BIONOMICS OF *HETERODERA GLYCINES* AND *PRATYLENCHUS PENETRANS* ASSOCIATED WITH MICHIGAN SOYBEAN PRODUCTION

By

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ABSTRACT

BIONOMICS OF *HETERODERA GLYCINES* AND *PRATYLENCHUS PENETRANS* ASSOCIATED WITH MICHIGAN SOYBEAN PRODUCTION

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Soybeans (*Glycine max* (L.) Merrill) are an important field crop throughout the world. In 2010, soybeans were planted on over 77 million acres and valued at 38.9 billion dollars in the United States (USDA-NASS, 2010). In Michigan, over 2 million acres of soybeans were planted in 2010 (USDA-NASS, 2010). Two important nematode species damage soybeans in Michigan, *Heterodera glycines* (Ichinohe, 1952) and *Pratylenchus penetrans* (Cobb, 1917; Filipjev & Schuurmans Stekhoven, 1941). The goal of this research was to discover information to improve management of these nematode species. A survey was conducted in 2011-2011 to determine the extent of *H. glycines* (soybean cyst nematode, SCN) and *P. penetrans* (penetrans lesion nematode, PLN) infestation in soybean-producing counties in Michigan (Chapter 2). A study was done from 2008-2010 to determine the effects of SCN-resistant varieties on field populations of SCN (Chapter 3). Finally, greenhouse studies were used to evaluate the effect of PLN on SCN reproduction on resistant varieties (Chapter 4). Improved management of SCN and PLN should increase soybean yields on infested acreage in Michigan.

DEDICATION

I would like to dedicate this thesis to my Grandpa “Shoey” Schumacher. He inspired me to pursue a graduate degree and achieve my academic goals.

ACKNOWLEDGEMENTS

I wish to thank my major professor, Dr. George W. Bird, for his encouragement and guidance throughout my degree program. I also want to thank the other members of my committee, Dr. Chris DiFonzo, Dr. Larry Gut, and Dr. Karen Renner, for their assistance in the writing and editing processes. I would like to thank John Davenport for his knowledge and assistance with planting and maintaining my research sites. Fred Warner and Angie Tenney of MSU Diagnostic services were of immense help to me during my time as a graduate student. I want to thank them for their patience and advice regarding my greenhouse trials. My student workers Ben Caswell, Cody Brown, Melissa Morgan, Holly Kole, Jamie Pereault, and Bethany Whitford were a huge help to me and I am thankful for their assistance throughout the years.

My research would not have possible without the support of Tom Kendle, Ken Arting, and John Rhein. I thank them for the use of their farms and for always helping me when needed. Last, and certainly not least, I wish to thank my Mom and Dad, my twin brother, Michael, as well as my husband, Josh, for their love and encouragement throughout this academic pursuit. Thank you all from the bottom of my heart.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1
Introduction and Literature Review

Heterodera glycines

The soybean cyst nematode (*Heterodera glycines* Ichinohe, 1952) is a soybean (*Glycine max* (L.) Merrill) pest of global significance. *Heterodera glycines* (SCN) is the most economically-damaging pest of soybeans in the United States and the second most economically-damaging pest of soybeans worldwide (Wrather & Koenning, 2006). Several approaches have been developed to manage SCN, including crop rotation, resistant varieties, sanitation, and chemical control (Schmitt *et al.*, 2004). Proper integrated nematode management plans increase soybean yields on infested farmland (Brown-Rytlewski *et al.*, 2009).

Soybean cyst nematode is believed to have originated in Asia. It was introduced to North America through imported soil used to inoculate soybean roots with nitrogen-fixing bacteria (Niblack & Tylka, 2002). Soybeans were introduced into North America in 1765, so the pest may have been undetected in the United States since that time (Schmitt *et al.*, 2004). SCN occurs in every major soybean production area worldwide, including North America, South America, and Asia (Niblack & Tylka, 2002). It was first discovered in the United States in North Carolina in 1954 (Winstead *et al.*, 1955). A SCN quarantine was promulgated in 1957. By 1972, however, SCN spread to Illinois and the quarantine was rescinded (Schmitt *et al.*, 2004).

In Michigan, SCN was first detected in Gratiot County in 1987. SCN exists in all but two major soybean-producing counties in the state (Warner *et al.*, 1994; Brown-Rytlewski *et al.*, 2009). Henry Ford was a soybean advocate who imported many germplasm lines and grew thousands of acres of the crop in Michigan in the 1930s (Bryan, 1990). It is unclear whether his involvements in the soybean industry lead to the accidental introduction of SCN to the state.

Soybean cyst nematode is currently classified according to the system described by De Ley and Blaxter in 2002 (Lee, 2002);

Kingdom Animalia

Phylum Nematoda

Class Chromadorea

Order Rhabditida (Chitwood, 1933)

Family Hoplolaimidae (Filipjev, 1934)

Genus *Heterodera* (Schmidt, 1871)

Species *H. glycines* (Ichinohe, 1952)

The SCN disease of soybeans causes both primary and secondary symptoms. Symptoms may be seen either above or below ground. Primary symptoms may be necrotic, hypoplastic (undergrowth), or hyperplastic (overgrowth). Necrotic symptoms of disease occur because SCN is a predisposition agent for other pathogens. Sudden death syndrome, caused by *Fusarium virguliforme*, can overwinter in SCN cysts (Rupe *et al.*, 1993). Hypoplastic symptoms occur because the nematode causes stunting of the soybean plant. Giant cell formations (syncytia) within the roots of the plant are hyperplastic symptoms (Perry & Moens, 2006). Plants infected with a high number of SCN have poorly developed root systems that cannot utilize nutrients and water efficiently (Niblack & Tylka, 2002). This causes the secondary symptoms of the disease, low yields and yellowing (chlorosis) of the leaves. Seed yields are low because fewer pods develop on infected plants (Niblack & Tylka, 2002). Above ground symptoms, in addition to chlorosis and stunting, include circular patches of plants that exhibit poorer growth than surrounding areas (Sardanelli & Ellison, 2005). Besides poorly-developed root systems, below ground SCN symptoms include inhibition of *Rhizobium* nodule formation on the roots (Sardanelli & Ellison, 2005).

SCN causes considerable damage to the soybean plant by utilizing nutrients that would normally be part of the plant's physiology. The disease process begins with SCN hatching from eggs, following a period of seasonal inactivity (winter). Soybean root exudates, temperature, and water trigger hatch of second-stage juveniles (J2) from eggs (Starr *et al*., 2002). Using their stylets, the J2 leave their eggs and enter the soil. Newly hatched J2 invade roots of soybean plants in the zone of elongation (Lee, 2002). Once inside roots, the J2 migrate intra-cellularly through cortical cells towards the vascular cylinder and use their stylets to move through to neighboring cells (Lee, 2002). After this period of exploration, a nematode chooses an initial cell for feeding. Chemical signals emitted by the nematode cause the plant's cell walls to deteriorate, forming syncytia in the vascular cylinder (Lee, 2002). A feeding tube is produced, allowing the nematode to obtain nutrients from the syncytia. The nematode swells into a "sausage shape" and molts again into a third-stage juvenile. Development continues until molting into a fourth-stage juvenile. One more molt occurs (into adulthood) and males leave the root (Lee, 2002). They no longer feed. Females feed from the J2 stage through the adult stage (about 21 days); whereas, males feed only for about 9 days as J2 and J3 (Colgrove & Niblack, 2005).

Following the final molt, the female SCN grows until she bursts through the root surface. A male will find her and mate. Under favorable environmental conditions, the female will produce its initial eggs in an external egg sac, which allows the eggs to hatch readily in water and result in a rapid population build-up (Perry & Moens, 2006). Subsequently, the female's body is filled with eggs. The cuticle tans, turns brown and leathery, and becomes a protective egg-filled cyst. The dead female's head detaches from its feeding site in the root and the cyst enters the soil where it remains until egg hatch. This occurs after diapause (a period of seasonal inactivity) and is an important aspect of the population biology of SCN, as it facilitates survival under adverse conditions and limits activity in the absence of a host (Schmitt *et al.*, 2004).

The typical life cycle of SCN is 21 days at 25°C (Perry & Moens, 2006). The optimum temperature for hatching is 25-30°C and 26-28°C for development. The number of days to mature female development is 14 (Starr *et al.*, 2002). Juvenile SCN are small, 1/64" (0.4 mm) or less (Purdue, 2007). Swollen females are larger, 1/25" (1 mm), and can be seen on soybean roots without magnification (Purdue, 2007). Dead females (cysts) are lemon-shaped and are capable of housing hundreds of live eggs, with approximately 150 or more eggs per healthy cyst. The male SCN has a single testis and the female has paired ovaries (Perry & Moens, 2006). Viable units are defined as the number of eggs and J2 found in a soil sample. This parameter is used when assessing the potential for a population to be infective.

The SCN host-parasite relationship is dynamic. Both egg production rates and life expectancy are genetically determined, but may be altered by host genetics, vigor, and other environmental factors (Schmitt *et al.*, 2004). SCN primarily affects soybeans in temperate regions. There can be up to six generations per year, depending on several factors: host, planting date, soil temperature, soil moisture, geographic location, soybean maturity group, and the length of the growing season (Niblack & Tylka, 2002). In Michigan, there are usually 2.5 generations per growing season (Bird & Warner, 2003). The horizontal spatial distribution pattern of SCN in soil is usually aggregated (Francl, 1986). The greatest population density (vertical spatial pattern) typically occurs in the upper 20-30 cm of the soil profile (Alston & Schmitt, 1987). Both growing season and over-winter changes in SCN densities are strongly density-dependent, with initial egg densities the most significant factor affecting population changes across rotations and years (Todd *et al.*, 2003).

Soil texture plays an important role in SCN population dynamics. SCN population density is related to the proportion of sand, clay, and silt in the soil (Avendaño *et al.*, 2004). Coarse-textured soils generally favor hatching and subsequent invasion of root systems, providing suitable conditions for aeration and nematode migration (Perry & Moens, 2006). Consequently, drought and water-logging may inhibit SCN hatch (Perry & Moens, 2006). In Michigan, SCN infection normally occurs from April to August, when soybean plants are actively growing in the soil.

Crop rotation is a very important component of managing SCN. Cysts can remain viable in the soil for 8-12 years in the absence of a host (Niblack & Tylka, 2002). Other host crops for SCN include: edible beans, common and hairy vetch, pea, and sweet clover (Tylka, 1994). Winter annual weeds like purple deadnettle, henbit, and field pennycress also serve as hosts for SCN (Creech *et al.*, 2007; Barnett in Brown-Rytlewski *et al.*, 2009). Rotation with non-host crops, therefore, is essential. Non-host crops for SCN include corn, wheat, and sugar beet. A three year rotation is generally recommended, with one year planted into a host crop and two consecutive years planted into non-host crops (Warner & Bird, 1997). Because SCN is an obligate parasite, it cannot successfully reproduce on non-host crops.

Damage caused by SCN has great economic significance. From 1999-2002, North Central states estimated a loss of 447 million bushels to SCN (Niblack & Tylka, 2002). Worldwide losses to this pathogen exceed $1 billion per year (Purdue University, 2007). Actual yield losses may range from 5-100% (Warner & Bird, 1997). An average soybean yield for Michigan is about 45 bu/acre, but heavily infested fields may only yield 15-25 bu/acre, or less.

Pest management strategies associated with SCN, such as the use of resistant soybean varieties, are commonly practiced on farms with SCN-infested acreage. A resistant soybean variety is one that yields well in a site that has an above threshold P_i (initial population density) and results in poor nematode reproduction as measure by P_f (final population density). A susceptible variety, on the other hand, is a variety that yields low in the presence of SCN and also supports a high population density of SCN.

There are 109 known sources of resistance (soybeans that have coevolved with SCN), but only seven have been commercialized (Niblack *et al.*, 2002). The seven commercialized sources are: PI 548402, PI 88788, PI 90762, PI 437654, PI 209332, PI 89772, and PI 548316. "PI" stands for "plant introduction", which includes sources of resistance originating from Asia. Varieties from three sources of resistance are currently available in Michigan: PI 548402, PI 88788, and PI 437654. PI 88788 is planted in ca. 95% of SCN-infested soybean acres in Michigan (Warner & Bird, 1997; Brown-Rytlewski *et al.*, 2009).

There are two known mechanisms of SCN resistance: horizontal and vertical resistance. Horizontal resistance is general and controlled by multiple minor genes. It is quantitative and effective against all pathogen variants (Starr *et al.*, 2002). Females reproducing on plants with this type of resistance are usually small and produce few eggs, resulting in a low P_f. Soybeans derived from PI 88788 have horizontal resistance. Vertical resistance is specific and controlled by up to three genes that result in no or poor nurse cell development. The P_f associated with this type of resistance is typically very low. The source of resistance differentiates intra-specific variants of SCN (Vanderplank, 1978). Some variants are highly aggressive whereas others are not aggressive. Soybeans derived from PI 548402 have vertical resistance. PI 437654-derived soybeans have both horizontal and vertical resistance, making them the most SCN-resistant soybeans available to Michigan soybean growers.

Aggressive field populations of SCN reproduce on and reduce yields of specific resistant varieties. This was first observed in 1957 by Ross and Brim, only three years after SCN was first detected in the United States (Winstead *et al.*, 1955). Golden *et al.* (1970) developed a SCN Race System to differentiate among field populations. The system was expanded in 1988 by Riggs and Schmitt. Because of deficiencies associated with the 1970 and 1988 race systems, the HG (*Heterodera glycines*) Type Test was developed by Niblack *et al.* in 2002. This test allows farmers to identify what SCN population types are in their fields and determine the most appropriate resistant variety to use. HG Type 0 (a non-aggressive population) will not reproduce or cause damage on any of the seven commercial sources of resistance. Planting any SCN resistant variety, therefore, should result in high bean yields. HG Type 1 reproduces on PI 548402, Type 2 on PI 88788, Type 3 on PI 90762, Type 4 on PI 437654 (a highly aggressive population), Type 5 on PI 209332, Type 6 on PI 89772, and Type 7 on PI 548316 (Niblack *et al.*, 2002). HG Type 2.5.7 is the most common HG Type in Michigan (personal communication, G.W. Bird, 2008). With this HG Type, SCN reproduction occurs on PI 88788, PI 209332, and PI 548316, resulting in significant soybean yield loss (Bird & Warner, 2003). In 2003, the system was truncated (SCN Type System) to use only the three current commercially-available sources of resistance (Niblack *et al.*, 2003; Brown-Rytlewski *et al.*, 2009).

Various cultural practices may be used to manage populations of SCN. These include no-tillage, late planting date, soil management (weeds and fertility), and the use of adequate irrigation. There is evidence that SCN population levels are lower and yield is higher in soil that receives little disturbance (no-till) with planting compared to soil receiving conventional tillage practices (Riggs & Wrather, 1992). In a study done in North Carolina, numbers of viable units at harvest were lower in no-till plots in four of six years (Noel & Wax, 2003). Furthermore, planting in late June to early July (rather than in May) impacts the population of SCN because its density declines in fallow soil from June through July (Riggs & Wrather, 1992). Organic amendments to the soil (e.g. animal manure) may reduce the population density of SCN by improving soil health and enhancing the activities of soil microorganisms that harm nematodes (Schmitt *et al.*, 2004). Also, using adequate irrigation with proper fertilization may help reduce plant stress. Combining all of these practices can increase a soybean plant's tolerance to SCN.

Sanitation is important to control SCN populations. SCN can be transferred to other fields on farm equipment; therefore, equipment should be cleaned thoroughly with a power washer. Soil movement should be kept to a minimum. Fields without SCN should always be worked and harvested before infested fields (Warner & Bird, 1997).

Nematode-predaceous fungi, endoparasitic fungi, parasites of females and eggs, fungi-producing antibiotic substances, and bacterial antagonists all show promise of becoming potential biological control agents for SCN (Carris and Glawe, 1989). Earlier this year I was involved in the discovery of a bacterial pathogen of SCN (*Pasteuria* spp.) associated with J2 from the research site used for Chapter 3 of this M.S. Thesis. This pathogen may explain the periodic SCN population crashes seen at the research site.

Pratylenchus penetrans

The penetrans lesion nematode (*Pratylenchus penetrans* Cobb, 1917; Filipjev & Schuurmans Stekhoven, 1941) is a common migratory endoparasite of plants. There are more than 70 species in the *Pratylenchus* genus. Some species are aggressive feeders and others are not. *Pratylenchus penetrans* (PLN) is an aggressive species with a very wide host range. PLN occurs in temperate regions around the world and causes damage to many important crops (Mai *et al.*, 1977). The nematode is named for the characteristic necrotic lesions it causes as it migrates through root tissue. It is the most common *Pratylenchus* spp. in Michigan.

PLN is currently classified according to the system described by De Ley and Blaxter in 2002 (Lee, 2002);

Kingdom Animalia

Phylum Nematoda

Class Chromadorea

Order Rhabditida (Chitwood, 1933)

Family Pratylenchidae (Thorne, 1949)

Genus *Pratylenchus* (Filipjev, 1936)

Species *P. penetrans* (Cobb, 1917; Filipjev & Schuurmans Stekhoven, 1941)

Unlike SCN, PLN hatching is not influenced by root exudates, but rather temperature and osmotic pressure (Mai *et al.*, 1977). Most members of the *Pratylenchus* genus reproduce via parthenogenesis. PLN, however, reproduce sexually, and the females lay up to 2 eggs per day (Perry & Moens, 2006). Eggs are laid in root tissue and along the root surface (Perry & Moens, 2006). All juvenile and adult stages of PLN are vermiform and less than 1/25" (1 mm) (Perry & Moens, 2006). Although PLN can be found in both soil and roots, higher population densities can be recovered from root tissue (Perry & Moens, 2006). The life cycle of PLN is 30 days at 30°C and 92 days at 15°C (Mai *et al.*, 1977).

The root cortex is affected by stylet thrusts and body movements of PLN (Mai *et al.*, 1977). As the cells decompose, they provide entry points for other pathogens like bacteria and fungi. If the root system of a soybean plant is affected by these other pathogens, the plant will not be able to absorb the necessary amount of water and nutrients. This causes affected plants to be stunted, chlorotic, and wilted (Mai *et al.*, 1977). Soybean yield losses of up to 20% may occur in sandy soils (Bridge & Starr, 2007).

Previous research suggested a possible interaction between PLN and SCN on soybeans. When the nematodes are present together, there is a decrease in soybean production (Melakeberhan, 1998). As the nematodes compete with each other, their population densities change. In a greenhouse study, infection rates of PLN increased with increasing rates of SCN (Melakeberhan, 2003). Bates (2006) reported that inoculating soybeans derived from the PI 88788 source of resistance with an equal amount of PLN and SCN resulted in enhanced SCN development.

Control of PLN is difficult due to its very wide host range. Research has shown that adding organic matter to the soil will reduce the number of PLN (Mai *et al.*, 1977). However, there is little information on species-specific control of PLN. Improved SCN management may also lead to lower population densities of other important plant parasites, like PLN, in soybean fields.

CHAPTER 2
Distribution of *Heterodera glycines* in Michigan

Abstract

The soybean cyst nematode (*Heterodera glycines* Ichinohe, 1952) is a pest of soybeans throughout most soybean-producing counties in Michigan. A 2010-2011 Michigan *H. glycines* (SCN) survey was designed and conducted to provide a comprehensive, statistically-valid analysis of the extent of SCN infestations throughout Michigan. The Michigan Soybean Promotion Committee grower database was used to randomly select five percent of Michigan soybean farms (n=558) from 46 counties. Sixty-six farms submitted soil samples for the survey early in the 2010 growing season. An additional 337 farms were visited during the growing season to collect soil samples. In 2010, 32 of 36 counties sampled were positive for SCN. Five of the seven highest soybean-producing counties in Michigan (Gratiot, Monroe, Saginaw, Shiawassee, and Tuscola) had SCN infestations above 50%. The average occurrence of SCN in Michigan was 50% in 2010.

Introduction

The soybean cyst nematode (*Heterodera glycines* Ichinohe, 1952) is a soybean (*Glycine max* (L.) Merrill) pest of global significance. *Heterodera glycines* (SCN) is considered the most economically-damaging pest of soybeans in the United States and the second most economically-damaging pest of soybeans worldwide (Wrather & Koenning, 2006). SCN is believed to have originated in Asia. It occurs in every major soybean production area worldwide, including North America (Figure 1), South America, and Asia (Niblack & Tylka, 2002). SCN was first detected in Michigan in 1987 (Gratiot County) and is thought to exist in all but two major soybean-producing counties in the state (Warner *et al.*, 1994; Brown-Rytlewski *et al.*, 2009). Figure 2 shows the dates SCN was first detected in Michigan soybean-producing counties (Tenney *et al.*, 2004).

The SCN host-parasite relationship is dynamic. Both egg production rates and life expectancy are genetically determined, but may be altered by host genetics, vigor, and other environmental factors (Schmitt *et al.*, 2004). SCN primarily affects soybeans in temperate regions. There can be up to six generations per year, depending on several factors: host, planting date, soil temperature, soil moisture, geographic location, soybean maturity group, and the length of the growing season (Niblack & Tylka, 2002). In Michigan, there are usually 2.5 generations per growing season (Bird & Warner, 2003).

Soil texture plays an important role in SCN population dynamics. SCN population density is related to the proportion of sand, clay, and silt in the soil (Avendaño *et al.*, 2004). Coarse-textured soils generally favor hatching and subsequent invasion of root systems, providing suitable conditions for aeration and nematode migration (Perry & Moens, 2006). Consequently, drought and water-logging may inhibit SCN hatch (Perry & Moens, 2006). In Michigan, SCN infection normally occurs from April to August, when soybean plants are actively growing in the soil.

Damage caused by SCN has great economic significance. From 1999-2002, North Central states estimated a loss of 447 million bushels to SCN (Niblack & Tylka, 2002). Worldwide losses to this pathogen exceed $1 billion per year (Purdue University, 2007). Actual yield losses may range from 5-100% (Warner & Bird, 1997). Michigan grows nearly 2 million acres of soybeans, which yield about 79 million bushels (Knudson and Peterson, 2011). Therefore, average bean yield in Michigan is approximately 39.5 bu/A, while SCN-infested fields may only yield 15-20 bu/A.

Pest management strategies associated with SCN, such as the use of resistant soybean varieties, are commonly practiced on farms with SCN-infested acreage. There are 109 known sources of resistance (soybeans that have coevolved with SCN), but only seven have been commercialized (Niblack *et al.*, 2002). The seven commercialized sources are: PI 548402, PI 88788, PI 90762, PI 437654, PI 209332, PI 89772, and PI 548316. "PI" stands for "plant introduction", which includes sources of resistance originating from Asia. Only three resistant varieties are currently available in Michigan: PI 548402, PI 88788, and PI 437654. PI 88788 is planted in 95% of SCN-infested soybean acres in Michigan (Warner & Bird, 1997; Brown-Rytlewski *et al.*, 2009).

Aggressive field populations of SCN reproduce on and reduce yields of specific resistant varieties. This was first observed in 1957 by Ross and Brim, only three years after SCN was first detected in the United States (Winstead *et al.*, 1955). In 1970, Golden *et al.* developed a SCN Race System to differentiate among field populations. The system was expanded in 1988 by Riggs and Schmitt. Because of deficiencies associated with the 1970 and 1988 race systems, the HG (*Heterodera glycines*) Type Test was developed by Niblack *et al.* in 2002. This allows farmers to identify what SCN population types are in their fields and determine the most appropriate resistant variety to use. HG Type 0 will not reproduce on any of the seven commercial sources of resistance. Planting any SCN resistant variety, therefore, should result in high bean yields. HG Type 1 reproduces on PI 548402, Type 2 on PI 88788, Type 3 on PI 90762, Type 4 on PI 437654, Type 5 on PI 209332, Type 6 on PI 89772, and Type 7 on PI 548316 (Niblack *et al.*, 2002). HG Type 2.5.7 is the most common HG Type in Michigan (personal communication with G. Bird, 2008). With this HG Type, SCN reproduction occurs on PI 88788, PI 209332, and PI 548316, resulting in significant soybean yield loss (Bird & Warner, 2003). In 2003, the system was truncated to use only the three current commercially-available sources of resistance (Niblack *et al.*, 2003; Brown-Rytlewski *et al.*, 2009).

A current pest survey is needed to determine the occurrence of SCN in Michigan soybean-producing counties. Previous SCN surveys have been biased, relying on extension educators to recruit participants (Warner *et al.*, 1994). In 2005, Diagnostic Services at Michigan State University received 873 samples from soybean farms throughout the state (Bird & Warner, 2005). Fifty-six percent of the samples were positive for SCN (Table 1). A survey conducted by Young (1990) was organized based on soybean acreage per county. In order to truly determine the level of SCN infestation in a region, a randomized survey must be performed. In Michigan, this can be accomplished by randomly selecting a specific number of soybean farms (five percent per county) from the Michigan Soybean Promotion Committee grower database.

Goal, Objective, and Hypotheses

The goal of this survey is to provide a statistically-valid analysis of the extent of SCN infestation throughout soybean acreage in Michigan. The main objective was to determine the occurrence of SCN and HG Type associated with Michigan soybean production. Other objectives were to relate the occurrence of SCN to *Pratylenchus penetrans* (penetrans lesion nematode) and *Fusarium virguiliforme* (sudden death syndrome pathogen). It was postulated that:

1) Fifty percent of Michigan soybean fields are infested with SCN.

2) Soybean-producing counties with the greatest acreage have the highest incidence of SCN.

3) HG Type 2 is the most common HG Type in Michigan.

4) *Pratylenchus penetrans* is widely distributed throughout Michigan soybean acreage.

5) *Fusarium virguliforme* (Mycota) is common in SCN-infested fields.

The project was funded by the Michigan Soybean Promotion Committee in 2010 using Michigan Soybean Checkoff dollars.

Materials and Methods

***Experimental Design* -** Ten percent of the soybean farms (n=1,116) in Michigan were randomly selected for the survey from a list obtained from the Michigan Soybean Promotion Committee grower database using a random number generator. An additional five percent of farms from each county were also randomly selected for alternates when farms from the ten percent list were unwilling to participate. The original ten percent were contacted by mail and asked to submit a soil sample to their local Michigan State University Extension Office from the third field they intended to plant in the 2010 growing season (Appendix 1). Only 66 farms responded to the mail request, so another postcard was sent out asking farms to mail in their samples. When no additional samples were received by mail, the protocol was changed. The number of farms was cut from ten percent down to five percent (n=558). Farms were visited by members of the survey team during the growing season (May-August) and a soil sample was taken from one of their fields.

***Sampling* -** In order for a site to be eligible for sampling, fields must have been planted to soybeans at least once between 2005 and 2010. Fields were sampled to determine the SCN population density using a cone-shaped soil probe (approximate volume of 2000 cm^3), 15.2 cm below the soil surface. Sampled fields ranged in size from less than 20 to greater than 80 acres. A zigzag pattern was used to sample a field, and the probe was emptied into 3.8 L plastic bags (Figure 3). The samples were stored at 7°C until processed.

Nematode Extraction, Identification - Before extraction, the soil in each sample bag was mixed thoroughly to ensure uniformity. A 100 cm^3 subsample from each sample was processed using a modified centrifugation-flotation technique (Jenkins, 1964). Each sample was examined microscopically to determine the presence or absence of SCN cysts or viable units (eggs and second-stage juveniles). To rule out false positives and false negatives, 50 soil samples were randomly chosen for a bioassay using a SCN-susceptible soybean variety, a *Heterodera schachtii* (sugar beet cyst nematode)-susceptible sugar beet variety, and a *Heterodera trifolii* (clover cyst nematode)-susceptible alfalfa variety. The bioassays were planted in clay pots with 600 cm^3 soil from each of the 50 selected farms. The pots were placed in the greenhouse under a 16 hour light period with a daytime temperature of 25°C and a nighttime temperature of 23°C. They were watered daily. After 30 days, the plants were removed from the pots and the root systems were evaluated for the presence of white females.

HG Type Testing – Seventeen soil samples were randomly selected for HG Type testing. The samples were sent to MSU Diagnostic Services.

Pratylenchus penetrans - In addition to SCN analysis, each sample was evaluated for occurrence of *Pratylenchus penetrans*. The results for *P. penetrans* are presented in Chapter 4.

Fusarium virguliforme - After each sample was read it was sent to Dr. Martin Chilvers (Department of Plant Pathology, Michigan State University) for *Fusarium virguliforme* analysis. Dr. Chilvers' lab is currently in the process of developing a PCR technique to identify *F. virguliforme* from processed soil samples. Results will be available in the future.

Results

In 2010, a total of 403 farms/fields were sampled from 36 counties in Michigan (Table 2). 155 farms remain to be sampled in 2011 (Table 3). Of the 36 counties sampled in 2010, 32 were positive for SCN. Counties were color-coded according to the following SCN-infestation levels: 0-24%; 25-49%; 50-74%; and 75-100% (Figure 4). The occurrence of SCN averaged among the counties sampled in 2010 was 50%.

Leading Michigan soybean-producing counties from 2008-2010 were Gratiot, Lenawee, Monroe, Saginaw, Sanilac, Shiawassee, and Tuscola (Figure 5). These counties had greater than 20 samples each and were 78, 45, 81, 91, 32, 79, and 54 percent positive for SCN, respectively.

White females were only observed on the susceptible soybean variety from the bioassays. Therefore, each nematode originally observed in the 50 samples was SCN and not another species of cyst nematode.

Six farms were processed for HG Type analysis in 2010. Based on the HG Type test results, 50% of farms had a HG Type 2 population.

Discussion

The survey conducted by Warner *et al.* in 1994 resulted in a SCN-infestation level of 53% in Michigan. Based on the 2010 findings from this survey, the level of SCN-infestation in Michigan is 50%. The incidence of SCN has not dramatically changed over the past 17 years. However, one county that had not been previously identified as SCN-positive (Oakland county) can now be added to the U.S. map seen in Figure 1.

Variations in the extent of SCN-infestation were observed within counties of similar soybean acreage. For example, highly-infested counties such as Gratiot (78%), Monroe (81%), Saginaw (91%), and Shiawassee (79%), had greater than 20 samples each. Huron (27%), Lenawee (45%), Sanilac (32%), and Tuscola (54%) counties, however, had greater than 20 samples each but lower levels of infestation.

There seems to be a relationship between number of bushels harvested and SCN occurrence. Five of the seven highest soybean-producing counties in Michigan (Gratiot, Monroe, Saginaw, Shiawassee, and Tuscola) had SCN infestations above 50%. The differences in SCN-infestation between counties may be due to soil type, tillage vs. no tillage, aggressiveness of the SCN populations, or time of year the sample was taken.

A HG Type 2 population means that SCN reproduction will occur on varieties derived from the PI 88788 source of resistance. This is alarming because soybean varieties with PI 88788 sources of resistance are planted on approximately 95% of infested acreage (Warner & Bird, 1997; Brown-Rytlewski *et al.*, 2009). Growers may have to find other means of controlling their SCN populations; such as, use of other SCN sources of resistance, or rotation to non-host crops.

Overall, the results of the 2010 portion of the survey indicate that SCN is a serious threat in many soybean-producing counties throughout Michigan. A comprehensive understanding of how many Michigan soybean farms are infested with SCN, as well as the SCN types present, is important for the design of future management strategies and education programs. It is necessary to learn more about SCN infestation to assure the future prosperity of the Michigan soybean industry.

CHAPTER 3

Effects of Soybean Cyst Nematode-Resistant Varieties on Michigan Field Populations of *Heterodera glycines* (Nematoda)

Abstract

The soybean cyst nematode (*Heterodera glycines* Ichinohe, 1952) is a pest of soybeans throughout most soybean-producing counties in Michigan. *Heterodera glycines* (SCN) management tactics, such as the use of resistant soybean varieties, are ever-increasing on farms with SCN-infested acreage. A resistant soybean variety is one that yields well in a site that has an initial SCN population density above threshold and results in low nematode reproduction. Commercial varieties from three sources of resistance are available for use in MI: PI 548402, PI 88788, and PI 437654. The objective of this research was to determine the effects of resistance sources on field populations of SCN within growing seasons. Three farms were selected, based on previous SCN history, for the project in 2008, 2009, and 2010; providing a total of nine site-year locations. Six soybean varieties were used each year. Selection was based on their maturity groups, sources of SCN resistance, and grower experience. The at-planting (P_i) and at-harvest (P_f) population densities were determined for each variety at each of the nine site-year locations. Annual SCN reproduction (RF=P_f/P_i) and bean yield were also determined for each site and growing season. It was hypothesized that in SCN-infested fields: 1) use of SCN-resistant varieties results in a decline in within-season nematode reproduction; 2) use of SCN-susceptible varieties results in an increase in within-season nematode reproduction; and 3) if the P_i is below damage threshold (less than 500 viable units [eggs and second-stage juveniles] per 100 cm^3), there will be no significant difference in yield response between susceptible and resistant soybean varieties. The P_i was not significantly different (P=0.05) among the various soybean

genetics at seven of the site-year locations, indicating that the P_i was relatively uniform among the varieties at the beginning of each growing season. The P_f was significantly different (P=0.05) at eight of the site-year locations, indicating that the varieties had an effect on either increasing or decreasing the SCN population within a growing season. At five site-year locations, yield was found to be statistically significant, with resistant varieties out-yielding susceptible varieties in many instances. The RF was significant (or marginally significant) at six site-year locations, showing that the level of SCN reproduction was affected by the source of resistance used. PI 548402 and PI 437654 varieties had significantly lower RFs than susceptible varieties. Appropriate use of SCN-resistant soybean varieties with good agronomic attributes can increase bean yields and lower SCN populations in infested sites under Michigan growing conditions.

Introduction

The soybean cyst nematode (*Heterodera glycines* Ichinohe, 1952) is a soybean (*Glycine max* (L.) Merrill) pest of global significance. *Heterodera glycines* (SCN) is the most economically-damaging pest of soybeans in the United States and the second most economically-damaging pest of soybeans worldwide (Wrather & Koenning, 2006). Several approaches have been developed to manage SCN, including crop rotation, resistant varieties, sanitation, and chemical control (Schmitt *et al.*, 2004). Proper integrated nematode management plans increase soybean yields on infested farmland (Brown-Rytlewski *et al.*, 2009).

Soybean cyst nematode is believed to have originated in Asia. It occurs in every major soybean production area worldwide, including North America, South America, and Asia (Niblack & Tylka, 2002). SCN was first detected in Michigan in 1987 (Gratiot County) and exists in all but two major soybean-producing counties in the state (Warner *et al.*, 1994; Brown-

Rytlewski *et al.*, 2009). A 2010-2011 Michigan SCN survey validated the extent of infestation in soybean-producing counties (Chapter 2).

The SCN host-parasite relationship is dynamic. Both egg production rates and life expectancy are genetically determined, but may be altered by host genetics, vigor, and other environmental factors (Schmitt *et al.*, 2004). SCN primarily affects soybeans in temperate regions. There can be up to six generations per year, depending on several factors: host, planting date, soil temperature, soil moisture, geographic location, soybean maturity group, and the length of the growing season (Niblack & Tylka, 2002). In Michigan, there are usually 2.5 generations per growing season (Bird & Warner, 2003). The horizontal spatial distribution pattern of SCN in soil is usually aggregated (Francl, 1986). The greatest population density (vertical spatial pattern) typically occurs in the upper 20-30 cm of the soil profile (Alston & Schmitt, 1987). Both growing season and over-winter changes in SCN densities are strongly density-dependent, with initial egg densities the most significant factor affecting population changes across rotations and years (Todd *et al.*, 2003).

Soil texture plays an important role in SCN population dynamics. SCN population density is related to the proportion of sand, clay, and silt in the soil (Avendaño *et al.*, 2004). Coarse-textured soils generally favor hatching and subsequent invasion of root systems, providing suitable conditions for aeration and nematode migration (Perry & Moens, 2006). Consequently, drought and water-logging may inhibit SCN hatch (Perry & Moens, 2006). In Michigan, SCN infection normally occurs from April to August, when soybean plants are actively growing in the soil.

Damage caused by SCN has great economic significance. From 1999-2002, North Central states estimated a loss of 447 million bushels to SCN (Niblack & Tylka, 2002).

Worldwide losses to this pathogen exceed $1 billion per year (Purdue University, 2007). Actual yield losses may range from 5-100% (Warner & Bird, 1997). An average soybean yield for Michigan is about 45 bu/acre, but heavily infested fields may only yield 15-25 bu/acre, or less.

Pest management strategies associated with SCN, such as the use of resistant soybean varieties, are commonly practiced on farms with SCN-infested acreage. A resistant soybean variety is one that yields well in a site that has an above threshold P_i (initial population density) and results in poor nematode reproduction (low P_f, final population density). A susceptible variety, on the other hand, is a variety that yields low in the presence of SCN and also supports a high population density of SCN. There are 109 known sources of resistance (soybeans that have coevolved with SCN), but only seven have been commercialized (Niblack *et al.*, 2002). The seven commercialized sources are: PI 548402, PI 88788, PI 90762, PI 437654, PI 209332, PI 89772, and PI 548316. "PI" stands for "plant introduction", which includes sources of resistance originating from Asia. Only three resistant varieties are currently available in Michigan: PI 548402, PI 88788, and PI 437654. PI 88788 is planted in ca. 95% of SCN-infested soybean acres in Michigan (Warner & Bird, 1997; Brown-Rytlewski *et al.*, 2009).

There are two known mechanisms of SCN resistance: horizontal and vertical resistance. Horizontal resistance is general and controlled by multiple minor genes. It is quantitative and effective against all pathogen variants (Starr *et al.*, 2002). Females reproducing on plants with this type of resistance are usually small and produce few eggs, resulting in a low P_f. Soybeans derived from PI 88788 have horizontal resistance. Vertical resistance is specific and controlled by up to three genes that result in no or poor nurse cell development. The P_f associated with this type of resistance is typically very low. The source of resistance differentiates intra-specific variants of SCN (Vanderplank, 1978). Some variants are highly aggressive whereas others are not aggressive. Soybeans derived from PI 548402 have vertical resistance. PI 437654-derived soybeans have both horizontal and vertical resistance, making them the most SCN-resistant soybeans available to Michigan soybean growers.

Aggressive field populations of SCN can reproduce on and reduce yields of resistant varieties. This was first observed in 1957 by Ross and Brim, only three years after SCN was first detected in the United States (Winstead *et al.*, 1955). Golden *et al.* (1970) developed a SCN Race System to differentiate among field populations. The system was expanded in 1988 by Riggs and Schmitt. Because of deficiencies associated with the 1970 and 1988 race systems, the HG (*Heterodera glycines*) Type Test was developed by Niblack *et al.* in 2002. This test allows farmers to identify what SCN population types are in their fields and determine the most appropriate resistant variety to use. HG Type 0 will not reproduce on any of the seven commercial sources of resistance. Planting any SCN resistant variety, therefore, should result in high bean yields. HG Type 1 reproduces on PI 548402, Type 2 on PI 88788, Type 3 on PI 90762, Type 4 on PI 437654, Type 5 on PI 209332, Type 6 on PI 89772, and Type 7 on PI 548316 (Niblack *et al.*, 2002). HG Type 2.5.7 is the most common HG Type in Michigan (personal communication with G. Bird, 2008). With this HG Type, SCN reproduction occurs on PI 88788, PI 209332, and PI 548316, resulting in significant soybean yield loss (Bird & Warner, 2003). In 2003, the system was truncated to use only the three current commercially-available sources of resistance (Niblack *et al.*, 2003; Brown-Rytlewski *et al.*, 2009).

Goal, Objective, and Hypotheses

The goal of this research was to discover information about SCN that will assist in development of improved SCN management practices. The objective was to determine the effects of SCN resistance source on field populations of SCN during the 2008-2010 growing seasons. It was hypothesized that in SCN-infested fields:

1) The use of SCN-resistant varieties results in a decline in within-season nematode reproduction.

2) The use of SCN-susceptible varieties results in an increase in within-season nematode reproduction.

3) If the P_i is below damage threshold (less than 500 viable units [eggs and second-stage juveniles] per 100 cm^3), there will be no significant difference in yield response between susceptible and resistant soybean varieties.

4) In Michigan, the HG Type of a field population of SCN can change within a single growing season.

The project was funded by the North Central Soybean Research Program (a twelve-state 2008-2010 SCN Project) and the Michigan Soybean Promotion Committee using Michigan Soybean Checkoff dollars.

Materials and Methods

***Research Site, Location, and Design* -** Four sites, one each in Cass, Monroe, Macomb, and Ingham counties, were used to demonstrate the effects of various SCN sources of resistance on field populations of SCN between 2008 and 2010. Fields were chosen based on their previous history of SCN infestation. Three fields were selected for 2008 research: one in Cass County (Edwardsburg, MI); one in Monroe County (Monroe, MI); and one in Ingham County (East Lansing, MI). Three fields were selected for 2009 research: one each in Cass, Ingham (same as in 2008), and Macomb (Richmond, MI) Counties. The same farms used in 2009 were also used for 2010 research. The USDA Web Soil Survey map was used to determine soil type for each location: the soil type was Oshtemo sandy loam for the Cass County site, Pewamo clay loam for the Monroe County site, Parkhill loam for the Macomb County site, and Capac loam and Marlette fine sandy loam for the Ingham County site.

A randomized complete block design (with the exception of Monroe County in 2008) was used and each variety replicated four times (one replicate/block). Six soybean varieties were used at each of the nine site-year trials (Table 4). Each replicate in the Cass, Monroe, and Macomb County trials consisted of four-row plots, each 250 ft (76.2 m)-long. Four replications of 100 ft (30.5 m)-long plots were used in the Ingham County trial each year. The soybeans were planted in a conventionally-tilled field using a 4-row planter in all sites except Ingham County (which used no-tillage planting). Different fields were used each year in the Macomb and Cass County trials. The Ingham County site, however, used the same sources of resistance planted in the exact same row location for the entire duration of the study. At the other sites, plots were re-randomized at the beginning of each growing season. Plots were harvested using a combine. The center two rows were harvested at each location. Soybean yields for each variety were taken and adjusted to 13% moisture.

Sampling - Two multiple-core soil samples were taken from each experimental unit throughout each growing season. Plots were sampled at-planting to determine the initial population density (P_i) using a cone-shaped soil probe (approximate volume of 2000 cm^3), 15.2 cm below the soil surface. A zigzag pattern was used to sub-sample each variety, and the probe was emptied into a numbered plastic bag when full (12 soil cores total per experimental unit). Approximately one liter of total soil was taken per variety per replicate. Soil was sampled at-harvest using the same method as at-plant sampling. In addition, soil samples were sent to Dr. Terry Niblack's laboratory at the University of Illinois for HG Type testing.

***Nematode Extraction* -** A 100 cm^3 subsample from each sample was processed using a modified centrifugation-flotation technique (Jenkins, 1964). Aliquots of 1.0 ml (20 ml total) were examined microscopically to determine the presence or absence of cysts. Once cysts were found, they were counted and crushed using a glass homogenizer. The number of viable units (eggs and second-stage juveniles) was counted using an inverted microscope. This number was then multiplied by 20 to obtain an estimated population density for 100 cm^3 soil.

***Data Analysis* -** The annual SCN reproduction factor (RF), which is determined by dividing the final SCN population density (P_f) by the initial SCN population density (P_i) was calculated for each experimental unit. If the RF was greater than one, the SCN population increased during the growing season. If the RF was equal to one, the SCN population remained constant throughout the growing season. Finally, if the RF was less than one, the SCN population was reduced during the growing season.

Analysis of variance (ANOVA) for RF, yield, and initial and final population densities was conducted using Minitab 15 Statistical Software using an alpha of 0.05. Levene's test was used to check for equality of variances. If variances were found to be unequal, a transformation was applied and ANOVA performed on the transformed data. SCN thresholds were used for transforming P_i or P_f. For this transformation, if the number of viable units was 0, category 1 was assigned; if the number of viable units was between 1 and 499, category 2 was assigned; if the number of viable units was between 500 and 2999, category 3 was assigned; and, if the number of viable units was greater than 3000, category 4 was assigned. A similar approach was used when analyzing RF. Rankings were used to transform RFs based on the best performing variety (lowest RF) to the worst performing variety (highest RF). Lowest RFs were given a ranking of 1 and highest RFs were ranked a 6 (ties were broken by taking an average of the closest ranking scores). Fisher's Least Significant Difference (LSD) multiple range analysis was used to examine pair wise comparisons.

An innovative color-metric system was developed by the North Central Soybean Research Program SCN project to show viable units and their appropriate threshold categories. The tables developed by this system are a valuable tool because they clearly show the increase or decrease of SCN populations within a growing season (Appendices 2-10).

Results

SCN Population Dynamics - The P_i establishes a base line for the population density of SCN at the beginning of each growing season. There were no significant differences (P=0.564, 0.931) among P_i for the individual plots (experimental units) in Ingham County in 2008 and 2010, respectively (Table 5). There were, however, significant differences (P=0.001) among P_i for the plots used in Ingham County in 2009. The experimental units for PI 437654 and PI 548402 sources of resistance were lower than those used for both susceptible varieties. No significant differences (P=0.405) were observed among P_i for the individual plots in Monroe County in 2008. Likewise, there were no significant differences (P=0.526, 0.282) among P_i for the experimental units used in Macomb County in 2009 and 2010, respectively. The P_i data for Cass County for 2008 are not available for statistical analysis. There were no significant differences (P=0.186, 0.920) among P_i for the plots in Cass County in 2009 and 2010, respectively. With the exception of the Ingham County site, plot P_i was independent of the variety planted at the beginning of each growing season.

The P_f provides the number of SCN viable units present in the soil at the end of each growing season. There were highly significant differences ($P<0.001$) among P_f for the varieties tested in Ingham County in 2008, 2009, and 2010 (Table 6). 75% of the SCN-resistant varieties used in 2008 significantly lowered the $P_{f,}$ compared with the susceptible varieties. One of the PI 88788 varieties performed similarly to the susceptible varieties. In 2009, only 50% of the SCN-resistant varieties used significantly lowered the P_f compared with the susceptible varieties. The PI 88788 varieties were not significantly different than the susceptible varieties. Similarly, in 2010, 50% of the SCN-resistant varieties used significantly lowered the P_f compared with the susceptible varieties. The PI 437654 and PI 548402 varieties were significantly lower than the susceptible varieties; whereas, the PI 88788 varieties were not different than one of the susceptible varieties.

There were significant differences ($P=0.013$) among P_f for the varieties tested in Monroe County in 2008. There were no differences between the PI 88788 varieties and the susceptible varieties. There were highly significant differences ($P<0.001$) among P_f for the varieties tested in Macomb County in 2009 and 2010. All resistant varieties were significantly lower than the susceptible varieties at this site during both years. There were also significant differences ($P=0.002$) among P_f for the varieties tested in Cass County in 2008, highly significant differences ($P<0.001$) in 2009, and no significant differences ($P=0.431$) among P_f for the varieties tested in Cass County in 2010. At this location in 2008 and 2009, the PI 88788 varieties were not significantly different than one or both of the susceptible varieties. The P_f associated with the resistant varieties was not always significantly lower than those of susceptible varieties.

The SCN population increase or decline (RF) within a growing season was a good indicator of the difference in host-parasite relationships among the varieties studied. There were highly significant differences ($P<0.001$) among RF for the varieties tested in Ingham County in 2008, no significant differences ($P=0.073$) among RF for the varieties tested in Ingham County in 2009, and significant differences ($P=0.013$) among RF for the varieties tested in Ingham County in 2010 (Table 7). At the Ingham County site in 2008, the PI 437654 and PI 548402 varieties were significantly different than one of the PI 88788 and both of the susceptible varieties. Marginally significant differences ($P=0.054$) were observed among RF for the varieties tested in Monroe County in 2008. There were highly significant differences ($P<0.001$) among RF for the varieties tested in Macomb County in 2009 and significant differences ($P=0.003$) among RF for the varieties tested in Macomb County in 2010. The RF data for the Cass County site in 2008 are not available for statistical analysis. There were highly significant differences ($P<0.001$) among RF for the varieties tested in Cass County in 2009, with significant differences between the susceptible varieties and the PI 437654 variety. No significant differences ($P=0.798$) among RF for the varieties tested in Cass County in 2010 were observed. All SCN-susceptible varieties showed a population increase at the end of the growing season at each site-year location.

Bean Yields - The impact of SCN on soybean yields from resistant and susceptible varieties varied among years and research locations. There were no significant differences ($P=0.535$, 0.985, 0.110) among the bean yields for the varieties tested in Ingham County in 2008, 2009, and 2010, respectively (Table 8). There were significant differences ($P=0.02$) among yield for the varieties tested in Monroe County in 2008. One of the PI 88788 varieties was significantly higher in yield than a susceptible variety. There were highly significant differences ($P<0.001$) among yield for the varieties tested in Macomb County in 2009 and significant differences ($P=0.047$) among yield for the varieties tested in Macomb County in 2010. In 2009, one of the PI 88788 varieties had a significantly higher yield than the other varieties tested. In 2010, the combined use of all varieties (multi-var) gave a significantly higher yield than the susceptible variety. There were highly significant differences ($P<0.001$) among yield for the varieties tested in Cass County in 2008, significant differences ($P=0.001$) among yield for the varieties tested in Cass County in 2009, and no significant differences ($P=0.472$) among yield for the varieties tested in Cass County in 2010. Use of SCN-resistant varieties resulted in high bean yields compared to susceptible varieties in some, but not all, site-year trials.

Discussion

Certain sources of resistance performed better than others in the presence of SCN. At seven of the site-year locations, the P_i was not statistically significant, indicating the SCN population was relatively uniform and not different among the plots (experimental units) at the beginning of each growing season. The only instance of a P_i being significantly different among the varieties was at the Ingham County site in 2009 ($P=0.001$). This was anticipated because of the experimental design. At this location, the same sources of resistance were used in the exact same row locations for multiple years. The PI 437654 and PI 548402 varieties significantly lowered the P_i compared with the susceptible varieties. It is also important to note at this location that the SCN population did not experience a high mortality rate during the winter of 2008. The P_i at the Cass County site in 2010 was very low. A possible explanation for the SCN population crash at this location was the discovery of a bacterial pathogen, *Pasteuria* spp., attached to SCN J2.

The at-harvest population density of SCN was statistically significant at eight of the site-year locations. This indicates that the varieties performed differently in the presence of SCN. In all site-year locations where the data set was complete, use of susceptible varieties resulted in an increase in P_f. One or both of the susceptible varieties always had a higher P_f than PI 437654 and PI 548402 varieties (with the exception of the Cass County site in 2010). This supports the original hypothesis that the use of SCN-susceptible varieties results in an increase in P_f (RF greater than one). The PI 88788 varieties, however, were not significantly different from one or both of the susceptible varieties at seven of the site-year locations. Different HG Types may explain this phenomenon. The field data strongly suggest the presence of HG Type 2 populations at the research locations. Since PI 88788-derived soybeans are planted in nearly 95% of SCN-infested sites in Michigan, growers may have to begin using other sources of resistance to reduce their SCN populations. We must reject the hypothesis that use of SCN-resistant varieties results in a decline in P_f (RF less than one), because SCN production occurred on all sources of resistance.

The RF was significant (or marginally significant) at seven site-year locations. Resistant varieties originating from PI 437654 lowered the SCN population throughout the growing season at six site-year locations (Ingham 2008, Ingham 2009, Ingham 2010, Macomb 2009, Macomb 2010, and Cass 2009). Resistant varieties originating from PI 548402 lowered the SCN population throughout the growing season at three site-year locations (Ingham 2009, Macomb 2009, and Macomb 2010). Resistant varieties originating from PI 88788 lowered the SCN population throughout the growing season in one location (Macomb 2009). SCN, therefore, is able to reproduce on varieties derived from all sources of SCN resistance currently available in Michigan.

At five site-year locations, yield was found to be statistically significant. We can neither accept nor reject the hypothesis that if below damage threshold (less than 500 viable units per 100 cm^3 soil), there will be no significant difference in yield response between susceptible and resistant soybean varieties. At the Macomb County site in 2010, where all varieties were combined (multi-var), yield was the highest. Even though a susceptible variety was included in the mixture, the genetic diversity of the soybeans caused this combination to significantly out-yield the use of individual SCN-resistant varieties. Researchers have shown that increased genetic diversity results in an increase in primary productivity (Bird, 2011; Reusch and Hughes, 2006; Kareiva and Marvier, 2011). Further research needs to be performed in the realm of yield response of susceptible versus resistant varieties. Such information would be valuable to growers trying to maximize their yields in fields under SCN pressure.

It is probable that the differences observed at the various sites resulted from environmental factors associated with the different growing seasons and locations. Bird *et al.* (2009) showed that rainfall impacted P_f, with the highest at-harvest SCN populations occurring during years with less than 40.6 cm of within-season precipitation. Furthermore, the highest yields were associated with years with greater than 50.8 cm of within-season precipitation (Bird *et al.*, 2009). At the Cass County site in 2008 where there was less than 40.6 cm of within-season precipitation, the P_f was high and yields were low (not over 22.5 bu/A). The opposite effect occurred in Cass County in 2010 when the amount of within-season precipitation was greater than 50.8 cm. The P_f was low and the yields were high (greater than 31.1 bu/A). This relationship suggests that precipitation plays a significant role in both SCN population density and soybean yield.

Variations in the aggressiveness of the SCN populations may account for differences between sites. The HG Type at the site in Monroe County in 2008 began as HG Type 2 (able to reproduce on PI 88788) in the spring, but ended up as HG Type 1.2 (able to reproduce on PI 88788 and PI 548402) at the end of the growing season. Similarly, at the site in Cass County in 2010, SCN reproduced on all resistant sources, which may be evidence of HG Type 1.2.4. This is a cause for significant concern; because it appears the nematode field population is becoming increasingly aggressive and can reproduce on and damage resistant varieties. Another possible explanation for these differences is the variations in agronomic properties and maturity groups that affect each variety differently. With constant changes in SCN population genetics, as well as advances in soybean genetics, it is difficult to test a wide selection of commercially-available resistant varieties. Therefore, understanding the intricacies of the soybean-SCN relationship is essential to the development of sound SCN management plans.

CHAPTER 4

Distribution of *Pratylenchus penetrans* in Michigan and the Influence of *Pratylenchus penetrans* on *Heterodera glycines* resistance

Abstract

Soybeans (*Glycine max* (L.) Merrill) are an important field crop throughout the world. Two important nematode species damage soybeans, *Heterodera glycines* (Ichinohe, 1952) and *Pratylenchus penetrans* (Cobb, 1917; Filipjev & Schuurmans Stekhoven, 1941). The goal of this research was to determine if *P. penetrans* (penetrans lesion nematode, PLN) allows *H. glycines* (soybean cyst nematode, SCN) to reproduce on two sources of resistance, PI 548402 and PI 437654. A 2010-2011 survey was conducted in Michigan to determine the extent of SCN and PLN infestations in soybean-producing counties. Of the 36 counties sampled in 2010, 32 were positive for SCN and 29 were positive for PLN. The occurrence of SCN averaged among the counties was 50%; whereas, the average occurrence of PLN was 46%. Two greenhouse trials were conducted to evaluate the effects that PLN had on SCN reproduction on susceptible, PI 548402, and PI 437654 soybean varieties. In trial 1, soybeans were inoculated with 250 PLN/conetainer. In trial 2, soybeans were inoculated with 500 PLN/conetainer. The inoculation level of SCN in both trials was 2000 SCN viable units/conetainer. Both SCN and PLN were able to reproduce on each variety. There was no difference in SCN development between PLN and non-PLN infested plants for the susceptible variety in both trials. In trail 1, PLN enhanced development of SCN males in roots on PI 437654 and SCN females on roots on PI 548402 ($P<0.05$). In trial 2, PLN enhanced development of SCN females in soil on PI 437654 and SCN viable units in soil on PI 548402 ($P\leq0.052$).

Introduction

Heterodera glycines - The soybean cyst nematode (*Heterodera glycines* Ichinohe, 1952) is a soybean (*Glycine max* (L.) Merrill) pest of global significance. *Heterodera glycines* (SCN) is considered the most economically-damaging pest of soybeans in the United States and the second most economically-damaging pest of soybeans worldwide (Wrather & Koenning, 2006). Several approaches have been developed to help manage SCN, including: crop rotation, resistant varieties, sanitation, and chemical control (Schmitt *et al.*, 2004). Proper integrated nematode management plans can increase soybean yields on infested farmland (Brown-Rytlewski *et al.*, 2009).

Soybean cyst nematode is believed to have originated in Asia. It occurs in every major soybean production area worldwide, including North America, South America, and Asia (Niblack & Tylka, 2002). SCN was first detected in Michigan in 1987 (Gratiot County) and is thought to exist in all but two major soybean-producing counties in the state (Warner *et al.*, 1994; Brown-Rytlewski *et al.*, 2009). A 2010-2011 Michigan SCN survey validated the extent of infestation in soybean-producing counties (Chapter 2).

The typical life cycle of SCN is 21 days at 25°C (Perry & Moens, 2006). The optimum temperature for hatching is 25-30°C and 26-28°C for development. The number of days to mature female development is 14 (Starr *et al.*, 2002). Juvenile SCN are small, 1/64" (0.4 mm) or less (Purdue, 2007). Swollen females are larger, 1/25" (1 mm), and can be seen on soybean roots without magnification (Purdue, 2007). Dead females (cysts) are lemon-shaped and are capable of housing hundreds of live eggs, with approximately 150 or more eggs per healthy cyst. The male SCN has a single testis and the female has paired ovaries (Perry & Moens, 2006). Viable units are defined as the number of eggs and J2 found in a soil sample. This parameter is used when assessing the potential for a population to be infective.

The SCN host-parasite relationship is dynamic. Both egg production rates and life expectancy are genetically determined, but may be altered by host genetics, vigor, and other environmental factors (Schmitt *et al.*, 2004). SCN primarily affects soybeans in temperate regions. There can be up to six generations per year, depending on several factors: host, planting date, soil temperature, soil moisture, geographic location, soybean maturity group, and the length of the growing season (Niblack & Tylka, 2002). In Michigan, there are usually 2.5 generations per growing season (Bird & Warner, 2003). The horizontal spatial distribution pattern of SCN in soil is usually aggregated (Francl, 1986). The greatest population density (vertical spatial pattern) typically occurs in the upper 20-30 cm of the soil profile (Alston & Schmitt, 1987). Both growing season and over-winter changes in SCN densities are strongly density-dependent, with initial egg densities the most significant factor affecting population changes across rotations and years (Todd *et al.*, 2003).

Soil texture plays an important role in SCN population dynamics. SCN population density is related to the proportion of sand, clay, and silt in the soil (Avendaño *et al.*, 2004). Coarse-textured soils generally favor hatching and subsequent invasion of root systems, providing suitable conditions for aeration and nematode migration (Perry & Moens, 2006). Consequently, drought and water-logging may inhibit SCN hatch (Perry & Moens, 2006). In Michigan, SCN infection normally occurs from April to August, when soybean plants are actively growing in the soil.

Crop rotation is a very important component of managing SCN. Cysts can remain viable in the soil for 8-12 years in the absence of a host (Niblack & Tylka, 2002). Other host crops for SCN include: edible beans, common and hairy vetch, pea, and sweet clover (Tylka, 1994). Winter annual weeds like purple deadnettle, henbit, and field pennycress also serve as hosts for

SCN (Creech *et al.*, 2007; Barnett in Brown-Rytlewski *et al.*, 2009). Rotation with non-host crops, therefore, is essential. Non-host crops for SCN include corn, wheat, and sugar beet. A three year rotation is generally recommended, with one year planted into a host crop and two consecutive years planted into non-host crops (Warner & Bird, 1997). Because SCN is an obligate parasite, it cannot successfully reproduce on non-host crops.

Pest management strategies associated with SCN, such as the use of resistant soybean varieties, are commonly practiced on farms with SCN-infested acreage. A resistant soybean variety is one that yields well in a site that has an above threshold P_i (initial population density) and results in poor nematode reproduction as measure by P_f (final population density). A susceptible variety, on the other hand, is a variety that yields low in the presence of SCN and also supports a high population density of SCN. Plant breeders have not incorporated resistance genes into the genome of susceptible varieties. There are 109 known sources of resistance (soybeans that have coevolved with SCN), but only seven have been commercialized (Niblack *et al.*, 2002). The seven commercialized sources are: PI 548402, PI 88788, PI 90762, PI 437654, PI 209332, PI 89772, and PI 548316. "PI" stands for "plant introduction", which includes sources of resistance originating from Asia. Varieties from three sources of resistance are currently available in Michigan: PI 548402, PI 88788, and PI 437654. PI 88788 is planted in ca. 95% of SCN-infested soybean acres in Michigan (Warner & Bird, 1997; Brown-Rytlewski *et al.*, 2009).

There are two known mechanisms of SCN resistance: horizontal and vertical resistance. Horizontal resistance is general and controlled by multiple minor genes. It is quantitative and effective against all pathogen variants (Starr *et al.*, 2002). Females reproducing on plants with this type of resistance are usually small and produce few eggs, resulting in a low P_f. Soybeans derived from PI 88788 have horizontal resistance. Vertical resistance is specific and controlled by up to three genes that result in no or poor nurse cell development. The P_f associated with this type of resistance is typically very low. The source of resistance differentiates intra-specific variants of SCN (Vanderplank, 1978). Some variants are highly aggressive whereas others are not aggressive. Soybeans derived from PI 548402 have vertical resistance. PI 437654-derived soybeans have both horizontal and vertical resistance, making them the most SCN-resistant soybeans available to Michigan soybean growers.

Aggressive field populations of SCN reproduce on and reduce yields of specific resistant varieties. This was first observed in 1957 by Ross and Brim, only three years after SCN was first detected in the United States (Winstead *et al.*, 1955). In 1970, Golden *et al.* developed a SCN Race System to differentiate among field populations. The system was expanded in 1988 by Riggs and Schmitt. Because of deficiencies associated with the 1970 and 1988 race systems, the HG (*Heterodera glycines*) Type Test was developed by Niblack *et al.* in 2002. This allows farmers to identify what SCN population types are in their fields and determine the most appropriate resistant variety to use. HG Type 0 (a non-aggressive population) will not reproduce or cause damage on any of the seven commercial sources of resistance. Planting any SCN resistant variety, therefore, should result in high bean yields. HG Type 1 reproduces on PI 548402, Type 2 on PI 88788, Type 3 on PI 90762, Type 4 on PI 437654 (a highly aggressive population), Type 5 on PI 209332, Type 6 on PI 89772, and Type 7 on PI 548316 (Niblack *et al.*, 2002). HG Type 2.5.7 is the most common HG Type in Michigan (personal communication, G.W. Bird, 2008). With this HG Type, SCN reproduction occurs on PI 88788, PI 209332, and PI 548316, resulting in significant soybean yield loss (Bird & Warner, 2003). In 2003, the system was truncated (SCN Type System) to use only the three current commercially-available sources of resistance (Niblack *et al.*, 2003; Brown-Rytlewski *et al.*, 2009).

Pratylenchus penetrans - The penetrans lesion nematode (*Pratylenchus penetrans* Cobb, 1917; Filipjev & Schuurmans Stekhoven, 1941) is a common migratory endoparasite of plants. There are greater than 70 species in the *Pratylenchus* genus. Some species are highly aggressive and others are not. *Pratylenchus penetrans* (PLN) is an aggressive species with a very wide host range. PLN occurs in temperate regions around the world and causes damage to many important crops (Mai *et al.*, 1977). The nematode is named for the characteristic necrotic lesions it causes as it migrates through root tissue. It is the most common *Pratylenchus* spp. in Michigan.

Unlike SCN, PLN hatching is not influenced by root exudates, but rather temperature and osmotic pressure (Mai *et al.*, 1977). Most members of the *Pratylenchus* genus reproduce via parthenogenesis. PLN, however, reproduce via sexual reproduction, and the female lays up to 2 eggs per day (Perry & Moens, 2006). Eggs are laid in root tissue and along the root surface (Perry & Moens, 2006). All juvenile and adult stages of PLN are vermiform and less than 1/25" (1 mm) (Perry & Moens, 2006). Even though PLN can be found in both soil and roots, higher population densities can be recovered from root tissue (Perry & Moens, 2006). At 30°C, the life cycle of PLN is 30 days; whereas, at 15°C, the life cycle is 92 days (Mai *et al.*, 1977).

Previous research suggests a possible interaction between PLN and SCN on soybeans. When the nematodes are present together, there is a decrease in soybean production (Melakeberhan, 1998). As the nematodes compete with each other, their population densities change. In a greenhouse study, infection rates of PLN increased with increasing rates of SCN (Melakeberhan, 2003). Bates (2006) reported that inoculating soybeans derived from the PI

88788 source of resistance with an equal amount of PLN and SCN resulted in enhanced SCN development.

Control of PLN is difficult due to its very wide host range. Research has shown that adding organic matter to the soil will reduce the number of PLN (Mai *et al.*, 1977). However, there is little information on species-specific control of PLN. Improved SCN management may also lead to lower population densities of other important plant parasites, like PLN, in soybean fields.

Goal, Objective, Hypotheses

The goal of this research was to gain a better understanding of PLN and SCN interactions. The objective of this research was to determine PLN occurrence in soybean-producing counties in Michigan. Another objective was to determine whether populations of PLN will enhance SCN reproduction on PI 437654 and PI 548402 sources of resistance. It was hypothesized that:

1) *Pratylenchus penetrans* is widely distributed throughout Michigan soybean acreage.
2) *Pratylenchus penetrans* has no effect on *H. glycines* development on a PI 437654 source of resistance.
3) *Pratylenchus penetrans* has no effect on *H. glycines* development on a PI 548402 source of resistance.

The survey portion of the project was funded by the Michigan Soybean Promotion Committee using Michigan Soybean Checkoff dollars. The greenhouse portion of the project was funded by Project GREEEN at Michigan State University.

Materials and Methods

***Survey Design* -** Ten percent of the soybean farms (n=1,116) in Michigan were randomly selected for the survey from a list obtained from the Michigan Soybean Promotion Committee grower database using a random number generator. An additional five percent of farms from each county were also randomly selected for alternates when farms from the ten percent list were unwilling to participate. The original ten percent were contacted by mail and asked to submit a soil sample to their local Michigan State University Extension Office from the third field they intended to plant in the 2010 growing season. Only 66 farms responded to the mail request, so another postcard was sent out asking farms to mail in their samples. When no additional samples were received by mail, the protocol was changed. The number of farms was cut from ten percent down to five percent (n=558). Farms were visited by members of the survey team during the growing season (May-August) and a soil sample was taken from one of their fields.

***Survey Sampling* -** In order for a site to be eligible for sampling, fields must have been planted to soybeans at least once between 2005 and 2010. Fields were sampled to determine the SCN population density using a cone-shaped soil probe (approximate volume of 2000 cm^3), 15.2 cm below the soil surface. Sampled fields ranged in size from less than 20 to greater than 80 acres. A zigzag pattern was used to sample a field, and the probe was emptied into 3.8 L plastic bags. The samples were stored at 7°C until processed.

***Survey Nematode Extraction, Identification* -** Before extraction, the soil in each sample bag was mixed thoroughly to ensure uniformity. A 100 cm^3 subsample from each sample was processed using a modified centrifugation-flotation technique (Jenkins, 1964). Each sample was examined microscopically to determine the presence or absence of SCN cysts or viable units (eggs and second-stage juveniles). Each sample was also examined for PLN (juveniles, females, and males).

Greenhouse PLN Extraction - PLN was obtained from greenhouse cultures grown on corn roots. The roots were processed using Bird's method (1971) with the substitution of Ethyl Mercuric Chloride solution for a 0.01% NaOCl solution. The root samples were examined microscopically and PLN were transferred into a beaker until a volume of 25 PLN/ml was reached.

SCN Extraction - Soil was collected from the Tom Kendle Farm in Edwardsburg, MI. This site has a well-documented SCN history. Soil (100 cm^3 increments) was processed using a modified centrifugation-flotation technique (Jenkins, 1964). Each sample was examined microscopically and SCN cysts were extracted. The cysts were crushed with a glass homogenizer to release the viable units until a volume of 200 SCN/ml was reached.

Soybean Development - Twenty soybean seeds each from susceptible (NK S17-B5), PI 437654 (Beck 298), and PI 548402 (Pioneer 92M75) varieties were planted in conetainers (approximate volume of 200 cm^3) filled with steam-sterilized sandy loam. The conetainers were placed in the greenhouse under a 16 hour light period with a daytime temperature of 25°C and a nighttime temperature of 23°C. They were watered daily.

PLN and SCN Inoculation, Trial 1 - After fourteen days, ten plants from each variety (susceptible, PI 437654, and PI 548402) were selected, based on uniform appearance, for nematode inoculation. Using a small metal spatula, a hole was formed near the root system of each plant, and a syringe was used to deliver either 2000 SCN viable units or 2000 SCN viable units + 250 PLN directly onto the roots. The holes were filled in with soil to cover the roots. Each treatment was replicated five times in a randomized complete block design (one replicate/block).

***PLN and SCN Inoculation, Trial 2* -** After seven days, eight plants from each variety (susceptible, PI 437654, and PI 548402) were selected, based on uniform appearance, for nematode inoculation. Using a small metal spatula, a hole was formed near the root system of each plant, and a syringe was used to deliver either 2000 SCN viable units or 2000 SCN viable units + 500 PLN directly onto the roots. The holes were filled in with soil to cover the roots. Each treatment was replicated four times in a randomized complete block design (one replicate/block).

***PLN and SCN Extraction* -** Thirty days after inoculation, the trials were terminated. The leaves and stems of the plants were removed using scissors. Root systems were carefully removed from the conetainers and examined for the presence of white females. Afterwards, the roots (1.0 g) were processed using Bird's method (1971) with the substitution of Ethyl Mercuric Chloride solution for a 0.01% NaOCl solution. A 100 cm^3 soil sample from each conetainer was processed using a modified centrifugation-flotation technique (Jenkins, 1964). Root and soil samples were examined microscopically for the presence of SCN females, males, and viable units as well as PLN juveniles, females, and males.

***Data Analysis* -** Two sample t-tests were performed for SCN and PLN population densities from PLN and non-PLN infested plants using Minitab 15 and an alpha of 0.05.

Results

In 2010, a total of 403 farms/fields were sampled from 36 counties in Michigan (Table 11). Of the 36 counties sampled in 2010, 32 were positive for SCN and 29 were positive for PLN. The average occurrence of SCN averaged among the counties was 50%; whereas, the average occurrence of PLN was 46%.

Both SCN and PLN were able to reproduce on the susceptible, PI 437654, and PI 548402 varieties in greenhouse trials 1 (Table 9) and 2 (Table 10). There were no significant differences in SCN development between PLN and non-PLN infested plants for the susceptible variety in both trials.

In trail 1, PLN enhanced development of SCN males in roots on PI 437654 (P=0.026). Furthermore, PLN enhanced development of SCN females on roots on PI 548402 (P=0.006) in trial 1.

In trial 2, PLN resulted in greater population densities of SCN females in soil on PI 437654 (P=0.024). Also, PLN resulted in greater population densities of SCN viable units in soil on PI 548402 (P=0.052) in trial 2.

Discussion

The 2010 survey gave a statistically-valid account of SCN and PLN infestations in Michigan soybean-producing counties. The overall infestation levels of SCN and PLN were 50% and 46%, respectively. It appears that PLN is widely distributed throughout soybean acreage in Michigan and there seems to be no direct correlation between SCN and PLN co-infestation.

Bates (2006) showed that in the presence of PLN, SCN had a higher rate of development on a PI 88788 source of resistance. Further research on this topic was necessary to determine if SCN can reproduce on two other sources of SCN resistance, PI 437654 and PI 548402, in the presence of PLN. Two greenhouse trials were performed to test the hypotheses that PLN has no effect on SCN development on PI 437654 and PI 548402 sources of resistance. A susceptible variety was used as the control. The inoculation rate of SCN was held at 2000 viable units/conetainer for both trials. The inoculation rate of PLN was 250 PLN/conetainer in trial 1 and 500 PLN/conetainer in trial 2.

The addition of PLN did not enhance SCN development on the susceptible variety in either trial. However, significant differences were observed between resistant varieties with PLN and without PLN in both trials. In each case where there was a difference, the SCN population densities were higher in the presence of PLN. Therefore, it does appear that the presence of PLN can enhance SCN reproduction on PI 437654 or PI 548402 resistant soybean varieties. It is unclear why the population densities of PLN and SCN were so low in trial 2 compared with trial 1. The nematodes experienced higher mortality in trial 2. It appears that doubling the amount of PLN did not have significant effects on SCN reproduction on resistant varieties. Additional inoculation rates of PLN and SCN should be evaluated to further support the original hypotheses.

CHAPTER 5
Concluding Remarks Regarding the Bionomics of *Heterodera glycines* and *Pratylenchus penetrans* Associated with Michigan Soybean Production

Overview

Two important nematode species damage soybeans (*Glycine max* (L.) Merrill) in Michigan, *Heterodera glycines* (Ichinohe, 1952) and *Pratylenchus penetrans* (Cobb, 1917; Filipjev & Schuurmans Stekhoven, 1941). The goal of this research was to discover information that will assist in improving management of these nematode species. A 2010-2011 Michigan survey was conducted to determine the extent of *H. glycines* (soybean cyst nematode, SCN) and *P. penetrans* (penetrans lesion nematode, PLN) infestation in soybean-producing counties (Chapter 2). A 2008-2010 study was done to determine the effects of SCN-resistant varieties on field populations of SCN (Chapter 3). Finally, greenhouse studies were used to evaluate the effects that PLN has on SCN reproduction on resistant varieties (Chapter 4). Improved management of SCN and PLN should increase soybean yields on infested acreage in Michigan.

Distribution of *Heterodera glycines* in Michigan

A 2010-2011 Michigan *H. glycines* (SCN) survey was designed and conducted to provide a comprehensive, statistically-valid analysis of the extent of SCN infestations throughout Michigan. It was hypothesized that:

1) Fifty percent of Michigan soybean fields are infested with SCN.

2) Soybean-producing counties with the greatest acreage have the highest incidence of SCN.

3) HG Type 2 is the most common HG Type in Michigan.

4) *Pratylenchus penetrans* is widely distributed throughout Michigan soybean acreage.

5) *Fusarium virguliforme* (Mycota) is common in SCN-infested fields.

The Michigan Soybean Promotion Committee grower database was used to randomly select five percent of Michigan soybean farms (n=558) from 46 counties. Soil samples from 403 farms were processed in 2010. 32 of 36 counties sampled during the 2010 growing season were positive for SCN. The average occurrence of SCN in Michigan was 50% in 2010. This directly supports the first hypothesis. Five of the seven highest soybean-producing counties in Michigan (Gratiot, Monroe, Saginaw, Shiawassee, and Tuscola) had SCN infestations above 50%, providing support for the second hypothesis. Three of the six samples processed for HG Type in 2010 were HG Type 2. Eleven samples remain to be analyzed for HG Type in 2011. More information is needed to accept or reject hypothesis 3. The results for the *Pratylenchus penetrans* portion of this research will be discussed in Chapter 4. Furthermore, *Fusarium virguliforme* analysis is being performed on the samples processed in 2010. Dr. Martin Chilvers is developing a PCR detection method for *F. virguliforme* and will have the results completed in the near future.

Effects of Soybean Cyst Nematode-Resistant Varieties on Michigan Field Populations of *Heterodera glycines*

The objective of this research was to determine the effects of resistant soybean sources on field populations of SCN within growing seasons. Commercial varieties from three sources of resistance are available for use in MI: PI 548402, PI 88788, and PI 437654. Three farms were selected, based on previous SCN history, for the project in 2008, 2009, and 2010; providing a total of nine site-year locations. Six soybean varieties were used each year. Selection was based on their maturity groups, sources of SCN resistance, and grower experience. The at-planting (P_i) and at-harvest (P_f) population densities were determined for each variety at each of the nine site-year locations. Annual SCN reproduction ($RF=P_f/P_i$) and bean yield were also determined for each site and growing season. It was hypothesized that in SCN-infested fields:

1) The use of SCN-resistant varieties results in a decline in within-season nematode reproduction.

2) The use of SCN-susceptible varieties results in an increase in within-season nematode reproduction.

3) If the P_i is below damage threshold (less than 500 viable units [eggs and second-stage juveniles] per 100 cm^3), there will be no significant difference in yield response between susceptible and resistant soybean varieties.

4) In Michigan, the HG Type of a field population of SCN can change within a single growing season.

We must reject the first hypothesis, because SCN reproduction occurred on all sources of resistance (PI 548402, PI 88788, and PI 437654). In all site-year locations where the data set was complete, use of susceptible varieties resulted in an increase in P_f. This supports the second hypothesis. There is some evidence to support the third hypothesis. At five site-year locations, yield was found to be statistically significant. The HG Type at the site in Monroe County in 2008 began as HG Type 2 (able to reproduce on PI 88788) in the spring, but ended up as HG Type 1.2 (able to reproduce on PI 88788 and PI 548402) at the end of the growing season. Similarly, at the site in Cass County in 2010, SCN reproduced on all resistant sources, which may be evidence of HG Type 1.2.4. These results support the fourth hypothesis that HG Types can change within a growing season.

Distribution of *Pratylenchus penetrans* in Michigan and the Influence of *Pratylenchus penetrans* on *Heterodera glycines* resistance

The goal of this research was to determine if *P. penetrans* (penetrans lesion nematode, PLN) allows *H. glycines* (soybean cyst nematode, SCN) to reproduce on two sources of resistance, PI 548402 and PI 437654. Another goal was to determine the extent of SCN and PLN infestations in soybean-producing counties throughout Michigan. It was hypothesized that:

4) *Pratylenchus penetrans* is widely distributed throughout Michigan soybean acreage.
5) *Pratylenchus penetrans* has no effect on *H. glycines* development on a PI 437654 source of resistance.
6) *Pratylenchus penetrans* has no effect on *H. glycines* development on a PI 548402 source of resistance.

Of the 36 counties sampled in 2010, 32 were positive for SCN and 29 were positive for PLN. The occurrence of SCN averaged among the counties was 50%; whereas, the average occurrence of PLN was 46%. This supports the first hypothesis that PLN is a prevalent nematode in Michigan soybean acreage. Two greenhouse trials were conducted to evaluate the effects that PLN had on SCN reproduction on susceptible, PI 548402, and PI 437654 varieties. Both SCN and PLN were able to reproduce on each variety. There were differences in SCN development between PLN and non-PLN infested plants for both sources of resistance. Therefore, we must reject the second and third hypotheses that PLN has no effect on SCN development on PI 437654 and PI 548402 sources of resistance.

Summary and Future Recommendations

Overall, the results of the 2010 portion of the survey indicate that SCN and PLN are both widely distributed throughout soybean-producing counties in Michigan. These two nematode species are responsible for yield loss in soybeans, which are a valuable crop in worldwide food and energy production. In order to better manage SCN and PLN, a comprehensive understanding of how many Michigan soybean farms are infested with these pests, as well as the HG types present, is important for the design of future management strategies and education programs.

Further research needs to be performed in the realm of yield response of susceptible versus resistant varieties. SCN and PLN are able to reproduce on all resistant sources currently available in Michigan. The 2008-2010 field studies show that proper use of resistant soybean varieties can increase yield and reduce SCN populations. Such information will be valuable to growers trying to maximize their yields in fields under SCN pressure. Soybean growers must gain a better understanding of how to control these pests to ensure the future prosperity of the Michigan soybean industry.

Table 1. Michigan soybean counties infested with *Heterodera glycines* in 2005 (Bird & Warner).

County	Farms Sampled	% of Samples Positive for SCN
Allegan	11	18
Bay	28	89
Berrien	32	63
Branch	11	36
Calhoun	1	0
Cass	1	100
Clinton	37	70
Eaton	5	20
Genesee	24	88
Gratiot	118	58
Huron	5	60
Ingham	29	34
Ionia	5	0
Isabella	5	40
Jackson	29	7
Kalamazoo	29	48
Lapeer	20	25
Lenawee	26	58
Livingston	17	35
Macomb	8	75
Midland	3	33
Monroe	79	76
Montcalm	11	45
Ottawa	2	50
Saginaw	112	90
St. Clair	30	57
St. Joseph	27	85
Sanilac	15	73
Shiawassee	130	80
Tuscola	16	94
Van Buren	6	83
Washtenaw	1	100
Total/Average	873	56

Table 2. Michigan soybean counties infested with *Heterodera glycines* in 2010.

County	Farms Sampled	% of Samples Positive for SCN
Allegan	8	25
Alpena	1	0
Arenac	5	20
Barry	15	40
Bay	15	47
Berrien	12	42
Branch*	3	100
Calhoun*	2	0
Cass	10	30
Eaton*	2	50
Genesee	12	42
Gratiot	27	78
Hillsdale*	2	100
Huron	22	27
Ingham*	3	67
Ionia*	2	100
Isabella	11	27
Kalamazoo	10	50
Kent	7	0
Lapeer	6	67
Lenawee	29	45
Macomb	7	29
Midland	7	57
Monroe	21	81
Montcalm	8	38
Oakland	1	100
Oceana	1	100
Ottawa	3	33
Presque Isle	1	0
Saginaw	32	91
St. Clair	11	18
St. Joseph	16	69
Sanilac	22	32
Shiawassee	38	79
Tuscola	26	54
Van Buren	5	60
Total/Average	403	50

*Sampling will be completed in 2011.

Table 3. Michigan soybean counties remaining to be sampled in 2011.

County	Number of Samples
Branch	12
Calhoun	17
Clinton	20
Eaton	18
Hillsdale	13
Ingham	9
Ionia	18
Iosco	1
Jackson	10
Livingston	3
Mason	1
Mecosta	1
Muskegon	2
Newaygo	2
Oakland	3
Ottawa	9
Washtenaw	13
Wayne	3
Total	155

Table 4. Soybean varieties and sources of resistance used by site and year for the Michigan component of the 2008-2010 NCSRP SCN Project.[1,2]

	Variety (Source of Resistance)							
Site (Year)	**Susceptible**	**Susceptible**	**PI 88788**	**PI 88788**	**PI 437654**	**PI 548402**	**PI 548402**	**Mutli-var[1]**
Ingham								
2008	NK S28-G1	NK S17-B5	NK S29-J6	NK S19-L7	Beck 298	SB 2979	--	--
2009	DF 8251	NK S17-B5	NK S29-J6	NK S19-L7	Beck 298	SB 2979	--	--
2010	DF 5191	DF 222	DF 8182	Beck 274	Beck 298	DF 181N	--	--
Monroe								
2008	NK S28-G1	NK S17-B5	NK S29-J6	NK S19-L7	Beck 298	SB 2979	--	--
Macomb								
2009	DF 8251	NK S17-B5	NK S26-P1	NK S19-L7	Beck 298	Pioneer 92Y20	--	--
2010	DF 5191	--	DF 8182	--	Beck 298	Pioneer 92M75	Pioneer 92Y53	Mutli-var[1]
Cass								
2008	NK S28-G1	NK S17-B5	NK S29-J6	NK S19-L7	Beck 298	SB 2979	--	--
2009	NK S28-G1	NK S17-B5	NK S29-J6	NK S19-L7	Beck 298	SB 2979	--	--
2010	DF 5191	DF 222	DF 8182	Beck 274	Beck 298	DF 181N	--	--

[1]Multi-var refers to an equal combination of all soybean varieties for the 2010 growing season in Macomb County.

[2]The varieties (DF 5191, DF 8182, Beck 298, Pioneer 92M75, and Pioneer 92Y53) were mixed together equally and added to the planter.

Table 5. At-planting population density (P_i) of *Heterodera glycines* associated with different soybean genetics at four locations in Michigan in 2008-2010.[1,2]

	H. glycines per 100 cm^3 soil								
Site (Year)	**Susceptible**	**Susceptible**	**PI 88788**	**PI 88788**	**PI 437654**	**PI 548402**	**PI 548402**	**Mutli-var[2]**	**P-value**
Ingham									
2008*	526 a	145 a	410 a	145 a	462 a	358 a	--	--	0.564
2009*	5169 a	2819 ab	551 bc	876 abc	133 c	129 c	--	--	0.001
2010*	74 a	53 a	50 a	21 a	61 a	50 a	--	--	0.931
Monroe									
2008	293 a	154 a	190 a	125 a	427 a	150 a	--	--	0.405
Macomb									
2009	696 a	678 a	680 a	831 a	1368 a	527 a	--	--	0.526
2010	888 a	--	754 a	--	1155 a	734 a	769 a	1929 a	0.282
Cass									
2008	--	--	--	--	--	--	--	--	--
2009	1113 a	1525 a	768 a	1188 a	3138 a	2090 a	--	--	0.186
2010	245 a	353 a	126 a	338 a	428 a	261 a	--	--	0.92

[1]Row means followed by the same letter are not significantly different (P=0.05) according to Fisher's LSD multiple range analysis. (N=6)

[2]The varieties (DF 5191, DF 8182, Beck 298, Pioneer 92M75, and Pioneer 92Y53) were mixed together equally and added to the planter.

*Data were transformed due to unequal variances: if the number of viable units was 0, category 1 was assigned; if the number of viable units was between 1 and 499, category 2 was assigned; if the number of viable units was between 500 and 2999, category 3 was assigned; and if the number of viable units was greater than 3000, category 4 was assigned.

Table 6. At-harvest population density (P_f) of *Heterodera glycines* associated with different soybean genetics at four locations in Michigan in 2008-2010.[1,2]

	H. glycines per 100 cm^3 soil								
Site (Year)	**Susceptible**	**Susceptible**	**PI 88788**	**PI 88788**	**PI 437654**	**PI 548402**	**PI 548402**	**Mutli-var[2]**	**P-value**
Ingham									
2008	10837 a	3692 ab	476 c	791 bc	30 c	201 c	--	--	<0.001
2009	6003 a	3183 a	1428 a	1868 a	25 b	78 b	--	--	<0.001
2010	1479 b	5253 a	512 bc	507 bcd	0 d	20 cd	--	--	<0.001
Monroe									
2008	6271 ab	14594 a	988 ab	1479 ab	914 b	1325 b	--	--	0.013
Macomb									
2009	3290 a	4174 a	188 b	539 b	279 b	311 b	--	--	<0.001
2010	6002 a	--	1098 b	--	18 d	60 cd	137 bcd	742 bc	<0.001
Cass									
2008	26714 a	16392 a	4947 ab	4878 ab	2158 bc	328 c	--	--	0.002
2009	6045 a	2401 ab	514 bc	759 bc	50 c	723 c	--	--	<0.001
2010	2137 a	2929 a	1818 a	2074 a	1053 a	2742 a	--	--	0.431

[1]Row means followed by the same letter are not significantly different (P=0.05) according to Fisher's LSD multiple range analysis. (N=6)

[2]The varieties (DF 5191, DF 8182, Beck 298, Pioneer 92M75, and Pioneer 92Y53) were mixed together equally and added to the planter.

*Data were transformed due to unequal variances: if the number of viable units was 0, category 1 was assigned; if the number of viable units was between 1 and 499, category 2 was assigned; if the number of viable units was between 500 and 2999, category 3 was assigned; and if the number of viable units was greater than 3000, category 4 was assigned.

Table 7. Influence of soybean genetics on reproduction factor (RF=P_f/P_i) of *Heterodera glycines* at four locations in Michigan in 2008-2010.[1]

	Reproductive Factor (RF=P_f/P_i)								
Site (Year)	**Susceptible**	**Susceptible**	**PI 88788**	**PI 88788**	**PI 437654**	**PI 548402**	**PI 548402**	**Mutli-var**[2]	**P-value**
Ingham									
2008*	27.75 ab	1517.81 a	1.12 cd	160.86 bc	0.11 d	1.41 d	--	--	<0.001
2009*	1.64 a	2.85 a	2.18 a	2.16 a	0.19 a	0.15 a	--	--	0.073
2010*	692.24 ab	2052.4 a	223.28 ab	141.06 ab	0 b	14.56 ab	--	--	0.013
Monroe									
2008*	32.71 ab	3153.88 a	650.56 ab	77.75 ab	2.43 b	17.52 ab	--	--	0.054
Macomb									
2009*	7.09 a	7.32 a	0.37 b	1.79 ab	0.17 b	0.82 ab	--	--	<0.001
2010*	38.94 a	--	4.18 ab	--	0.01 b	0.06 b	0.27 ab	1.03 ab	0.003
Cass									
2008*	--	--	--	--	--	--	--	--	--
2009*	14.38 a	2.72 ab	1.13 bc	1.85 b	0.02 c	1.78 bc	--	--	<0.001
2010*	1133.83 a	391.78 a	297.38 a	800.34 a	106.3 a	105.12 a	--	--	0.798

[1]Row means followed by the same letter are not significantly different (P=0.05) according to Fisher's LSD multiple range analysis. (N=6)
[2]The varieties (DF 5191, DF 8182, Beck 298, Pioneer 92M75, and Pioneer 92Y53) were mixed together equally and added to the planter.
*Data were transformed due to unequal variances: rankings were used to transform RFs based on the best performing variety (lowest RF) to the worst performing variety (highest RF). Lowest RFs were given a ranking of 1 and highest RFs were ranked a 6 (ties were broken by taking an average of the closest ranking scores).

Table 8. Soybean yield associated with various sources of *Heterodera glycines* resistance-susceptibility at four locations in Michigan in 2008-2010.[1]

	Yield (bu/A)								
Site (Year)	**Susceptible**	**Susceptible**	**PI 88788**	**PI 88788**	**PI 437654**	**PI 548402**	**PI 548402**	**Mutli-var[2]**	**P-value**
Ingham									
2008	16.2 a	19.7 a	15.2 a	17.5 a	14.7 a	15.7 a	--	--	0.535
2009	28.9 a	34.7 a	34.9 a	32.8 a	32 a	29.5 a	--	--	0.983
2010	33.21 a	34.57 a	37.86 a	37.54 a	27.39 a	32.76 a	--	--	0.11
Monroe									
2008	32.5 ab	27.9 b	42.2 a	38.5 ab	37.3 ab	34 ab	--	--	0.02
Macomb									
2009	35.17 bc	26.62 c	45.03 a	25.42 c	34.4 b	39.98 ab	--	--	<0.001
2010	24.48 b	--	29.59 ab	--	28.3 ab	28.79 ab	30.45 ab	32.9 a	0.047
Cass									
2008	18.8 ab	13.1 c	22.5 a	15.5 bc	18.5 ab	20.2 a	--	--	<0.001
2009	14.7 ab	8.2 b	17 a	8.8 b	19.3 a	17.3 a	--	--	0.001
2010	39 a	35.3 a	35.2 a	37.4 a	31.1 a	36 a	--	--	0.472

[1]Row means followed by the same letter are not significantly different (P=0.05) according to Fisher's LSD multiple range analysis. (N=6)
[2]The varieties (DF 5191, DF 8182, Beck 298, Pioneer 92M75, and Pioneer 92Y53) were mixed together equally and added to the planter.

Table 9. Trial 1 population densities of *Heterodera glycines* (SCN) and *Pratylenchus penetrans* (PLN) associated with susceptible, PI 437654, and PI 548402 soybean varieties.[1]

	Population Densities (Means)					
Trial 1	PI 437654 w/ SCN	PI 437654 w/ SCN and PLN	PI 548402 w/ SCN	PI 548402 w/ SCN and PLN	Susceptible w/ SCN	Susceptible w/ SCN and PLN
SCN females on roots	0.2	0.4	0.2	2.8	70.8	65.3
P-values	0.545		0.006		0.897	
SCN females in roots	0.8	3.4	0.7	2.2	112.5	186.7
P-values	0.178		0.475		0.187	
SCN VU in roots	2.2	9.6	2.3	6.6	61.0	326.7
P-values	0.247		0.156		0.394	
SCN males in roots	1.4	8.0	2.0	3.0	277.5	173.3
P-values	0.026		0.782		0.441	
SCN females in soil	12.0	6.0	8.0	14.0	64.0	50.0
P-values	0.094		0.217		0.663	
SCN VU in soil	252.0	168.0	266.0	326.0	350.0	490.0
P-values	0.072		0.398		0.367	
SCN males in soil	12.0	14.0	6.0	6.0	176.0	74.0
P-values	0.724		1.000		0.174	

[1]SCN was inoculated at a rate of 2000 viable units/conetainer. PLN was inoculated at a rate of 250 PLN/conetainer.

Table 10. Trial 2 population densities of *Heterodera glycines* (SCN) and *Pratylenchus penetrans* (PLN) associated with susceptible, PI 437654, and PI 548402 soybean varieties.[1]

	Population Densities (Means)					
Trial 2	PI 437654 w/ SCN	PI 437654 w/ SCN and PLN	PI 548402 w/ SCN	PI 548402 w/ SCN and PLN	Susceptible w/ SCN	Susceptible w/ SCN and PLN
SCN females on roots	1.0	2.3	2.5	1.8	7.3	6.0
P-values	0.194		0.387		0.418	
SCN females in roots	0.2	1.5	2.8	5.8	5.8	4.0
P-values	0.215		0.158		0.452	
SCN VU in roots	1.0	1.5	5.5	8.0	15.5	19.8
P-values	0.791		0.399		0.314	
SCN males in roots	0.8	4.3	8.3	8.3	1.3	1.8
P-values	0.116		1.000		0.628	
SCN females in soil	0.5	2.0	3.3	5.0	4.8	5.8
P-values	0.024		0.316		0.413	
SCN VU in soil	5.0	6.3	28.3	47.3	22.0	32.3
P-values	0.712		0.052		0.146	
SCN males in soil	2.5	1.3	6.0	9.5	0.3	2.3
P-values	0.344		0.246		0.129	

[1] SCN was inoculated at a rate of 2000 viable units/conetainer. PLN was inoculated at a rate of 500 PLN/conetainer.

Table 11. Michigan soybean counties infested with *Heterodera glycines* and *Pratylenchus penetrans* in 2010.

County	Farms Sampled	% of Samples Positive for SCN	% of Samples Positive for PLN
Allegan	8	25	63
Alpena	1	0	0
Arenac	5	20	20
Barry	15	40	67
Bay	15	47	47
Berrien	12	42	42
Branch*	3	100	100
Calhoun*	2	0	0
Cass	10	30	50
Eaton*	2	50	100
Genesee	12	42	42
Gratiot	27	78	70
Hillsdale*	2	100	100
Huron	22	27	41
Ingham*	3	67	100
Ionia*	2	100	50
Isabella	11	27	45
Kalamazoo	10	50	40
Kent	7	0	0
Lapeer	6	67	33
Lenawee	29	45	24
Macomb	7	29	0
Midland	7	57	0
Monroe	21	81	43
Montcalm	8	38	88
Oakland	1	100	100
Oceana	1	100	100
Ottawa	3	33	66
Presque Isle	1	0	0
Saginaw	32	91	41
St. Clair	11	18	0
St. Joseph	16	69	38
Sanilac	22	32	32
Shiawassee	38	79	18
Tuscola	26	54	42
Van Buren	5	60	60
Total/Average	403	50	46

*Sampling will be completed in 2011.

Figure 1. Distribution of *Heterodera glycines* in the United States (Data from Riggs and Tylka, 2008). For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

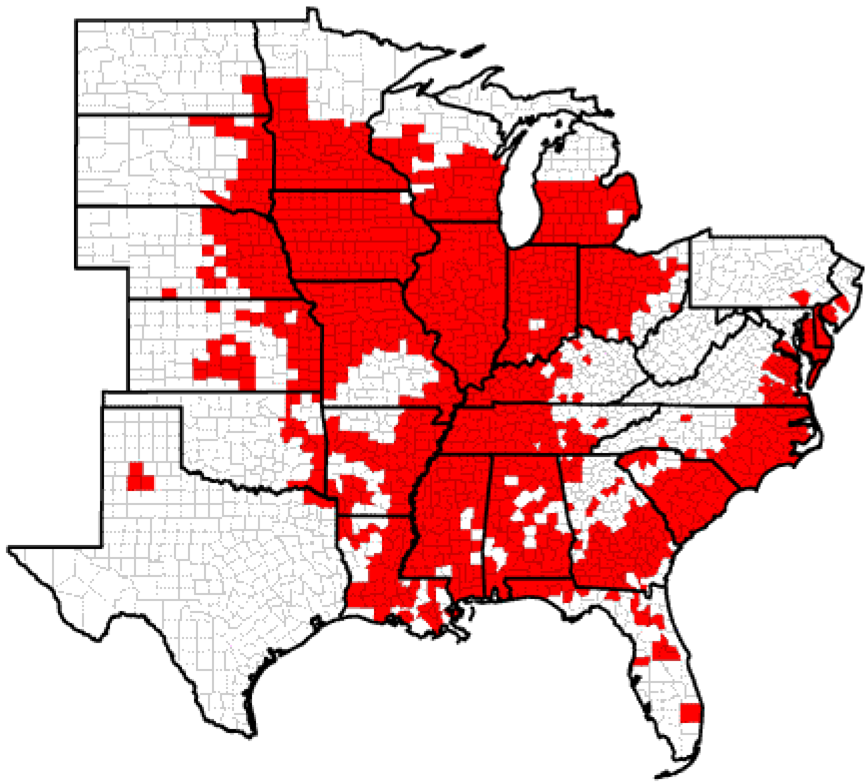
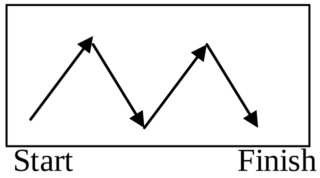


Figure 2. Dates of *Heterodera glycines* initial identification in Michigan soybean-producing counties from 1987-2005 (Tenney *et al.*, 2004).

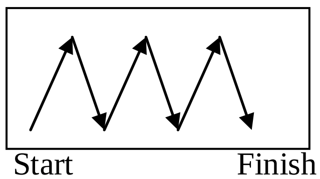
1987-Gratiot
1988-Van Buren
1989-Bay, Saginaw
1991-Monroe
1992-Berrien, Cass, Clinton, Midland, St. Joseph, Shiawassee
1993-Genesee, Lenawee, Tuscola
1994-Montcalm
1995-Hillsdale
1996-Sanilac
1997-Branch, Calhoun, Livingston, Macomb
1998-Ionia, Isabella, Lapeer, Washtenaw, Wayne
1999-Allegan, Barry, St. Clair
2000-Arenac, Ingham, Kalamazoo, Kent
2001-Muskegon
2002-Mecosta
2003-Jackson, Ottawa
2004-Huron
2005-Eaton

Figure 3. Sampling design for the 2010-2011 Michigan *Heterodera glycines* survey.

Small field (less than 40 acres):
2 rounds, 10 cores per side, 20 per round (40 cores total)



Medium field (40-80 acres):
3 rounds, 10 cores per side, 20 per round (60 cores total)



Large field (more than 80 acres):
4 rounds, 10 cores per side, 20 per round (80 cores total)

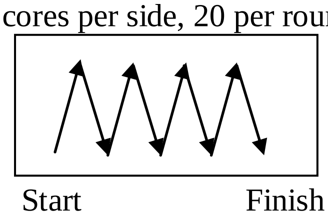


Figure 4. Distribution of *Heterodera glycines* in soybean-producing counties in Michigan based on the 2010 survey (Michigan Soybean Promotion Committee).

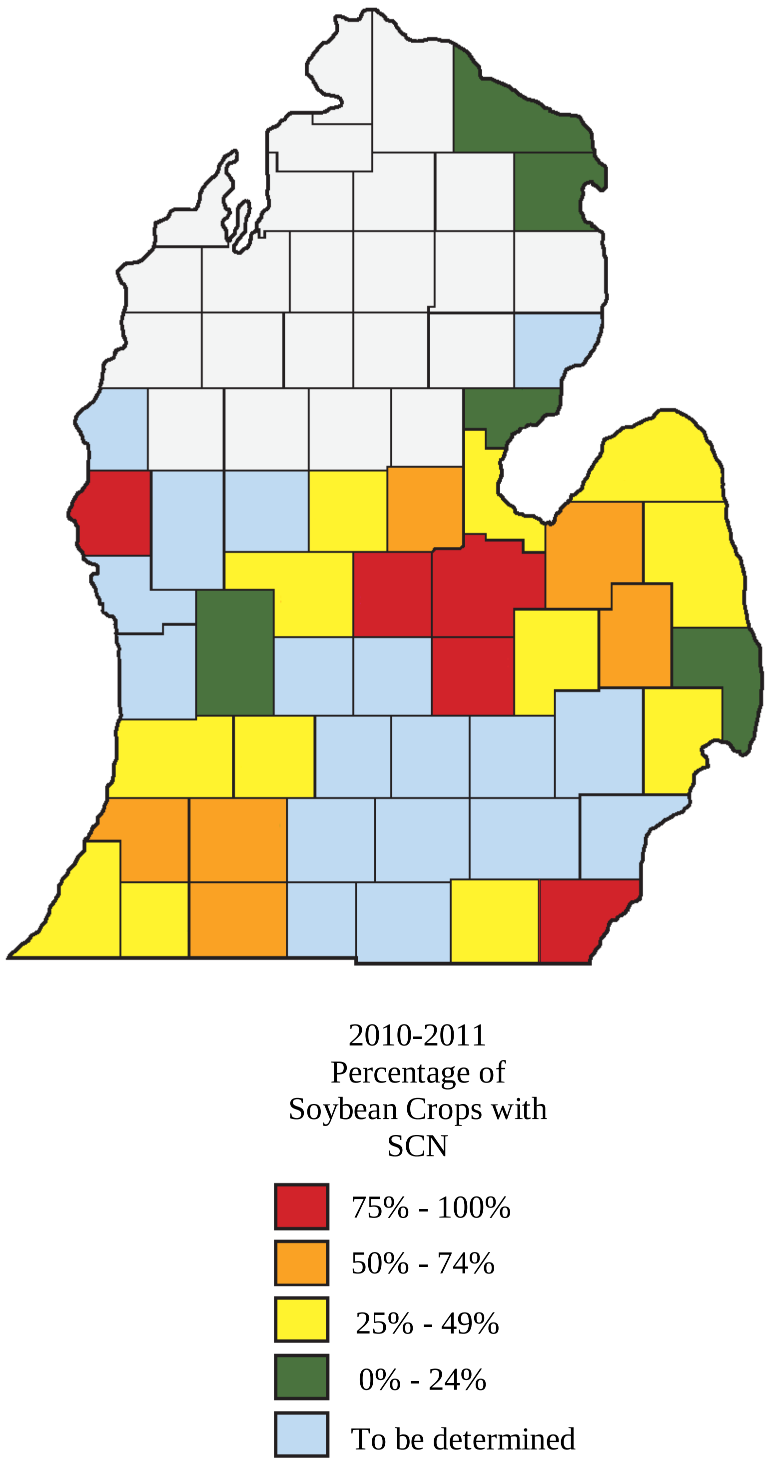
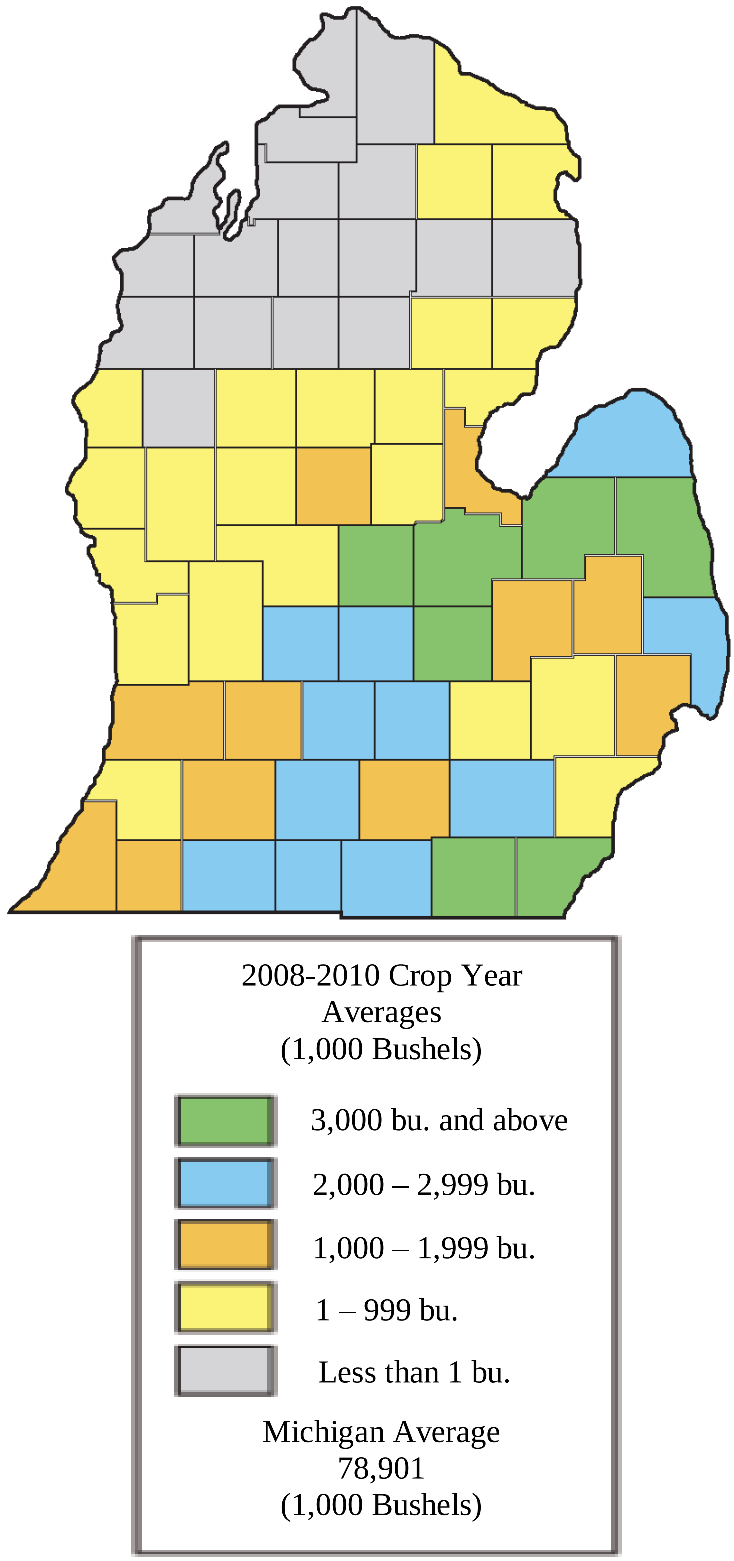


Figure 5. 2008-2010 Michigan soybean production by county and region (Michigan Soybean Promotion Committee).



APPENDICES

Appendix 1. Letter sent to randomly-selected soybean farms (n=1,116) in March 2010.

March 25, 2010

To: Michigan Soybean Grower
Topic: 2010 Soybean Cyst Nematode Survey Participation Request

The Michigan Soybean Promotion Committee has approved and funded a 2010 Statewide Soybean Cyst Nematode Survey to be conducted by the Michigan State University G.W. Bird Nematology Laboratory. Your farm has been selected at random to participate in this important research project. We sincerely hope you will agree to collaborate.

As a participating soybean grower, you will be responsible for submitting a soil sample from the third field you intend to plant for the 2010 growing season. The sample is to be dropped off at your local county extension office April 19-21. The protocol for taking and submitting the sample is on the back of this letter. The samples will be brought to Michigan State University and processed. The results for your farm will be forwarded to you and kept confidential.

We are writing to inform you about this project and ask for your participation. Kindly fill out the enclosed reply card and return it as soon as possible, since the beginning of the growing season is upon us. If you have any questions about the survey please contact Lesley Schumacher-Lott at schuma75@msu.edu. We thank you in advance for your cooperation.

Thank you,

George W. Bird, Professor

Lesley Schumacher-Lott, Research Assistant

Appendix 2. Comparative color-metric analysis of the at-plant (P_i) and at-harvest (P_f) SCN population densities associated with the 2008 Ingham County, Michigan NCSRP SCN Project.

Figure 6. Category, color, and viable unit (eggs+J2) key to at-plant (P_i) and at-harvest (P_f) SCN population densities associated with the 2008-2010 NCSRP SCN project.

Category	Color	Eggs+J2s
4	red	>3000
3	orange	500-2999
2	yellow	1-499
1	white	0

Table 12. At-plant (P_i) SCN population density associated with the 2008 Ingham County, Michigan NCSRP SCN Project.

Rep.	**P_i**					
IV	864	330	340	40	280	42
III	200	40	300	40	90	140
II	720	210	640	500	900	1384
I	321	0	360	0	160	280
Average	526	145	410	145	358	462
r gene	Sus	Sus	88788	88788	548402	437654
Variety	NK S28G1	NK S17B5	NK S29J6	NK S19L7	SB 2979	Beck 298

Table 13. At-harvest (P_f) SCN population density associated with the 2008 Ingham County, Michigan NCSRP SCN Project.

Rep.	**P_f**					
IV	10360	3200	580	44	0	0
III	5890	2124	400	320	124	0
II	8610	3450	924	2170	0	0
I	18486	5992	0	630	679	120
Average	10837	3692	476	791	201	30
r gene	Sus	Sus	88788	88788	548402	437654
Variety	NK S28G1	NK S17B5	NK S29J6	NK S19L7	SB 2979	Beck 298

Appendix 3. Comparative color-metric analysis of the at-plant (P_i) and at-harvest (P_f) SCN population densities associated with the 2009 Ingham County, Michigan NCSRP SCN Project.

Table 14. At-plant (P_i) SCN population density associated with the 2009 Ingham County, Michigan NCSRP SCN Project.

Rep.			**P_i**			
IV	1536	3240	600	282	0	79
III	5088	600	380	252	0	100
II	7560	6656	315	1364	516	352
I	6490	780	910	1606	0	0
Average	5169	2819	551	876	129	133
r gene	Sus	Sus	88788	88788	548402	437654
Variety	DF 8251	NK S17B5	NK S29J6	NK S19L7	SB 2979	Beck 298

Table 15. At-harvest (P_f) SCN population density associated with the 2009 Ingham County, Michigan NCSRP SCN Project.

Rep.			**P_f**			
IV	5770	3610	80	400	0	50
III	2210	5530	730	830	0	0
II	4200	3130	610	520	310	50
I	11830	460	4290	5720	0	0
Average	6003	3183	1428	1868	78	25
r gene	Sus	Sus	88788	88788	548402	437654
Variety	DF 8251	NK S17B5	NK S29J6	NK S19L7	SB 2979	Beck 298

Appendix 4. Comparative color-metric analysis of the at-plant (P_i) and at-harvest (P_f) SCN population densities associated with the 2010 Ingham County, Michigan NCSRP SCN Project.

Table 16. At-plant (P_i) SCN population density associated with the 2010 Ingham County, Michigan NCSRP SCN Project.

Rep.			**P_i**			
IV	40	110	0	0	0	120
III	80	40	30	63	110	0
II	175	60	96	20	0	0
I	0	0	75	0	90	125
Average	74	53	50	21	50	61
r gene	Sus	Sus	88788	88788	548402	437654
Variety	DF 5191	DF 222	DF 8182	Beck 274	DF 181N	Beck 298

Table 17. At-harvest (P_f) SCN population density associated with the 2010 Ingham County, Michigan NCSRP SCN Project.

Rep.			**P_f**			
IV	86	5400	875	30	58	0
III	1350	4040	123	78	0	0
II	1740	3570	0	1460	0	0
I	2740	8000	1050	460	20	0
Average	1479	5253	512	507	20	0
r gene	Sus	Sus	88788	88788	548402	437654
Variety	DF 5191	DF 222	DF 8182	Beck 274	DF 181N	Beck 298

Appendix 5. Comparative color-metric analysis of the at-plant (P_i) and at-harvest (P_f) SCN population densities associated with the 2008 Monroe County, Michigan NCSRP SCN Project.

Table 18. At-plant (P_i) SCN population density associated with the 2008 Monroe County, Michigan NCSRP SCN Project.

Rep.			**P_i**			
IV	200	390	60	40	300	360
III	140	200	700	260	270	588
II	390	27	0	200	30	720
I	440	0	0	0	0	40
Average	293	154	190	125	150	427
r gene	Sus	Sus	88788	88788	548402	437654
Variety	NK S28G1	NK S17B5	NK S29J6	NK S19L7	SB 2979	Beck 298

Table 19. At-harvest (P_f) SCN population density associated with the 2008 Monroe County, Michigan NCSRP SCN Project.

Rep.			**P_f**			
IV	10062	9750	1200	4175	1072	2163
III	9292	20304	169	966	2512	1248
II	3960	16443	1358	575	1716	192
I	1770	11880	1224	200	0	52
Average	6271	14594	988	1479	1325	914
r gene	Sus	Sus	88788	88788	548402	437654
Variety	NK S28G1	NK S17B5	NK S29J6	NK S19L7	SB 2979	Beck 298

Appendix 6. Comparative color-metric analysis of the at-plant (P_i) and at-harvest (P_f) SCN population densities associated with the 2009 Macomb County, Michigan NCSRP SCN Project.

Table 20. At-plant (P_i) SCN population density associated with the 2009 Macomb County, Michigan NCSRP SCN Project.

Rep.			**P_i**			
IV	1485	1173	858	2366	260	2240
III	378	264	160	357	240	1764
II	600	580	1280	120	942	605
I	320	693	420	480	666	864
Average	696	678	680	831	527	1368
r gene	Sus	Sus	88788	88788	548402	437654
Variety	DF 8251	NK S17B5	NK S26P1	NK S19L7	92Y20	Beck 298

Table 21. At-harvest (P_f) SCN population density associated with the 2009 Macomb County, Michigan NCSRP SCN Project.

Rep.			**P_f**			
IV	3952	9512	90	371	70	702
III	1380	4505	100	1178	512	230
II	1666	810	371	387	300	50
I	6160	1870	190	220	360	132
Average	3290	4174	188	539	311	279
r gene	Sus	Sus	88788	88788	548402	437654
Variety	DF 8251	NK S17B5	NK S26P1	NK S19L7	92Y20	Beck 298

Appendix 7. Comparative color-metric analysis of the at-plant (P_i) and at-harvest (P_f) SCN population densities associated with the 2010 Macomb County, Michigan NCSRP SCN Project.

Table 22. At-plant (P_i) SCN population density associated with the 2010 Macomb County, Michigan NCSRP SCN Project.

Rep.	P_i					
IV	1235	2291	1672	1335	1392	2409
III	297	1440	60	935	693	1071
II	70	640	774	456	330	600
I	1950	3344	510	210	660	539
Average	888	1929	754	734	769	1155
r gene	Sus	Mix	88788	548402	548402	437654
Variety	DF 5191	Mix	DF 8182	92M75	92Y53	Beck 298

Table 23. At-harvest (P_f) SCN population density associated with the 2010 Macomb County, Michigan NCSRP SCN Project.

Rep.	P_f					
IV	3720	150	40	115	60	72
III	8372	312	621	126	220	0
II	8600	2430	1440	0	212	0
I	3315	76	2289	0	55	0
Average	6002	742	1098	60	137	18
r gene	Sus	Mix	88788	548402	548402	437654
Variety	DF 5191	Mix	DF 8182	92M75	92Y53	Beck 298

Appendix 8. Color-metric analysis of the at-harvest (P_f) SCN population density associated with the 2008 Cass County, Michigan NCSRP SCN Project.

Table 24. At-harvest (P_f) SCN population density associated with the 2008 Cass County, Michigan NCSRP SCN Project.

Rep.	**P_f**					
IV	36465	12780	1323	13080	0	0
III	20240	17220	12915	1958	140	87
II	29440	9430	2040	3675	780	8471
I	20710	26136	3510	800	390	75
Average	26714	16392	4947	4878	328	2158
r gene	Sus	Sus	88788	88788	548402	437654
Variety	NK S28G1	NK S17B5	NK S29J6	NK S19L7	SB 2979	Beck 298

Appendix 9. Comparative color-metric analysis of the at-plant (P_i) and at-harvest (P_f) SCN population densities associated with the 2009 Cass County, Michigan NCSRP SCN Project.

Table 25. At-plant (P_i) SCN population density associated with the 2009 Cass County, Michigan NCSRP SCN Project.

Rep.	**P_i**					
IV	500	340	120	700	360	4180
III	630	960	1290	780	2580	4380
II	3150	3360	1380	360	4440	1830
I	171	1440	280	2910	980	2160
Average	1113	1525	768	1188	2090	3138
r gene	Sus	Sus	88788	88788	548402	437654
Variety	NK S28G1	NK S17B5	NK S29J6	NK S19L7	SB 2979	Beck 298

Table 26. At-harvest (P_f) SCN population density associated with the 2009 Cass County, Michigan NCSRP SCN Project.

Rep.	**P_f**					
IV	5115	2405	350	520	2500	75
III	5330	1260	200	105	345	60
II	7500	4080	1385	2340	0	40
I	6235	1860	120	70	45	25
Average	6045	2401	514	759	723	50
r gene	Sus	Sus	88788	88788	548402	437654
Variety	NK S28G1	NK S17B5	NK S29J6	NK S19L7	SB 2979	Beck 298

Appendix 10. Comparative color-metric analysis of the at-plant (P_i) and at-harvest (P_f) SCN population densities associated with the 2010 Cass County, Michigan NCSRP SCN Project.

Table 27. At-plant (P_i) SCN population density associated with the 2010 Cass County, Michigan NCSRP SCN Project.

Rep.			**P_i**			
IV	0	0	95	960	10	0
III	51	240	360	0	750	600
II	930	780	50	390	240	1110
I	0	390	0	0	43	0
Average	245	353	126	338	261	428
r gene	Sus	Sus	88788	88788	548402	437654
Variety	DF 5191	DF 222	DF 8182	Beck 274	DF 181N	Beck 298

Table 28. At-harvest (P_f) SCN population density associated with the 2010 Cass County, Michigan NCSRP SCN Project.

Rep.			**P_f**			
IV	201	1540	0	2465	3948	216
III	1617	3840	5724	0	5688	2310
II	2431	3996	380	2640	672	1480
I	4300	2340	1166	3192	658	204
Average	2137	2929	1818	2074	2742	1053
r gene	Sus	Sus	88788	88788	548402	437654
Variety	DF 5191	DF 222	DF 8182	Beck 274	DF 181N	Beck 298

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LITERATURE CITED

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