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Distribution and Characterization of the Soybean Cyst Nematode, Heterodera glycines (HG) Types in South Dakota

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DISTRIBUTION AND CHARACTERIZATION OF THE SOYBEAN CYST NEMATODE, *HETERODERA GLYCINES* (HG) TYPES IN SOUTH DAKOTA

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

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ABBREVIATIONS

^{0}C = degree Celcius

ANOVA = analysis of variance

b = billions

bu = bushel

cm = centimeter

df = degree of freedom

ft = feet

FI = female index

HG = *Heterodera glycines*

in = inches

LSD = least significant difference

m = meters

MG = maturity group

P = probability

spp = species

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ABSTRACT

DISTRIBUTION AND CHARACTERIZATION OF THE SOYBEAN CYST NEMATODE, *HETERODERA GLYCINES* (HG) TYPES IN SOUTH DAKOTA

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The soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is an endo-parasitic nematode and one of the major pests of soybean (*Glycine max* L.) in the United State and all over the world where soybean is grown. SCN is ranked first among the biological factors that cause yield loss in soybean. The estimated yield loss by this nematode is $1b annually in the United States alone. SCN is thought to have been first found in China. It was first identified in the United States in 1954 in North Carolina and in South Dakota in 1995. To date, SCN continues to spread in South Dakota having been detected in 29 counties. SCN is spread through soil movement when the soil is infested with SCN. The females (cyst) and also eggs remaining outside the cyst are the dispersal units of SCN. The second stage juveniles (J2) (worm-like, infecting stage) hatch from the eggs after stimulation from soybean roots, and infects the soybean roots. SCN not only attacks soybean, but also invades several other leguminous crops such as common bean (*Phaseolus vulgaris L.*), black bean (*Phaseolus vulgaris L.*), kidney bean (*Phaseolus vulgaris L.*), pinto (*Phaseolus vulgaris L.*) and navy (*Phaseolus vulgaris L.*) and forage legumes such as vetch (*Vicia sativa*), lespedeza (*Kummerowia sp.*), and lupine (*Lupinus perennis*). Several winter weeds like common chickweed (*Stellaria media*), henbit (*Lamium amplexicaule*), purple deadnettle (*Lamium purpureum*), small-flowered bitter

cress (*Cardamine hirsuta*), shepherd's-purse (*Capsella bursa-pastoris*), and field pennycress (*Thlaspi arvense*) are also hosts for SCN. The losses to soybean production caused by SCN vary mainly with the degree of infestation (population density), susceptibility of the cultivar grown, and other biotic and abiotic factors such as soil type and weather conditions. SCN can cause yield loss of up to 30 % without showing any visible symptoms in the plants and losses can go up to 75% in heavily infested fields. SCN completes its life cycle within 3 to 4 weeks depending upon the environmental factors mainly soil temperature. SCN population genetic diversity is the most challenging aspect for the management of this nematode. This study examined the distribution of *Heterodera glycines* and characterized HG types prevalent in South Dakota by monitoring the present status, population density, and determining HG types. This study also assessed the resistance of few of the available commercial soybean cultivars against prevalent HG types occur in South Dakota. A total of 250 soil samples were arbitrarily collected from different counties of South Dakota that had previously been found positive for SCN. Analysis of the soil samples showed a high prevalence of SCN infestation (32%) of the soil samples collected for this study and SCN population density varied from 250 to 62,500 per 100 cm^3 of soil. The extracted SCN populations were subjected to HG type determination under greenhouse conditions. A total of eight types of *Heterodera glycines* population were found in South Dakota. Among the eight HG types, HG types 0, 2.5.7, and 7, were most prevalent. Assessment of commercial cultivars for resistance showed varied reaction from susceptible to resistant. Most of the commercial soybean cultivars tested were resistant to HG type 0 and moderately resistant to HG type 0, 2.5.7, and 7 and only one cultivar showed susceptibility to all three populations tested in

greenhouses conditions. These results indicate that host resistance is effective in managing in SCN in South Dakota. However, with almost all SCN resistant cultivars having similar source of SCN resistance genes (PI 88788), and some populations of SCN having > 10% reproduction on PI 88788, there is a need to integrate host resistance with crop rotation, nematicide seed treatments, and rotation within resistant cultivars for sustainable SCN management in South Dakota.

CHAPTER 1

1. Literature Review

1.1. Soybean (*Glycine max*) history and production in the USA

Soybean [*Glycine max* L. (Merr.)] is one of the oldest cultivated crops in the world. It originated from the northern and central regions of China (Hymowitz 1970; Hymowitz and Newell 1981). Soybean was first introduced in the United States in 1765 by Samuel Bowen, a seaman in Georgia (Hymowitz and Harlan 1983) and has been grown all over the country for several purposes including oil, meal, animal feed, nutrient source, and others. In addition, soybean has been used for biodiesel production. About 1,750 million gallons of soybean biodiesel were produced in the United States in 2014 (ASA 2015). It is commonly grown as a rotational crop mainly with corn and wheat.

Currently, soybean is the second most grown crop in the United States following corn in terms of production and planted acres (Holcomb 2012). About 80 % of worlds total soybean production is grown by North and South Americas (Chang et al. 2015). In the year 2014, 34% of the world soybean was produced in the United States followed by 30% in Brazil, and 18% in Argentina. Out of the total 3,969 million bushels production in the United States, South Dakota alone produced about 230 million bushels (8%), indicating that this is an important crop in South Dakota (ASA 2015).

1.2. Constraints to soybean production

Several biotic and abiotic factors reduce soybean yield and seed quality. Among abiotic factors include low temperature, salt toxicity, water stress, and nutritional deficiencies. Biological factors that negatively impact soybean yield include weeds,

insect pests, and diseases (Hartman et al. 2011). The soybean cyst nematode (SCN) (*Heterodera glycines,* Ichinohe), Phytophthora root and stem rot (*Phytophthora sojae*), soybean rust (*Phakopsora pachyrhizi*), brown spot (*Septoria glycines*), charcoal rot (*Macrophomina phaseolina*), and a few others are the most common diseases that reduce soybean yield in the United States. Among all biological constraints, the soybean cyst nematode (SCN) is the most important factor in causing soybean yield loss in the United States (Koenning and Wrather 2010; Wrather and Koenning 2006 and 2009).

1.3. History and distribution of the soybean cyst nematode (*Heterodera glycines*)

Soybean cyst nematode (SCN), *Heterodera glycines,* Ichinohe is an obligatory, sedentary, endo-parasitic nematode (Niblack et al. 2006). It was first identified in northeast China in 1899 (Li et al. 2011b) and later in Japan in 1915, in Korea in 1936, in Manchuria in 1938, and in the United States in 1954 in North Carolina (Riggs 1977; Winstead et al. 1955). It is thought to have been introduced in the U.S. from China through the introduction of Rhizobia of soybeans in the first half of the 19th century (Noel 1986).

SCN is spread through several means including soil movement by machinery, wind, and soil attached to human and animal feet. SCN has now been detected in 90% of the soybean producing states in the U.S. (Tylka and Marett 2014) (Fig. 1.1). In South Dakota, SCN was first detected in1995 in Union County (Smolik and Draper 1998) and as of 2015, SCN has been found in 29 South Dakota counties (Fig. 1.2).

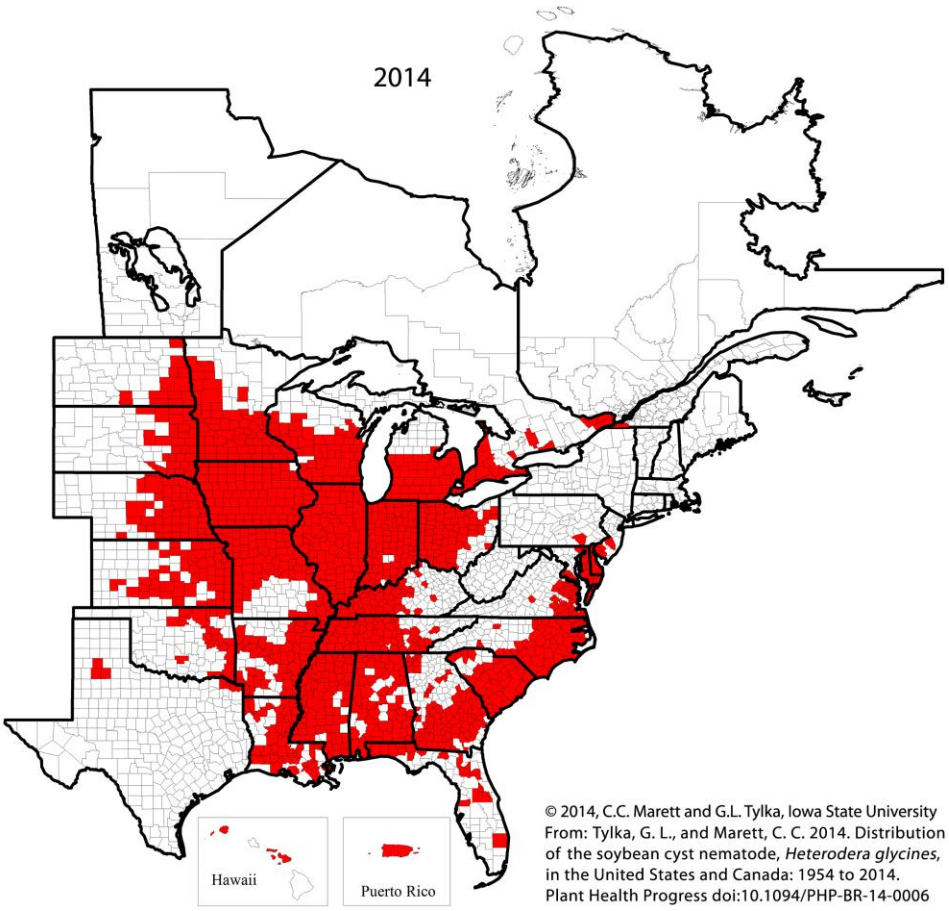

Fig. 1.1. Distribution of the soybean cyst nematode, *Heterodera glycines*, in the United States and Canada: 1954 to 2014 (Tylka and Marett 2014).

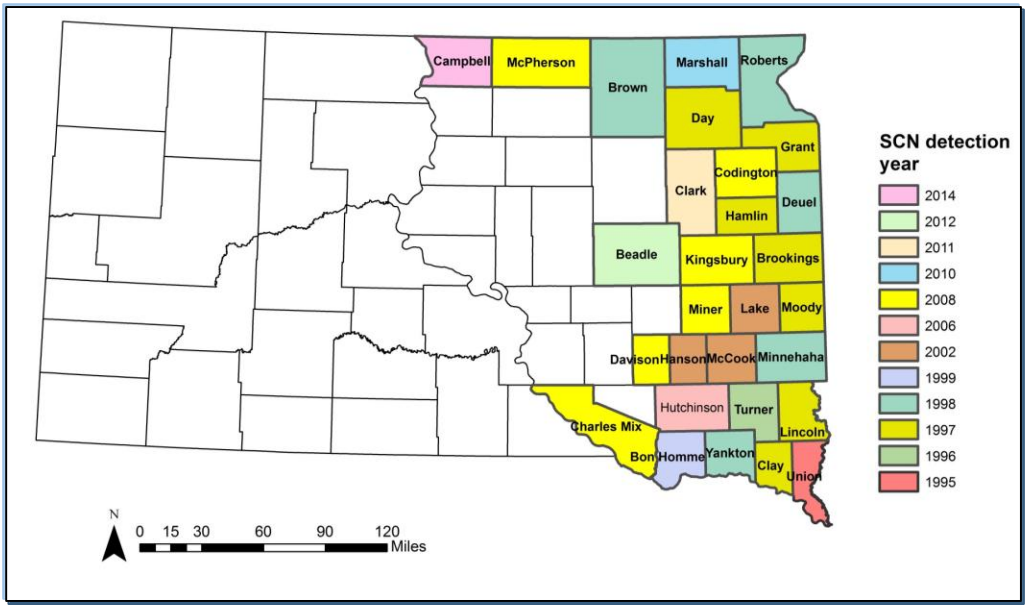

Fig. 1.2. Distribution of *H. glycines* in South Dakota counties and time periods in which the presence of the SCN was confirmed (Dr. Emmanuel Byamukama)

1.3.1. Host range

Besides soybean, SCN also infects several beans such as kidney (*Phaseolus vulgaris* L.), navy (*Phaseolus vulgaris* L.), pinto (*Phaseolus vulgaris* L.), and black beans (*Phaseolus vulgaris* L.) (Pormarto et al. 2011; Poromarto and Nelson 2009). SCN also infects a few winter weeds including common chickweed (*Stellaria media*), henbit (*Laminum amplexicaule*), purple deadnettle (*Laminum purpureum*), small-flowered bittercress (*Cardamine hirsuta*), shepherd's-purse (*Capsella bursa-pastoris*), and field pennycress (*Thlaspi arvense*) (Creech et al. 2007a; Creech et al. 2007b; Johnson et al. 2008; Venkatesh et al. 2009; Werle et al. 2015). A greenhouse study showed that some native legumes such as strawberry clover (*Trifolium fragiferum* L.), Canada tick clover (*Desmodium canadense* [L.] DC.) and hairy vetch (*Vicia villosa* Roth) and cultivated legumes such as string bean (*Phaseolus vulgais* L.), tendergreen bean (*Phaseolus vulgais*

L.), lima bean (*Phaseolus vulgais* L.) and little marvel pea (*Pisum sativum* L.) are also good hosts for *H. glycines* (Jones 1997).

1.3.2. Life cycle of Heterodera glycines

SCN has three main life stages, the egg, juvenile and the adult. The juvenile stage is further divided into four juvenile stages. The J2s (which is the infective stage of this nematode) hatch from the eggs, having already molted from J1 to J2 while in the eggs. (Niblack 2005). The J2 infects susceptible plants by penetrating roots. After penetration, the J2 moves towards the vascular system, especially to the cortex, endodermis or pericycle from where it induces the host cells to form permanent feeding site called syncytium. At this time, the juvenile becomes sedentary and changes into the third stage juveniles (J3) and it undergoes sex differentiation (Riggs and Wrather 1992). Most of the time, the ratio of female to male remained 1:1, but this ratio is influenced by the host resistance and other environmental conditions (Colgrove and Niblack 2005).

After sex differentiation, the J3 females continue feeding and molt to J4 stage and become adult females. The female starts to produce eggs and its body expands to give female cyst the characteristic lemon shape and bursts through the root epidermis. Some of the eggs are enclosed by the gelatinous matrix outside of the body. The males after J3 stage undergo metamorphosis and exit the roots after 10 to 15 days of infection (Triantaphyllou and Hirschmann 1962). The males are attracted to mate with females through female secretions. After fertilization, the males become free living in the soil and the female dies and its body develops into a hard structure (cyst) which is composed of some antimicrobial compounds like chitinase and polyphenoloxidase (Niblack et al. 2006). The encased cyst provides protection for the eggs and the eggs inside the cyst can

remain viable for a long time and if suitable moisture is available they can remain viable up to 9 years (Inagaki and Tsutsumi 1971; Melito et al. 2010; Niblack et al. 2006).

SCN takes about 3 to 4 weeks to complete its life cycle, but this is greatly influenced by temperature and other environmental factors, the optimum temperature ranges from 15 to 30^{o} C (Riggs and Wrather 1992). Under controlled environment with temperature of 25^{0} C, SCN can complete its life cycle within 21 days (Lauritis et al. 1983). SCN can complete up to 4 life cycles in a single soybean growing season in South Dakota, depending upon the maturity group of soybean planted. A female contains about 200-600 eggs in its life cycle (Niblack 2005; Schmitt et al. 2004).

1.3.3. SCN infection of soybean

The infection of soybean by *H. glycines* starts when the second juvenile stage is attracted to soybean roots through roots exudates. The J2 uses its strong stylet to probe into epidermal cells of fine young soybean roots. Once inside the epidermal cells, the J2 move to the vascular bundle. The probing of cortical cells results into physical and mechanical damage of root cells (Schmitt et al. 2004). The physiological damage on soybean plants starts with the syncytium formation (the nematode feeding site), as the compatible interaction takes place between the enzymes from host plant and nematode (Riggs and Wrather 1992).

In most cases, SCN infestation may go undetected due to lack of visual symptoms when the SCN population density is low and other factors are not stressing the plants (such as drought) (Wang et al. 2003; Young 1996), but at high SCN population density, soybean plants are stunted and yellow. Symptoms produced by the soybean cyst nematode can be misinterpreted for other stresses like drought stress, nutritional

deficiency such as iron, potassium, nitrogen, and attack by other pests and soil-borne pathogens (Niblack et al. 2006).

Initial SCN population density affects SCN reproduction throughout the growing season, for example, low initial population favors rapid SCN reproduction than high initial population since low populations produce higher number of SCN populations at the end of soybean growing season (Alston and Schmitt 1987).

1.3.4. Interaction of SCN with other diseases and insects

Soybean crop is attacked by several pest and pathogens throughout its growing season and these may interact among each other. Like other parasites, *H. glycines* interacts differently with different pests and pathogens while infecting a soybean plant. For instance, when soybean plants were infected with *H. glycines* and *Fusarium solani f. sp. glycines*, both reduced the growth of soybean plants and high inoculum of *F. solani f. sp. glycines* suppressed the reproduction of *H. glycines*. However, population density of *H. glycines* did not affect the colonization of the roots by *F. solani* (Gao et al. 2006). *H. glycines* infection along with abiotic stress support the infection of *Phytophthora sojae* causal agent of Phytophthora root and stem rot disease of soybean (Kaitany et al. 2000). Infection by *H. glycines* in soybean plant can increase the severity of the brown stem rot of soybean caused by *Cadophora gregata* (*Phialophora gregata*): a greenhouse study showed the increment in colonization of the stem by *C. gregata* with increases in *H. glycines* population density (Tabor et al. 2006).

Feeding by the soybean aphid (*Aphis glycines* Matsumura) on soybean plants supports *H. glycines* attack, but at a higher population density of the aphids, the number of nematodes decreases due to the decrease in resource quantity of host plants

(McCarville et al. 2014). Moisture stress produced by disruption of vascular bundles in tandem with the infection of the *H. glycines* provides the plant stress suitable for the infection and colonization of soybean roots by *Macrophomina phaseolina,* causal agent of the fungal disease charcoal rot of soybean (Todd et al. 1987). *H. glycines* also interact with *Diaporthe phaseolorum* var. *caulivora,* causative agent of stem canker disease of soybean and *Pseudoplusia includens,* soybean looper, a lepidopterous defoliator of soybean plants. A greenhouse study showed the antagonistic effect of *D. phaseolorum* on *H. glycines* population, while soybean looper had the additive effect on *H. glycines* population in both soybean roots and soil (Russin et al. 1989).

Some research studies reported that *H. glycines* eggs and juveniles can play as a vector for the viruses. Four different types of negative sense RNA virus distantly related to the groups such as nyaviruses and bornaviruses, rhabdoviruses, bunyaviruses and tenuiviruses have been sequenced and identified from the both egg and juvenile stage of *H. glycines* (Bekal et al. 2011).

1.3.5. Effects of abiotic factors on H. glycines reproduction

The life cycle of *H. glycines* is highly influenced by different abiotic factors such as temperature, pH, soil types and other chemical compounds, which affect directly and indirectly *H. glycines* reproduction and race determination (Alston and Schmitt 1988; Duan et al. 2009; Lehman et al. 1971; Palmateer et al. 2000; Pedersen et al. 2010; Perez-Hernandez 2013; Young and Heatherly 1990; Zheng et al. 2010).

Temperature is one of the most important factor for development of SCN. Temperatures below 10^{o} C and above 30^{o} C are considered unfavorable for the infectivity of soybean roots by juveniles (J2) (Riggs and Wrather 1992). High soil pH is favored by

the *H. glycines*. A research study showed the positive correlation between the soil pH and *H. glycines* population densities for both susceptible and resistant soybean cultivars (Pedersen et al. 2010).

Different metal ions have also been found to have effects on SCN. A study performed in-vitro showed that the hatching SCN eggs increases when eggs were treated with zinc compound $ZnSO_4$, but the hatching rate decreases when treated with $ZnCl_2$. However, there was no recordable effect when the same treatments were applied under the field conditions (Behm et al. 1995). Other positive metal ions known to have inhibition effects on hatching of SCN eggs include higher concentration of positive of copper, manganese, sulfur, and iron ions. Concentrated nitrogenous compounds of ammonium ion (NH_4^+) and nitrate ions (NO_2^- and NO_3^-) were reported to increase the mortality of J2 *in-vitro* (Duan et al. 2009).

1.3.6. HG typing

Heterodera glycines type (from here on abbreviated at HG type) test is a relatively new method used to characterize *H. glycines* phenotypes based upon the reproduction on soybean differential lines relative to the susceptible check. Race determination is based on only resistance or susceptible reaction to four SCN differential lines Peking, Picket, PI 88788, and PI 90763 and standard susceptible check Lee 74 (Riggs and Schmitt 1988). Because new resistance sources have been developed, race determination based on seven differential lines became more complicated and confusing. HG typing is the revised classification of SCN, which considers phenotypic diversity and documentation of SCN reproduction differences on soybean lines PI 548402 (Peking), PI 88788, PI 90763, PI 437654, PI 209332, PI 89772, and PI 548316 (Cloud) with respect to

standard susceptible check (Niblack et al. 2002; Wang et al. 2013). The differential lines were assigned numbers 1-7 depending on when the resistance was first identified. HG type determination can inform of resistance sources that are effective against SCN. For instance, a study showed about 70% population of SCN has adapted to resistant source PI 88788 to some extent because of the continued planting of resistant variety with same source of resistant genes (Niblack et al. 2008). In Minnesota, the *H. glycines* populations were found to be virulent on PI 548316 i.e. relatively higher susceptibility than in any other differential line, so this line would not be recommended as a source of resistance for the breeding for resistance to SCN (Zheng et al. 2006).

In Wisconsin, 109 SCN populations were analyzed for HG types from 2006 to 2010 and several SCN populations were found to be adapted to (PI 88788) i.e. HG type 2 were found more frequently than any other HG type (MacGuidwin 2012). More than 70% of Missouri SCN population were virulent to differential lines PI 88788, PI 209332, and PI 548316 (Cloud), and 30% of the SCN were also virulent to PI 548402 (Peking). Other differential lines PI 90763, PI 437654, PI 89772, or PI 438489B were found to be resistant to SCN (<10% reproduction) (Mitchum et al. 2007). In Kentucky, 20 *H. glycines* populations from eight counties were subjected to HG type testing and all 20 populations were found to be virulent on three differential lines PI 88788, PI 209322, and PI 548316 (Hershman et al. 2008).

The pathogenicity of the SCN on PI 88788 had increased after more than 5 years, either in monoculture or rotation with other resistant or non-host crop like corn, and the race 3 (HG type 0 and 7) changed to race 1 (HG type 2.5.7) and this race was adapted to PI 88788 with a female index more than 60% (Zheng and Chen 2011). In case of South

Dakota, race typing was performed ten years ago using only two populations of *H. glycines* from Union and Turner county, and they were identified as race 3 (Jones 1997).

1.3.7. Molecular detection of H. glycines infection

Detection of the pathogen by using molecular techniques is becoming more popular these days because of its efficiency, specificity, effectiveness and informative to develop proper management strategies (Sankaran et al. 2010). Enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (common PCR and Real-time PCR), immunofluorescence (IF), fluorescence in-situ hybridization (FISH) and DNA microarrays, etc. are the commonly used molecular detection techniques for different plant pathogens and these have greatly improved the detection ability by understanding the host-pathogen interaction and disease epidemiology (López et al. 2003; Martin et al. 2000). Different detection techniques such as PCR, amplified fragment length polymorphism (AFLP), and others have been used for plant parasitic nematode detection including cyst and root knot nematode (Hooper et al. 2005).

1.3.8. Management of soybean cyst nematode

Soybean cyst management strategies vary from place to place, but the most common practices are using SCN resistant cultivars and crop rotation in the United States and in South Dakota. These two methods have been found to be more effective than others tactics (Niblack et al. 2003; Oyekanmi and Fawole 2010; Schmitt et al. 2004).

1.3.8.1. Host resistance

Different resistance genes have been exploited from different soybean germplasm for developing SCN resistant soybean cultivars (Concibido et al. 2004). The resistance genes to *H. glycines* were explored for the first time in soybean germplasm Peking, which has

three recessive genes *rhg1*, *rhg2*, and *rhg3* in 1960 (Caldwell et al. 1960). Later the fourth new dominant gene *Rhg4* resistant to *H. glycines* was also identified in Peking, which was closely linked to *i locus* and responsible for distribution of pigmentation of seed coat (Matson and Williams 1965). Another dominant resistant gene was discovered in PI 88788 germplasm and later named *Rhg*5 (Rao-Arelli 1994; Rao-Arelli et al. 1992). For developing resistant cultivars against *H. glycines*, seven differential line PI 548402 (Peking), PI 88788, PI 90763, PI 437654, PI 209332, PI 89772, PI 548316 (Cloud) are considered. Out of these seven differential lines, PI 88788 constitute more than 90% of the SCN resistant cultivars in the United States (Concibido et al. 2004; Schmitt et al. 2004; Shannon et al. 2004). Other PI lines such as PI 548402 and PI 437654 also been used for breeding a few resistant cultivars. A novel QTL was identified on soybean line PI 567516C and showed a broad range of resistance for multiple HG types (Vuong et al. 2010).

Second stage juveniles (J2) can enter the roots of the resistant cultivar or non-host crop such as potato and others due to the roots exudates that attract the J2s, but they can't reproduce on a resistant cultivar or non-hosts crops because the nematode cannot form the permanent feeding site inside the roots of resistant cultivars and non-host crops (Davis et al. 2004; Davis et al. 2000; Johnson et al. 1993).

SCN infected soybean plants have reduced plant biomass and pod and seed development between 12 to 14 weeks after planting for the resistant cultivar. In susceptible cultivar biomass production remains unchanged up to 10 weeks. Reduction in pod and seed development occurs in reproductive stage of soybean plants. Reduction in

pods and seeds leads to significant reduction in soybean yield in susceptible cultivars (Wang et al. 2003).

Most of the research conducted on the effect of SCN resistant cultivar on soybean yield component showed yield benefit from resistant cultivars than the susceptible cultivars when grown in *H. glycines* infested areas. In Minnesota, 28.4% more yield was obtained from resistant cultivars than the susceptible cultivars grown in six different *H. glycines* infested fields. Likewise in North Carolina, resistant cultivars produced higher yield and reduced number of *H. glycines* than susceptible cultivars (Chen et al. 2001; Koenning 2004). In Iran, SCN resistant cultivars in combination with nematicides showed 48% greater yield than susceptible cultivars when planted in two fields infested with HG 0 populations (Heydari et al. 2012). Growers are encouraged to practice crop rotation along with the rotation among the SCN resistant cultivars because continuous planting of the same resistance cultivar helps the SCN to adapt to the resistance source being used because of the selection pressure (Niblack 2005; Tylka and Mullaney 2015).

1.3.8.2. Cultural control methods

There are different cultural methods used to control the soybean cyst nematode including crop rotation, tillage, spacing, and planting dates. Out of these, crop rotation is one of the more commonly used method, in which soybean crop is rotated with non-host or poor host of SCN. Annual rotation between resistant soybean and corn has been found to be effective for reducing SCN population along with higher yield of both crops (Chen et al. 2001). Rotation of soybean with sun hemp (*Crotalaria juncea*), Illinois bundle flower (*Desmanthus illinoensis*), oilseed rape (*Brassica napus*), perennial ryegrass (*Lolium perenne*), red clover (*Trifolium pretense*), and corn (*Zea mays*) were also tested

for the management of SCN and sun hemp and red clover were found to be more effective in managing soybean cyst nematode. These crops stimulated SCN hatching and hence decreased the number of SCN for next soybean growing season due to the unavailability of host plant for infection by hatched J2 (Warnke et al. 2008).

Besides the crop rotation, tillage also plays an important role in SCN management. Conventional tillage and reduced tillage help to decrease the population density, but no-tillage and ridge tillage support increase in SCN populations (Gavassoni et al. 2001). Other cultural practices like the planting of the early maturity cultivar in mid-to -late June can also help to control the *H. glycines* population up to some level (Schmitt 1991). Different trap crops such as *Crotalaria juncea* and *C. sepectabilis* have been tested for soybean cyst nematode management and reduced *H. glycines* numbers significantly by inducing hatching and subsequent SCN penetration of the roots (Kushida et al. 2003).

1.3.8.3. Chemical control

Different chemical products have been developed for the control of plant parasitic nematodes, but application of the chemical compounds as nematicides to control nematodes is not considered economical and eco-friendly method because of the cost and toxicity to handlers and ground water pollution (Matthiessen and Kirkegaard 2006; Oka 2010). Aldicarb, a granular carbamate compound which is commonly used as a nematicide in SCN management in the Midwest region of the United States has some level of control of the nematode population (Grabau 2013). Another nematicide tested in the Midwest is Telone C-35 (Dow Agrosciences, Indianapolis, IN), which is a fumigant with 1, 3 dichloroprene and chloropicrin as active ingredients with effects on both nematodes and fungi. A study conducted at three field sites in Iowa showed that Telone

increased soybean yield by 10% and decreased SCN egg populations by 42% on an average (De Bruin and Pedersen 2008). Application of the Benzyl isothiocynate (BITC) showed multiple effects on the reproduction of *H. glyicnes* by affecting the hatching of eggs, embryonic development and movement of the infective juveniles in the soil (Wu et al. 2014). Use of these chemical compounds in the management of SCN has been limited due to the cost and non-target effects as well as environmental concerns. Some herbicides such as Acifluorfen, bentazon, lactofen, and surfactants such as crop oil concentrate (COC), and nonionic surfactant (NIS) have been used for SCN management and found to be effective in reducing SCN eggs population density because they induced the production of glyceollin compound which might increase the host resistance to soybean cyst nematode (Levene et al. 1998). Another study was performed for the effect of commonly used herbicides such as Atrazine (atrazine), Basagran (bentazon), Bladex (cyanazine), Blazer (acifluorfen), Command (clomazone), Lasso (alachlor), Sonalan (ethalfluralin), and Treflan (trifluralin) on hatching of *H. glycines*, but only Blazer (acifluorfen) showed the suppression in *H. glycines* hatching (Wong et al. 1993)

The effectiveness of the two new seed treatments Avicta (fermented product abamectin, derived from an actinomycetes and released by Syngenta crop protection) and Aeris (mixture of neonicotinoid insecticide inidacloprid and thiocarb, a carbamate insecticide/nematicide and released by Bayer Crop Science) were evaluated for the management of the SCN. Although population of the nematode were not reduced, still some yield benefits were observed due to abemectin treatment (Frye 2009). Different fungicides (Cleary 3336F, Azoxystrobin, Cholorothalonil, and Metalaxyl) were also tested for the effects on the *H. glycines* development, out of these fungicides, Cleary

3336F was the most effective suppressor to the *H. glycines* population (Faghihi et al. 2007).

Recently, Syngenta has released a nematicidal seed treatment named Clariva™ based on a bacteria *Pasteuria nishizawae* (Sharma et al. 2015), some trial reports by Iowa State University stated that there was some effects of Clariva™ in reducing nematode population and increase in soybean yield (Fawcett et al. 2014). Another seed treatment is also available on the market by Bayer Crop Science named IleVO® based on Fluropyram and was tested for the control of SCN in the greenhouse conditions and showed some effects on reducing numbers on treated seed than in untreated control (Zaworski 2014).

1.3.8.4. Application of plant extracts

Different biological products have been tested for the control of soybean cyst nematode and considered as a promising management strategy, as an alternative to chemical control (nematicide). Kernel of neem (*Azadirachta. indica*), dried de-fattened meal of brassica (*Brassica chinensis*), resin of acacia (*Acacia nilotica*) and seaweed (*Eclonia maxima*) have been used as bio-pesticides control measures to reduce the number of nematode populations while increasing yield of soybean (Auwal et al. 2014). Annual ryegrass, *Lolium multiflorum* residue was found to reduce the *H. glycines* population by increasing the hatching of eggs of nematodes in the absence of the host and depletion of lipid reserved in juvenile. The ryegrass residue also decreased the parasitism of the nematode (Mock et al. 2009; Riga et al. 2001). Application of bean sprout residue in a soybean field showed stimulator and subsequent decrease in soybean cyst nematode reproduction and juvenile hatching and significantly reduced the nematode population (Toyota et al. 2013). The swine manure enriched with two compounds volatile fatty acids

(VFA) and ammonium nitrogen (NH+4) inversely affects the SCN cyst count in a linear manner up to 35 days (Xiao et al. 2007).

1.3.8.5. Biological control

Several bacterial and fungal species have been evaluated for SCN management. *Hirsutella rhossiliensis*, an endoparasitic fungus, is a promising biological control of the soybean cyst nematode. The fungus infect nematode at juvenile stage and can greatly reduce the infection of roots by J2 (Chen 2007; Chen and Liu 2005; Chen et al. 2000; Zhang et al. 2006). Another biological control agent evaluated is *Sinorhizobium fredii.* Soybean seedlings infected with endophytic bacterium, *Sinorhizobium fredii* strain Sneb183 showed systemic resistance to *H. glycines* and reduced the juveniles and cyst numbers by increasing the development period of nematode (Tian et al. 2014). Another plant growth promoting fungus, *Pirformospora indica,* can reduce the *H. glycines* eggs and juveniles populations and also promotes soybean flowering, when applied to the field as a soil amendment (Bajaj et al. 2015).

1.3.8.6. Biotechnology

Use of biotechnology in pest management has been on the increase, but so far, not so much progress has been made in SCN management. RNAi induced suppression of the numerous genes associated with the nematode development, reproduction and parasitism can be implemented for the sustainable nematode management and study of the gene expression on feeding site syncytia (Li et al. 2011a). Silencing of the aldolase gene of SCN greatly reduces the number of SCN females to reach maturity and this process is also becoming one of the promising step for incorporating resistance in plants against plant parasitic nematode (Youssef et al. 2013). Bean pod mottle virus (BPMV) based

virus-induced gene silencing (VIGS) method has been developed and used for the functional analysis of gene involved in the resistance to soybean cyst nematode and reveal the interaction between soybean-nematode infecting soybean roots (Kandoth et al. 2013). Recent research showed that the candidate gene of *SAMT* genes from soybean (*GmSAM1*) was found to be involved in the defense mechanism for *H. glycines*, and the overexpression of this gene in the hairy roots soybean cultivar reduced SCN development (Lin et al. 2013). These tools are still in development but have great potential for managing SCN.

Integration of resistant cultivar, crop rotation with non-host crop, tillage practices, and knowledge of virulent genotypes (HG types) provides better control of SCN population and help to maximize soybean yield (Conley et al. 2011), but management strategies recommended for different states in the United States are not giving the good result for controlling *H. glycines* population and significant increase in soybean yield (Niblack et al. 2006).

1.4. Justification of the study

The soybean cyst nematode (SCN), *Heterodera glycines* is most important soybean production constraint in South Dakota and in the United States. It is ranked number one among the biological yield-limiting factors for soybean. In South Dakota, SCN was first detected in South Dakota in 1996 in Union County and has since been found in 29 counties as of 2015. This nematode not only infects soybean, but also invades several leguminous crops and weed species. Management of this nematode is challenging because limited aboveground symptoms. So visual assessment for this nematode is almost misleading because it can cause up to 30% yield loss without any visual

symptoms in the field. Analysis of soil samples to know about the presence and estimation population density is recommended.

Although there is significant level of infestation by *H. glycines* in South Dakota, characterization of the virulent phenotypes (HG types) has not been performed. Knowledge about the HG types and their distribution is important in deploying and rotating resistant cultivars that have varying resistance source for SCN. The goal of this study is to improve strategies to manage SCN on soybean in South Dakota through characterization of SCN HG types that occur in South Dakota. Another aspect of this research project is to screen the commercial soybean cultivars for resistance against the popular HG types occurring in South Dakota.

The results obtained from this study will provide information to help producers in making SCN management decisions. Knowledge about the SCN HG types occurring in South Dakota will be recommended for selecting sources of resistance genes in breeding of SCN resistant cultivars. Cultivar screening for resistance against popular HG types will give the performance of commercial resistant cultivar for prevalent SCN HG types in South Dakota and guide growers in planting suitable resistance cultivars. The specific objectives of this research were to:

1. Monitor the occurrence of SCN in soybean of South Dakota.
2. Determine HG types of SCN that occur in South Dakota.
3. Screen major commercial soybean cultivars for resistance against HG types prevalent in South Dakota.

Literature cited

Alston, D., and Schmitt, D. 1987. Population density and spatial pattern of *Heterodera glycines* in relation to soybean phenology. J. Nematol. 19:336-345.

Alston, D., and Schmitt, D. 1988. Development of *Heterodera glycines* life stages as influenced by temperature. J. Nematol. 20:366-362.

ASA. 2015. Soystats: a reference guide to important soybean facts and figures. The American Soybean Association, online ed. [http://www. http://soystats.com/wp-content/uploads/SoyStats-2015.pdf]

Auwal, H. M., Wang, H., Li, L., Kakar, K.U., and Zheng, J. 2014. Utilization of plant materials for control of soybean cysts nematode., Section B, Soil Plant Sci. 64:392-397.

Bajaj, R., Hu, W., Huang, Y., Chen, S., Prasad, R., Varma, A., and Bushley, K. E. 2015. The beneficial root endophyte Piriformospora indica reduces egg density of the soybean cyst nematode. Biol. Control 90:193-199.

Behm, J., Tylka, G., Niblack, T., Wiebold, W., and Donald, P. 1995. Effects of zinc fertilization of corn on hatching of *Heterodera glycines* in soil. J. Nematol. 27:164-171.

Bekal, S., Domier, L. L., Niblack, T. L., and Lambert, K. N. 2011. Discovery and initial analysis of novel viral genomes in the soybean cyst nematode. J. Gen. Virol. 92:1870-1879.

Caldwell, B. E., Brim, C., and Ross, J. 1960. Inheritance of resistance of soybeans to the cyst nematode, *Heterodera glycines*. Agron. J. 52:635-636.

Chang, W.S., Lee, H.I., and Hungria, M. 2015. Soybean Production in the Americas. in Lugtenberg, B.(Eds). Principles of Plant-Microbe Interactions. 393-400, Springer, US.

Chen, S. 2007. Suppression of *Heterodera glycines* in soils from fields with long-term soybean monoculture. Biocontrol Sci. Technol. 17:125-134.

Chen, S., and Liu, X. 2005. Control of the soybean cyst nematode by the fungi *Hirsutella rhossiliensis* and *Hirsutella minnesotensis* in greenhouse studies. Biol. Control 32:208-219.

Chen, S., Liu, X., and Chen, F. 2000. *Hirsutella minnesotensis sp*. nov., a new pathogen of the soybean cyst nematode. Mycologia. 92 :819-824.

Chen, S., Porter, P., Orf, J., Reese, C., Stienstra, W., Young, N., Walgenbach, D., Schaus, P., Arlt, T., and Breitenbach, F. 2001. Soybean cyst nematode population development and associated soybean yields of resistant and susceptible cultivars in Minnesota. Plant Dis. 85:760-766.

Chen, S., Porter, P. M., Reese, C. D., and Stienstra, W. C. 2001. Crop sequence effects on soybean cyst nematode and soybean and corn yields. Crop Sci. 41:1843-1849.

Colgrove, A. L., and Niblack, T. L. 2005. The effect of resistant soybean on male and female development and adult sex ratios of *Heterodera glycines*. J. Nematol. 37:161-167.

Concibido, V. C., Diers, B. W., and Arelli, P. R. 2004. A decade of QTL mapping for cyst nematode resistance in soybean. Crop Sci. 44:1121-1131.

Conley, S., Gaska, J., Pedersen, P., and Esker, P. 2011. Soybean yield and *Heterodera glycines* response to rotation, tillage, and genetic resistance. Agron. J. 103:1604-1609.

Creech, J. E., Faghihi, J., Ferris, V. R., Westphal, A., and Johnson, W. G. 2007a. Influence of intraspecific henbit (*Lamium amplexicaule*) and purple deadnettle (*Lamium purpureum*) competition on soybean cyst nematode reproduction. Weed Sci. 55:665-670.

Creech, J. E., Webb, J. S., Young, B. G., Bond, J. P., Harrison, S. K., Ferris, V. R., Faghihi, J., Westphal, A., and Johnson, W. G. 2007b. Development of soybean cyst nematode on henbit *(Lamium amplexicaule)* and purple deadnettle *(Lamium purpureum)*. Weed Technol. 21:1064-1070.

Davis, E. L., Hussey, R. S., and Baum, T. J. 2004. Getting to the roots of parasitism by nematodes. Trend Parasitol. 20:134-141.

Davis, E. L., Hussey, R. S., Baum, T. J., Bakker, J., Schots, A., Rosso, M.-N., and Abad, P. 2000. Nematode parasitism genes. Ann. Rev. Phytopathol. 38:365-396.

De Bruin, J. L., and Pedersen, P. 2008. Soybean cultivar and planting date response to soil fumigation. Agron. J. 100:965-970.

Duan, Y. X., Zheng, Y. N., Chen, L.J., Zhou, X. M., Wang, Y.Y., and Sun, J. S. 2009. Effects of abiotic environmental factors on soybean cyst nematode. Agr. Sci. China 8:317-325.

Faghihi, J., Vierling, R., Santini, J., and Ferris, V. 2007. Effects of selected fungicides on development of *soybean cyst nematode*. Nematropica 37:259-265.

Fawcett, Jim; Koopman, Zack; Miller, Lance; Roush, Wayne; and Sievers, Josh. 2014. "On-Farm Soybean Seed Treatment Trials" . Iowa State Research Farm Progress Reports. Paper 2246.http://lib.dr.iastate.edu/farms_reports/2246

Frye, J. W. 2009. Efficacy of Novel Nematicide Seed Treatments for the Control of *Heterodera glycines* in Soybean Production. Online http://www.lib.ncsu.edu/resolver/1840.16/46.

Gao, X., Jackson, T., Hartman, G., and Niblack, T. 2006. Interactions between the soybean cyst nematode and *Fusarium solani* f. sp. *glycines* based on greenhouse factorial experiments. Phytopathology 96:1409-1415.

Gavassoni, W. L., Tylka, G. L., and Munkvold, G. P. 2001. Relationships between tillage and spatial patterns of *Heterodera glycines.* Phytopathology 91:534-545.

Grabau, Z. 2013. Management strategies for control of soybean cyst nematode and their effects on the nematode community. Retrieved from the University of Minnesota Digital Conservancy, http://purl.umn.edu/157428

Hartman, G. L., West, E. D., and Herman, T. K. 2011. Crops that feed the World 2. Soybean, worldwide production, use, and constraints caused by pathogens and pests. Food Security 3:5-17.

Hershman, D., Heinz, R., and Kennedy, B. 2008. Soybean cyst nematode, *Heterodera glycines*, populations adapting to resistant soybean cultivars in Kentucky. Plant Dis. 92:1475-1475.

Heydari, R., Maafi, Z. T., and Pourjam, E. 2012. Yield loss caused by soybean cyst nematode, *Heterodera glycines*, in Iran. Nematology 14:589-593.

Holcomb, R. 2012. Agricultural Statistics 2011. Government Printing Office.

Hooper, D. J., Hallmann, J., and Subbotin, S. A. 2005. Methods for extraction, processing and detection of plant and soil nematodes. Plant parasitic nematodes in subtropical and tropical agriculture 2:53-86.

Hymowitz, T. 1970. On the domestication of the soybean. Econ. Botany 24:408-421.

Hymowitz, T., and Newell, C. 1981. Taxonomy of the genus *Glycine*, domestication and uses of soybeans. Econ. Bot. 35: 272-288.

Hymowitz, T., and Harlan, J. R. 1983. Introduction of soybean to North America by Samuel Bowen in 1765. Econ. Bot. 37:371-379.

Inagaki, H., and Tsutsumi, M. 1971. Survival of the soybean cyst nematode, *Heterodera glycines* Ichinohe (Tylenchida: *Heteroderidae*) under certain spring conditions. Appl. Entol. and Zool. 6:156-162.

Johnson, A., Kim, K., Riggs, R., and Scott, H. 1993. Location of *Heterodera glycines* induced syncytia in soybean as affected by soil water regimes. J. Nematol. 25:422-426.

Johnson, W. G., Earl Creech, J., and Mock, V. A. 2008. Role of winter annual weeds as alternative hosts for soybean cyst nematode. Crop Management: http://dx.doi.org/10.1094/CM-2008-0701-01-RV.

Jones, J. L. 1997. Studies on a South Dakota population of *Heterodera glycines* (Thesis), South Dakota State University.

Kaitany, R., Melakeberhan, H., Bird, G. W., and Safir, G. 2000. Assocation of *Phytophthora sojae* wtih *Heterodera glycines* and nutrient stressed soybeans. Nematropica 30:193-200.

Kandoth, P. K., Heinz, R., Yeckel, G., Gross, N. W., Juvale, P. S., Hill, J., Whitham, S. A., Baum, T. J., and Mitchum, M. G. 2013. A virus-induced gene silencing method to study soybean cyst nematode parasitism in *Glycine max*. BMC research notes 6:255.

Koenning, S. 2004. Resistance of soybean cultivars to field populations of *Heterodera glycines* in North Carolina. Plant Dis. 88:942-950.

Koenning, S. R., and Wrather, J. A. 2010. Suppression of soybean yield potential in the continental United States by plant diseases from 2006 to 2009. Plant Health Prog. http://dx. doi. org/10.1094/PHP-2010-1122-01-RS.

Kushida, A., Suwa, N., Ueda, Y., and Momota, Y. 2003. Effects of *Crotalaria juncea* and *C. spectabilis* on hatching and population density of the soybean cyst nematode, *Heterodera glycines* (Tylenchida: *Heteroderidae*). Appl. Entomol. Zool. 38:393-399.

Lauritis, J., Rebois, R., and Graney, L. 1983. Development of *Heterodera glycines* Ichinohe on soybean, *Glycine max* (L.) Merr., under gnotobiotic conditions. J. Nematol. 159:272-281.

Lehman, P., Barker, K., and Huisingh, D. 1971. Effects of pH and inorganic ions on emergence of *Heterodera glycines*. Nematologica 17:467-473.

Levene, B. C., Owen, M. D., and Tylka, G. L. 1998. Response of soybean cyst nematodes and soybeans (*Glycine max*) to herbicides. Weed Sci. 46:264-270.

Li, J., Todd, T. C., Lee, J., and Trick, H. N. 2011a. Biotechnological application of functional genomics towards plant-parasitic nematode control. Plant Biotech. J. 9:936-944.

Li, Y.-H., Qi, X.-T., Chang, R., and Qiu, L.-J. 2011b. Evaluation and utilization of soybean germplasm for resistance to cyst nematode in China. Soybean, molecular aspects of breeding. Intech Publishers:373-396.

Lin, J., Mazarei, M., Zhao, N., Zhu, J. J., Zhuang, X., Liu, W., Pantalone, V. R., Arelli, P. R., Stewart, C. N., and Chen, F. 2013. Overexpression of a soybean salicylic acid methyltransferase gene confers resistance to soybean cyst nematode. Plant Biotech. J. 11:1135-1145.

López, M. M., Bertolini, E., Olmos, A., Caruso, P., Gorris, M. T., Llop, P., Penyalver, R., and Cambra, M. 2003. Innovative tools for detection of plant pathogenic viruses and bacteria. Int. Microbiol. 6:233-243.

MacGuidwin, A. 2012. Distribution of SCN HG types in Wisconsin. 2011, Proc. of the 2012 Wisconsin Crop Management Conference, Vol. 51:107.

Martin, R. R., James, D., and Lévesque, C. A. 2000. Impacts of molecular diagnostic technologies on plant disease management. Ann. Rev. Phytopathol. 38:207-239.

Matson, A. L., and Williams, L. F. 1965. Evidence of a fourth gene for resistance to the soybean cyst nematode. Crop Sci. 5:4407-4414.

Matthiessen, J. N., and Kirkegaard, J. A. 2006. Biofumigation and enhanced biodegradation: opportunity and challenge in soilborne pest and disease management. Critical Reviews in Plant Sci. 25:235-265.

McCarville, M. T., Soh, D. H., Tylka, G. L., and O'Neal, M. E. 2014. Aboveground feeding by soybean aphid, *Aphis glycine*s, affects soybean cyst nematode, *Heterodera glycines*, reproduction belowground. PloS one 9:e86415.

Melito, S., Heuberger, A. L., Cook, D., Diers, B. W., MacGuidwin, A. E., and Bent, A. F. 2010. A nematode demographics assay in transgenic roots reveals no significant impacts of the Rhg1 locus LRR-Kinase on soybean cyst nematode resistance. BMC Plant Biol. 10:104. doi:10.1186/1471-2229-10-104.

Mitchum, M. G., Wrather, J. A., Heinz, R. D., Shannon, J. G., and Danekas, G. 2007. Variability in distribution and virulence phenotypes of *Heterodera glycines* in Missouri during 2005. Plant Dis. 91:1473-1476.

Mock, V. A., Creech, J. E., Davis, V. M., and Johnson, W. G. 2009. Plant growth and soybean cyst nematode response to purple deadnettle (*Lamium purpureum*), annual ryegrass, and soybean combinations. Weed Sci. 57:489-493.

Niblack, T. 2005. Soybean cyst nematode management reconsidered. Plant Dis. 89:1020-1026.

Niblack, T., Lambert, K., and Tylka, G. 2006. A model plant pathogen from the kingdom animalia: *Heterodera glycines*, the soybean cyst nematode. Annu. Rev. Phytopathol. 44:283-303.

Niblack, T., Wrather, J., Heinz, R., and Donald, P. 2003. Distribution and virulence phenotypes of *Heterodera glycines* in Missouri. Plant Dis. 87:929-932.

Niblack, T. L., Colgrove, A. L., Colgrove, K., and Bond, J. P. 2008. Shift in virulence of soybean cyst nematode is associated with use of resistance from PI 88788. Online. Plant Health Progress doi:10.1094/PHP-2008-0118-01-RS.

Niblack, T., Arelli, P., Noel, G., Opperman, C., Orf, J., Schmitt, D., Shannon, J., and Tylka, G. 2002. A revised classification scheme for genetically diverse populations of *Heterodera glycines*. J. Nematol. 34:279.

Noel, G. R. 1986. The Soybean Cyst Nematode. Pages 257-268 in: Cyst nematodes. Springer.

Oka, Y. 2010. Mechanisms of nematode suppression by organic soil amendments, a review. Appl. Soil Ecol. 44:101-115.

Oyekanmi, E. O., and Fawole, B. 2010. 15 Nematodes of Soybean and Their Management in: Singh, G. (Ed.). (2010). The soybean: botany, production and uses. CABI.

Palmateer, A., Schmidt, M., Stetina, S., and Russin, J. 2000. Temperature effects on race determination in *Heterodera glycines*. J. Nematol. 32:349-355.

Pedersen, P., Tylka, G., Mallarino, A., Macguidwin, A., Koval, N., and Grau, C. 2010. Correlation between soil pH, population densities, and soybean yield. Crop Sci. 50:1458-1464.

Perez-Hernandez, O. 2013. Multifactorial analysis of mortality of soybean cyst nematode (*Heterodera glycines* Ichinohe) populations in soybean and in soybean fields annually rotated to corn in Nebraska. University of Nebraska – Lincoln.

Pormarto, S. H., Nelson, B. D., and Helms, T. C. 2011. Reproduction of soybean cyst nematode on dry bean cultivars over multiple generations. Plant Dis. 95:1239-1243.

Poromarto, S. H., and Nelson, B. D. 2009. Reproduction of soybean cyst nematode on dry bean cultivars adapted to North Dakota and northern Minnesota. Plant Dis. 93:507-511.

Rao-Arelli, A. 1994. Inheritance of resistance to *Heterodera glycines* race 3 in soybean accessions. Plant Dis. 78:898-900.

Rao-Arelli, A., Anand, S. C., and Wrather, A. J. 1992. Soybean resistance to soybean cyst nematode race 3 is conditioned by an additional dominant gene. Crop Sci. 32:862-864.

Riga, E., Topp, E., Potter, J., Welacky, T., Anderson, T., and Tenuta, A. 2001. The impact of plant residues on the soybean cyst nematode, *Heterodera glycines*. Can. J. Plant Pathol. 23:168-173.

Riggs, R. 1977. Worldwide distribution of soybean cyst nematode and its economic importance. J. Nematol. 9:34-39.

Riggs, R., and Schmitt, D. 1988. Complete characterization of the race scheme for *Heterodera glycines*. J. Nematol. 20:392-395.

Riggs, R. D., and Wrather, J. A. 1992. Biology and Management of the Soybean Cyst Nematode. American Phytopathological Society, St. Paul Minnesota, USA.

Russin, J., Layton, M., Boethel, D., McGawley, E., Snow, J., and Berggren, G. 1989. Development of *Heterodera glycines* on soybean damaged by soybean looper and stem canker. J. Nematol. 21:108-114.

Sankaran, S., Mishra, A., Ehsani, R., & Davis, C. 2010. A review of advanced techniques for detecting plant diseases. Computers and Electron. Agric. 72, 1-13.

Schmitt, D. 1991. Management of *Heterodera glycines* by cropping and cultural practices. J.Nematol. 23:348-352.

Schmitt, D. P., Wrather, J. A., and Riggs R. D. (eds.) 2004. Biology and Management of Soybean Cyst Nematode, Second Edition. Walsworth Publishing Company, Marceline, MO.

Shannon, J.G., Arelli, P.R., Young, L.D. 2004. Breeding for soybean cyst nematode resistance. In: Schmitt, Wrather, Riggs, editors. Biology and Management of Soybean Cyst Nematode. Second Edition. p. 155-180.

Sharma, K., Singh, U., Sharma, P., Kumar, A., and Sharma, L. 2015. Seed treatments for sustainable agriculture-A review. J. Appl. and Nat. Sci. 7:521-539.

Smolik, J. D., and Draper, M. A. 1998. Soybean Cyst Nematode. Cooperative Extension Service, South Dakota State University

Tabor, G., Tylka, G., and Bronson, C. 2006. Soybean stem colonization by genotypes A and B of *Cadophora gregata* increases with increasing population densities of *Heterodera glycines*. Plant Dis. 90:1297-1301.

Tian, F., Wang, Y., Zhu, X., Chen, L., and Duan, Y. 2014. Effect of *Sinorhizobium fredii* strain Sneb183 on the biological control of soybean cyst nematode in soybean. J. Basic Microb. 54:1258-1263.

Todd, T., Pearson, C., and Schwenk, F. 1987. Effect of *Heterodera glycines* on charcoal rot severity in soybean cultivars resistant and susceptible to soybean cyst nematode. J. Nematol. 19:35.

Toyota, K., Goto, K., and Ito, D. 2013. Effect of bean sprout residue on the hatching and density of the soybean cyst nematode *Heterodera glycines* in soil. Nematology 15:923-929.

Triantaphyllou, A., and Hirschmann, H. 1962. Oogenesis and mode of reproduction in the soybean cyst nematode, *Heterodera glycines*. Nematologica 7:235-241.

Tylka, G. L., and Marett, C. C. 2014. Distribution of the soybean cyst nematode, *Heterodera glycines*, in the United States and Canada: 1954 to 2014. Plant Health Progress. Doi: 10.1094/PHP-BR-14-0006.

Tylka, G. L., and Mullaney, M. P. 2015. Soybean cyst nematode-resistant soybean varieties for Iowa. Iowa State University, University Extension, Online. http://www.plantpath.iastate.edu/tylkalab/files/page/files/2015%20ISU%20SCN-Resistant%20Variety%20Trials.pdf.

Venkatesh, R., Harrison, S. K., and Riedel, R. M. 2009. Weed Hosts of soybean cyst nematode (*Heterodera glycines*) in Ohio. Weed Technol. 14: 156-160.

Vuong, T. D., Sleper, D. A., Shannon, J. G., and Nguyen, H. T. 2010. Novel quantitative trait loci for broad-based resistance to soybean cyst nematode (*Heterodera glycines* Ichinohe) in soybean PI 567516C. TAG. Theor. Appl. Genetics. 121:1253-1266.

Wang, D., Zhu, X. F., Wang, Y. Y., Luo, X., Song, P., Zhu, F., Wang, F., Chen, J. S., Chen, L. J., and Duan, Y. X. 2013. A reassessment of virulence phenotypes of soybean cyst nematode (*Heterodera glycines*) in China with HG typing method. Plant Dis. 98:702-702.

Wang, J., Niblack, T., Tremain, J., Wiebold, W., Tylka, G., Marett, C., Noel, G., Myers, O., and Schmidt, M. 2003. Soybean cyst nematode reduces soybean yield without causing obvious aboveground symptoms. Plant Dis. 87:623-628.

Warnke, S. A., Chen, S., Wyse, D. L., Johnson, G. A., and Porter, P. M. 2008. Effect of rotation crops on hatch, viability and development of *Heterodera glycines.* Nematology 10:869-882.

Werle, R., Giesler, L. J., Bernards, M. L., and Lindquist, J. L. 2015. Likelihood of soybean cyst nematode (*Heterodera glycines*) reproduction on henbit (*Lamium amplexicaule*) roots in Nebraska. Weed Technol. 29:35-41.

Winstead, N., Skotland, C., and Sasser, J. 1955. Soybean cyst nematode in North Carolina. Plant Dis. Reporter 39:9-11.

Wong, A., Tylka, G., and Hartzler, R. 1993. Effects of eight herbicides on in vitro hatching of *Heterodera glycines.* J. Nematol. 25:578-584.

Wrather, J., and Koenning, S. 2006. Estimates of disease effects on soybean yields in the United States 2003 to 2005. J. Nematol. 38:173-180.

Wrather, J., and Koenning, S. 2009. Effects of diseases on soybean yields in the United States 1996 to 2007. Online. Plant Health Progress doi:10.1094/PHP-2009-0401-01-RS.

Wu, H., Masler, E. P., Rogers, S. T., Chen, C., and Chitwood, D. J. 2014. Benzyl isothiocyanate affects development, hatching and reproduction of the soybean cyst nematode *Heterodera glycines*. Nematology 16:495-504.

Xiao, J., Zhu, J., Chen, S., Ruan, W., and Miller, C. 2007. A novel use of anaerobically digested liquid swine manure to potentially control soybean cyst nematode. J. Environ. Sci. Health 42:749-757.

Young, L. 1996. Yield loss in soybean caused by *Heterodera glycines*. J. Nematology 28:604-607.

Young, L. D., and Heatherly, L. G. 1990. *Heterodera glycines* invasion and reproduction on soybean grown in clay and silt loam soils. J. Nematol. 22:618-619.

Youssef, R. M., Kim, K.-H., Haroon, S. A., and Matthews, B. F. 2013. Post-transcriptional gene silencing of the gene encoding aldolase from soybean cyst nematode by transformed soybean roots. Exp. Parasitology. 134:266-274.

Zaworski, E. R. 2014. Effects of ILeVO® on soybean sudden death syndrome and soybean cyst nematode. Graduate Theses and Dissertations. Paper 14261. http://lib.dr.iastate.edu/etd/14261

Zhang, L., Liu, X., Zhu, S., and Chen, S. 2006. Detection of the nematophagous fungus *Hirsutella rhossiliensis* in soil by real-time PCR and parasitism bioassay. Biol. Control 36:316-323.

Zheng, J., and Chen, S. 2011. Estimation of virulence type and level of soybean cyst nematode field populations in response to resistant cultivars. J. Entomol. Nematol. 3:37-43.

Zheng, J., Li, Y., and Chen, S. 2006. Characterization of the virulence phenotypes of *Heterodera glycines* in Minnesota. J. Nematol. 38:383-390.

Zheng, Y., Duan, Y., Chen, S., Sun, J., and Chen, L. 2010. Response of soybean cyst nematode, *Heterodera glycines* to macroelement and microelement compounds. Bulg. J. Agric. Sci. 16:172-180.

CHAPTER 2

2. Determination of *Heterodera glycines* Virulence Phenotypes Occurring in South Dakota

Abstract

The soybean cyst nematode (SCN); *Heterodera glycines*, is the most important yield-limiting factor of soybean in the United States. In South Dakota, SCN has been found in 29 counties so far and continues to spread. Determining the virulence phenotypes (*H. glycines* [HG] types) of the SCN populations can reveal the diversity of the SCN populations and the sources of resistance that would be most effective for SCN management. To determine the HG types prevalent in South Dakota, 250 soil samples were collected from at least 3 arbitrarily selected fields in each of the 28 counties previously found positive for SCN and were tested for SCN. SCN was detected in 82 (33%) fields, and combined egg and juvenile counts ranged from 200 to 65,200 per 100 cm^3 of soil. The SCN population in each soil sample was then grown on the seven SCN HG type test indicator soybean lines and Williams 82 as a susceptible check. A female index (FI) was calculated based on the number of females found on each differential line relative to the susceptible check. Female indices equal to or greater than 10% in any line was assigned as that HG type. Out of 73 SCN populations for which HG type tests were done, 63% had FI ≥10% on PI 548316 or Cloud (indicator line #7), 25% on PI 88788 (#2), 19% on PI 209332 (#5), 7% on Peking (#1), 4% on PI 90736 (#3), and 4% on PI 89722 (#6). None of the SCN populations had FI ≥10% on PI 437654 (indicator line #4). The most prevalent HG types were 0, 2.5.7, and 7. These accounted for 81% of all the HG types determined for the samples tested. HG types with ≥10% reproduction on

indicator lines PI 88788, PI 209332, and PI 548317 were most prevalent in these soil samples, suggesting that the use of these sources of resistance for developing SCN, resistant cultivars should be avoided. For sustainable SCN management, use of resistant cultivars should be integrated with non-host crop rotations and rotation within SCN-resistant cultivars.

Key words: *Heterodera glycines*, HG type, soybean cyst nematode, female index, resistance, management.

2.1. Introduction

Soybean [*Glycine max* L. (Merr.)] is the most important leguminous crop and is ranked second after corn in terms of production in the United States (Holcomb 2012). About 80% of the world's soybean production is produced by North and South America and it is used as oilseed crop, animal feed, biodiesel, and other products (Chang et al. 2015). Soybean production is limited by different abiotic and biotic factors. These include low temperature, salt toxicity, water stresses, nutritional deficiency, and biotic factors include weeds, insect pests, soybean cyst nematode (SCN), and diseases (Hartman et al. 2011). Of the biotic factors, SCN is the top most factor for soybean production in the United States (Koenning and Wrather 2010; Wrather and Koenning 2009).

The soybean cyst nematode, *Heterodera glycines* (Ichinohe 1955) is an obligatory, sedentary, endoparasitic nematode that infects soybean roots. It was first discovered in northeast China in 1899 (Li et al. 2011b) and was identified in Japan in 1915, in Korea in 1936, in Manchuria in 1938, and in the United States in 1954 in North Carolina (Riggs 1977; Winstead et al. 1955). SCN is thought to have been inadvertently introduced into the U.S. with the rhizobia inoculum from China in the first half of the 19th century (Noel 1986). SCN has now been detected in 90 % of the soybean producing states (Tylka and Marett 2014) and was first detected in South Dakota in Union County in 1995 (Smolik et al. 1996).

The extent of yield loss caused by SCN depends upon the level of infestation, environmental factors, and the cultivar planted. SCN can cause yield loss of up to 30% without showing any visible symptoms in the soybeans. Yield loss can go up to 75% in the heavily infested fields (Wang et al. 2003; Young 1996). Different management

practices have been used for SCN, such as host resistance, chemical control, cultural methods, biological control, and biotechnology (Auwal et al. 2014; Chen and Liu 2005; Concibido et al. 2004; Conley et al. 2011; Grabau 2013; Li et al. 2011a). Growing resistant soybean cultivars and nonhost crops in rotation are the most effective and currently available and affordable methods of SCN management (Niblack et al. 2003; Oyekanmi and Fawole 2010; Schmitt et al. 2004). Recently, a few nematode-protectant seed treatments have been labelled on soybean that show some effect on SCN numbers (De Bruin and Pedersen 2008: Frye 2009: Grabau 2013: Wu et al. 2014).

There are many soybean cultivars that are resistant to SCN available in the United States, but more than 90% of the resistant cultivars have been derived from the resistance source PI 88788, PI 548402, and PI 437654 (Concibido et al. 2004; Schmitt et al. 2004; Shannon et al. 2004). Continued planting of varieties with same SCN resistance genes can result in SCN populations that are adapted to these sources of resistance (Niblack 2005; Tylka and Mullaney 2015). Several research reports suggested that commonly used resistance source PI 88788, PI 548402, and PI 437654 are being overcome by *H. glycines* population in different states such as Kansas (Rzodkiewicz 2010), Kentucky (Hershman et al. 2008), Missouri (Mitchum et al. 2007), Minnesota (Zheng et al. 2006), Wisconsin, and others (MacGuidwin 2012). Knowledge of the occurrence and distribution of virulent phenotypes (HG types) can provide valuable information regarding sustainable and effective use of resistant cultivars (Conley et al. 2011).

Knowledge about the population diversity adapted to specific area is important for the SCN management. Distribution and characterization of *H. glycines* populations in South Dakota are necessary for developing improved management strategy by using

resistance cultivar derived from durable resistance source. The analysis of soil samples submitted to Plant Diagnostic Clinic, South Dakota State University show varying level of infestation in soybean fields in South Dakota (Acharya et al. 2014). Many factors can lead to high SCN population densities in fields infested with SCN, including adaptation of the nematode population to SCN resistant cultivars. Thus, the objective of this study was to determine level of SCN infestation and *Heterodera glycines* HG types occurring in South Dakota.

2.2. Material and methods

A study to determine *Heterodera glycines*, HG types of soybean cyst nematode was conducted in controlled greenhouse conditions during the year 2014 and 2015. For *H. glycines* populations, soil samples were collected after harvesting of the soybean crop during the 2013 and 2014 across the east part of the state.

2.2.1. Soil sample collection.

Soil sampling was conducted across 28 counties of South Dakota in which soybean cyst nematode had been detected through voluntary SCN testing at the Plant Diagnostic Clinic in South Dakota State University, Brookings, SD. At least 8 and up to 21 soybean fields were arbitrarily selected from each of the counties, and in each field, up to 2 gallons of soil were collected from field entrances, low spot areas, and along the fence line to obtain the representative soil sample for each field that was most likely to have SCN. Soil was then put in a plastic bag and labeled with field number and collection date, then kept in the cooler before transportation and storage in the cold room at 4^{o} C until SCN extraction was done. Before SCN extraction, each soil sample was manually mixed well and 100 cm^3 of soil was selected for extraction of cysts using

mechanical elutriation (Byrd Jr et al. 1976) Fig. 2.1, 2.2). Extraction of eggs and juveniles from cysts followed procedures by Faghihi and Ferris, (2000). For samples with the sufficient number of eggs and juveniles (>10,000 per 100 cm^3), extracted SCN eggs and juveniles (J2) were subjected to HG typing, while samples with less than required population density (< 10,000) were increased by inoculating susceptible cultivar Williams 82 in the greenhouse, before performing HG type testing.

2.2.2. HG type determination.

To determine HG types occurring in SD, HG type indicator lines PI 548402 (Peking, indicator #1), PI 88788 (#2), PI 90763 (#3), PI 437654(#4), PI 209332 (#5), PI 89772 (#6), PI 548316 (Cloud, #7), and a susceptible check (Williams 82) were used (Niblack et al. 2002). Seeds of each differential line were directly planted into the cone-tainer (3.8-cm diam. and 21-cm high), Stuewe and Sons, Inc., Tangent OR) filled with sterilized soil mixture (2 parts of sand and 1 parts of soil) by volume and each line (differentials and susceptible check) was replicated four times for each of the *H. glycines* populations. Inoculation of the HG type differential lines and the susceptible check with SCN was done following procedures by Niblack et al (2002). Inoculation was done at the time of planting (Fig. 2.3). After planting, cone-tainers (7 differential and one susceptible check) for each soil sample were put in a 2 gallon bucket filled with sand and were placed in the water bath in the greenhouse (Fig. 2.4). The water bath in the greenhouse was maintained at 27-28^o C temperature and day light length of 16 hours until 35 days. After 35 days, the cones were taken out of the bucket and soaked in water for 15 minutes and the plants were uprooted gently and sprayed with a strong stream of water to dislodge the SCN females from the roots (Fig. 2.6). The females were collected

in a 250-µm-pore sieve nested under a 710-µm-pore sieve. Released females were collected from each of the roots and counted for the determination of female index. The number of females present on the roots of each of the differential lines and susceptible line were counted and used for calculating of the female index. The female index for each line was calculated as follows:

$$\text{Female Index} = \frac{\text{Average no.of cysts found on differential line}}{\text{Average no.of cysts found on susceptible line}} * 100$$

Female indices equal to or greater than 10% on any line was assigned as that HG type. To use Williams 82 as susceptible check in place of Lee 74, we tested for the difference in the number of females on the roots of these two susceptible checks. We did not detect significant differences in the number of females in the roots ($P \leq 0.05$) between Williams 82 and Lee 74 (Table 2.1), hence we used Williams 82 because of better germination than Lee 74.

2.2.3. Data analysis.

Data were analyzed using SAS 9.3 (SAS Institute, Cary, NC). General linear model (GLM) was used to obtain descriptive statistics for the SCN population of each of the soybean differentials. Pearson's product moment correlation (CORR) was used for determining association between the female indices for differential lines. Average number of cysts from four replications were used for the calculation of female index for each soybean line.

Fig. 2.1. Mechanical elutriator used for extraction of females (cysts) from soil samples collected from the soybean fields.

Fig. 2.2. Crushed cyst showing eggs and juveniles which were used for the inoculation to soybeans plants

Fig. 2.3. Inoculation by eggs and juveniles to soybean differentials and susceptible checks after soybean planting.

Fig. 2.4. Experimental setup in the greenhouse for both HG type testing where the plants were kept for 35 days in controlled environment.

Fig. 2.5. Soybean roots showing SCN females (Cysts) after 35 days of inoculation.

2.3. Results

SCN prevalence. Out of the 250 samples collected from the different counties previously known to be infested with SCN, eighty-two (33%) were positive for SCN. These came from 16 counties out of 28 counties sampled. Combined egg and juvenile counts from four replications of each samples ranged from 200 to 65,200 per 100 cm^3 of soil (Table 2.2). Differences in the population densities of the nematode were found between the samples within a county. Most of the SCN positive counties were from southeast part of the state (Fig. 1.2). The highest average SCN population density was found in Moody County, followed by Union and Roberts Counties.

Of the 82 samples that were positive for *H. glycines*, 73 samples had sufficient eggs and juveniles or were increased to obtained sufficient eggs and juveniles to

conduct HG type tests. Nine samples did not have sufficient SCN numbers even after increase on Williams 82 in the greenhouse.

SCN reproduction on differential lines. Peking (HG type indicator line #1) had three SCN populations reared on it with FI of 48, 47 and 44%. These came from Turner and Brookings counties (Table 2.3). This line had only two other SCN populations with FI of 20% (from Deuel County) and 10% (from Bon Homme County). PI 88788 (HG type indicator line #2) had the second highest number of SCN populations with FI>10%. The highest FI among SCN populations reared on PI 88788 of 65% was from Clay County. The only other high SCN population had an FI of 41% on PI 88788, the rest of the SCN populations had between 10 and 19% for this indicator line. PI 90736 (HG type indicator line #3) had three SCN populations with >10% FI (20, 31 and 35%). None of the SCN populations tested on PI 437654 (HG type indicator line #4) had more than 10% FI, with the highest FI on this indicator line being 1.7%. On PI 209332 (HG type indicator line #5), three SCN populations had FI of 33, 23 and 21%, and rest of the SCN populations had a FI of >10% but < 19%. PI 89722 (HG type indicator line #6) had three SCN populations with FI of 61%, 31%, (both from Brookings county) and 27% (from Turner county). PI Cloud (HG type indicator line #7) had the highest number of SCN populations with FI >10%. The majority of the populations had FI between 10 and 20%. Four SCN populations had FI between 37 and 51% on this PI line.

Most of the *H. glycines* populations reproduced on all the differential lines, that is, none of the differentials were 100% immune. However, PI 437654 (HG type

indicator line #4) had the lowest FI. Overall, 63% of SCN population tested had FI ≥10% on Cloud, 25% on PI 88788, 19% on PI 209332, 7% on Peking, 4% on PI 90736, and 4% on PI 89722 (Table 2.4).

HG type determination. Among the SCN populations tested, eight different HG types were found in South Dakota: HG type 0, 1, 2, 7, 2.7, 5.7, 1.3.6, and 2.5.7. (Table 2.5). HG type 7 (≥10% FI on this line, HG type indicator line #7) was the most predominant (36%) followed by HG type 0 (29%), and HG type 2.5.7 (16%). These HG types collectively accounted for 80 % of *H. glycines* population in South Dakota in the year of 2013 and 2014 (Table 2.2, 2.6). The diversity of the *H. glycines* populations varied between and within the counties. Brookings, Clay, Turner, and Union county had more diverse SCN population than any other counties (Table 2.7).

HG type 7 was the most predominantly detected phenotype alone, followed by HG type 2 and 1. Although several SCN populations had FI >10% on HG type indicator line #5, these populations had also FI >10 on HG type indicator line #2 and HG type indicator line #7. Similarly, SCN populations that had FI >10% on HG type indicator line #6 had also FI>10% on HG type indicator line #3 and HG type indicator line #1. There was also a tendency for HG type 2 to be found with HG type indicator line #5 and/or #7. A positive correlation ($P \leq 0.05$) was found between the female indices of soybean differential lines used in the HG type experiment. Differential lines PI 88788, PI 209332, and cloud were correlated with each other and Peking, PI 90736, and PI 89722 were also correlated (Table 2.7).

2.4. Discussion

The first time of SCN detection in South Dakota was observed in 1995 in Union county (Smolik et al. 1996), since then, the number of counties with SCN have increased to 29 (Byamukama et al. 2015). This shows the expanded risk of SCN in South Dakota. In this study high level of SCN infestation was observed in southeast part of South Dakota. Although all the soil samples were collected from counties previously detected with SCN, not all the counties were found positive for SCN in our study, probably due to low SCN prevalence in these counties. Our results showed that SCN populations in soil samples collected in 2013 and 2014 had total of eight different HG types namely 0, 1, 2, 7, 2.7, 5.7, 1.3.6, and 2.5.7. HG types 0, 2.5.7, and 7 were the most prevalent among the different HG types suggesting that most of the *H. glycines* populations in the collected samples are virulent on PI 548316, PI 88788, and PI 209332. Similar results were observed in Minnesota and Kentucky, where PI 548316 was found to be most supportive for SCN reproduction. However, in Missouri and Kansas, HG type 2 (virulent on PI 88788) was most prevalent compared to other HG types (Hershman et al. 2008; Mitchum et al. 2007; Niblack et al. 2003; Rzodkiewicz 2010; Zheng et al. 2006). These results suggest that some of the SCN populations in South Dakota have adapted to the commonly used SCN resistance source PI 88788 and there is a risk further adapted SCN populations with the continued use of this resistance.

The frequencies of virulence phenotypes varied among the counties. SCN populations in some counties such as Brookings, Turner, Union, and Clay had more than 3 HG types detected. This may be due partly to the numbers of soil samples tested from those counties and longer history of SCN infestation. Moreover, these counties

may be planting resistant cultivars hence they are likely to have high SCN diversity as SCN adapts to SCN resistant cultivars (Kim et al. 1997).

We observed a significant correlation for FI between HG type indicator line #1 (PI 548402), HG type indicator line #3 (PI 90763), and HG type indicator line #6 (PI 89772). And a correlation between HG type indicator line #2 (PI 88788), HG type indicator line #5 (PI 209332), and HG type indicator line #7 (PI 89772). Similar results have been reported for two HG types 1.3.6 and 2.5.7 (Colgrove and Niblack 2008).

The available resistance sources for SCN are categorized into two groups: Peking and PI 88788 groups based on their different resistance response. HG type indicator line # 3, (PI 90763) and HG type indicator line #6 (PI 89772) are considered part of the Peking group and their resistance induces early plant tissue response resulting in necrosis. HG type indicator lines #2 (PI 88788), #5 (PI 209332), and #7 (PI 548316) are considered as PI 88788 group and their resistance induces slower nuclear degradation of plant cells at the nematode feeding site (Colgrove and Niblack 2008: Kim et al. 1987). These groups indicate the risk of using a similar type of resistance source. Rotating within sources of resistance may prolong the adaption of SCN on these lines.

All the differential lines showed some level of SCN adaptation except PI 437654, which was the only differential line that showed 100% resistance to all the populations of *H. glycines* tested in this study. Due to the high level of resistance to most of the population of *H. glycines* in the United States, PI 437654 was used for the developing resistant cultivar Hartwig (Anand 1992). However, after few generations, *H.*

glycines populations adapted to the resistance varieties derived from line PI 437654 (Colgrove and Niblack 2008). This underscores the need for an integrated approach to effectively manage SCN as no one method can sustainably manage SCN populations within a field.

Considering frequency of *H. glycines* adapted on soybean differential lines, Cloud may not be preferred for breeding for SCN resistance because of higher adaptability (more than about 63% FI). PI 88788 and Peking had 25% and 7% respectively of *H. glycines* populations tested with FI≥10%, indicating good level of resistance to most *H. glycines* populations tested. While these sources of resistance can still be used in SCN management, rotation with nonhost crop, rotation between the different resistant cultivars derived from different resistance sources is highly recommended. Continuous monitoring for the incidence and determining of the HG types of *H. glycines* population for incidence and virulence changes is necessary for sustainable SCN management.

Literature cited

Acharya, K., Byamukama, E., and Tande, C. 2014. The status of soybean cyst nematode, *Heterodera glycines*, in South Dakota. Phytopathology. 104:Sx

Anand, S. 1992. Registration of 'Hartwig'soybean. Crop Sci. 32:1069-1070.

Auwal, H. M., Wang, H., Li, L., Kakar, K.U., and Zheng, J. 2014. Utilization of plant materials for control of soybean cysts nematode. Soil and Plant Sci. 64:392-397.

Byamukama, E., Acharya, K., and Tylka, G. L. 2015. Determning *Heterodera glycines* HG types to improve soybean cyst nematode management in South Dakota. Phytopathology 105: Sx.

Byrd Jr, D., Barker, K., Ferris, H., Nusbaum, C., Griffin, W., Small, R., and Stone, C. A. 1976. Two semi-automatic elutriators for extracting nematodes and certain fungi from soil. J. Nematol. 8:206-212.

Chang, W.S., Lee, H.I., and Hungria, M. 2015. Soybean Production in the Americas. In: Lugtenberg, B.(Eds). Principles of Plant-Microbe Interactions. 393-400, Springer, US.

Chen, S., and Liu, X. 2005. Control of the soybean cyst nematode by the fungi, *Hirsutella rhossiliensis* and *Hirsutella minnesotensis* in greenhouse studies. Biol. Control. 32:208-219.

Colgrove, A., and Niblack, T. 2008. Correlation of female indices from virulence assays on inbred lines and field populations of *Heterodera glycines*. J. Nematol. 40:39-45.

Concibido, V. C., Diers, B. W., and Arelli, P. R. 2004. A decade of QTL mapping for cyst nematode resistance in soybean. Crop Sci.44:1121-1131.

Conley, S., Gaska, J., Pedersen, P., and Esker, P. 2011. Soybean yield and *Heterodera glycines* response to rotation, tillage, and genetic resistance. Agron. J. 103:1604-1609.

De Bruin, J. L., and Pedersen, P. 2008. Soybean cultivar and planting date response to soil fumigation. Agron. J. 100:965-970.

Faghihi, J., and Ferris, J. 2000. An efficient new device to release eggs from *Heterodera glycines*. J. Nematol. 32:411-413.

Frye, J. W. 2009. Efficacy of novel nematicides seed treatments for the control of *Heterodera glycines* in soybean production. http://www.lib.ncsu.edu/resolver/1840.16/46.

Grabau, Z. 2013. Management strategies for control of soybean cyst nematode and their effect on the nematode community. Retrieved from the University of Minnesota Digital Conservancy, http://purl.umn.edu/157428.

Hartman, G. L., West, E. D., and Herman, T. K. 2011. Crops that feed the world. Soybean worldwide production, use, and constraints caused by pathogens and pests. Food Security 3:5-17.

Hershman, D., Heinz, R., and Kennedy, B. 2008. Soybean cyst nematode, *Heterodera glycines*, populations adapting to resistant soybean cultivars in Kentucky. Plant Dis. 92:1475-1475.

Holcomb, R. 2012. Agricultural Statistics 2011. Government Printing Office.

Kim, D., Riggs, R., Robbins, R., and Rakes, L. 1997. Distribution of races of *Heterodera glycines* in the central United States. J. Nematol. 29:173-179.

Kim, Y. H., Riggs, R. D., and Kim, K. S. 1987. Structural changes associated with resistance of soybean to *Heterodera glycines*. J. Nematol. 19:177-187.

Koenning, S. R., and Wrather, J. A. 2010. Suppression of soybean yield potential in the continental United States by plant diseases from 2006 to 2009. Plant Health Prog. http://dx.doi.org/10.1094/PHP-2010-1122-01-RS.

Li, J., Todd, T. C., Lee, J., and Trick, H. N. 2011a. Biotechnological application of functional genomics towards plant-parasitic nematode control. Plant Biotech. J. 9:936-944.

Li, Y. H., Qi, X. T., Chang, R., and Qiu, L. J. 2011b. Evaluation and utilization of soybean germplasm for resistance to cyst nematode in China. Soybean molecular aspects of breeding. Intech Publishers:373-396.

MacGuidwin, A. 2012. Distribution of SCN HG types in Wisconsin. 2011, Proc. of the 2012 Wisconsin Crop Management Conference, Vol. 51:107.

Mitchum, M. G., Wrather, J. A., Heinz, R. D., Shannon, J. G., and Danekas, G. 2007. Variability in distribution and virulence phenotypes of *Heterodera glycines* in Missouri during 2005. Plant Dis. 91:1473-1476.

Niblack, T. 2005. Soybean cyst nematode management reconsidered. Plant Dis. 89:1020-1026.

Niblack, T., Wrather, J., Heinz, R., and Donald, P. 2003. Distribution and virulence phenotypes of *Heterodera glycines* in Missouri. Plant Dis. 87:929-932.

Niblack, T., Arelli, P., Noel, G., Opperman, C., Orf, J., Schmitt, D., Shannon, J., and Tylka, G. 2002. A revised classification scheme for genetically diverse populations of *Heterodera glycines*. J. Nematol. 34:279-288.

Noel, G. R. 1986. The Soybean Cyst Nematode. in: Lamberti, F., Taylor, C.E. (Eds), Cyst nematodes. Springer, US. P: 257-268.

Oyekanmi, E. O., and Fawole, B. 2010. 15 Nematodes of Soybean and Their Management in Singh, G. (Ed.). (2010). The soybean: botany, production and uses. CABI.

Riggs, R. 1977. Worldwide distribution of soybean-cyst nematode and its economic importance. J. Nematol. 9:34-39.

Rzodkiewicz, P. A. 2010. Characterization of soybean cyst nematode diversity in Kansas. Kansas State University.

Schmitt, D. P., Wrather, J. A., and Riggs R. D. (eds.) 2004. Biology and Management of Soybean Cyst Nematode, Second Edition. Walsworth Publishing Company, Marceline, MO

Shannon, J. G., Arelli, P. R., Young, L.D. 2004. Breeding for Soybean Cyst Nematode Resistance. In: Schmitt, Wrather, Riggs, editors. Biology and Management of Soybean Cyst Nematode. Second Edition. p. 155-180.

Smolik, J., Jones, J., Gallenberg, D., and Gille, J. 1996. First report of *Heterodera glycines* on soybean in South Dakota. Plant Dis. 80:224

Tylka, G. L., and Marett, C. C. 2014. Distribution of the soybean cyst nematode, *Heterodera glycines*, in the United States and Canada: 1954 to 2014. Plant Health Progress. Doi: 10.1094/PHP-BR-14-0006.

Tylka, G. L., and Mullaney, M. P. 2015. Soybean cyst nematode-resistant soybean varieties for Iowa. Iowa State University, University Extension, Online. http://www.plantpath.iastate.edu/tylkalab/files/page/files/2015%20ISU%20SCN-Resistant%20Variety%20Trials.pdf.

Wang, J., Niblack, T., Tremain, J., Wiebold, W., Tylka, G., Marett, C., Noel, G., Myers, O., and Schmidt, M. 2003. Soybean cyst nematode reduces soybean yield without causing obvious aboveground symptoms. Plant Dis. 87:623-628.

Winstead, N., Skotland, C., and Sasser, J. 1955. Soybean cyst nematode in North Carolina. Plant Dis. Reporter 39:9-11.

Wrather, J., and Koenning, S. 2009. Effects of diseases on soybean yields in the United States 1996 to 2007. Online. Plant Health Progress doi:10.1094/PHP-2009-0401-01-RS.

Wu, H., Masler, E. P., Rogers, S. T., Chen, C., and Chitwood, D. J. 2014. Benzyl isothiocyanate affects development, hatching and reproduction of the soybean cyst nematode *Heterodera glycines*. Nematology 16:495-504.

Young, L. 1996. Yield loss in soybean caused by *Heterodera glycines*. J. Nematol. 28:604-607.

Zheng, J., Li, Y., and Chen, S. 2006. Characterization of the virulence phenotypes of *Heterodera glycines* in Minnesota. J. Nematol. 38:383-390.

Table 2.1. Comparing number of *Heterodera glycines* reproduction (female cysts) on two susceptible soybean cultivar checks Williams 82 and Lee 74.

	SCN females (cysts)	
Rep	**Williams 82**	**Lee 74**
1	145	160
2	158	112
3	183	131
4	167	123
5	188	143
6	125	160
7	120	98
8	160	193
Average	156	140

t-value = 1.133736, *P*-value= 0.137973. Null hypothesis of no difference between the two samples is accepted, indicating that the mean number of cysts on two susceptible checks Williams 82 and Lee 74 in controlled greenhouse conditions.

Table 2.2. Frequency and population densities of *Heterodera glycines* in the samples collected from different counties of South Dakota during 2013 and 2014.

County	Total soil samples [a]	Positive samples % [b]	Maximum no. of eggs and juveniles[c]	Average no. eggs and juveniles [d]
Bon Homme	8	37.5	19,900	10,267
Brookings	13	46.2	12,000	6,875
Clay	21	71.4	65,200	10,827
Davison	8	25.0	2,300	2,300
Day	5	20.0	10,000	10,000
Deuel	10	50.0	16,850	4,850
Grant	10	20.0	6,550	7,425
Lake	3	33.3	350	350
Lincoln	14	64.3	8,800	2,711
McCook	8	25.0	7,600	4,175
Minnehaha	8	50.0	1,100	738
Moody	12	50.0	28,600	17,250
Roberts	8	25.0	23,750	12,775
Turner	15	66.7	7,100	2,920

Union	12	84.6	50,000	15,042
Yankton	8	37.5	1,600	1,233

[a] Total number of soil samples collected.

[b] Percent of samples positive for *H. glycines.*

[c] Maximum number of *H. glycines* eggs/100 cm^3 of soil.

[d] Average number of eggs and juveniles/100 cm^3 of soil in positive soil samples from each county.

Table 2.3. *Heterodera glycines* types in soils samples collected from different counties of South Dakota during 2013 and 2014.

County	Female index [a]							Females on William 82 [b]	HG type
	1 (Peking)	2 (PI88788)	3 (PI90736)	4 (PI437654)	5 (PI209332)	6 (PI89722)	7 (Cloud)		
Bon Homme	1.3	4	0.5	0.5	1.3	0.2	10.7	143	**7**
Bon Homme	0.4	1.1	0	0.2	1.1	0.4	3.5	142	**0**
Bon Homme	10.3	0.1	2.6	0.0	0.0	0.1	6.1	172	**1**
Brookings	0	3.4	0	0	3.8	0.7	11.7	303	**7**
Brookings	46.9	6.5	31	1.7	1.8	31.1	8.5	176	**1.3.6**
Brookings	47.8	4.1	35.4	1	4.8	61.4	3.7	269	**1.3.6**
Brookings	0	9.5	0.3	0	8.4	0	36	92	**7***
Brookings	1.7	11.1	1.9	0.1	11.9	1.9	26.1	189	**2.5.7**
Brookings	0	12.7	0.1	0.2	8.3	0.2	37.3	456	**2.7**
Brookings	1.3	2.6	0	0	1.7	0	15.4	59	**7***
Clay	0.3	6.3	0	0	1.9	0.2	3.5	158	**0**
Clay	3	0.4	0	0.2	1.5	1.5	4.9	118	**0**
Clay	0.2	11.8	0.3	0	6.9	0.1	11.2	217	**2.7**
Clay	0.1	5	0	0.1	6.9	0.1	15.4	423	**7**
Clay	0.8	1.4	0.3	0.2	1.4	1.6	12.3	440	**7**
Clay	0.3	0.4	0.9	0	0	0.2	1.8	279	**0**
Clay	0.9	65.2	0	0	12.9	0	50.9	111	**2.5.7**
Clay	0.8	41.3	0.8	0.4	36	0	90.2	66	**2.5.7***
Clay	0	0	0	0	0	0	0	96	**0***
Clay	2.1	1.7	1.5	0	5.4	1	8.7	353	**0**
Clay	0	1.1	0	0	0.5	0.1	2.3	393	**0**
Clay	1	5.4	2	0	9.3	0.5	7.4	233	**0**
Davison	0	3.6	0	0	4	0.5	10.4	106	**7**
Davison	0	7.5	0	0	8	0	13.4	97	**7***
Day	0.6	21	0	0	18.5	0.2	49	131	**2.5.7**
Deuel	0.7	7.3	0.2	0	9.5	0	17.9	134	**7**
Deuel	3.3	5.2	0.9	0	3.5	2	13.1	114	**7**
Deuel	0.2	13	0.9	0	4.7	0.2	6.7	112	**2**

Deuel	19.5	2.5	8	0.5	0	3.5	1	50	**1***
Deuel	9.3	3.1	1.8	0.3	2.8	3.3	14.6	429	**7**
Grant	0	0	0	0	0	0	4.5	73	**0***
Grant	0	4.3	0	0	1.2	0	8.8	226	**0**
Lincoln	1	11.4	0.1	0	22.7	2.2	22.9	169	**2.5.7**
Lincoln	4.2	0.8	3.3	0.1	3.4	3.8	6.7	183	**0**
Lincoln	1	17.8	0	0	21.4	0.3	23	329	**2.5.7**
Lincoln	0.8	2	0	0.4	2.4	0.6	13.8	125	**7**
Lincoln	0.4	6.7	0.1	0.1	14.9	0	13.8	432	**5.7**
McCook	0	2.3	0	0	0	0	5.3	296	**0**
McCook	0	0.7	0	0	2	0.1	11	205	**7**
Minnehaha	1.2	0.7	0	0	1.4	0	7.7	107	**0**
Moody	4.8	11.2	0	0.2	13	0.1	18.8	154	**2.5.7**
Moody	3.6	2.2	0	0	10.3	0.7	10.3	192	**5.7**
Moody	0.7	2.2	0	0	1.7	0	5	100	**0**
Moody	0.0	1.5	0.0	0.0	2.5	0.0	19.4	188	**7**
Moody	7.4	19.2	1.7	0.2	10.6	5.1	23.9	249	**2.5.7**
Moody	0	3.7	0	0	4.6	0	21.8	328	**7**
Moody	0.1	10.1	0	0.1	4.6	0.1	12.9	404	**2.7**
Roberts	2.6	5.6	0.3	0.4	4.6	0.2	19.8	244	**7**
Roberts	3.6	10.3	3.1	0.2	10.5	2.1	10.1	221	**2.5.7**
Turner	1.2	1.7	0	0.5	6.5	0.2	13.2	100	**7**
Turner	0.2	1	0	0	0	0.2	4.3	153	**0**
Turner	0	8.5	0	0.1	5	0.1	15.3	237	**7**
Turner	0	0.3	0	0	0.4	0.2	0.8	358	**0**
Turner	0.5	17.7	0	0.1	12.4	0.1	24.3	343	**2.5.7**
Turner	0	0.7	0	0.1	0.7	0	2.6	246	**0**
Turner	44.2	1.8	20.2	0	0.7	26.6	9	191	**1.3.6**
Turner	0	0	0.2	0	0.2	0	1	156	**0**
Turner	7.9	4.5	0.5	0	2	0.2	13.8	401	**7**
Turner	0.4	2.0	0.0	0.0	3.3	0.2	14.2	127	**7**
Union	0.1	14.7	0	0	5.5	0.4	26.2	183	**2.7**
Union	6.9	7	2.8	0.2	4.7	6.8	16.8	202	**7**
Union	5.8	26.7	1.2	0.4	33.4	3.7	42.1	168	**2.5.7**
Union	1.3	1.7	0.2	0	0.8	0.2	1.9	159	**0**
Union	8.8	21	1.3	0.2	12.9	0.3	26.2	250	**2.5.7**
Union	4.3	3.3	0.4	0.2	3.1	0.8	13.5	250	**7**
Union	5.9	2.8	3.3	0.2	1.2	3.1	4.7	207	**0**

Union	0.3	2.1	0.2	0	3.4	0.2	16.4	154	**7**
Union	2.1	2.3	1.5	0.4	3.7	1.5	11.4	260	**5.7**
Union	0.2	10.5	0	0	7.9	0	22.4	145	**2.7**
Union	0.2	4.1	0.0	0.0	1.6	0.0	11.1	153	**7**
Yankton	0.2	1.1	0	0	1.1	1.1	5.2	140	**0**
Yankton	1.2	3.5	0	0	1.9	0.3	17.3	145	**7**
Yankton	0.3	4.9	0.2	0	2.5	0.2	14.6	152	**7**

[a] Female index = (mean number of females on differentia/mean number of females on Williams 82) x 100 (average of four reps)

[b] Female on Williams 82 = mean number of females on Williams 82.

*HG types where average number of cysts on Williams 82 was less than 100.

Table 2.4. Univariate analysis of female indices (FI) of 73 greenhouse populations of *H. glycines* that produced FI > 10 on soybean differential lines with resistance to *H. glycines.*

Soybean differential lines	**Percentage of populations having > 10% FI**	**Population with Female index (FI) > 10 %**			
		Minimum observed	**Maximum observed**	**Mean**	**Standard deviation**
1 (Peking)	6.8	10.3	47.8	33.7	17.5
2 (PI 88788)	24.66	10.1	65.2	19.2	13.8
3 (PI 90736)	4.1	20.2	35.4	28.8	7.8
4 (PI 437654)	0	0	0	0	0
5 (PI 209332)	19.1	10.3	36	17.2	8.4
6 (PI 89722)	4.1	26.6	61.4	39.7	19
7 (Cloud)	63	10.1	90.2	20.8	14.3

Table 2.5. Frequencies of *H. glycines* HG types in soil samples collected in 2013 and 2014 in South Dakota.

HG Type	**Number of Population**	**% Frequency**	**Females/plants on Williams 82[a]**
0	21	28.8	199 (73-393)
1	2	2.7	111 (50-172)
1.3.6	3	4.1	212 (176-269)
2	1	1.4	112 (112)
2.7	5	6.8	281 (145-456)
5.7	3	4.1	295 (192-432)
7	26	35.6	206 (59- 440)
2.5.7	12	16.4	198 (66-343)

[a] The mean values are mean of the number of females on Williams 82 and the number in the parenthesis is range.

Table 2.6. County wise distribution of HG types of *H. glycines* populations in the soil samples collected from different counties of South Dakota in the year 2013 and 2014.

County	**No. of population tested**	**HG types**
Bon Homme	3	0, 1, 7 (3)
Brookings	7	1.3.6, 2.5.7, 2.7, 7 (4)
Clay	12	0, 2.5.7, 2.7, 7 (4)
Davison	2	7 (1)
Day	1	2.5.7 (1)
Deuel	5	1, 2, 7 (3)
Grants	2	0 (1)
Lake*	0	nd
Lincoln	5	0, 2.5.7, 5.7 (3)
Mc Cook	2	0, 7 (2)
Minnehaha	1	0 (1)
Moody	7	0, 2.5.7, 2.7, 7 (4)
Roberts	2	7, 2.5.7 (2)
Turner	10	0, 1.3.6, 2.5.7, 7 (4)
Union	11	0, 2.5.7, 2.7, 5.7, 7 (5)
Yankton	3	0, 7 (2)

Number in the parenthesis refers to the number of *Heterodera glycines* populations found on that county.

* Lake County had not enough numbers of SCN eggs and juveniles for the HG type test.

nd Not determined.

Table 2.7. Correlation coefficient among soybean differential lines with resistance to *Heterodera glycines* based on female indices (FI)[a] from 73 population of South Dakota populations collected in 2013 and 2014.

Differential lines	**Peking**	**PI 88788**	**PI90736**	**PI437654**	**PI209332**	**PI89722**
PI 88788	-0.049 ns					
PI 90736	0.959 **	-0 .057 ns				
PI 437654	0.683**	0.045 ns	0.73108**			
PI 209332	-0.084 ns	0.705**	-0.088ns	0.069ns		
PI 89722	0.900**	-0.049 ns	0.958**	0.645**	-0.05287 ns	
Cloud	-0.124 ns	0.800**	-0.137ns	0.043ns	0.796**	-0.118 ns

[a] Female Index = (mean number of females on differential line/mean number of female on Williams 82) x100.

** = $P < 0.001$ (Pearson's correlation coefficient)

[c] ns = not significant $P > 0.05$

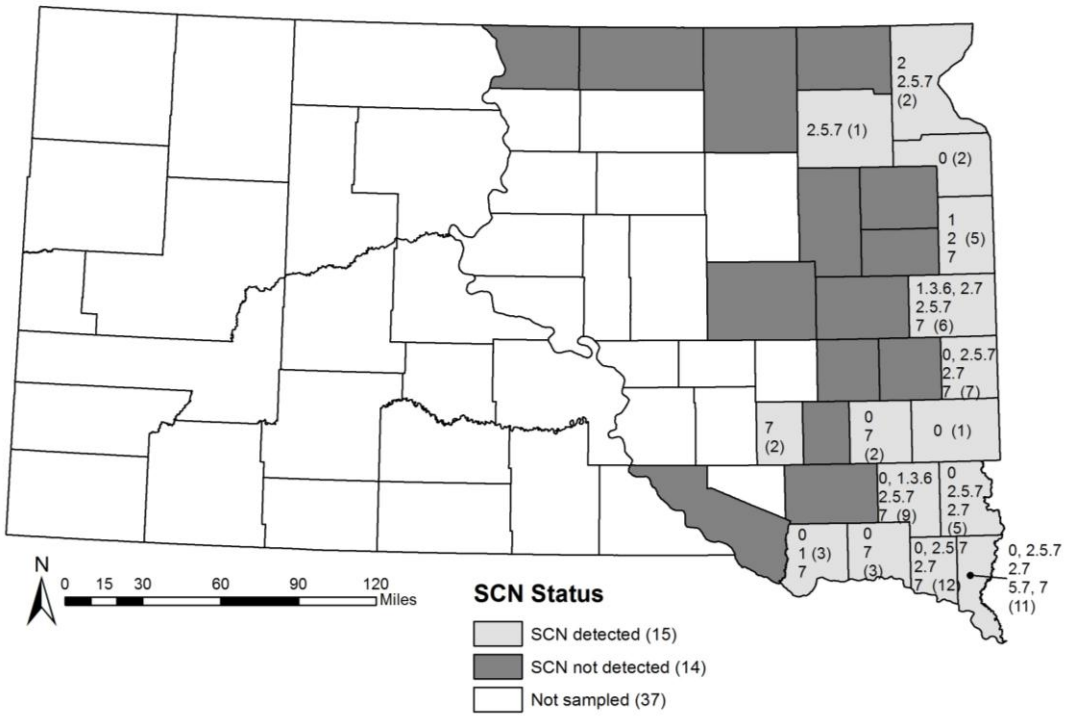

Fig. 2.6. Distribution of *Heterodera glycines* (HG) types in South Dakota for soil samples collected in 2013 and 2014. The number in parenthesis refers to the number of soil samples screened for HG types.

CHAPTER 3.

3. Assessment of Commercial Soybean Cultivars for Resistance against Prevalent *Heterodera glycines* Populations of South Dakota

Abstract

Soybean cyst nematode (SCN, *Heterodera glycines*) is the most important yield limiting factor of soybean production in South Dakota and the main management practice is the planting SCN resistant cultivars. The effectiveness of host resistance is often limited by the diversity of SCN populations. A greenhouse study was set up to determine the response of 34 SCN-resistant commercial soybean cultivars to three commonly found SCN HG types in South Dakota. To screen the soybean cultivars, SCN eggs and juveniles of prevalent HG types 0, 2.5.7, and 7 were each inoculated to 34 commercial soybean cultivars and also three differential lines PI 88788 (#2), PI 209332 (#5), and PI 548316 (#7) and two susceptible Lee 74 and Williams 82 as controls. Each of the treatment was replicated four times in complete randomized design and the experiment was repeated once. A female index (FI) was calculated based on the average number of females (cysts) found on each cultivar relative to the susceptible check after 35 days. Resistant reaction (R) for a cultivar was when FI was < 10%, moderately resistant (MR), FI =10-30%, moderately susceptible (MS), FI = 30-60% and FI >60% was classified as susceptible(S). Soybeans cultivars showed a varied response to *H. glycines* populations with 21 % showing resistance response, 67% showing moderate resistant, 10% showing moderately susceptible, and only 2% showing susceptible response. These results indicate that host resistance is effective in managing SCN in South Dakota. However, with almost all SCN resistant cultivars having similar source of SCN resistance genes, use of these cultivars

should be combined with crop rotation with nonhost crops and rotation within soybean cultivars for sustainable SCN management.

Key words; *Glycine max*, resistance, soybean cyst nematode, *Heterodera glycines*, HG type, cultivar screening.

3.1. Introduction

Soybean (*Glycine max*) is one of the important leguminous crop in the United States and is ranked second in terms of planted acres and productivity (Holcomb 2012). North America and South America alone contributes about 80% of the world soybean production (Chang et al. 2015). Yield limiting factors for soybean production includes both biotic and abiotic factors, and among the biological factors soybean cyst nematode (SCN) is ranked first (Hartman et al. 2011; Koenning and Wrather 2010).

Soybean cyst nematode, *Heterodera glycines* Ichinohe, is obligatory endo-parasitic nematode that infects soybean through roots and leads to poor soybean growth and development (Schmitt et al. 2004). It is widely distributed in the United States and neighboring countries. SCN was first reported in the U.S. in 1954 in North Carolina and has spread through Midwest and now has been identified most of the soybean field in the United States (Tylka and Marett 2014). In South Dakota, SCN was first detection in Union county in 1995 (Smolik et al. 1996) and currently has been found in 29 counties (mainly eastern-most part). Different management strategies have been used to manage soybean cyst nematode in different parts of the United States, however, host resistance and crop rotation are the most common and affordable methods. These methods also found to be effective than chemical control (Niblack et al. 2003; Oyekanmi and Fawole 2010; Schmitt et al. 2004).

The problem associated with the SCN management in South Dakota is little knowledge about the SCN resistant cultivars and virulence genotype of *Heterodera glycines* population. These are important for increasing soybean yield and durability of the resistance sources used for resistant cultivar breeding. Additionally, lack of the

information about the resistant cultivars source of resistance or races these cultivars are effective against in seed catalogs can limit producer choices (Niblack et al. 2009). Some greenhouse studies that screened resistance of commercial cultivars against the different HG type populations of SCN were performed in Illinois, Iowa, and Missouri. Cultivars labeled with resistance to specific races or HG types also showed different resistance reaction when they were assessed with traditional method (Faghihi 2006; Hershman et al. 2008; Koenning 2004; Tylka et al. 2015).

Although there is variability of resistance response of the commercial cultivars, yield benefit can still be obtained by planting resistant cultivars. A recent study done in Minnesota, planting of resistant soybean cultivar showed 28.4% yield benefit than susceptible cultivar (Chen et al. 2001). In South Dakota, resistant soybean cultivars showed 23 to 63% yield benefit in resistance cultivar as compared to susceptible cultivars (J. D. Smolik, *Unpublished data*).

Performance of the commercial cultivars against prevalent HG types in South Dakota can provide growers with information to guide them in selecting resistant cultivars. Our goal is to improve soybean cyst nematode management in South Dakota by using cultivars with good resistance to *H. glycines* populations. The objective of this study was to screen the SCN resistant commercial cultivars of different maturity group against prevalent HG type 0, 2.5.7, and 7, the most prevalent *H. glycines* populations present in South Dakota.

3.2. Materials and methods

A study to assess the commercial soybean cultivars for resistance against prevalent *Heterodera glycines* populations of South Dakota was conducted under controlled greenhouse conditions.

3.2.1. Cultivars selection

A total of 34 commercial soybean cultivars were selected from Asgrow, Prairie Brand, and Syngenta based upon the availability, relative maturity, and SCN resistance sources. Three differential lines PI 88788 (#2), PI 209332 (#5), and PI 548316 (#7) including two susceptible Lee 74 and Williams 82 were used as susceptible checks.

3.2.2. H. glycines populations

The prevalent HG types were selected based upon a study by (K. Acharya et al. *Unpublished*). HG types 0, 2.5.7, and 7 were used for screening for resistance because of their high prevalence in South Dakota. The populations of the three HG types were increased by inoculating susceptible cultivar Williams 82 in the greenhouse. Cysts for each HG type were processed by extraction of eggs and juveniles from cyst following procedures by Faghihi and Ferris (2000). Naturally infested field was selected and HG type was determined and confirmed to be HG type 0 at South East Research Farm (SERF) of South Dakota State University.

3.2.3. Cultivar assessment

3.2.3.1. Greenhouse study

Seeds of each line were directly planted into the cone filled with sterilized soil mixture (2 parts of sand and 1 part of soil) by volume. Extra seedlings were thinned from each container immediately after germination such that one seedling per cone was

left. Each line (commercial, differentials, and susceptible checks) was replicated four times for each HG type of the *H. glycines* populations. Eggs and juveniles of each HG type were counted under the scope (Faghihi and Ferris 2000), and each of the lines was inoculated with 3000 eggs and juveniles. Inoculation was done at the time of planting. After planting, cones were put in a 2 gallons bucket filled with sand and kept in the water bath in the greenhouse. The greenhouse was maintained at 27-28^{o} C and day length of 16 hours. After 35 days, the containers were taken out of the water bath and soaked in water for 15 minutes and then plants were uprooted gently and washed in tap shower to release the females (cysts). Released cysts were collected from each of the roots and counted for the determination of female index. The number of cysts present on the roots of each of the commercial, differential, and susceptible lines were counted and used for calculating the female index. The female index for each cultivar was calculated as follows.

$$\text{Female Index} = \frac{\text{Average no.of cysts found on commercial line}}{\text{Average no.of cysts found on susceptible line}} * 100$$

The labeling of the resistance for commercial cultivars was done by standard protocol developed by Schmitt and Shannon (1992)

3.2.3.2. Field study

The South Dakota State University Southeast Research Farm (SERF), was selected for field study after determining the population HG type in this field.

HG type 0 was confirmed by HG type experiment performed in the greenhouse. All of 34 SCN resistant commercial cultivars including susceptible cultivar Williams 82 were planted on the field infested with the soybean cyst nematode HG type 0. They were

planted in two row plots of 6 m length and 4 m width with row spacing was of 30 inches. Plant stand counts, yield, test weights, spring SCN and fall SCN numbers were determined for each plot. Soil samples were collected two times, first, at the time of planting to know the initial population density of each plot and second, after harvesting for the final population. Soil samples were collected by using standard soil sampling method for SCN and at least 12 cores were taken for each plot. The design of the experiment was randomized complete block (RCB) with four replications. Each soil samples were mixed well and 100 cm^3 of soil was taken for the extraction of SCN by using mechanical elutriator and eggs and juveniles were counted on the stereoscope by using nematode counting slide after grinding the cysts. A reproduction factor of *H. glycines* populations on each of the cultivar and susceptible check was calculated as follows.

$$\text{Reproduction Factor} = \frac{\text{No.of eggs and juveniles in soil of the cultivar at harvesting}}{\text{No.of eggs and juveniles in soil of the cultivar at planting}}$$

3.2.4. Data analysis

Data were analyzed with SAS 9.3 (SAS Institute, Cary, NC). Average number of cyst from four replications were used for the calculation of female index on each soybean lines. Analysis of variance was used to determine differences in number of cysts produced on each cultivars in the greenhouse study and reproduction factor and yield in the field study. Pearson's product moment correlations (CORR) was used for determining correlation between yield and final SCN population in the field after harvesting of soybean crop and also between the maturity groups of soybean cultivars and reproduction factor of *H. glycines* population on each cultivar.

3.3. Results

3.3.1. Greenhouse study

3.3.1.1. Reproduction of *H. glycines* population on commercial cultivar

In both greenhouse tests, a total of 34 commercial soybean cultivars and soybean differentials PI 88788 (#2), PI 209332 (#5), PI 548316 (#7) and Lee 74 and Williams 82 were planted (Table 3.2). Female indices for each cultivar were calculated with respect to susceptible check Williams 82 (Table 3.3 and 3.4) for both greenhouse tests.

In first greenhouse test, significant differences were observed in mean number of females produced on the cultivars tested for HG type 0, 7 and 2.5.7 ($P \leq 0.05$). Out of 34 cultivars tested with HG type 0, 19 cultivars were resistant, 13 were moderately resistant, 2 were moderately susceptible, and none of the cultivar showed susceptible reaction. For Hg type 7, out of 34 cultivars, one cultivar was resistant, 28 cultivars were moderately resistant, 5 cultivars were moderately susceptible, and one cultivar showed susceptible reaction. For HG type 2.5.7 populations, out the 34 cultivars, 2 cultivars were resistant, 30 cultivars were moderately resistant, one cultivar was moderately susceptible, and one cultivar showed susceptible reaction (Fig. 3.1).

In second greenhouse test, similar results as first run were observed in mean number of females produced on the cultivars tested for HG type 0, 7 and 2.5.7 ($P \leq 0.05$). Out of 34 cultivars tested for HG type 0, 17 cultivars were resistant, 15 were moderately resistant, one cultivar was moderately susceptible, and one cultivar showed susceptible reaction to HG type 0. For Hg type 7, out of 34 cultivars, 3 cultivars were resistant, 24 cultivars were moderately resistant, 6 cultivars were moderately

susceptible, and one cultivar showed susceptible reaction. For HG type 2.5.7, out the 34 cultivar, none of the cultivars showed resistant reaction, 28 cultivars were moderately resistant, 5 cultivars were moderately susceptible, and one cultivar showed susceptible reaction (Fig. 3.2).

The overall greenhouse results showed a varied response of SCN resistant cultivars to all three *H. glycines* populations with 21 % showing resistant response, 67% showing moderate resistant, 10% showing moderately susceptible and only 2% showing susceptible response (Fig. 3.3).

3.3.2. Field study

The resistance response of these cultivars in the field infested with HG type 0 showed varied reproduction factor ranged from 0.7 to 12.5. HG type 0 populations showed reproduction factor greater than 1 for most of the cultivars tested in the field (Table 3.5), but we did not detect the significant difference among the reproduction factors for the cultivars tested ($P > 0.05$). We did not see the significance difference in the yield of all 34 cultivars tested in field ($P > 0.05$). We did not see any correlation between the final SCN eggs and juveniles counts and yields ($P > 0.05$).

3.4. Discussion

The results obtained from the greenhouse assessment of commercial soybean cultivars for resistance against the prevalent HG types occurring in South Dakota indicated that most of the commercial cultivars showed resistant reaction to HG type 0 and moderately resistant reaction to HG type 2.5.7, and 7. Response of SCN resistant cultivar to *H. glycines* population suggested that the host resistance is effective in SCN

management in South Dakota. This finding agrees with other research studies in different states in the United States (Chen et al. 2001; Davis et al. 1996; Koenning 2004). However, a similar study done in Iran indicated that HG type 0 had increased reproduction on resistant cultivars (Heydari et al. 2009). Some of the cultivars tested showed susceptible reaction that might be due to continuous planting cultivars with the same source of resistance for a long period of time in the same field (Niblack et al. 2008).

Knowledge of SCN resistant source and *Heterodera glycines* HG types is very important in managing SCN, which facilitates the grower in selecting resistant cultivars for effective SCN management. Although the resistance source was same for all the cultivars (PI 88788), variation in the female indices was observed. This might be due to the differences in parents from which they were derived from. Some previous results suggested that the maturity group of the soybean cultivars also affects in the resistance and susceptible reaction in the field condition as illustrated in a research study by Koenning et al. (1993), but in our study, we did not detect any correlation between the maturity group and the resistance reaction against all the three *H. glycines* populations.

In the field study, we did not see any correlation between the final SCN population of HG type 0 and yield, this may be due to high variability of *H. glycines* population in the field and yield is a complex trait which is affected by different biotic and abiotic factors in the fields. Unfortunately, we weren't able to test for other two HG types, 7 and 2.5.7 populations in the field because of the unavailability of the fields naturally infested with these populations.

The results obtained from this study showed good resistant response to all the three *H. glycines* populations under greenhouse testing. Further research is needed to determine the response of commercial cultivars to other HG types and effect of SCN on yield components of commercial soybean cultivars.

Literature cited

Chang, W.S., Lee, H. I., and Hungria, M. 2015. Soybean Production in the Americas. Pages 393-400 in: Principles of Plant-Microbe Interactions. Springer, USA.

Chen, S., Porter, P., Orf, J., Reese, C., Stienstra, W., Young, N., Walgenbach, D., Schaus, P., Arlt, T., and Breitenbach, F. 2001. Soybean cyst nematode population development and associated soybean yields of resistant and susceptible cultivars in Minnesota. Plant Dis. 85:760-766.

Davis, E., Koenning, S., Burton, J., and Barker, K. 1996. Greenhouse evaluation of selected soybean germplasm for resistance to North Carolina populations of *Heterodera glycines*, *Rotylenchulus reniformis*, and *Meloidogyne species*. J. Nematol. 28:590-598

Faghihi, J. 2006. Soybean cyst nematode. Purdue University, Cooperative Extension Service.

Faghihi, J., and Ferris, J. 2000. An efficient new device to release eggs from *Heterodera glycines*. J. Nematol. 32:411-413.

Hartman, G. L., West, E. D., and Herman, T. K. 2011. Crops that feed the World 2. Soybean worldwide production, use, and constraints caused by pathogens and pests. Food Security 3:5-17.

Hershman, D., Heinz, R., and Kennedy, B. 2008. Soybean cyst nematode, *Heterodera glycines*, populations adapting to resistant soybean cultivars in Kentucky. Plant Dis. 92:1475-1475.

Heydari, R., Pourjam, E., Tanha, M. Z., And Safaei, N. 2009. Evaluation of Some Common Soybean Cultivars to The Major Type of The Soybean Cyst Nematode of Iran, *Heterodera glycines* HG Type 0. Iranian J. Plant Pathol. 44: 319-329.

Holcomb, R. 2012. Agricultural Statistics 2011. Government Printing Office.

Koenning, S. 2004. Resistance of soybean cultivars to field populations of *Heterodera glycines* in North Carolina. Plant Dis. 88:942-950.

Koenning, S. R., and Wrather, J. A. 2010. Suppression of soybean yield potential in the continental United States by plant diseases from 2006 to 2009. Plant Health Prog http://dx. doi. org/10.1094/PHP-2010-1122-01-RS.

Koenning, S., Schmitt, D., and Barker, K. 1993. Effects of cropping systems on population density of *Heterodera glycines* and soybean yield. Plant Dis. 77:780-786.

Niblack, T. L., Colgrove, A. L., Colgrove, K., and Bond, J. P. 2008. Shift in virulence of soybean cyst nematode is associated with use of resistance from PI 88788. Online. Plant Health Progress doi:10.1094/PHP-2008-0118-01-RS.

Niblack, T., Wrather, J., Heinz, R., and Donald, P. 2003. Distribution and virulence phenotypes of *Heterodera glycines* in Missouri. Plant Dis. 87:929-932.

Niblack, T., Tylka, G. L., Arelli, P., Bond, J., Diers, B., Donald, P., Faghihi, J., Ferris, V. R., Gallo, K., Heinz, R. D., Lopez Nicora, H., Von Qualen, R., Welacky, T., and Wilcox, J. 2009. A standard greenhouse method for assessing soybean cyst nematode resistance in

soybean: SCE08 (standardized cyst evaluation 2008). Online. Plant Health Progress doi: 10.1094/PHP-2009-0513-01-RV.

Oyekanmi, E. O., and Fawole, B. 2010. 15 Nematodes of Soybean and Their Management in Singh, G. (Ed.). (2010). The soybean: botany, production and uses. CABI.

Schmitt, D., and Shannon, G. 1992. Differentiating soybean responses to *Heterodera glycines* races. Crop Sci. 32:275-277.

Schmitt, D. P., Wrather, J. A., and Riggs R. D. (eds.) 2004. Biology and Management of Soybean Cyst Nematode, Second Edition. Walsworth Publishing Company, Marceline, MO.

Smolik, J. D., Jones, J. L., Gallenberg, D. L.,and Gille, J. P. 1996. First report of *Heterodera glycines* on soybean in South Dakota. Plant Dis. 80:224.

Tylka, G. L., Gebhart, G. D., Marett, C. C., and Mullaney, M. P., 2015. Evaluation of Soybean Varieties Resistant to Soybean Cyst Nematode in Iowa in 2015. Iowa State University University Extension.

Table 3.1. Classification of the soybean cultivars for resistance to the soybean cyst nematode was done by evaluating the female index on each of the cultivar (Schmitt and Shannon 1992).

Female Index (%)	**Rating**	**label**
< 10	Resistant	R
10 -30	Moderately Resistant	MR
31-60	Moderately Susceptible	MS
>60	Susceptible	S

Table 3.2. List of the cultivars used for screening for resistance against prevalent HG types of South Dakota.

Name of cultivars	Relative maturity	Source of SCN resistance	Company/ industry
AG0835	0.8	R3	Asgrow
AG0934	0.9	R3	Asgrow
AG1135	1.1	R3	Asgrow
AG1234	1.2	R3	Asgrow
AG1435	1.4	R3	Asgrow
AG1733	1.7	R3	Asgrow
AG1935	1.9	R3	Asgrow
AG2035	2	R3	Asgrow
AG2136	2.1	R3	Asgrow
AG2336	2.3	R3	Asgrow
AG2433	2.4	MR3	Asgrow
AG2535	2.5	R3	Asgrow
AG2636	2.6	R3	Asgrow
AG2733	2.7	MR3	Asgrow
AG2836	2.8	R3	Asgrow

AG2935	2.9	R3	Asgrow
PB-0598R2	0.3	PI 88788	Prairie Brand
PB-0676R2	0.6	PI 88788	Prairie Brand
PB-0777R2	0.7	PI 88788	Prairie Brand
PB-0863R2	0.8	PI 88788	Prairie Brand
PB-0879R2	0.8	Resistant	Prairie Brand
PB-0966R2	0.9	PI 88788	Prairie Brand
PB-1147R2	1.1	PI 88788	Prairie Brand
PB-1234R2	1.2	PI 88788	Prairie Brand
PB-1466R2	1.4	PI 88788	Prairie Brand
PB-1586R2	1.5	PI 88788	Prairie Brand
PB-1794R2	1.7	PI 88788	Prairie Brand
PB-1822R2	1.8	PI 88788	Prairie Brand
PB-1956R2	1.9	PI 88788	Prairie Brand
PB1947R2	1.9	PI 88788	Prairie Brand
S09-K4	0.9	R3, MR14/ PI 88788	Syngenta
S06-H5	0.6	R3, MR14/ PI 88788	Syngenta
S22-S1	2.2	R3, MR14/ PI 88788	Syngenta
S26-P3	2.6	R3, MR14/ PI 88788	Syngenta

PI 88788 **(2)**	3	PI 88788	Differential
PI 209332 **(5)**	4	PI 209332	Differential
PI 548316 **(7)**	3	PI 548316	Differential
Lee 74	-	Susceptible	Susceptible
Williams 82	3.9	Susceptible	Susceptible

Table 3.3. Number of cysts, Female index and resistance classification for commercial soybean cultivars inoculated with soybean cyst nematode HG types 0, 7, and 2.5.7 under greenhouse conditions during the first run.

	HG type 0			**HG type 7**			**HG type 2.5.7**		
Cultivars	**No. of cysts[a]**	**Female index (%)**	**Label**	**No. of cysts[a]**	**Female index (%)**	**Label**	**No. of cysts[a]**	**Female index (%)**	**Label**
AG0835	17	10.41	MR	49	30.63	MS	35	23.61	MR
AG0934	15	9.04	R	30	18.44	MR	25	16.53	MR
AG1135	18	10.72	MR	52	32.66	MS	31	20.91	MR
AG1234	25	15.01	MR	54	33.44	MS	47	31.87	MS
AG1435	18	10.87	MR	24	14.84	MR	16	10.79	MR
AG1733	11	6.58	R	25	15.31	MR	21	13.83	MR
AG1935	12	7.04	R	19	12.03	MR	32	21.25	MR
AG2035	10	5.82	R	28	17.34	MR	23	15.68	MR
AG2136	7	4.44	R	17	10.63	MR	21	14.17	MR
AG2336	13	7.81	R	18	11.25	MR	24	16.36	MR
AG2433	11	6.89	R	31	19.38	MR	23	15.18	MR
AG2535	21	12.71	MR	33	20.31	MR	25	16.86	MR
AG2636	23	13.94	MR	49	30.78	MS	35	23.78	MR
AG2733	22	13.32	MR	46	28.59	MR	34	22.60	MR
AG2836	8	5.05	R	25	15.47	MR	24	16.02	MR
AG2935	10	5.97	R	31	19.06	MR	23	15.35	MR
PB-0598R2	13	7.66	R	47	29.22	MR	39	26.14	MR
PB-0676R2	15	9.19	R	50	30.94	MR	43	29.01	MR
PB-0777R2	17	10.41	MR	34	21.09	MR	26	17.20	MR
PB-0863R2	68	41.65	MS	170	106.09	S	125	84.15	S
PB-0879R2	30	18.38	MR	41	25.63	MR	56	37.94	MS
PB-0966R2	24	14.85	MR	37	23.28	MR	39	25.97	MR

PB-1147R2	5	2.76	R	17	10.63	MR	15	9.95	R
PB-1234R2	16	9.95	R	41	25.47	MR	27	17.88	MR
PB-1466R2	25	15.16	MR	52	32.66	MS	44	29.85	MR
PB-1586R2	14	8.73	R	30	18.59	MR	12	8.26	R
PB-1794R2	16	9.49	R	31	19.53	MR	29	19.39	MR
PB-1822R2	25	15.31	MR	35	21.88	MR	43	28.84	MR
PB-1956R2	26	15.93	MR	26	16.25	MR	33	22.43	MR
PB1947R2	15	9.19	R	27	16.88	MR	39	25.97	MR
S09-K4	56	34.15	MS	25	15.31	MR	35	23.27	MR
S06-H5	13	7.66	R	5	2.97	R	26	17.71	MR
S22-S1	8	5.05	R	34	20.94	MR	20	13.49	MR
S26-P3	16	9.80	R	32	20.16	MR	33	22.43	MR
PI 88788 **(2)**	4	2.30	R	25	15.78	MR	21	14.17	MR
PI 209332 **(5)**	2	1.38	R	13	8.13	R	22	14.84	MR
PI 548316 **(7)**	6	3.83	R	39	24.38	MR	35	23.61	MR

[a] Average of four replications.

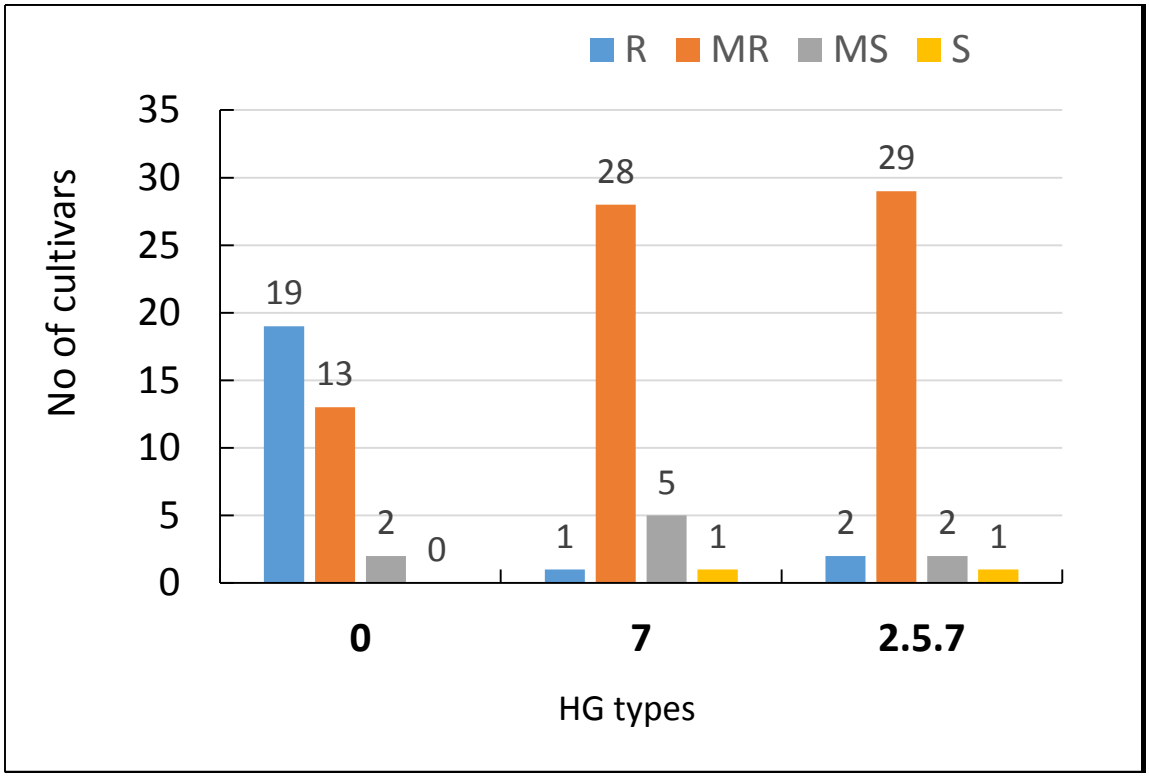

Fig. 3.1. Frequency of cultivars assessed with resistant (<10% FI), moderately resistant (FI= 10-30 %), moderately susceptible (FI= 31-60%), or susceptible (FI > 60%) reaction against three types of *Heterodera glycines* populations, under greenhouse conditions during the first run.

Table 3.4. Number of cysts, Female index and resistance classification for commercial soybean cultivars inoculated with soybean cyst nematode HG types 0, 7, and 2.5.7 under greenhouse conditions during the second run.

	HG type 0			**HG type 7**			**HG type 2.5.7**		
Cultivars	**No. of cysts [a]**	**Female index (%)**	**Label**	**No. of cysts [a]**	**Female index (%)**	**Label**	**No. of cysts [a]**	**Female index (%)**	**Label**
AG0835	22	11.37	MR	50	21.47	MS	50	20.95	MR
AG0934	13	6.39	R	20	8.55	R	49	20.63	MR
AG1135	20	10.22	MR	31	13.14	MS	67	28.25	MR
AG1234	22	10.99	MR	28	11.75	MS	46	19.47	MS
AG1435	12	6.01	R	36	15.17	MR	49	20.53	MR
AG1733	15	7.79	R	26	11.22	MR	30	12.59	MR
AG1935	14	6.90	R	28	11.86	MR	45	19.15	MR
AG2035	10	5.11	R	29	12.39	MR	38	16.19	MR
AG2136	12	6.26	R	22	9.19	R	35	14.71	MR
AG2336	13	6.39	R	29	12.39	MR	44	18.73	MR
AG2433	15	7.67	R	28	11.75	MR	50	21.16	MR
AG2535	22	11.37	MR	45	19.34	MR	76	32.17	MS
AG2636	15	7.79	R	78	33.12	MS	74	31.43	MS
AG2733	13	6.52	R	45	19.34	MR	60	25.29	MR
AG2836	22	11.12	MR	37	15.92	MR	76	31.96	MS
AG2935	8	3.96	R	37	15.81	MR	62	26.03	MR
PB-0598R2	13	6.39	R	45	19.23	MR	51	21.69	MR
PB-0676R2	25	12.78	MR	35	15.06	MR	57	24.23	MR
PB-0777R2	17	8.43	R	29	12.39	MR	85	35.77	MS
PB-0863R2	125	63.88	S	238	101.82	S	153	64.66	S
PB-0879R2	65	33.09	MS	120	51.39	MS	57	24.13	MS
PB-0966R2	27	13.54	MR	46	19.55	MR	60	25.29	MR

PB-1147R2	12	6.13	R	14	5.88	R	25	10.48	MR
PB-1234R2	25	12.78	MR	31	13.03	MR	51	21.59	MR
PB-1466R2	34	17.38	MR	32	13.57	MS	56	23.70	MR
PB-1586R2	30	15.08	MR	32	13.57	MR	46	19.37	MR
PB-1794R2	25	12.65	MR	30	12.61	MR	44	18.41	MR
PB-1822R2	39	19.68	MR	33	13.89	MR	57	23.92	MR
PB-1956R2	26	13.29	MR	37	15.81	MR	26	11.01	MR
PB-1947R2	15	7.54	R	30	12.93	MR	45	18.84	MR
S09-K4	16	8.18	R	34	14.53	MR	26	11.01	MR
S06-H5	19	9.45	R	25	10.68	MR	31	13.12	MR
S22-S1	21	10.73	MR	28	11.86	MR	35	14.81	MR
S26-P3	13	6.52	R	41	17.31	MR	42	17.78	MR
PI 88788 **(2)**	11	5.49	R	21	8.76	R	43	18.20	MR
PI 209332 **(5)**	4	2.04	R	38	16.13	MR	44	18.52	MR
PI 548316 **(7)**	14	6.90	R	35	14.85	MR	54	22.72	MR

[a] Average of four replications

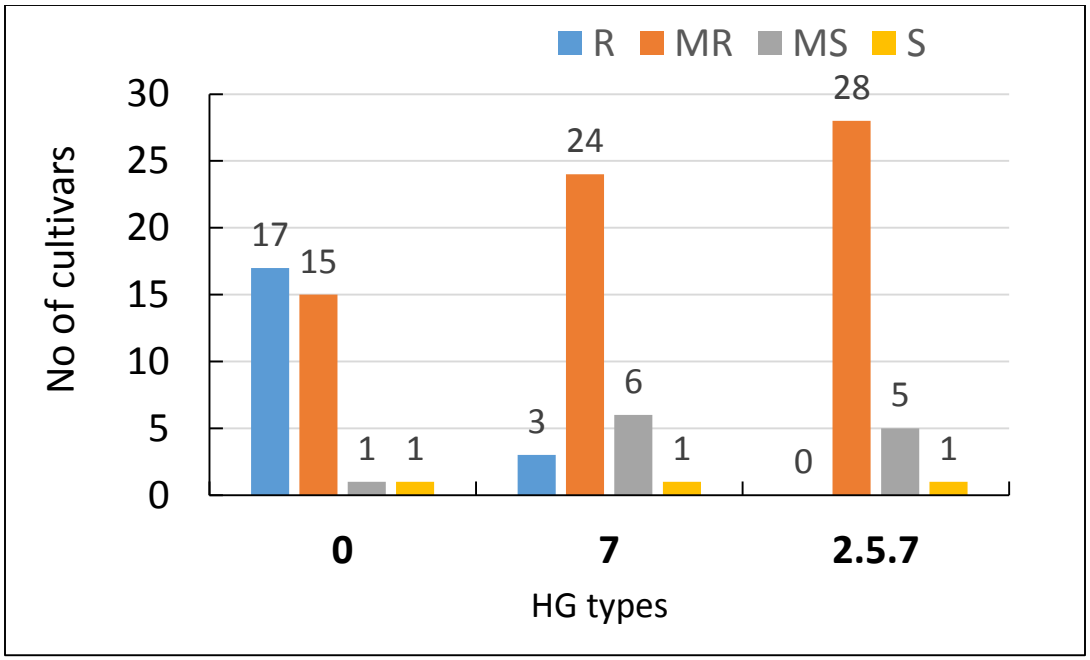

Fig. 3.2. Frequency of cultivars assessed with resistant (<10% FI), moderately resistant (FI= 10-30 %), moderately susceptible (FI= 31-60%), or susceptible (FI > 60%) reaction against three types of *Heterodera glycines* populations, under greenhouse conditions during the second run.

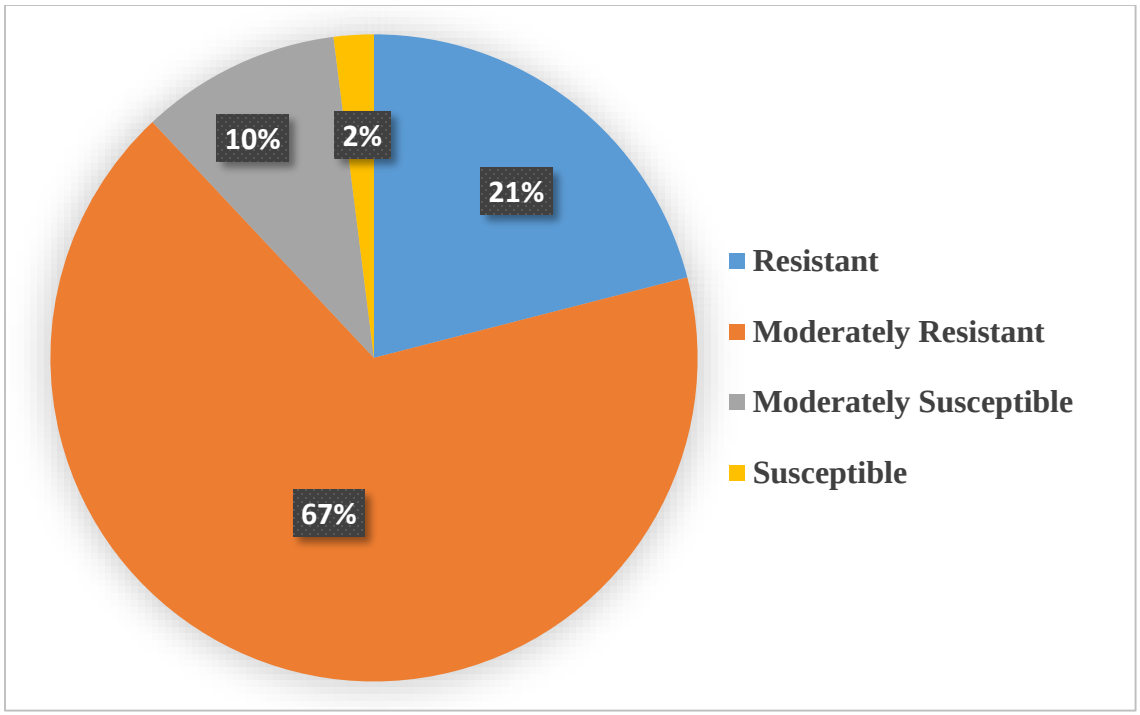

Fig. 3.3. Performance of all cultivars tested combined across the three HG types. Cultivar performance was classified as resistant if Female Index (FI) was <10 %, moderately resistant if FI= 10-30, moderately susceptible if FI= 30- 60 or susceptible if FI= >60% based on the female index.

Table 3.5. Average reproduction factor of *Heterodera glycines*, relative maturity, and average yield of commercial soybean cultivars grown at Southeast Research Farm field infested with HG type 0 in South Dakota.

Cultivars	Relative maturity	Average Rf[a]	Yield (bu/acre)	Resistance race on the label
AG0835	0.8	0.7	62.77	R3
AG0934	0.9	2.3	73.17	R3
AG1135	1.1	5.7	75.32	R3
AG1234	1.2	2.0	74.50	R3
AG1435	1.4	1.0	62.47	R3
AG1733	1.7	1.6	65.50	R3
AG1935	1.9	2.2	69.64	R3
AG2035	2	5.4	69.26	R3
AG2136	2.1	2.5	72.62	R3
AG2336	2.3	8.3	106.33	R3
AG2433	2.4	1.9	71.37	MR3
AG2535	2.5	2.1	71.35	R3
AG2636	2.6	4.6	75.82	R3
AG2733	2.7	2.5	76.64	MR3
AG2836	2.8	1.9	79.00	R3
AG2935	2.9	1.8	76.28	R3
PB-0598R2	0.3	6.2	58.89	PI 88788
PB-0676R2	0.6	2.0	55.45	PI 88788

PB-0777R2	0.7	1.0	60.92	PI 88788
PB-0863R2	0.8	2.4	63.45	PI 88788
PB-0879R2	0.8	3.9	57.50	Resistant
PB-0966R2	0.9	3.3	62.78	PI 88788
PB-1147R2	1.1	1.4	68.85	PI 88788
PB-1234R2	1.2	1.6	61.79	PI 88788
PB-1466R2	1.4	1.3	64.53	PI 88788
PB-1586R2	1.5	3.8	63.56	PI 88788
PB-1794R2	1.7	12.5	60.64	PI 88788
PB-1822R2	1.8	1.9	69.89	PI 88788
PB-1956R2	1.9	3.8	70.69	PI 88788
PB-1947R2	1.9	1.1	74.79	PI 88788
S 09- K4	0.9	0.7	53.68	R3, MR14/ PI 88788
S 06- H5	0.6	3.9	75.33	R3, MR14/ PI 88788
S 22- S1	2.2	2.7	74.05	R3, MR14/ PI 88788
S 26- P3	2.6	2.3	76.03	R3, MR14/ PI 88788
Williams 82	3.9	6.7	51.91	Susceptible check

[a] Reproduction factor (Rf) = no. of eggs and juveniles on the soil samples of cultivar at harvest / no. of eggs and juveniles on the soil samples of cultivar at planting in spring in a site having HG 0 type *H. glycines*.

CHAPTER 4.

4. Conclusions

These research studies reported in this thesis were on distribution and characterization *Heterodera glycines*, HG types to improve the soybean cyst nematode management in South Dakota. In this research, we worked at the three aspects of the soybean cyst nematode management: monitoring the present status, characterizing the population of *H. glycines* based upon the HG type test, and evaluation of the commercial cultivars resistant against the prevalent *H. glycines* population in South Dakota. Although we did not detect SCN from all 28 counties previously found to have SCN, varying level of infestation was observed in 16 southeast counties with population density ranging from 200 to 65,200 eggs and juveniles per $100cm^3$ of soil. Out of 16 counties which were positive for SCN, Brookings, Clay, Turner, and Union were found to be the most prevalent counties based upon the soil samples collected for this study. These counties have also had a long history of SCN occurrence. A total of eight types of *H. glycines* populations were identified. Of these types, HG type 7, 2.5.7, and 0 were most prevalent accounting for 80% in the total populations tested. Interestingly, we observed correlation between the female indices of HG type 1.3.6 and that of HG type 2.5.7.

Greenhouse assessment of the 34 commercial cultivars showed varying level reproduction to three *H. glycines* populations 0, 2.5.7, and 7. Cultivars were classified as resistant, moderately resistant, moderately susceptible, and susceptible based upon the female index with respect to susceptible cultivar Williams 82. Most of the cultivars tested were found resistant to HG type 0 and moderately resistant to HG type 2.5.7, and 7 and few showed susceptible reaction in both greenhouse tests. PB-0863R2 with average

female index of 77 % and cultivar PB-1147R2 with female index of 7.5% were the most susceptible and resistant cultivars to all the three *H. glycines* populations, respectively.

All of the 34 cultivars including Williams 82 were also tested in the field naturally infested with HG type 0 of *H. glycines* population. Varied resistance response was observed based upon the reproduction factor of *H. glycines* on each cultivar and reproduction factor ranged from 0.7 to 12.5. The information obtained from this research will be helpful in understanding the status of soybean cyst nematode in South Dakota, *H. glycines* types and response of commercial cultivars to the prevalent HG types in South Dakota. Because of limited diversity of resistance genes, future management strategies for sustainable SCN management should include an integrated approach of rotation with non-hosts, rotation within resistant cultivars and possibly use of nematicidal seed treatments in South Dakota.

In this study, we determined the present status, average population densities in different counties, diversity of *H. glycines* population in different counties, and response of commercial resistant cultivars to prevalent *H. glycines* in South Dakota. There are other management strategies currently used in SCN management such as management of alternative hosts, use of nematicidal seed treatments, and tillage practices, etc. Research on above mentioned fields will be helpful in further improvement in SCN management in South Dakota.

Appendices

Appendix 1. Analysis of variance table of number of HG type 0 cysts on soybean commercial cultivars under greenhouse conditions during the first run.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	34	102101.1000	3002.9735	5.16	<.0001
Error	105	61099.5000	581.9000		
Corrected Total	139	163200.6000			

R-Square	Coeff Var	Root MSE	cyst Mean
0.625617	105.3389	24.12260	22.90000

LSD = 33.821

Appendix 2. Analysis of variance table of number of cysts produced by HG type 7 on soybean commercial cultivars under greenhouse conditions during the first run.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	34	150012.4714	4412.1315	13.08	<.0001
Error	105	35415.5000	337.2905		
Corrected Total	139	185427.9714			

R-Square	Coeff Var	Root MSE	cyst Mean
0.809007	45.25107	18.36547	40.58571

LSD = 25.75

Appendix 3. Analysis of variance table of number of cysts produced by HG type 2.5.7 on soybean commercial cultivars under greenhouse conditions during the second run.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	34	99246.9000	2919.0265	9.45	<.0001
Error	105	32425.5000	308.8143		
Corrected Total	139	131672.4000			

R-Square	Coeff Var	Root MSE	cyst Mean
0.753741	48.54451	17.57311	36.20000

LSD = 24.639

Appendix 4. Analysis of variance table of number of cysts produced by HG type 0 on soybean commercial cultivars under greenhouse conditions during the second run.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	34	144694.5181	4255.7211	4.88	<.0001
Error	103	89904.4167	872.8584		
Corrected Total	137	234598.9348			

R-Square	Coeff Var	Root MSE	cyst Mean
0.616774	109.5111	29.54418	26.97826

Appendix 5. Analysis of variance table of number of cysts produced by HG type 7 for soybean commercial cultivars under greenhouse conditions during the second run.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	34	310259.2878	9125.2732	7.66	<.0001
Error	104	123963.0000	1191.9519		
Corrected Total	138	434222.2878			

R-Square	Coeff Var	Root MSE	cyst Mean
0.714517	74.03467	34.52466	46.63309

Appendix 6. Analysis of variance table of number of cysts produced by HG type 2.5.7 soybean commercial cultivars under greenhouse conditions during the second run.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	34	201163.9714	5916.5874	15.43	<.0001
Error	105	40254.0000	383.3714		
Corrected Total	139	241417.9714			

R-Square	Coeff Var	Root MSE	cyst Mean
0.833260	33.76672	19.57987	57.98571

LSD = 27.452

Appendix 7. Analysis of variance table of yield of commercial soybean cultivars planted at the South east research farm field naturally infested with HG type 0.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	34	13472.33975	396.24529	1.49	0.0635
Error	105	27852.64195	265.26326		
Corrected Total	139	41324.98170			

R-Square	Coeff Var	Root MSE	Yield Mean
0.326010	23.92991	16.28690	68.06086

LSD = 22.835

Appendix 8. Analysis of variance table of reproduction factor on each of the 34 soybean commercial cultivar planted at the Southeast research farm field naturally infested with HG type 0.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	34	597.945166	17.586623	0.79	0.7846
Error	97	2169.870733	22.369801		
Corrected Total	131	2767.815899			

R-Square	Coeff Var	Root MSE	Reproduction factor Mean
0.216035	156.8674	4.729672	3.015076

LSD = 6.885