MANAGING SOYBEAN CYST NEMATODE BY UTILIZING COVER CROPS AND RESISTANT SOURCES FROM EARLY MATURING SOYBEAN ACCESSIONS

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Title

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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

DOCTOR OF PHILOSOPHY

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ABSTRACT

Greenhouse and microplot studies were conducted for understanding the effects of cover crop species/cultivars for hosts and population reduction of soybean cyst nematode (SCN; *Heterodera glycines*) from the fields of North Dakota. Moreover, early-maturing soybean [*Glycine max* (L.)] accessions from different countries of origin were screened for resistance against two common SCN populations for finding new sources of resistance. Thirty-eight cover crop species/cultivars were evaluated for their hosting ability of two SCN populations (SCN103 and SCN2W) from two fields of North Dakota in greenhouse experiments. The majority of the tested crops were non-hosts for both SCN populations. However, a few of them, such as Austrian winter pea (*Pisum sativum* L.), crimson clover (*Trifolium incarnatum* L. cv. Dixie), crambe (*Crambe abyssinica*, cv. BelAnn), field pea, cvs. Aragorn and Cooper, hairy vetch (*Vicia villosa* Roth), turnip (*Brassica rapa* L. cv. Purple top), and white lupine (*Lupinus albus* L.) were poor-hosts/hosts of both SCN populations. Furthermore, thirteen of them were tested for the SCN population reduction either or both in the greenhouse and microplot experiments. Out of 13, at least four crops, such as annual ryegrass (*Lolium multiflorum* L.), brown mustard (*Brassica juncea* L. cv. Kodiak), daikon radish (*Raphanus sativus* L.), and turnip cv. Pointer showed more than 50% population reduction compared with initial population densitiy, consistently in the greenhouse or microplot experiments. The resistance screening of 152 early-maturing soybean accessions showed that a majority of the accessions were susceptible/moderately susceptible to both SCN populations (SCN HG type 0 and 2.5.7), while a few (*n*=18) showed good resistance responses to both or either of the SCN populations.

The cover crops, which were non-hosts/poor-hosts and have a greater ability for the SCN population reduction have great potential to be included in an integrated SCN management strategy. The novel resistant accessions identified in this study have the potential to be used in soybean breeding for developing SCN-resistant cultivars after confirming their resistance response and identifying the resistance genes/loci. The results obtained from this study helps in developing a sustainable SCN management strategy in the northern Great Plains.

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DEDICATION

This work is dedicated to my parents Mr. Lok Nath Padhya and Mrs. Laxmi Devi Padhya. I also like to dedicate this work to my wife, Mrs. Tara Dhungana Acharya, my Daughter, Prapti Acharya and brother, Kamal Acharya and sisters, Amrita Acharya and Sita Acharya.

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LIST OF ABBREVIATIONS

CRD	Completely Randomized Design
FI	Female Index
GPS	Global Positioning System
ITS	Internal Transcribed Spacer
LSD	Least Significance Difference
MN	Minnesota
MSD	Minimum Significant Difference
MSU	Michigan State University
MT	Montana
ND	North Dakota
NDSU	North Dakota State University
PPNs	Plant-Parasitic Nematodes
RF	Reproductive Factor
SBCN	Sugarbeet Cyst Nematode
SCN	Soybean Cyst Nematode
UMN	University of Minnesota
US	United States
US$	United States Dollar
USA	United States of America
USDA-ARS	United States Department of Agriculture-Agriculture Research Service

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DISSERTATION ORGANIZATION

This dissertation describes about the interaction of soybean cyst nematode (SCN; *Heterodera glycines*) and cover crops in the northern Great Plains. The research describes about the abilities of diverse cover crops for SCN reproduction and population reduction in SCN-infested soil. In addition to that, diverse soybean accessions were screened for finding new resistance source against virulent SCN phenotypes. There are six chapters in this dissertation. Chapter 1 is the introduction and literature review. Chapter 2 describes about the hosting ability of diverse cover crops for two SCN populations prevalent in North Dakota. Chapter 3 examines three industrial crops for hosting and population reduction of two SCN populations prevalent in North Dakota. Chapter 4 contains experiments on the effects of cover crops on two SCN populations in the greenhouse and microplot study. Chapter 5 describes the screening of diverse soybean accessions for resistance against two SCN populations. Lastly, chapter 6 is a summary of the findings from these research projects.

CHAPTER 1. LITERATURE REVIEW

History and production of soybean in the USA

Soybean [*Glycine max* L. (Merr.)] is believed to be originated from northern and central regions of China in the late nineties (Hymowitz 1970; Hymowitz and Newell 1981). Samuel Bowen, a seaman in the state of Georgia introduced soybean into the United States in 1765 (Hymowitz and Harlan 1983). Soybean has been grown for oil and meal purpose and planted as a common rotational crop with corn (*Zea mays* L.) and wheat (*Triticum aestivum* L.) (Hartman 2011). It is now the second most grown crop in the United States following corn (Holcomb 2012). Soybean is a rich source of protein and seed oil output, giving it a high-value commercial crop (Lijuan and Singh 2010; Singh and Shivakumar 2010). More than 80% of the world's soybean is produced by North and South America and is used for nutrient sources for both human and animal food (Chang et al. 2015; ASA 2019). In addition to oil and meal (Schmitt 2004), soybean also has been used to produce biodiesel and other industrial products (Kumar et al. 2002). About 7,014 million liters of biodiesel were produced in the United States in the year 2018 (ASA 2019). The United States ranked the first for soybean production in the world (ASA 2019). In the year 2018, The United States contributed 34% of the world soybean production followed by 32% in Brazil, and 15% in Argentina. Out of the total 123.7 million metric tons soybean production in the United States, North Dakota alone produced about 6.6 million metric tons (5%) in 2018 (ASA 2019).

Constraints of soybean production in the USA

Both biotic and abiotic factors can negatively impact soybean yield and seed quality. The abiotic factors include low temperature, salt toxicity, water stress, and nutrient deficiency, whereas weeds, insect pests, and diseases are the biotic factors (Hartman et al. 2011). Among biotic factors, soybean cyst nematode (SCN), *Phytophthora* root and stem rot (*Phytophthora sojae*), soybean rust (*Phakopsora pachyrhizi*), brown spot (*Septoria glycines*), charcoal rot (*Macrophomina phaseolina*), and others are the most important diseases for reducing soybean production in the United States. Among those, SCN remains on the top to cause yield loss than any other soybean diseases in the United States since last two decades (Allen et al. 2017; Koenning and Wrather 2010; Wrather and Koenning 2006, 2009).

Soybean Cyst Nematode (SCN; *Heterodera glycines*)

History and worldwide distribution

Soybean cyst nematode (*Hetrodera glycines* Ichinohe) (Ichinohe 1955) is an obligate, sedentary, and endo-parasitic nematode (Niblack et al. 2006). It was discovered for the first time in northeast China in 1899 (Li et al. 2011), the continued to be identified in Japan in 1915, in Korea and Manchuria in 1930s, and in the United States in 1954 in North Carolina (Riggs 1977; Winstead et al. 1955; Wrather et al. 1954). It is considered that the SCN was introduced to the United States from China with the introduction of rhizobia in soybeans in the middle of the 20th century (Noel 1986). The SCN continued to be detected in soybean fields in Tennessee and Missouri in 1956, then in Mississippi, Kentucky, and Arkansas in 1957, and Virginia in 1958. Until now, SCN has been detected in Alabama, Delaware, Florida, Georgia, Illinois, Indiana, Iowa, Kansas, Louisiana, Maryland, Minnesota, Michigan, Nebraska, New Jersey, North Dakota, Ohio, Oklahoma, Pennsylvania, South Carolina, South Dakota, Texas, and Wisconsin (Riggs 1975; Yu 2011), including New York in 2016 (Tylka and Marett 2017). It was first detected in North Dakota (ND) in 2003 in Richland County (Bradley et al. 2004), since then it has become one of the major threats to soybean production in North Dakota.

Spreading of SCN

The SCN can be spread to non-infested area with the movement of nematode infested soil by farm machinery, contaminated seed, and plant parts, water movement, wind, animals and human beings (Riggs 1977; Chen 2011). Various cultivation practices, such as leveling lands and sharing farm machinery between farmers help the spreading of SCN from infested fields to non-infested fields. The soil particles containing SCN egg or cyst mixed with the seeds while harvesting soybean from infested field help in the spreading in non-infested areas. Evidence has been found that bird's digestive system can also transmit SCN without losing the viability (Riggs 1977; Chen 2011). Black bird species, such as the brown-headed cowbird (*Molothrus ater*), grackle (*Quiscalus quiscula*), and the brown starling (*Sturnis vulgaris*) were able to transport SCN cysts with viable eggs inside them (Riggs 1977).

Life cycle of SCN

The SCN has mainly three life stages, egg, juvenile, and the adult male or female (cyst). Eggs are the survival unit of SCN that are encased in a protective structure cyst (dead female). The first molting (J1) of this nematode takes place inside the egg and second molting leads to second stage juveniles (J2), which is the infective stages of SCN (Niblack 2005). Eggs survive inside the cyst in a dormant state until the favorable environment, such as temperature, moisture, and host root exudates inducing for J2 hatching (Masler and Rogers 2011; Niblack 2005; Tefft et al. 1982; Turner and Subbotin 2013). The J2 migrates to soybean roots with the help of gradient formed by the root exudates and then penetrates by releasing root-degrading enzymes, such as cellulase with the help of the hollow stylet (Papademetriou and Bone 1983; Smant et al. 1998). The J2 migrates intercellularly towards the vascular tissues, such as cortex, endodermis or pericycle, then selects one cell to form a permanent feeding site, known as syncytium (Johnson et al. 1993; Riggs and Wrather 1992). The juvenile releases effector proteins, which mimics endogenous plant cell signals that lead to the reduction of plant defenses inducing that plant cell to turn into a nutrient sink (Kandoth et al. 2011). The cell wall surrounding the feeding site dissolves and a large multinucleated cell forms from dissolving many adjacent plant cells, then the nematode starts to feed (Mitchum et al. 2013). While feeding, the juvenile remains sedentary and molts up to three times to become an adult male or female. The vermiform male (J3) undergoes metamorphosis and forms an adult male, then leaves the root after 10 to 15 days after infection by J2, while the female continues to feed and molts to J4 and mature to an adult female (Lauritis et al. 1983; Triantaphyllou and Hirschmann 1962). In general, the female to male ratio remains 1:1 that is thought to be determined by genetics of nematodes and influenced by other conditions such as temperature, infection density, and host resistance to nematode (Colgrove and Niblack 2005; Koliopanos and Triantaphyllou 1972; Melton et al. 1986). After two weeks of initial infection, the posterior end of the female emerges and attracts male for mating through secretion. After mating, the female produces hundreds of eggs inside the body and some are enclosed outside the female body with gelatinous matrix (Sipes et al. 1992). The SCN female dies after fertilization of the eggs, the outer covering of female body develops into a protective hard structure cyst (dead female) composed of anti-microbial compounds chitinase and polyphenoloxidase, which protects the SCN eggs inside the cyst (Melito et al. 2010; Niblack et al. 2006). The SCN-eggs enclosed inside the cyst can remain viable for almost a decade (Inagaki and Tsutsumi 1971). A single SCN-female usually contains 200 - 600 eggs (Niblack 2005; Schmitt et al. 2004). Normally, SCN completes its life cycle in about 3 to 4 weeks, but this is greatly influenced by temperature and moisture conditions. The optimum temperature of SCN development ranges from 15 to 30°C (Riggs and Wrather 1992). It is found that SCN can complete its life cycle in 21 days in a controlled environment of 25℃ (Lauritis et al. 1983). The SCN can have four generations in a growing season in North Dakota. Initial population density affects in SCN reproduction throughout the soybean growing season. It has been found that, lower initial population increases SCN population greater than with higher initial population (Alston and Schmitt 1987).

Host range of SCN

The SCN has a narrow host range compared with other plant-parasitic nematodes such as root-knot, root-lesion, and others. The SCN also infects other leguminous crops such as kidney, navy, pinto and black bean (*Phaseolus vulgaris* L.) (Poromarto et al. 2011; Poromarto and Nelson 2009). Some weeds, such as common chickweed (*Stellaria media* L.), henbit (*Lamium amplexicaule* L.), purple deadnettle (*Lamium purpureum* L.), small-flowered bittercress (*Cardamine parviflora* L.), shepherd's-purse (*Capsella bursa-pastoris* L.), and field pennycress (*Thlapsi arvense* L.) act as alternate host for SCN (Creech et al. 2007; Johnson et al. 2008; Poromarto et al. 2015; Venkatesh et al. 2009; Werle et al. 2015). In South Dakota, some native legume crops were screened and found to be hosts for SCN population. The crops which supported SCN reproduction include strawberry tick clover (*Trifolium fragiferum* L.), Canada tick clover (*Desmodium canadense* L.), hairy vetch (*Vicia villosa* L.), and some cultivated species such as string bean, tender-green bean (*Phaseolus vulgaris* L.), lima bean (*Phaseolus lunatus* L.) and little marvel pea (*Pisum sativum* L.) (Jones 1997).

Symptoms of SCN

Soybean cyst nematode not necessarily produce obvious above-ground symptoms in infested fields. More than 30% yield loss in soybean can be accrued in infested fields without any visual symptoms or may be confused with other bacterial, fungal, or herbicide injury

(Mueller et al. 2016). High SCN population density may cause stunting and yellowing of soybean plants. In most cases, SCN infection may go undetected if other factors do not stress the host plants (such as drought) (Wang et al. 2003; Young 1996). Many other factors may confuse SCN infestation, include drought stress, nutritional deficiency such as iron, nitrogen, potassium, and other compounds, including the infection by other soil pathogens (Niblack et al. 2006).

Interaction of *H. glycines* with other diseases and insect pests

Many pests and pathogens interact with SCN while infecting the soybean crop. The interaction may be synergistic or antagonistic to each other between interacting pathogen/pest and *H. glycines*, but often leading to greater damage to soybeans. The co-infection of *Fusarium solani* f. sp *glycines* and *H. glycines* reduce the growth of soybean plants, where higher infection of *F. solani* f. sp. *glycines* have negative impacts on *H. glycines* reproduction, but *H. glycines* does not affect *F. solani* infection and colonization of roots (Gao et al. 2006). The similar interaction has been observed between *H. glycines* and *Fusarium virguliforme*, a causative agent of sudden death syndrome of soybean (McLean and Lawrence 1993). Infection of *H. glycines* along with abiotic stress supports the infection of *Phytophthora sojae*, causative agent of root and stem rot of soybean (Kaitany et al. 2000). The soybean aphid (*Aphis glycines*) infestation on soybean plant supports the *H. glycines* infestation, but the antagonistic effect has been observed between them, where at high aphid population density SCN numbers decrease by reducing the available resource quantity of host plant (McCarville et al. 2014). Another fungal pathogen *Cadophora gregata*, causative agent of brown stem rot on soybean supports the infection of *H. glycines* (Tabor et al. 2006). Similarly, *Macrophomina phaseolina*, causative agent charcoal rot of soybean colonization is increases when both *M. phaseolina* and *H. glycines* infect the host plant (Todd et al. 1987). Moreover, *H. glycines* has been found to interact with other plant-parasitic nematode groups. The presence of other nematodes impacts on *H. glycines* reproduction and possibly by competing for nutrient from the host plant (Melakeberhan and Dey 2003). *Heterodera glycines* has been found to be associated with transmitting four different types of negative sense RNA viruses from nyaviruses, bornaviruses, rhabdoviruses, bunyaviruses, and tenuiviruses. Both egg and juvenile of *H. glycines* may carry and transmit these viruses (Bekal et al. 2011).

Effects of abiotic factors on *H. glycines* reproduction

Various abiotic factors, such as soil temperature, pH, soil types, including different chemical compounds affect *H. glycines* reproduction and determining the SCN race (Alston and Schmitt 1988; Duan et al. 2009; Lehman et al. 1971; Palmateer et al. 2000; Pedersen et al. 2010; Perez-Hernandez 2013; Young and Heatherly 1990; Zheng et al. 2010). Temperature is one of the important factors for SCN development, the second stage juvenile (J2) cannot infect and proceed for further life stages if the soil temperature falls below 10℃ and above 33℃ (Alston and Schmitt 1988; Riggs and Wrather 1992). The rate of female development becomes linear at 20 to 28°C. The soil pH above 6.0 and SCN population densities showed positive correlation in the experiments conducted in greenhouse and field conditions (Pederson et al. 2010; Teff et al. 1982; Wiggs 2019), but such correlation did not impact the soybean yield of both resistant and susceptible soybean cultivars (Pedersen et al. 2010). Although the mechanism is not clear yet, *H. glycines* infestation may increase the iron deficiency chlorosis symptoms on soybeans in soils with pH > 7.0 (Chen et al. 2007). The interaction of *H. glycines* and soil texture is still unclear, but a research report showed that a higher population density was observed in loamy sand soil compared with a sandy clay loam soil (Avendano et al. 2004).

Diversity of SCN populations

Before 2002, there was a race system to characterize the SCN population based on the reproduction on four soybean differential lines, such as Peking, Picket, PI88788, and PI90763 comparing with standard susceptible check, Lee 74 (Riggs and Schmitt 1988). *Heterodera glycines* (HG) type system is the revised system to classify the SCN field populations based on reproduction on seven SCN indicator lines compared with a standard susceptible check. The HG type designation is given each SCN population based on female index (FI = mean number of SCN females on indicator line/mean number of SCN females in susceptible check x 100) (Niblack et al. 2002). Seven soybean indicator lines including, PI548402 (Peking), PI88788, PI90763, PI437654, PI209332, PI89772, PI548316 (Cloud), and susceptible soybean (Lee74) are inoculated and phenotypes designation is given based on the level of reproduction on the seven indicator lines compared with the susceptible check. The HG type system not only categorizes the SCN population, but also consider genetic diversity of nematode populations and documentation of population difference on indicator lines (Niblack et al. 2002). The HG type performed in different states of the United States showed the virulence changes in the field SCN populations. In Minnesota, SCN populations were able to reproduce with more than 10% female index (FI) on PI548316, suggesting that this differential line was more susceptible than other HG type differential lines tested in field populations (Zheng et al. 2006). Similar results were obtained when the SCN populations from Wisconsin fields were tested, where the majority of field populations were able to reproduce on PI88788 with FI higher than 10% (MacGuidwin 2012). In South Dakota, about 63% of SCN populations reproduced with higher FI than 10% on PI548316, 25% were able to reproduce on PI88788, 19% on PI209332, 7% on PI548402, 4% on PI90736, and 4% on PI89722, but none of the population had FI more than 10% on PI437654

(Acharya et al. 2016). In Missouri, about 70% of the SCN populations were reproduce on PI88788, PI209332, and PI548316 (Cloud), 30 % of the populations were able to reproduce on PI58402 (Peking), while other differential PI90763, PI437654, PI89772, or PI438489B had FI less 10% suggesting that they were resistant to SCN populations (Mitchum et al. 2007; Niblack et al. 2003). Twenty SCN populations from soybean fields from Kentucky were tested, and all the populations were able to reproduce on PI88788, PI209322, and PI548316 (Hershman et al. 2008). Studies have shown that about 70% of the SCN populations have adapted to the major SCN-resistant source PI88788 because of the continued planting of cultivars with the same source of resistance (Niblack et al. 2008; Zheng and Chen 2011).

Economic importance of SCN

Plant-parasitic nematodes are one of the major problems on crops worldwide. The economic loss associated with these nematodes is estimated more than 100 billion US dollars annually (Opperman and Bird 1998). The soybean cyst nematode is the major pest of soybean worldwide including the United States and considered to be a billion-dollar soybean pest in the United States. It ranks first to cause more yield loss compared with any othe diseases of soybean (Allen et al. 2017; Koenning and Wrather 2010; Niblack et al. 2006; Wrather and Koenning 2009). The average yield loss estimated due to SCN from 2010 to 2012 in the northern soybean growing states was 31% (Allen et al. 2017). Although there is less information about yield loss in dry bean by SCN, dry bean is a good host of SCN (Poromarto and Nelson 2009; Poromarto et al. 2010).

Management of SCN

Soybean cyst nematode management includes different tactics, such as host resistance, crop rotations, biological control, chemical control, and others. Among those, host resistance and crop rotations are more popular because of their effectiveness and environmentally friendly nature (Niblack et al. 2005; Oyekanmi and Fawole 2010; Schmitt et al. 2004).

Host resistance

The resistant genes have been identified from different soybean accessions and utilized for developing soybean cultivars resistant to SCN (Concibido et al. 2004). The screening of many plant introduction lines was performed by Ross and Brim (1957), they found only four resistance PIs, including Peking and later Caldwell et al (1960) discovered three recessive genes *rhg1*, *rhg2*, and *rhg3* from Peking conferring resistance to SCN populations. In addition to that, a fourth novel dominant gene *Rhg4* was identified from Peking, the gene was closely linked to the *i* locus associated with the distribution of pigmentation in the soybean seed coat (Matson and Williams 1965). The genetic regions *Rhg1* and *Rhg4* have been identified in most of the QTL mapping experiments and the molecular markers linked to these regions are commonly used in marker assisted breeding for SCN-resistant cultivars (Concibido et al. 2004). About 90% of the SCN-resistant cultivars are derived from *rhg1-b* allele from PI88788 (Concibido et al. 2004; Schmitt et al. 2004; Shannon et al. 2004; Tylka and Mullaney 2018).

The resistance mechanism for SCN has been elucidated as a complex mechanism, since researchers found that the effectiveness of the resistance to SCN from PI88788 and Peking is controlled by the copy number of *rhg1* genes within the locus (Cook et al. 2012; Mitchum 2016). There are other SCN-resistant sources, which carry different resistance loci than *rhg1* and *Rhg4* suggesting that there are possibilities of identifying novel sources of resistance alternative to PI88788 or Peking (Li et al. 2011). In addition to this, screening of soybean accessions from diverse origin for SCN resistance would be recommended to find novel sources of resistance.

Crop rotation

The rotation of soybean with non-host crops of SCN is one of recommended method to control the population increase in SCN-infested fields. The SCN has a narrow host range, so soybean can be rotated with nonhost crops such as corn, wheat, and other non-hosts crops. Soybean is commonly rotated with corn, and SCN juveniles may enter the roots or starve resulting in a decrease in SCN population over the years (Warnke et al. 2008). A study suggested that the annual rotation of resistant soybean and corn decreased the SCN population with an increase in yield of both crops (Chen et al. 2001). Farmers are encouraged to adapt crop rotations with non-host crops, such as cereals and crops from the Brassicaceae family. In addition to that, the rotation between the SCN-resistant cultivars derived from different resistant sources is recommended. The continuous planting of the same variety with the same source of resistant aids SCN to overcome the resistant gene being used (Niblack 2005; Tylka and Mullaney 2018).

Chemical control

Application of the chemical compounds is one of the management methods for SCN. Chemical control is not considered economical and eco-friendly method because of the cost, toxicity to handlers, and ground water pollution (Matthiessen and Kirkegaard 2006; Oka 2010). Aldicarb, a granular carbamate has been used in SCN management in the Midwest region of the United States, the chemical showed some level of control over the nematode population (Grabau 2013). Similarly, Telone C-35 (Dow Agrosciences, Indianapolis, IN), a fumigant with 1, 3 dichloroprene and chloropicrin as active ingredients found to have an effect on both nematodes and fungi. In Iowa, application of Telone C-35 decreased SCN egg numbers by 42% and increased the soybean yields by 10% in three field trials (De Bruin and Pedersen 2008). Wu et al. (2014) reported that the application of benzyl isothiocynate (BITC) had the multiple effects on the SCN reproduction by affecting on egg hatching, embryonic development, and movement of the infective juveniles in the soil. (Wu et al. 2014). Some herbicides were also tested for effects on SCN population, Acifluorfen (2-nitrobenzoic acid), bentazon (Sodium bentazon), lactofen (Easter of acifluorfen), crop oil concentrate (COC), and nonionic surfactant (NIS) found to be effective to reduce SCN numbers by production of glyceollin that may increase the resistance of soybean crop against SCN (Levene et al. 1998).

Nematicidal seed treatments with chemical or biological active ingredients have been released from the industries. Nematicidal seed treatments limit the damage in soybean from nematode infection by killing them or preventing soybean roots from infection (Munkvold et al. 2014). The commercial use of nematicidal seed treatment started in the early 2000s, since it has been considered one of the economic methods to integrate in SCN management strategies. Although the seed treatments are used for managing SCN, their performance on increasing soybean yield and reduction of SCN numbers has not been consistent in field conditions (Chilvers et al. 2012; Gaspar et al. 2014; Wheeler et al. 2013). Fluopyram is one of the popular seed treatments reported to have antagonistic effects on SCN cyst and eggs. It has been found to reduce the reproductive rate, root penetration, ultimately reducing SCN number, which helps to reduce mortality of soybean plant compared with the untreated control in controlled environment and field experiments (Zaworski 2014; Faske and Hurd 2015; Beeman 2017; Beeman and Tylka 2018). Two seed treatments, such as Avicta (released by Syngenta crop protection) and Aeris (released by Bayer Crop Science) were tested for the effects on SCN population. Although they were not able to reduce SCN numbers, yield benefits were observed from treated seeds (Frye 2009). Another seed treatment, abamectin was tested alone and in combination with thiabendazole for effects on SCN population. Abamectin alone was able to reduce the SCN population, but was not effective, if used in low dose combined with thiabendazole (Vitti et al. 2014). Different nematicidal seed treatments, such as Activa Complete a, N-Hibit, VOTiVO, Clariva Complete, ILeVO, NEMASTRIKE, and AVEOEZ are available in the market for soybean growers in Minnesota, but their effectiveness in controlling SCN is not consistent or in need of evaluation in field trials (Bissonnette and Tylka 2017). An experiment performed in Iowa showed that ILeVO was able to reduce the SCN juveniles (J2) when applied as seed treatment, but the effects were limited to an area nearby the treated seed (Beeman et al. 2019).

Biological control

Biological control is an ecologically sound method of managing SCN in infested fields. An endoparasitic fungus, *Hirsutella rhossiliensis* infects SCN juvenile and egg and reduces the infection to soybean roots (Chen 2007; Chen and Liu 2005; Chen et al. 2000; Zhang et al. 2006). An endoparasitic bacterium, *Sinorhizobium fredii* strain Sneb 183 was tested for effects on SCN population in fields, the soybean seedlings infected by the bacterium showed the systemic resistance to SCN and was able to reduce the nematode numbers (Tian et al. 2014). Three fungal species, *Fusarium oxyporum, F. solani,* and *Exophiala pisciphila* in Alabama and two, *Nematophthora gynophila* and *Catenona auxilaris* in Tennessee found to infect some SCN eggs and cysts (Wrather et al. 1984). Moreover, *Pasteuria nishizawae, Bacillus amyloliquefaciens*, and *Bacillus firmus* have been registered as biological products for suppressing SCN population in infested fields (PMRA 2011, PMRA 2015). Dried defatted meal from *Brassicas* (*Brsssica chinensis* L.), kernel of the neem plant (*Acacia indica* L.), resin of the acacia (*A. nilotica* L.), and seaweed (*Ecklonia maxima* L.) have been tested and found effective in suppressing SCN numbers in an infested field with the increase in soybean yield (Auwal et al. 2014). Bean sprouts residing in soybean field stimulate the SCN egg hatching and subsequent decrease in SCN numbers in field conditions (Toyota et al. 2013). An experiment conducted to evaluate the effects of swine manure enriched with volatile fatty acids (VFA) and ammonium nitrogen ($NH^{+}{}_{4}$) showed the reduction in SCN counts in a linear manner up to 35 days of its application in the infested soil (Xiao et al. 2007).

Cover crops

The integration of cover crops into the cropping system has been increased over the past decades throughout the United States, including northern Great Plains. The cover crops provide multiple benefits that farmers can get by adopting this practice (CTIC 2017). The historical aspect of cover crops started from the first president of the United States, George Washington, who supported the idea of growing crops to eat and sell and to replenish the soil (Groff 2015). For many years, farmers have found the benefits of cover crops as they increase soil nutrients. After World War II, advanced technology helped synthesize many nitrogen fertilizers that led to decrease the use of cover crops (Groff 2015). Specifically, from the 1940s to 2010s, the use of the nitrogen fertilizers increased tremendously in the northern Corn Belt (Cao et al. 2018). The extensive use of the fertilizers led to the leaching of nutrients into ground water and watersheds causing detrimental impacts on the water reservoir, ecosystem, and human health (Billen et al. 2013). Because of this situation, cover crops have integrated into cropping system to reduce nutrient leaching, soil erosion, and increase soil organic matter (Clark 2007). Besides the agronomic benefits, cover crops may have effects on plant pathogens, including plant-parasitic nematodes (PPNs).

Interaction of cover crops and plant-parasitic nematodes

The interactions between cover crops and plant-parasitic nematodes have been stated in several research papers and extension bulletins. Cover crops can be the host or non-host of plant-parasitic nematodes (PPN). However, some of the crops can be integrated into plant-parasitic nematode management because of their ability to reduce SCN population (Kruger et al. 2013; Halbrendt 1996). Cover crops may reduce plant-parasitic nematode populations by producing volatile and non-volatile biochemical compounds released from their roots and crop residues, which induces the egg hatching of PPNs, including SCN (Abawi and Thurston 1994; Kushida et al. 2003; Riga et al. 2001). Different crops, such as sunnhemp (*Crotalaria juncea* L.), velvet bean [*Mucuna pruriens* (L.) DC], and *Brassica* species were tested and found to have suppressive effect on root-knot nematode (*Meloidogyne sp.)* in different vegetable crops (Anita 2012; Guerena 2006; Wang et al. 2004). Moreover, Brassica crops were tested for potato cyst nematode (PCN; *Globodera rostochiensis*) in Europe, their reports suggested up to 90% of PCN population reduction was observed in infested fields (Valdes et al. 2011). Marigold (*Tagetes spp.)*, acts as a trap crop for cyst nematodes and reduces nematodes number by interrupting the nematode life cycle (Hooks et al. 2010). Moreover, marigold planted in potato (*Solanum tuberosum L.*) and pea fields infested with the root-lesion nematode (*Pratylenchus penetrans*) reduced the nematode population in field conditions (Kimpinski et al. 2000; Pudasaini et al. 2006). Castor bean (*Ricinus communis* L.) and sorghum-sudangrass [*Sorghum bicolor* x *S. bicolor* var. *sudanense*] were tested for population reduction of two PPNs, root-knot (*Meloidogyne incognita*) and root-lesion (*P. penetrans*) nematodes in infested fields, both of the crops were able to reduce *M. incognita* numbers, while only sorghum-sudangrass was able to reduce *P. penetrans* numbers compared with fallow ground in a susceptible soybean rotation (Everts et al. 2006). In Michigan, oilseed radish (*Raphanus sativus* L.) planted before sugarbeet (*Beta vulgaris* L.), reduced the sugarbeet cyst nematode (SBCN; *Heterodera schachtii*) population by acting as a trap crop, where juveniles were only able to penetrate and enter into the roots but was unable to complete their life cycle (Poindexter 2011).

Cover crops as host for SCN

Soybean cyst nematode can reproduce on different plant species, such as dry bean, and weed species (Poromarto et al. 2015; Poromarto et al. 2009; Poromarto and Nelson 2010; Venkatesh et al. 2000). Cover crops, such as hairy vetch, red clover (*Trifolium pratense* L.), and alfalfa (*Medicago sativa* L.) were tested for SCN reproduction. Hairy vetch supported SCN reproduction, while SCN juveniles were able to penetrate the roots of red clover and alfalfa but did not produce mature females (Schmitt and Riggs 1991). In another study, sunnhemp and showy rattlebox (*Crotalaria spectabilis* L.) induced SCN-egg hatching and allowed penetration by SCN juveniles, but did not result in mature SCN females, suggesting that these two crops may act as trap crops (Kushida et al. 2003). Alfalfa, Austrian winter pea, berseem clover (*Trifolium alexandrinum* L.), cowpea (*Vigna unguiculata.* L), crimson clover, field pea, hairy vetch (two different cultivars), red clover (three different cultivars), and white clover (two different cultivars) were poor-hosts with a very low level of SCN reproduction compared with susceptible soybean (Kobayshi et al. 2017).

Cover crops effects on SCN populations

The effects of cover crops on SCN populations is not well understood, but a few studies have shown the potentiality of cover crops on SCN population reduction. In a study, sunnhemp and showy rattlebox were tested for SCN reproduction, and these crops induced SCN-egg hatching and allowed subsequent penetration by SCN juveniles, but also did not result in mature SCN females (cyst), suggesting that these two have a potential to reduce SCN population in the infested fields (Kushida et al. 2003). Rotation of soybean with sunnhemp, Illinois bundle flower

(*Desmanthus illinoensis*), oilseed rape (*Brassica napus*. L.), perennial ryegrass (*Lolium perenne*. L.), red clover, and corn were tested for the management of SCN. Among those sunnhemp and red clover were found to be effective in managing SCN by stimulation of SCN egg hatching, hence decreased in the SCN numbers (Warnke et al. 2008). Annual ryegrass residue is found to be effective to reduce the *H. glycines* population by increasing the hatching of eggs in the absence of the host and depletion of the lipid reserved in juveniles and decreases the parasitism of the pathogen (Mock et al. 2009; Riga et al. 2001).

Other control measures

Soybean cyst nematode spreads through machinery, which moves infested soil from one place to another. Sanitation is highly recommended to prevent the SCN infestation in uninfected areas. The proper cleaning of farm machineries, preventing contamination of crop seeds while harvesting, and washing root crops harvested from infested fields are necessary to control the SCN spreading (Giesler and Wilson 2011). The proper agronomic practices such as optimum soil fertility, effective weed and pest control measures in soybean are always good to reduce the stress and yield reduction by SCN (Niblack 2005).

Justification of study

The Soybean cyst nematode is one of the major soybean pests in the United States, including North Dakota. Managing SCN is always a challenge job because almost impossible to eradicate once the field is infested with this nematode. Host resistance and crop rotations are commonly practiced methods for managing SCN. In addition to soybean, SCN can infect and reproduce on many other leguminous crops, weed species, and some cover crops. Although a considerable number of crops and weed species have been tested for the hosting ability for SCN, many others still need to be tested for SCN populations. The hosting and population reduction ability of commonly or potential to be grown cover crops, including industrial crops from different plant families for prevalent SCN populations are not well documented in the northern Great plains. On the other hand, majority of SCN resistant cultivars for the northern Great Plains are derived from single resistant source PI88788. The use of the single source of resistance for a long run is not recommended because the sexually reproducing SCN population can rapidly adapt to resistant cultivar by overcoming the resistance. The goal of this study is to evaluate the different crops for hosts and effects on SCN populations. In addition, this study tries to and find the novel resistance sources against SCN populations from early maturing soybean accessions.

The results obtained from this study will provide information on host status of different crops and their effects for SCN populations that is important for selecting potential cover crops for integrating them in SCN management strategy. The novel resistance sources from early maturing soybean accessions identified in this study will have potential to be used in SCN-resistant soybean breeding programs in northern Great Plains. The specific objectives of this research were to:

1. Evaluate diverse cover crops species/cultivars for the hosting ability for SCN populations in greenhouse experiments.
2. Evaluate three industrial crops for hosting and population reduction abilities for SCN populations in greenhouse experiments.
3. Determine the effects of cover crops on population reduction of SCN in greenhouse and microplot experiments.
4. Screen the early maturing soybean accessions for resistance against two SCN populations from North Dakota.

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CHAPTER 2: EVALUATION OF DIVERSE COVER CROPS AS HOSTS OF TWO POPULATIONS OF SOYBEAN CYST NEMATODE, *HETERODERA GLYCINES*[1]

Abstract

Soybean cyst nematode (SCN; *Heterodera glycines*) reproduces on a wide range of plants, including some cover crops. However, reproduction of SCN populations on a number of cover crops has not been investigated in the northern Great Plains. Thirty-five cover crop species/cultivars from four plant families were evaluated as hosts for SCN. Greenhouse evaluations were done with two common SCN populations, SCN103 (HG type 0) and SCN2W (HG type 7) under controlled conditions. The sources of two SCN populations were soil from two soybean [*Glycine ma*x (L.)] fields of North Dakota. After 35 days of growth, white SCN females were extracted from root and soil of individual plant and counted to determine a female index (FI = average number of females on a tested crop/average number of females in a susceptible check x 100) for each crop. Out of the 35 cover crop species/cultivars tested, at least one of the SCN populations reproduced on seven crops/cultivars but did not reproduce on the other 28 crops/cultivars. Out of these seven crops, only white lupine (*Lupinus albus* L.) was a suitable host (FI ≥ 10) for both SCN populations in all the experiments, while others showed varied responses from poor host to suitable host for the SCN populations. The host crops were from the family *Brassicaceae* or *Fabaceae*, while all the crops in the *Linaceae* or *Poaceae* family were non-hosts. The non-host crops can be planted in SCN-infested fields without the

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[1] The material in this chapter was co-authored by Krishna Acharya (Department of Plant Pathology, North Dakota State University), Guiping Yan (Department of Plant Pathology, North Dakota State University) and Marisol T. Berti (Department of Plant Sciences, North Dakota State University). Krishna Acharya had primary responsibility for collecting soil samples from the fields and perform greenhouse experiments. Krishna Acharya was the primary developer of the conclusions that are advanced here. Marisol T. Berti helped by providing seeds of cover crops for this experiment and served as proofreader. Guiping Yan served as principle investigator and corresponding author and checked the statistical analysis conducted by Krishna Acharya. This paper was submitted to Journal, Crop Protection.

concern of increasing SCN populations, while poor hosts with low female index should be evaluated for effects on reduction of SCN numbers in the fields before they are used as cover crops in a soybean cropping system.

Key words: *Heteroderea glycines*, reproduction, female index, cover crops, hosting ability.

Introduction

Soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe), is an obligatory endo-parasitic nematode distributed worldwide and one of the major yield- suppressing pathogens of soybean in the United States, including North Dakota (Allen et al. 2017; Wrather and Koenning 2009). Soybean cyst nematode was first detected in the United States in 1954 in North Carolina (Riggs 1977; Winstead et al. 1955) and has now been detected in 90% of the U.S. soybean production states (Tylka and Marett 2017). In North Dakota, since SCN first detected in 2003 in Richland County (Bradley et al. 2004), it has been spreading rapidly in the southeastern counties and confirmed in at least 19 counties in 2017 (Tylka and Marett 2017). The has a wide host range, which includes soybean and other leguminous crops including weed and cover crop species from different families (Poromarto et al. 2009; Poromarto and Nelson 2010; Venkatesh et al. 2000). Different management strategies, such as host resistance, crop rotations, biological control agents, and chemical control have been utilized for controlling SCN. Among these, host resistance and crop rotations with nonhost crops are considered more effective than chemical and other methods due to their cost-effective and environment friendly nature (Miller et al. 2006; Niblack et al. 2006; Oyekanmi and Fawole 2010).

Different cover crops, such as sunnhemp (*Crotalaria juncea L.),* velvet bean [*Mucuna pruriens* (L.) DC], and *Brassica* species were tested and found to have a suppressive effect on root-knot nematode (*Meloidogyne sp.)* in different vegetable crops (Anita 2012; Guerena 2006;

Wang et al. 2004). Moreover, similar results were obtained when different crops in the Brassicaceae family, henceforth Brassica crops, were tested for potato cyst nematode (PCN; *Globodera rostochiensis*) and up to 90% PCN population reduction was observed (Valdes et al. 2011). Marigold (*Tagetes spp.)* is used as a cover crop in horticultural crops, acting as a trap crop for reducing plant-parasitic nematodes numbers in infested fields (Hooks et al. 2010). In Michigan, oilseed radish (*Raphanus sativus* L.) planted before sugarbeet (*Beta vulgaris* L.) significantly reduced the sugarbeet cyst nematode (SBCN; *Heterodera schachtii*) population by acting as a trap crop (Poindexter 2011).

A cropping sequence of soybean and corn (*Zea mays* L.) was tested to determine its effect on SCN population and corn and soybean yields. Although rotation of SCN-resistant soybean and corn was able to reduce the SCN numbers, yields benefit was not observed for both soybean and corn (Chen et al. 2001). Many other crops, such as cabbage (*Brassica oleracea* L.), tobacco (*Nicotiana tabacum* L.), cotton (*Gosyppium hirsutum* L.), hairy vetch (*Vicia villosa* Roth), red clover (*Trifolium pratense* L.), alfalfa (*Medicago sativa* L.), field and sweet corn, and wheat, including soybean cultivars were tested for SCN reproduction. Only soybean and hairy vetch supported SCN reproduction, while red clover and alfalfa were only penetrated by juveniles and did not produce mature SCN females (Schmitt and Riggs 1991). In another study, sunnhemp and showy rattlebox (*Crotalaria spectabilis* L.) were tested for SCN reproduction, and these crops induced SCN egg hatching and allowed subsequent penetration by SCN juveniles, but also did not result in mature SCN females (cyst), suggesting that these two crops act as trap crops (Kushida et al. 2003). Other studies also have shown that soybean cyst nematode can reproduce on diverse plant species, such as dry bean (*Phaseolus vulgaris* L.), weed species, and other crops from different plant families (Poromarto et al. 2015; Poromarto et al., 2009; Poromarto and

Nelson 2010; Venkatesh et al., 2000). Some of these plant species studied also were shown to be capable of reducing plant-parasitic nematode numbers of different nematode groups when planted in nematode-infested fields (Guerena 2006; Kushida et al. 2003; Poindexter 2011).

Many studies have shown that some soybean resistant cultivars are not showing complete effectiveness against the SCN HG types, including HG types found in the northern Great Plains (Acharya et al. 2017, 2016; Niblack 2005; Niblack et al. 2008; Zheng et al. 2006). These results suggest that management of SCN by host resistance alone is at risk, so effective integrated practices, including crop rotations and cover crops are needed to improve SCN management in this region.

Cover crops are becoming popular in the northern Great Plains because of their role on crop profitability mainly from improved soil properties (Carr et al. 2012; Dagel et al. 2014; Karlen et al. 2006; Reeves 2017; Snapp et al. 2005). Farmers are, however, concerned about the hosting ability of these cover crops to major diseases and pests of field crops, such as soybean cyst nematode in soybean fields. Many crops and weed species have been tested with different plant-parasitic nematodes including SCN (Poromarto et al. 2015; Poromarto and Nelson 2010), but other crops have not been tested for common SCN populations (HG type 0 and 7) (Chowdhury et al. 2017), so the hosting ability of the cover crops for prevalent SCN populations is not fully understood in the northern Great Plains. The objective of this study was to evaluate the host suitability of diverse cover crops to two common SCN populations (HG type 0 and 7) of North Dakota in controlled greenhouse conditions to select appropriate cover crops for SCN-infested fields.

Materials and methods

Cover crop species and cultivars collection

Thirty-five cover crop species/cultivars from four plant families, *Brassicaceae, Fabaceae, Linaceae*, *and Poaceace*, were selected based on their current or potential use as cover crops in the northern Great Plains, and two rotational crops corn and wheat (*Triticum aestivum* L.) were also included (Table 2.1). The seeds were acquired from Forage and Biomass Crop Production Program (North Dakota State University, Fargo, ND), Allied Seed (Nampa, ID), and Great Northern AG (Plaza, ND). Two local soybean cultivars (Barnes and Sheyenne), both susceptible to SCN, were used as controls.

Soybean cyst nematode populations

Two SCN populations SCN103 and SCN2W described in Acharya et al. (2019) were used for this study. To perform the greenhouse experiments, large amount of SCN-infested soils were collected from the two North Dakota fields as described before. The soil samples from each field were mixed thoroughly to uniformly distribute SCN populations in the soil. The initial SCN population densities for SCN103 were 5,000 and 3,100 and for SCN2W were 10,000 and 5,000 eggs and juveniles per 100 cm$_3$ of soil for experiments with naturally infested soil. To repeat each experiment with artificial infestation, both the SCN populations were increased on susceptible soybean (Barnes) using naturally SCN-infested soil in controlled greenhouse conditions to obtain enough infestation numbers and inoculated with 2,000 eggs and juveniles per 100 cm$_3$ of soil.

Table 2.1. List of cover crops and rotational crops tested for soybean cyst nematode reproduction under controlled greenhouse conditions.

Crop (Cultivar or Cultivar Not Stated= CNS)	**Scientific name**	**Family**
Alfalfa (Bullseye)	*Medicago sativa* L.	*Fabaceae*
Annual ryegrass (CNS)	*Lolium multiflorum* L.	*Poaceae*
Austrian winter pea (CNS)	*Pisum sativum* L. *subsp. arvense*	*Fabaceae*
Balansa clover (CNS)	*Trifolium michelianum* Savi	*Fabaceae*
Berseem clover (CNS)	*Trifolium alexandrinum* L.	*Fabaceae*
Camelina (Joelle)	*Camelina sativa* (L.) Crantz	*Brassicaceae*
Carinata (CNS)	*Brassica carinata* L.	*Brassicaceae*
Corn (DKC44-13)	*Zea mays* L.	*Poaceae*
Cowpea (CNS)	*Vigna unguiculata.* L.	*Fabaceae*
Crimson clover (Dixie)	*Trifolium incarnatum* L.	*Fabaceae*
Ethiopian cabbage (CNS)	*Brassica carinata* L.	*Brassicaceae*
Faba bean (Petite)	*Vicia faba* Roth	*Fabaceae*
Faba bean 1 (CNS)	*Vicia faba* Roth	*Fabaceae*
Faba bean 2 (CNS)	*Vicia faba* Roth	*Fabaceae*
Field pea (Aragorn)	*Pisum sativum* L.	*Fabaceae*
Field pea (Cooper)	*Pisum sativum* L.	*Fabaceae*
Flax (Carter)	*Linum usitatissimum* L.	*Linaceae*
Forage oat (CNS)	*Avena sativa* L.	*Poaceae*
Foxtail millet (Siberian)	*Setaria italica* subspp. R*ubofructa* (L.) P. Beauv.	*Poaceae*
Hairy vetch (CNS)	*Vicia villosa* Roth	*Fabaceae*
Japanese millet (CNS)	*Echinochloa esculenta* L.	*Poaceae*
Oilseed radish (Concorde)	*Raphanus sativus* L.	*Brassicaceae*
Oilseed radish (Control)	*Raphanus sativus* L.	*Brassicaceae*
Oilseed radish (Image)	*Raphanus sativus* L.	*Brassicaceae*
Radish Daikon (CNS)	*Raphanus sativus* L.	*Brassicaceae*
Radish Daikon (Eco-Till)	*Raphanus sativus* L.	*Brassicaceae*
Rape (Dwarf Essex)	*Brassica napus* L.	*Brassicaceae*
Red clover (Allington)	*Trifolium pratense* L.	*Fabaceae*
Sunn hemp (CNS)	*Crotolara juncea* L.	*Fabaceae*
Sweetclover (CNS)	*Melilotus officinalis* L.	*Fabaceae*
Triticale (Winter 336)	X *Triticosecale* Wittmack.	*Poaceae*
Turnip (Purple Top)	*Brassica rapa subsp. rapa* L.	*Brassicaceae*
Wheat (Glenn)	*Triticum aestivum* L.	*Poaceae*
White lupine (CNS)	*Lupinus albus* L.	*Fabaceae*
White mustard (Master)	*Sinapis alba* L.	*Brassicaceae*
White proso millet (CNS)	*Panicum miliaceum* L.	*Poaceae*
Winter rye (ND Dylan)	*Secale cereale* L.	*Poaceae*

Host range evaluation

To determine the capability of the cover crops to host SCN, two naturally SCN-infested soils (SCN103 and SCN2W) were used as described earlier. Seeds for each crop, including susceptible soybeans (Barnes and Sheyenne) were directly planted into the cones (3.8-cm in diameter and 21-cm tall, Stuewe and Sons, Inc., Tangent, OR) that had been filled with 100 cm^3

of the SCN-infested soil and replicated five times for each crop entry and each SCN population. After planting, cones were placed in 14 x 7 -cell plastic racks in a completely randomized design (CRD) and kept in a growth chamber (GR64, Conviron, Winnipeg, Manitoba, Canada) maintained at a temperature of 27-28°C and daylight period of 16 h. Extra seedlings were thinned out to maintain a single plant in each cone. After 35 days, the cones were taken out of the growth chamber and SCN white females were extracted from each plant. White females were dislodged from the roots and soil using a high-pressure water shower. The white females were collected in a 250-µm-pore sieve nested under a 710-µm-pore sieve by following the sieving and decanting method described by Krusberg et al. (1994). White females for each individual crop, including susceptible soybean, were counted under the microscope (SM 100 Series, Swift Optical Instrument, Inc. TX, USA) by placing them on gridded Petri plates. The female index (FI) for each crop was calculated by using the following formula.

$$\text{Female Index (FI)} = \frac{\text{Average no.of white females found on tested crop}}{\text{Average no.of white females found on susceptible soybean}} \text{ x } 100$$

The FI was used to determine the host status of the crops. The crops with FI values equal/greater than 10 were considered as suitable hosts, while crop with FI less than 10 and greater than 0 as poor-hosts. The crops that had FI value of 0 or no any SCN white females on them were considered as non-hosts (Niblack et al. 2002; Poromarto et al. 2015; Poromarto and Nelson 2010).

The above host range evaluation experiments were repeated once with the greenhouse experiments under the same growth conditions with artificial inoculation. The same SCN populations (SCN103 and SCN2W) were increased on the susceptible soybean cultivar Barnes. White females obtained from Barnes for each SCN population were collected and crushed to obtain eggs and juveniles following the procedure described by Faghihi and Ferris (2000). Each of the crops and the two soybean cultivars were planted in a cone (3.8-cm in diameter and 21-cm tall), Stuewe and Sons, Inc., Tangent, OR) filled with 100 cm^3 of autoclaved river sand soil, then inoculated with 2,000 eggs and juveniles of each SCN population and replicated four times. Inoculation was done at the time of planting by making 2 to 3 holes of 3-5 cm depth around the root zone, then 5 mL of SCN egg and juvenile suspension was placed and covered with moist soil. Cones were arranged in a completely randomized design in a 14 x 7 -cell plastic rack and kept in the same growth chamber. A single plant in each cone was maintained by thinning out the extra seedlings immediately after germination. After 35 days, SCN white females from each crop were extracted and counted under the microscope to determine female index (FI), as described above.

Soybean cyst nematode eggs per white female produced on cover crops

The newly developed white females on cover crops roots were evaluated and compared to white females produced on the susceptible checks (Barnes and Sheyenne). White females collected from each crop were crushed to determine the average number of eggs per white female in each cover crop to compare with the eggs per female obtained from the susceptible soybeans (Faghihi and Ferris 2000). The average number of eggs per female was determined for each SCN population in artificial infestation.

Experimental design and data analysis

Experimental design for the experiments was a completely randomized design (CRD) with five replicates (one plant per replicate) for the naturally infested soil, and each of these experiments was repeated with artificially SCN-infested soil and the same seed lot in four replicates for both SCN populations, SCN103 and SCN2W. The data from individual experiments were analyzed separately by using SAS 9.4 (SAS Institute, Cary, NC). The average number of white females from five replicates were used to calculate of the female index on each crop in naturally SCN-infested soil, while the average number of white females from four replicates were used to calculate the female index in each crop for artificial infestation of each SCN population. General linear model (GLM) with *F*-protected LSD mean separation with a significance level of 5% was used to determine the difference in the average number of white females in each crop, and the difference in the average number of eggs per white female on different crops.

Results

Soybean cyst nematode reproduction

The majority of the tested crops that supported SCN reproduction were from the *Fabaceae* family for both SCN populations. The significant difference ($P < 0.0001$) was observed in average number of white females produced on each cover crop species/cultivar supported SCN reproduction in both runs of the experiments. For SCN103, average number of white females produced on field pea cv. Aragorn were 13 and 2 for natural and artificial infestation, respectively, while field pea cv. Cooper and Austrian winter pea did not support reproduction of the SCN103 population. Crimson clover from the *Fabaceae* family and turnip cv. Purple Top from the *Brassicaceae* family also supported reproduction of SCN103. Crimson clover had an average of 13 and 2 white females and Purple Top turnip had an average of 2 and 7 white females for natural and artificial infestation, respectively. White lupine and hairy vetch also supported reproduction of SCN103, where white lupine had an average number of 60 and 55 and hairy vetch had 25 and 11 white females for natural and artificial infestation, respectively (Table 2.2).

Similar results were obtained for SCN2W population, where significant differences ($P < 0.0001$) was observed in average number of white females produced on each cover crop species/cultivar supported SCN reproduction in both runs of the experiments. Austrian winter pea and field pea cv. Cooper, which supported reproduction only for the SCN2W population. The average number of white females produced on field pea cv. Aragorn were 136 and 33; on field pea cv. Cooper were 21 and 9; and on Austrian winter pea were 64 and 8 for natural and artificial infestation, respectively. Crimson clover and turnip cv. Purple Top also supported reproduction of SCN2W. Crimson clover had an average of 1 and 4 white females and turnip cv. Purple Top had 8 and 5 white females for natural and artificial infestation, respectively. White lupine and hairy vetch also supported reproduction of SCN2W, where white lupine had an average number of 177 and 73 white females and hairy vetch had 17 and 10 white females for natural and artificial infestation, respectively (Table 2.3).

Table 2.2. Reproduction of soybean cyst nematode on five of the tested cover crops planted in infested soils with a SCN population (SCN103) from Richland county, ND, under controlled greenhouse conditions. All of the other 30 cover crops/cultivars tested did not produce SCN white females in both runs of the experiments.

	Number of white females/plant[x]	
Crop (Cultivar or Cultivar Not Stated = CNS)	**First run** **Natural infestation**	**Second run** **Artificial infestation**
White lupine (CNS)	60 (20-88) A	55 (37-76) a
Hairy vetch (CNS)	25 (2-49) B	11 (4-14) b
Crimson clover (Dixie)	13 (1-54) B	2 (0-2) b
Field pea (Aragorn)	13 (3-25) B	2 (1-3) b
Turnip (Purple Top)	4 (2-8) B	7 (2-11) b
LSD	25	12

[x] Average number of white females produced on each plant with the range of white females from five replicates in each experiment. Experiments were performed in two runs. Average number of white females on the susceptible soybean check Barnes for first run was 716 (450-995) and for second run was 122 (103-140). Numbers in the parenthesis refer to the range of white females produced on each crop. ($P < 0.0001$, LSD mean separation), means with same letters are not significantly different from each other.

Table 2.3. Reproduction of soybean cyst nematode on seven of the tested cover crops planted in infested soils with a different SCN population (SCN2W) from Cass county, ND, under controlled greenhouse conditions. All of the other 28 cover crops/cultivars tested did not produce SCN white females in both runs of the experiments.

	Number of white females/plant[x]	
Crop (Cultivar or Cultivar Not Stated = CNS)	**First run** **Natural infestation**	**Second run** **Artificial infestation**
White lupine (CNS)	177 (83-300) A	73 (61-90) a
Field pea (Aragorn)	136 (96-177) A	33 (9-61) b
Austrian winter pea (CNS)	64 (3-163) B	8 (2-20) c
Field pea (Cooper)	21 (16-28) BC	9 (7-12) c
Hairy vetch (CNS)	17 (2-72) BC	10 (7-18) c
Turnip (Purple Top)	8 (3-16) C	5 (1-12) c
Crimson clover (Dixie)	1 (0-4) C	4 (2-7) c
LSD	56	16

[x] Average number of white females produced on each plant with the range of white females from five replicates in each experiment. Experiments were performed in two runs. Average number of white females on the susceptible soybean check Barnes for first run was 1,070 (780-1,318) and for second run was 260 (202-295). Numbers in the parenthesis refer to the range of white females produced on each plant. ($P < 0.0001$, LSD mean separation), means with same letters are not significantly different from each other.

Hosting abilities of cover crops to soybean cyst nematode

Soybean cyst nematode SCN103 population reproduced on five out of the 35 cover crop species/cultivars tested, which were suitable hosts (FI of 10 or greater) or poor hosts (FI less than 10 but greater than 0) in both natural infestation and artificial infestation experiments. White lupine was a suitable host with FI values of greater than 10 while other crops that supported SCN103 reproduction had FI values lower than 10 suggesting poor hosts (Fig. 2.1). The remaining 30 cover crop species/cultivars were non-hosts, with FI values of 0 for SCN103.

For SCN2W, seven out of 35 cover crop species/cultivars supported reproduction in both natural and artificial infestation experiments. Field pea cv. Aragorn and white lupine had FI values greater than 10, thus were classified as suitable hosts, while other five cover crops were poor hosts with FI values less than 10 (Fig. 2.2). The remaining 28 cover crop species/cultivars were non-hosts, with FI values of 0 for SCN2W. As expected, the two rotational crops corn (DKC44-13) and wheat (Glenn) were non-hosts with FI values of 0 for both populations SCN103 and SCN2W.

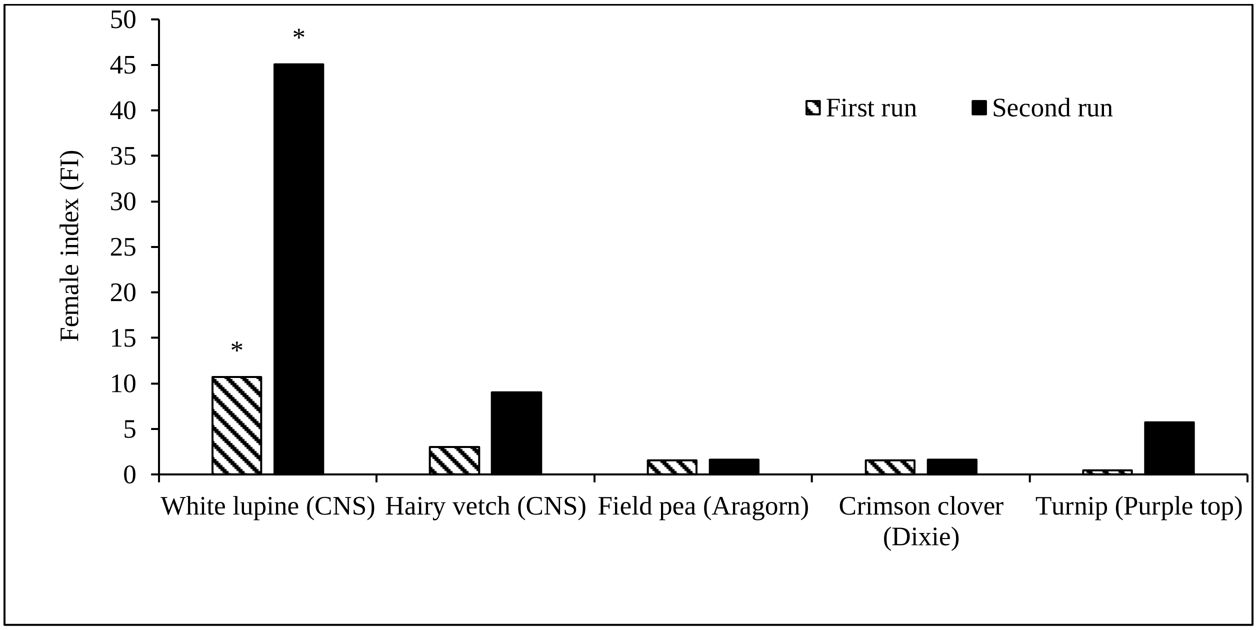

Fig. 2.1. Female index for five cover crops under natural (first run) and artificial (second run) infestation of a soybean cyst nematode population (SCN103) planted in greenhouse conditions. All of the other 30 cover crops/cultivars tested had no production (0) of SCN white females. Female index = (average no. of white females on a tested crop/average no. of white females in the susceptible soybean, Barnes) x 100. Crops labelled with * were considered suitable hosts and others were poor hosts for SCN.

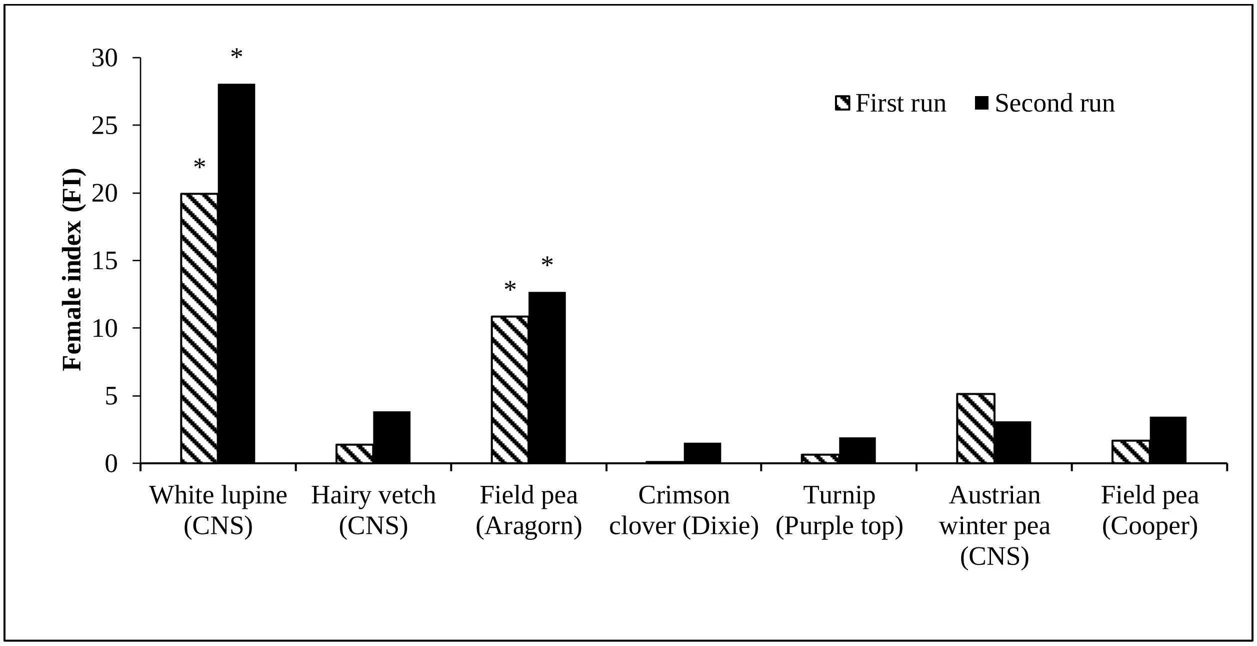

Fig. 2.2. Female index for seven cover crops under natural (first run) and artificial (second run) infestation of a different soybean cyst nematode population (SCN2W) planted in greenhouse conditions. All of the other 28 cover crops/cultivars tested had no production (0) of SCN white females. Female index = (average no. of white females on a tested crop/average no. of white females in the susceptible soybean, Barnes) x 100. Crops labelled with * were considered suitable hosts and others were poor hosts for SCN.

Soybean cyst nematode eggs per white female produced on cover crops

For the crops that produced white females to SCN103, there was no significant difference ($P > 0.05$) between the average egg numbers (125 to 199) in each white female obtained from soybean with those obtained from other crops (Fig. 2.3). However, for SCN2W white females, significant differences ($P < 0.05$) were observed between the average egg numbers per white female in susceptible soybean (Barnes and Sheyenne) and those obtained from other crops, except for white lupine, which did not differ in the average egg numbers as compared with susceptible soybean. Susceptible soybeans (Barnes and Sheyenne), and white lupine had significantly ($P < 0.05$) higher number of eggs per white female (247 to 280) compared with other crops, with average eggs per white female of 130 to 197 (Fig. 2.4).

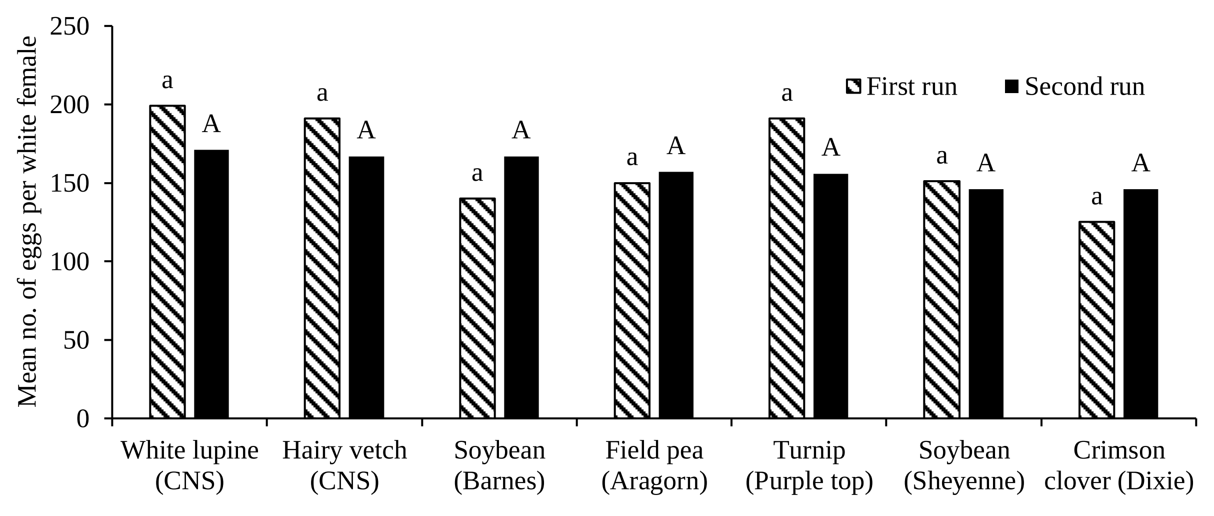

Fig. 2.3. Average number of eggs per white female produced on different cover crops and susceptible soybean cultivars (Barnes and Sheyenne) for the soybean cyst nematode population, SCN103 in artificial infestation. Average number of eggs produced on the cover crops were not significantly different from Barnes and Sheyenne for SCN103 with same letters (*P* > 0.05) for both run of the experiment with artificial inoculation.

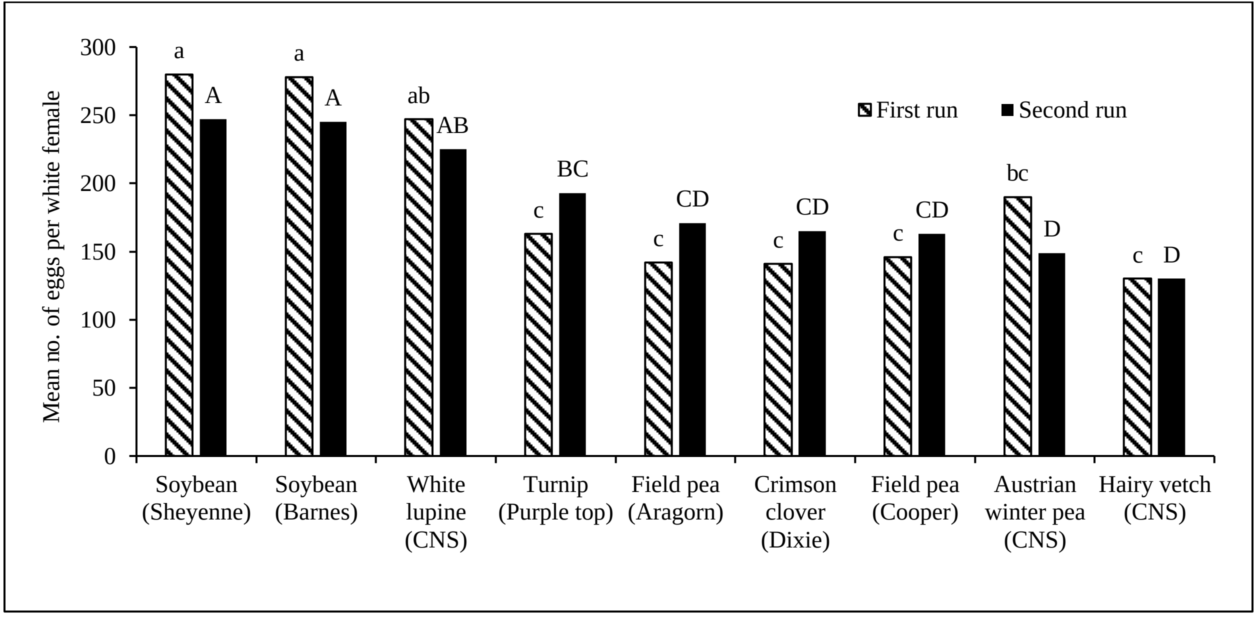

Fig. 2.4. Average number of eggs per white female produced on different cover crops and susceptible soybean cultivars (Barnes and Sheyenne) for the soybean cyst nematode population, SCN2W in artificial infestation. Average number of eggs produced on the cover crops were significantly different from Barnes and Sheyenne for SCN2W with different letters (*P* < 0.05) for both runs of the experiment with artificial inoculation.

Discussion

Out of thirty-five cover crops species/cultivars tested, SCN developed white females only in seven crops/cultivars, from two plant families, *Brassicaceae* and *Fabaceae.* None of the crops from *Linaceae* and *Poaceae* supported SCN reproduction. Our results are consistent with other studies which evaluated crimson clover, hairy vetch, and white lupine cultivars. These crops and cultivars were tested and confirmed as hosts for a SCN population (Donald et al. 2007; Poromarto and Nelson 2010). Their results showed that other species such as red clover, sweetclover (*Melilotus officinalis* L.), and field pea cultivars Admiral, Eclipse, Majoret, Miami, Mozart and Striker, and camelina cultivars Blaine Creek, Boha, Calena, Celine, Ligena, and Suneson were classified as nonhosts for SCN population. We found one different cultivar of field pea (Aragorn) to support some reproduction of SCN103 and was a host for SCN2W population.

The reproduction of SCN differs among the host cultivars and resistant soybean cultivars derived from the same resistant sources (Acharya et al. 2017; Poromarto and Nelson 2009). Similar results were observed in our experiments, where Austrian winter pea and field pea cv. Cooper only supported reproduction of SCN2W but did not support SCN103 in both naturally SCN-infested soil and artificially infested soil. Although these results suggest that SCN populations and crop cultivars may influence the host status for SCN, further research is necessary to confirm it by using diverse cultivars and SCN populations on same experiments. Turnip cv. Purple Top supported reproduction of both SCN populations. This is the first report confirming that SCN populations can produce mature cysts on a turnip cultivar. In a previous study by Riggs, (1987), juveniles (J2) of SCN race 3 were able to penetrate the turnip, but development was confined up to J3, so further research will be needed to confirm turnip as host/poor host for different SCN populations. Majority of the previous studies have used a single

SCN population, for evaluating the hosting ability (Poromarto et al. 2015; Poromarto and Nelson 2010; Riggs 1987; Schmitt and Riggs 1991; Venkatesh et al. 2000), while in this study, two SCN populations SCN103 and SCN2W were used.

A study by Kobayashi et al. (2017) showed that alfalfa, Austrian winter pea, berseem clover (*Trifolium alexandrinum* L.), cowpea, crimson clover, field pea, hairy vetch (two different cultivars), red clover (three different cultivars), and white clover (two different cultivars) supported SCN reproduction at very low level, suggesting poor hosts. Our results support their findings that Austrian winter pea, crimson clover, field pea (Aragorn and Cooper), and hairy vetch were poor host/suitable hosts, but alfalfa, cowpea, and berseem clover were non-hosts for both SCN populations in our study. This suggests that, evaluating all the available cultivars for each crop is always important to understand the host status of crops for SCN populations. White mustard *(Sinapis alba* L.) and rapeseed (*Brassica napus* L.) have been found to suppress plant-parasitic nematodes (Hoorman 2011) but have not been tested for hosting ability for SCN. White mustard cv. Master and rape cv. Dwarf Essex were tested in our experiments and both of them were non-hosts for both SCN populations. For the rape cv. Dwarf Essex, our results were supported by the results from Bernard and Montgomery (1993) on winter rapeseed cultivars Bridger, Gorzanski, and Viking. In their study, these three cultivars tested did not support SCN reproduction, except for a rare root penetration by J2s and an occasional mature SCN male development.

In our experiments, the number of white females on the roots of the diverse crop species could have been affected by different root size as compared with susceptible soybean. In order to account for root size and be consistent throughout the experiment, we used a single plant for each crop. White females were mature when observed under the microscope, suggesting that eggs were viable for further infections. This indicates that even a very low level of SCN reproduction in these poor hosts and suitable hosts could build up SCN populations in the field over time. Significant differences in the average number of eggs per white female of SCN2W were observed in cover crops compared with those of susceptible soybean. This was not observed for SCN103. We determined the average number of eggs per white female only in artificially infested soil experiments for both SCN populations because in naturally infested soil, both cysts (dead females from field) and white females were present together at the end of the experiments. Further research will be required to understand the effect of SCN populations on eggs per white female.

The screening for host status of diverse crops for two SCN populations was performed by using both naturally SCN-infested soil and artificially infested soil. Our results suggested that host status of the crops evaluated is not influenced by the inoculation methods, but differences were observed in the number of white females produced on the same crops. Naturally infested soil had high SCN white females on the test plants, even when the level of initial inoculum was not significantly higher than the artificially infested soil. This may be due to the stress to the nematode population during extraction and crushing of white females to obtain eggs and juveniles as inoculum while in naturally infested method, SCN infested soil from the field was directly used as inoculum. Although differences were observed in the numbers of white females in two soils, all the crops showed the same trend of host responses. Two rotational crops corn and wheat known as non-hosts of SCN were included and classified as non-hosts in this study as expected.

Cover crop acreage is increasing in the northern Great Plains with the increase in awareness of improving soil quality and providing ecosystem benefits (Conservation Technology

Information Center (CITIC 2017). Balansa clover (*Trifolium michelianum* Savi) (cultivar not stated, CNS), Ethiopian cabbage (*Brassica carinata* L.) (CNS), faba bean (*Vicia faba* Roth) (CNS and forage type cv. Petite), forage oat (*Avena sativa* L.) (CNS), Japanese millet (*Echinochloa esculenta* L.) (CNS), flax (*Linum usitatissimum* L.) (cv. Carter), foxtail millet [*Setaria italica* subspp. *rubofructa* (L.) P. Beauv.] (cv. Siberian), white mustard (*Sinapis alba* L.) (cv. Master), oilseed radish (cvs. Concorde, Control, and Image), sunnhemp (CNS), turnip (cv. Purple Top), white proso millet (*Panicum mileaceum* L.) (CNS), and winter rye (cv. ND Dylan) (Table 1) had never been evaluated for the reproduction of SCN populations from North Dakota fields before, and all were classified as non-hosts for SCN in this study. Crops such as alfalfa, winter rye, plants in the *Brassicaceae* family and other crops are planted as cover crops in soybean fields (Clark 2007; Villamil et al. 2006). In addition to improving soil quality and ecosystem, if crops are non- or poor-SCN hosts and have the potential to reduce SCN numbers in infested fields, that would be an additional benefit for farmers.

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CHAPTER 3: CAN WINTER CAMELINA, CRAMBE, AND BROWN MUSTARD REDUCE SOYBEAN CYST NEMATODE POPULATIONS?[2]

Abstract

Industrial oilseeds have a great potential in the northern Great Plains both as oilseeds and as cover crops sown following wheat (*Triticum aestivum* L.) harvest and before soybean [*Glycine max* (L.) Merr.] sowing in the following spring. One of the most important biotic stresses in soybean production is soybean cyst nematode (*Heterodera glycines* Ichinohe, SCN), a serious pest that affects 90% of the soybean producing areas in the U.S. The objective of this study was to evaluate the host status of and the SCN population reduction by, winter camelina [*Camelina sativa* (L.) Crantz, cv. Joelle], crambe (*Crambe abyssinica* Hochst. Ex R.E.Fr., cv. BelAnn), and brown mustard (*Brassica juncea* L. cv. Kodiak). The experiments were performed in a growth chamber at 27°C for 35 days by planting the crops in soil naturally infested with SCN and autoclaved sandy soil artificially inoculated with two SCN populations from two fields in North Dakota. Soybean cyst nematode did not reproduce on brown mustard or camelina with a female index (FI) of 0, suggesting these are non-hosts, while it reproduced on crambe. The numbers of white females on crambe ranged from 1 to 13 per plant with FI of 0.2 to 1.1 in naturally infested soils, and 1 to 4 per plant with FI of 1.2 to 2.5 in artificially infested soils, thus crambe would be classified as a poor-host (FI < 10). Brown mustard and winter camelina reduced the SCN populations by an average of 51% and 48%, respectively, while crambe only reduced the

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[2] The material in this chapter was co-authored by Krishna Acharya (Department of Plant Pathology, North Dakota State University), Guiping Yan (Department of Plant Pathology, North Dakota State University) and Marisol Berti (Department of Plant Sciences, North Dakota State University). Krishna Acharya had primary responsibility for collecting soil samples from the fields and perform greenhouse experiments. Krishna Acharya was the primary developer of the conclusions that are advanced here. Marisol Berti helped by providing seeds of cover crops for this experiment and served as proofreader. Guiping Yan served as the principle investigator and corresponding author and checked the statistical analysis conducted by Krishna Acharya. This paper was published in Industrial Crops and Products. Vol.140: 111637 (Reproduced with permission).

populations by an average of 24%, across all the experiments with naturally infested soils when compared with the initial population levels. Both brown mustard and camelina consistently reduced the SCN populations but crambe did not steadily reduce the SCN populations when compared with the non-planted control (fallow). Further understanding the effects of these crops on SCN populations under natural field conditions is needed to determine if cover crops can be used for sustainable SCN management in SCN-infested soybean fields.

Key words: *Heterodera glycines*; reproduction; hosting ability; cover crops; SCN population reduction.

Introduction

Camelina, crambe, and brown mustard are emerging oilseeds in the Midwest region of the U.S. The oil and meal from these crops are potential feed-stocks for biofuel and bio-based products as an alternative to fossil-fuels derived products (Berti et al. 2016; Carlsson 2009; Righini et al. 2016; Zanetti et al. 2013).

Winter camelina has a potential as a winter annual in double- and relay-cropping systems or intersown into standing soybean in the U.S. northern Great Plains (Berti et al. 2015, 2017b; Gesch et al. 2014). Winter camelina is very winter-hardy which makes it an ideal cash cover crop in this region. Camelina provides soil cover, reduces soil NO_3-N preventing nitrate leaching and nutrient run-off (Berti et al. 2017a), provides pollen and nectar for pollinators (Eberle et al. 2015), and can be harvested early enough to allow a second crop after its harvest (Berti et al. 2015). Crambe and brown mustard do not survive the winter in the Midwest, but they can play a role when included as rotational crops in wheat-soybean rotations as full-season oilseeds or fall-sown cover crops after wheat harvest. Camelina, mustard, and crambe residues can be incorporated into the field after harvested. The residues contain glucosinolates and their chemical breakdown products isothiocyanates act as a bio-fumigant for controlling different pathogens, including plant-parasitic nematodes (Anita 2012).

Soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe), is an obligatory sedentary endo-parasitic nematode, which infects and colonizes soybean roots. It is one of the major yield-limiting pathogens of soybean worldwide (Niblack et al. 2006; Riggs 1977; Wrather and Koenning 2009). It has now been detected in 90% of the soybean producing states in the U.S. (Tylka and Marett 2017). In North Dakota, SCN was first confirmed in 2003 in Richland County (Bradley et al., 2004) and has been confirmed in at least 19 counties (Yan et al. 2015). Soybean cyst nematode has a wide host range; many leguminous crops, including oilseed crops, cover crops, and weed species, support its reproduction (Poromarto and Nelson 2010; Poromarto and Nelson 2009). The hosting ability of SCN varies with the host species and their cultivars (Venkatesh et al. 2000; Poromarto and Nelson 2010). Although many crops already have been confirmed as hosts for SCN, other crops suppress/reduce plant-parasitic nematode populations by producing volatile and non-volatile biochemical compounds from their roots and crop residues that inhibit the parasitic nematodes including SCN (Kushida et al. 2003; Riga et al. 2001). Crop rotation with a non-host crop is one of the common management practices commonly integrated with other practices that helps to reducing the SCN egg population densities in the infested fields (Chen et al. 2001; Miller et al. 2006; Schmitt and Riggs 1991).

Research in other countries has reported the reduction of sugarbeet cyst nematode (SBCN) (*Heterodera schachtii* L.) populations with brown mustard and fodder radish (*Raphanus sativus* L.) (Hauer et al. 2016; Hemayati et al. 2017), and the reduction of SCN with crambe in Brazil (Nascimento et al. 2016). Growing brown mustard and incorporating into the soil is a common management practice in Germany to reduce SBCN. However, reduction of SBCN by brown mustard or radish does not mean it will be effective against SCN. Mighty mustard™ brown mustard cv. Kodiak is marketed as a bio-fumigant against soil borne pathogens such as *Sclerotinia*, *Verticillium*, *Rhizoctonia, Fusarium*, *Aphanomyces*, and plant-parasitic nematodes in the U.S. It contains high level of glucosinolates, a chemical that acts a bio-fumigant agent similar to synthetic commercial products such as metam sodium and others.

Some crops such as cabbage (*Brassica oleraceae* L.), tobacco (*Nicotinana tabacum* L.), cotton (*Gossypium hirsutum* L.), hairy vetch (*Vicia villosa* Roth), red clover (*Trifolium pratense* L.), alfalfa (*Medicago sativa* L.), field and sweet corn (*Zea mays* L.), wheat, and soybean cultivars were tested for hatching, penetration, and production of mature SCN white females. The results suggested that soybean and hairy vetch were able to support SCN reproduction, while in red clover, alfalfa, and others, SCN juveniles failed to continue development in the roots (Schmitt and Riggs 1991). Some other crops and weeds were also tested for the reproduction of a SCN population (HG type 0) in North Dakota and SCN showed varied reproduction on those crops. Dry bean (*Phaseolus vulgaris* L.), lupine (*Lupinus albus* L.), henbit (*Lamium amplexicaule* L.), and field pennycress (*Thlaspi arvense* L.) were good hosts; however, crambe, pea (*Pisum sativum* L.), and other species were poor hosts for the SCN population (Poromarto et al. 2015; Poromarto and Nelson 2010; Poromarto and Nelson 2009). Recently, two field populations of SCN were tested for reproduction in brown mustard cv. Kodiak and other cover crops in greenhouse conditions in Iowa, and both SCN populations were not able to reproduce or had very limited reproduction on those crops (Kobayashi-Leonel et al. 2017). However, the effect of those crops on SCN population reduction has not been reported in the northern Great Plains.

Cover crops and industrial oilseeds preceding or interseeded into soybean are becoming common in the northern Great Plains. Most studies have concentrated on soil health benefits of cover crops, but not on the effect of cover crops on soil nematodes. Selection of the effective rotational crops or cover crops in the soybean growing regions is very important, and selection of such crops should be relied on responses of the crops to targeted nematodes. Hosting ability and population reduction by industrial oilseeds for SCN populations could provide guidelines to select appropriate cover crops or rotational crops in soybean-based systems to manage SCN populations.

The objectives of this study were to determine the hosting ability of winter camelina, crambe, and brown mustard for two SCN populations and to evaluate the ability of population reduction of SCN by these crops in greenhouse conditions.

Materials and methods

Winter camelina [*Camelina sativa* (L.) Crantz, cv. Joelle], crambe (*Crambe abyssinica* Hochst. Ex R.E.Fr., cv. BelAnn), Mighty Mustard ™ brown mustard (*Brassica juncea* L. cv. Kodiak), and a susceptible soybean [*Glycine max* (L.) Merr., cv. Barnes] were evaluated for hosting ability and population reduction of SCN.

Soybean cyst nematode populations

Populations of SCN were obtained from two fields, one in Cass County and the other in Richland County, ND. The soil was analyzed for physical properties and the density and HG type of the SCN populations were determined (Table 3.1).

Table 3.1. Soil properties of the naturally infested soils from Richland and Cass counties of North Dakota and the soybean cyst nematode virulence (HG) types for the soils.

County	% Sand	% Silt	% Clay	Textural Class	% OM	pH	SCN HG Type
Richland	65	23	12	Sandy Loam	2.0	7.9	0
Cass	43	37	20	Loam	2.7	7.7	7

OM = organic matter

The SCN HG type was determined by inoculating seven indicator lines in greenhouse conditions (Niblack et al., 2002). The lines PI548402 (Peking, indicator line 1), PI88788 (2), PI90763 (3), PI437654 (4), PI209332 (5), PI89772 (6), PI548316 (Cloud, 7), and the susceptible check (Barnes) were used. The SCN population from Richland County (SCN103) was confirmed as HG type 0, and the SCN population from Cass County (SCN2W) was confirmed as HG type 7. For artificial infestation, both SCN populations (SCN103 and SCN2W) were increased by inoculating susceptible soybean (Barnes) plants in controlled greenhouse conditions. White females for each of the populations were collected from the susceptible soybean roots by following the sieving and decanting method described by Krusberg et al. (1994) and were crushed to obtain eggs and juveniles by following the procedures described by Faghihi and Ferris (2000). The white females were crushed by rubber stopper of a motorized stirrer (MasterForce Drill Press, Menards, Fargo, ND) and the eggs and juveniles were released and collected on a 20-µm-pore sieve nested under a 75-µm-pore sieve, then used as inoculum in experiments with artificial inoculation.

Growth chamber experiments

Seeds of each crop were directly planted into a cone-type container (3.8-cm in diameter and 21-cm in height) (Stuewe and Sons, Inc., Tangent OR) filled with naturally infested soils with each of the nematode populations (SCN2W and SCN103) from two counties, Cass and Richland. The soil was thoroughly mixed to have a uniform nematode population for planting and infection. A single plant was grown in each container. Each entry (crop, susceptible check, non-planted naturally infested soil) was replicated five times for each SCN population in a completely randomized design in a plastic rack with 14 x 7 wells and kept in a growth chamber (GR64, Conviron, Winnipeg, Manitoba, Canada). The growth chamber was constantly maintained at 27-28$_{o}$ C and day length of 16 h. After 35 days of growth, the plants were removed and females and cysts from both roots and soil in each container were then extracted using the sieving and decanting method described by Krusberg et al. (1994). Roots of the plants and soil surrounding the roots were sprayed with a high-pressure water shower to dislodge all the newly formed females and old cysts in the infested soil in to a 4-liter bucket filled with 3/4 full of water, and then it was stirred and poured into the sieves immediately. The females and cysts were collected in a 250-µm-pore sieve nested under a 710-µm-pore sieve, and were counted under a dissecting microscope (SM 100 Series, Swift Optical Instrument, INC. TX, USA) in a gridded Petri-plate with the nematode suspension. Only white or light yellow females from each plant were counted to determine the host status. A female index was calculated for each crop by using the following formula.

$$\text{Female Index (FI)} = \frac{\text{Average no.of white females found on a cover crop}}{\text{Average no.of white females found on a susceptible check}}\, x100$$

Female index 10 or greater was considered as a host for SCN, less than 10 FI was considered as a poor-host, and FI value of 0 was considered as a non-host for SCN (Poromarto and Nelson, 2010; Poromarto et al., 2015). After counting white females, all the newly formed white females and old cysts from previous years in the soil for each plant and non-planted control were crushed to release eggs and juveniles by following the procedures of Faghihi and Ferris (2000) described previously. The eggs and juveniles were then counted under the microscope (Zeiss Axiovert, 25, Carl Zeiss Microscopy, NY, USA) to determine final population densities. Percentage of population reduction and reproductive factor for each crop were calculated as follows.

$$\text{Population reduction (\%)} = \frac{\text{(Initial} - \text{final) SCN egg and juveniles population}}{\text{Initial SCN egg and juveniles population}}\ x\ 100$$

$$\text{Reproductive factor (RF)} = \frac{\text{Final SCN eggs and juveniles population on a crop}}{\text{Initial SCN eggs and juveniles population on the crop}}$$

The experiment was repeated once to confirm both hosting ability and population reduction by using naturally SCN-infested soils. The hosting ability of the crops was also confirmed by using an artificial inoculation method. Each cover crop and susceptible soybean was planted in a small container containing about 100 cm_3 of sterilized sandy soil and was then inoculated with 2,000 eggs and juveniles of each SCN population. Inoculation was performed at the time of planting by making 2 to 3 holes of 3-5 cm depth around the root zone, then 5 mL of SCN egg and juvenile suspension was added and covered with moist soil. Each entry was replicated four times in a completely randomized design in a plastic rack with 14 x 7 wells, then kept in a growth chamber at 27-28°C. After 35 days of growth, white females were extracted using the same procedure as described above, and counted under the dissecting microscope (SM 100 Series, Swift Optical Instrument, Inc. TX, USA) in gridded Petri-plates with the nematode suspensions, and a female index for each crop was calculated as described previously. After counting, white females were crushed to release the eggs, and the numbers of eggs and juveniles per white female were determined. The egg and juvenile numbers were compared with numbers of eggs and juveniles per white female obtained from the susceptible soybean check.

Data analysis

Data were analyzed using SAS 9.4 (SAS Institute, Cary, NC). Because the initial SCN population in each of the experiments was different, the data were analyzed separately for each run of the experiments. The general linear model (GLM) with *F*- protected LSD mean separation with a significance level of 5% was used to determine the difference in average number of white females, average reproductive factor, and population reduction of crambe, winter camelina, and brown mustard and then compared with the non-planted control. Similar procedure was used in the experiments with artificial inoculation and differences were determined for average numbers of white females and final SCN populations in soil for different crops.

Results

In all the four greenhouse experiments with naturally infested soils, camelina cv. Joelle and brown mustard cv. Kodiak did not produce any white females on their roots for both SCN populations, suggesting that these crops do not support SCN reproduction, while crambe cv. BelAnn supported the reproduction of both SCN populations (Table 3.2 and 3.3). In the first run of the experiment, the average numbers of SCN white females on the individual crambe plants ranged from 1 to 5 (FI: 0.2 to 0.6), compared with the susceptible soybean 560 to 888. In the second run of the experiment, the average numbers of SCN white females on the individual crambe plants ranged from 3 to 4 (FI: 0.7 to 1.1), compared with the susceptible soybean 376 to 411. In artificial infestation, both SCN populations did not reproduce (FI = 0) on camelina and brown mustard, but reproduced on crambe (Table 3.4). The average numbers of white females on the individual crambe plants for both populations were 3 (FI: 1.2 to 2.5) as compared with the susceptible soybean with 122 to 260 white females.

White females obtained from both crambe and susceptible soybean were crushed to release eggs and counted under the microscope. The white females produced on crambe had an average of 138 eggs per white female for the two populations as compared with an average of 208 eggs per white female on the susceptible soybean. Poromarto and Nelson (2010) also identified crambe as a poor host for a soybean cyst nematode population (HG type 0) and showed that white females produced on crambe had viable eggs inside the females. The results of these experiments confirmed that crambe is a poor host (FI less than 10) for SCN103 (HG type 0), and demonstrated for the first time that crambe is also a poor host for a different SCN population SCN2W (HG type 7), and mature eggs formed inside the white females.

In the experiment with naturally infested soils, the cover crops were evaluated for population reduction of SCN. Camelina and brown mustard consistently had lower reproductive factors than the non-planted control for both SCN populations (Fig. 3.1 and 3.2), suggesting that these two crops were able to reduce SCN populations in greenhouse conditions compared with the non-planted natural soil serving as fallow. Crambe only had a lower reproductive factor for SCN103 compared with the non-planted control in one of the experiments (Fig. 3.1 and 3.2), suggesting that this crop was not able to consistently reduce SCN populations.

In the first run of the experiments with naturally infested soil for SCN103, crambe and brown mustard significantly reduced the SCN population by an average of 51.0% and 41.9%, respectively, when compared to 18.7% in the non-planted control, but winter camelina did not reduce the SCN population significantly from the control at $P < 0.05$ (Table 3.2). For SCN2W, camelina and brown mustard were able to significantly reduce the SCN population by 61.2% and 50.0%, respectively, while crambe did not reduce the SCN population compared to the non-planted control (Table 3.3).

In the second run of the experiments with naturally infested soil, both brown mustard and camelina were able to reduce significantly ($P < 0.05$) the two SCN populations compared with the non-planted natural soil (Table 3.2 and 3.3). With SCN103, brown mustard and camelina reduced the SCN population by an average of 53.0% and 36.6%, respectively, when compared with 17.1% in the non-planted control, but crambe did not reduce the SCN population compared with the non-planted control (Table 3.2). For the SCN2W, brown mustard and camelina significantly reduced the SCN population by 57.2% and 55.5%, respectively, while crambe did not reduce the SCN population compared with the non-planted control (Table 3.3).

Table 3.2. Reproduction and population reduction of soybean cyst nematode by different crops planted in naturally infested soil from Richland County, ND (SCN103) in greenhouse conditions.

Crop	**No. of white females**[w]	**Female index**[x]	**Final population**[y]	**Population reduction (%)**[z]
First run:				
Camelina cv. Joelle	0 B	0.0	1,940 AB	37.4 AB
Crambe cv. BelAnn	1 A	0.2	1,520 B	51.0 A
Brown mustard cv. Kodiak	0 B	0.0	1,800 B	41.9 A
Non-planted control	0 B	0.0	2,520 A	18.7B
LSD	1		604	19.5
Second run:				
Camelina cv. Joelle	0 B	0.0	3,320 B	36.6 B
Crambe cv. BelAnn	4 A	1.1	4,480 C	14.4 C
Brown mustard cv. Kodiak	0 B	0.0	2460 A	53.0 A
Non-planted control	0 B	0.0	4,340 C	17.1 C
LSD	4		563	10.8

Average initial SCN population (eggs and juveniles) in 100 cm^3 of soil was 3,100 for first run and 5,233 for second run of the experiments.

[w] Average number of white females produced on each plant.

[x] Female index calculated as,

$$\text{Female Index (FI)} = \frac{\text{Average no.of white females found on a cover crop}}{\text{Average no.of white females found on a susceptible check}} x 100$$

Average number of white females on the susceptible soybean check Barnes was 560 and final SCN population (eggs and juveniles) of 122,880 in 100 cm^3 of soil for the first run of the experiment and 376 and final SCN population (eggs and juveniles) of 54,300 in 100 cm^3 of soil for the second run of the experiment.

[y] Average final SCN eggs and juveniles population in 100 cm^3 of soil.

[z] Average population reduction for each crop, calculated as,

$$\text{Population reduction (\%)} = \frac{\text{(Initial − final) SCN egg and juveniles population}}{\text{Initial SCN egg and juveniles population}} x\ 100$$

($P < 0.05$, LSD mean separation), means with same letters are not significantly different from each other.

Table 3.3. Reproduction and population reduction of soybean cyst nematode by different crops planted in naturally infested soil from Cass County, ND (SCN2W) in greenhouse conditions.

Crop	**No. of white females** [w]	**Female index** [x]	**Final population** [y]	**Population reduction (%)** [z]
First run:				
Camelina cv. Joelle	0 B	0.0	1,940 B	61.2 A
Crambe cv. BelAnn	5 A	0.6	4,980 A	0.4 B
Brown mustard cv. Kodiak	0 B	0.0	2,500 B	50.0 A
Non-planted control	0 B	0.0	4,340 A	13.2 B
LSD	4		934	18.7
Second run				
Camelina cv. Joelle	0 B	0.0	2,640 A	55.5A
Crambe cv. BelAnn	3 A	0.7	4,060 B	31.6 B
Brown mustard cv. Kodiak	0 B	0.0	2,540 A	57.2 A
Non-planted control	0 B	0.0	3,980 B	32.9 B
LSD	3		799	13.5

Average initial SCN population (eggs and juveniles) in 100 cm^3 of soil was 5,000 for first run and 5,933 for second run of the experiments.

[w] Average number of white females produced on each plant.

[x] Female index calculated as,

$$\text{Female Index (FI)} = \frac{\text{Average no.of white females found on a cover crop}}{\text{Average no.of white females found on a susceptible check}} x 100$$

Average number of white females on the susceptible soybean check Barnes was 888 with final SCN population (eggs and juveniles) of 155,220 in 100 cm^3 of soil for the first run of the experiment and 411 and final SCN population (eggs and juveniles) of 62,280 in 100 cm^3 of soil for the second run of the experiment.

[y] Average final SCN population (eggs and juveniles) in 100 cm^3 of soil.

[z] Average population reduction for each crop, calculated as,

$$\text{Population reduction (\%)} = \frac{\text{(Initial − final) SCN egg and juveniles population}}{\text{Initial SCN egg and juveniles population}} x\ 100$$

($P < 0.05$, LSD mean separation, means with same letters are not significantly different from each other).

Table 3.4. Reproduction of soybean cyst nematode by different crops planted in artificially infested soil with SCN103 and SCN2W in greenhouse conditions.

Crop	**No. of white females** ^{w}	**Female index** ^{x}	**Final population** ^{y}
SCN103:			
Camelina cv. Joelle	0 B	0.0	0 B
Crambe cv. BelAnn	3 A	2.5	350 A
Brown mustard cv. Kodiak	0 B	0.0	0 B
Non-planted control	0 B	0.0	0 B
LSD	1		99
SCN2W:			
Camelina cv. Joelle	0 B	0.0	0 B
Crambe cv. BelAnn	3 A	1.2	475 A
Brown mustard cv. Kodiak	0 B	0.0	0 B
Non-planted control	0 B	0.0	0 B
LSD	1		203

Average initial SCN population (eggs and juveniles) was 2,000 in 100 cm^3 of soil for both SCN103 and SCN2W.

^{w} Average number of white females produced on each plant.

^{x} Female index based on the susceptible soybean Barnes calculated as,

$$\text{Female Index (FI)} = \frac{\text{Average no.of white females found on a cover crop}}{\text{Average no.of white females found on a susceptible check}} x100$$

Barnes was a susceptible check for calculating female index of other crops. Average number of white females on Barnes was 122 for the experiment using SCN103, and 260 for the experiment using SCN2W.

^{y} Average final SCN population (eggs and juveniles) in 100 cm^3 of soil.

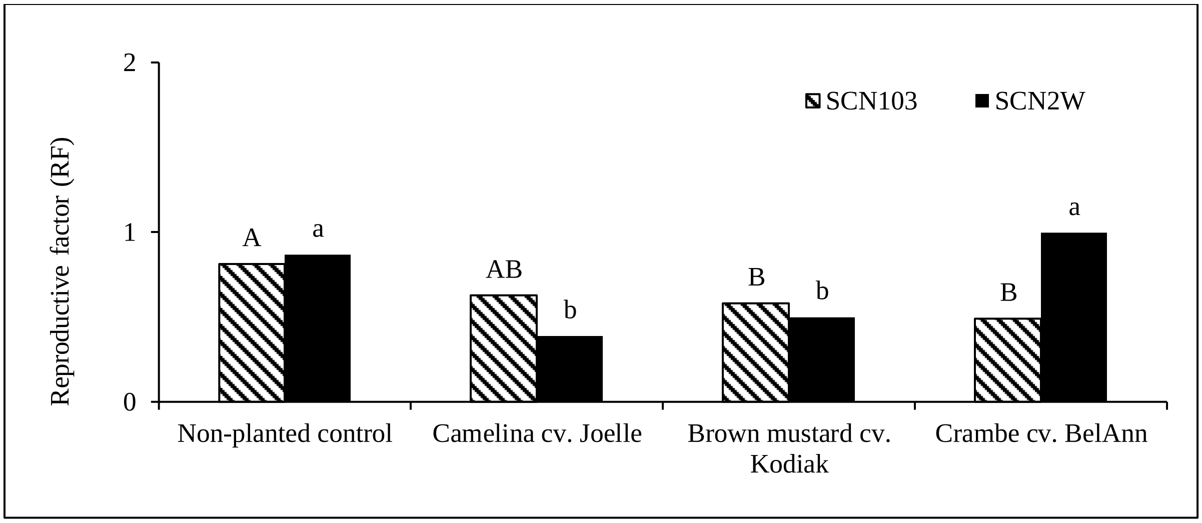

Fig. 3.1. Reproductive factor of soybean cyst nematode populations (SCN103 and SCN2W) on different cover crops and non-planted control in naturally infested soils in greenhouse conditions for first run of the experiments. The reproductive factor was calculated as (RF: final/initial SCN egg numbers). Means with same capital letters are not significantly different for SCN103 ($P < 0.05$, LSD: 0.20), while means with same small letters are not significantly different for SCN2W ($P < 0.0001$, LSD: 0.19).

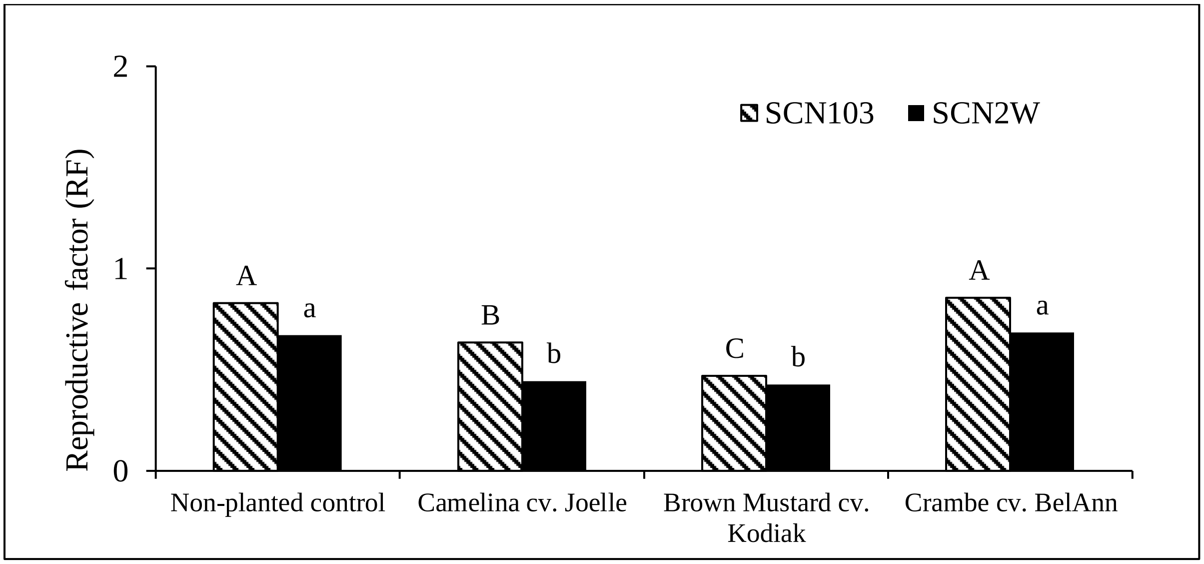

Fig. 3.2. Reproductive factor of soybean cyst nematode populations (SCN103 and SCN2W) on different cover crops and non-planted control in naturally infested soils in greenhouse conditions for second run of the experiments. The reproductive factor was calculated as (RF: final/initial SCN egg numbers). Means with same capital letters are not significantly different for SCN103 ($P < 0.0001$, LSD: 0.11), while means with same small letters are not significantly different for SCN2W ($P < 0.0009$, LSD: 0.14).

Discussion

When compared with the fallow (non-planted control), crambe did not reduce the SCN2W population in both experiments and did not reduce the SCN103 population in one experiment, and brown mustard and camelina reduced the two SCN populations across all the experiments under the controlled greenhouse conditions. This may be due to the fact that crambe is a poor-host and camelina and brown mustard are non-hosts for both SCN populations (SCN103 and SCN2W). To our knowledge, winter camelina cv. Joelle has not been reported for its abilities for both SCN hosting and population reduction. A different crambe cv. FMS Brilhante was reported to reduce the SCN populations compared with the initial field populations in Brazil but the information was not available for the comparison with a fallow treatment (Nascimento et al. 2016). Similar to the present study, brown mustard cv. Kodiak was reported to be a non-host of SCN in Iowa (Kobayashi-Leonel et al. 2017). Additionally, Wen et al. (2017) tested a different brown mustard cv. Pacific Gold for SCN population reduction in field trials in Illinois and found that this cultivar did not consistently reduce SCN numbers across different locations and years.

In recent years, SCN virulence diversity, the ability of nematode populations to reproduce on different resistance sources, has been observed in many states of the northern Great Plains of the U.S. (Acharya et al. 2016; Howland et al. 2018; McCarville et al. 2017; Niblack et al. 2008; Zheng et al. 2006). Soybean cyst nematode ability to reproduce on different crops suggests that these crops could increase or maintain the nematode populations in the field conditions (Poromarto et al. 2015; Poromarto and Nelson 2010; Venkatesh et al. 2000). Recommendation from these greenhouse experiments may be early regarding selection of these crops in soybean cropping system, but further research on the effects of these crops on SCN populations under natural conditions in the field would provide the information about suitability of these crops in sustainable SCN management.

In conclusion, cover crops tested for hosting ability of two SCN populations indicated that crambe cv. BelAnn was able to support very low level of reproduction for both SCN103 and SCN2W populations compared with the susceptible soybean, while winter camelina cv. Joelle and brown mustard cv. Kodiak did not show SCN reproduction. The results suggest that crambe is a poor-host and winter camelina and brown mustard are non-hosts for both SCN populations. All crops except crambe in second run were able to reduce SCN egg numbers in both runs of experiments for SCN103, while in both runs of experiments for SCN2W only winter camelina and brown mustard reduced the SCN egg numbers compared with the non-planted control (fallow) in the naturally infested field soils under greenhouse conditions. Therefore, different SCN populations from fields need to be tested with additional cultivars of crambe, camelina, and brown mustard to determine if these plants have any potential for use in reducing SCN populations in infested fields. Further research under natural field conditions will strengthen the information about the suitability of these industrial crops as cover crops or rotational crops in soybean growing areas of the northern Great Plains.

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CHAPTER 4: EFFECTS OF COVER CROPS ON POPULATION REDUCTION OF SOYBEAN CYST NEMATODE, *HETERODERA GLYCINES* [3]

Abstract

Greenhouse and microplot experiments were conducted to evaluate the effects of cover crops on population reduction of a major soybean pest, soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe). Ten crop species, including annual ryegrass (*Lolium multiflorum* L), Austrian winter pea (*Pisum sativum* L. *subsp. arvense*), carinata (*Brassica carinata* A. Braun), faba bean (*Vicia faba* Roth), foxtail millet [*Setaria italica* (L) P. Beauvois], daikon radish (*Raphanus sativus* L.), red clover (*Trifolium pratense* L.), sweetclover (*Melilotus officinalis* L.), turnip (*Brassica rapa subsp. rapa* L.), and winter rye (*Secale cereale* L.) were planted along with susceptible soybean [*Glycine max* (L.) Merr., cv. Barnes] in soil naturally infested with each of two SCN populations (SCN103 and SCN2W) from two North Dakota soybean fields. Crops were grown at 27$_o$ C for 35 days in greenhouse experiments, while they were grown for 75 days in an external environment (Microplot). Soil samples were collected from each pot for nematode extraction and SCN eggs were counted to determine the final SCN egg density. The SCN reproductive factor (RF: final/initial SCN egg numbers) and population reduction [PR: (Initial-final density)/initial density x 100 %] were determined for each crop. In greenhouse experiments, no significant differences were observed in RF and PR by any crop compared to fallow (non-planted control) for both SCN populations. However, in microplot

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[3] The material in this chapter was co-authored by Krishna Acharya (Department of Plant Pathology, North Dakota State University), Guiping Yan (Department of Plant Pathology, North Dakota State University) and Addison Plaisance (Department of Plant Pathology, North Dakota State University). Krishna Acharya had primary responsibility for collecting soil samples from the fields and perform greenhouse experiments. Krishna Acharya was the primary developer of the conclusions that are advanced here. Addison Plaisance helped in soil sample collection and microplot preparation. Guiping Yan served as principle investigator and corresponding author and checked the statistical analysis conducted by Krishna Acharya. This paper is to be submitted to the journal Plant Disease.

experiments, all tested cover crops and non-planted control had significantly ($P < 0.0001$) lower RF for SCN populations compared to susceptible soybean (Barnes) in 2016 and 2017. Also, a significant difference ($P < 0.0001$) was observed in SCN population reduction by all the tested cover crops compared to non-planted control (fallow). All the tested cover crops except Austrian winter pea, carinata, faba bean, and foxtail millet consistently reduced SCN egg numbers compared with fallow in both years of microplot experiments. The population reductions of SCN by the cover crops in microplot experiments ranged from 44 to 67% of the initial population. Annual ryegrass and daikon radish reduced more SCN egg numbers than others, and had an average of 65 and 67% reduction from initial population density, respectively for both years. The results suggested that cover crops reduced the SCN populations in external microplot conditions, and their use has great potential for improving SCN management in infested fields.

Key words: *Heterodera glycines*, cover crops, external environment, reproductive factor, population reduction, management.

Introduction

The soybean cyst nematode, (SCN; *Heterodera glycines*, Ichinohe) is an economically damaging pest of soybean in the United States (Allen et al. 2017; Mitchum 2016; Niblack et al. 2006). SCN has been identified as the disease causing the most yield and economic loss in soybeans in the United States, with annual economic loss from this nematode estimated at more than 1 billion US dollars (Allen et al. 2017; Koenning and Wrather 2010; Wrather and Koenning 2009). This nematode infects soybean, but its host range includes other important leguminous crops such as common beans (*Phaseolus vulgaris* L.) and different weed species (Poromarto and Nelson 2009, 2010; Poromarto et al. 2015). The SCN has been detected in all soybean producing states of the United States (Tylka and Marett 2017). In North Dakota, it was first detected in

2003 in Richland County (Bradley et al. 2004) and since then has been detected in at least 19 counties (Yan et al. 2015).

Host resistance and crop rotation are the commonly adopted management practices to control SCN because of their eco-friendly and economical nature (Miller et al. 2006; Niblack et al. 2006; Oyekanmi and Fawole 2010). However, virulence population diversity is becoming a major challenge for managing this nematode. Recent research reports on SCN virulence changes have been published in many soybean-producing states in the United States (Acharya et al. 2016; Niblack et al. 2008; Zheng et al. 2006). Research reports indicated that SCN-resistant cultivars derived from resistant source PI88788 may have reduced effectiveness in controlling *H. glycines* populations (Acharya et al. 2017; Hershman et al. 2008; McCarville et al. 2017). This situation is mainly due to the continuous planting of the SCN-resistant soybean cultivars derived from single resistance source PI88788. A very few cultivars are derived from Peking and Hartwig, but they are not widely used (Mitchum 2016). Although host resistance is the most effective and economic measure for nematode management, it requires lengthy breeding processes and is limited by few available resistance gene sources. To better manage SCN in soybean fields, an alternative integrated approach is necessary, an approach which can slow down the virulence changes in nematode population as well as reduce the nematode numbers in the infested fields. Integrating cover crops is such an approach for SCN management. Previous research studies have suggested integrating cover crops in management strategies to help reduce plant-parasitic nematode numbers in infested fields (Halbrendt 1996; Kruger et al. 2013). In addition to reduction of nematode numbers, cover crops provide multiple benefits, such as improving the soil quality for plant growth, improvement in the nutrient cycle, and preventing soil degradation (Dagel et al. 2014; Hunter et al. 2019; Karlen et al. 2006; Reeves 2017; Snapp et al. 2005).

Another in vitro trial showed the greater mortality of sugarbeet cyst nematode (SBCN; *Heterodera schachtii*) when treated with the liquid chromatography fraction derived from marigold seed exudates as compared to the water control (Riga et al. 2005). In Michigan, when oilseed radish (*Raphanus sativus* L.) was planted before planting sugarbeet (*Beta vulgaris* L.), the radish acted as a trap crop and reduced the sugarbeet cyst nematode population, a major nematode that infects sugarbeet crop (Poindexter 2011).

Various research reports have been published for the hosting ability of diverse crops and plant species for SCN populations but the effects of cover crops on SCN population reduction is not well documented. Different crops, such as alfalfa (*Medicago sativa* L.), cabbage (*Brassica oleracea* var. *capitata* L.), hairy vetch (*V. villosa* L.), red clover, field corn (*Zea mays* L.), sweet corn (*Z. mays* L.), and wheat (*Triticum aestivum* L.) were compared with soybean cultivars for hatching, penetration, and production of mature SCN cysts. Only soybean and hairy vetch supported SCN reproduction, while SCN juveniles barely penetrated red clover and alfalfa (Schmitt and Riggs 1991).

Root exudates from diverse cover crops species were tested for SCN egg hatching and nematicidal effect on juveniles; annual ryegrass and white clover (*Trifolium repens* L.) showed a significant increase in SCN egg hatching and decreased the juvenile's numbers by depleting lipid reserves in their body in the absence of host plants (Riga et al. 2001). Furthermore, SCN eggs were able to hatch and penetrate roots of sunnhemp and showy rattlebox but were not able to form mature SCN females and were thus unable to continue their life cycle (Kushida et al. 2003). Based on the several reports of cover crops and SCN interaction, it is possible that different mechanisms, such as non-host, nematicidal activity, and acting as trap crops, are responsible for reducing nematode numbers when the crops were planted in SCN infested fields (Niblack and

Chen 2004; Tylka 2014). A study conducted in Minnesota by Chen et al. (2006) showed that inter-seeded red clover and alfalfa as cover crops may reduce the SCN numbers in the infested fields. They also suggested that yield reduction may be accrued due to competitions, so later planting date of those cover crops may be appropriate for soybean-corn rotation in Minnesota. Most of the fields in the northern Great Plains remain fallow for about 2-3 months after soybean/wheat harvesting before the snow covers in the winter. There is a possibility of planting cover crops for providing the cover on fallow land before the winter kills. However, the effects of commonly grown or potential to be grown cover crops on SCN population reduction have not been well- documented in the northern Great Plains, so the current research focuses on utilizing suitable cover crops for managing of *H. glycines* in the northern Great Plains. The objective of this study was to evaluate the effects of cover crops on population reduction of SCN populations from North Dakota in infested soils.

Materials and methods

Cover crops

The cover crops include different species from three plant families including Brassicaceae, Fabaceae, and Poaceae. The crops were selected based on their current or potential use as cover crops in the northern Great Plains (Table 1). A soybean cultivar (Barnes) susceptible to SCN was used as a positive control. The seeds were acquired from the Forage and Biomass Crop Production Program at North Dakota State University, Fargo, ND.

Table 4.1. List of the cover crop species tested in greenhouse and microplot experiments.

Crop (Cultivar or Cultivar Not Stated = CNS)	Scientific name	Family
Annual ryegrass (CNS)	*Lolium multiflorum* L.	*Poaceae*
Austrian winter pea (CNS)	*Pisum sativum* L. *subsp. arvense*	*Fabaceae*
Carinata (CNS)	*Brassica carinata* A. Braun	*Brassicaceae*
Faba bean (CNS)	*Vicia faba* Roth	*Fabaceae*
Foxtail millet (Siberian)	*Setaria italica* (L.) P. Beauvois	*Poaceae*
Daikon radish (CNS)	*Raphanus sativus* L.	*Brassicaceae*
Red clover (Allington)	*Trifolium pratense* L.	*Fabaceae*
Sweetclover (CNS)	*Melilotus officinalis* L.	*Fabaceae*
Turnip (Pointer)	*Brassica rapa subsp. rapa* L.	*Brassicaceae*
Winter rye (ND Dylan)	*Secale cereale* L.	*Poaceae*

Soybean cyst nematode populations

Two SCN populations, SCN103 and SCN2W were used as described on Acharya et al. (2019). Large amount of naturally infested soils for both SCN103 and SCN2W was collected from respective fields. For the year 2016, the initial SCN population (eggs and juveniles) per 100 cm_3 of soil was 4,540 and 7,540 for SCN103 and SCN2W, respectively at planting. For 2017, initial SCN population (eggs and juveniles) per 100 cm_3 of soil was 6,500 and 6,600 for SCN103 and SCN2W, respectively at planting.

Greenhouse experiments

Experiments were conducted to evaluate the population reduction abilities of the selected cover crops for two SCN populations (SCN103 and SCN2W) in controlled greenhouse conditions at the Agricultural Experiment Station Research Greenhouse Complex - NDSU Agriculture and Extension. Ten cover crops and susceptible soybeans (Barnes) were planted in naturally SCN-infested soils from two fields. Naturally SCN-infested soils were collected from above mentioned fields and used for experiments after analyzing soil properties, initial SCN egg densities, and virulence phenotypes. Seeds were directly sown in cone type containers (3.8-cm in diameter and 21-cm in height) (Stuewe and Sons, Inc., Tangent OR) each containing 100 cm^3 of soil for each soil type. Cone-tainers were arranged on a 14 x 7 -cell plastic rack in a completely randomized design (CRD) with five replicates and kept in a growth chamber (GR64, Conviron, Winnipeg, Manitoba, Canada) maintained at a temperature of 27-28°C and daylight period of 16 h. Plants were grown for 35 days with watering regularly to keep required moisture and fertilized once with Peter's 20-10-20 water-soluble fertilizer. After 35 days, SCN white females were extracted from soil and roots of individual plants by using decanting and sieving procedures by Krusberg et al. (1994). The white females were dislodged by rubbing of roots with a high-pressure water shower, then all cysts (white females and old cysts) collected by pouring suspension from a 4-L bucket filled with 3/4 full of water into a 250-µm-pore sieve nested under a 710-µm-pore sieve (Krusberg et al. 1994). The host status of these crops was already determined in a previous study (Table 2) (Acharya et al. *Under review*).

Entire cyst and white females collected for each crop, including non-planted control were crushed by a rubber stopper attached to a motorized drill press (MasterForce Drill Press, Menards, Fargo, ND) to release the eggs and juveniles as described by Faghihi and Ferris (2000). These were then counted under the microscope (Zeiss Axiovert, 25, Carl Zeiss Microscopy, NY, USA) to determine the final egg and juvenile numbers for each SCN population on each crop. The reproductive factor (RF) of each SCN population on each crop was calculated as RF = the final SCN population divided by the initial SCN population. The population reduction of SCN by each crop and in non-planted control was calculated as follows:

Population reduction (%) =

$$\frac{\text{Initial SCN population on tested crop} - \text{final SCN population on tested crop}}{\text{Initial SCN population on tested crop}} * 100$$

The significant difference between population reduction (%) for each crops and natural population reduction (%) was in non-planted naturally infested soils were compared for both SCN populations.

Microplot experiments

Experiments were conducted in a research field site at North Dakota State University during a time period of the second week of August to the first week of November of the years 2016 and 2017. In 2016, the average soil temperature from August to November ranged from 23 to 6°C, while in 2017 the average soil temperature from August to November ranged from 21 to 1°C (NDAWN 2019). Naturally SCN-infested soils were collected from each field of Cass and Richland counties in North Dakota. The soil samples from each field were mixed thoroughly to distribute SCN populations uniformly. The SCN population density of each soil was determined by extracting nematode from soil subsamples of 100 cm^3 of soil and, then crushed to obtain eggs and juveniles and counted under the microscope. For setting up the microplot, holes were dug in the field to hold the plastic pots so that there was about 8-cm of the pot above the soil surface. The other remaining areas of the field plot area were covered with plastic mesh [weed barrier (ECO gardener premium, 5OZ Pro garden weed barrier landscape fabric)] to prevent the weeds and contamination of the pots from surrounding soil. An external fence was built to prevent the entry of wild animals. Large plastic pots (22.86-cm in diameter and 20.30-cm in height) (High Performance 200, Haviland, OH, USA) were used for the microplot evaluations. Selected crops were planted in each plastic pot containing about 5 kg of soil from each field. Standard seeding rate of each crop was used for maintaining numbers of plants per pot for each crop, including non-planted control (Midwest Cover Crops Council. 2014). After emergence, the plant numbers per pot were: five plants for carinata and turnip, three plants for annual ryegrass, sweetclover, red clover, foxtail millet, winter rye, and daikon radish, two plants for Austrian winter pea and soybean, and one plant for faba bean. Pots were kept in a greenhouse room for two weeks for better establishment, then moved to the external environment (microplot). The experiments were conducted in randomized complete block design (RCBD) with 5 replicates. The microplot pots were regularly watered to keep required moisture for two weeks to prevent drying and fertilized twice with Peter's 20-10-20 water-soluble complete fertilizer. Seventy-five days after pots were placed in field, three soil cores were collected from each pot by taking soil across the roots of the plants. These three cores were thoroughly mixed, and then sub-sample of 100 cm^3 of soil was used to determine the final population density by extracting SCN cysts from the soil using a standard method described previously (Krusberg et al. 1994). The cysts were then crushed by using a procedure described by Faghihi and Ferris (2000). The resultant SCN eggs were counted under the microscope (Zeiss Axiovert, 25, Carl Zeiss Microscopy, NY, USA). The SCN reproductive factor and population reduction (%) for each crop and non-planted control were calculated by using formulas previously described in the greenhouse experiments.

Data analysis

Data for each experiment were analyzed [using SAS 9.4 (SAS Institute, Cary, NC)] separately, because the initial SCN population in each of the experiments was different for each year. The general linear model (GLM) with Tukey's honestly significant difference (HSD) mean separation at 5%, was used to determine the significant difference in final SCN population, average population reduction (PR%), and average reproductive factor (RF) for each crop and non-planted control in both microplot and greenhouse experiments. For each crop, the reproductive factor was compared with susceptible soybean (Barnes) and population reduction percentage was compared with fallow (non-planted control).

Results

Greenhouse experiments

Significant differences in reproductive factor (RF) for each crop, when compared with susceptible soybean cultivar Barnes, were observed for both SCN populations SCN103 and SCN2W. Population SCN103 had a significantly lower average reproductive factor (RF: ranged from 0.14 to 0.54) for all the tested cover crops and non-planted natural soil when compared with susceptible soybean ($P < 0.0004$). The SCN2W had significantly lower average reproductive factor (RF: ranged from 0.17 to 0.64) for all the tested cover crops and non-planted natural soil compared with susceptible soybean ($P < 0.05$) (Table 4.2). In evaluation of cover crop effects on the reduction of the SCN population, only Austrian winter pea, faba bean, red clover, foxtail millet, sweetclover, and carinata significantly ($P < 0.05$) reduced the population of SCN103 (range of reduction from 62 to 86%) compared with the non-planted control. In case of SCN2W, none of the crops significantly reduced SCN population compared to the non-planted control (Fig. 4.1).

Microplot experiments- Reproductive factor

In 2016, the SCN103 population had significantly ($P < 0.0001$) lower reproductive factor (RF: 0.28 to 0.66) on all tested crops compared with the susceptible soybean (Barnes RF: 0.91), except for Austrian winter pea, faba bean, and foxtail millet (Table 4.3). The population SCN2W had a significantly ($P < 0.0001$) lower average reproductive factor (RF: 0.32 to 0.76) on all tested cover crops compared with susceptible soybean (Barnes, RF: 1.50). For the SCN2W population, the average reproductive factor of Austrian winter pea and non-planted control were significantly higher than all other tested cover crops but significantly lower than the susceptible soybean (Barnes) (Table 4.3).

Table 4.2. Reproductive factor of soybean cyst nematode for cover crop species, a susceptible soybean, and for non-planted controls in naturally infested soils (populations SCN103 and SCN2W) under greenhouse conditions.

Crop (Cultivar or Cultivar Not Stated = CNS)	**Reproductive factor** [x]		**Host status (Acharya et al. *Under review*)** [y]
	SCN103	**SCN2W**	
Soybean (Barnes)	42.13 A	34.86 a	Host
Non-planted natural soil	0.54 B	0.55 b	Non-host
Winter rye (Dylan)	0.42 B	0.36 b	Non-host
Turnip (Pointer)	0.39 B	0.43 b	Poor-host
Daikon radish (CNS)	0.38 B	0.29 b	Non-host
Annual ryegrass (CNS)	0.34 B	0.34 b	Non-host
Sweetclover (CNS)	0.24 B	0.18 b	Non-host
Carinata (CNS)	0.24 B	0.34 b	Non-host
Foxtail millet (Siberian)	0.23 B	0.28 b	Non-host
Red clover (Allington)	0.20 B	0.26 b	Non-host
Faba bean (CNS)	0.18 B	0.17 b	Non-host
Austrian winter pea (CNS)	0.14 B	0.64 b	Poor-host
MSD	5.65	3.20	-

[x] Average reproductive factor calculated as (initial SCN population/final SCN population) of each crop from five replicates for SCN103 (Richland County, ND) and SCN2W (Cass County, ND). Initial SCN population (eggs and juveniles) per 100 cm^3 of soil was 5,000 and 10,000 for SCN103 and SCN2W, respectively at planting. Means with same letters are not significantly different from each other in each column [$P < 0.0001$, minimum significant difference (MSD) mean separation].

[y] Host status of crops for SCN population (unpublished data), determined based on the female index. The crop with female index greater than 10 was considered as a host, less than 10 but > 0 as a poor host, and a female index of 0 considered a non-host for soybean cyst nematode.

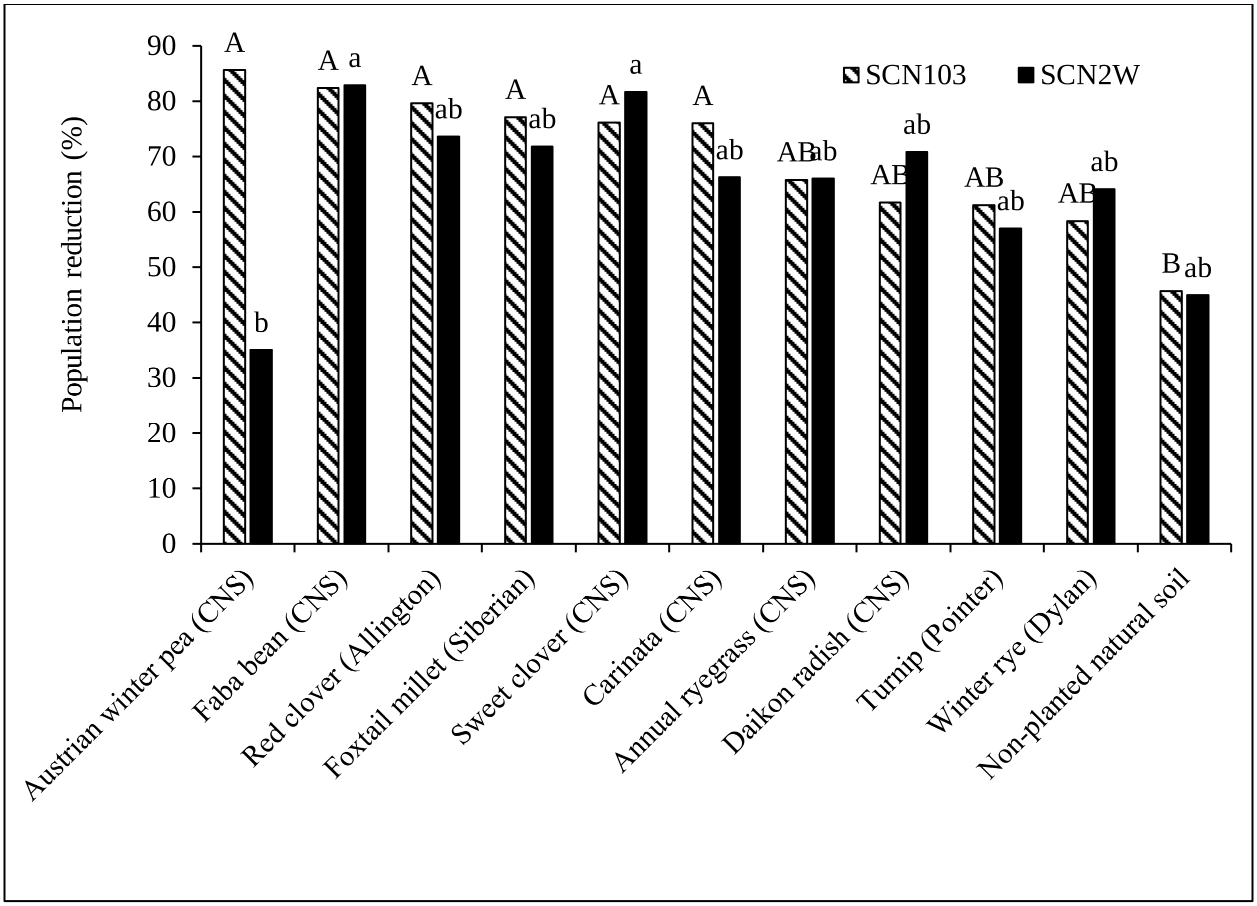

Fig. 4.1. Population reduction of two SCN populations (SCN103 and SCN2W) by different cover crops and non-planted control in naturally infested soils. The experiments had five replicates. Means with same capital letters are not significantly different for SCN103 ($P <$ 0.0004, MSD: 28.62), while means with same small letters are not significantly different for SCN2W ($P < 0.029$, MSD: 45.90).

Table 4.3. Reproductive factor for SCN103 and SCN2W populations on cover crop species, including a susceptible soybean, and non-planted control in microplot experiment in 2016.

Crop (Cultivar or Cultivar Not Stated = CNS)	**SCN103**		**SCN2W**	
	Final population in 100 cm^3 of soil [x]	**Reproductive factor** [y]	**Final population in 100 cm^3 of soil** [x]	**Reproductive factor** [y]
Austrian winter pea (CNS)	4,522 a	0.99 A	5,474 b	0.73 B
Non-planted natural soil	4,495 a	0.99 A	5,730 b	0.76 B
Soybean (Barnes)	4,113 ab	0.91 AB	11,250 a	1.50 A
Faba bean (CNS)	3,341 abc	0.74 ABC	3,966 bc	0.53 BC
Foxtail millet (Siberian)	2,996 bcd	0.66 BCD	2,729 c	0.36 C
Winter rye (Dylan)	2,388 cde	0.53 CDE	3,242 c	0.43 C
Sweetclover (CNS)	2,370 cde	0.52 CDE	2,895c	0.38 C
Red clover (Allington)	2,324 cde	0.51 CDE	3,514 c	0.47 C
Carinata (CNS)	1,979 de	0.44 DE	3,830 bc	0.51 BC
Turnip (Pointer)	1,725 e	0.38 E	3,076 c	0.41 C
Daikon radish (CNS)	1,344 e	0.30 E	2,398 c	0.32 C
Annual ryegrass (CNS)	1,262 e	0.28 E	2,518 c	0.33 C
MSD	1,192	0.26	1,927	0.25

[x] Average final SCN population (eggs and juveniles) per 100 cm^3 of soil on each crop at harvesting (five reps). Initial SCN population (eggs and juveniles) per 100 cm^3 of soil for each crop was 4,540 and 7,540 for SCN103 and SCN2W, respectively at planting.

[y] Average reproductive factor calculated as (initial SCN population/final SCN population) of each crop from five replicates. ($P < 0.0001$, MSD) mean separation, means with same letters are not significantly different from each other in each column.

In the 2017 microplot experiments, SCN103 had a significantly ($P < 0.0001$) lower average reproductive factor (RF: 0.28 to 1.02) on all tested crops compared with non-planted control, Austrian winter pea, and susceptible soybean (Barnes) (RF: 0.95, 1.02, 4.15, respectively) (Table 4.4). The SCN2W population had a significantly ($P < 0.0001$) lower average reproductive factor (RF: 0.42 to 1.02) on all tested cover crops and non-planted natural soil compared with susceptible soybean (Barnes, RF: 3.21). None of the cover crops had a lower reproductive factor than the non-planted soil.

Table 4.4. Reproductive factor for SCN103 and SCN2W populations on different cover crop species, including a susceptible soybean and non-planted control in microplot experiment 2017.

	SCN103		**SCN2W**	
Crop (Cultivar or Cultivar Not Stated = CNS)	**Final population in 100 cm3 of soil** [x]	**Reproductive factor** [y]	**Final population in 100 cm3 of soil** [x]	**Reproductive factor** [y]
Soybean (Barnes)	26,960 a	4.15 A	21,180 a	3.21 A
Austrian winter pea (CNS)	6,620 b	1.02 B	6,720 b	1.02 B
Non-planted natural soil	6,180 b	0.95 B	6,380 b	0.96 B
Sweetclover (CNS)	3,620 c	0.56 C	3,620 b	0.55 B
Winter rye (Dylan)	3,600 c	0.55 C	3,480 b	0.53 B
Red clover (Allington)	3,420 c	0.53 C	3,640 b	0.55 B
Turnip (Pointer)	3,420 c	0.53 C	2,800 b	0.42 B
Faba bean (CNS)	3,240 c	0.50 C	3,140 b	0.48 B
Foxtail millet (Siberian)	2,920 c	0.45 C	4,320 b	0.66 B
Carinata (CNS)	2,640 c	0.41 C	3,920 b	0.59 B
Annual ryegrass (CNS)	2,060 c	0.32 C	3,040 b	0.46 B
Daikon radish (CNS)	1,820 c	0.28 C	2,880 b	0.44 B
MSD	2,140	0.32	4,469	0.39

[x] Average final SCN population (eggs and juveniles) per 100 cm3 of soil on each crop at harvesting (five reps). Initial SCN population (eggs and juveniles) per 100 cm3 of soil for each crop was 6,500 and 6,600 for SCN103 and SCN2W, respectively at planting.

[y] Average reproductive factor calculated as (initial SCN population/final SCN population) of each crop (five reps). (P <0.0001, MSD) mean separation, means with same letters are not significantly different from each other in each column.

Microplot experiments- Population reduction of SCN

The Significant population reduction was observed in infested soil when planted with different cover crop species in microplot conditions in 2016 and 2017. In 2016, all the tested crops, except Austrian winter pea and faba bean, reduced the SCN103 numbers compared to the initial population density and reduction was significantly (P < 0.0001) higher (PR: 33% to 73%) than in non-planted naturally infested soil (PR: 1%). The high SCN number was reduced by annual ryegrass (72%), 70% by daikon radish, 62% by turnip, 56 % by carinata, and 46% to 34% by red clover, winter rye, and foxtail millet (Fig. 2). For SCN2W, all the tested crops except Austrian winter pea, carinata, and faba bean reduced SCN numbers compared to the initial population density and reduction was significantly (P < 0.0001) higher (PR: 53 to 68%) than in non-planted naturally infested soil (PR: 24%). The highest of 68% population reduction was by daikon radish, 67% by annual ryegrass, 64% by foxtail millet, 62% by sweetclover, 60% by turnip, and 53 to 57% by, red clover, and winter rye (Fig. 4.2). The population reduction by Austrian winter pea was 1% and 28% for SCN103 and SCN2W, respectively.

In 2017, all the tested crops except Austrian winter pea reduced SCN103 numbers compared to the initial population density and the reduction was significantly ($P < 0.0001$) higher (PR: 44 to 72%) than in non-planted naturally infested soil (PR: 5%). Daikon radish resulted in the highest PR of 71%, then 68% by annual ryegrass, 57% by carinata, 55% by foxtail millet, and 50 to 44% by faba bean, turnip, red clover, winter rye, and sweetclover (Fig. 4.3). For SCN2W, all the tested crops except Austrian winter pea and foxtail millet reduced SCN numbers compared to the initial population density and the reduction was significantly ($P < 0.0001$) higher (PR: 41 to 58%) than in non-planted control (PR: 4%). The highest of 58% population was reduced by turnip, then 56% by daikon radish, 54% by annual ryegrass, and 52 to 45% by faba bean, winter rye, sweetclover, red clover, and carinata, but while foxtail millet did not significantly reduce SCN population compared with non-planted control. Austrian winter pea increased the SCN103 and SCN2W population by about 1%. The highest population reduction was observed in the soil planted with Annual ryegrass and daikon radish with an average PR of 65 and 67% compared to initial numbers, respectively, for both years.

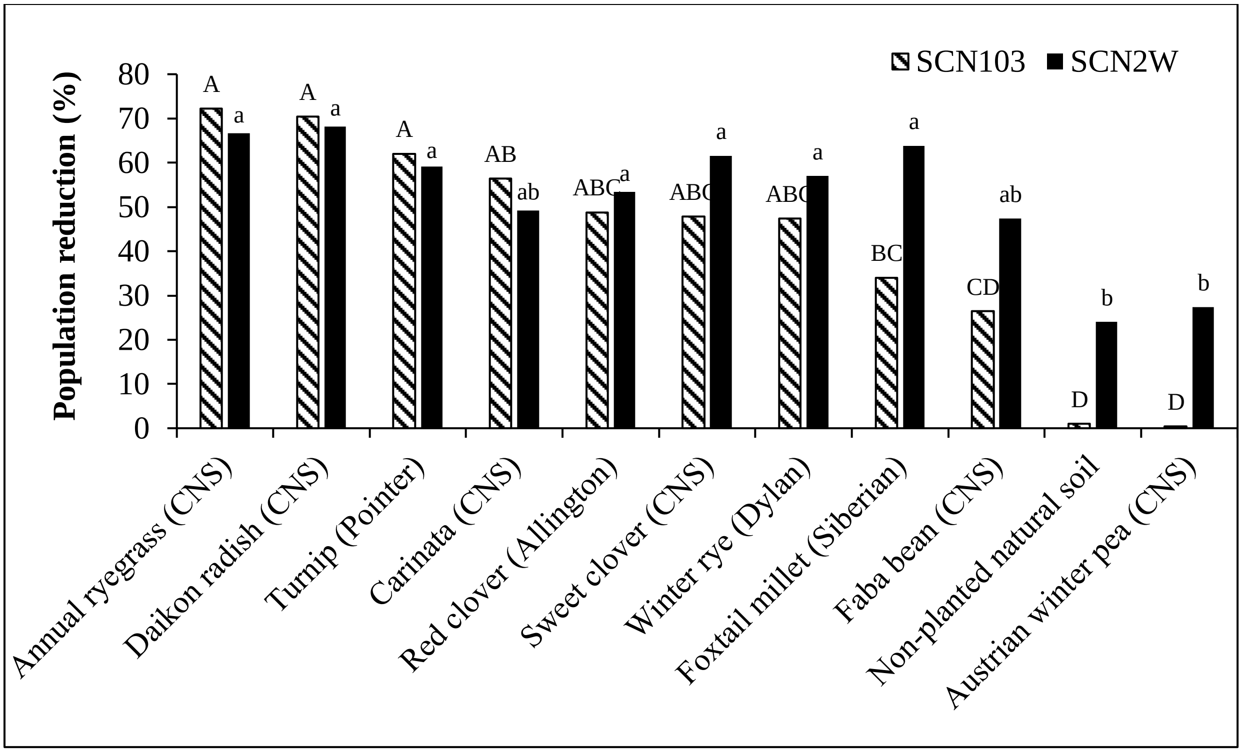

Fig. 4.2. Microplot experiments 2016: Population reduction of two SCN populations (SCN103 and SCN2W) by different cover crops and non-planted control in naturally infested soils in 2016. The experiments had five replicates. Treatment means with same capital letters are not significantly different for SCN103 (*P* < 0.0001, MSD: 26.58) while treatment means with same small letters are not significantly different for SCN2W (*P* < 0.0001, MSD: 25.78).

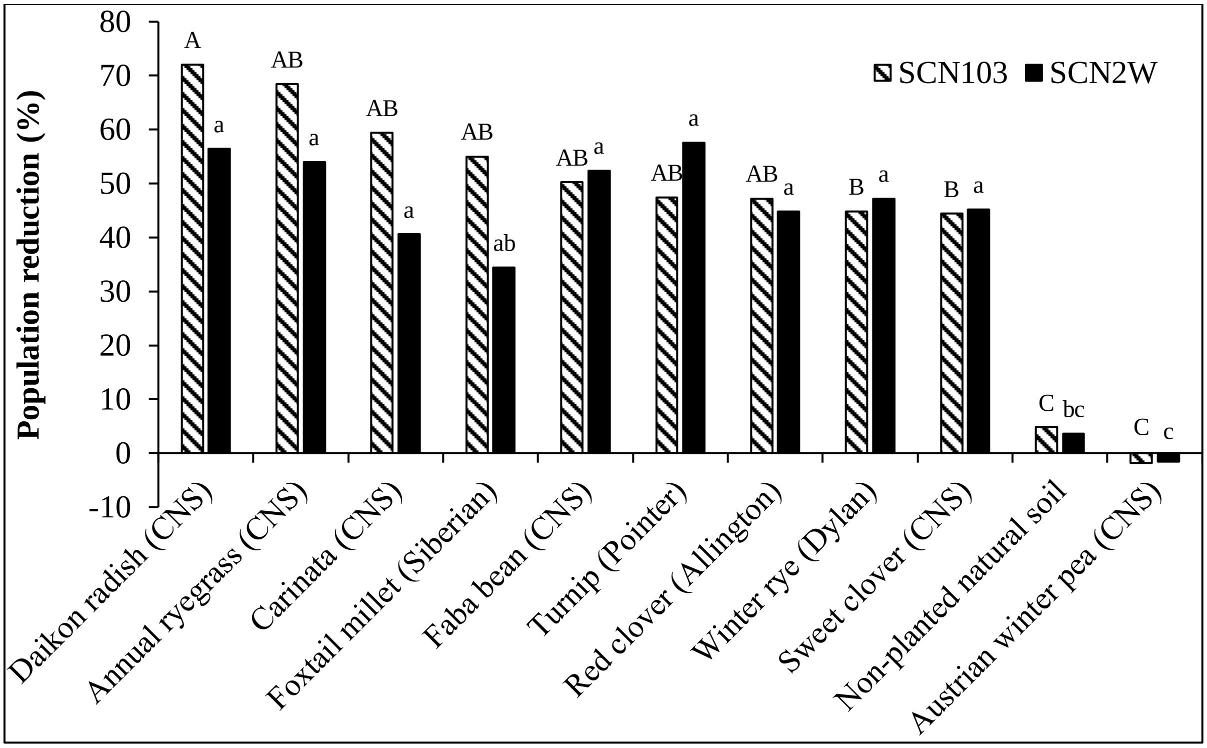

Fig. 4.3. Microplot experiments 2017: Population reduction of two SCN populations (SCN103 and SCN2W) by different cover crops and non-planted control in naturally infested soils. The experiments had five replicates. Treatments means with same capital letters are not significantly different for SCN103 (P < 0.0001, MSD: 25.48), while treatment means with same small letters are not significantly different for SCN2W (*P* < 0.0001, MSD: 32.34).

Discussion

The greenhouse experiment did not indicate that any of the cover crops resulted in a lower reproductive factor than observed in the non-planted natural soil. The greenhouse experiment showed inconsistent responses of the cover crops for population reduction of both SCN103 and SCN2W. The inconsistent results may have occurred because the crops were grown for only 35 days in the greenhouse conditions. In the short time experiments, the effects of the crops on SCN population might not be enough to show significant differences, so we considered them as preliminary results for further experiments. Previous work had indicated that turnip and Austrian winter pea were poor-hosts, while the other cover crops tested were non-hosts (Acharya et al. unpublished). However, in microplot experiments, most cover crops tested except Austrian winter pea, faba bean, carinata and foxtail millet consistently and significantly reduced SCN populations across the two years of microplot experiments and the two SCN populations. Austrian winter pea had a poor-host response to SCN populations (Acharya et al, unpublished) and was able to maintain SCN population when planted in infested soils. These results support the concept that rotating non-host crops is one of the effective strategies for SCN management (Chen et al. 2001; Hunter et al. 2019; Miller et al. 2006; Neher et al. 2019; Wranke et al. 2006).

Cover crops were grown for more than two and half months in infested soils in the external environment. Consistent responses were observed in the population reduction of SCN by some cover crops such as annual ryegrass, daikon radish, red clover, sweetclover, turnip, and winter rye in microplot experiments. However, inconsistency was observed for carinata, faba bean, and foxtail millet for reducing SCN egg numbers even though they were able to reduce SCN numbers for at least a SCN population or in a year experiment. Our results support the results from a greenhouse experiment conducted by Chen et al. (2008), where they found that red clover was effective in reducing SCN number in infested soils compared to control. In Brassicas, daikon radish, turnip, and carinata reduced SCN populations in both years of the microplot studies except carinata for SCN2W in 2016. Our results support the results from previous studies, where many *Brassica* species such as yellow mustard (species), rapeseed, oilseed radish and others were able to reduce the potato cyst nematode (*G. rostochiensis*) number up to 90 % when crops were incorporated into potato fields (Valdes et al. 2011). Acharya et al. (2019) screened three industrial crops, winter camelina [*Camelina sativa* (L.) Crantz, cv. Joelle], brown mustard (*Brassica juncea* L. cv. Kodiak), and crambe (*Crambe abyssinica* Hochst. Ex R.E.Fr., cv. BelAnn) for host status and population reduction of SCN in controlled greenhouse conditions. Both brown mustard and winter camelina were non-hosts and able to reduce the more

SCN egg numbers compared with the non-planted natural soil, while crambe was a poor-host to SCN and did not show significant reduction in SCN numbers compared to non-planted control. Radish cultivars (Colonel, Defender, Comet, Luna, Adagio, and Metex) were able to reduce nematode numbers in the infested soils with two nematodes *M. chitwoodi* and *H. schachtii* compared with fallow (Hafez et al. 2009). These studies suggested that Brassicas can have a potential role in SCN management, if included in an integrated SCN management tactics (Avato et al. 2013; Dutta et al. 2019; Fourie et al. 2016; Ngala et al. 2015). Annual ryegrass, foxtail millet, and winter rye were from the family *Poaceae* and the three species reduced more SCN numbers compared with fallow for both SCN population except foxtail millet, which did not reduce SCN2W in an experiment in 2017. Previous research by Pedersen and Rodriguez (1991) and Riga et al. (2001) supports our results that annual ryegrass reduces the SCN population when planted in infested soils. To our knowledge, no published reports are available for foxtail millet and winter rye regarding SCN population reduction when planted in infested soils.

The clear mechanism of population reduction by cover crops is still unknown; some experiments have attempted to explore the mechanism by testing root exudates from different cover crops. Root exudates were tested for SCN egg hatching and nematicidal effect on juveniles; annual ryegrass and white clover showed the significant increase in SCN egg hatching and decreased the juvenile's number by depleting lipid reserve in their body and starving to death in the absence food (Riga et al. 2001). Brassica crops root exudates not only act as biofumigants, but also reduce the size of the dorsal pharyngeal gland nucleus of potato cyst nematode (*G. rostochiensis*) (Aries et al. 2009), and they have an antagonistic effect on pale potato cyst nematode, *G. pallida* (Dossey 2011; Lord et al. 2011). The population reduction of cyst nematode is associated with high level of two compounds, 2-phenthyl and 2-propenyl glucosinolates released by Brassicas crops (Aires et al. 2009). Crops in the Fabaceae, Austrian winter pea, faba bean, red clover, and sweetclover were tested here, and it is possible that these crops induced SCN egg hatching (Aiba and Mitsui 1995; Schmitt and Riggs 1991; Warnke et al. 2006). A natural hatching inducing compound, glycinoeclepin A, was isolated from root exudates of dry bean (kidney bean) and soybean (Miwa al. 1987) and later dry beans were found to be a good host of SCN (Poromarto et al. 2009). Other legumes, such as sunnhemp and showy rattlebox, were tested as trap crops for SCN. Results showed that these crops induced SCN egg hatching and subsequent penetration into roots by juveniles, but the nematode was not able to form mature SCN females (Kushida et al. 2003; Poindexter 2011). However, these crops may not be suitable to our area because they required a warm season for optimum growth. Another study by Miller et al. (2006) suggested that non-host/ poor-host leguminous crops may reduce SCN number in infested field but planting a single season may not have significant reduction, if susceptible soybean is planted after those crops. For the *Poaceae* crops tested in this study which were non-hosts for SCN, they may have induced SCN egg hatching and subsequent dying of SCN juveniles (J2) due to degradation of lipid content in the juveniles in the absence of a host crop (Riga et al. 2001). Further research will be needed to confirm if the juveniles are able to infect but fail to develop into mature white females. Populations of both SCN103 and SCN2W were considerably reduced in non-planted natural soil. The juveniles hatched in non-planted soils eventually die by not getting a host to infect and feed upon. It is also obvious that hatching of SCN eggs is influenced by temperature and other environmental conditions (Tefft et al. 1982). The population reduction by these crops may be associated with the hatching and subsequent dying of SCN juveniles (J2) (unpublished data), but further studies such as analysis of root exudates, SCN penetration and subsequent development of SCN females by root staining, and visualization for nematodes inside the root tissue will be required to confirm the mechanisms of population reduction of these crops. These mechanisms include allelochemical or biofumigant effects and non-host or trap crop effects.

This study suggests that the cover crops have effects on SCN population reduction, when planted in infested soils. Our results support other studies, which found that cover crops were able to reduce different plant-parasitic nematodes such as, *M. incognita, P. penetrans, G. rostochiensis, H. schachtii, R. reniformis*, and many others in microplot and field conditions (Everts et al. 2007; Jones et al. 2006; Oliveira et al. 2011; Riga et al. 2005; Valdes et al. 2011). Another comprehensive study on investigating the interaction between SCN and cover crops showed inconsistent results when they were tested in greenhouse and field conditions, but soybean crop followed by annual ryegrass, radish, mustard, mix 1 (annual ryegrass + crimson clover + daikon radish), or tomato (*Lycopersicon esculentum*. L.) produced significantly lower nematode numbers than the soybean grown in non-planted control in a greenhouse experiment (Harbach 2019). Our study showed that annual ryegrass, daikon radish, and turnip consistently reduced SCN populations by more than 50% for both years; suggesting cover crop from families *Poaceae* and *Brassicaceae* families has potential use in northern Great Plains soybean growing systems. However, planting of these crops after soybean harvesting in the field may not provide enough time to grow to full maturity and will grow as good as they grow in the normal growing season. For example, foxtail millet is a summer cover crop and requires relatively higher temperature for its optimum growth (Doust et al. 2009). Planting cover crops after soybean/wheat harvesting is appropriate if enough time is available for their growth compared to planting as inter-seeded crops, because in inter-seeding cover crops may reduce crop yield due to competition (Chen et al. 2006). Further studies such as determining mechanisms of population reduction, appropriate planting time, and economic and agronomical benefits of cover crops should be performed to understand the applicability of these crops in an integrated SCN management strategy.

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CHAPTER 5: SCREENING EARLY MATURITY SOYBEAN ACCESSIONS AGAINST TWO POPULATIONS OF SOYBEAN CYST NEMATODE, *HETERODERA GLYCINES*

Abstract

Soybean cyst nematode (SCN; *Heterodera glycines*) is one of the devastating soybean pests worldwide. Resistant cultivars combined with crop rotation are the primary methods for managing SCN. The SCN is known to be genetically diverse populations and can develop new virulent forms over time due to continuous use of the same source of resistance. Thus, identifying novel SCN resistant sources is important. In this study, we screened many early maturity soybean [*Glycine max* (L.)] accessions to identify new resistant sources to SCN HG types 2.5.7 and 0, which are two common SCN populations in North Dakota. White SCN females were extracted from individual plant after 35 days of growth in greenhouse conditions. The SCN white females were counted to determine a female index (FI = average number of females on a tested line/average number of females in Barnes, susceptible check x 100). The resistance response of each soybean line was categorized as FI < 10%: as resistant (R), 10-30 %: moderately resistant (MR), ≥ 30-60 %: moderately susceptible (MS), and ≥ 60 %: susceptible (S). Out of 152 soybean lines tested against HG type 0, five lines were resistant, 38 were moderately resistant, 64 were moderately susceptible, and 45 were susceptible. Out of 149 soybean accessions tested against HG type 2.5.7, 17 lines were resistant, 35 were moderately resistant, 38 were moderately susceptible, and 59 were susceptible. Majority of soybean accessions screened were susceptible/moderately susceptible, while 18 of them showed good resistance for both or one of the SCN populations. These lines have potential to be used in breeding SCN-resistant cultivars after confirmation of the resistance reaction and elucidation of the resistance genes.

Key words: Accessions, *Heterodera glycines*, resistance, female index, management.

Introduction

Soybean [*Glycine max* (L.) Merr.] is one of the important leguminous crops in the world and in the United States. It ranked second after corn in the United States behind corn with the production of 36.1-million ha planted in 2018 (ASA 2019). Various biotic and abiotic factors limit the yield of soybean in the United States, including North Dakota (Hartman et al. 2011). Among biotic factors, soybean cyst nematode (SCN; *Heterodera glycines*) is a major pest in soybean distributed worldwide and ranks first to reduce more yield than other soybean diseases in the United States (Allen et al. 2017; Koenning and Wrather 2010; Riggs 1977). Currently, SCN has been detected in almost every soybean producing state in the United States (Tylka and Marett 2017; Wang et al. 2017). In North Dakota, SCN has been confirmed in at least 19 counties since it was first detected in Richland County in 2003 (Bradley et al. 2004; Yan et al. 2015). This nematode is spreading into non-infested areas by means that are involved in the movement of SCN infested soil, such as farming tools, animals, birds, infested seed, plant parts, and human beings (Chen 2011; Riggs 1997). The major challenge for managing SCN is the absence of the above-ground symptoms which makes it difficult to confirm SCN infestation until the SCN reaches a high population density and causes yellowing patches in the fields and up to that it can have a yield loss of 30% in soybean (Mueller et al. 2016). Analyzing the soil samples from fields and observing the soybean roots for SCN white females are used to confirm the SCN infestation in soybean fields. It can be performed before or after planting, or during soybean growing season (Niblack et al. 2006). Recent survey and studies in North Dakota and Minnesota fields showed that, dry beans also host this devastating soybean pest, SCN (Poromarto et al. 2011; Poromarto and Nelson 2009: Yan et al. 2017).

Although SCN numbers can be controlled or suppressed through different measures, it can be challenging because none of the measures can eradicate SCN completely from infested fields (Inagaki and Tsutsumi 1971). The SCN resistant cultivars and rotation with non-host crops are most common and effective methods used in SCN management in infested fields (Niblack 2005; Niblack et al. 2006). The screening of soybean accession for SCN resistance started as early as 1957, after SCN was first detected in North Carolina in 1954 in the United States (Kim et al. 2016; Winstead et al. 1955). PI88788 has been used extensively in soybean breeding for developing SCN-resistant cultivars due to its desirable traits, including yield (Concibido et al. 2004). Two resistance genes *rhg1* and *Rhg4* in PI88788 and Peking and their mechanism of resistance were elucidated. The resistance mechanism in soybean is based on copy number differences in *rhg1* gene within the locus (Lee et al. 2015). The PI88788 type resistance has high copy of *rhg1* gene, which shows resistance reaction alone, whereas Peking type requires additional gene *Rhg4* for resistance because it has very low copy number of *rhg1* gene in resistance locus (Cook et al. 2012; Mitchum 2016).

Large number of soybean accessions have been screened by several research groups and 158 of them were confirmed resistant to at least one of the SCN race/phenotypes (Li et al. 2011; Rincker et al. 2017). A total of 461 soybean accessions of different maturity groups were screened for SCN HG 0, about 90 showed the resistant responses and the majority of them were from maturity group greater than II (Tran et al. 2019). Acharya et al. (2017) screened 34 commercial resistant cultivars in South Dakota developed by private seed companies, and the majority of cultivars had the same resistance source PI88788. The cultivars were still effective in managing about 75% of the field populations in South Dakota, but it is recommended to rotate with other resistance source for preventing rapid virulence changes in nematode populations.

Another report from Iowa indicated that the 95% of the commercial cultivars from different companies are derived from the resistance source PI88788, while a very few from other resistance sources, such as Peking and PI437654 (Tylka and Mullaney 2018).

Continuous planting of SCN resistance cultivars derived from single or a very few resistance sources will facilitate the nematode population to overcome the resistance (Mitchum 2016). In recent years many survey reports in different states have revealed the virulence change in SCN populations and their adaptation on the commonly used resistance sources in many states in the United State (Acharya et al. 2016; Chowdhury et al. 2017; Niblack et al. 2008: Tylka and Mullaney 2018). This situation urges that new SCN-resistant accessions from different genetic backgrounds than PI88788 and Peking need to be examined from different soybean maturity groups for finding novel resistant sources. Until now, majority of identified resistance sources have been from higher maturity groups ranged II to IV, but a very few studies have included the lower maturity group accessions. In this study, we targeted soybean accessions from early-maturity groups, ranging from 00 to 1 from different countries of origin. Early-maturing-resistant soybean accessions will be helpful for finding durable resistance accessions alternative to already used resistant accessions from a higher maturity group and will be suitable for cold region like North Dakota. With the paramount importance of novel resistance source for breeding soybean with resistance against virulent SCN phenotypes, the objective of this study was to identify new early maturing soybean accessions resistant to two SCN HG types 0 and 2.5.7 in controlled greenhouse conditions.

Materials and methods

Soybean accessions

A total of 152 early maturing soybean accession (maturity group from 00 to 1) were acquired from the United States Department of Agriculture Soybean Germplasm Collection Center, Illinois. These accessions were from different origin diversity from nine countries (Fig. 5.1), with a large group (n=61) from China, from where the soybean was domesticated (Hymowitz 1970). To our knowledge, all the accessions have not been screened previously for resistance against SCN HG 0 and HG 2.5.7. The 152 accessions, along with three known resistant cultivars (Fayette, Ina, and Hartwig), and a susceptible check (Barnes) were evaluated in the greenhouse conditions. Moreover, the seven SCN HG type indicator lines [Peking (1), PI88788 (2), PI90763 (3), PI437654 (4), PI209332 (5), PI89772 (6), and PI548316 (7)] were also included for confirming the virulence phenotypes of the used SCN populations (Niblack et al. 2002).

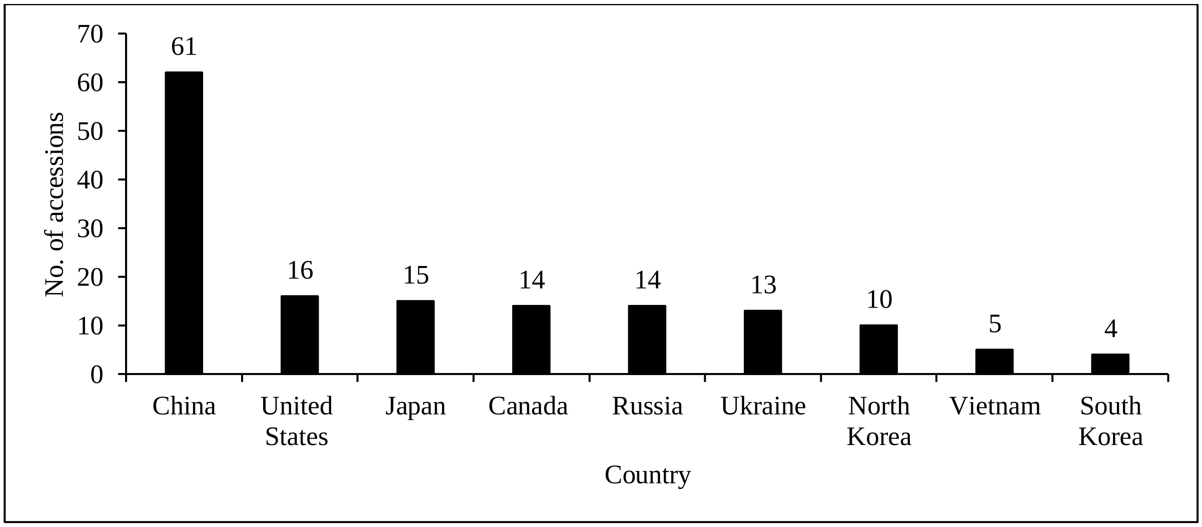

Fig. 5.1. Early maturity soybean accessions collected from USDA-ARS Germplasm Collection Center, Illinois with different country of origin.

Soybean cyst nematode populations

Nematode populations were collected from two fields of ND. The HG type 0 was from Richland County, whereas HG type 2.5.7 was from Traill County. These populations were previously examined for their virulence phenotypes by Choudhury et al. (2017). These two SCN populations were increased in greenhouse conditions by inoculating a susceptible soybean cultivar, Barnes. The white females of each HG type were collected using a standard protocol by Krusberg et al. (1994) and further eggs and juveniles were extracted following procedures by Faghihi and Ferris (2000) to prepare SCN inoculum for screening soybean accession.

Screening for resistance in the greenhouse

Four pregerminated seedlings of each soybean accession were planted into each cone-tainers (3.8 cm wide, 21 cm tall; Stuewe and Sons Inc., Tangent, OR) filled with 100 cm_3 of a pasteurized river-sand soil. Each of the accessions, indicator lines and susceptible check, was replicated four times for each HG type population and placed in a 14 x7 -cell plastic rack in completely randomized design (CRD). Each of the plants was inoculated with 2,000 eggs and juveniles for each HG type at the time of planting, then kept in a growth chamber maintained at 27 °C and daylight of 16 h. After 35 days, the plants were taken out of the growth chamber, then white females were extracted from each plant by using a standard protocol by Krusberg et al. (1994). A female index for each accession was calculated as, Female Index (FI) =

$$\frac{\text{Average no.of white females found on tested line}}{\text{Average no.of white females found on susceptible line}} * 100$$

Soybean accessions were classified for their resistance reactions based on the female index, FI < 10 [resistant (R), 10% ≤ FI < 30% [moderately resistant (MR), 30% ≤ FI < 60% [moderately susceptible (MS), and FI ≥ 60% [susceptible (S)] as described by Schmitt and Shannon (1992) (Table 5.1).

Table 5.1. Classification of the resistance response of soybean accessions to SCN (Schmitt and Shannon 1992).

Female Index (%)	Rating	Label
< 10	Resistant	R
10 – 30	Moderately Resistant	MR
≥ 30 - 60	Moderately Susceptible	MS
≥ 60	Susceptible	S

Data analysis

Data were analyzed using SAS 9.4 (SAS Institute, Cary, NC). The general linear model (GLM) with Tukey's honestly significant difference (HSD) for mean separation, significance level of 5% was used to determine the difference in the average number of white females in among accessions. Average number of white females from four replications was used to calculate the FI of each soybean accession. Because of the large number of accessions and time limits, the experiments were repeated only for accessions that showed resistant reaction for at least one of the SCN HG types for confirming their resistance levels.

Results

The significant differences ($P < 0.0001$) were observed in average number of white females produced on each soybean accession for both SCN populations. The average number of white females of 152 accessions was 120 with a range of 3 to 504 for SCN HG types 0 (Table 5.2), while the average number of white females of 149 accessions was 96 with a range of 0 to 438 for SCN HG 2.5.7 (Table 5.2). For SCN HG 0, the mean FI of 152 accessions was 46.6 with a range of 0.9 to 152.7. Five accessions were resistant (R) with FI less than 10%, 38 accessions were moderately resistant (MR) with a FI between 10 to 30%. Additionally, 65 accessions were moderately susceptible (MS) with FI between 30 to 60% and 45 accessions were susceptible with FI greater than 60%. Moreover, all the seven HG type indicator lines and known resistance cultivars (Fayette, Ina, and Hartwig) were showed resistant response with FI less than 10% (Table 5.3). Of the five resistant accessions, two accessions with history of origin from China, one from Japan, and two from North Korea. However, for SCN HG 2.5.7, the mean FI of 149 accessions was 54.5 with a range of 0.0 to 254.7. Of the 149 accessions screened, 16 accessions were resistant (R) with FI less than 10%, 35 accessions were moderately resistant (MR) with a FI between 10 to 30%. Additionally, 38 accession were moderately susceptible (MS) with FI between 30 to 60% and 59 accessions were susceptible with FI greater than 60% (Table 5.2). Moreover, the known resistance cultivars Ina, and Hartwig, including four indicator lines (1, 3, 4, 6) showed resistant response with FI less than 10%, while Fayette and three indicator lines (2, 5, 7) showed moderately resistance reaction (MR) with a FI between 10 to 30% (Table 5.3). Of the resistant 16 accessions, five were with history of origin from China, four from Canada, four from Japan, three from North Korea, and one from Ukraine (Table 5.2). The experiment is repeated for 18 accessions those were resistant to either HG 0 or HG 2.5.7, including three previously known resistant cultivars. We were able to get the similar response for all repeated entries except a few accessions, where four accessions had greater FI for SCN HG 0 and SCN HG 2.5.7 compared to previous experiment with moderately resistant or moderately susceptible response reaction (Table 5.4).

Table 5.2. Resistance reactions of diverse early maturing soybean accession to SCN HG type 0 and 2.5.7 from North Dakota soybean fields, tested in controlled greenhouse conditions.

				HG type 0			**HG type 2.5.7**		
Accession ID	**Country**	**Cultivar/line**	**MG** ^{x}	**Average no. of white females**	**Female index** ^{y}	**Resistance label**	**Average no. of white females**	**Female index**	**Resistance label**
PI548642	Canada	Maple Donovan	0	78	31.2	MS	3	1.5	R
PI548644	Canada	OAC Musca	0	49	19.6	MR	312	181.5	S
PI548648	Canada	Canatto	00	353	107	S	191	110.8	S
PI548649	Canada	Nattawa	0	72	28.8	MR	99	57.3	MS
PI548650	Canada	Nattosan	00	74	29.6	MR	4	2.2	R
PI549054	Japan	Ao 75	00	78	54.2	MS	317	184.0	S
PI549061	Japan	Ichiriki	00	96	66.7	S	-	-	-
PI556749	Canada	KG30	00	56	38.9	MS	132	76.5	S
PI556751	Canada	KG20	00	70	48.6	MS	92	53.6	MS
PI556926	Canada	TK 89	I	90	62.5	S	179	103.8	S
PI561211	United States	3172	I	63	43.8	MS	52	29.9	MR
PI561389A	Japan	Okura Natto	00	31	21.5	MR	182	105.7	S
PI561389B	Japan	(Okura Natto)	0	67	46.5	MS	325	189.1	S
PI567177	Japan	Line 9970	0	62	43.1	MS	438	254.7	S
PI567283	Japan	Shirojisi	00	36	25	MR	130	75.4	S
PI567782	Canada	OAC Dorado	I	82	32.8	MS	5	3.1	R
PI567783	Canada	OAC Eclipse	0	47	18.8	MR	3	1.7	R
PI567784	Canada	OAC Frontier	00	69	27.6	MR	76	43.9	MS
PI567785	Canada	OAC Shire	I	78	31.2	MS	164	95.1	S
PI572241	Canada	Brock	I	209	63.3	S	167	97.1	S
PI572243	Canada	Harovinton	I	394	119.4	S	116	67.6	S
PI587091	United States	Council	0	296	89.7	S	115	67.0	S
PI593655	United States	Danatto	0	500	151.5	S	124	71.8	S
PI593997	South Korea	-	0	60	41.7	MS	228	132.6	S

Table 5.2. Resistance reactions of diverse early maturing soybean accession to SCN HG type 0 and 2.5.7 from North Dakota soybean fields, tested in controlled greenhouse conditions (continued).

				HG type 0			HG type 2.5.7		
Accession ID	**Country**	**Cultivar/line**	**MG**[x]	**Average no. of white females**	**Female index**[y]	**Resistance label**	**Average no. of white females**	**Female index**	**Resistance label**
PI594016	South Korea	Keun daedu	I	27	18.8	MR	75	66.7	S
PI594021	South Korea	Keunolkong	I	42	29.2	MR	40	35.7	MS
PI594245A	Japan	Okuhara 1-B	00	90	62.5	S	2	2.0	R
PI594245B	Japan	(Okuhara 1-B)	0	58	40.3	MS	0	0.0	R
PI594261	Japan	Shika 4	0	73	50.7	MS	53	47.3	MS
PI594279	Japan	Shinsei	0	113	78.5	S	3	2.2	R
PI594296	Japan	Tokachi kuro	I	197	60.7	S	40	21.0	MR
PI594301	Japan	Toyomusume	I	27	18.8	MR	1	0.9	R
PI594304A	Japan	Tsurukogane	I	26	13.8	MR	-	-	-
PI594314	Japan	Wase suzunari	I	6	3.2	R	15	12.1	MR
PI594319	Japan	Yuuhime	I	20	10.6	MR	30	26.8	MR
PI596541	United States	Traill	0	163	65.2	S	121	70.2	S
PI597391A	Ukraine	Kievskaya 27	00	42	22.3	MR	152	80.0	S
PI597391B	Ukraine	(Kievskaya 27)	00	19	10.1	MR	29	25.9	MR
PI597391C	Ukraine	(Kievskaya 27)	0	21	11.2	MR	4	3.8	R
PI597393	Ukraine	Kirovogradskaya 5	0	24	12.8	MR	31	27.5	MR
PI597394	Ukraine	Kharkovskaya Zernokormovaya	00	29	15.4	MR	21	19.0	MR
PI597395	Ukraine	Solnechnaya	0	48	25.5	MR	44	38.8	MS
PI597398	Ukraine	Yug 40	0	24	12.8	MR	32	28.8	MR
PI597399	Ukraine	Yug 30	00	20	10.6	MR	19	16.5	MR
PI597405A	Ukraine	Zaporozhie 46-96	0	24	12.8	MR	22	19.9	MR
PI597405B	Ukraine	(Zaporozhie 46-96)	I	25	13.3	MR	42	37.5	MS
PI597405C	Ukraine	(Zaporozhie 46-96)	I	23	12.2	MR	29	25.9	MR
PI597405D	Ukraine	(Zaporozhie 46-96)	I	23	12.2	MR	20	17.6	MR

Table 5.2. Resistance reactions of diverse early maturing soybean accession to SCN HG type 0 and 2.5.7 from North Dakota soybean fields, tested in controlled greenhouse conditions (continued).

				HG type 0			**HG type 2.5.7**		
Accession ID	**Country**	**Cultivar/line**	**MG** [x]	**Average no. of white females**	**Female index** [y]	**Resistance label**	**Average no. of white females**	**Female index**	**Resistance label**
PI597487	South Korea	Hwaseongputkong	I	33	17.6	MR	121	64.0	S
PI599299	United States	Stride	I	89	35.6	MS	142	74.9	S
PI599300	United States	Surge	0	126	50.4	MS	125	72.8	S
PI602896	United States	Daksoy	00	227	68.8	S	109	63.1	S
PI602897	United States	Jim	00	504	152.7	S	177	102.8	S
PI603148	North Korea	Oh won No. 1	I	22	11.7	MR	0	0.0	R
PI603150	North Korea	-	00	38	20.2	MR	58	51.6	MS
PI603151A	North Korea	-	I	17	9.0	R	10	8.5	R
PI603153	North Korea	-	0	23	12.2	MR	18	16.3	MR
PI603169	North Korea	-	00	13	6.9	R	4	3.6	R
PI603424A	China	Ben di huang dou	0	16	8.5	R	7	5.8	R
PI603426B	China	(Ben di yuan huang dou)	0	137	40.7	MS	9	8.3	R
PI603426D	China	(Ben di yuan huang dou)	0	215	63.8	S	43	38.2	MS
PI603426E	China	(Ben di yuan huang dou)	0	164	48.7	MS	24	21.2	MR
PI603429A	China	Cha dou	0	141	41.8	MS	26	23.4	MR
PI603429B	China	(Cha dou)	0	257	76.3	S	26	23.0	MR
PI603432C	China	(Huang dou)	I	304	90.2	S	28	25.2	MR
PI603438A	China	Da hei qi	I	40	11.9	MR	6	5.4	R
PI603438B	China	(Da hei qi)	I	57	16.9	MR	19	17.0	MR
PI603439	China	74-2	I	219	65.0	S	140	125.2	S
PI603440B	China	(Nong yan da bai qi)	I	245	72.7	S	113	101.1	S
PI603440C	China	(Nong yan da bai qi)	I	249	73.9	S	156	139.5	S

Table 5.2. Resistance reactions of diverse early maturing soybean accession to SCN HG type 0 and 2.5.7 from North Dakota soybean fields, tested in controlled greenhouse conditions (continued).

				HG type 0			HG type 2.5.7		
Accession ID	**Country**	**Cultivar/line**	**MG** [x]	**Average no. of white females**	**Female index** [y]	**Resistance label**	**Average no. of white females**	**Female index**	**Resistance label**
PI603443A	China	Ke qi xiao hei dou	I	3	0.9	R	2	1.6	R
PI603444B	China	(Hei dou)	I	159	47.2	MS	17	15.4	MR
PI603445A	China	Da li hei dou	I	118	35.0	MS	36	31.9	MS
PI603447	China	Ka qi mao yan dou	I	173	51.3	MS	20	17.6	MR
PI603900	United States	Norpro	0	49	19.6	MR	83	48.4	MS
PI612610	North Korea	Musan-1	I	35	10.4	MR	15	13.2	MR
PI612615	North Korea	Bochon	0	137	42.4	MS	22	19.9	MR
PI612617A	North Korea	Kapsan	I	220	65.3	S	49	43.5	MS
PI612617B	North Korea	(Kapsan)	0	173	51.3	MS	-	-	-
PI612705	China	He feng No. 9	I	162	48.1	MS	16	14.1	MR
PI612706A	China	Bayan 32	I	123	38.0	MS	83	53.3	MS
PI612706B	China	(Bayan 32)	0	170	52.5	MS	44	28.4	MR
PI612707A	China	Bei 8709	0	99	35.7	MS	46	29.7	MR
PI612707B	China	(Bei 8709)	0	162	58.5	MS	22	13.9	MR
PI612708B	China	(K 89-9081)	I	144	51.9	MS	48	30.8	MS
PI612708C	China	(K 89-9081)	I	134	48.4	MS	78	50.2	MS
PI612708D	China	(K 89-9081)	I	143	51.6	MS	54	35.0	MR
PI612709A	China	K 87-104	0	104	32.0	MS	57	36.6	MS
PI612709B	China	(K 87-104)	I	204	73.7	S	49	31.3	MS
PI612709C	China	(K 87-104)	I	236	85.2	S	43	27.9	MR
PI612711A	China	K 93-89	I	118	42.6	MS	28	18.2	MR
PI612711B	China	(K 93-89)	I	118	42.6	MS	29	18.9	MR
PI612713A	China	He feng 910	I	243	87.7	S	22	14.0	MR
PI612713B	China	(He feng 910)	I	250	90.3	S	62	40.2	MS
PI612714A	China	He feng 1538	I	95	34.3	MS	41	26.3	MR

Table 5.2. Resistance reactions of diverse early maturing soybean accession to SCN HG type 0 and 2.5.7 from North Dakota soybean fields, tested in controlled greenhouse conditions (continued).

Accession ID	Country	Cultivar/line	MG x	HG type 0			HG type 2.5.7		
				Average no. of white females	Female index y	Resistance label	Average no. of white females	Female index	Resistance label
PI612714B	China	(He feng 1538)	I	151	54.5	MS	89	57.4	MS
PI612715	China	Hei nong 40	I	214	77.3	S	70	44.8	MS
PI612716	China	Harbin 93-6349	I	237	77.9	S	38	24.2	MR
PI612717	China	Harbin 94-2508	I	148	48.7	MS	152	80.0	S
PI612718	China	Harbin 92-1062	I	228	75.0	S	90	57.7	MS
PI612719	China	Harbin 91-6065	I	159	52.3	MS	75	48.1	MS
PI612720A	China	Jilin 26	0	118	38.8	MS	145	76.3	S
PI612720B	China	(Jilin 26)	I	199	65.5	S	98	62.8	S
PI612721A	China	Jilin 33	I	117	38.5	MS	111	58.4	MS
PI612721B	China	(Jilin 33)	0	171	56.3	MS	114	60.0	S
PI612722	China	Jilin 34	I	93	30.6	MS	94	60.3	S
PI612723	China	Jilin 35	I	108	35.5	MS	64	41.0	MS
PI612724	China	Jilin 36	I	94	30.9	MS	105	67.3	S
PI612725	China	Jilin 37	I	166	54.6	MS	74	47.4	MS
PI612726	China	Jilin 8966-25	I	128	42.1	MS	68	43.6	MS
PI612727	China	Jilin 8966-35	I	125	38.5	MS	-	-	-
PI612728	China	Jilin 8978-6	0	114	37.5	MS	86	55.1	MS
PI612732	China	Zhao shu 18	I	119	39.1	MS	84	53.9	MS
PI612734	China	Jihei 45	I	124	38.3	MS	130	112.6	S
PI612735	China	Jiunong 21	I	50	16.4	MR	64	41.0	MS
PI612736	China	Yi No. 3	I	226	74.3	S	120	76.9	S
PI612737	China	Hefeng 21	I	115	37.8	MS	50	32.1	MS
PI612738	China	67803	I	202	66.5	S	118	75.6	S
PI612739	China	67562	0	195	60.3	S	93	59.6	MS
PI612740	China	CM048	0	275	90.5	S	127	81.4	S

Table 5.2. Resistance reactions of diverse early maturing soybean accession to SCN HG type 0 and 2.5.7 from North Dakota soybean fields, tested in controlled greenhouse conditions (continued).

				HG type 0			**HG type 2.5.7**		
Accession ID	**Country**	**Cultivar/line**	**MG**[x]	**Average no. of white females**	**Female index**[y]	**Resistance label**	**Average no. of white females**	**Female index**	**Resistance label**
PI612743	China	Bianjing	I	202	66.5	S	108	56.8	MS
PI612744	China	89445	I	167	54.9	MS	144	92.3	S
PI612745	China	Bonwand	0	226	74.3	S	2	1.3	R
PI612746	China	Fushuali	I	268	82.7	S	126	64.7	S
PI613561	North Korea	Nui 2 hu	I	200	65.8	S	40	25.6	MR
PI615585	United States	Sargent	0	98	39.2	MS	280	162.5	S
PI615586	United States	Walsh	0	121	48.4	MS	87	50.3	MS
PI631437	United States	Nornatto	0	90	36.0	MS	117	67.9	S
PI631438	United States	Nannonatto	0	85	34.0	MS	259	150.6	S
PI634813	United States	LaMoure	0	85	34.0	MS	234	135.8	S
PI638510	United States	Pembina	00	73	29.2	MR	127	73.5	S
PI638511	United States	ProSoy	0	127	50.8	MS	249	144.5	S
PI639539A	North Korea	-	I	49	16.1	MR	179	94.2	S
PI639554	Russia	-	0	250	82.2	S	113	72.4	S
PI639559A	Ukraine	VYTKA 2	00	108	83.7	S	197	66.0	S
PI639560A	Russia	KOREISKAYA MESTNAYA	I	98	75.9	S	245	82.1	S
PI639560B	Russia	(KOREISKAYA MESTNAYA)	I	68	52.7	MS	222	74.3	S
PI639590B	Russia	(KZ 659)	00	94	72.9	S	158	53.1	MS
PI639628	Russia	LADA	00	113	87.6	S	169	56.8	MS
PI639630B	Russia	(ARMAVIRSKAYA 2)	I	92	71.3	S	81	27.0	MR
PI639631	Russia	ARMAVIRSKAYA 4	0	57	44.2	MS	148	49.5	MS
PI639632A	Russia	RENTA	0	74	57.4	MS	136	72.0	S
PI639632B	Russia	(RENTA)	0	70	54.3	MS	118	62.0	S

Table 5.2. Resistance reactions of diverse early maturing soybean accession to SCN HG type 0 and 2.5.7 from North Dakota soybean fields, tested in controlled greenhouse conditions (continued).

				HG type 0			HG type 2.5.7		
Accession ID	**Country**	**Cultivar/line**	**MG** x	**Average no. of white females**	**Female index** y	**Resistance label**	**Average no. of white females**	**Female index**	**Resistance label**
PI639632C	Russia	(RENTA)	I	51	39.5	MS	188	63.1	S
PI639633A	Russia	GARMONIYA	00	26	20.2	MR	201	67.5	S
PI639633B	Russia	(GARMONIYA)	00	115	35.5	MS	133	70.0	s
PI639634	Russia	DAURIYA	00	34	26.4	MR	154	81.0	s
PI675764A	Vietnam	dau tuong vang	I	71	55.0	MS	238	79.8	S
PI675764B	Vietnam	(dau tuong vang)	I	16	12.4	MR	59	19.6	MR
PI675891	Vietnam	DTRTQ8	I	94	72.9	S	246	82.6	S
PI675914	Vietnam	Soybean dal 2	I	95	73.6	S	269	90.1	S
PI675917	Vietnam	SB1 gen	I	113	87.6	S	246	82.5	S
MSD (*P* < 0.0001)				171			119		

x MG- maturity group.

y Female index for each of the accession was calculated by using a susceptible check Barnes. Female index = (average no. of white females on a tested accession /average no. of white females in the susceptible soybean, Barnes) x 100. For SCN HG 0, the average no. of white females on Barnes was from 144 to 271. For SCN HG 25.7, the average no. of white females on Barnes was 112 to 298. Because of the large number of accessions, the experiments were performed in batches and each experiment had 4 replications of susceptible soybean, Barnes.

Table 5.3. Resistance response of soybean accession with known resistance source and SCN HG type indicator lines to SCN HG type 0 and 2.5.7 from North Dakota soybean field, tested in controlled greenhouse conditions.

Accession ID	Resistance source	MG [x]	HG type 0			HG type 2.5.7		
			Average no. of white females	**Female index [y]**	**Resistance label**	**Average no. of white females**	**Female index**	**Resistance label**
PI518674	Fayette	III	22	6.7	R	48	28.1	MR
PI606749	Ina	IV	16	4.8	R	4	2.3	R
PI209332	Differential	IV	17	3.8	R	29	17.0	MR
PI437654	Differential	III	0	0.0	R	0	0.0	R
PI543795	Hartwig	V	0	0.1	R	0	0.1	R
PI548316	Cloud	III	16	3.7	R	27	15.6	MR
PI548402	Peking	IV	0	0.1	R	1	0.3	R
PI88788	Differential	III	8	1.9	R	29	16.6	MR
PI89722	Differential	IV	0	0.0	R	0	0.0	R
PI90736	Differential	IV	0	0.0	R	0	0.0	R

[x] MG- maturity group.

[y] Female index for each of the accession was calculated by using a susceptible check Barnes. Female index = (average no. of white females on a tested accession /average no. of white females in the susceptible soybean, Barnes) x 100. For HG type 0, the average no. of white females on Barnes was from 144 to 271. For HG type 2.5.7, the average no. of white females on Barnes was 112 to 298. Because of the large number of accessions, the experiments were performed in batches and each experiment had 4 replications of susceptible soybean, Barnes.

Table 5.4. Confirmation of resistance responses of diverse soybean accessions to two SCN populations (HG type 0 and HG type 2.5.7) isolated from North Dakota soybean fields, tested in controlled greenhouse conditions.

Accession ID	Country	Cultivar	MG[x]	HG type 0		HG type 2.5.7	
				Average no. of white females	Female index[y]	Average no. of white females	Female index[y]
PI518674	-	Fayette	III	12	5.3	48	19.3
PI543795	-	Hartwig	V	1	0.2	1	0.4
PI606749	-	Ina	IV	1	0.6	3	1.3
PI548642	Canada	Maple Donovan	0	64	28.6	5	2.1
PI548650	Canada	Nattosan	00	62	27.9	3	1.0
PI567782	Canada	OAC Dorado	I	43	19.1	7	2.8
PI567783	Canada	OAC Eclipse	0	32	14.4	4	1.4
PI594245A	Japan	Okuhara 1-B	00	31	13.9	5	2.1
PI594245B	Japan	(Okuhara 1-B)	0	32	14.2	4	1.6
PI594279	Japan	Shinsei	0	54	24.1	2	0.6
PI594301	Japan	Toyomusume	I	46	20.5	5	1.8
PI594314	Japan	Wase suzunari	I	31	13.8	32	12.9
PI597391C	Ukraine	(Kievskaya 27)	0	33	14.8	3	1.2
PI603148	North Korea	Oh won No. 1	I	35	15.7	4	1.7
PI603151A	North Korea	-	I	55	24.4	80	32.1
PI603169	North Korea	-	00	26	11.8	95	38.3
PI603424A	China	Ben di huang dou	0	48	21.3	66	26.7
PI603426B	China	(Ben di yuan huang dou)	0	31	13.9	56	22.4
PI603438A	China	Da hei qi	I	38	16.8	22	8.9
PI603443A	China	Ke qi xiao hei dou	I	5	2.4	2	0.8
PI612745	China	Bonwand	0	52	23.1	3	1.2

[x] MG- maturity group.

[y] Female index for each of the accession was calculated by using a susceptible check Barnes. Female index = (average no. of white females on a tested accession /average no. of white females in the susceptible soybean, Barnes) x 100. The average no. of white females on Barnes was 223 and 248 for HG type 0 and HG type 2.5.7, respectively.

Discussion

This study evaluates the resistance response 152 early-maturing soybean accessions for two SCN population HG type 0 and 2.5.7 from two fields of North Dakota. To our knowledge, the accessions screened in this study were never tested for resistance against for any SCN populations, including HG 0 and HG 2.5.7. The FI of these early-maturing accessions showed a large variation with a range of 0.9 to 152.7 for SCN HG 0 and 0.0 to 254.7 for SCN HG 2.5.7. The majority of the accessions had more FI more than 30% for both SCN populations, which were considered moderately susceptible and susceptible. The previously known resistant cultivars (Fayette, Ina, and Hartwig) showed the similar responses as described for their resistant sources such as PI88788, Peking, and PI543795 for both SCN populations (Bernard et al. 1988; Kazi et al. 2010; Nickell et al. 1999). The susceptible check and seven indicator lines also showed the expected results and confirmed the respective HG types for assigned HG types (Niblack et al. 2002). Majority of the accessions in our study showed the moderately susceptible to susceptible reaction for both SCN populations, a few about 12% of accessions showed resistant response to either HG 0 or HG 2.5.7 suggesting it is difficult to find early maturing resistant accessions. Similar response was observed when Tran et al. (2019) screened 461 accessions, only 90 showed resistant response with FI less than 10%. Among those resistant accessions, only three were from early maturity group 00 to I and those were different from what we found resistant in our experiment.

Many soybean accessions were screened in the past for resistance against SCN populations, and majority of the identified resistant accessions were from higher maturity group. Among those, seven resistance lines are now used as indicator lines HG type experiment (Niblack et al. 2002). Two indicator lines, Peking and PI88788 with maturity group IV and III, respectively are widely used soybean accessions, specifically the PI88788 in QTL mapping and breeding program for SCN resistant soybean cultivars (Concibido et al. 2004). Many other resistant sources were identified from different research group, but the majority of them possess the same resistance mechanisms by Peking or PI88788 type of resistance against SCN populations (Concibido et al. 2004: Kandoth et al. 2011; Mitchum 2016; Zheng and Chen 2011). To date, most of the soybean breeding programs relied on these two resistance sources to develop SCN resistant cultivars. About 95 % of the commercially grown cultivars in the northern Great Plains are labeled with a resistant source as PI88788, and a few with Peking (Tylka and Mullaney 2018) leading to limited genetic backgrounds in SCN resistant cultivars. The impacts of the use of single resistance source resulted in virulence changes in SCN populations in many soybean producing states by overcoming the resistance from either of PI88788 or Peking (Niblack et al. 2008). Recent reports on virulence phenotypes of the SCN population of South Dakota, Minnesota, Missouri, Nebraska, and North Dakota showed about 20 to 70% of the field populations were able to reproduce on PI88788 with a FI more than 10% (Acharya et al. 2016; Choudhury et al. 2017; Hershman et al. 2008; Niblack et al. 2008). Many efforts are made to elucidate the resistance mechanism on different group of soybean accessions using different methods such as QTL mapping, genome wide association mapping, and others for finding novel resistance gene or locus. Some progress has been made to find resistance locus/gene other than *Rhg1* and *Rhg4* (Zhang et al. 2016; Zhang et al. 2017), but none of those resistant sources are utilized yet for developing commercial cultivars due to the long time and negative agronomical traits (Mitchum 2016). In addition to exploring existing resistance by utilizing advanced technology, it is always recommended to continue screening more soybean accessions from different maturity group to find novel and durable resistance source against soybean cyst nematode.

Based on our results, majority of early-maturing soybean accessions were susceptible, while a few (n=18) were resistant to both or either HG 0 or 2.5.7 with FI less than 10. Interestingly, some of these accessions had FI more than 10 for HG 0 but FI less than 10 for HG type 2.5.7, so further research is necessary to understand resistance on these lines. The identified resistant accessions have never been evaluated or confirmed to be resistant to either of the SCN populations, so have a potential to be a good alternative to known resistance sources, PI88788, Peking, and others. Further confirmation of resistant response and elucidation of resistance locus/loci is necessary before using them in the soybean breeding program for developing SCN resistant cultivars.

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CHAPTER 6: SUMMARY

The aim of this dissertation "Managing soybean cyst nematode by utilizing cover crops and resistant sources from early maturing soybean accessions" is to help in deciding sustainable soybean cyst nematode (SCN; *Heterodera glycines*) management strategy for the northern Great Plains. The acres of cover crops have been increased over the past decades in the United States, including the northern Great Plains. Cover crop provides multiple benefits for farmers by improving the soil organic matters, soil nutrients, and reducing the soil erosions. In addition, cover crops may have effects on SCN by acting as hosts/non-hosts or may reduce the nematode number in infested fields. Moreover, the majority of utilized known resistant sources have been overcome by SCN populations, so identification of new sources of resistance and elucidation of their resistance gene is of paramount importance for breeding soybean for resistance against virulent SCN populations.

The first greenhouse experiment assessed the hosting ability of 35 cover crop species/cultivars for two SCN populations, SCN103 and SCN2W. Only seven of the crops showed poor-host or host response, while a majority (n=28) of the crops were non-host for SCN populations. Poor-host or host crop includes, Austrian winter pea, crimson clover, field peas, hairy vetch, turnips, and white lupine. The second greenhouse experiment assessed three industrial crops, such as brown mustard, camelina, and crambe for hosting and population reduction abilities for two SCN populations. Results suggested that brown mustard and camelina were non-host and have a potential to reduce SCN population, while crambe was a poor host and did not reduce SCN populations compared with the non-planted control. The third experiment assessed the SCN population reduction abilities of 10 cover crop species/cultivars in the greenhouse and microplot conditions. The results suggested that annual ryegrass, daikon radish, and turnip showed the reduction of SCN numbers in infested soils by an average greater than

50% while compared to initial population density in two years of microplot experiments. Overall results indicate that cover crops may have a potential to be used in integrated SCN management strategy.

Moreover, the fourth experiment evaluated the resistance of 152 early-maturing soybean accessions to two SCN populations, HG 0 and HG 2.5.7. The screening results indicated that the majority of early maturing soybean accessions were susceptible to both SCN populations, while a few of them showed good resistant response to both or either of the nematode populations. The resistant accessions have a potential to be used in the soybean breeding program after further confirmation of their resistance and elucidation of the resistance genes/loci.

The results obtained from this study will help deciding sustainable SCN management strategy by integrating suitable cover crops and identifying novel resistant sources for SCN resistance breeding programs in the northern Great Plains.

APPENDIX A. SOYBEAN CYST NEMATODE PICTURES, AND GREENHOUSE AND MICROPLOT EXPERIMENTS

(a)

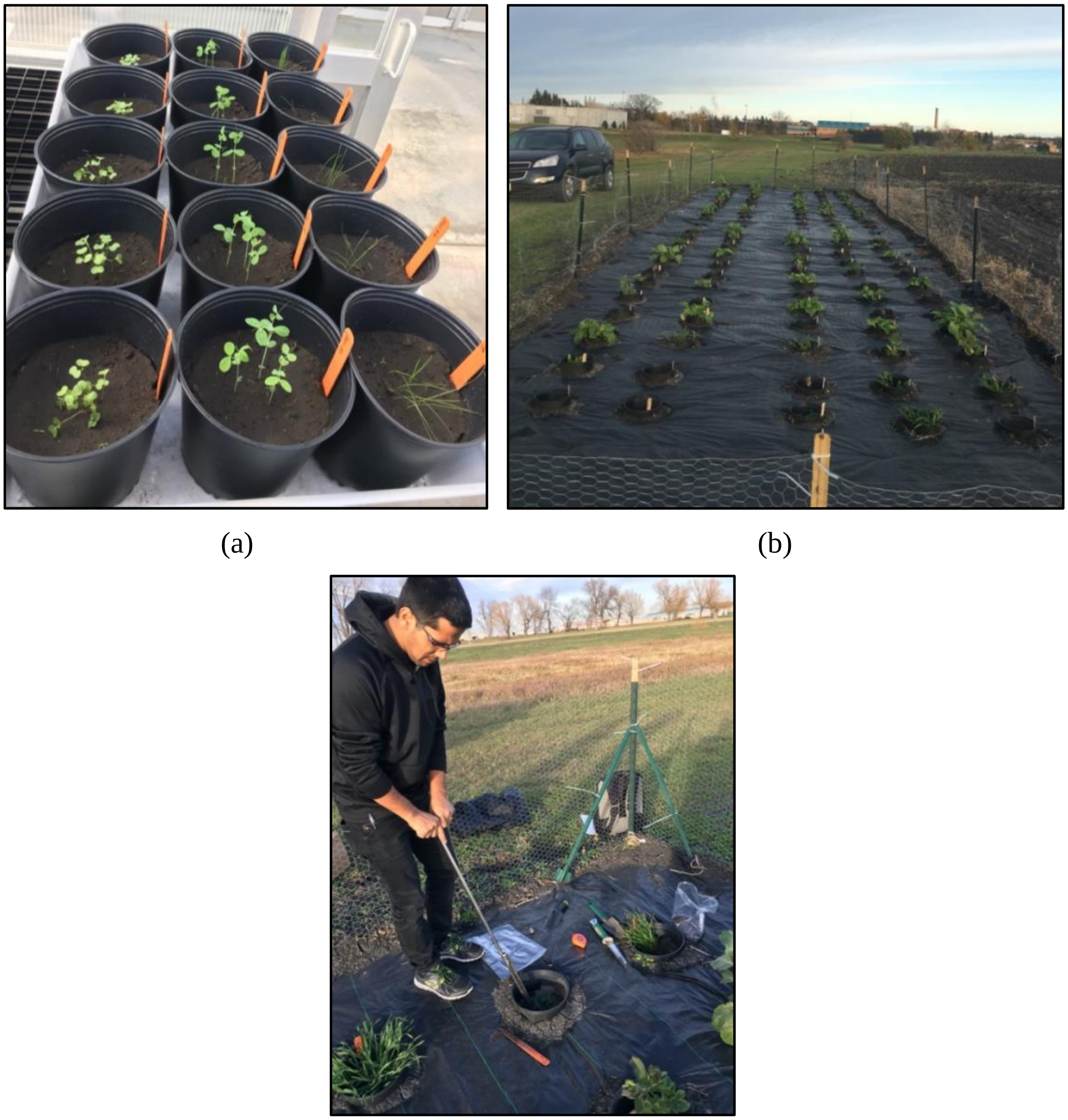
(b)

Fig. A1. Soybean cyst nematode white females attached to soybean roots (a), a single second state juvenile (J2) coming out of the egg (b).

Fig. A2. Soybean cyst nematode (Male).

Fig. A3. Host range study for two soybean cyst nematodes SCN103 and SCN2W in a growth chamber maintained at 27℃ at 16 h. of daylight.

Fig. A4. Susceptible soybean (Barnes) showing soybean cyst nematode white females attached to soybean roots after 35 days of growth in greenhouse conditions.



(a)

(b)

(c)

Fig. A5. Setting up microplot experiment in external environment and collecting soil samples by using soil probe. (a) Plants grown in greenhouse conditions for 15 days before they moved to microplot; (b) Growing plants in microplot conditions for 75 days; (c) Collecting soil samples using soil probe.

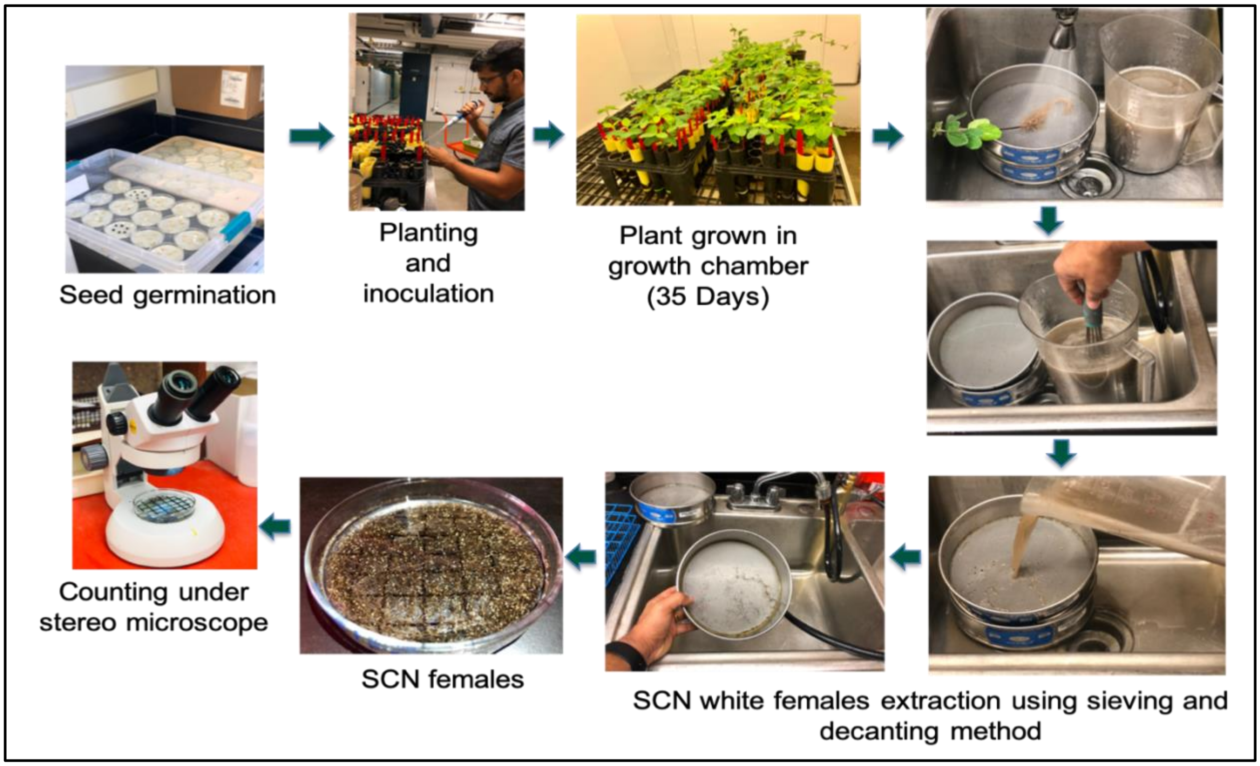


Fig. A6. Procedure for inoculation, extraction, and counting of soybean cyst nematode for greenhouse experiments.

APPENDIX B. TABLES

Table B1. Information about the soybean cyst nematode populations used in the greenhouse and microplot studies.

Sample ID	County	GPS coordinates		Cropping system	HG type	Chapter in dissertation
		North	West			
SCN103	Richland	46.06878	96.79391	Corn-Soy Rotations	0	#2, #3, and #4
SCN2W	Cass	47.05009	97.13320	Corn-Soy Rotations	7	#2, #3, and #4
SCN101	Richland	46.38527	96.92546	Corn-Soy Rotations	0	#5
SCN330	Traill	47.57041	97.41068	Corn-Soy Rotations	2.5.7	#5