (Allington)	1,406*	-25	950*	-32	1,413*	-25	1,250	-12
Corn (DKC44-13)	1,398*	-26	1,175	-16	1,369*	-27	906*	-34
Daikon radish (Eco-Till)	1,311*	-30	1,281	-2	1,394*	-26	1,256	-10
Turnip (Purple Top)	1,281*	-32	3,546*	177	1,363*	-28	1,889*	39
Oilseed radish (Concorde)	1,275*	-32	539*	-58	1,119*	-41	806	-28
Faba bean (Petite)	1,106*	-41	1,125	2	1,075*	-43	1,181	10
Soybean (Barnes)	1,050*	-44	68,213*	6,396	1,006*	-47	70,863*	6,944
Oilseed radish (Control)	1,021*	-46	1,125	10	963*	-49	1,144	19
Winter rye (ND Dylan)	1,011*	-46	1,125	11	1,031*	-45	1,156	12
Foxtail millet (Siberian)	976*	-48	1,250*	28	1,194*	-37	1,306	9
Alfalfa (Bullseye)	975*	-48	1,100	13	1,213*	-36	881*	-27
White mustard (Master)	861*	-54	1,063	23	1,238*	-34	1,175	-5

[v] First trial was conducted in September 2020 with initial population density of 1,877 (eggs + juveniles) and second trial was started in December 2021 with initial population density of 1,883 (eggs + juveniles) per cone-container.
[w] FPD (final population density) 15 DAP is the mean final population density of four replicates for each treatment at 15 DAP. It was calculated by adding total SCN eggs in soil to total hatched juveniles (J2s) in the soil 15 DAP. FPD 15 DAP with asterisk (*) are significantly different ($P \leq 0.05$) from pre-plant SCN population of respective treatment for each trial.
[x] Percentage change (15DAP - 0DAP) is the mean percentage change in FPD from beginning of the trial to 15 DAP from four replicates for each treatment. It was calculated by formula; (FPD at 15 DAP - pre-plant population density)/pre-plant population density × 100. Negative (-) sign indicates the FPD at 15 DAP is lower than pre-plant population.
[y] FPD 30 DAP is the mean final population density of four replicates for each treatment at 30 DAP. It was calculated by adding total SCN eggs in soil to total hatched juveniles (J2s) in the soil 30 DAP. FPD 30 DAP with asterisk (*) are significantly different ($P \leq 0.05$) from FPD at 15 DAP of respective treatment for each trial.
[z] Percentage change (30DAP - 15DAP) is the mean percentage change in FPD from 15 DAP to 30 DAP from four replicates for each treatment. It was calculated by formula; (FPD at 30 DAP - FPD at 15 DAP)/FPD at 15 DAP ×100. Negative (-) sign indicates the FPD at 30 DAP is lower than FPD at 15 DAP. Positive (+) sign indicates the FPD at 30 DAP is greater than FPD at 15 DAP.

The mean FPD 30 DAP for most crops and controls were statistically similar ($P > 0.05$) to FPD 15 DAP (Table 5.4). However, red clover, turnip, oilseed radish (cv. Concorde), soybean, and foxtail millet showed significant differences between FPD 15 and 30 DAP in the first trial. In the second trial, four treatments, including corn, turnip, soybean, and alfalfa, showed significant differences between FPD 15 and 30 DAP. We observed the formation of white females in two crops, turnip, and soybean at 30 DAP in both trials. Turnip had an average of five

and one white female in the first and second trials, respectively. Similarly, soybean had an average of 248 and 184 white females in the first and second trials, respectively.

Discussion

The present study investigated the potential of ten different cover crops as trap crops for managing SCN. Faba bean (Petite) had the most SCN juveniles in the roots and stimulated the greatest SCN hatching among the tested crops. The numbers of hatched J2s and SCN inside faba bean 15 DAP were similar to susceptible soybean. However, faba bean had a significantly lower number of SCN inside the roots at 30 DAP than 15 DAP, suggesting mortality of penetrated SCN. That was further proved by root staining observations as we did not find fully developed adult females in faba bean. Root staining images 15 DAP showed that juveniles in roots of both faba bean and soybean appeared to be drawing nutrients. However, those juveniles transitioned into white females only in susceptible soybean, whereas possible degeneration of those juveniles was observed inside faba bean roots 30 DAP. These results demonstrated that faba bean had the best potential to serve as a trap crop for SCN among the tested crops in our experiments. This crop was reported as a non-host to two SCN populations (Acharya et al. 2020), and we also observed population reduction of SCN in faba bean. To our knowledge, this is the first study to show the potential of faba bean as a trap crop for SCN.

All tested cover crops and controls had statistically lower mean final population densities (FPD) of SCN 15 DAP than the initial population densities in both trials. However, the mean FPD of SCN 30 DAP were statistically similar to population densities at 15 DAP for most crops, except for turnip and soybean which had significantly greater mean FPD 30 DAP than 15 DAP. This suggested the SCN population increased in turnip and soybean, which was also supported by white females present in both crops. As all the cover crops tested, except turnip, were non-

hosts, the greater population densities 30 DAP than 15 DAP for some crops may be due to two different plant sets for two harvesting points (15 and 30 DAP). This pattern of significant reduction of SCN within 15 days after planting could have an important impact on SCN management strategy using cover crops. It suggests that even a short growth of effective cover crops in SCN infested soil can reduce up to 50% of the initial population. This result is significant for geographical regions, including the northern Great Plains, where a limited period for plant growth after soybean or wheat harvest occurs due to early cold weather. Cover crops, red clover (cv. Allington), winter rye (cv. ND Dylan), and foxtail millet (cv. Siberian) showed a significant reduction in SCN103 population compared with fallow treatment in microplot experiments (Acharya et al. 2021). Similarly, alfalfa (cv. Bullseye), oilseed radish (cvs. Concorde and Control), daikon radish (cv. Eco-Till), faba bean (cv. Petite), and white mustard (cv. Master) had a greater reduction of SCN103 compared with fallow in other microplot experiments (Neupane et al. unpublished data). These crops need to be tested in field trials as their performance may vary in natural field conditions due to weather and soil factors.

Another leguminous crop, red clover, showed significantly greater hatching in both trials than unplanted infested soil. However, we observed very few SCN inside the roots of red clover, 15 and 30 DAP, in both trials. Chen et al. (2008) reported significant hatching of SCN in red clover (cv. Marathon) with minimal development of SCN inside the roots. However, we did not find further growth of SCN J2s after penetration. Kushida et al. (2003) observed similar penetration of red clover (cv. Sapporo) by SCN J2s as in sunnhemp (*Crotalaria* spp.), which was the most promising trap crop in their study. Similarly, white clover (*T. repens* cv. Ladino) and alsike clover (*T. hybridum*) induced significant hatching of SCN and reduced the neutral lipid content of juveniles (Riga et al. 2001). Another clover species, crimson clover (*T. incarnatum*),

was the best potential crop to serve as a trap crop for SCN (Harbach et al. 2021). These studies demonstrated the effects of several clover species to enhance the hatching of SCN juveniles. They also indicated that some clover species allowed the penetration of their roots by SCN juveniles without supporting the complete development of nematodes. The cultivar Allington of red clover used in our study enhanced the significant hatching of SCN but allowed the least number of SCN to penetrate the roots. In our study, alfalfa had a similar number of SCN J2s in its roots compared with red clover 15 DAP, with no further development. Riggs (1987) also found penetration in alfalfa cultivars by SCN with no or slight transition into advanced stages. However, in our study, the hatching was not consistent for alfalfa in two trials compared to unplanted infested soil. Riga et al. (2001) had observed significantly more SCN hatching on alfalfa (cv. Apollo Supreme) than in the water control.

We evaluated two cover crops, foxtail millet and winter rye, belonging to the Poaceae family. They had a similar number of hatched SCN compared with unplanted natural soil. However, winter rye had significantly more SCN than the rest of the cover crops inside the roots at 15 DAP, except for faba bean. Winter rye also had significantly less SCN inside the roots 30 DAP than 15 DAP, suggesting its restriction to SCN development. This result may indicate that winter rye could serve as a trap crop for SCN despite smaller hatching numbers than with susceptible soybean. Harbach et al. (2021) found very few numbers of SCN juveniles in the roots of winter rye (cvs. Aroostook and Guardian) despite a similar hatching population to unplanted soil control. This difference could be due to the different cultivars used in their study. A rotation crop, corn, used in this study had a variable effect on the hatching of SCN eggs compared with unplanted infested soil. Also, we observed a discrepancy in the number of SCN penetrated in corn roots in two trials. Nevertheless, corn did not allow the further development of penetrated

J2s. Our finding of corn as a non-host agrees with previously published studies (Chen et al. 2008; Riggs 1987; Sortland and MacDonald 1987). Chen et al. (2008) reported similar hatching on REs of corn and in the fallow control. Additionally, they found no further development of J2s inside the roots after penetration. Riggs (1987) did not observe SCN penetration in corn.

We noticed less SCN hatching in the second trial than the first trial for most treatments. The hatching of SCN eggs is affected by many factors, including temperature, soil moisture, pH, and root exudates (Duan et al. 2009; Tefft et al. 1982). The possibly higher number of dormant eggs in the second trial could have resulted in less SCN hatching (Chen et al. 2008). Additionally, the SCN population used in the second trial could be a different generation than population used in the first trial, which could affect the hatching (Masler and Perry 2018; Niblack et al. 2006). The reduced vigor and hatching of the SCN could explain the overall reduced penetration of roots for most of the crops in the second trial. While most non-host crops had significantly lower or equal juveniles inside the roots 30 DAP compared with 15 DAP, two non-host cover crops, alfalfa and red clover, allowed significantly greater juveniles inside the roots 30 DAP. This observation could be due to the significant production of water-soluble nematode attractants in these crops in the latter half of the experiments, but the attractants were not evaluated in this study. Production of attractants could be plant-specific and growth stage dependent, and the interactions between these chemical cues and nematode species remain a complex phenomenon (Ochola et al. 2020).

We found that SCN juveniles penetrated the roots of all Brassica crops evaluated in this study. The number of SCN inside the roots 15 and 30 DAP varied between the trials for these crops. Both tested radish cultivars had significantly lower SCN at 30 DAP than 15 DAP, whereas white mustard and turnip had increased numbers of SCN inside their roots 30 DAP. However,

only turnip produced a few white females at the end of the experiment showing its ability to support SCN reproduction as a poor host. Turnip also induced significant hatching of SCN in both trials. It promoted extensive hatching, but only a few juveniles penetrated and developed inside the roots. This finding supports the previous observation for turnip (Purple Top) as a poor host for SCN (Acharya et al. 2020). Other studies have reported root penetration of the Brassica crops, including turnip, by SCN juveniles (Chen et al. 2008; Riggs 1987), but the number of nematodes penetrated was not mentioned. Harbach et al. (2021) reported the quantitative data for root penetration of some Brassica crops, including daikon radish and oilseed radish. They also stated that some Brassica crops could serve as trap crops for SCN. In our study, the numbers of hatched SCN in the non-host Brassica crops were not significantly greater than unplanted soil, except for daikon radish in the second trial. Additionally, the non-host Brassica crops had significantly lower SCN penetrated in their roots than susceptible check. Therefore, no significant effects of the non-host Brassica crops were observed on the hatching of SCN or their root penetration by J2s. Their SCN population reductions could be due to their well-known biofumigation property, as this activity was reported to be also effective during the active plant growth stage (Ngala et al. 2015).

In the present study, the effect of crops on hatching of SCN was evaluated using the number of hatched juveniles 15 DAP present in the soil and root. In most of the previous studies, crops were evaluated for their effect on hatching of SCN using the soil leachates (SLs) and root exudates (REs) extracted after 4-9 weeks of plant growth (Chen et al. 2008; Harbach et al. 2021; Kushida et al. 2003; Sikora and Noel 1996). That *in vitro* method can accurately measure the proportion of hatched juveniles compared with the inoculated eggs as the arrangement allows convenient observation of the hatching process in the lab. However, many factors, including the

concentration of SLs and REs, the age of the plants (Masler and Perry 2018; Ochola et al. 2020; Sikora and Noel 1996; Tefft et al. 1982), and the microorganisms and their hatching factors (Lettice and Jones 2015, 2016; Ryan and Jones 2003) influence the cyst nematode hatching. In our method, the nematode was exposed to root diffusates all the time in the soil until harvesting. Also, we intended to best mimic the field conditions, including the effects of microorganisms and their products by using the naturally infested soil from the field in the experiments. This factor would aid in the validity of the results obtained for hatching. However, our method could not quantify the number of hatched juveniles that might have perished before 15 DAP, which indicates that the hatched juvenile number in our trials could have been underestimated. Additional observation points could be added before 15 DAP and after 30 DAP to assess the effect of short-term and prolonged exposure of SCN to cover crops on hatching and root penetration.

Our study has demonstrated that faba bean has the greatest potential as a trap cover based on the number of hatched and root penetrated juveniles. Red clover showed significantly more hatching than the unplanted control and had the least J2s in their roots. The hatched J2s eventually die due to starvation (Riga et al. 2001), ultimately reducing the SCN population. Similarly, winter rye showed the potential to be a trap crop with a significant number of juveniles inside their roots. These are cool-season crops for the northern Great Plains (Brummer et al. 2015; Wick et al. 2018) and some studies have shown potential utilization of faba bean and winter rye cultivars in our region for different agronomic aspects (Andersen et al. 2020; Johnson et al. 2021). Field evaluation is necessary for possible integration of these effective cover crops into current cropping systems to determine if the results obtained in our study are valid and consistent in the field conditions. As the growing season in the northern Great Plains is short,

further work would be beneficial to optimize their integration time, growing length, and other agronomic aspects to make this approach economically viable.

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APPENDIX A. ACTIVITIES DURING MICROPLOT TRIALS

Fig. A1. Cover crops and controls in ND Agriculture Experimental Station greenhouse before transferring them to microplot.

Fig. A2. Cover crops and controls in microplot at a research field site, NDSU, Fargo.

Fig. A3. Cover crops growth in microplot.

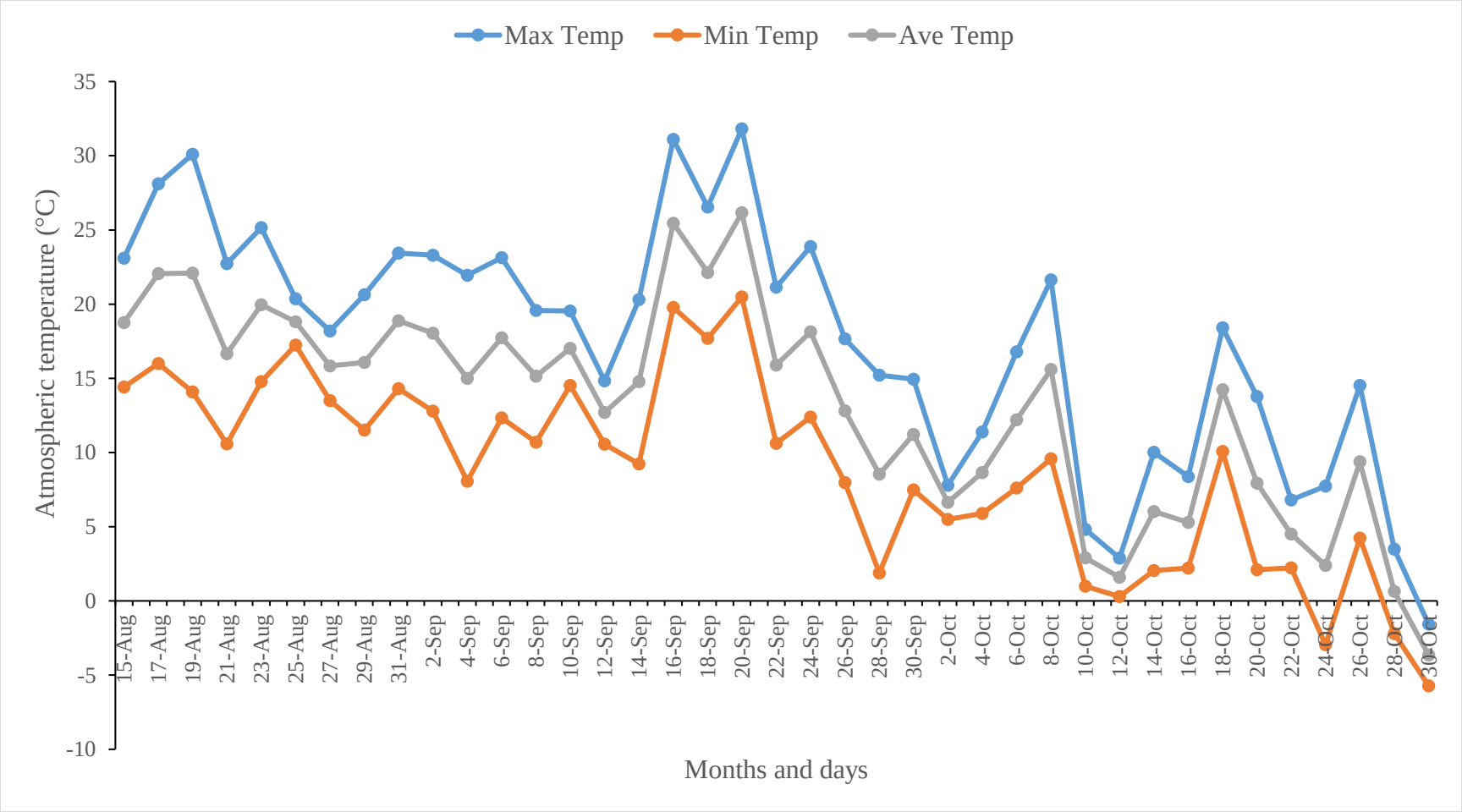


Fig. A4. Maximum, minimum and average atmospheric temperature during the microplot experiment 2019.
Source: North Dakota Agricultural Weather Network (NDAWN)
https://ndswn.ndsu.nodak.edu

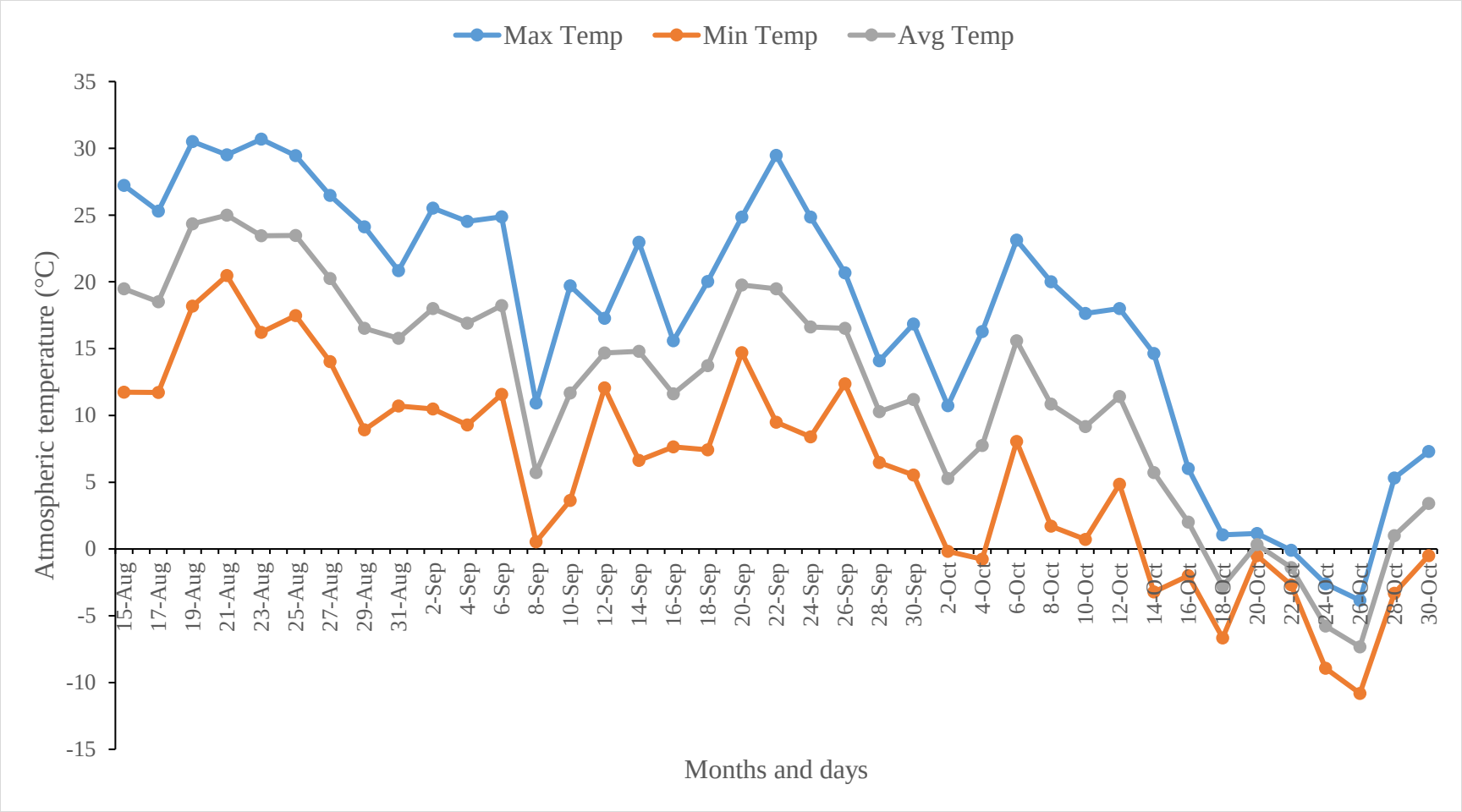


Fig. A5. Maximum, minimum and average atmospheric temperature during the microplot experiment 2020.
Source: North Dakota Agricultural Weather Network (NDAWN)
https://ndswn.ndsu.nodak.edu

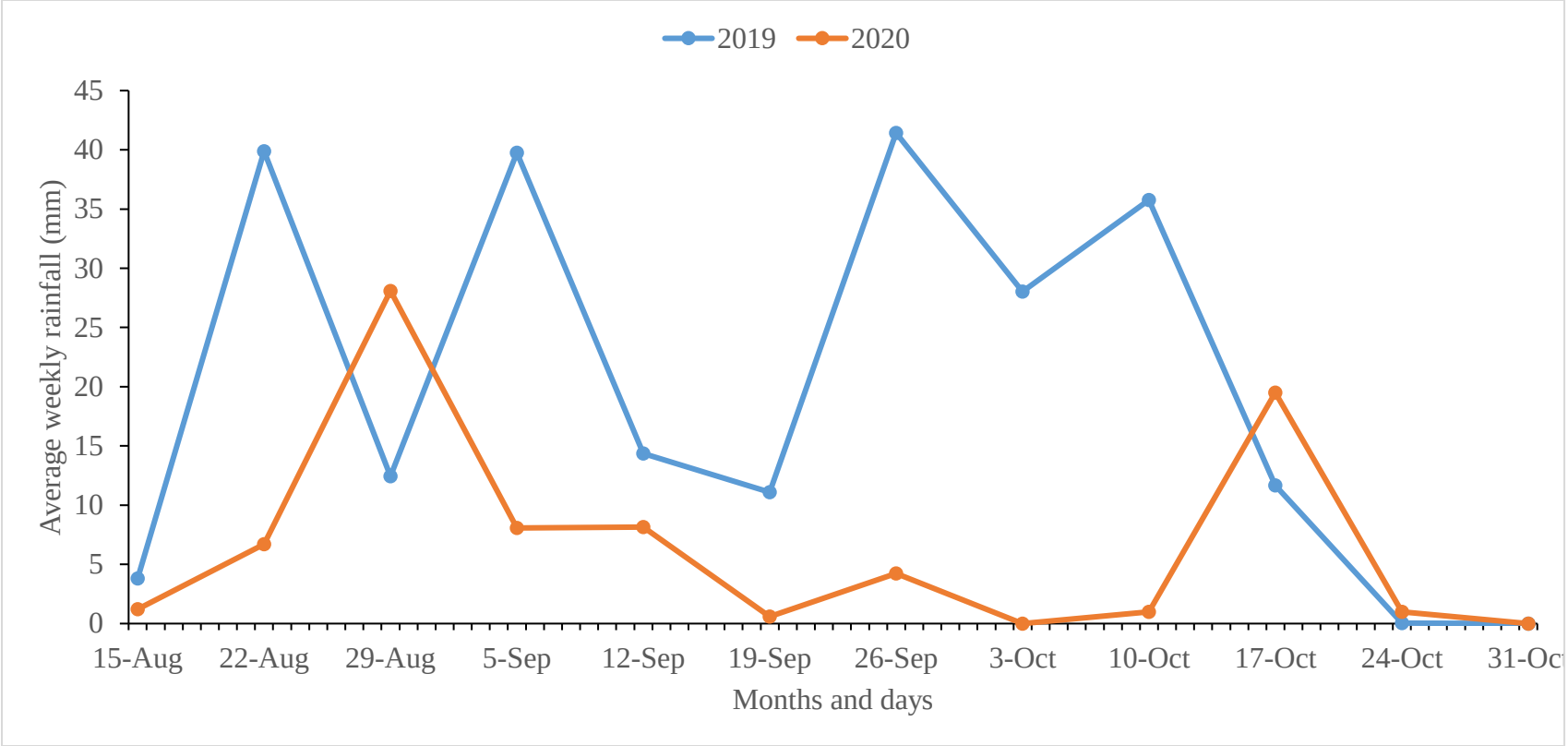


Fig. A6. Average weekly rainfall during microplot experiment 2019 and 2020.
Source: North Dakota Agricultural Weather Network (NDAWN)
https://ndswn.ndsu.nodak.edu

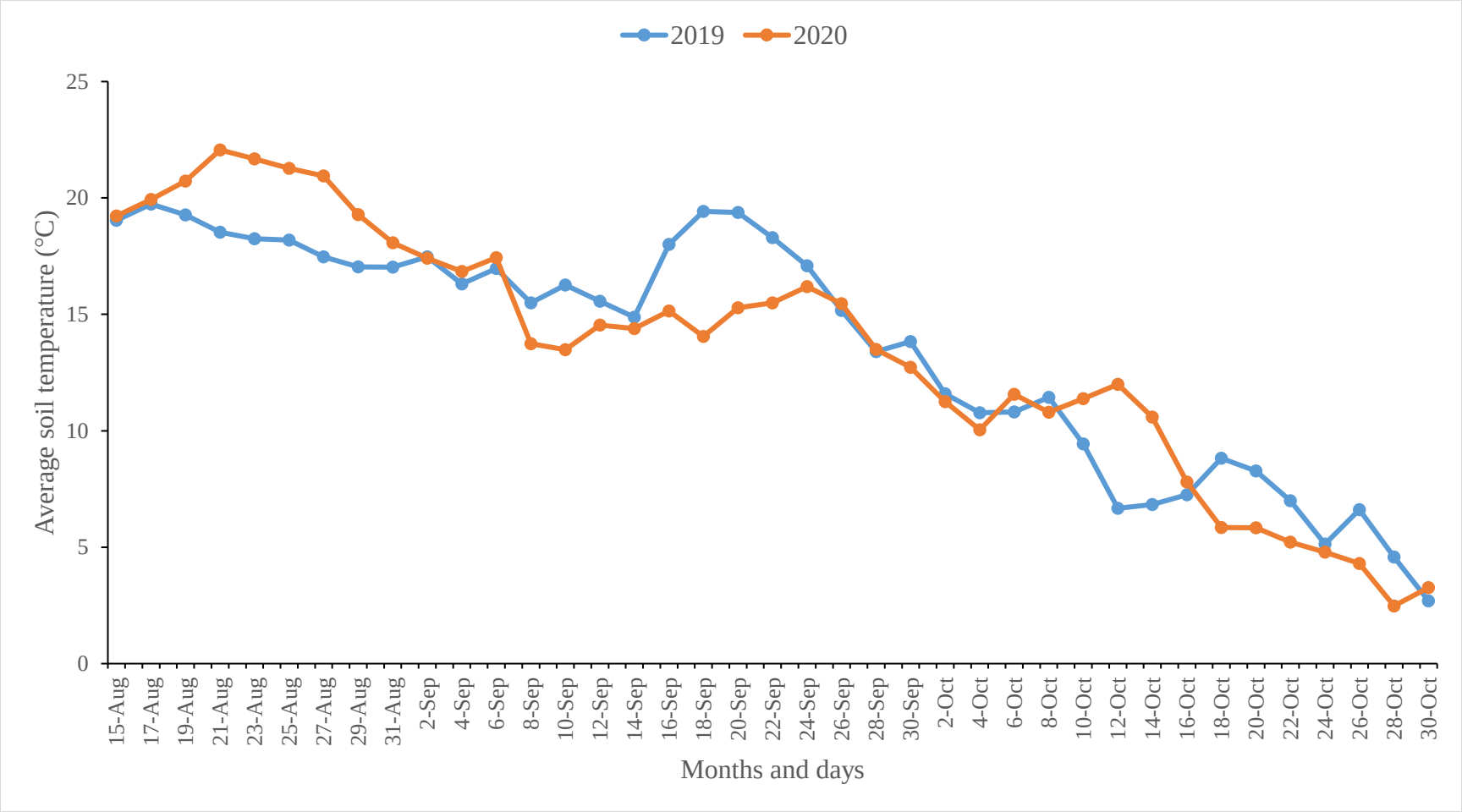
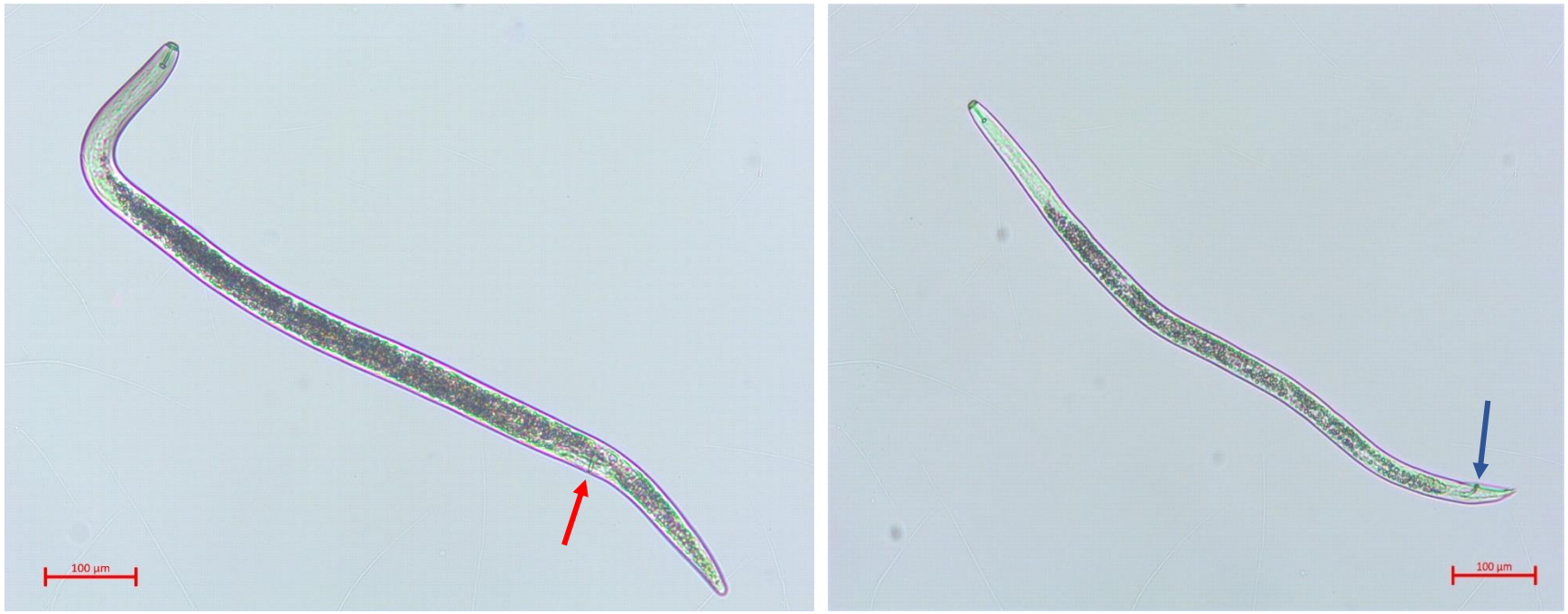


Fig. A7. Average soil temperature during the microplot experiment 2019 and 2020.
Source: North Dakota Agricultural Weather Network (NDAWN)
https://ndswn.ndsu.nodak.edu

APPENDIX B. ACTIVITIES DURING AND AFTER GREENHOUSE EXPERIMENTS FOR HOSTING ABILITY OF COVER CROPS TO *PRATYLENCHUS PENETRANS*

Fig. B1. Potato planted for population maintenance of root-lesion nematode, *Pratylenchus penetrans*.

Fig. B2. Cover crops growing in ND Agriculture Experimental Station greenhouse.



A **B**

Fig. B3. A. Female of *P. penetrans*. Red arrow shows the vulva slit, a diagnostic character of female. **B.** Male of *P. penetrans*. Blue arrow shows the spicules, a diagnostic character of male.

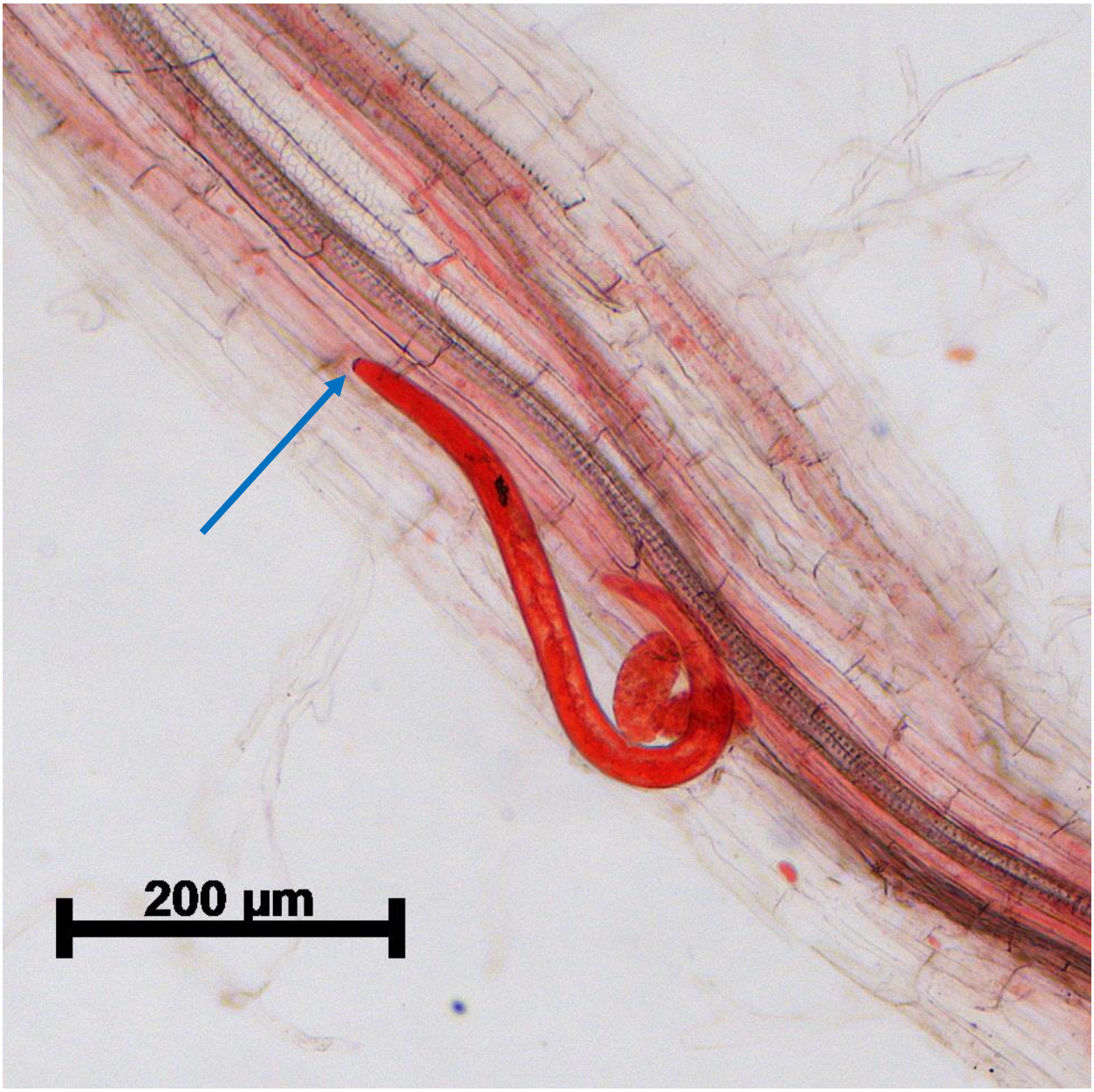


Fig. B4. A *P. penetrans* with eggs inside the root of radish (Concorde) stained with food coloring dye. Blue arrow shows the head region with sclerotized lips.

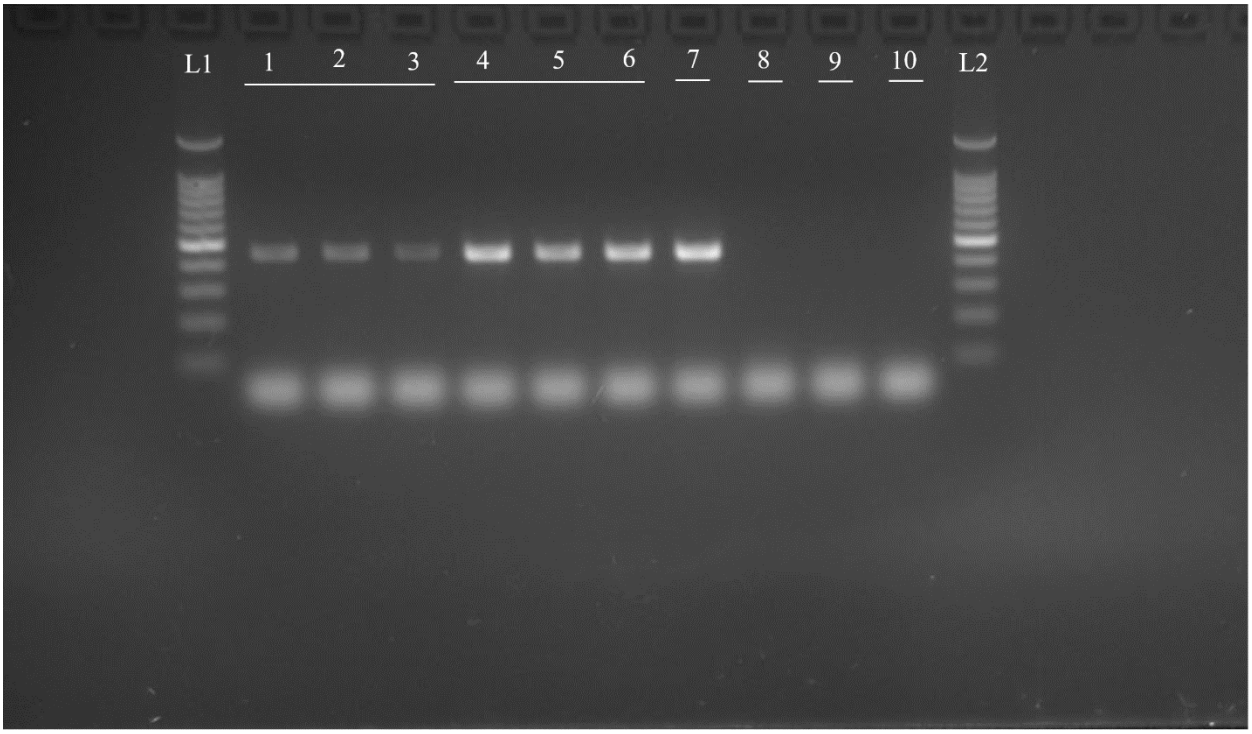


Fig. B5. Identification of *P. penetrans* by conventional polymerase chain reaction using species-specific primers (PP5F/PP5R) pair (approx. 520 bp).
L= Ladder (100 bp)
1, 2, 3 = DNAs from single lesion nematode individuals
4, 5, 6 = DNAs from two lesion nematodes
7 = Positive control (*Pratylenchus penetrans*) DNA
8 = Negative control (*Pratylenchus scribneri*) DNA
9 = Negative control (HG51) DNA
10 = No template control (Double-distilled water)

APPENDIX C. ACTIVITIES DURING AND AFTER GROWTH CHAMBER EXPERIMENTS ON COVER CROPS AND THEIR IMPACTS ON HATCHING AND PENETRATION OF SCN

Fig. C1. Cover crops and controls growing in growth chamber.

Fig. C2. Developing SCN inside the roots of soybean (Barnes). Blue arrow shows the developing SCN.

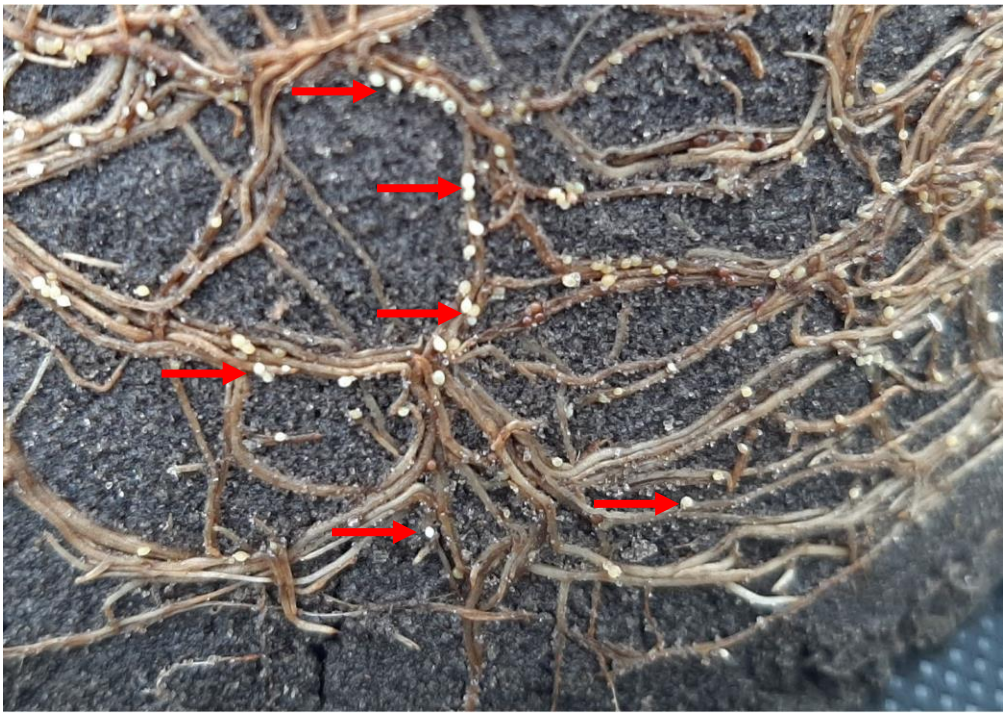
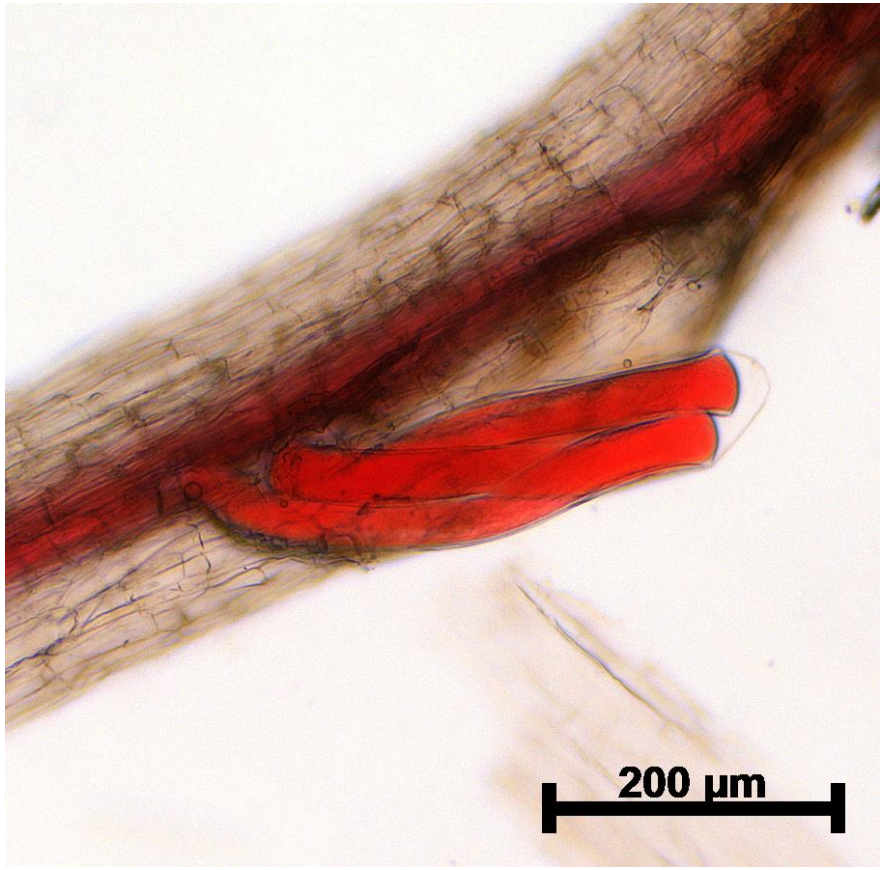


Fig. C3. SCN white females still attached to soybean (Barnes) roots 30 DAP. Red arrows show the SCN white females.



A **B**

Fig. C4. A. A developing SCN male still attached to soybean (Barnes) root 30 DAP. **B.** An adult SCN male. Blue arrow shows the spicules, a diagnostic character of male.