DIVERSITY AND VIRULENCE OF SOYBEAN CYST NEMATODE (*Heterodera glycines* Ichinohe) IN NEBRASKA

Kyle C. Broderick
*University of Nebraska-Lincoln*

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DIVERSITY AND VIRULENCE OF SOYBEAN CYST NEMATODE
(Heterodera glycines Ichinohe) IN NEBRASKA

by
Kyle C. Broderick

A THESIS

Presented to the Faculty of
The Graduate College at the University of Nebraska
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Major: Agronomy

Under the Supervision of
Professor Loren J. Giesler

Lincoln, Nebraska

August, 2016
Soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe) is one of the most economically important soybean pathogens in the United States. Best management practices are the use of resistant cultivars and crop rotation. Though there are several genetic sources of SCN resistance, most of the SCN-resistant cultivars are derived from a single resistance source (PI 88788). Other states have reported an increase in virulence to PI 88788 due to prolonged use of this resistance. In this thesis, two studies were conducted to characterize the diversity and virulent phenotypes of SCN populations in Nebraska.

The first study assessed the virulent phenotypes of SCN field populations and their diversity in Nebraska by conducting HG type tests on 118 populations from 36 soybean-producing counties. 46.6%, 29.7%, and 88.1% of populations were virulent on PI 88788, Peking, and PI 548316 resistance respectively. No populations were virulent on PI 437654 (Hartwig). Virulence to PI 88788, PI 209332, and PI 548316 was common and found in nearly every county. Many counties also had populations virulent on Peking, PI 90763, and PI 89772.
The second study investigated the mitochondrial diversity of SCN in Nebraska as well as the diversity within a field. Previous work examining the haplotype diversity of SCN using CO1 mitochondrial markers found low diversity and two primary haplotypes – one common and found throughout the U.S. while the second, the MNNE haplotype, was only found in Minnesota and northeast Nebraska. Markers were developed to determine if there is association of the MNNE haplotype with HG type and the incidence of the MNNE haplotype in Nebraska. No association was found between the MNNE haplotype and HG type. Populations from the original Nebraska field did not contain the MNNE haplotype, however it was confirmed to be in the original isolates suggesting the MNNE haplotype is found at very low frequencies in the field. Information on virulence and diversity of SCN in Nebraska will provide insight for development and selection of SCN resistant cultivars.
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DEDICATION

To my family for their love and support
   Dad and Mom,
   Erik, Sarah, and Luca

Other friends and family who shared in the journey –
   this one’s for you.
   You probably know what you did.
Acknowledgments

Happiness does not depend on outward things, but on the way we see them. That said, my happiness and mental health during the course of my studies were highly dependent on outward things, primarily in the assistance and support I received from others. While I am sure to forget somebody, know that your support didn’t go unnoticed.

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Heartfelt thanks to my family for their love, support, and encouragement. To my parents, Jack and Lynette, for instilling a love of learning and showing me the wonders that nature has to offer. Thanks to my brother, Erik and his family for providing needed distractions that remind me of the things that are actually important in this world. With any luck, Luca will be diagnosing diseases himself before long.

Big thanks to all of my friends, who kept me grounded and engaged in non-pathology discussions that were always a massive relief at the end of the day. Jeremy Wortmann decided to join the research efforts and was a great relief on stressful days. I’d also like to thank Hanna Hofer for tolerating me and understanding that my schedule revolves around plants and the weather and doesn’t adhere to “normal” hours.

I have been extremely fortunate to find great friends in some of my coworkers. Lisa Sutton and Josh Miller were always there to help me talk through my research and results, or just discuss family and life. I’d like to acknowledge Nick Arneson for putting up with me, both in and out of the lab. Our countless times around a fire were one of the only things keeping me sane. I’m not sure I’ll find another crew who automatically goes silent upon hearing the opening chords of Purple Rain; nor one that will wait to get out of the car until the song is finished.

Thanks to the Plant Pathology Extension Group. Debbie Pederson was the therapist I was too cheap to see. If she charged a nickel every time she solved one of my
mental crises, I’d owe her thousands of dollars. I had assistance from more student workers than I have time to thank here, but I wouldn’t have been able to complete anything without them.

I would also like to thank members of Dr. Powers’ research group for their assistance in the haplotype diversity study. To Tim Harris, Kris Powers – I doubt I could have spelled _haplotype_ (let alone define it) without them. I would also like to thank Mathew Lodema for conducting a lot of the preliminary research looking at nematode haplotype diversity.

Finally, I would like to thank the Nebraska Soybean Board for providing the funds necessary to conduct this research. I would also like to thank all of the professors and other educators who helped me along the way. Without those dedicated to turning dilettantes into professionals, none of this would have been possible.

Thanks again to all those who helped me along this journey,

To get through this thing called “life.”
1.1 Origin and Distribution

The soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe) is a soilborne pathogen known to affect many plant species (Riggs, 1992). Due to morphological similarities of different species of *Heterodera*, confusion regarding identification was common (Ichinohe, 1952) and SCN was often misidentified as other species of *Heterodera*. In 1951, the nematode was classified as the pea cyst nematode, *Heterodera gottingiana* (Goffart, 1951) but was described as a new species—*Heterodera glycines* in 1952 (Ichinohe, 1952).

SCN was first reported to cause damage on soybeans in Japan in 1915 (Hori, 1915). At the time, the disease was known as “yellow dwarf” and attributed to the sugar beet cyst nematode (*Heterodera schachtii*, SBCN). Evidence suggests that farmers in northeastern China reported the nematode in 1880 (Noel, 1992; Liu et al., 1997), though this is unconfirmed. In 1936 the nematode was first reported in Korea and then found in the United States in 1954 (Winstead et al., 1955). In the late 1960’s SCN was found in
Egypt (Riggs, 1975). In the early 1980’s the nematode was found in Columbia and spread to Brazil in 1992 (Mendes and Dickson, 1993; Noel et al., 1994) and Argentina in 1997 (Dias et al., 1998). SCN has also been reported in Canada, Italy, and Iran (Yu, 2011).

The first report of SCN in the United States was in New Hanover County, North Carolina in 1954 (Winstead et al., 1955). In the late 1930’s symptoms similar to nutrient deficiencies were noted in southeastern North Carolina, though fertilizers were not solving the problem. Soil containing SCN is the most likely source of introduction into the USA. In 1893 the beneficial effect of inoculating soybeans with dust from Japan containing Bradyrhizobium spp. was demonstrated and soil infested with the bacterium was shipped throughout the United States (Hymowitz, 1990). In addition to soybean, flower bulbs were also produced in this area and a shipment of bulbs from Japan may have brought the nematode as well (Spears, 1955). Within four years of first detection, SCN was found in soy-producing states across the country – Missouri and Tennessee in 1956, Arkansas, Kentucky, and Mississippi in 1957, and Virginia in 1958 (Riggs, 1975) and can currently be found in nearly all soybean-producing states. SCN was first found in Richardson County, Nebraska in 1986 (Powers et al., 1989). After being identified in southeast Nebraska, SCN spread throughout the state and was found in 56 counties responsible for 93% of Nebraska’s soybean production as of 2016 (Giesler, unpublished data).

There is some debate as to the origin of H. glycines in the United States. While many believe that both soybeans and the nematode originated in China, an argument can
be made for the nematode being indigenous to the United States – primarily due to it being found over a large geographic area within a few years of first detection. One explanation for this could be parallel evolution of *H. schachtii*, which was documented in both North America and Asia prior to SCN being found in North America. Another theory is that migratory Indians traveling from Asia to the southern United States may have brought it with infested material (Canby, 1979).

SCN is spread though anything that can move SCN-infested soil and plant tissue, such as water, wind, and agricultural equipment (Lehman, 1994). Cysts have also been recovered from the digestive tracts and of blackbirds 48 hours after feeding (Epps, 1971) and migratory birds may spread the nematodes as they stop to feed (Thorne and Malek, 1968). Through these and other means, SCN continues to spread over both long and short distances.

1.2 Biology

1.2.1 Life Cycle and Infection Process

The life cycle of SCN can be broken into three main stages: egg, juvenile, and adult. First-stage juveniles (J1) develop within the eggs and molt into second-stage juveniles (J2) prior to hatching. J2 nematodes have a stylet allowing them to penetrate root tissue and infect suitable hosts (Lauritis et al., 1983). Egg hatch is stimulated by many factors including temperature and root exudates, at which point hatched J2 nematodes emerge from the cysts (Niblack, 2005). The juveniles then locate the root, likely via chemolocation (Perry, 1996), and penetrate the epidermal tissue. Without
forming a feeding site, juvenile nematodes migrate towards the central cylinder of the root, destroying the epidermis and cortex through mechanical means as well as the production of cellulases (Ross, 1958; Wang et al., 1999). Once near the vascular bundle, the nematode injects a variety of secretions into the initial feeding cell. These secretions result in significant cytoplasmic changes – cell walls of adjacent cells begin to dissolve, forming a dense mass of joined cytoplasm (Endo, 1998). After dissolution of the cell walls the protoplasts fuse creating a multinucleate feeding site known as a syncytium (Davis et al., 2004). Male and female second-stage juveniles initiate syncytia at different locations, with males feeding near the protophloem and females feeding near protoxylem (Endo, 1964).

After an initial feeding period, the J2 will begin to swell before molting into the next stage, the J3 juvenile nematode. Six to seven days after establishing a feeding site, the J3 molts again becoming the fourth-stage juvenile (J4). At this point sexual dimorphism becomes evident, with some of the J4’s beginning to elongate within the cuticle (the males), while others continue to feed and swell (the females). Eight to nine days after infection, the final molt occurs and the J4’s become adult nematodes. Adult males regain their vermiform shape and emerge from the root, while the females continue to swell until they are too large to be contained within the root, at which point they rupture the surface. The neck and head of the adult female remains embedded in the root, with her vulva exposed outside of the root. Males then respond to a sex pheromone (Jaffe et al., 1989) to locate the females. After fertilization the males die and several hundred viable eggs begin to develop within the female, with a portion of the developed
eggs being released into gelatinous matrix outside of her body. The rest of the eggs remain inside the female as she dies and her body turns into the cyst. The SCN life cycle lasts from 21 to 22 days under optimal conditions and there can be several completed generations in a given year (Lauritis et al., 1983; Niblack et al., 2006).

1.2.2 Host Range

Though cultivated soybean is the most economically important host of SCN, the nematode is known to reproduce on other cultivated and noncultivated hosts. Over 140 genera of plants from a range of different families, including Fabaceae and 22 non-legume families are known hosts of the nematode (Riggs, 1992). Female nematodes are also able to undergo reproduction on many cultivars of dry bean (*Phaseolus vulgaris* L.), including black, kidney, and pinto beans (Poromarto and Nelson, 2009). This suggests that SCN could potentially become a problem in dry bean production where overlap with soybean acres occurs.

Many winter annual weeds are known hosts of SCN (Johnson et al., 2008; Mock et al., 2009). Field pennycress (*Thlaspi arvense* L.), henbit (*Lamium amplexicaule* L.), and shepherds’ purse (*Capsella bursa-pastoris* (L.) Medik) are some of the winter annuals which can be a host for SCN. Sugar beets and tomatoes have been found to be experimental hosts of the nematode as well (Miller, 1983).
1.3 Impact on Soybean Production

1.3.1 Response to Infection

In the field SCN causes few descript symptoms, and most can be associated with poor root health. Classic symptoms include stunting and chlorosis and plants with high levels of infestation will have poor root systems. As juvenile nematodes migrate through the epidermal and cortical cells of the root, cells are damaged, disrupting nutrient and water uptake (Niblack and Tylka, 2010). Occasionally, cream-colored to light-brown females rupturing the root surface can be seen after gently washing roots, but they often sluff off during the washing. Stunting and chlorosis may appear on aboveground parts and often resemble nutrient deficiencies such as iron or nitrogen. Infected plants may also have a reduced number of pods per plant and seeds per pod (Mueller, 1984), though many times there are no obvious aboveground symptoms (Wang et al., 2003).

1.3.2 Yield Reduction

SCN suppresses yield more than any other soybean disease (Koenning and Wrather, 2010). Yield reduction due to SCN is highly variable and can be caused by a number of small factors that together may lead to significantly decreased yields. In asymptomatic fields, yield loss is often the only indication of SCN infestations. These symptomless fields may see a 15% decrease in yield (Wang et al., 2003; Noel, 1992) due to a number of factors including decreases in photosynthetic rate, nodulation, and pod number, as well as the syncytium acting as a metabolic sink (Barker et al., 1993; Huang et al., 1984; Mueller, 1984; Gommers and Dropkin, 1977; Poskuta et al., 1986). Fields
with heavy infestations of SCN, displaying classic symptoms of such as stunting and chlorosis, can experience anywhere from a slight to 90% yield loss (Sinclair, 1982).

In susceptible hosts both photorespiration and photosynthesis decreased, while photosynthesis was not affected in a resistant host this reduction in the rate of photosynthesis is not correlated to plant growth or yield (Barker et al., 1993). One thing that was correlated with yield loss was the amount of starch content after the end of the dark cycle of photosynthesis. Starch content was positively correlated with initial inoculum pressure, and negatively correlated with yield. Huang et al. (1984) demonstrated that SCN can decrease nodulation of *Bradyrhizobium japonicum*. This decreased nodulation led to an increase of isoflavonoids in the plant and reduced leghemoglobins in the nodules (Huang and Barker, 1983; Kennedy et al., 1999). Leghemoglobin is necessary for nitrogen fixation by facilitating oxygen supply to the bacteria (Virtanen, 1948).

Another factor influencing yield is the syncytium acting as a metabolic sink, providing all of the nutrients necessary for nematode development. Nutrients such as potassium, calcium, magnesium, and glucose concentrate in the roots (Gommers and Dropkin, 1977; Poskuta et al., 1986) and are unavailable to be used in seed fill. Reduced seed and pod development is known to decrease yield and has been observed throughout the reproductive stages in fields infested with SCN (Mueller, 1984; Wang et al., 2003), though SCN can cause yield reduction with little or no changes in plant growth (Young, 1996).
1.3.3 Disease Interactions

In addition to causing yield loss due to stolen photosynthates, SCN is also known to interact and may even increase incidence of other soilborne pathogens. Interactions between SCN and numerous other soil-borne organisms have been reported (Bond and Wrather, 2004) and several soybean root diseases are intensified in the presence of SCN.

1.3.3a – Brown Stem Rot of Soybeans

*Phialophora gregata* (Allington & D. W. Chamberlain) W. Grams, the causal agent of brown stem rot (BSR) is a soil-borne fungal pathogen of soybeans. Both SCN and BSR are common throughout the Midwestern U. S. (Workneh et al., 1999) and primarily managed through the use of resistant varieties combined with rotation to a nonhost (Niblack, 2005; Yang and Lundeen, 1997). BSR colonization causes a discolored, segmented pith and foliar symptoms can develop in the mid-reproductive stages. When both foliar and internal discoloration are present, yield losses of up to 30% may occur (Megnistu and Grau, 1987).

In greenhouse studies, Tabor et al. (2003) found that both the incidence and severity of BSR was greater in the presence of SCN than in the absence of the nematode, regardless of resistance or susceptibility to either pathogen. These researchers also found that, when in the presence of *H. glycines*, root colonization of *P. gregata* occurred earlier in both resistant and susceptible varieties. Yield loss due to BSR is the result of reduction in seed size rather than seed number (Weber et al., 1966), so earlier colonization of the fungus may have a greater impact on yield.
The relationship between SCN and BSR is complicated by the fact that some sources of SCN resistance may confer resistance to BSR while others do not (Oplinger et al., 1999). Hughes et al. (2004) found that soybean cultivars with PI 88788 resistance performed equal to or better than BSR-resistant checks in preventing infestation of *P. gregata*. Greenhouse trials showed soybeans with Peking resistance performing at a level similar to standard BSR-susceptible checks (Kurtzweil et al., 1999) though the results were confounded when studied in the field, suggesting that additional environmental factors play a role in BSR development (Hughes et al., 2004).

1.3.3b – Sudden Death Syndrome of Soybeans

Sudden death syndrome (SDS) of soybeans is caused by a group of soil-borne fungi in *Fusarium solani* species complex (Aoki et al., 2003; Aoki et al., 2005). In North America *Fusarium virguliforme*, formerly *Fusarium solani* f.sp. *glycines* (Aoki et al., 2003) is the causal agent of SDS. In South America *F. brasiliencse*, *F. cuneirostrum*, and *F. tucumaniae* also cause SDS symptoms (Aoki, et al., 2005). SDS can be found in most soybean-producing areas of the United States and Canada and can cause severe yield loss (Wrather and Koenning, 2006). Early in the season, SDS infects the soybean root but foliar symptoms do not occur until early reproductive stages when there is adequate soil moisture (Luo et al., 1999). Foliar symptoms are the result of toxins produced in the root that are translocated to the above ground parts of the plant (Jin et al., 1996).

As with SCN, best management practices of SDS include the use of resistant varieties, however crop rotation is not always effective (Rupe et al., 1997). SDS appears
in low, wet, compacted areas of the field, often in areas where *H. glycines* has been a problem (Roy et al., 1997). Though *F. virguliforme* can cause disease on its own, the fungus is often found in association with SCN (Roy et al., 1989) and results regarding the relationship between the two pathogens have been inconsistent. Many studies have found a positive correlation between the fungus and nematode (McLean and Lawrence, 1993; Xing and Westphal, 2006), while others have found the interactions to have weak or no correlation (Roy et al., 1993; Gao et al., 2006). Marburger et al. (2014) surveyed fields in Wisconsin and found a negative correlation between finding both *H. glycines* and *F. virguliforme* and that fields with heavy SCN pressure are not at greater risk of SDS. However another recent study found that SCN combined with SDS led to more root-branching in the greenhouse and co-infected plants consistently had greater symptom development than plants only infested with *F. virguliforme*, especially on younger plants (Tatalovic et al., 2014), suggesting that SCN pressure may increase the age-related susceptibility to SDS.

Though the relationship between SDS and SCN has been studied extensively, the inconsistent and sometimes contradictory results require that more research be done to fully understand the relationship between the two pathogens. However researchers agree that when the two pathogens occur together there may be a synergistic relationship and farmers should manage for both (Xing and Westphal, 2013).
1.4 SCN Biotype

1.4.1 Race System

Prior to the release of the first resistant soybean varieties, a variation in the ability of field populations to parasitize different cultivars was noticed (Niblack and Riggs, 2004). When originally classifying the virulence phenotypes of SCN, Golden et al. (1970) proposed a race system. A race is a genetically distinct mating group that infects a given set of plant variables (Agrios, 2005). Races were determined by measuring the nematode’s ability to reproduce on a resistant host, also known as the Female Index (FI). If the number of females produced by a nematode population on a soybean differential line is equal to or greater than 10% of the number of females produced on a susceptible line, the nematode population is considered virulent to that resistance source. Four races were introduced, based on the cultivars Peking and Pickett, and plant introductions PI 88788 and PI 90763 (Golden et al., 1970). As phenotype testing became more widespread, more races were described – eventually describing all 16 possible races from the originally selected differential lines (Inagaki, 1979; Chen et al., 1998; Riggs and Schmitt, 1988; Niblack, 1992).

1.4.2 HG Type Scheme

As more sources of SCN-resistance were discovered, a need arose to modify the race system. Since the races were based off of reproduction on each differential, adding one new line exponentially increased the number of possible races. In 2002 (Niblack et al.) a revised classification system called the “HG type” test was introduced. The “HG”
portion of the name represents the first letters of the genus and species names of SCN.

The newly classified test used seven differential lines (Table 1) (Niblack et al., 2002).

Table 1. Differential lines used in the HG type test and their associated HG Number.

<table>
<thead>
<tr>
<th>HG Line</th>
<th>Indicator Line</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Peking (PI 548402)</td>
</tr>
<tr>
<td>2</td>
<td>PI 88788</td>
</tr>
<tr>
<td>3</td>
<td>PI 90763</td>
</tr>
<tr>
<td>4</td>
<td>PI 437654 (Hartwig)</td>
</tr>
<tr>
<td>5</td>
<td>PI 209332</td>
</tr>
<tr>
<td>6</td>
<td>PI 89772</td>
</tr>
<tr>
<td>7</td>
<td>PI 548316 (Cloud)</td>
</tr>
</tbody>
</table>

As with the race scheme, HG types depend on a population’s ability to reproduce on each of the differential lines compared to a standard susceptible – normally Lee, however Williams 82 may be used due to germination problems with Lee (Niblack et al., 2009). If a population has a FI greater than 10 reproduction compared to the standard susceptible, it is that type – if a population can reproduce on line 1, it is type 1; if a population can reproduce lines 2, 5, and 7, it is type 2.5.7. HG type 0 means that the population is not virulent on any of the resistant lines. The current HG type classification scheme more adequately defines the diversity in virulence phenotypes (Niblack et al., 2002). Another benefit to the current scheme is that as new sources of resistance are defined, the line only need be added to the list. A modification of the HG type test, the “SCN type test” only incorporates the three commonly used sources of resistance used in commercial soybean varieties: Peking, PI 88788, and PI 437654 (Hartwig).
1.4.3 **Distribution of HG Types**

As growers began to manage for SCN through host resistance, the distribution of HG types across North America changed (Niblack et al., 2003). Shortly after discovering the nematode in the United States, type 0 (race 3, avirulent to all resistance sources) was widely distributed across the Midwest and type 2 (race 1, virulent to PI 88788) was prevalent in North Carolina (Niblack and Riggs, 2004). As resistance to these races was planted, field populations were able to overcome the resistance and new races began to emerge.

With PI 88788 being the most common source of SCN-resistance in the United States, shifts in HG types have been observed (Table 2). An Illinois survey sampled fields in 1991 and found 35% of SCN populations were virulent to PI 88788; that number had increased to 70% by 2005 (Niblack et al., 2008). As more populations gain virulence to PI 88788, deploying Peking and other sources of resistance will become more important to growers. Throughout the region, there has been a shift with populations becoming virulent to PI 88788 resistance and, to a lesser extent, Peking.
Table 2. Percentage of virulence phenotypes described in publications in the North Central soybean growing region of the United States.

<table>
<thead>
<tr>
<th>State of Survey (year sampled) (Citation)</th>
<th>Resistance Source</th>
<th>Peking (PI 548402)</th>
<th>PI 88788</th>
<th>PI 437654 (Hartwig)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Missouri (2005) (Mitchum, et al, 2007)</td>
<td></td>
<td>29%</td>
<td>78%</td>
<td>0%</td>
</tr>
<tr>
<td>Kansas (2008) (Rzodkiewicz, P., 2010)</td>
<td></td>
<td>33%</td>
<td>64%</td>
<td>0%</td>
</tr>
<tr>
<td>Illinois (2005) (Niblack et al, 2008)</td>
<td></td>
<td>8%</td>
<td>70%</td>
<td>0%</td>
</tr>
<tr>
<td>Indiana (2008) (Faghihi et al, 2010)</td>
<td></td>
<td>0%</td>
<td>56%</td>
<td>0%</td>
</tr>
<tr>
<td>Ontario (2008) (Faghihi et al, 2010)</td>
<td></td>
<td>15%</td>
<td>27%</td>
<td>0%</td>
</tr>
<tr>
<td>Wisconsin (2011) (MacGuidwin, 2012)</td>
<td></td>
<td>26%</td>
<td>78%</td>
<td>0%</td>
</tr>
<tr>
<td>Minnesota (2002) (Zheng et al., 2006)</td>
<td></td>
<td>1%</td>
<td>15%</td>
<td>0%</td>
</tr>
</tbody>
</table>

As more extensive testing of virulence phenotypes occurred, correlations between female indices on certain sources of resistance became evident (Colgrove and Niblack, 2008). FI from populations virulent on PI 88788 (type 2) are highly correlated with PI 209332 (type 5) and PI 548316 (type 7). Likewise, FI on Peking (type 1), PI 90763 (type 3), PI 89772 (type 6), and PI 438489B are highly correlated. Further studies into the genetic basis for SCN resistance found that *rhg1*, the gene thought to be of the most importance to breeding SCN resistance, is found in nearly all sources of SCN resistance (Concibido et al, 1996; Concibido et al. 1997; Webb et al, 1995; Yue et al, 2001) and instead of different genes being responsible for SCN resistance, it is primarily dependent on the number of copies of the gene (Cook et al, 2012).
1.4.4 Population Structure of Introduced Nematodes

Though there is some debate as to the origin of *H. glycines* in the United States; the prevailing thought is that it was introduced from Asia. As many sources of SCN resistance are native to China, this hypothesis is reasonable. Genetic diversity of many non-indigenous species (NIS) is dependent on the number of founder events. If a population is founded from a single event, only a small portion of the original genetic diversity will be retained and successive bottlenecks can reduce genetic diversity even more (Crisescu et al. 2001; Sakai et al., 2001). Conversely, multiple introductions from genetically distinct populations may result in high diversity (Colautti et al., 2005; Kolbe et al., 2004). Mitochondrial markers have proven useful to studying population structure by detecting phylogeographic patterns, the effects of genetic drift, and isolation patterns due to their high mutation rate and lack of recombination (Avise et al., 1987). The mitochondrial cytochrome *c* oxidase subunit 1 (CO1) gene has been widely used to study the geographic and genetic diversity for both native and introduced species (Cristescu et al., 2001; May et al., 2006).

Mitochondrial sequence analysis of a European NIS, *Globodera pallida* (potato cyst nematode) found that haplotype diversity of populations in Western Europe is fairly low and the allelic richness of the European populations was only one-third the diversity observed in the nematode’s native Peru (Plantard et al., 2008). Though the European populations had low diversity compared to those in the *G. pallida*’s native region, the European populations still had a relatively high level of genetic variability, making management more difficult than if the pest had a narrower gene pool. The evolutionary
potential for this pathogen is reflected in its relatively diverse population structure – increasing the likelihood of overcoming genetic resistance (McDonald and Linde, 2002). Studies of another European NIS, *H. schachtii*, found that cyst nematodes often have a fairly low level of genetic differentiation among fields, even those separated by over 150 kilometers, yet they have a relatively high level of gene flow within a field due to transportation of cysts via equipment and water (Plantard and Porte, 2003). This gene flow within a field is likely due to the large field populations that can be found with certain cyst nematodes and their passive dispersal (Plantard and Porte, 2003). When populations are high, as can be found with certain cyst nematode species, even low migration rates can still facilitate gene flow preventing differentiation among fields. As with SCN, *H. schachtii* has limited active dispersal in soil which favors inbreeding due to juveniles from the same cyst penetrating the same root leading to an increase in inbreeding, which may affect the evolution of virulence phenotypes due to an increase of homozygous individuals (Plantard and Porte, 2003). Cyst nematodes require assistance in long-range dispersal, such as movement through water, equipment, or infected material. This passive dispersal helps explain the relatively low genetic diversity across the globe of different cyst nematodes when compared to native nematode populations (Koch et al., 2000).
1.5 Management

1.5.1 Host Plant Resistance

The use of resistant varieties has been an effective method to manage SCN (Niblack, 2005; Tylka, 2008). As SCN-infested fields have become more common across the United States, seed companies have produced a number of high-yielding, SCN-resistant varieties for growers to incorporate into their SCN management programs. Shortly after finding SCN in North Carolina (Winstead et al., 1955), work to identify host resistance began and resistant cultivars were introduced (Ross and Brim, 1957). Bradley and Duffy (1982) estimate a $405 million profit increase from 1973 to 1982 that was due to the deployment of the resistant cultivar ‘Forrest.’

Many of the plant introductions (PI) used to breed for SCN-resistance came to the U.S. through China in order to utilize the soybean’s natural genetic variation. Over 118 soybean PIs with resistance to SCN have been identified from the USDA-ARS soybean germplasm collection (Arelli et al., 2000), however only two PIs are commonly used in SCN-resistance breeding programs (PI 88788 and PI 548402 or Peking). For this reason, the genetic basis of host resistance remains narrow (Concibido et al., 2004).

Through crosses of resistant and susceptible soybeans, three independently, inherited recessive quantitative trait loci (QTLs) were found – \( r_{hg1} \), \( r_{hg2} \), and \( r_{hg3} \) responsible for Peking (PI 548402) resistance (Cadwell et al., 1960). A fourth QTL, \( r_{hg4} \) was identified by Matson and Williams (1965). An additional, dominant, resistance gene in PI 88788 – \( r_{hg5} \) has been identified as well (Rao-Arelli et al., 1992). More detailed mapping revealed that \( r_{hg1} \) is actually three genes and the number of gene
copies determines resistance, with a susceptible variety having only one copy and resistant varieties having multiple repeats of the rhg1 cluster (Cook et al., 2012). Between 90% and 95% of commercially marketed SCN-resistant soybeans contain the rhg1-b allele, which was derived from PI 88788 (Cregan et al., 1999; Kim et al., 2010).

The mechanism of SCN resistance in soybean is preventing or disrupting formation of the syncytium. Plants with Peking-type resistance develop a layer of necrotic tissue around the nematode head, at which point the initially parasitized cell dies (Kim et al., 1987). Plants with PI 88788 resistance only experience necrosis of the initial cell (Endo, 1991). Additionally, soybeans derived from Peking-type resistance lines have physical and chemical barriers called cell wall appositions that are absent in PI 88788-type resistance (Mahalingam and Skorupska, 1996).

SCN-resistance is a quantitative trait, meaning that it occurs in genetically variable (segregating) populations that do not fit typical Mendelian segregation ratios (Concibido et al., 2004). Like other quantitative traits, SCN resistance is multigenic – controlled by any number of genes that interact with each other and the environment. For this reason, quantitative, multigenic resistance is not as well understood as qualitative, monogenic resistance.

1.5.2 Crop Rotation

Rotation to a nonhost crop has been recommended to manage SCN since the early 1960s (Ross, 1960; Thorne, 1961). Though SCN has a broad host range, many crops are either non- or poor hosts, and can therefore be used in a rotation or as a cover crop
(Niblack & Chen, 2004). In the Midwest, corn is the crop most commonly rotated with soybean (Noel and Edwards, 1996; Chen et al., 2001; Pérez-Hernández, 2013) and has been shown that annual, biennial, and longer rotation schemes of corn and soybeans results in significantly lower SCN population densities compared to continually growing a susceptible soybean (Young and Hartwig, 1992; Noel, 2008). Though the mechanisms responsible for SCN population reductions are not fully understood, Warnke et al (2008) studied SCN egg hatch on different crops such as hemp, oilseed rape, corn, red clover, and susceptible soybeans and found that stimulating egg hatch of J2s and preventing subsequent infection was the main mechanism responsible for decreasing SCN population density. While a year of a nonhost may decrease populations, Miller et al. (2006) found that only one year of rotation away from the host was not an effective means to manage SCN. They also found that leguminous non- or poor hosts, as a group, were the most effective in decreasing SCN populations while corn was among the least effective. Rotating to corn, in addition to using nematicides as soil fumigants, successfully controlled for SCN in the field (Sasser and Grover, 1991). A three year study in Nebraska found SCN mortality during a corn rotation year ranged from 0 – 94%, with an average decline of 51% (Pérez-Hernández, 2013).

Some plants also have nematicidal properties which can be used to control SCN (Chitwood, 2002). Brassica spp. such as cabbage and mustard produce chemicals during decomposition that are toxic to nematodes (Donkin et al., 1995) and have potential to be used as a cover crop. These plants are known to contain glucosinolate, which by itself is non-toxic to nematodes (Jing and Halbrendt, 1994) however the decomposition product
of glucosinolate is isothiocyanate, a compound with strong nematicidal properties (Donkin, et al., 1995). Crops such as wheat (*Vicia faba*) and other cereals produce phenolic acids which may reduce nematode populations (Hershman, et al., 1995). Wight et al. (2011) found planting a cover crop of winter wheat and poultry manure did not result in a significant decrease in egg populations. Cereal rye (*Secale cereale*), a widely planted cover crop produces benzoazinoids that are toxic to two common agricultural pests, the root knot nematode (*Meloidogyne incognita*) and the American dagger nematode (*Xiphinema americanum*) (Zasada et al, 2005). Small plot research has suggested that planting ryegrass after harvest can reduce SCN populations (Pedersen and Rodríguez-Kábana, 1990).

### 1.5.3 Tillage and Irrigation

While there have been many studies looking at the effect of tillage on SCN populations, much of the data is inconsistent. One reason for this could be that tillage results are very specific to the field or region (Noel and Wax, 2003; Niblack and Chen, 2004; Westphal et al., 2009). A regional study of fields in the Midwest found that tillage can increase SCN populations, especially when dealing with finer textured soils (Workneh et al., 1999). However, when looking at coarser soils, tillage appeared to have no effect on SCN population densities. Gavassoni et al. (2007) confirmed that tillage can move SCN and it is believed that tillage is associated with the nematode’s regional spread. Westphal et al. (2009) studied intensity of tillage and found that reducing intensity resulted in lower SCN populations in rotated experimental plots in fine-textured soils.
soil. Multifactorial analysis of eight predictors found that soil type, and not tillage, was the largest predictor of SCN mortality in annually rotated fields in Nebraska (Pérez-Hernández, 2013; Pérez-Hernández and Giesler, 2014).

Little is known about the effect of irrigation on SCN populations. Koenning and Barker (1995) found greater SCN population density in non-irrigated plots than in irrigated plots, but that may have been a function of a more favorable water content/oxygen content ratio than the moisture levels alone. When experiencing drought stresses, soybeans respond by increasing root biomass (Huck et al., 1986), which may favor SCN reproduction. Since soil-water and soil-type affects the amount of available oxygen in the soil, soil-oxygen levels may be a limiting factor to SCN (Koenning and Barker, 1995), as the nematode’s life cycle requires aerobic respiration. Fine-textured soils drain more slowly than coarse soils and may allow anaerobic conditions to persist, which may impede nematode activity. (Vrain, 1986). If moisture isn’t a limiting factor, soybeans may be able to partially compensate for nematode damage; however yield suppression due to *H. glycines* cannot be avoided by increasing irrigation (Heatherly et al, 1992).

### 1.5.4 Weed Suppression

Winter annual weeds are those that typically emerge after harvest, overwinter as seedlings, and resume growth during the spring (Radosevish et al., 1997). Since these weeds often complete their lifecycle prior to planting, their management is often overlooked. Venkatesh et al. (2000) found that SCN could reproduce on several winter
annuals including henbit, purple deadnettle, and field pennycress in the greenhouse. Reproduction on both henbit and purple deadnettle have been observed in the field as well (Creech et al., 2007), which could elevate SCN reproduction. Failing to manage for these weeds may provide an additional niche for SCN development in the absence of soybeans (Johnson et al., 2008).

Creech et al. (2007) found that a majority of *H. glycines* reproduction on winter annuals occurs during the fall, suggesting that fall management of winter annual weeds may be an effective way to minimize SCN reproduction after harvest. Studies looking at the use of herbicides to control SCN on henbit found that applications of glyphosate reduces the number cysts and the number of eggs per cyst when compared to 2,4-D, especially at early application times (Werle et al., 2013).

### 1.5.5 Biological and Chemical Control

Other means of managing SCN include the use of biological and chemical controls. Nematicides are not commonly used due to the environmental and health concerns associated with their use. These concerns have forced researchers to look for other, less-toxic pesticides to use. Thiophate-methyl, a methyl benzimidazole carbamate fungicide was shown to suppress SCN, but was ineffective when moved to field trials (Faghihi et al., 2007). Another fungicide, fluopyram, a succinate dehydrogenase inhibitor has been investigated for nematicidal potential against root-knot (*M. incognita*) and reniform nematode (*Rotylenchulus reniformis*). Faske and Hurd (2015) found that fluopyram is nematistatic and low concentrations effectively managed nematode
infection of tomato roots. In greenhouse trials seed treatments containing fluopyram had significantly fewer SCN females and eggs per gram of root compared to those without the fungicide (Zaworski, 2014; Broderick et al., 2015).

In fields that have been infested with *H. glycines* for some time, SCN populations are naturally regulated by macro- and microorganisms in the soil through predation, parasitism, and other organisms outcompeting SCN for the same resources (Chen, 2004). Many different species of fungi and bacteria, such as *Hirsutella minnesotensis*, *H. rhossiliensis*, *Cylindrocarpon heteronema*, *Lysobacter* spp., and *Variovorax* spp. have been found to be antagonistic to SCN. *H. minnesotensis* and *H. rhossiliensis* were found to cause high levels of parasitism to J2 juveniles (Chen and Reese, 1999).

Currently there are two commercial biological control agents available to for SCN management. Syngenta® released a seed treatment with the bacterium *Pasteuria nishizawai*e Pn1 as the active ingredient. *P. nishizawai*e is an endospore-forming organism and endoparasite of SCN that effectively reduced the number of cysts and eggs/cyst in a micorplot study of soil naturally infested with the bacterium (Sayre et al, 1991; Noel et al, 2005). *Bacillus firmus* I-1582 is an endospore-forming bacterium that Bayer CropScience® has released as a seed treatment. *B. firmus* colonizes the root and consumes exudates, preventing juvenile nematodes from locating the root and feeding (Crow, 2014). In both greenhouse and small plot trials, treatments of *B. firmus* reduced galling caused by root knot nematodes (*Meloidogyne* spp.) as much as an application of the nematicide cadufafos (Rugby) (Karen-Zur et al, 2000). While effective in greenhouse and small plot trials, field studies examining the effect of *P. nishizawai*e and *B. firmus*
against SCN have been inconclusive (Tylka et al., 2014; Musil et al., 2015; Robertson et al., 2016).

1.5.6 Challenges

SCN brings about novel management challenges due to the presence of a cyst stage, a high reproductive capacity, a diverse group of virulence phenotypes, and the pervasiveness of corn/soybean rotations. In addition to cultural controls and using host resistance, efforts to genetically modify soybeans to prevent SCN infection have yet to be successful.

The ability for females to become a cyst to enhance survival and persistence is one challenge of managing SCN. Within the cyst, eggs remain viable for several years, even with consecutive years of crop rotation (Warnke et al., 2008). Additionally, cyst nematodes are able to enter periods of dormancy when the environment becomes less favorable (Yen et al., 1995). *H. glycines* eggs do not hatch at the same time, making it more difficult to determine the relationship between SCN egg densities and yield loss. Hatching is mediated by root diffusates (Tefft and Bone, 1985), temperature, and/or time – some eggs will hatch when the temperature rises to a certain point, others will hatch when in the presence of host exudates, and still others hatch when a certain amount of time has passed. (Yen et al., 1995). Hatching mechanisms are not well understood, but likely involve a combination of enzymatic activity and physical force (Niblack, 2005). The persistence of the cyst and the non-synchronicity of egg hatch allow SCN to persist and spread in fields with diverse environments.
The reproductive capacity (the time required to complete one life cycle and the number of progeny per female) of SCN is quite high. In a single growing season, SCN may go through three to six generations depending on the location and length of growing season. Factoring this with the 200 – 600 eggs produced by each cyst (Davis and Tylka, 2000), even fields with low population densities can increase by several orders of magnitude in a given year. This high reproductive capacity helps SCN compensate for losses that occur due to nonhost rotations, predation, parasitism, and competition with other nematode species (Agrios, 2005).

Another challenge facing growers managing for SCN is the ubiquity of corn/soybean rotations, especially in the North Central region of the U.S. (Noel and Edwards, 1996; Niblack, 2005; Giesler and Wilson, 2011). Due to the lack of diverse crop rotations, many SCN-infested fields will only be rotated away from soybeans to prevent the issues that arise from continuous corn. Often, those one or two years of a nonhost are not sufficient to drop SCN populations to a non-damaging level (Miller et al., 2006).

A final challenge associated with managing for SCN is a high level of diversity among virulent phenotypes. While use of resistant varieties decreases egg densities, resistance is incomplete and a number of individuals will be able to reproduce on any given variety, regardless of the source of host resistance. SCN is multivoltine, meaning it can have more than two generations in a single year and these individuals can reinfect the resistant host which increases the possibility of a resistant population emerging in a
single year. Adding to the problem is that a vast majority of resistant varieties are derived from one of two ascensions, either Peking or PI 88788 resistance.

1.6 Research Objectives

The objectives of this thesis were: 1) to characterize the distribution and diversity of the virulence phenotypes of SCN in Nebraska fields; and 2) to explore the haplotype diversity of SCN within Nebraska and within a single field.
1.7 Literature Cited


Chapter II

Characterization of Virulence Phenotypes of Soybean Cyst Nematode in Nebraska

2.1 Introduction

Soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe has become one of the most economically important soybean pathogens in the United States and Nebraska. It is estimated that SCN causes over 100 million bushels in yield loss each year in the United States (Koenning and Wrather, 2010). SCN was first reported in southeast Nebraska in 1986 (Powers and Wisong, 1987) and has since spread across the eastern third of the state into 56 counties responsible for producing over 91% of the soybeans in Nebraska (NASS, 2016). Yield loss due to the nematode is estimated to have cost Nebraska growers over $40 million dollars each year with previous surveys estimating ~25% of fields in Nebraska are infested with SCN (Wilson and Gielser, 2014). In addition to the yield suppression, SCN is also known to increase incidence of other root rot pathogens such as brown stem rot (*Philopha gregata*) and sudden death syndrome of soybeans (*Fusarium virguliforme*) (Tabor et al., 2003; Roy et al., 1989).

While there are some seed treatments that have shown to be antagonistic to SCN (Vitti et al., 2014), crop rotation along with deployment of resistant varieties remains the
best tool to manage the nematode (Niblack and Chen, 2004). Adaptation allows *H. glycines* to overcome resistant cultivars and decreases their effectiveness in the field as a management tool. Farmers are recommended to rotate their resistance to slow shifts in virulence phenotypes of SCN populations (Niblack, 2005). There are many lines with resistance to SCN in the USDA germplasm collection (Diers et al., 1997), but few have been used in programs breeding for SCN resistance (Niblack et al., 2002). While breeders are working on incorporating new lines, only two are widely available to Nebraska growers – PI 88788 and Peking (PI 548402). An assessment of soybean varieties available to Nebraska farmers in 2015 found PI 88788 being the resistance source in over 95% of varieties resistant to SCN and Peking in less than 4% of marketed soybean varieties. PI 437654 (Hartwig) is known to have good resistance to SCN but is not common in commercial breeding programs due to having lower yields than other SCN-resistant lines (Tylka and Mullaney, 2015; Giesler et al., 2016).

With few choices for genetic resistance to SCN available, many states have seen shifts in SCN virulence phenotypes, with more populations overcoming PI 88788 resistance. A 1991 survey of Illinois fields found 35% of SCN populations were virulent on PI 88788; by 2005, 70% of SCN-infested Illinois fields were virulent to PI 88788 (Niblack, et al., 2008). Additional surveys have found that 78% and 29% of Missouri SCN populations (Mitchum et al., 2007) and 64% and 33% of Kansas populations were virulent on PI 88788 or Peking, respectively (Rzodkiewicz, 2010). Conversely, only 15% of populations in Minnesota are virulent on PI 88788 (Zheng et al., 2006). In 2002 the HG type scheme was proposed for biotyping SCN populations and determining virulent
phenotypes (Niblack et al., 2002) and is currently the most accepted method. Few of Nebraska’s SCN populations have been HG typed and little is known about the virulent phenotypes in the state. Knowledge of the dominant virulent phenotypes in a state is important when making broad management recommendations to growers. Therefore, the objective of this study was to characterize the virulence phenotypes of SCN populations across the state of Nebraska.

2.2 Materials and Methods

A total of 118 soil samples from 36 counties across the state were selected from a pool of *H. glycines*-infested soil samples submitted by soybean farmers between 2008 and 2014. These samples were submitted for nematode diagnostic tests as a part of a state-wide survey program aimed at determining the spread of the nematode across Nebraska. Samples were stored at 4°C and randomly selected from each county that had previously tested positive for SCN. The number of samples selected from each county was determined by taking ~10% of the total number of samples received from that county. Only soil samples with moderate to high SCN populations were used for this survey with samples randomly selected from each county. To decrease sampling bias caused by growers submitting multiple soil samples over multiple years, only one sample was selected from a grower. Populations from each county were assigned a number and a random number generator picked which populations would be used in the HG type survey. If multiple populations from a single grower were randomly selected, only the first was used. Each population was increased for at least one generation on the SCN-
susceptible variety Williams 82 to decrease variability in hatching rates and ensure adequate egg numbers for testing.

Virulence was determined by the HG type test with indicator lines Peking, PI 88788, PI 90763, PI 437654, PI 209332, PI 89772, and PI 548316 (Cloud) (Niblack et al., 2002). Lee 74 was used as the susceptible check, though it often failed to germinate and Williams 82 was used as another positive control (Niblack et al., 2009). Each replication included all indicator lines and a standard susceptible. All soybean lines were obtained from the USDA Soybean Germplasm Collection in Urbana, Illinois. Seeds were germinated on moist, sterile paper towels at room temperature. After 48 – 72 hours seedlings with 2 – 3 cm radicles were selected for the HG-type test and transplanted into pasteurized, sandy loam soil from an area that has never been in agricultural production and was free of SCN or other cyst nematodes. PVC tubes (3.2-cm-diameter and 15-cm-high) were filled with sandy loam soil and a 3-cm-deep hole was made in the center of each soil-filled PVC tube and freshly harvested SCN eggs were deposited along one side of the hole. SCN egg density for inoculation was between 1,000 and 2,000 eggs with each test having a uniform egg density. The seedlings were placed in the hole with the radicle downward and touching the side that received the inoculum. Each line was replicated 6 times and PVC tubes for each soybean line were placed in larger containers (19-cm-diameter and 21-cm-high) that could hold 2 complete replications each. Plants were maintained in a ~25°C greenhouse and a 28°C water bath to keep soil and roots at a constant temperature.
At 28-30 days after inoculation, PVC tubes were removed and roots extending beyond the 15-cm tube were discarded to normalize the amount of root material between populations. Soil and roots were soaked in water for ~30 minutes. Females were collected from soil and dislodged from roots on nested sieves (850-μm pore over 250-μm pore sieve) with a high pressure water spray.

All females were collected, counted under magnification, and the female index (FI) was calculated. FI was determined for each soybean line by dividing the number of females on the indicator line by the female count on the susceptible control and multiplying by 100. FI measures the ability of a SCN population to reproduce on a resistance line. A FI of greater than 10 means that the SCN population can successfully reproduce on a given resistant line (Niblack et al., 2002). If the average number of females on Lee 74 or Williams 82 were less than 100, the data was discarded and the population was tested again.

### 2.3 Results

Of the 118 populations tested, 13 unique HG types were found. Types 7 (race 6) and 2.5.7 (race 1) appeared the most frequently with each type being found in 35 out of 118 of populations (29.7%) (Figure 1, Table 1). HG type 1.3.6.7 was the third most common virulence phenotype, comprising 9.3% of the sampled populations. Forty-six point six per cent of populations were virulent on PI 88788 though every population tested was able to produce at least one female on this source of resistance (Table 1). Virulence on Peking resistance was identified on 29.7% of populations (Table 1).
Virulence to PI 548316 (type 7) was identified in 88% of all populations. While there were no populations virulent to PI 437654, 20.3% of all populations tested had low levels of reproduction with the highest FI being 2 (Table 1). HG type 0 (race 3), meaning the populations were not virulent to any of the resistance sources, was rare, making up only 4.2% of the populations that were typed (Figure 1).

Only 11% of the typed populations had a FI greater than 30 on PI 88788 (24% of populations virulent to PI 88788) (Table 2). The range of FI of populations virulent to PI 88788 was 10 – 60. Ten per cent of populations had a FI greater than 30 on Peking (40% of all populations virulent to Peking) with a range of 10 – 72 (Tables 1 and 2). Only 12.7% of the sampled populations were virulent on both Peking and PI 88788 resistance and no populations were identified to have a FI greater than 30 on both Peking and PI 88788 resistance.

In addition to determining the common HG types across Nebraska, there was also the question as to where certain HG types were appearing. SCN populations from six of Nebraska’s eight agricultural reporting districts were included in this survey. These six districts are responsible for 97.2% of Nebraska’s harvested soybean acres (NASS, 2016). There did not appear to be a difference in HG type distribution among agricultural reporting districts, though type 2.5.7 and/or type 7 were the most common in each (Figure 2). No populations virulent to Peking were found in the Central crop reporting district, but we believe this is a function of having a low number of samples instead of an actual lack of virulence to Peking resistance. Virulence to Peking resistance was identified in the North and South Central agricultural reporting districts. Fourteen of 36
(39%) represented counties had populations virulent to both Peking and PI 88788 resistance (Figure 3).

While there are several other PIs used in the HG type scheme, most are not common in commercial soybean varieties. Though these PIs are not commonly used in programs selecting for SCN resistance, many populations were virulent to PI 90763 (25%, FI range of 0 – 51), PI 209332 (44%, FI range of 0 – 44), and PI 89772 (25%, FI range of 0 – 70) (Table 1).

2.4 Discussion

Though many populations in Nebraska were found to be virulent on PI 88788, the frequency of virulent populations is not as high as has been observed in other states. While some populations have adapted to Peking resistance (PI 548402), it still confers resistance to most SCN populations in Nebraska. In addition, few populations were virulent to PI 88788 and Peking, suggesting that if farmers are able to rotate resistance source, both can still be used to manage SCN population density. Though no populations were able to successfully reproduce on PI 437654, the associated yield drag continues to be a problem in commercial breeding programs trying to utilize this resistance source (Vierling et al., 2000). Alternative sources of resistance entering the market will benefit farmers, allowing those with fields having high SCN populations to reduce the speed at which populations are able to overcome a given resistance source. Even though rotating sources of resistance is difficult for many farmers, it is recommended that they rotate the specific resistant variety, which will also slow the nematode’s ability to adapt (Niblack,
2005). Genetic resistance to SCN is quantitative, meaning resistance is conferred by both the specific resistance gene and the number of times those genes are copied in the soybean (Cook et al., 2012). By rotating the resistant cultivar, farmers increase the likelihood that a given SCN population won’t be subject to same resistance genes and copy number of each in consecutive years.

The percentage of populations virulent to PI 88788 was not as high in populations tested in this study as in other states (Illinois, Indiana, Missouri) (Niblack et al., 2008; Faghihi et al., 2010; Mitchum et al., 2007), nor was it as low as found in Minnesota (Zheng et al., 2006). Kansas, Minnesota, Missouri, and Wisconsin all had comparable numbers to the percentage of Nebraska populations virulent to Peking resistance (Zheng et al., 2006; Rzodkiewicz, 2010; MacGuidwin, 2012). The frequency of populations virulent to Peking resistance in Nebraska, 29.7% was comparable to Wisconsin (26%) (MacGuidwin, 2012), Missouri (29%) (Mitchum et al., 2007), and Kansas (33%) (Rzodkiewicz, 2010), but higher than that found in Minnesota (1.1%) (Zheng et al., 2006).

In Missouri, SCN populations virulent to PI 88788 increased from 58% to 78% from 1998 to 2005 (Niblack et al., 2003; Mitchum et al., 2005). In Illinois just over 30% of populations were virulent on PI 88788 in a 1989 – 1990 survey (Sikora and Noel, 1991), but that number had increased to 70% by 2005 (Niblack et al., 2008). In addition to 70% of Illinois fields containing SCN populations virulent to PI 88788, the range in FI is greater in Illinois than in Nebraska. A FI between 10 and 30 indicates that the test line is moderately resistant. A FI greater than 30 represents that the line is at least moderately
susceptible to the SCN population and a FI of 60 or higher the test line is considered susceptible to SCN (Schmitt and Shannon, 1992). Illinois had fields from nearly every region with an FI greater than 30 and had a high FI of 98 on PI 88788 resistance (Niblack et al., 2008). The highest FI on PI 88788 resistance recorded from a Nebraska field was 60 and the highest FI on Peking resistance was 72 (Table 2).

Certain sources of resistance appeared to be linked, with 90% of populations virulent to PI 90763 and PI 89772 also being virulent to Peking. 87% of populations virulent to PI 88788 were virulent on PI 209332. These results agree with a study by Colgrove and Niblack (2008) that found a high correlation of FI on PI 88788, PI 209332, and PI 548316 and a correlation of FI on Peking, PI 90763, and PI 89772. There were also populations virulent to Peking and/or PI 88788 even though there had been no history of planting resistant soybeans in the sampled fields.

While the frequency of populations virulent to PI 88788 was similar in Nebraska to that in other states in the region, Nebraska did not have many populations with a high FI (greater than 30). One reason for this could be that Nebraska soybean producers have not been managing for SCN as long as growers to the east and south. SCN was first found in Nebraska in 1986 (Powers and Wysong, 1987), was identified in Missouri in 1956 (Hegge, 1957) and Illinois in 1961 (Spears, 1964). While it is probable that *H. glycines* was present in Nebraska fields prior to 1986, it wasn’t until the nematode was officially identified by a diagnostics lab that Nebraska growers began to manage for the nematode, at the same time selecting for populations virulent to the most common source of resistance, PI 88788. Historically soybeans acres were in the east, northeast, and
southeast reporting districts and is where the majority of the samples used in this survey were collected (Figure 3). The low frequency of type 0 populations, (4.2%) suggests that the initial introductions of SCN to Nebraska may have contained populations virulent to either Peking or PI 88788 resistance. This idea is supported by type 7 and type 2.5.7 populations being found in all counties (Figure 3).

With the race test, farmers need to consult a key in order to know which race corresponds to a nematode’s ability to overcome a given source of resistance. With the HG type test, producers need only know the sources of resistance used in the HG type test, which reduces the level of ambiguity that is associated with the race system. Race 1 refers to a SCN population that is type 2.5.7, but race 3 could be either 5.7 or 7. One problem with the HG type test is that there is no way to differentiate between low, moderate, and high FIs (unless it is reported). HG type 2- populations can have a FI that varies from 11 to 99. PI 88788 resistance may still be effective against the population with an FI of 11 but will fail against a population with an FI of 60 or more.

The results of this study show the need for an increase in soybean varieties with other than PI 88788. With almost half of the Nebraska’s SCN populations already virulent to the most common source of commercial resistance and every population producing at least one female on it, we expect more populations to become virulent to PI 88788. An increase of Peking and other sources of resistance to the market will allow farmers to rotate resistance sources, providing tools for those managing fields with HG type 2- SCN populations and improve sustainable soybean production.
2.5 Literature Cited:


2.6 Figures

Figure 1. Frequency distribution of 118 Nebraska SCN Populations HG types\(^x\) identified from 2008 - 2014.

\[^x\] HG type is determined by the ability of a nematode population to reproduce on a set of indicator lines, Peking, PI 88788, PI 90763, PI 437654, PI 209332, PI 89772, and PI 548316. This is known as the Female Index (FI). Female Index = ((number of females on differential line) / (number of females on susceptible line)) x 100. A FI greater than 10 means the nematode population was virulent to that resistance source – a type 0 cannot reproduce on any lines; a type 1 can reproduce on Peking; a type 2 can reproduce on PI 88788; a type 2.5.7 can reproduce on PI 88788, PI 209332, and PI 548316.
Figure 2. Number of SCN HG types\(^x\) by agricultural reporting districts\(^y\) with significant soybean production in Nebraska.

\(^x\) HG type is determined by the ability of a nematode population to reproduce on a set of indicator lines, Peking, PI 88788, PI 90763, PI 437654, PI 209332, PI 89772, and PI 548316. This is known as the Female Index (FI). Female Index = ((number of females on differential line) / (number of females on susceptible line)) x 100. A FI greater than 10 means the nematode population was virulent to that resistance source – a type 0 cannot reproduce on any lines; a type 1 can reproduce on Peking; a type 2 can reproduce on PI 88788; a type 2.5.7 can reproduce on PI 88788, PI 209332, and PI 548316.

\(^y\) Total number of samples from each reporting district were: Northeast (37), East (52), Southeast (15), South Central (8), Central (4), and North (2)
Figure 3. Map of the eastern portion of Nebraska showing the agricultural reporting districts and HG types identified in each county surveyed.
2.7 Tables

Table 1. Virulence phenotypes of 118 SCN populations collected in Nebraska from 2008 through 2014.

<table>
<thead>
<tr>
<th>Resistance Source</th>
<th>Peking (PI 548402)</th>
<th>PI 88788</th>
<th>PI 90763</th>
<th>PI 437654 (Hartwig)</th>
<th>PI 209332</th>
<th>PI 89772</th>
<th>PI 548316</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total percent of Virulent Populations</td>
<td>29.7%</td>
<td>46.6%</td>
<td>25.4%</td>
<td>0.0%</td>
<td>44.1%</td>
<td>25.4%</td>
<td>88.1%</td>
</tr>
<tr>
<td>Female Index Range&lt;sup&gt;x&lt;/sup&gt;</td>
<td>10 – 72</td>
<td>10 – 60</td>
<td>10 – 51</td>
<td>0 – 2</td>
<td>10 – 44</td>
<td>10 – 70</td>
<td>10 – 63</td>
</tr>
<tr>
<td>Number of Virulent Populations (118 total)</td>
<td>35</td>
<td>55</td>
<td>30</td>
<td>0</td>
<td>52</td>
<td>30</td>
<td>104</td>
</tr>
</tbody>
</table>

<sup>x</sup> The Female Index (FI) is calculated from taking the mean number of SCN females produced on an indicator line divided by the mean number of females on a standard susceptible (Lee 74 or Williams 82) and multiplied by 100.
Table 2. SCN populations with a Female Index\(^x\) (FI) greater than 30 on a given HG indicator line (resistance source is considered at least moderately susceptible) out of 118 populations.

<table>
<thead>
<tr>
<th>Resistance Source</th>
<th>Peking (PI 548402)</th>
<th>PI 88788</th>
<th>PI 90763</th>
<th>PI 437654(^y) (Hartwig)</th>
<th>PI 209332</th>
<th>PI 89772</th>
<th>PI 548316</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Populations (FI &gt; 30)</td>
<td>12</td>
<td>13</td>
<td>6</td>
<td>0</td>
<td>9</td>
<td>8</td>
<td>31</td>
</tr>
<tr>
<td>Per cent of Virulent Populations(^z)</td>
<td>40.0%</td>
<td>24.1%</td>
<td>23.1%</td>
<td>-</td>
<td>17.7%</td>
<td>30.8%</td>
<td>29.8%</td>
</tr>
<tr>
<td>Per cent of All Tested Populations with a FI &gt; 30</td>
<td>10.2%</td>
<td>11.0%</td>
<td>5.1%</td>
<td>-</td>
<td>7.6%</td>
<td>6.8%</td>
<td>26.3%</td>
</tr>
</tbody>
</table>

\(^x\) The Female Index (FI) is calculated from taking the mean number of SCN females produced on an indicator line divided by the mean number of females on a standard susceptible (Lee 74 or Williams 82) and multiplied by 100.

\(^y\) No populations were found to be virulent on PI 437654.

\(^z\) Per cent of tested populations with an FI > 30 compared to the total number of virulent populations on a given resistance source.
Chapter III

Haplotype Diversity of Soybean Cyst Nematode

3.1 Introduction

While there are some seed treatments that have shown antagonism to SCN (Vitti et al., 2014), crop rotation along with deployment of resistant varieties remain the most effective tools to manage the nematode (Niblack and Chen, 2004). Though there are over 118 plant introduction lines with resistance to SCN in the USDA-ARS soybean germplasm collection (Arelli et al., 2000), only two (PI 88788 and Peking) are commonly used in SCN-resistance breeding programs. Of these two sources of resistance, PI 88788 is found in a large majority of cultivars resistant to SCN and populations virulent to PI 88788 have become more prevalent (Niblack et al., 2008; Mitchum et al., 2007; Rzodkiewicz, 2010; Chapter II).

Genetic studies have indicated that resistance and susceptibility of soybeans to SCN is polygenic and determined by the presence or absence of QTLs \textit{rgh1}, \textit{rgh2}, \textit{rgh3}, \textit{rgh4}, and \textit{rgh5} (Cadwell et al., 1960; Matson and Williams, 1965; Rao-Arelli et al., 1992). QTL linkage group \textit{rgh1} is considered to be the most important in cultivated soybeans and has been mapped in most SCN resistance found in breeding programs – PI 88788, Peking, PI 90763 (Concibido et al., 1997), PI 437654 (Webb et al., 1995), PI
209332 (Concibido et al., 1996), and PI 89772 (Yue et al., 2001). SSR markers close to the \textit{rhg1} locus, have been developed (Mudge et al., 1997; Cregan et al., 1999; Ruben et al., 2006) to aid in development of resistant soybean lines. Cook et al. (2012) found that resistance is tied to the number of gene copies of \textit{rhg1}, with a susceptible variety having one copy and resistant varieties having as many as ten copies. Studies of virulent SCN populations in Illinois showed that there was a correlation between Peking, PI 90763, and PI 89772 resistance as well as a correlation between PI 88788, PI 209332, and PI 548316 resistance (Colgrove and Niblack, 2008) and virulent phenotypes can be broken down into two main types – those virulent to Peking resistance and those virulent to PI 88788 resistance.

As SCN populations virulent to PI 88788 become more common, there will be an increased need for soybean growers to ensure that they are deploying the correct resistance package. If a farmer uses a resistant soybean to which the field population of SCN is virulent, there may not be an increase in yield (Giesler et al., 2015; Giesler et al., 2016). As such, growers need to determine the HG type (formerly known as race) (Niblack et al., 2002).

Since 2002, the HG type test has been used to determine virulence phenotypes of SCN populations by comparing the number of females on a standard susceptible line to the number of females on a given source of resistance (Niblack et al., 2002). While an effective means of determining virulent SCN phenotypes, this test can take more than two months and has a high degree of variability. For this reason a test to quickly and accurately determine the virulent phenotype of a SCN population is needed.
Virulent phenotypes are able to overcome specific resistance genes in the plant, as well as the innate resistance common to many plants (Smant and Jones, 2011). The complex nature of how plant parasitic nematodes evade or suppress host plant resistance suggests specific virulence genes that are yet to be identified in *H. glycines*. In *Globodera pallida*, the potato cyst nematode, the effector protein Gp_RBP-1 induced a specific hypersensitive response when co-expressed with the potato nematode resistance gene *Gpa-2* (Sacco et al., 2012). In tomatoes (*Lycopersicon esculentum*) containing the resistance genes *Cr-2* and *Rcr3pim* genes, cell death was triggered by the venom allergen produced by *G. rostochiensis*, Gr-VAP1 (Lozano-Torres et al., 2012; Lozano-Torres et al., 2014). Research to understand the allelism of SCN virulence loci has been confounded by the quantitative, multigenic nature of SCN resistance (Bekal et al., 2015).

As part of a nematode diversity study, the University of Nebraska-Lincoln sequenced a 701 bp region of the cytochrome *c* oxidase 1 (CO1) mitochondrial gene region to look for polymorphisms (Western Association of Agricultural Experiment Directors, 2013). CO1 has been widely used to study geographical and genetic diversity of both native and introduced species (Cristescu et al., 2001; May et al., 2006). Examination of 82 SCN populations from 8, soybean-producing states found 8 polymorphic sites and 4 haplotypes. However, 3 of the haplotypes were only a single nucleotide different and were only found in one sample each which is indicative of a random, non-conserved mutation. The other haplotype had 4 polymorphic sites which were found in multiple SCN samples. This suggests 2 primary haplotypes. One of these haplotypes is common throughout the United States (referred to as ‘A’ from this point
forward), and the other haplotype (referred to as ‘MNNE’ from this point forward) was restricted to Minnesota and one field in northeastern Nebraska (Western Association of Agricultural Experiment Directors, 2013).

Mitochondrial DNA (mtDNA) is maternally inherited and SCN infestations can begin with a single adult female. The objectives of this study were to develop a primer to rapidly and accurately identify the MNNE haplotype and to determine if the two SCN haplotypes found on the cytoplasmic DNA were related to the two main virulent phenotypes. The haplotype was localized to Minnesota and a single northeastern Nebraska field, and its incidence within a field was also investigated.

3.2 Materials and Methods

Diagnostic Tool Development

In order to develop a diagnostic tool that would quickly and precisely identify the MNNE haplotype of SCN, two methods were explored – using restriction enzymes and using a multiplex marker. Due to the similarities between different species of cyst nematode, the ideal diagnostic tool would allow one to accurately identify SCN and/or the MNNE haplotype in a given SCN population. The target polymorphism was a 4 bp palindrome, GCGC that is found in the MNNE haplotype (GTGC in the A haplotype). The $Hha1$ restriction endonuclease recognizes and cleaves the sequence GCG$^C$, allowing detection of the MNNE haplotype through restriction digests. mtDNA templates were amplified with two sets of SCN-specific CO1 markers, one set that bound to a 701 bp strand and another that bound to a 321 bp strand. Following amplification,
restriction digests were performed using the *Hha*1 restriction endonuclease. Restriction digests were performed on amplified mtDNA from 30 different SCN populations – 10 populations from the field in northeast Nebraska that were found to previously contain the MNNE haplotype, 5 populations from fields in Minnesota found to previously contain the MNNE haplotype, and 15 populations from across Nebraska that had not previously been tested. Digests were also done on 10 cleaned and reamplified templates from fields that originally detected the MNNE haplotype. Each digestion tube contained 10.0 μl of amplified product, 1.2 μl of 10x Tango buffer, and 1.0 μl of the *Hha*1 enzyme and was incubated at 37 °C for 2 hours.

The second method involved the use of a multiplex marker. To cut down on costs, the original 701 bp SCN-specific CO1 mitochondrial markers were shortened to target a 321 bp region within the CO1 gene. The external flanking primers were specific to *H. glycines*, while a second, internal reverse primer was developed that would bind at a polymorphic site with a perfect match to the MNNE haplotype. If successful, this primer set would result in two bands (one at 321 bp and the other at 201 bp). PCR reactions were done in 50 μl tubes using a Techne® Prime thermocycler. Reaction mixture contained 3 μl of mtDNA template, 2.4 μl of the outside primers F1a (5’-TTTTGGTTATTAGGAATAATTACGC-3’) and R1 (5’-CCACATAATAAGAATCATGCAAAAC-3’), 1.2 μl of the inside primer R2 (5’-GAGAGCCGTAAACCCTTATTAGC-3’), 15 μl of RedTaq polymerase, and 6 μl deionized water for a total volume of 30.0 μl. The PCR amplification profile consisted of 50 cycles of 94 °C for 30 sec, 61 °C for 30 sec, and 72 °C for 90 seconds. Product was
stored at 4 °C. The multiplex marker was tested on 83 SCN populations from across the state of Nebraska.

Correlation of MNNE haplotype to SCN Virulence Phenotype

To test if the MNNE haplotype was related to the SCN virulence on either PI 88788 or Peking resistance, segregating greenhouse populations of the nematode were developed. These segregating lines were developed by serially transferring populations on a given SCN resistance source and monitoring their reproduction on other sources of resistance. Segregating lines were serially transferred on the same source of SCN resistance for nine generations. KB-H reproduces on PI 88788, but not Peking or PI 437654. KB-M reproduces well on Peking, but not PI 88788 or PI 437654. KB-321 reproduces well on both PI 88788 and Peking. mtDNA was obtained by crushing juveniles and eggs, which ruptured cells containing mtDNA. Reaction tubes containing mtDNA were stored at 4 °C.

Field Incidence

The Nebraska field originally found to contain the MNNE haplotype was located in Cedar County, seven miles north of Laurel, NE. The field was sampled in the late winter of 2014 following a year of soybeans. Due to low SCN pressure in the field, sampling was targeted to areas in which the nematode was likely to be present (Figure 1). These areas included low-lying areas, entry ways, and areas with evidence of water movement. Ten, 3 x 3-m sampling grids were identified as likely to have SCN pressure.
Twenty, 2.5-cm-diameter, 15-to-20-cm deep cores, spaced at 60-cm were collected and mixed for a single composite sample. Samples were stored at 4 °C until processed.

Each soil sample was processed for presence of SCN. In the laboratory each composite sample was crushed and thoroughly mixed. Soil was soaked in water for approximately 30 minutes and was decanted over a 710-μm-pore sieve stacked over a 250-μm-pore sieve to obtain cysts. If the samples revealed “healthy” cysts, some were broken open and juveniles and eggs were crushed to rupture cells containing mtDNA template. Each template – the region of DNA that serves as a binding site for PCR primers, was prepared from a single juvenile or egg. A subset of all samples were increased in the greenhouse on SCN-susceptible line Williams 82 to continue the population and obtain fresh cysts for mtDNA extraction. Two to three mtDNA templates were obtained from each of 30 cysts at each locations throughout the field that “healthy” cysts were found. Templates were stored at 4 °C.

Additionally, DNA from 35 different isolates was cleaned and amplified using an IBI PCR clean-up and extraction kit (IBI Scientific, USA) and sent to the sequencing facility at University of California, Davis which uses an ABI Prism® automated sequencer. Sequences were edited using CodonCode Aligner 6.0.2 (Li-Cor Inc, 2015). The 35 sequenced isolates consisted of: 30 from the northeast Nebraska field; 3 from Minnesota fields that previously tested positive for the MNNE haplotype; 1 cleaned and reamplified template that had previously tested positive for the MNNE haplotype; and 1 cleaned and reamplified templated that had previously been identified as the A haplotype.
3.3 Results

*Diagnostic Tool Development*

Digestion with the *Hha1* restriction endonuclease was unsuccessful and resulted in a single band for all but 2 of the previously tested, cleaned, and reamplified templates. Due to incomplete records, it was known that all cleaned and reamplified templates were from a field that contained the MNNE haplotype, but not if the specific template contained the MNNE haplotype. The *Hha1* enzyme did not cut any of the templates prepared from field populations (Figure 2). Detection of the MNNE haplotype using the *Hha1* restriction enzyme allowed for the development of a positive control for the multiplex markers.

The multiplex marker was tested on 83 field populations designed to target the 321 bp region within CO1. Fortunately, the positive controls developed from cleaned and reamplified templates ensured that the reactions were functioning correctly. Aside from the positive controls, each test resulted in gels with a single band indicating that the A haplotype (Figure 3).

*Correlation of MNNE haplotype to SCN Virulence Phenotype*

Performing PCR on the segmenting greenhouse populations of *H. glycines* using the multiplex marker targeting the MNNE haplotype did not reveal evidence of a correlation between virulent phenotypes and the MNNE haplotype (Figure 4). All segregating lines tested were negative for the MNNE haplotype.
Field Incidence

Evidence of SCN was detected in 8 of the 10 locations sampled, but not found in location 5 or 8 (Figure 1). Screening of cysts from the northeast Nebraska field with the multiplex marker did not identify the MNNE haplotype (Figure 5). After sequencing and editing the 321 bp sequence, the GCGC palindrome was only present in the cleaned and reamplified positive controls. None of the templates from the northeast Nebraska field contained the MNNE haplotype.

3.4 Discussion

Extensive sampling and screening of the northeast Nebraska field known to have the MNNE haplotype only identified the A haplotype found throughout the United States. There was no evidence to suggest that the MNNE haplotype is associated with HG type after screening the segregating SCN populations. There was, however, confirmation that the MNNE haplotype exists as it was confirmed in a sample previously identified to contain it. This suggests that the MNNE haplotype exists in extremely low frequencies in a field setting. Previous research identifying the haplotype may have had their results skewed due to random chance or sampling error, such as multiple positive ID’s coming from the same cyst and thus containing the same cytoplasmic DNA. While the MNNE haplotype exists, it is not found at high enough levels to warrant the development of a diagnostic test.

Analysis of the diversity of the CO1 mitochondrial region provides insight into the origin of SCN. Though there is some debate as to whether H. glycines is indigenous
to the United States or if it was introduced from Asia, the low amount of genetic diversity in the United States could be explained by passive dispersal associated with agricultural expansion across the globe. This recent distribution is observed in many nematode species (Koch et al., 2000). Studies of the *G. pallida* mitochondrial genome found that allelic richness of populations introduced to Europe was only one-third that which was observed in the nematode’s native Peru (Plantard et al., 2008). The low diversity can further be explained by suggesting that SCN was introduced to the United States in a small number of founder events. Single events are characterized by successive bottlenecks that reduce genetic diversity (Crisescu et al., 2001; Sakai et al., 2001) while multiple introductions may result in a high amount of genetic diversity (Colautti et al., 2005; Kolbe et al., 2004). Further research into the cytoplasmic genetic diversity of SCN in Asia is needed to support this argument. Looking at 8 microsatellite markers, Wang et al. (2015) genotyped 13 SCN populations from north China and found a high level of genetic diversity. Examination of the diversity of the CO1 mitochondrial region in *H. glycines* populations from different regions in China would provide a direct comparison to the data in this study. Higher levels of mitochondrial diversity would be expected if the nematode is native to China. Due to the passive dispersal of cyst nematodes certain patterns of diversity might be scrambled, but the overall diversity between regions may be much greater. However, there could be selection for certain genotypes associated with modern soybean breeding programs that greatly decreases the amount of variation.

Though we were unable to detect a relationship of SCN virulence to the MNNE haplotype or find the haplotype in high levels in the field, this research provides a deeper
understanding of the genetic makeup of SCN. By sequencing the length of the CO1 mitochondrial genome, and also a smaller area that was found to contain polymorphisms, we successfully showed that new diagnostic tools to determine SCN virulence should focus on other regions of the nematode’s DNA. Additionally, we found that the MNNE haplotype is found at much lower levels than previously thought.
3.5 Literature Cited


3.6 Figures

**Figure 1.** Map of Nebraska field in Cedar County in which the MNNE haplotype was identified. Each number represents a targeted SCN sampling location. The MNNE haplotype was originally found in location 10. Evidence of SCN was found in all locations except 5 and 8.
Figure 2: HhaI restriction digest on SCN isolates from a Minnesota field previously found to have the MNNE haplotype. Columns 1, 7, 8, and 14 were 20 bp ladders. Templates on the left (2–6) were 701 bp long and those on the right (9–13) were 321 bp. Only columns 4 and 11 contained the GCGC palindrome.
Figure 3. Check gel of 25 Nebraska SCN populations using the multiplex markers. All Nebraska populations were negative for the MNNE haplotype. If any of the populations tested contained the MNNE haplotype, there would have been a second, smaller band.
Figure 4. Gel of SCN populations segregating for virulence to PI 88788 and Peking resistance. 1) KB-H reared on PI 88788; 2) KB-H reared on Susceptible; 3) KB-M reared on Peking; 4) KB-M reared on susceptible; 5) KB-321 reared on PI 88788; 6) KB-321 reared on Peking; 7) KB-321 reared on susceptible; 8) MNNE negative control; 9) 50 bp Ladder; 10-11) inner primer control; 12-13) MNNE positive controls 1 and 2. There was no association of the MNNE haplotype with segmenting SCN greenhouse populations regardless of virulence or host resistance source.
Figure 5. Gel of SCN DNA templates from the Cedar County, Nebraska field originally found to contain the MNNE haplotype. 1) 50 bp ladder; 2) Location 1; 3) Location 2; 4) Location 3; 5) Location 4; 6) Location 6; 7) Location 7; 8) Location 9; 9) Location 10; 10) MNNE haplotype positive control. The GCGC palindrome was not found in any of the sampled locations from the Cedar County field.
Chapter IV

Thesis Conclusions

Soybean cyst nematode (*Heterodera glycines* Ichinohe) is one of the most economically important pests of soybeans in the United States as well as the rest of the world. While crop rotation and the use of resistant varieties have helped to manage the nematode, a lack of multiple sources of host resistance has led to an increase in the percentage of virulent populations in Nebraska and other soybean-producing states. As the frequency of virulent SCN populations increases, host resistance will become less effective. Due to the potentially hidden impact of SCN, Nebraska soybean farmers not only need tools to manage the nematode, but they also need to ensure they are utilizing the tools correctly.

The frequency of SCN populations virulent to PI 88788 was in the middle of those found in surrounding states – with some states having a higher frequency and others being lower. This is true even though Nebraska was one of the last states in the region to confirm SCN in a field. The high frequency of virulent populations in Nebraska and the presence of HG types 2.5.7 and/or 7 within all agricultural reporting districts suggests that many of the populations initially introduced to Nebraska may have already been
virulent to certain sources of resistance. Another point that supports this theory is that of the populations virulent to PI 88788, the frequency of populations with a Female Index (FI) greater than 30 is fairly low. Populations with a FI greater than 30 are considered hyper-virulent. Illinois and other states have found hyper-virulent populations spread throughout, but only 11% of the populations virulent to PI 88788 were hyper-virulent in Nebraska.

As more growers become aware of SCN and begin managing for the nematode, the shifts in virulence profiles will continue with more populations being able to overcome PI 88788 resistance. As this happens and yields associated with PI 88788 resistance drop, industry will be forced to shift away from the overuse of PI 88788 and towards other sources of resistance – primarily Peking resistance, or perhaps cultivars stacked with resistance from multiple plant introduction lines. Increasing the number of cultivars with Peking resistance alone would only be a temporary fix, since many populations are already known to be virulent to Peking resistance (close to 30% of Missouri, Kansas, and Nebraska SCN populations).

Only 11% of Nebraska populations were virulent on both Peking and PI 88788, suggesting that stacking cultivars with Peking and PI 88788 resistance could be an effective means to not only manage SCN, but also to slow shifts in virulence. While they each contain the \textit{rhg1} resistance gene (though many more copies are in PI 88788), the addition Peking resistance provides physical barriers (such as cell wall appositions) that will prevent less vigorous nematodes from forming a syncytium, even if they are virulent
to many copies of the *rhg1* resistance gene. These thickened cell walls are not evident in
the PI 88788 resistance reaction.

PI 437654 (Hartwig) is still a very effective source of host resistance and no
populations in Nebraska, nor the rest of the North Central soybean production region,
were virulent to it. However, due to yield drag associated with PI 437654, use of this
resistance source continues to be a problem and few breeding programs are utilizing it.
Sequencing the soybean genome and the increase of marker-assisted selection breeding
programs, breeders will gain a better understanding of the relationship between low
yields and PI 437654 resistance. If a commercial soybean variety containing PI 437654
resistance, but not the associated yield drag is released, growers around the United States
and the world would immediately adopt it into their SCN-management programs. Aside
from providing control against SCN populations already virulent to PI 88788 and Peking
resistance, the introduction of a high-yielding PI 437654 variety would also slow the rate
at which the nematode is able to overcome the common forms of host resistance.

Originally, it was thought that the discovery and characterization of a second SCN
haplotype (MNNE) would aid farmers managing virulent populations of SCN. Initial
testing found that the MNNE haplotype is not found at high enough levels in the field to
be of a concern to farmers and warrant the development of a diagnostic test. Had there
been association between the MNNE haplotype and virulence to either PI 88788 or
Peking resistance, a diagnostic test would have been helpful despite the low frequency in
the field. Our intensive study of the CO1 region of mitochondrial DNA showed that
further research to develop a diagnostic test to determine HG type of field populations of
SCN should focus on other regions of the nematode’s DNA. Non-indigenous nematodes, such as the potato cyst nematode (*Globodera pallida*), have low levels of genetic diversity. The low amount of haplotype diversity found within the mitochondrial genome of SCN provides evidence that SCN is not native to the United States and offers insight into the nematode’s spread.

Looking into the abundance and spread of the MNNE haplotype highlighted the need to check previously completed research for accuracy and not just take a previous report as canon. We were unable to find the MNNE haplotype in any of the Minnesota fields, nor the field in northeast Nebraska that first tested positive for the off-haplotype. Many things can go into inaccurately reporting species information. Through no fault of those who originally classified the MNNE haplotype, the results were skewed to show that the MNNE haplotype was found in a relatively high abundance in the Minnesota fields that had been tested. In reality, this haplotype was rare and the previous results were most likely an anomaly due to sampling bias.

The goal of this research was to determine the diversity and virulence of SCN in Nebraska through two objectives: 1) to characterize the distribution and diversity of the virulence phenotypes of SCN in Nebraska; and 2) to explore the haplotype diversity of SCN within Nebraska and within a single field. In conclusion, though the shifts in virulence phenotypes are not as severe as have been reported elsewhere, Nebraska farmers should continue to monitor their SCN populations to ensure that their resistance is still effective. Furthermore, as with other non-indigenous nematodes, the haplotype diversity of SCN is quite low and farmers need not be concerned with the MNNE
haplotype. As SCN HG types and virulence becomes better understood, farmers in Nebraska and around the world will be able to expand the toolbox necessary to successfully manage this nematode.