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Evaluation of Alternative Weed Hosts and Management Strategies for Soybean Cyst Nematode in South Dakota

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EVALUATION OF ALTERNATIVE WEED HOSTS AND MANAGEMENT STRATEGIES FOR SOYBEAN CYST NEMATODE IN SOUTH DAKOTA

BY

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This thesis is approved as a creditable and independent investigation for the Masters in Plant Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ABBREVIATIONS

ANOVA = analysis of variance

bi = billions

bu = bushel

^{0}C = degree Celsius

cm = centimeter

df = degree of freedom

FI = female index

ft = feet

ha = hectares

HG = *Heterodera glycines*

in = inches

LSD = least significant difference

m = meters

mi = millions

Mmt = Million metric tons

Mt = Metric tons

P = probability

Spp. = species

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ABSTRACT

EVALUATION OF ALTERNATIVE HOSTS AND MANAGEMENT STRATEGIES FOR SOYBEAN CYST NEMATODE IN SOUTH DAKOTA

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The soybean cyst nematode (SCN; *Heterodera glycines*, Ichinohe) is an important pathogen of soybean in South Dakota which causes significant yield losses. SCN has been found in 30 South Dakota counties as on 2017 and is estimated to cause yield loss of 1.9 Metric tons (Mt) annually. SCN has diverse crop and weed hosts as it can reproduce in several crops and weeds. The presence of *Heterodera glycines* (HG) types can reduce the effectiveness of SCN-resistant cultivars and the HG types reproduction on weed hosts can negate the effectiveness of crop rotation by continued build-up in the field. This study examined the alternative weed hosts of SCN in South Dakota based on field and greenhouse studies. Out of 63 weed species studied, field pennycress and purple deadnettle were determined to be the good hosts whereas white clover, common mallow, shepherd's purse, Canada thistle and cocklebur were determined to the poor hosts of SCN in South Dakota.

This research also investigated the reproduction of three commonly found HG types 0, 2.5.7, and 7 on three major weed hosts of SCN in the United States: purple deadnettle,

field pennycress, and henbit relative to a susceptible check, Williams-82, under greenhouse conditions. SCN reproduction was found to be influenced by the type of weed species, HG types and their interaction. SCN reproduction was highest for HG type 2.5.7 (FI = 6.4) followed by HG type 7 (FI = 6.1) and HG type 0 (FI = 5.9). Similarly, among weed species, henbit (*Lamium amplexicaule*) was found to harbor highest SCN cysts followed by purple deadnettle (*Laminum purpureum*) (FI = 6.9) and field pennycress (*Thalpsi arvense*) (FI = 4.8). These results indicate that all the three weeds considerably supported SCN HG types tested and hence these weeds should be managed proactively as an important component of SCN management strategies.

Another aspect of this research was to determine the effects of flooding on SCN development with or without nematicide seed treatment. Flooding days and seed treatment affected the number of SCN cysts on the susceptible cultivar. The greatest number of cysts developed when plants were flooded for 2 days (Cyst = 36) followed by treatments flooded for 0, 4, and 6 days. The number of cyst was lowest for the plants flooded for 8 days (Cysts = 26). This study also indicated that ILeVO seed treatment lowered SCN reproduction on a SCN susceptible soybean cultivar and promoted root development.

This study indicated that a few weeds support SCN reproduction which is impacted by the presence of different HG types of SCN, flooding and seed treatments. All these aspects should be integrated with other management strategies in order to manage SCN effectively.

Keywords: *Heterodera glycines*, HG types, SCN, winter annual weeds, SCN weed hosts, female index, flooding, ILeVO, management, reproduction

CHAPTER 1

1. Literature review

1.1. Soybean history

Soybean [*Glycine max* (L.) Merr.] originated from Southeast Asia (Hymowitz, 1970; Hymowitz and Newell, 1981). It was first domesticated in Northeast China about 1100 BC, and this area is regarded as its primary gene center. Soybean was introduced and developed as landrace into several countries including Japan, Indonesia, Philippines, Vietnam, Thailand, Malaysia, Burma, Nepal and India which are regarded as secondary gene centers (Hymowitz, 1990). Samuel Bowen (a seaman in East India Company) from Savannah, Georgia first introduced soybean to the United States in 1765 AD (Harlan and Hymowitz, 1983). Later, Benjamin Franklin also sent soybean seeds from London to Philadelphia, Pennsylvania in 1770 (Harlan and Hymowitz, 1983). Soybean was introduced to Illinois in 1851 and subsequently to the corn-belt (Hymowitz, 1990). Initially, it was grown for the manufacture of soy sauce, vermicelli (soybean noodles), coffee berries to brew coffee (Harlan and Hymowitz, 1983), forage crop (Probst and Judd, 1973), poultry feed, soybean oil, vegetable protein meat and other uses (Hymowitz, 1990). However, its status as grain crop became prominent after 1920 AD (Probst and Judd, 1973).

1.2. Production and economy

Soybean is second most important crop in terms of acreage and production in the United States (USDA NASS-ERS, 2017). North America and South America contribute around 80% of total soybean produced in the world (Chang et al. 2015). United States of

America contributed around 35% of world's total soybean production and was worth about $40.8 billion in 2017 (http://SoyStats.com, 2017) with a production of 119.5 million metric tons (Mmt) for the year 2017 (USDA ERS, 2017). This makes the USA first in soybean production in the world and is followed by Brazil, Argentina, and China, which contribute 33%, 14%, and 4% of the world soybean supply, respectively (USDA-FAS, 2017-2018).

Global soybean production is estimated to reach 354.5 million metric ton by 2018 suggesting an increase in production around the globe (USDA-FAS, 2017-2018). In South Dakota, soybean is second most important crop grown in the eastern half of the state in an area of 2.1 million hectares producing 6.6 Million metric tons and worth around $2 billion in 2017 (USDA NASS, 2017).

1.3.1. Soybean cyst nematode

Soybean production is affected by numerous plant pathogens (Hartman and Sinclair, 1999) and among all the biological factors causing soybean yield loss, soybean cyst nematode (*Heterodera glycines*, Ichinohe) ranks first (Niblack, 2005; Wrather and Koenning, 2006). In a three-year study, it was found that soybean cyst nematode (SCN) caused an estimated annual loss of $1.3 billion (Koenning and Wrather, 2010). Thus, soybean cyst nematode is an important pathogen which continuously threatens soybean production all around the globe (Mitchum, 2016). Soybean cyst nematode is a soilborne, obligatory, sedentary, endoparasitic nematode which parasitizes soybean roots (Niblack et al. 2006).

Soybean cyst nematode belongs to phylum Nematode, order Tylenchida, and family Heteroderidae. The genus *Heterodera* comprises cyst-forming nematodes which are diagnosed by their ability to form cysts (thick walled dead female shielding eggs) on the roots (Agrios, 2005). Furthermore, the genus *Heterodera* is one of the three genera of sedentary endoparasites which is considered as the most economically important group of plant-parasitic nematodes (Williamson and Gleason, 2003).

1.3.2. Origin and distribution of SCN

SCN was first identified and reported in Japan in 1915 AD (Hori, 1915). There had been reports of SCN being present before 1915 AD, however, it was misidentified as sugar beet cyst nematode, *Heterodera schachtii* (Ishikawa, 1916). Later it was named 'soybean yellow dwarf disease' in 1921 (Ito, 1921). In 1880, there had been reports of SCN in Northeastern China which remained unconfirmed (Noel, 1992; Liu et al.1997). It was later reported from Korea (Yokoo, 1936), China (Nakata and Asuyana, 1938), and the United States of America (Winstead et al. 1955). Other reports of SCN from Canada, Italy, and Iran followed (Yu, 2011).

In the United States, SCN was first detected in Hanover County of North Carolina in 1954 (Winstead et al. 1955). It is believed to have been introduced from Japan through soil infested with *Bradyrhizobium* spp. (Hymowitz, 1990) while some scientists advocate the view that SCN evolved from *H. schachtii*. Some scientists even believe that it had been introduced through soybean seeds and flower bulbs of narcissus and gladiolus from

Japan (Spears, 1955). SCN was detected in Missouri and Tennessee in 1956, Arkansas, Kentucky and Mississippi in 1957, and Virginia in 1958 (Riggs, 1975) and quickly spread to the corn-belt. SCN was first detected in South Dakota in Union county in 1995 (Smolik and Draper, 1998) and has since been found in 30 counties of South Dakota (Acharya, 2015) causing estimated yield loss of 1.9 Metric tons (Mt) annually. https://www.sdsoybean.org.

1.3.3. Life cycle and infection process of SCN

The life cycle of SCN is comprised of three main stages: egg, juvenile, and adult which begin with the fertilized eggs. Embryogenesis and molting result in the formation of the first stage juvenile (J1) inside the egg (Niblack, 2005). The J1s continue molting to form second-stage juvenile (J2). Egg hatching is influenced by several factors such as soil temperature (Wallace, 1955; Slack and Hamblen, 1961; Clarke et al. 1978; Tefft et al. 1982), soil texture (Hillel, 2004), host root exudates (Tefft and Bone, 1985), pH (Tefft et al. 1982) and sometimes egg hatching is age-mediated (Niblack, 2005). The J2 locate the root through chemo-location (Perry, 1996) and infects the susceptible host with the help of specialized penetrating organ called stylet (Lauritis et al. 1983). Failure in finding the root ultimately results in nematode starvation and death (Hershman, 1997). The J2 then migrates towards the vascular system (Davis et al. 2004) and destroys cortical and epidermal cells by cellulase enzyme (Ross, 1958; Wang et al. 1999). The juveniles then produce pathogenesis factors that dissolve cell wall which results in substantial cytoplasmic changes forming a dense mass of cytoplasm (Endo, 1998). These dissolved cells undergo morphological changes and enlarge to metabolic sink known as a

syncytium (Davis et al. 2004) which remains intact throughout the life of nematode (Koenning and Sipes, 1998). The juveniles now become sedentary and continue drawing nutrients from the syncytium and molt into next stage, the third stage juvenile (J3). At this development stage, the J3s undergo sexual differentiation (Riggs and Wrather, 1992; Wrather et al. 1984). J3 males undergo metamorphosis regaining their vermiform shape and exit the root (Triantaphyllou and Hirschman, 1962). These adult males become free living and move in the soil searching for the females to mate with the help of sex pheromone (Jaffe et al. 1989) and consequently die after mating. J3 females continue to draw nutrients and eventually change into a lemon-shaped structure called cyst that break externally through the root surface. Each cyst contains 40-600 eggs with an average of 200 eggs (Sipes et al. 1992). Most of the eggs are present inside the cyst but few eggs are present outside in the gelatinous secretion as well (Koenning, 2004). Upon death, the cyst produces antimicrobial compounds like chitinase and polyphenol oxidase (Niblack et al. 2006) to guard eggs against desiccation and microbial infection. Eggs can remain viable in optimal conditions for up to 9 years (Inagaki and Tsutsumi, 1971; Melito et al. 2010; Niblack et al. 2006). Typically, SCN takes around 3 to 4 weeks to complete its life cycle but this is influenced by several environmental factors such as temperature, moisture, and pH (Riggs and Wrather, 1992). However, under the controlled environment of 25 °C, it takes 21 days for SCN to complete its life cycle (Lauritis et al. 1983). In South Dakota, depending upon the soybean maturity group planted, SCN can complete up to 3-4 life cycles in a single growing season (Acharya, 2015).

1.3.4. Symptoms and detection measures

SCN infection starts when second stage juvenile penetrates into fine epidermal root of young soybean plants. The injury causes morphological and physiological damage to the root (Schmitt et al. 2004) but the extent of damage in terms of yield and growth is dependent on the number of second stage juveniles feeding the roots (Endo, 1992). These syncytia (metabolically active nematode feeding sites in the roots) interfere with the secondary root growth (Noel, 2004) leading to root system reduction and the blockage of the vascular bundles. A reduced root system with clogged vascular bundles affects the transport of minerals and nutrients from the soil. Sometimes, it also reduces nodule formation resulting in fewer nodules that supply nitrogen to the plant and is an important factor for soybean growth (Noel, 2004). Typical symptoms of SCN include stunting, yellowing, midday wilting, and ultimately resulting in yield reduction (Niblack, 2005). However, foliar symptoms are not confirmatory symptoms and sometimes, the infestation may not be detected due to lack of visual symptoms (Wang et al. 2003; Young, 1996). Foliar symptoms produced by SCN can also be misinterpreted for other problems like nutrient deficiency (iron, potassium, and nitrogen), drought stress, herbicide injury, other pests and disease interactions (Niblack et al. 2006). Soil sampling for SCN is the most reliable means of confirming and monitoring SCN infestations and there are also several molecular techniques which can detect SCN effectively (Sankaran et al. 2010; Baidoo et al. 2017). Polymerase chain reaction (PCR) and Real-time PCR, Enzyme-Linked Immunosorbent Assay test (ELIZA), Immunofluorescence (IF), Fluorescence in-situ hybridization (FISH) and DNA microarrays, Amplified Fragment Length Polymorphism (AFLP) are commonly used techniques (Lopez et al. 2003; Martin et al. 2000; Hooper et al. 2005).

1.3.5. Race and HG type

SCN are highly heterozygous in nature, which results in the variability in parasitism that differs among soybean cultivars. After the development of the first resistant cultivars against SCN, several reports of variability in pathogenesis were also reported (Ross, 1962; Niblack and Chen, 2004). To explain the variability of SCN to growers and breeders, the race concept was developed in 1970. To characterize the race, resistance or susceptibility of SCN against four SCN differential lines PI 88788, PI 90763, Peking and Picket were utilized (Golden et al. 1970; Riggs and Schmitt, 1988). Race classification was expanded again into 16 different races utilizing the original four differential lines (Riggs and Schmitt, 1988). SCN population was determined to be virulent to a resistant source if the relative number of females developed was equal or greater than 10% of the number of females developed in SCN susceptible check Lee 74 (Schmitt and Shannon, 1992). Constantly changing pathogenicity of SCN and the development of new resistant sources of soybean, the race system became very complex using the race formula to determine the diversity of SCN population. Thus, the race system was then replaced by HG type system (HG: *Heterodera glycines*) (Niblack et al. 2002). HG type is ameliorated classification system of SCN by considering the reproduction of SCN on seven soybean differential lines which are PI 548402 (Peking) (1), PI 88788 (2), PI 90763 (3), PI 437654 (4), PI 209332 (5), PI 89772 (6), and PI 548316 (Cloud) (7) as compared to the standard susceptible check (Niblack et al. 2002; Wang et al. 2013). Unlike the race system, HG type system does not use Picket as this differential line was derived from Peking and has 4 additional differential lines.

Peking and PI 88788 are the two important sources of resistance against SCN in North America (Concibido et al. 2004). PI 88788 alone constituents more than 95% of the share in commercial resistant cultivars (Mitchum, 2016; Joos et al. 2013; Tylka and Mullaney, 2015). This has led to the adaptation of SCN to PI 88788 source of resistance which is supported by reports of shifts and breakdown of PI 88788 resistance from different soybean growing states (Mitchum et al. 2007; Niblack et al. 2008; MacGuidwin, 2012; Hershman et al. 2008; Zheng and Chen, 2011; Acharya et al. 2017b). In South Dakota, race determination was done in 2005 using two populations of SCN from Turner and Union county which was identified as race 3 (HG type 0) (Jones, 1997) while a recent report suggests that HG type 0, 2.5.7 and 7 are more prevalent in South Dakota (Acharya et al. 2017a).

1.3.6. SCN crop hosts

Soybean is the major host of SCN but it can also infest a wide range of other crops and weeds. Over 140 genera of plants can be infected by SCN with a majority of hosts belonging to the family Leguminosae while 22 non-legume families have been found to be the hosts (Riggs and Wrather, 1992). Riggs (1987) reported the penetration and development of SCN in crops such as turnip (*Brassica rapa* L.), alfalfa (*Medicago sativa* L.), lima bean (*Phaseolus lunatus* L.), common bean (*P. vulgaris* L.), hairy vetch (*Vicia villosa* Roth.), cowpea (*Vigna unguiculata*), tomato (*Lycopersicon esculentum* Mill.), white lupine (*Lupinus albus L.*), purple bush bean (*Macroptilium atropurpurea*), wild bush bean (*Macroptilium lathyroides*), tepary bean (*P. acutifolius* A.), and adzuki bean (*Vigna angularis* Willd.) (Riggs, 1987). Legumes such as Canada tick clover

(*Desmodium canadense* L.), hairy vetch (*Vicia villosa* Roth.), lima bean (*Phaseolus lunatus* L.), little marvel pea (*Pisum sativum* L.), strawberry clover (*Trifolium fragiferum* L.), string bean (*Phaseolus vulgaris* L.) and tender bean (*Phaseolus vulgaris* L.) were reported to be good hosts of SCN in a greenhouse study (Jones, 1997). Dry beans such as Black bean (*Phaseolus vulgaris* L.), Kidney bean (*Phaseolus vulgaris* L.), Navy bean (*Phaseolus vulgaris* L.), and Pinto bean (Phaseolus vulgaris L.) were also reported to be good hosts of SCN (Poromarto et al. 2011; Poromarto and Nelson, 2009; Yan et al. 2017).

1.3.7. Weed hosts

Several winter annual weeds were determined to be alternative hosts of SCN and therefore pose significant impact on its management in the field (Duncan and Noling, 1998; Thomas et al. 2005). Resistant cultivars, seed treatments, and crop rotation are important measures to manage the SCN (Hartman and Sinclair, 1999) but weed hosts supporting high reproduction of SCN could reduce the effectiveness of these management measures (Poromarto et al. 2015). Riggs and Hamblen conducted a the detailed study on the alternative weed hosts of SCN where 164 weed species were listed as poor hosts from the collection of 286 weed species from 22 families. The weed species were declared hosts if SCN were able to form at least 1 cyst in the weed roots (Riggs and Hamblen, 1966a). In 1987, a comprehensive list of crops and weed species was determined and categorized as 1) plants not penetrated by SCN, 2) plants penetrated but no development occurred, 3) plants penetrated and slight development occurred and 4) plants penetrated and development occurred to maturity (Riggs, 1987). In another study,

Poromarto et al. (2015) detected 26 new alternative weed species from 11 plant families from different collections. However, henbit (*Lamium amplexicaule* L.) (Epps and Chambers, 1958; Riggs and Hamblen, 1962; Poromarto et al. 2015; Werle et al. 2015; Creech et al. 2007b), common purslane (*Portulaca oleracea L*.) (Riggs, 1992; Riggs and Hamblen, 1966a; Poromarto et al. 2015), purple deadnettle (*Lamium purpureum* L.) (Riggs and Hamblen, 1962; Poromarto et al. 2015; Werle et al. 2015; Creech et al. 2007b), and field pennycress (*Thalpsi arvense* L.) (Venkatesh et al. 2000; Poromarto et al. 2015) are the major alternative weed hosts commonly reported from different states in the United States.

Winter annual weeds have added daunting challenges to crop production in the recent years (Nice and Johnson, 2005). Different types of tillage practices adopted by growers have contributed to the weed establishment in the field. For an instance, conservation tillage practice contributed around 27% of total tillage practices and no-till contributed around 37% of the total cultivable land (USDA NASS, 2017) which has resulted in the reduction in soil disturbance and has facilitated weed establishment and seed production. Another important cause for the resurgence in weed populations is the adoption of herbicide-resistant soybean cultivars. In 2017, herbicide tolerant soybean constituted around 94% of total soybean acreages in the United States (USDA ERS, 2017). Increased use of herbicide-tolerant soybean has led to a reduction in the use of soil residual herbicides that has also promoted weed seed bank (USDA ERS, 2017). Mild winters experienced in recent years also has reduced winter killing of the weeds (Krausz et al. 2003). Soybean growers who use more intensive tillage practices as a measure to control

weeds can promote the SCN spread and multiplication as a result of these soil disturbances (Koenning and Barker, 1995; Young, 1987). Meanwhile, herbicides used to manage the weeds also facilitate SCN growth by altering the soil temperature (Creech et al. 2007b).

1.3.8. Role of weeds in HG type diversity

Reproduction of SCN HG types on different weed hosts is an important aspect of SCN management. This knowledge can help to determine whether a particular SCN HG type is able to reproduce on specific weed hosts along with the ability of the weed hosts to support different SCN HG types. Furthermore, several reports have documented that weed species are responsible for the selection pressure of SCN in a particular region (Poromarto et al. 2015).

The most conventional measure for SCN management is planting of resistant cultivars and when a resistant cultivar derived from a single source of resistance is continuously planted over subsequent cropping seasons, SCN HG types which are able to reproduce on the resistance source may be developed. Similarly, several weed hosts in the field may be able to support a particular SCN HG type and maintain the source of inoculum for the subsequent cropping season. This, in turn, can favor particular SCN HG type to dominate in the field. This phenomenon is witnessed by major soybean producing states where shifts in the SCN HG types had been observed. The reason behind the shift is not well known but may be due to alternative weed hosts influencing development of different HG types in the field further complicating SCN management. HG type diversity and

interaction with different hosts can impart important information on HG type shifts, selection pressure, and abundance of new HG types which in turn can inform about devising appropriate management strategies against SCN.

1.4. SCN interaction with other pathogens

SCN and brown stem rot (BSR) are commonly found diseases throughout the soybean growing region of the United States (Workneh et al. 1999). Incidence and severity of BSR of soybean were highly correlated with the presence of SCN regardless of the resistance or susceptibility of cultivars to BSR. Some sources of SCN resistance also confer resistance to BSR (Oplinger et al. 1999), which is validated by the fact that PI 88788 source of resistance of SCN performed better than BSR resistant check in preventing the infection of BSR, whereas Peking performed similarly to standard BSR susceptible check under in the greenhouse conditions (Kurtzweil et al. 1999).

Sudden Death Syndrome (SDS) and SCN are generally prominent in the low-lying parts of the field where the soil has higher moisture (Roy et al. 1997). Though SDS and SCN are unrelated in the infection mechanism, they are often found occurring together (Roy et al. 1989) but the relationship among these pathogens have been reported to be inconsistent. Some research reported positive correlation between the fungus and SCN (McLean and Lawrence, 1993; Xing and Westphal, 2006; Brzostowski et al. 2014, Westphal et al. 2014) while other studies reported weak interaction or no correlation between the pathogens (Roy et al. 1993; Gao et al. 2006; Marburger et al. 2014).

An additive interaction was observed between SCN and the *Phytophthora* rot pathogen *P. sojae* where the lesion length caused by *P. sojae* was significantly higher in presence of SCN (Adeniji et al. 1975). In another study, *P. sojae* incidence was increased on the SCN infected soybean plants (Kaitany et al. 2000). *P. sojae* and SCN were found to possess damaging effect on all the growth variables of soybean plants when combined as compared to the single pathogen (Chowdhury, 2017). Increased lesion length was observed for *P. sojae* in presence of SCN whereas SCN population was significantly reduced in presence of *P. sojae* (Chowdhury, 2017).

It was reported that the presence of SCN can reduce the lesion length of stem canker pathogen *Diaporthe caulivora* by 40% on soybeans (Russin et al. 1989) depicting the negative effect of SCN on the fungal pathogen on SCN (Raven and Johnson, 2002; Russin et al. 1989). Reduction in the cyst and juvenile numbers in presence of *D. caulivora* has also been reported (Russin et al. 1989). In another study, it was reported that co-infestation of both SCN and *D. phaseolorum caulivora* did not significantly reduce yield as compared to non-inoculated control (Pacumbaba, 1991). In a recent study, SCN reproduction was also found to be reduced by 90% or greater when the fungus was inoculated earlier to the plants than SCN whereas the lesion length was increased by 76% or more when SCN was inoculated earlier (Posch, 2017).

Soybean aphid and SCN possess an indirect effect on one another if soybean is infected with both of pests (McCarville et al. 2014). In the presence of soybean aphid, SCN reproduction increased on the SCN resistant cultivar whereas reproduction decreased on

the susceptible cultivar (McCarville et al. 2014) which indicated that the aphid feeding favors the SCN but at higher aphid population densities, this effect is compensated by decrease in resources for both pests (McCarville et al. 2014). Moreover, it was also reported that the cultivars containing soybean aphid resistant Rag1 gene do not confer protection against SCN (Macintosh et al. 2009). In a recent study, it was also found that the number of SCN cysts increased when soybean aphids were increased for SCN resistant cultivars while this pattern was not observed for SCN on the susceptible cultivar (Knodel et al. 2016).

1.5. SCN management

SCN management is a challenge to soybean growers because of SCN ability to survive for a long period of time even in absence of hosts, its ability to break down the resistance of commonly grown SCN resistant cultivars and its ability to survive in a wide range of deleterious environmental conditions (Niblack and Chen, 2004). The most commonly practiced SCN management measures are crop rotation, seed treatment, and planting of resistant cultivars (Niblack et al. 2003; Faghihi and Ferris, 2006; Niblack, 2005; Tylka, 2008; Oyekanmi and Fawole, 2010).

1.5.1. Host resistance

Resistant varieties are a promising management tool to manage SCN (Niblack et al. 2003; Faghihi and Ferris, 2006; Niblack, 2005; Tylka, 2008; Oyekanmi and Fawole, 2010). In

resistant cultivars, second stage juveniles fail to form a permanent feeding site i.e. syncytia (Davis et al. 2004; Davis et al. 2000; Johnson et al. 1993). After the discovery of SCN in North Carolina (Winstead et al. 1955), efforts to identify SCN resistance genes began and subsequently, a resistant cultivar was introduced in 1957 (Ross and Brim, 1957). From 1973-82, $405 million profit was obtained by the deployment of a resistant cultivar called Forrest (Bradley and Duffy, 1982). Around 118 PI lines resistant to SCN were identified from USDA-ARS soybean germplasm collection (Arelli et al. 2000). Among these 118 PI lines, only two (PI 88788 and PI 548402/Peking) have been utilized in breeding programs depicting the narrow genetic base of today's host resistance (Concibido et al. 2004). In soybean PI line Peking, 3 recessive QTLs': rhg1, rhg2, and rhg3 were found which confer resistance on Peking germplasm (Caldwell et al. 1960). A fourth dominant gene linked to I-locus, Rhg4 has been identified from Peking source of germplasm responsible for seed coat pigmentation as well as SCN resistance (Matson and Williams, 1965). Dominant gene Rhg5 was also discovered from PI 88788 (Rao-Arelli, 1994; Rao-Arelli et al. 1992). However, it was determined that LRR-kinase gene (classical gene family conferring resistance) at rhg1 and Rhg4 were independent of resistance to SCN which led the researchers to refine the linkage maps (Liu et al. 2011; Melito et al. 2010). In another study, it was reported that the 3 dissimilar genes in 10 tandem copies of 31 kb segment are responsible for PI 88788 type of resistance (Cook et al. 2012) whereas the gene serine hydroxy-methyltransferase (SHMT) was responsible for Peking-type resistance (Liu et al. 2012). Moreover, genetic mapping studies suggested that PI 88788 type resistance requires Rhg1 whereas Peking-type resistance requires Rhg4 to confer resistance to SCN (Concibido et al. 2004; Meksem et al. 2001).

Seven PI lines PI-548402 (Peking), PI-88788, PI-90763, PI-437654, PI-209332, PI-89772 and PI-548316 (Cloud) are usually utilized for development of SCN resistant varieties in which PI-88788 constituents more than 95% of resistant sources in the United States (Concibido et al. 2004; Schmitt et al. 2004; Shannon et al. 2004; Mitchum, 2016). Remaining 5% is shared by Peking, PI-437654 or by their combinations (Joos et al. 2013; Tylka and Mullaney, 2015). A QTL was identified on PI-567516C conferring resistance to multiple HG types (Vuong et al. 2010). Resistant cultivars were found to reduce SCN infestation along with increasing soybean yield (Chen et al. 2001b). The yield benefit was about 48% when resistant cultivar was combined with nematicides (Heydari et al. 2012).

SCN resistance is found to be governed by the group of dissimilar genes at multiple loci and the variation of their copy numbers, therefore, more in-depth research is needed beyond a single gene model (Mitchum, 2016). Another alternative strategy for imparting resistance is through turning-off or modifying susceptibility genes (Fosu-Nyarko and Jones, 2015) which were first demonstrated by Guo et al. (2015). Approaches that can stack R and S genes could be promising for improving SCN resistance in soybean along with sustaining natural resistance. For now, a combination of crop rotation with non-host crops along with planting SCN resistant cultivars differing in their resistant gene sources are followed to manage the disease (Niblack, 2005; Tylka and Mullaney, 2015; Mitchum, 2016).

1.5.2. Crop rotation

Though SCN has broad host range consisting of many cultivated crops and weed hosts, crop rotation is an important measure to manage the SCN (Ross, 1960; Niblack and Chen, 2004). Crop rotation is the most sustainable and environmentally friendly measure to check the SCN population increase in the field.

In the Midwestern region, corn is one of the commonly rotated crops with soybean (Noel and Edwards, 1996; Chen et al. 2001a; Perez-Hernandez, 2013). It has been found that corn rotation with soybean in the form of annual, biennial or longer crop rotations reduces SCN population densities (Young and Hartwig, 1992; Noel, 2008) but the underlying mechanism is not precisely known. In another study, the average decline of SCN was 51% during a three-year crop rotation study in Nebraska (Perez-Hernandez, 2013). However, stimulation of egg hatching but failure to get subsequent infections is found to reduce the number of SCN in such rotation (Warnke et al. 2008). However, one year of rotation was not found to be effective in reducing the number of nematodes (Miller et al. 2006). Rotation with corn coupled with soil fumigation with nematicides was found to be effective in controlling SCN. However, fumigation is no longer recommended a as most fumigants have been removed from the market because of threat to the environment (Sasser and Grover, 1991).

1.5.3. Use of cover crops

Several cover crops are found to reduce nematodes in the field either in rotation or when inter-seeded with the major cash crops (Duncan and Noling, 1998; Abawi et al. 2000). However, the effect of cover crops on the nematodes depends upon species of cover

crops and the nematodes present in the field (Phatak, 1998; McSorley, 1998; Abawi et al. 2000). Several research studies have shown there is a potential of cover crops to help in SCN management (Niblack and Chen, 2004). For instance, *Brassica* spp. which contain glucosinolates that decompose to form isothiocyanate, which has a strong nematicidal property is effective against nematodes (Donkin et al. 1995; Chitwood, 2002; Jing and Halbrendt, 1994). Crops such as wheat and other cereals were found to produce phenolic compounds responsible for reducing nematode populations (Hershman and Bachi, 1995), although winter wheat coupled with poultry manure did not reduce SCN population significantly (Wight et al. 2011). Cereal rye produces toxic compound benzoxazinoids, which have been found to reduce nematode populations (Zasada et al. 2005). Another study reported the use of annual ryegrass after soybean harvest reduced SCN (Pedersen and Rodriguez-Kabana, 1991) as the residue of annual ryegrass (*Lolium multiflorum*) stimulated hatching of SCN eggs in absence of soybean, and thus reducing the subsequent SCN population and parasitism (Mock et al. 2009; Riga et al. 2001). Bean sprout residue was also found to reduce SCN population when applied in the field (Toyota et al. 2013). In a greenhouse study, SCN cyst development was not found in cover crops such as annual ryegrass, *Camelina*, carinata, Ethiopian cabbage, faba bean, foxtail millet, radish, rape dwarf Essex, red clover, sweet clover, triticale and winter rye, indicating their use in the form of crop rotation would not increase SCN in the soil (Acharya et al. 2017a).

1.5.4. Effect of irrigation and tillage on SCN

The impact of irrigation on SCN is not well documented. However, it was found that SCN population density was lowered in irrigated plots than in non-irrigated plots (Koenning et al. 1995) but it was unknown whether it was due to the water content or oxygen content ratio. Nevertheless, water is crucial for the spread of SCN from the previously infested field to the new fields (Faghihi et al. 2010). Drought stress facilitates soybean to increase root mass to absorb more water and nutrients and the increased root mass also favors SCN infection (Huck et al. 1986). Since SCN nematode requires aerobic respiration in its life cycle, the soil oxygen level also plays an important role in SCN survival (Koenning and Barker, 1995). Coarse-textured soil has larger pores and drains more easily than fine-textured soils, and favors nematode activity by allowing longer periods of aerobic conditions (Vrain, 1986).

Many studies have been conducted to determine the effect of tillage on SCN populations but the results obtained are inconsistent. This may be attributed to the fact that the results obtained from tillage types were field specific (Noel and Wax, 2003; Niblack and Chen, 2004; Westphal et al. 2009). It was also found that the tillage, especially of fine-textured soil, could increase the nematode population in the field (Workneh et al. 1999). Another study confirmed that tillage could be responsible for the regional spread of SCN (Gavassoni et al. 2007). Reduced tillage intensity in fine-textured soils was found to lower the SCN population in rotated experimental plots (Westphal et al. 2009). In another multifactorial analysis of 8 predictors, it was found that soil type was the important factor in predicting the SCN mortality in annually rotated plots in Nebraska (Perez-Hernandez, 2013; Perez-Hernandez and Giesler, 2014).

1.5.5. Biological control measures

Several studies were conducted to determine the effect of different fungi and bacteria for SCN management. Fungi such as *Hirsutella minnesotensis*, *H. rhossiliensis*, *Cylindrocarpon heteronema* and bacteria such as *Pseudomonas spp*., *Lysobacter* spp., and V*ariovorax* spp. had been found to possess antagonistic effects on SCN. Endoparasitic fungal species such as *Hirsutella minnesotensis* and *H. rhossiliensis* were reported to infest SCN juveniles and thus reduce the infection on the roots (Chen, 2007; Chen and Liu, 2005; Chen et al. 2000; Zhang et al. 2006). *Sinorhizobium fredii* strain Sneb183 is reported to reduce SCN cysts and juveniles by lengthening their developmental period (Tian et al. 2014). *Pirformospora indica*, a plant growth promoting fungi suppresses SCN eggs and juveniles along with enhancing flowering in soybean (Bajaj et al. 2015).

Pasteuria nishizawae, an endospore-forming endoparasite of SCN reduces SCN juveniles and eggs considerably (Sayre et al. 1991; Noel et al. 2005). Syngenta has developed a nematicidal seed treatment Clariva^{TM} based on *Pasteuria nishizawae* (Sharma et al. 2015) which is found to reduce nematode population along with enhancing yield in soybean (Fawcett et al. 2014). *Bacillus firmus* is the other bio-control agent which is an endospore-forming bacteria that feeds on root exudates and thus, reduces the possibility of SCN juveniles infestation of roots (Crow, 2014). However, the effect of *Pasteuria nishizawae* and *Bacillus firmus* has been inconclusive under field conditions (Tylka and Marett, 2014; Musil et al. 2015; Robertson et al. 2016). Poncho/ VOTiVO is another nematicidal seed treatment developed by Bayer CropSciences (2018). It is derived from

the bacteria *Bacillus firmus* which shields young roots against SCN juveniles by creating a living barrier.

Research studies have shown that seven strains of *Pseudomonas* were found to suppress the activity of SCN through 15 independent sets of greenhouse assays (Taylor, 2017). It has also been speculated that microorganisms naturally regulate SCN populations in the infected field for some period (Chen, 2004). Though several bio-control agents for managing SCN have been identified, the cost-effective integration of these biological control agents in conventional soybean production still challenges the SCN research community and commercial producers.

1.5.6. Chemical control measures

Several chemical compounds (nematicides, herbicides, fungicides) have been found to impart some control over SCN along with enhancing plant health. Aldicarb, a carbamate group of nematicide, has some level of control on SCN but is a restricted use nematicide in Midwest region (Grabau, 2013). Its efficacy, however, is not consistent in different states (Niblack, 1992; Noel, 1987; Rotundo et al. 2010; Smith et al. 1991). Telone C-35 is another multi-purpose liquid fumigant and nematicide developed by Dow AgroSciences having chloropicrin as an active ingredient reported to control all types of nematodes including SCN and soil-borne pathogens. In a research study in Iowa, Telone 15 was found to enhance soybean yield by 10% while reducing SCN egg population by 42% (De Bruin and Pedersen, 2008). However, the chemical product is federally restricted-use pesticide and is not registered for sale and use in all states (Dow AgroSciences, 2018).

In another study, benzyl isothiocyanate prepared in 1% methanol was effective in reducing juvenile movement, SCN egg hatching, and reproduction (Wu et al. 2014). Though isothiocyanates have immense potential in crop protection, its biological effects are not well known but these compounds have been the focus of attention for many researchers (Brown and Morra, 1997). ILeVO® is another nematicidal seed treatment developed by Bayer Crop Science, which is based on Fluropyram and is reported to possess direct impact on SCN in seed zone along with a season-long reduction in nematode numbers in the field (Bender, 2017). ILeVO® is recommended to be applied with Poncho/VOTiVO for triple action protection. Avicta (actinomycetes derived product) developed by Bayern Crop Sciences was found to provide yield benefits when coupled with Aeris (a neonicotinoid insecticide) but was not found to reduce SCN population (Frye, 2009).

Among different commonly used herbicides, Blazer (Acifluorfen) was reported to suppress hatching of SCN eggs (Wong et al. 1993). Among different fungicides tested for the effect on SCN, Cleary 163336F was reported to suppress SCN significantly (Faghihi et al. 2007). Although chemical nematicides are effective in controlling the nematode to a certain extent, they also pose a serious threat to the environment and are restricted or fail to make it to market. Application of chemical compounds for control of SCN is not eco-friendly as they pose threats to the handlers and have the potential to affect aquatic organisms. Besides, chemical nematicides are not economical when considered for SCN management because of their high market prices and the high cost of application (Matthiessen and Kirkegaard, 2006; Oka, 2010).

1.5.7. Plant extracts

Several nematicide fumigants and nematicides were banned due to their harmful effect on the environment and human health (Rich et al. 2004). This has led to developing and testing different types of organic compounds and organic amendments for nematode control (Rodriguez-Kabana, 1986). Many plant extracts have been tested and found to be effective for managing SCN and other nematodes along with isolation of nematicidal compounds from them (Gommers and Bakker, 1988; Chitwood, 2002). Leaf extracts, oil cakes and kernel oils from Neem (*Azadirachta indica*) were tested for their nematicidal activity (Silva et al. 2008; Mojumder and Mittal, 2003; Akhtar, 2000). Nematicidal compounds such as limonoids, azadirachtin, nimbin, salanin and many others were identified (Devkumar et al. 1985; Akhtar, 2000). In a recent study, 120 µg/ml leaf extract from neem was found to reduce egg hatching of SCN by about 72%, caused 100% juvenile mortality within 84 hours of treatment, and reduced cyst development in susceptible soybean cultivar, Wiliam-82 by 83% (Hassan et al. 2013). Many plants species of Asteraceae family like *Tagetes, Artemisia* spp., *Chrysanthemum spp., Gaillardia spp., Inula viscosa*, and *Rudbeckia hirta* were studied for their nematicidal activity (Gommers an Bakker, 1988; Timchenko and Maiko, 1989; Dias et al. 2000; Ploeg, 2000; Debprasad et al. 2000; Natarajan et al. 2006; Bar-Eyal et al. 2006). However, no information regarding testing of plant extracts from these species have been reported on SCN.

Glucosinolates are one of the main compounds secreted by most of the plant species from the *Brassicacea*e. These chemicals hydrolyze to release compounds such as

isothiocyanates, thiocyanates, nitriles, and oxazolidine-2-thiones (Matthiessen and Kirkgaard, 2006; Mumm et al. 2008; Oka, 2010). Many attempts have incorporated rapeseed (*Brassica napus*) and Indian mustard (*Brassica juncea*) for nematode management worldwide (Mojtahedi et al. 1991, 1993; Walker and Morey, 1999; Ploeg and Stapleton, 2001; Stirling and Stirling, 2003; Zasada and Ferris, 2004; Rahman and Somer, 2005). However, only Chinese cabbage (*Brassica chinensis*) from the *Brassicaceae* family has been studied to test its activity against SCN. The Chinese cabbage leaf extract at 120 µg/ml was found to reduce egg hatching of SCN by around 63%, caused 68% juveniles mortality at 84 hours of treatment and reduced cyst development in susceptible soybean cultivar, Wiliam-82 by 66% (Hassan et al. 2013). Thus, numerous plant-derived extracts and compounds have been found to exhibit efficacy against different plant-parasitic nematodes (Bones and Rossiter, 2006; Kabeh and Jalingo, 2007; Elbadri et al. 2009; Khan et al. 2009; Sultana et al. 2011; Hassan et al. 2013) but their field effectiveness is yet to be determined.

1.5.8. Biotechnology

Novel sources of SCN resistance deployed through molecular mechanisms or through plant breeding expands the genetic basis of SCN resistance. Biotechnological tools such as RNAi gene silencing and effector proteins mechanism are being tested for SCN management. RNAi induced suppression of genes associated with physiological functions of SCN can be useful in gene expression studies on feeding sites (Li et al. 2011). Similarly, silencing or overexpression of genes associated with the SCN pathogenesis has

shown a great potential for engineering resistance against SCN. For instance, silencing of aldolase gene of SCN was found to reduce the number of SCN cysts (Youssef et al. 2013) and eighteen virus-induced gene silencing based on Bean pod mottle virus (BPMV) was used in the functional analysis of genes involved in SCN resistance (Kandoth et al. 2013). It had also been reported that the resistance against SCN was enhanced in soybean by silencing putative CLE (CLAVATA3/EMBRYO SURROUNDING REGION) receptors emphasizing the novel means of engineering resistance in soybeans (Guo et al. 2015). The same research group also discovered that the silencing of cytokinin synthesizing isopentenyl transferase gene in cyst nematode caused a significant reduction in virulence which further demonstrated the ability of cyst nematode to synthesize a plant hormone in order to control the host system for prolonged parasitic activity (Siddique et al. 2015). On the other hand, overexpression of candidate gene, salicylic acid methyl transferase (SAMT) which promotes the formation of methyl salicylate from salicylic acid was found to confer resistance against SCN (Lin et al. 2013).

Recently, SCN research community has been focused on the parasitism mechanism influenced by effector proteins where more than 80 *H. glycines* effector proteins have already been documented (Gao et al. 2001, Gao et al. 2003; Wang et al. 2003; Noon et al. 2015). Effector protein with N terminal secretion signal peptides were found to be released in the plant via mouth spear (Mitchum et al. 2013) and play an important role in suppression or activation of plant innate immunity (Hewezi and Baum, 2013; Mitchum et al. 2013; Goverse and Smant, 2014; Hewezi, 2015). HgGLAND18 effector protein was reported to suppress plant innate immunity and thus was responsible for pathogenicity

obtained from the compatible interactions with the soybean plant (Baum et al. 2016). Signal transduction study from *Arabidopsis thaliana* showed that the cyst nematode parasitism may require suppression of salicylic acid signaling in the roots (Wubben et al. 2008). Time course RNA gel blot analysis revealed that *Arabidopsis* homologs gene at 17.1 was found to be closely associated with cyst nematode parasitism of plants (Baum et al. 2004). A gene expression study revealed that the active ethylene-signaling pathway reduced the vulnerability of soybean roots by SCN and the authors concluded that there are important roles for ethylene pathways during pathogenicity at early and later parasitic stages (Li et al. 2017). Even though several molecular mechanisms governing resistance of soybean and pathogenesis have been discovered, there is still a lot of complexity yet to be resolved. However, molecular tools possesses immense potential for SCN management through the development of novel SCN resistant soybean cultivars.

1.6. Justification of the study

SCN is an important pathogen of soybean in South Dakota and is ranked number one among yield limiting biological factors in soybean production worldwide. It was first detected in South Dakota in Union county in 1996 (Smolik and Draper, 1998) and has since been found in 30 soybean growing counties (Acharya, 2015). SCN is found to primarily infect soybean, however, no studies have been carried out to determine alternative weed hosts of SCN in South Dakota and their role in influencing SCN reproduction in absence of soybean. Reports from other states suggest that several winter annual weeds act as the alternative hosts of SCN posing a significant impact on SCN

management in the field (Duncan and Noling, 1998; Thomas et al. 2005). Most commonly practiced measures for SCN management are crop rotation coupled with the planting of resistant cultivars (Niblack et al. 2003; Faghihi and Ferris, 2006; Niblack, 2005; Tylka, 2008; Oyekanmi and Fawole, 2010). Crop rotation is one of the ineluctable measures to manage SCN (Hartman and Sinclair, 1999) but weed hosts might negate its effect supporting reproduction of SCN and thus reducing the effectiveness of crop rotation (Poromarto et al. 2015). Weeds such as henbit (*Lamium amplexicaule*), common purslane (*Portulaca oleracea*), purple deadnettle (*Lamium purpureum*), and field pennycress (*Thlaspi arvense*) have been commonly reported from other states as SCN weed hosts. However, there is no information on the weed species that might serve as alternative weed hosts of SCN in South Dakota. Furthermore, very little is known about the interaction of weed hosts and SCN HG types yet this is important in order to determine the role of alternative weed hosts in influencing different HG types in the soil. Among all the environmental factors, soil moisture is an important factor impacting SCN activity in the field. However, there is a paucity of information on the impact of excessive moisture on SCN infectivity and effectiveness of nematicide seed treatment.

Thus, the objectives of this study were to:

I. Determine weeds serving as alternative hosts for SCN in South Dakota
II. Assess the reproduction of most prevalent HG types (HG type 0, 7 and 2.5.7) on major three SCN weed hosts (field pennycress, henbit, and purple deadnettle)
III. Determine the effects of flooding on SCN infectivity and effectiveness of nematicide seed treatments.

CHAPTER 2

2. Determination of Alternative Weed Hosts of Soybean Cyst Nematode in South Dakota

Abstract

Soybean cyst nematode (SCN; *Heterodera glycines*) causes an estimated $1 billion losses in revenue annually in the USA and consistently ranks as the most threatening pathogen for soybean. Winter annual weeds can further exacerbate the SCN problem by harboring SCN in the absence of soybean in the field. These weeds have become widespread in recent years due to an increase in conservational tillage practices and the reduction in the use of residual herbicides. Identification and evaluation of alternative weed hosts of SCN is important to provide effective management strategies against SCN. To determine alternative annual winter weed hosts in South Dakota, 670 whole weed samples were collected from 48 SCN infected fields from 13 counties during fall 2016 and spring 2017 comprising 63 weed species. The weed species were soaked in water for 48 hours to separate the adhered soil from the roots. The roots were dissected into smaller pieces of 2 cm length, macerated in a blender at 12,000 rpm and the resulting suspension was passed through the series of 250, 150 and 45 µm sieves. Using a dissecting microscope, the filtrate was then examined within a counting dish.

Based on the morphological features, SCN juveniles were detected and greenhouse confirmation study was conducted for the development of cysts on the weed hosts and the female index (FI) were determined. Twelve weeds out of 63 were found to harbor SCN

juveniles in the field. Female index (FI) was highest for purple deadnettle (FI=36.3) followed by field pennycress (FI=29), common mallow (FI=3.07), Canada thistle (FI=1.88), shepherd's purse (FI=3.08), white clover (FI=1.15) and cocklebur (FI=1.15). Field pennycress and purple deadnettle were found to be good hosts of SCN whereas the other weed species were poor hosts of SCN in South Dakota. All the weed species determined as hosts from this study were similar to the previous studies except common mallow, which was weed hosts only in this study.

Keywords: SCN, *Heterodera glycines*, winter annual weeds, weed hosts, female index, reproduction

2.1. Introduction

Soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe) is a major pest of soybean in the soybean growing regions (Niblack et al. 2006, Winter et al. 2006). Among all the biological yield-limiting factors, SCN ranks first in the U.S. and has been reported from most of the soybean growing states of the USA (Niblack, 2005, Wrather and Koenning, 2006; Wrather et al. 2010; Tylka and Marett, 2014). Nearly 36% yield losses had been reported from 1996 to 2014 from SCN which accounted for $1.2 billion losses per annum in the revenue in the United States (Koenning and Wrather, 2009; Nguyen et al. 2016). In South Dakota, soybean is the second most important crop after corn and is grown in an area of 2.1 million hectares with a total production of 6.9 million metric tons generating $2.33 billion revenue (USDA NASS, 2017). SCN has been reported from 30 counties of South Dakota and is continuously spreading to other soybean growing counties (Acharya, 2015) causing estimated revenue loss of 1.9 Metric tons (Mt) annually (www.sdsoybean.org).

Soybean and some other legumes are the crop hosts of SCN. However, weeds can also be the alternative hosts of SCN in the field and can play a critical role in the development of the continuous SCN inoculum in the field (Duncan and Noling, 1998; Thomas et al. 2005). Generally, winter annual weeds play an important role in harboring SCN (Gibson et al. 2005; Nice et al. 2005). Winter annual weeds emerge during fall, overwinter as seedlings and then complete their life cycle in the spring. There have been reports of the proliferation of winter annual weeds in different states attributed to factors such as adoption of conservation tillage practices, increased use of herbicide for the weed

management and relatively mild winters (Buhler, 1995; Cardina et al. 2002; Thomas et al. 2004; Wicks et al. 1994; Barnes et al. 2003; Krausz et al. 2003). Resistant cultivars and crop rotations with non-host crops are commonly practiced techniques for SCN management (Hartman and Sinclair, 1999; Niblack, 2005; Faghihi and Ferris, 2006; Tylka, 2008; Okekanmi and Fawole, 2010; Mitchum, 2016). However, a weed host can negate the effects of crop rotation (Poromarto et al. 2015) and can continuously support SCN population in the field if present. Additionally, because SCN is capable of reproducing on the alternative weed hosts, this can possibly influence the HG type (*Heterodera glycines*) by the development of the selection pressure favoring particular SCN HG type reproduction, along with possibilities of development of new HG types in a long run. This is supported by the fact that many major soybean producing states have witnessed changes in the SCN HG types (Mitchum et al. 2007; Niblack et al. 2008; MacGuidwin, 2012; Hershman et al. 2008; Zheng and Chen, 2011; Acharya et al. 2017b). Hence, weed hosts complicate SCN management by reducing the effectiveness of other management measures.

Several studies have been conducted to evaluate the status of weeds as alternative hosts, which is important in devising long-term SCN and weed management strategies. However, identification of alternative weed host species is complicated due to complexity in the weed host species, the development of different HG types of SCN in response to the localized environment, and selection pressures associated with the production practices (Koskinen and McWhorter, 1986; Riggs, 1987; Riggs and Schmitt, 1988). Although considerable genetic variability exists within single weed species and the SCN

types (Radosevich et al. 1997; Niblack, 1992), very few research studies have included weed genetic variations, weed HG types preferences and weed field variations in the weed host research. Riggs and Hamblen, 1962 conducted a first detailed study on the alternative weed host of SCN where 146 weed species were listed as hosts from the collection of 286 weed species from 22 families based on their ability to form at least 1 cyst (Riggs et al. 1992; Riggs and Hamblen 1966a, 1966b).

Sixty-six weed species belonging to nine plant families were reported to be the hosts of the soybean cyst nematode. The nine plant families found to be SCN hosts were Capparidaceae, Caryophyllaceae, Cruciferae, Geraniaceae, Labiatae, Leguminosae, Phytolaccaceae, Portulacaceae, and Scrophulariaceae. Species classified in the family Leguminosae were the most numerous, followed by Scrophulariaceae Furthermore, the plant species were divided as plants not penetrated by SCN, plants penetrated but no SCN development occurred, plants penetrated where slight SCN development occurred and plant penetrated and SCN development occurred to the maturity (Riggs, 1987). Only plants penetrated and where SCN development occurred to the maturity are considered hosts of SCN. In a recent study, 51 weed species from the Northern Great Plains were evaluated for their host/ nonhost status where cysts formed on 31 weed species but only henbit and field pennycress allowed significant cyst development (Poromarto et al. 2015). However, SCN weed hosts may depends on the types of HG type present in the field and also on the weed biotype which needs further research.

Environmental factors play important roles in the development of SCN on the winter annual weeds. Though SCN can hatch, penetrate roots and develop into mature cysts over a wide range of temperatures, the rate of SCN growth and development is highly temperature dependent (Creech et al. 2007b). The optimum temperature suitable for the proper development of SCN is 25 °C (Alston and Schmitt, 1988). However, the rate increases linearly from 15 to 30 °C (Creech et al. 2007b). SCN reproduction has also been reported to be higher in autumn than in the spring which may be attributed to a favorable environment suitable for the nematode to complete its life cycle. In addition, number of J2, J3, and J4 inside the weeds were found to be higher in the spring than in the autumn season. This is found to be reasonable as the rate of SCN egg hatch and development of J2 declines in the autumn due to a reduction in soil temperature whereas increases in the spring soil temperature increases will decrease dormancy (Bonner and Schmitt, 1985, Hill and Schmitt, 1989; Ross, 1963). It had also been found that the risk of the infection and continuous development of SCN juveniles inside the root of winter annual weeds in the spring is the major factor responsible for the development of continuous inoculum in the field which ensures that these weeds should be removed from the field before planting (Creech et al. 2007a). Thus, it is important to manage these winter annual weeds effectively during the autumn to disrupt the SCN life cycle.

Commonly found weed hosts of SCN in different soybean producing states in the United States include burclover, alsike clover, crimson clover, scarlet clover, common chickweed, mouse-ear chickweed, common mullein, field pennycress, henbit and purple deadnettle (Wrather and Mitchum, 2015; Giesler and Wilson, 2011; Tylka, 2012; Noel,

2015; Faghihi and Ferris, 2017; Poromarto et al. 2015; Chen, 2012; Niblack, 2013, Mock et al. 2007). However, fewer weeds are reported as hosts only in some state and host/ non-host status of weeds varies from states to states. For an instance, shepherd's purse is reported as hosts only in Indiana (Faghihi and Ferris, 2017) and Ohio (Niblack, 2013), small flower bittercress only in Indiana (Faghihi and Ferris, 2017) and wild mustard in Illinois (Mock et al. 2007). This shows that weed host determination in a particular region is attributed to several factors such as HG type diversity, weeds distribution, climate and soil conditions and farming practices.

Nematode populations obtained from root and soil are useful for weed host evaluation studies of the endoparasitic nematode. However, most of the research studies related to weed host determination are conducted under controlled condition by infesting the host with SCN inoculum and determining the respective female index (percentage of the average number of cysts found on the weed species compared to a susceptible soybean check). Moreover, it is important to assess whether the weed hosts really follow the similar trend in the field as there are several factors which affect the SCN pathogenicity such as the differences in HG types, the presence of different species of the nematodes, interactions among different soilborne pathogens, and different environmental factors. In addition, there had also been contrasting reports of plants species penetrated by SCN from multiple locations where cyst development has the varying trend among the same weed species (Poromarto et al. 2015). Hence, it is important to address field and greenhouse variations and weed hosts complexity in weed hosts determination study.

Thus, the objective of this research was to determine the weed hosts of SCN in South Dakota through field and greenhouse study and also to determine weed hosts distribution in the soybean-producing region of South Dakota.

2.2. Methodology

2.2.1. Sample collection

SCN infected fields in 13 different counties of SD were identified from the research study conducted by Acharya, 2015. Whole weed samples along with soil from 0- 30 cm depth were collected from the infected field using a shovel. Weed species were collected based on the abundance of the weed species in each particular field but generally, at least two samples for each weed species were collected at each field. Weed samples were collected in the fall of 2016 from September to November and in the spring of 2017. A total of 48 fields were sampled, 11 fields in the fall 2016 and 37 fields during spring 2017. Samples were collected in the ziplock bags of 10 by 13 cm size and placed in the cooler with ice bags and the coordinates of each field in each county were noted at the entry point. Additionally, a gallon of soil sample was collected from every sampled field in zig-zag pattern out of which 100 cc of soil was used to confirm the presence of SCN in the sampled field.

2.2.2. Sample processing and SCN extraction

Samples were placed in a bucket filled with water without disturbing the roots. They were left for 48 hours to facilitate easy release of soil from the roots. SCN cysts and eggs were extracted from the 100 cc of the representative soil sample taken from 10 different

parts of each field by soil cores to confirm the presence of SCN in the field following the extraction procedure by Faghihi and Ferris, 2000. Roots were separated from the individual sampled weed species and stored for further analysis.

2.2.3. Juvenile extraction

The root of each weed was cut into small pieces (1-2 cm length) and was macerated in a blender in 100 ml at 12000 rev/min for 1 minute (EPPO, 2013). The resulting suspension was passed through the two set of sieves with 250 µm at the top and 25 µm at the bottom. The suspension was collected in 50 ml beaker and then analyzed in a counting slide for the presence of juveniles through the dissecting microscope. Based on the morphology of the juveniles obtained, nematodes from the Genus *Heterodera* were identified and confirmed.

2.2.4. Greenhouse assay

Twelve weed species positive for the presence of juvenile from the field sample were collected and grown in the greenhouse. The experiment was set up to determine the cyst development in previously identified positive weed species from the field study. Weed seeds were pre-germinated at the room temperature in the petri-dish in a filter paper. Pre-germinated seeds of each weed species and susceptible soybean cultivar Williams 82 were transplanted into individual cone-tainer (3.8 cm diameter and 21 cm height, Stuewe and Sons Inc., Tangent OR) filled with sterilized clay-sand mixture (2 parts of sand and 1 part of clay by volume). The weed species and the susceptible soybean cultivar were placed in a 7.6-litre bucket filled with sand and was placed in a water bath in the

greenhouse. The water bath was maintained at the temperature of 27-28 °C and at day length of 16 hours. Individual cone-tainers were inoculated 3 cm below the soil surface with 1ml egg-water solution containing egg density of 2000 eggs per ml through a pipette. All the treatments were arranged in completely randomized design with 8 replications and a susceptible Williams 82 check and were repeated twice.

After 40 days post-inoculation, the cone-tainers were taken out of the bucket, soaked in water for 20 min and the treated plant species were gently uprooted and removed. Cysts were collected in 210 µm pore sized sieve nested under 710 µm pore sized sieve sprayed with a strong stream of water to dislodge the cysts from the roots where the roots were weighed and stored at 4°C in 5 by 7.6 cm ziplock bags.

2.2.5. Data analysis

Field data comprised of the total number of weeds found in each location, weeds distribution frequency and the number of juveniles found from each weed species in fields sampled. Greenhouse data included the total number of cysts developed on the root of the weed species and their respective female index determined relative to the cysts formed in the susceptible check.

2.3. Results

2.3.1. Distribution of weed samples

A total of 670 whole weed samples were collected from the fields during fall 2016 and spring 2017. Canada thistle (*Cirsium arvense*) was the most abundant with a distribution frequency of 69% (33 out of 48) sampled locations (Table 2.1). Common lamb squarters (*Chenopodium album*), field pennycress (*Thlaspi arvense*), dandelion (*Taraxacum officinale*), white clover (*Trifolium repens*), field bindweed (*Convolvulus arvensis*), cocklebur (*Xanthium strumarium*) and kochia (*Bassia scoparia*) were found in 59%, 56%, 50%, 46%, 44%, 42%, 42% of the sampled fields respectively, (Table 2.1). Weeds such as alfalfa (*Medicago sativa*), bittercress (*Cardamine parviflora*), broadleaf plantain (*Plantago rugelii*), chickweed (*Stellaria media*), field horsetail (*Equisteum spp*.), moth mullein (*Verbascum blattaria*), musk mallow (*Malva moschata*), purple poppy mallow (*Callirhoe involucrate*), prostrate knotweed (*Polygonum aviculare*), wild onion (*Allium ascalonicum*), and yellow spine thistle (*Cirsium ochrocentrum*) were observed once among the 48 sampled fields. However, the low abundance of the weed species does exclude them from being an alternative host of SCN.

2.3.2. SCN confirmation from the infected field

Cysts and eggs from each infected field were extracted from the representative 100 cc soil sample soil collected which confirmed the presence of SCN in the majority of the sampled fields. Only 3 out of 48 fields were not infected with SCN. SCN populations ranged from 700 to 100,000 per 100 cc soil sample suggesting highly variable SCN distribution in the infected fields in South Dakota (Table 2.2).

2.3.3. Juvenile extraction and identification

Among all the weed species, juveniles were obtained from the field pennycress and white clover in the majority of the fields (50% and 24% respectively). In addition, juveniles were obtained from cocklebur, leafy spurge and Canada thistle from more than five fields. Juveniles were also obtained from the other weed species such as Venice mallow, horseweed, small flower bittercress, shepherd's purse, purple poppy mallow, purple deadnettle and common mallow from few fields (Figure 2.1).

2.3.4. Greenhouse confirmation of weed hosts

Cyst development on weed hosts was assessed through the greenhouse confirmation experiment from the weeds which were found to be positive for the presence of juveniles from the field samples. Field pennycress and purple deadnettle were found to support considerable cyst development in the greenhouse with the female index were 29.03 and 36.31, respectively, and hence considered as strong hosts of SCN. Female indices were considerably lower for Canada thistle (FI=1.88), common mallow (FI=3.07), shepherd’s purse (FI=3.08), cocklebur (FI=1.15) and white clover (FI=1.15) which showed that these weeds hosts supported SCN development poorly and thus can be considered as the poor hosts of SCN in South Dakota. However, horseweed, Venice mallow, and leafy spurge did not support cyst development in the greenhouse while small flowered bittercress was not tested in the greenhouse due to unavailability of seeds.

2.4. Discussion

The results from this study suggest that there are few winter annual weed hosts species which support SCN cyst development in South Dakota. Although weeds such as field pennycress, purple deadnettle, common mallow, shepherd's purse, white clover, cocklebur and Canada thistle supported SCN cyst development in the greenhouse, there was variation in the number of cyst that developed for each of these weed species. Results from this research study also showed that field pennycress and purple deadnettle supported SCN development significantly in the field and the greenhouse suggesting these weed species to be the good hosts of SCN in South Dakota.

In this study, 38 commonly found weed species in the soybean fields in South Dakota were assessed as hosts of SCN in the field and the greenhouse. Out of these, only 12 weed species were found to harbor juveniles in their roots from the field samples. This is supported by the previous findings which have shown that the SCN presence in the field conditions is highly variable because of different environmental factors such as soil temperature, soil texture, soil pH, number of inoculum, seasons, weed density and abundance (Wallace, 1955; Slack and Hamblen, 1961; Clarke et al. 1978; Tefft et al. 1982; Hillel, 2004; Tefft et al. 1982; Mock et al. 2007). Additionally, most of the research studies conducted to determine the weed hosts were limited to the greenhouse as it is extremely difficult to control all the variables in field conditions. However, this study investigated weed hosts supporting SCN in the field conditions as well as the greenhouse conditions.

Greenhouse studies for determining alternative weed hosts species of SCN in different states showed varying results. In a research study conducted by Wong and Tylka (1994), cocklebur and Canada thistle were found to be the non-hosts of SCN in Iowa. Similarly, Venkatesh et al. (2000) determined shepherd's purse, field pennycress, henbit and purple deadnettle as the hosts of SCN in Ohio. Similarly, purple deadnettle was determined to be an alternative weed host of SCN in Nebraska (Werle et al. 2015; Werle, 2012) and in Indiana (Creech et al. 2007a). In a recent study, 31 weed species were determined to support SCN development out of which only henbit and field pennycress allowed substantial reproduction while similar SCN development was determined for shepherd's purse, Canada thistle, horseweed, Venice mallow, henbit, leafy spurge and field pennycress (Poromarto et al. 2015). In our study, common mallow was the weed species to support SCN development that has not been reported in other studies.

This study confirmed 7 weed species that supported SCN cyst growth in the greenhouse and the field conditions. This suggests that the weed species which support juveniles and cyst development fall in the host range as determined previously from the study by Riggs et al. (1987). Interestingly, all the weed species except purple deadnettle and field pennycress did not have cysts in all the replicates in the greenhouse. This indicates that some biotype may be present within a weed species which may not support cyst development while others are able to support cyst development (Poromarto et al. 2015). Weeds such as purple poppy mallow, horseweed, Venice mallow and leafy spurge failed to support cyst growth in the greenhouse conditions. This might be due to fact that these species only allow penetration and juvenile development but do not allow completion of

the cyst development which was also reported by Riggs (1987). Surprisingly, white clover was found with SCN juveniles from the field samples but never developed cysts under greenhouse conditions. This could indicate that this weed species may act as a trap crop.

Although most of the SCN management practices followed by the soybean growers in South Dakota are based on non-host crop rotation and host resistance, growers should also be aware of the weed hosts and their abundance to implement effective management practices. The greenhouse confirmation study suggests that field pennycress is an important SCN weed host from the SCN management point of view as it can support SCN reproduction. The abundance of field pennycress in the soybean growing fields in South Dakota further indicate its importance as abundant alternative SCN weed host. This research findings suggest that the weed hosts determined to be SCN hosts should be proactively managed as a part of effective SCN management strategies.

2.5 Acknowledgements

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Table 2.1. Distribution frequency (%) of different weed species sampled from previously soybean cyst nematode positive fields during fall 2016 and spring 2018. (Total number of fields = 48)

Weed species	**Scientific names**	**Frequency (%)**
Canada thistle	*Cirsium arvense*	69.4
Common lambsquarters	*Chenopodium album*	58.9
Field pennycress	*Thlaspi arvense*	56.8
Dandelion	*Taraxacum officinale*	50.5
White clover	*Trifolium repens*	46.3
Field bindweed	*Convolvulus arvensis*	44.2
Cocklebur	*Xanthium strumarium*	42.1
Kochia	*Kochia scoparia*	42.1
Horseweed	*Conyza Canadensis*	37.9
Rumex	*Rumex crispus*	37.9
Knotweed	*Polygonum aviculare*	35.8
Ragweed	*Ambrosia artemisifolia*	35.8
Milkweed	*Asclepias syriaca*	33.7
Shepherd’s purse	*Capsella bursa-pastoris*	31.6
Flixweed	*Descurainia sophia*	29.5
Velvetleaf	*Abutilon theophrasti*	27.4
Giant ragweed	*Ambrosia trifida*	25.2
Waterpod	*Hydrolea quadrivalvis*	25.2

Wild lettuce	*Lactuca virosa*	25.2
Catnip	*Nepeta cataria*	23.1
Leafy spurge	*Euphorbia esula*	23.1
Water hemp	*Amaranthus tuberculatus*	23.1
Common mallow	*Malva neglecta*	21.0
Marijuana	*Cannabis indica*	21.0
Purslane speedwell	*Veronica peregrina*	21.0
Silvery cinquefoil	*Potentilla argentea*	18.9
Alsike Clover	*Trifolium hybridum*	16.8
Russian thistle	*Salsola spp.*	16.8
Catchweed	*Galium aparine*	16.8
Buffalobur	*Solanum rostratum*	14.7
Stinging nettle	*Urtica dioica*	14.7
Burdock	*Arctium lappa*	14.7
Common groundsel	*Senecio vulgaris*	12.6
Spiny thistle	*Sonchus asper*	12.6
Venice mallow	*Hibiscus trionum*	12.6
Wild rose	*Rosa acicularis*	12.6
Black mustard	*Brassica nigra*	10.5
Motherwort	*Leonurus cardiaca*	10.5
Wild mustard	*Sinapis arvensis*	10.5
Pigweed	*Amaranthus retroflexus*	8.4
Blue violet	*Viola sororia*	8.4

Indian mustard	*Brassica juncea*	8.4
Red clover	*Trifolium pratense*	8.4
Smartweed	*Polygonum pensylvanicum*	8.4
Wormweed	*Artemisia absinthium*	8.4
Pinneaple weed	*Matricaria discoidea*	6.3
Plantain	*Plantago major*	6.3
Wild garlic	*Allium vinale*	6.3
Hedge bindweed	*Calystegia sepium*	4.2
Horsetail	*Equisetum arvense*	4.2
Woolyleaf bursage	*Ambrosia eriocentra*	4.2
Purple deadnettle	*Lamium purpureum*	2.1
Alfalfa	*Medicago sativa*	2.1
Bittercress	*Cardamine parviflora*	2.1
Blackseed plantain	*Plantago rugelii*	2.1
Chickweed	*Stellaria media*	2.1
Field horsetail	*Equisetum spp.*	2.1
Moth mullein	*Verbascum blattaria*	2.1
Musk mallow	*Malva moschata*	2.1
Purple poppy mallow	*Callirhoe involucrata*	2.1
Prostrate knotweed	*Polygonum aviculare*	2.1
Wild onion	*Allium ascalonicum*	2.1
Yellowspine thistle	*Cirsium ochrocentrum*	2.1

Table 2.2. Soybean cyst nematode (SCN) population density (expressed in eggs per 100 cc of soil samples) obtained from the soil samples sampled counties previously confirmed to have SCN in South Dakota in fall 2016 and spring 2017

Field Sampled	**County**	**SCN eggs/ 100 cc soil**
1	Turner	8500
2	Turner	5900
3	Turner	100000
4	Turner	4600
5	Lincoln	7000
6	Lincoln	2900
7	Brookings	2600
8	Brookings	2400
9	Brookings	2200
10	Brookings	2500
11	Brookings	2500
12	Brookings	2200
13	Turner	2600
14	Turner	1200
15	Lincoln	3400
16	Lincoln	2900
17	Clay	1500

18	Clay	1900
19	Clay	1900
20	Turner	2700
21	Deuel	1600
22	Hamlin	1400
23	Brookings	2500
24	Hamlin	1600
25	Turner	2900
26	Bon Homme	3300
27	Bon Homme	-
28	Bon Homme	2500
29	Bon Homme	1800
30	Bon Homme	1900
31	Hutchinson	900
32	Hutchinson	1600
33	Hutchinson	2700
34	Hanson	1200
35	Hanson	2300
36	Hanson	1500
37	McCook	2600
38	Minnehaha	1800
39	Minnehaha	-
40	Roberts	-

41	Roberts	1500
42	Roberts	2500
43	Grant	700
44	Turner	4000
45	Turner	6700
46	Brookings	11500
47	Brookings	3500
48	Brookings	5800

Table 2.3. Average number of soybean cyst nematode cysts formed on weed roots after inoculation with SCN eggs and the respective female index for weed samples in the greenhouse SCN host confirmation experiment

Weed Species	**Number of fields found**	**Average number of cysts**	**Female Index (%)**
Field Pennycress	27/48	9	29.03
Cocklebur	20/48	0.375	1.15
Common Mallow	10/48	0.875	3.07
Canada Thistle	33/48	0.5	1.88
Purple Deadnettle	1/48	10.71	36.31
Shepherd’s Purse	15/48	1.25	3.08
White Clover	22/48	0.375	1.15

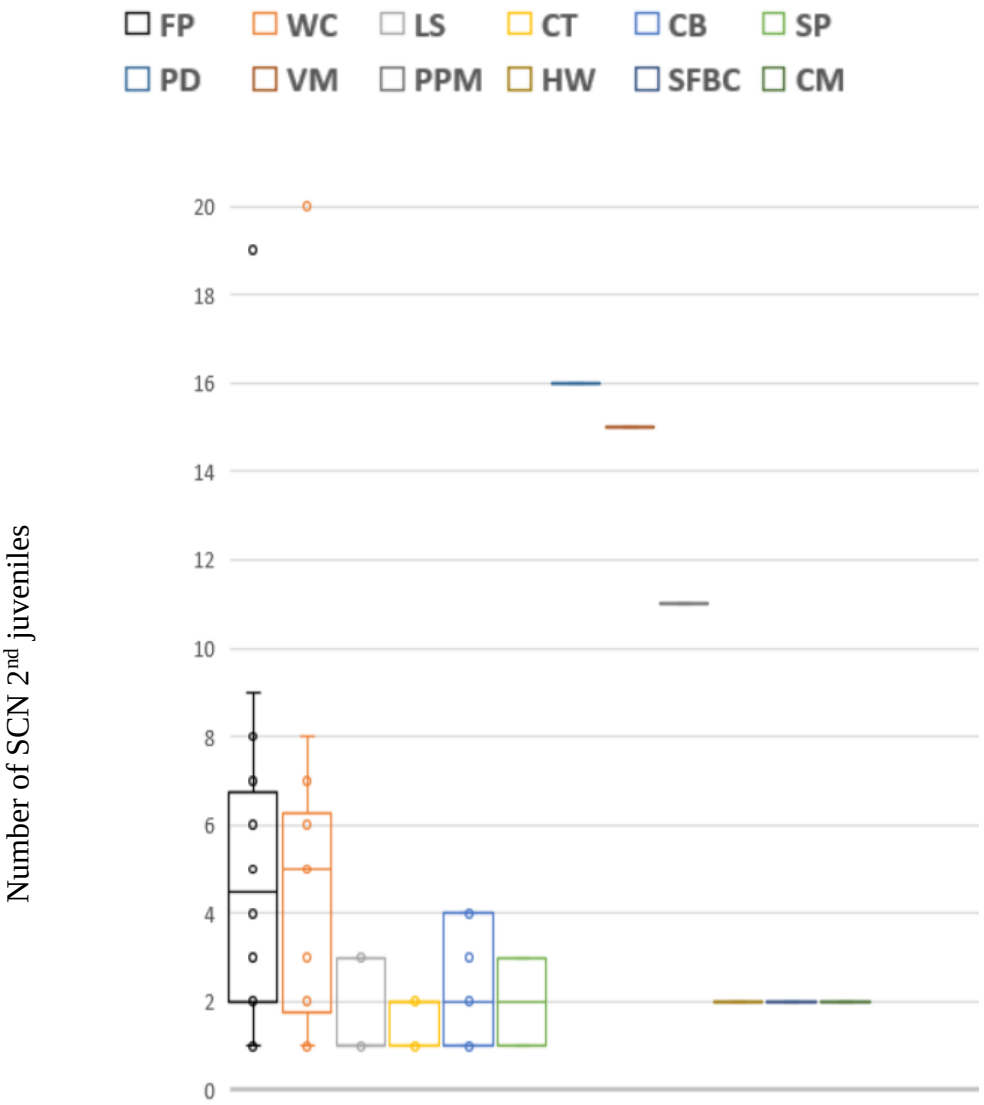

Figure 2.1. Number of juveniles obtained from the roots of each weed species after maceration. Weeds were sampled from the previously SCN positive soybean fields

FP: Field pennycress; CM: Common mallow; CB: Cocklebur; PPM: Purple poppy mallow; PD: Purple deadnettle; CT: Canada thistle; VM: Venice mallow; HW: Horseweed; SFBC: Small-flowered bittercress; LS: Leafy spurge; WC: White clover; SP: Shepherd's purse

CHAPTER 3

3. Reproduction of *Heterodera glycines* Types on Field Pennycress, Henbit and Purple Deadnettle in South Dakota

Abstract

The soybean cyst nematode (SCN; *Heterodera glycines*, Ichinohe) is an important pathogen of soybean in South Dakota causing significant yield losses. SCN has been found in 30 South Dakota counties as on 2017 and is estimated to cause yield loss of 1.9 Metric tons (Mt) annually. The presence of *Heterodera glycines* (HG) types can limit the performance of SCN-resistant cultivars, moreover, HG types reproduction on weed hosts can negate the effectiveness of crop rotation by a continued build-up in the field. This study was conducted to determine the reproduction of three commonly found HG types 0, 2.5.7, and 7 on three major weed hosts of SCN: purple deadnettle, field pennycress, and henbit compared to a susceptible check, Williams-82, under greenhouse conditions. Two ml suspension of eggs and juveniles having egg density of approximately 2000 eggs per ml of each HG type were inoculated to each weed species and the plants were kept in a water-bath at 27-28 °C. SCN reproduction was found to be influenced by the type of weed species, HG types and their interaction. SCN reproduction was highest for HG type 2.5.7 (FI = 6.4) followed by HG type 7 (FI = 6.1) and HG type 0 (FI = 5.9). Similarly, among weed species, henbit (*Lamium amplexicaule*) was found to harbor highest SCN cysts (FI = 7.1) followed by purple deadnettle (*Laminum purpureum*) (FI = 6.9) and field pennycress (*Thalpsi arvense*) (FI = 4.8). The number of cysts that developed on purple deadnettle and henbit was statistically similar whereas the field pennycress had relatively

lower cyst numbers. These results indicate that all the three weeds differentially support HG types development in South Dakota and hence these weeds should be managed proactively as an important component of SCN management strategies.

Keywords: *Heterodera glycines*, HG types, SCN, winter annual weeds, SCN weed hosts, female index, management, reproduction

3.1. Introduction

Soybean [*Glycine max* (L.) Merr.] is second most important crop in terms of acreage and production in the United States (NASS ERS, 2017). In South Dakota, soybean is grown in over 2.1 million hectares producing 6.9 million metric tons and fetching over $2 billion (USDA NASS, 2017). Several biotic and abiotic constraints affect soybean production in the United States (Hartman et al. 2011; Nguyen et al. 2016). However, among all the biological constraints, soybean cyst nematode (SCN; *Heterodera glycines*, Ichinohe) is the most important yield-limiting factor (Niblack, 2005; Wrather and Koenning, 2006; Wrather, 2009). Soybean cyst nematode has been reported in 30 main soybean producing counties in South Dakota (Acharya, 2015) causing yield loss estimated at 1.9 Metric tons (Mt) annually (www.sdsoybean.org).

Soybean cyst nematode is an obligate and sedentary endoparasitic cyst forming nematode which causes chlorosis, stunting, premature defoliation, root damage and generally a yield reduction of 10-20 % (Niblack et al. 2006; Winter et al. 2006). SCN occurs throughout the soybean production areas of the United States (Niblack, 2005; Wrather and Koening, 2006, Tylka and Marret, 2014, Wrather et al. 2010) except West Virginia. Average yield loss of around $1.2 billion is estimated annually in the United States (Koenning and Wrather, 2009) which makes it the most devastating pathogen of soybean.

Resistant soybean cultivars and crop rotation with non-host crops are the commonly practiced measures for SCN management (Hartman and Sinclair, 1999; Niblack, 2005; Faghihi and Ferris, 2006; Mitchum, 2016). Crop rotation with the non-host crops has

been found to reduce SCN population by 55% (Faghihi, 2012). However, weed hosts can negate the effects of crop rotation (Poromarto et al. 2015). Winter annual weeds play an important role in the biology of the plant parasitic nematodes (Norton, 1978) as they can serve as alternative hosts of SCN facilitating them to continue their life cycle even in the absence of the major host, soybean (Duncan and Noling, 1998; Thomas et al. 2005).

Although several research studies have reported that winter annual weed species may be alternative weed hosts of soybean cyst nematode, the most commonly known weed species determined to be alternative weed hosts of SCN are purple deadnettle (*Lamium purpureum L.*) (Riggs and Hamblen, 1962; Creech et al. 2005; Creech and Johnson, 2006; Werle et al. 2015; Poromarto et al. 2015), henbit (*Lamium amplexicaule L.*) (Epps et al. 1958; Riggs et al. 1962; Creech and Johnson, 2006; Creech et al. 2007b; Werle et al. 2015; Poromarto et al. 2015) and field pennycress (*Thalpsi arvense* L.) (Venkatesh et al. 2000; Creech et al. 2005; Creech et al. 2007a; Poromarto et al. 2015).

The HG type of SCN population is an important factor that indicates the level of reproduction on a given SCN resistant soybean. However, there is a paucity of information regarding the reproduction of SCN population (HG) types on common weed hosts. Previous studies only tested the reproduction of race 3 or HG type 0 (Venkatesh et al. 2000; Poromarto et al. 2015) which does not take into account the diversity of SCN population types present in the field. Different states with a long history of use of resistant cultivars like Iowa and Missouri have been shown to possess greater diversity in virulence of SCN populations linked to selection pressure (Niblack et al. 2002; Niblack et

al. 2008). Thus, it is important to determine the reproduction of HG types on major alternative weed hosts found in the region to understand and devise effective management strategies for the SCN type.

The objective of this study was to determine the reproduction of three prevalent HG types of SCN in South Dakota, HG type 0, 2.5.7, and 7 on three major weed hosts: purple deadnettle, field pennycress and henbit under greenhouse conditions. This information would elucidate the roles of alternative weed hosts in influencing HG type diversity.

3.2. Materials and methods

3.2.1. Source of inoculum

Three HG types prevalent in South Dakota, i.e. 0, 2.5.7 and 7 (Acharya, 2015), were used in this study. The HG types were obtained from soybean fields in South Dakota and were increased in the greenhouse by inoculating susceptible cultivar Williams 82. Cysts from roots of Williams 82 were processed following the SCN eggs extraction procedure by Faghihi and Ferris (2000).

3.2.2. Weed source

Seeds of field pennycress were collected from different locations in South Dakota and were bulked and stored at 4°C until planting. Henbit seeds were obtained from collections by Dr. Rodrigo Werle from the University of Nebraska Lincoln and the purple

deadnettle seeds were obtained from the field collection of Dr. Bruce A. Ackley from The Ohio State University.

3.2.3. Experiment set up

Pre-germinated seeds of each weed species and a susceptible cultivar Williams 82 were transplanted into individual cone-tainer (3.8 cm diameter and 21 cm height, Stuewe and Sons Inc., Tangent OR) filled with sterilized soil mixture (2 parts of sand and 1 part of soil by volume). A replicate (one bucket) contained each of three weed species (single plant in a cone) and the susceptible soybean check treated inoculated with each of the three HG types placed in 18.9-litre bucket filled with sand. SCN inoculum was prepared from the three HG types cysts maintained on susceptible soybean cultivar in the greenhouse. Cysts of the three HG types were individually crushed to a solution containing approximately 2000 eggs per ml. The cone-tainers were inoculated with 2 ml of the egg solution 3 cm below the soil surface. All the treatments were arranged in a completely randomized design with 6 replications and the experiments were repeated once. The buckets were then placed in a water bath that was maintained at the temperature of 27-28 °C and daylight length of 16 hours. After 35 days, the plants in cone-tainers were taken out from the bucket, soaked in water for 20 min and the plants were gently uprooted and washed to collect cysts. Cysts were collected in 210 µM pore sized sieve nested under 710 µM pore sized sprayed with a strong stream of water to dislodge them from the roots. The number of cysts on weed species and the susceptible check Williams-82 was to calculate the female index (FI) using the formula:

$$\text{Female Index (FI)} = \frac{\text{Average number of cysts found on the Weed species}}{\text{Average number of cysts found on the susceptible check}} * 100$$

Six cysts from each replication of individual treatment were randomly selected using a 2ml plastic transfer pipette which were placed on a glass slide in a drop of water and burst with the help of a teasing needle. Eggs from each cyst were counted using a hemocytometer under the inverted microscope.

3.2.4. Data analysis

Number of cysts that developed on the root, female index, number of cysts per gram root weight and number of eggs per cyst were analyzed with R studio version 3.4.3 (The R Foundation for Statistical Computing Platform). An average number of cysts and the female index were determined from 6 replications and a repetition pooled after Bartlett's homogeneity test. Analysis of variance was used to test interactions and the main effect of the HG types on the weed species. Duncan's multiple range test for multiple pairwise comparisons was used to separate means at $P \leq 0.05$ using R package "Agricolae" through R studio version 3.4.3.

3.3. Results

3.3.1. SCN Reproduction

The total number of cysts formed varied among the three weeds species and across the HG types (Table 3.1, 3.2, 3.3). Among HG types, HG type 0 2.5.7 had the highest reproduction (Cysts = 11.8) whereas HG type 0 had lowest reproduction (Cysts = 9.7). HG type 7 had statistically similar cyst formation (Cysts = 9.8) as that of HG type 0.

Among weed species, henbit supported the highest reproduction (Cysts = 11.8) followed by purple deadnettle (Cysts = 11.4) across all the HG types whereas field pennycress (Cysts = 9.6) were found to support statistically similar cyst reproduction across all the HG types (Table 3.2). All three HG types reproduced statistically higher on henbit and purple deadnettle, and lower on field pennycress. Interaction effect was significant between HG types and weed species where cyst reproduction was observed to be highest for HG type 2.5.7 followed by 7 and 0. Among weed species, henbit was observed to have higher cyst number followed by purple deadnettle and field pennycress. However, in purple deadnettle, HG 0 had numerically slightly higher reproduction on than the other HG types.

3.3.2 Female Index

The female index for all HG types on field pennycress, henbit and purple deadnettle varied significantly. HG type 2.5.7 had the highest female index (FI = 6.4) on three weed species. HG type 7 (FI = 6.1) and HG type 0 (FI = 5.9) had statistically similar female index on all the three weed species (Table 3.5).

Among the weed species, henbit had the highest female index (FI = 7.1) which insinuates that it supports highest cyst reproduction when inoculated with all the HG types. Meanwhile, purple deadnettle (FI = 6.9) and field pennycress (FI = 4.8) had statistically lower female index than henbit (Table 3.4).

3.3.3. Cysts per gram root weight

Total number of cyst per gram root weight were determined from cysts formed on weed species per unit root weight. HG types and the weed species had significant impact on the cyst per gram root weight which varied across all the treatments. HG type 2.5.7 had the highest cyst per gram root weight (cyst/gram root weight = 21.7). HG type 7 (cyst/gram root weight = 16.5) and HG type 0 (cyst/gram root weight = 18.3) (Table 3.6).

Similarly, among weed species, henbit (cyst/gram root weight = 23.6) had highest cyst per gram root weight followed by purple deadnettle (cyst/gram root weight = 20.4) and field pennycress (cyst/gram root weight = 12.5) which indicates that henbit and purple deadnettle had ability to support more cyst per gram root weight than field pennycress (Table 3.7).

3.3.4. Eggs count per cyst

The number of eggs in cysts on all the three weeds species varied significantly vary among HG types. An average number of eggs contained in cyst among different treatments ranged from 294 to 397 (Table 3.8). Meanwhile, eggs count per cyst was found to be statistically similar on each weed species for all the HG types.

3.4. Discussion

The knowledge of reproduction of HG types on weed hosts is important to determine whether a weed host significantly supports certain HG types in a particular region. Furthermore, it can also help to determine whether a weed species can cause a selection

pressure in SCN over a particular region (Poromarto et al. 2015). This knowledge can motivate the soybean growers for the proper management of particular weed species that facilitate continuous development of SCN inoculum in the field. The results suggested that all the three weeds studied supported cyst development.

The number of cysts on the field pennycress, henbit, and purple deadnettle followed a similar pattern as the susceptible check cultivar Williams-82 where HG type 2.5.7 had significant reproduction than HG type 0 and 7. In addition, HG type 0 had less SCN reproduction in both susceptible soybean check and weed species which might be attributed to HG type 0 being less aggressive than HG type 2.5.7 and 7. Cyst development on weeds also revealed that HG type 7 is less aggressive than HG type 2.5.7 for the three weed species tested. The results also suggested that the purple deadnettle and henbit had a similar tendency to support SCN growth whereas field pennycress was had a lesser number of cysts when compared to the purple deadnettle and henbit. This might be due to the fact that purple deadnettle and henbit belongs to the same family Lamiaceae (mint family) whereas field pennycress belongs to the family Brassicaceae (mustard family). However, all the three weed species were determined to be good hosts of SCN in South Dakota.

The results from HG type reproduction on weeds are consistent with the research studies conducted by Venkatesh et al. (2000) and Poromarto et al. (2015). Cyst formation in field pennycress, henbit, and purple deadnettle were 73, 155 and 510 respectively for race 3 in a research study conducted by Venkatesh et al. (2000). Similarly, in another research

study conducted by Poromarto et al. (2015) for HG type 0, the female index for field pennycress and henbit were 34 to 42 and 15.5 to 45.5 respectively. The results from both these research reports are comparable with the findings from our findings. Our study also showed variation in cyst formations in the treatments among the weed species but the variations were not significantly different for each species treated with a particular HG type. The difference with the Poromarto et al. (2015) study may be due to the use of weed species from multiple collections. However, this research addressed the effect of three prominent HG type of SCN in South Dakota on three important major weed hosts.

Although all the HG types varied in the number of SCN reproduction on the weed species tested, it is also important to note that the abundance of the weed species in a particular field varied in South Dakota (Chapter 2). Since the research was conducted in the greenhouse in controlled conditions, there might be some variation in the results under the field condition. Previous research studies had also shown that the environmental factors play an important role in the development of SCN on the winter annual weeds. SCN can hatch, penetrate roots and develop into mature cyst over a wide range of temperatures and the rate of SCN growth and development is highly temperature dependent (Creech et al. 2007a). Similarly, SCN reproduction is higher in the autumn than in the spring periods which may be attributed to favorable environmental conditions suitable for the SCN.

Use of a resistant cultivar with the same source of resistance continuously may limit some SCN HG type reproduction on such cultivars. However, SCN HG types having the

ability to reproduce differentially on the alternative weed hosts in the field can be the source of inoculum for the next cropping season. Additionally, SCN capable of reproducing on the alternative weed hosts may influence the HG type development through exhibiting the selection pressure favoring particular SCN HG type reproduction along with possibilities of development of new HG types in a long run. This is supported by the fact that many major soybean producing states had witnessed the changes in the SCN HG types. The reason is not well known but weed hosts might be a crucial factor. Findings from our other research study conducted to determine the weed hosts abundance in the field showed that field pennycress is found in more than 50% of the fields sampled in soybean growing regions of South Dakota whereas purple deadnettle was found only in 4% of the sampled fields (Chapter 2). This shows that field pennycress is the most important weed host prominent in the soybean growing regions of the South Dakota which is crucial from SCN management point of view. Although HG types reproduction varied on field pennycress, it is important to note that the female index was significantly higher for all HG types which insinuate that irrespective of HG types of SCN, field pennycress should be managed early in the soybean fields in South Dakota for effective SCN management.

3.5. Acknowledgments

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Table 3.1. Effect of weed species on reproduction of *Heterodera glycines* across the three HG type 0, 2.5.7 and 7 under greenhuse conditons.

Weed species	**Mean cysts [a]**
Henbit	11.8 a
Purple deadnettle	11.4 a
Field Pennycress	8.0 b

[a] Data were transformed using square root transformation before being subjected to analysis of variance (ANOVA). The values are pooled mean from 6 replications and two repetitions after homogeneity test using Bartlett's homogeneity test. Values followed by the same letter are not significantly different according to least significant difference test at $P \leq 0.05$.

Table 3.2. Reproduction of HG types 0, 2.5.7 and 7 across three weed species under greenhouse conditions

HG types	Mean cysts [b]
2.5.7	11.8 a
7	9.8 b
0	9.7b

[b] Data were transformed using square root transformation before being subjected to analysis of variance (ANOVA). The values are pooled mean from 6 replications and across three weed species (purple deadnettle, henbit and field pennycress) and two repetitions after homogeneity test using Bartlett's homogeneity test. Values followed by the same letter are not significantly different according to least significant difference test at $P \leq 0.05$.

Table 3.3. Effect of weed species and HG types on reproduction of *Heterodera glycines* under greenhouse conditions

Weed species	**HG types**	**Mean cysts [c]**
Henbit	0	10.4 cd
	2.5.7	13.2 a
	7	11.9 abc
Purple deadnettle	0	11.6 bcd
	2.5.7	12.2 ab
	7	10.5 cd
Field Pennycress	0	6.9 e
	2.5.7	10.1 d
	7	6.9 e

[c] Data were transformed using square root transformation before being subjected to analysis of variance (ANOVA). The values are pooled mean from 6 replications and 2 repetitions after homogeneity test using Bartlett's homogeneity test. Values followed by the same letter are not significantly different according to least significant difference test at $P \leq 0.05$.

Table 3.4. Effect of weed species on the female index of field pennycress, henbit and purple deadnettle after being inoclulated with HG type 0, 2.5.7 and 7

Weed species	**Female index [d]**
Henbit	7.1 a
Purple deadnettle	6.9 a
Field pennycress	4.8 b

[d] Data were transformed using square root transformation before being subjected to analysis of variance (ANOVA). The values are pooled mean from 6 replications and 2 repetitions after homogeneity test using Bartlett's homogeneity test. Values followed by the same letter are not significantly different according to least significant difference test at $P \leq 0.05$.

Table 3.5. Effect of HG types of *Heterodera glycines* on the femalee index across field pennycress, henbit and purple deadnettle

HG types	**Female index [e]**
2.5.7	6.4 a
7	6.1 b
0	5.9 b

[e] Data were transformed using square root transformation before being subjected to analysis of variance (ANOVA). The values are pooled mean from 6 replications and 2 repetitions after homogeneity test using Bartlett's homogeneity test. Values followed by the same letter are not significantly different according to least significant difference test at $P \leq 0.05$.

Table 3.6. Effect of HG types on cyst per gram root weight of field pennycress, henbit and purple deadnettle after being inoclulated with HG type 0, 2.5.7 and 7

HG types	**Cyst per gram root weight [f]**
2.5.7	21.7 a
7	16.5 b
0	18.3 b

[f] Data were transformed using square root transformation before being subjected to analysis of variance (ANOVA). The values are pooled mean from 6 replications and 2 repetitions after homogeneity test using Bartlett's homogeneity test. Values followed by the same letter are not significantly different according to least significant difference test at $P \leq 0.05$.

Table 3.7. Effect of weed species on cyst per gram root weight of henbit, field pennycress and purple deadnettle after being inoclulated with HG type 0, 2.5.7 and 7

Weed species	**Cyst per gram root weight [g]**
Henbit	23.6 a
Purple deadnettle	20.4 b
Field pennycress	12.5 c

[g] Data were transformed using square root transformation before being subjected to analysis of variance (ANOVA). The values are pooled mean from 6 replications and 2 repetitions after homogeneity test using Bartlett's homogeneity test. Values followed by the same letter are not significantly different according to least significant difference test at $P \leq 0.05$.

Table 3.8. Number of eggs per cyst on field pennycress, henbit, purple deadnettle and susceptible check soybean cultivar after being inoculated with soybean cyst nematode HG types 0, 2.5.7 and 7

HG types	**Eggs count per cyst [h]**			
	Soybean check	**Purple Deadnettle**	**Henbit**	**Field Pennycress**
0	386.3 a	369.2 ab	394.0 a	293.8 b
2.5.7	361.3 ab	385.7 a	390.0 a	330.8 ab
7	339.5 ab	317.2 ab	396.7 a	309.5 ab

[h] Values followed by a similar letter are not significantly different according to the least significant difference P ≤0.05. The values are the mean of 6 replications

CHAPTER 4

4. Effect of Flooding on the Reproduction of Soybean Cyst Nematode on Soybean

Abstract

The soybean cyst nematode (SCN; *Heterodera glycines*, Ichinohe) is an important soil-borne pathogen whose survival and infection process is affected by several biotic and abiotic factors. Among abiotic factors, soil moisture is one of the crucial factors for SCN juvenile movement in the soil hence the infection process. Soil moisture may also affect the effectiveness of nematicide seed treatments and physiology of the plant. This study was conducted to determine the effects of 2, 4, 6, and 8 days of flooding of soybean seedlings on SCN reproduction or both nematicide seed treated (ILeVo nematicide treated) and non-treated in the greenhouse. Non-flooded plants were the check. Each experimental unit consisted of 10 plants: 5 plants for susceptible treated and susceptible untreated which were inoculated with approximately 2000 SCN eggs and were arranged in randomized block design. After 5 days of inoculation, the plants were flooded for a period of 2, 4, 6, and 8 days which were later kept in a water-bath at 27-28 °C. The number of cysts after 35 days post-inoculation was counted, eggs were obtained by crushing cysts and dry root weight was taken from each treatment. Flooding days and seed treatment affected the number of SCN cysts on the susceptible cultivar. The highest number of cysts developed when plants were flooded for 2 days (Cyst = 36) followed by treatments flooded for 0, 4, and 6 days. The number of cyst was lowest for the plants flooded for 8 days (Cysts = 26). This study also indicated that ILeVO seed treatment

lowered SCN reproduction on a SCN susceptible soybean cultivar and promoted root development.

Keywords: SCN, *Heterodera glycines*, resistant, susceptible, flooding, ILeVO seed treatment, reproduction

4.1. Introduction

Soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe) is an important pathogen which continuously threatens soybean production all around the globe (Mitchum, 2016). Among the soybean yield-limiting factors, SCN is the most important constraint for the soybean production (Niblack, 2005; Wrather and Koenning, 2006). SCN is a soil-borne pathogen which is affected by several abiotic and biotic factors incumbent on soil status of a particular region. Abiotic factors such as soil temperature, soil pore size, soil aeration, water content, and pH have been documented to affect the SCN life cycle and infection process (Riggs and Wrather, 1992; Heatherly and Young, 1991). However, very little is known about the impact of these abiotic factors on SCN.

Soybean is the dominant oilseed which accounts for a significant share of 14% of the total harvested irrigated acres in the United States (USDA ERS, 2018). Upper Midwest contributes more than 80% of the US total soybean acreage where non-irrigated soybean production is comparably abundant than the irrigated ones. Though there is an increasing trend of soybean yield in US, soybean is still vulnerable to erratic yearly variations in weather conditions (NCA, 2014). Among all the weather parameters affecting soybean production and SCN activity, precipitation is the most important and fickle factor. The rise in the annual rainfall by 20% in some regions of the Midwest is proof that the region generally experiences the greatest precipitation and the heavy downpour is common (USGCRP, 2014) which might impact soybean growth as well as SCN infectivity.

Impact of excessive moisture on SCN infectivity and effectiveness of nematicide seed treatment is not well known. However, it was found that SCN population density was lowered in irrigated plots than in non-irrigated plots (Koenning and Barker, 1995) but it was unknown whether it was due to moisture content or oxygen content ratio. It was also found that the hatched juveniles of *Meloidogyne hapla* and *Heterodera schachtii* were unable to migrate without moisture in the soil (Couch and Bloom, 1960; Wallace, 1955). Furthermore, well-irrigated soil was found to maintain the juveniles of *Heterodera glycines* for a longer period of time although the excessive wet soil was found to hinder the cyst development (Heatherly et al. 1982). Nevertheless, water is crucial for the spread of SCN from the previously infested field to new fields (Faghihi et al. 2010). Drought stress facilitates soybean to increase root mass to absorb more water and nutrients and the increased root mass also favors SCN infection (Huck et al. 1986). Since the nematode requires aerobic respiration in its life cycle, soil oxygen level also plays an important role in SCN survival (Koenning and Barker, 1995). In addition, coarse-textured soil drains more easily than fine-textured which favors nematode activity by allowing aerobic conditions (Vrain, 1986). However, increasing irrigation was not found to reduce yield suppression by SCN (Heatherly et al. 1992). Nematodes affect plant roots and thus the plant's ability to uptake water and nutrients. Hence, yield loss due to nematodes is often higher when moisture is limited. In some cases, alleviating water stress with irrigation or other practices was found to reduce yield loss, although it did not reduce nematode populations (Windham, 1998).

Soybean is grown mostly in the Midwestern region of the United States which experiences erratic weather changes in terms of rainfall and drought conditions. Besides, water is an important factor impacting SCN activity in the field. Hence, it is very important to determine the impact of the flooding on the SCN infectivity. Thus, the objective of this research was to determine the effect of flooding on SCN reproduction in both untreated and nematicide seed-treated susceptible cultivar.

4.2. Materials and Methods

SCN susceptible soybean cultivar Williams-82was selected for the experiment. Seeds were treated with the standard dose of ILeVO, 0.125 mg ai/seed or left untreated. ILeVO seed treatment contains fluopyram as an active ingredient and is labelled as broad-spectrum fungicide and nematicide reported to reduce SCN population (Bayer Crop Science, 2018).

Pre-germinated seeds treated or not treated with ILeVO were transplanted into individual cone-tainer (3.8 cm diameter and 21 cm height, Stuewe and Sons Inc., Tangent OR) filled with sterilized soil mixture (2 parts of sand and 1 part of soil by volume). Each experimental unit consisted of 10 plants: 5 plants treated and untreated inoculated with 1ml of the egg solution 3 cm below the soil surface. The egg solution was obtained by crushing cysts from the maintained population of the SCN HG type 0 and standardized as 2000 eggs per ml. Five days after inoculation, all the plants except the day 0 plants were flooded with water in a plastic bucket. Individual plants were removed after 2, 4, 6 and 8 days of flooding. All plants were placed in a bucket filled with sand and placed in a water

bath which was maintained at the temperature of 27-28 °C and daylight length of 16 hours. All the treatments were arranged in a partially randomized block design with 6 replications and the experiments were repeated once.

After 35 days of the inoculation, the plants in cone-tainers were taken out from the bucket, soaked in water for 20 minutes, gently uprooted, washed to collect cysts and the roots were kept in blotting paper for 24 hours and weighted. Cysts were collected in 210 µM pore sized sieve nested under 710 µM pore sized sprayed with a strong stream of water to dislodge them from the roots and eggs were extracted from the cysts following the SCN eggs extraction procedure by Faghihi and Ferris (2000).

4.2.1. Data analysis

Data comprising the total number of cysts developed on the roots, number of eggs, and the dry root weight were analyzed with R studio version 3.4.3 (The R Foundation for Statistical Computing Platform). An average number of cysts and a total number of eggs were determined from 6 replications and a repetition. Analysis of variance was used to test interactions and the main effects. Duncan's multiple range test for multiple pairwise comparisons was used to separate means at $P \leq 0.05$ using R package "Agricolae".

4.3. Results

The total number of cysts formed varied among the untreated and treated susceptible cultivar as well with the flooding (Table 4.1, 4.2). The number of cysts developed in the susceptible cultivar was impacted significantly by both seed treatment and flooding

period (Table 4.1, 4.2) while the interaction of seed treatment and the flooding days was not significant (Table 4.1). The number of cysts that developed on the non-treated (cysts = 36.5) was significantly higher than that of seed treatment (cysts = 26.8) (Table 4.2). Days of flooding period also impacted the cyst development significantly. Maximum cyst development was observed on the plants subjected to 2 days of flooding (cysts = 35.7) (Table 4.2). Cyst development was statistically similar in the treatments subjected to flooding period of 4 days (cysts = 31.6) and 6 days (cysts = 29.9) and lowest on the treatments flooded for 8 days (cysts = 26.2) (Table 4.2).

The total number of eggs formed across the treatments were significantly impacted by seed treatment, days of flooding and their interactions across both the repetitions (Table 4.3 and Table 4.4). Total number of eggs formed on the non-treated plants were significantly higher than the seed treatment plants. The total number of eggs formed on the non-treated plants subjected to flooding period of 2, 4, 6 and 8 days were statistically similar. However, the unflooded plants had lower eggs number in both the repetitions (Table 4.3 and table 4.4). Contrastingly, the total number of eggs formed on the seed-treated showed that the total number of eggs were significantly higher on the unflooded plants in the first run which were statistically similar to the eggs in the rest of the flooding days (Table 4.3). In the second run of the experiment, total number of eggs formed was statistically similar for unflooded and the 8 days flooding period (Table 4.4).

Fresh root weight from each treatment varied across treated and the non-treated (check). Root weight for the non-treated plants was significantly lower while the root weight was

higher for the seed-treated plants across both the runs of the experiment (Table 4.5 and table 4.6).

4.4. Discussion

Results from this study suggest that the SCN development in susceptible soybean cultivar was affected by both ILeVO seed treatment and the flooding days. Seed treatment with ILeVO had statistically fewer cysts than the non-treated seed. This is similar to a research study conducted by Heatherly et al. 1992 where the seed treatment lowered cyst development.

The number of cysts in the susceptible soybean cultivar was influenced by different days of flooding. Number of cysts was statistically higher in the treatment subjected to 2 days of flooding and this was statistically similar to the unflooded treatment. The treatment subjected to 4 and 6 days of flooding showed similar cyst development. This finding is similar to where irrigation for soils having lower moisture level were found to have a positive effect on the nematode density while the effect becomes negative in presence of excess moisture (Vandegehuchte et al. 2015). In our study, the flooding for 2 days promoted SCN development while the flooding for 8 days had negative impact on the cyst number.

Similarly, the total number of eggs formation was significantly impacted by the seed treatment and the flooding days. Seed treatment was found to be effective in lowering the total number of eggs on treated susceptible cultivars as compared to the non-treated.

Contrastingly, on the treated susceptible cultivar, unflooded cultivars had higher eggs number. This indicated that flooding did not significantly impact total number of eggs on untreated susceptible cultivar however, seed treatments reduced the total number of eggs on the treated susceptible cultivars. Additionally, seed treatment had significant impact on the dry root weight. It indicates that ILeVO seed treatment impact root development on the susceptible cultivar. This finding contrasts with another research study conducted by Heatherly et al. 1992 where the irrigation did not significantly affect the yield among the treatments.

Nematode development depends on the water availability for their activities and their density was found to decrease in drought condition (Landesman et al. 2011, Stevnbak et al. 2012) and increased with water addition (Smolik and Dodd, 1983). However, it had also been found that the nematode population is less sensitive to short-term changes in the water availability (Stevnbak et al. 2012) while they were negatively correlated with the excess soil moisture for a longer period of time (Freckman et al. 1987). Increased precipitation was not found to increase nematode population, neither decreased precipitation reduced nematode population (Vandegehuchte et al. 2015).

Our research study was to determine the effect of flooding and the seed treatment on susceptible and resistant cultivar in the greenhouse. The results from this research study demonstrated that the ILeVO seed treatment significantly reduce cyst formation and also increase root biomass in flooded conditions. Further, cyst formation in susceptible soybean cultivar was higher on the unflooded and the treatment flooded for 2 days while

reduced on the treatments subjected for 8 days of flooding implying that short period flooding can increase cyst formation while long period gradually reduces the cyst development. In addition, flooding was not found to countermine the resistance of SCN resistant cultivar.

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Table 4.1. Total number of SCN cysts on seed treated and untreated susceptible cultivar after being subjected to flooding period of 0, 2, 4, 6 and 8 days

Treatments	**Cysts [a]**
Non-treated	36.55 a
Treated	26.81 b

[a] The values are pooled mean from 6 replications and 2 repetitions after homogeneity test using Bartlett's homogeneity test. Values followed by the same letter are not significantly different according to least significant difference test at $P \leq 0.05$.

Table 4.2. Cysts developed on susceptible cultivar after being subjected to 0, 2, 4, 6 and 8 days of flooding

Treatments (Days of flooding)	**Cysts [b]**
0	34.8 a
2	35.7 a
4	31.6 b
6	29.9 b
8	26.2 c

[b] The values are pooled mean from 6 replications and 2 repetitions after homogeneity test using Bartlett's homogeneity test. Values followed by the same letter are not significantly different according to least significant difference test at $P \leq 0.05$.

Table 4.3. Total number of eggs on seed-treated and non treated susceptible cultivars after being subjected to 0, 2, 4, 6 and 8 days of flooding

Cultivar * Days of flooding	**Total number of eggs [c]**
Non-treated: 0	14.7 b
Non-treated: 2	15.8 a
Non-treated: 4	15.5 a
Non-treated: 6	15.7 a
Non-treated: 8	13.0 d
Treated: 0	14.7 b
Treated: 2	13.7 c
Treated: 4	13.0 d
Treated: 6	12.7 de
Treated: 8	12.2 e

[c] Data were transformed using square root transformation before being subjected to analysis of variance (ANOVA). Values followed by similar letter are not significantly different according to least significant difference test at $P \leq 0.05$. The values are the mean of treatments from 6 replications from the first run of the experiment.

Table 4.4. Total number of eggs on seed-treated and non treated susceptible cultivar after being subjected to 0, 2, 4, 6 and 8 days of flooding on the repetition experiment

Cultivar * Days of flooding	**Total number of eggs [d]**
Non-treated: 0	15.4 bc
Non-treated: 2	16.1 a
Non-treated: 4	16.1 a
Non-treated: 6	15.7 ab
Non-treated: 8	15.5 ab
Treated: 0	14.8 d
Treated: 2	13.8 e
Treated: 4	13.9 e
Treated: 6	13.9 e
Treated: 8	14.9 cd

[d] Data were transformed using square root transformation before being subjected to analysis of variance (ANOVA). Values followed by similar letter are not significantly different according to least significant difference test at $P \leq 0.05$. The values are the mean of treatments from 6 replications from the second run of the experiment.

Table 4.5. Dry root weight of treated and untreated susceptible cultivar after being subjected to flooding period of 0, 2, 4, 6, and 8 days

Treatments	**Root weight (g)** [e]
Non-treated	1.04 b
Treated	1.11 a

[e] Data were transformed using square root transformation before being subjected to analysis of variance (ANOVA). Values followed by similar letter are not significantly different according to least significant difference test at $P \leq 0.05$. The values are the mean of treatments from 6 replications from the first run of the experiment

Table 4.6. Dry root weight of treated and untreated susceptible cultivar after being subjected to flooding period of 0, 2, 4, 6, and 8 days on the repetition experiment

Treatments	**Root weight (g)** [f]
Non-treated	1.08 b
Treated	1.11 a

[f] Data were transformed using square root transformation before being subjected to analysis of variance (ANOVA). Values followed by similar letter are not significantly different according to least significant difference test at $P \leq 0.05$. The values are the mean of treatments from 6 replications from the second run of the experiment.

General Conclusions

This thesis encompasses research studies on alternative weed hosts of SCN, reproduction of different HG types of SCN on three weed hosts: henbit, field pennycress, and purple deadnettle, and the effect of flooding on the reproduction of SCN for seed-treated and non-treated resistant and susceptible cultivars. All the research studies conducted aimed to improve the SCN management strategies in South Dakota.

SCN has diverse crop and weed hosts as it can reproduce in several crops and weeds. This study examined the alternative weed hosts of SCN in South Dakota based on field and greenhouse data. Field pennycress and purple deadnettle were determined from both field and greenhouse studies to be the good hosts of SCN in South Dakota whereas white clover, common mallow, shepherd's purse, Canada thistle, and cocklebur were determined to the poor hosts of SCN in South Dakota. All the weed species determined as hosts from this study were similar to the previous studies except common mallow, which was found to be weed host only in this study.

This research also determined the reproduction of SCN HG types on common weed hosts: purple deadnettle, field pennycress, and henbit relative to a susceptible check, Williams-82, under greenhouse conditions. Though all the three weed hosts supported SCN HG types differentially, purple deadnettle and henbit were found to have higher number of cysts developed whereas field pennycress was found to support lower cyst development. These results indicate that all three weeds considerably support SCN

development in South Dakota and hence these weeds should be managed proactively as an important component of SCN management strategies.

This thesis has also determined the effects of flooding on SCN resistant and susceptible cultivars with and without an ILeVO seed treatment. Water being a crucial factor for SCN survival and the infection process and nematicide seed treatment being effective and common for soybean growers in South Dakota, it was an important study from growers point of view regarding SCN development. Susceptible cultivar untreated and treated with ILeVO showed varying SCN development whereas the resistant cultivar did not show cyst development irrespective of ILeVO treatment and flooding. Though ILeVO treatment and flooding were found to reduce SCN development significantly, the practical significance was barely observed suggesting the need for further research in the field. Thus, this research study suggests that the integration of the various strategies such as weed management, crop rotation, seed treatment and the planting of resistant cultivars based on HG type of SCN are crucial for the effective management of SCN in South Dakota.

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