STUDIES ON THE BIOLOGY OF SOYBEAN CYST NEMATODE

A Dissertation
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Susilo Hambeg Poromarto

In Partial Fulfillment
for the Degree of
DOCTOR OF PHILOSOPHY

Major Department:
Plant Pathology

December 2011

Fargo, North Dakota

North Dakota State University

Graduate School

Title

<u>Studies on the Biology of Soybean Cyst Nematode</u>

By

<u>Susilo Hambeg Poromarto</u>

The Supervisory Committee certifies that this *disquisition* complies with North Dakota
State University's regulations and meets the accepted standards for degree of

<u>DOCTOR OF PHILOSOPHY</u>

SUPERVISORY COMMITTEE:

Dr. Berlin D. Nelson
Chair

Dr. Marcia P. McMullen

Dr. Luis M. Del Rio

Dr. Gary K. Clambey

Approved:

12-13-2011   Jack B. Rasmussen
Date   Department Chair

ABSTRACT

Soybean cyst nematode (SCN), *Heterodera glycines*, is a threat to soybean production in North Dakota. Studies on the biology of SCN were conducted to improve my understanding and management of this plant parasitic nematode. The objectives of the research were to; (1) determine if SCN reproduces on crops commercially grown or being tested for production in North Dakota, (2) evaluate the effects of SCN on growth of dry bean, (3) determine if there could be a shift in the SCN population toward greater ability to reproduce on dry bean, and (4) characterize the spatial distribution of SCN in research size field experiments. Canola, clover, lentil, and sunflower were nonhosts while borage, camelina, chickpea, crambe, cuphea, field pea, nyjer, and safflower were poor hosts for SCN with female indices (FI) less than 8. Lupines were susceptible hosts with FI's of 42 to 57. FI's of dry bean cultivars varied from 5 to 117. Kidney beans averaged the highest FI at 110 followed by navy, pinto and black at FI's 41, 39, and 16, respectively. Pod number (PN), pod weight (PW), seed number (SN), and seed weight (SW) of GTS-900 (pinto bean) were significantly less at 5,000 and 10,000 eggs/100 cm^3 soil compared with the control by 44 to 56% averaged over the two years. Significant reduction in growth of Montcalm (kidney bean) and Mayflower (navy bean) was observed at 2,500 and 5,000 eggs/100 cm^3 soils in 2009, but not in 2008. There was no evidence that SCN was increasing reproduction during two 11 month periods of continual reproduction on roots of dry bean cultivars Premiere and Cirrus (navy), Buster and Othello (pinto), and Eclipse and Jaguar (black). The spatial distribution of SCN in field plots was aggregated in nine of ten field sites with large differences in egg numbers between plots. Lloyd's index of patchiness

ranged from 1.09 to 3.34. Spatial distribution of SCN can be an important factor affecting

the results of field experiments.

ACKNOWLEDGEMENTS

Last but not least, I heartily honor my lovely wife, Yuni E. Armawati, my wonderful daughter, Adlina A. Paramarta, and my astonishing son, Dienul A. Paramarta for their continuous help, support, encouragement, understanding, and company during my study. This honor is extended to my mother in law, sisters, brothers, sisters-in-law, brothers-in-law, nieces, and nephews for their untiring care, love, and encouragement. I am indebted and greatly appreciate my American family in Fargo, ND, the Kooren's family, Dennis, Karen, Leah, Jana, and Joel for their unreserved help, support, and care during my life in USA.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1. GENERAL INTRODUCTION

Soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe (Tylenchida:

Heteroderidae), is the most damaging pathogen of soybean (*Glycine max*). About 31% of

soybean yield losses due to diseases during 2006 to 2009 was due to SCN; losses averaged

about 128.6 million bushels with a value of $1.286 billion (Koenning and Wrather, 2010).

The nematode lowers soybean yield by feeding on plant nutrients, retarding root growth,

reducing water and nutrient uptake and transport from roots to shoots, and inhibiting

rhizobial nodulation (Ko et al., 1984; Koenning and Barker, 1995; Postuka et al., 1986;

Riggs and Schmidtt, 1987; Ross, 1969).

SCN was first observed in China and Japan in the 1880s (Hartman et al., 1999).

Since then, SCN has been reported in Asia, Africa, South America and Europe (EPPO,

2009). In the USA, SCN was first reported in 1954 in North Carolina (Winstead et al.,

1955) and subsequently spread to 30 states and into Canada with the extension and

intensification of soybean cultivation. In Minnesota, SCN was first reported in 1978

(MacDonald et al., 1980). Since then, the nematode has been detected in most counties in

southern and central Minnesota where soybean is grown. In 1995, the nematode (race 3)

was reported in South Dakota (Smolik et al., 1996). In North Dakota, the nematode (HG

type 0) was discovered in 2003 in Richland Co. in the southeast corner of the state (Bradley

et al., 2004).

SCN has a broad host range (McSorley, 1988; Noel et al., 1982; Riggs, 1992; Riggs

and Hamblen, 1966; Smith and Young, 2003). However, there is a wide variation in

reproduction of SCN within a species of host plants (Chen et al., 2001; Donald et al., 2006;

Melton et al., 1985; Mitchum et al., 2007; Riggs and Hamblen; 1962, 1966; Schmitt and Shannon, 1992; Smith and Young, 2003).

SCN is a threat to soybean, dry bean, and potentially other crops produced in North Dakota and northern Minnesota. The reasons are: (1) SCN has been reported in these regions (Bradley et al., 2004; MacDonald et al., 1980) and is easily disseminated from field to field due to agricultural practices (Lal and Lal, 2006), especially practices used in the production of sugar beets and potatoes; (2) SCN survival in soil is favored by the long periods of cold soil temperature which minimize microbial degradation of eggs; and (3) this region is a major producer of soybean with approximately 11.2 million ha, about 13.4% of the total USA soybean production in 2011. In addition, North Dakota and Minnesota is the number one dry edible bean production area in USA with 520 thousand ha, about 37.8% of the total USA dry bean production (NASS, 2011). There are also specialty oilseed crops grown in this area such as cuphea, camelina and nyjer, which are hosts of SCN and might be damaged by the nematode (Warnke et al. 2006).

Resistance and crop rotation are the core management practices used to maximize seed yield of susceptible cultivars and to lower the density of *H. glycines* in the soil (Conley et al., 2011; Hershman, 2010; Meese et al., 1991; Miller et al., 2006; Pedersen and Lauer, 2003; Warnke et al., 2008). Resistance to SCN, however, can break down since virulence shifts in SCN populations have been shown to occur when using a single source of resistance (Conley et al., 2011; Mitchum et al., 2007; Niblack et al., 2008; Tylka et al., 2010). Managing SCN requires a broad knowledge of the biology of this important nematode.

This study examined several different aspects of the biology of SCN. The objectives were: (1) determine if SCN can reproduce on crops grown in ND other than soybean; (2) measure effects of SCN on growth of dry bean; (3) determine if there could be a shift in the SCN population toward greater ability to reproduce on dry bean; and (4) study spatial distribution of SCN in research plots. The final objective was to improve my understanding of how to interpret field studies on this nematode.

Literature Cited

Bradley, C. A., Biller, C. R., and Nelson, B. D. 2004. First report of soybean cyst nematode (*Heterodera glycines*) on soybean in North Dakota. Plant Dis. 88:1287.

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

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

Donald, P. A., Pierson, P. E., St.Martin, S. K., Sellers, P. R., Noel, G. R., Macguidwin, A. E., Faghihi, J., Ferris, V. R., Grau, C. R., Jardine, D. J., Melakerberhan, H., Niblack, T. L., Stienstra, W. C., Tylka, G. L., Wheeler, T. A., and Wysong, D. S. 2006. Assessing *Heterodera glycines*-resistant and susceptible cultivar yield response. J. Nematol. 38:76-82.

EPPO. 2009. *Heterodera glycines*. Bulletin OEPP/EPPO 39:59-61.

Hartman, G. L., Sinclair, J. B., and Rupe, J. C. (eds.). 1999. Compendium of soybean diseases. American Phytopathological Society, St. Paul, MN. 100 pp.

Hershman, D. E. 2010. Soybean Cyst Nematode (SCN) Management Recommendations for Kentucky. Plant Pathology Fact Sheet. UK Cooperative Extension Service. PPFS-AG-S-24.

Ko, M. P., Barker, K. R., and Huang, J. S. 1984. Nodulation of soybeans as affected by half-root infection with *Heterodera glycines*. J. Nematol. 16:97-105.

Koenning, S. R., and Barker, K. R. 1995. Soybean photosynthesis and yield as influenced by *Heterodera glycines,* soil type and irrigation. J. Nematol. 27:51-62.

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

Lal, R., and Lal, A. 2006. Plant parasitic nematodes intercepted from seeds, soil clods and packing material under import quarantine. J. New Seeds 8:49-60.

MacDonald, D. H., Noel, G. R., and Lueschen, W. E. 1980. Soybean cyst nematode, *Heterodera glycines*, in Minnesota. Plant Dis. 64:319-321.

McSorley, R. 1988. Population dynamics. pages 109-133 in: K. R. Barker, G. A. Pederson, and G. L. Windham (Ed.), Plant and nematode interactions. Madison. American Society of Agronomy.

Meese, B. G., Carter, P. R. Oplinger, E. S. and Pendleton, J. W. 1991. Corn/soybean rotation effect as influenced by tillage, nitrogen, and hybrid/cultivar. J. Prod. Agric. 4:74-80

Melton, T. A., Noel, G. R., Jacobsen, B. J., and Hagedorn, D. J. 1985. Comparative host suitabilities of snap beans to the soybean cyst nematode (*Heterodera glycines*). Plant Dis. 69:119-122.

Miller, D. R., Chen, S. Y., Porter, P. M., Johnson, G. A., Wyse, D. L., Stetina, S. R., Klossner, L. D., and Nelson, G. A. 2006. Rotation crop evaluation for management of the soybean cyst nematode in Minnesota. Agron. J. 98:569-578.

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

NASS. 2011. Agricultural Statistics Database. United States Department of Agriculture. Online publication. http://www.nass.usda.gov/QuickStats/index2.jsp.

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

Noel, G. R., Jacobsen, B. J., and Leeper, C. D. 1982. Soybean cyst nematode in commercial snap bean. Plant Dis. 66:520-522.

Pedersen, P., and Lauer, J. G. 2003. Corn and soybean response to rotation sequence, row spacing, and tillage system. Agron. J. 95:965-971.

Postuka, J. W., Dropkin, V. H., and Nelson, C. J. 1986. Photosynthesis, photorespiration, and respiration of soybean after infection with root nematodes. Photosynthetica 20:405-410.

Riggs, R. D. 1992. Host Range. Pages 107–114 in R. D. Riggs and J. A. Wrather, eds. Biology and Management of the Soybean Cyst Nematode. St. Paul, MN: American Phytopathological Society.

Riggs, R. D., and Hamblen, M. L. 1962. Soybean cyst nematode studies in the family Leguminosae. Ark. Agr.. Expt. Sta. Rep. Series 110:1-8.

Riggs, R. D., and Hamblen, M. L. 1966. Further studies on the host range of the soybean cyst nematode. Ark. Agr.. Expt. Sta. Series 110:2-19.

Riggs, R. D., and Schmitt, D. P. 1987. Nematodes. Pages 757-7788. In J. R. Wilcox, ed. Soybean: Improvement, production, and uses. 2nd ed. American Society of Agronomy, Inc., Crop Science Society of American, Inc., Soil Science Society of American, Inc., Madison.

Ross, J. P. 1969. Effect of *Heterodera glycines* on yields of nonnodulating soybeans grown at various nitrogen nevels. J. Nematol. 1:40-42.

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

Smith, J. R., and Chavarría-Carvajal, J. A. 2002. Host Suitability of pigeon pea for *Heterodera glycines* race 2. Nematropica 32:125-130.

Smith, J. R., and Young, L. D. 2003. Host suitability of diverse lines of *Phaseolus vulgaris* to multiple populations of *Heterodera glycines*. J. Nematol. 35:23-28.

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

Tylka, G. L., Gebhart, G. D., and Marett, C. C. 2010. Evaluation of soybean varieties resistant to soybean cyst nematode in Iowa in 2009. IPM 52. Iowa State Univ. Ext. Serv., Ames.

Venkatesh, R., Harrison, S. K., and Riedel, R. M. 2000. Weed hosts of soybean cyst nematode (*Heterodera glycines*) in Ohio. Weed Technol. 14:156-160.

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

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

CHAPTER 2. LITERATURE REVIEW

Biology of *Heterodera glycines*

Nomenclature

Heterodera glycines Ichinohe, is the scientific name for soybean cyst nematode. SCN is the international common name in English. SCN also has other international names such as "Heterodera de la soja or nematodo de la soya" in Spanish, and "nématode du soja" in French. In addition there are local common names as "Aelchen Sojabohnenzysten" or "Sojabohnenzystennematode" in Germany, "anguillula de la soia" in Italy, "daizu-iwo-byo, daizu-sisuto-sentyu", or tsukiyobo in Japan, and "nematoda kista kedelai" in Indonesia.

Taxonomy

SCN was first described and then classified as the new species *Heterodera glycines* Ichinohe, in 1952. It was first reported in Japan as *Heterodera schachtii* in 1915 and had previously been referred to as one of the strains of *Heterodera gottingiana* (Ichinohe, 1952). SCN belongs to Domain Eukaryote, Kingdom Animalia, Phylum Nemata, Class Secernentea, Order Tylenchida, Family Heteroderidae, and Genus *Heterodera* Schmidt, 1871 (Maggenti, 1991). This genus has the following synonyms: *Tylenchus* A. Schmidt, 1871, *Heterobolbus* Railliet, 1896, *Bidera* Krall' & Krall', 1978, and *Ephippiodera* Shagalina & Kral', 1981 (Baldwin and Mundo-Ocampo, 1991).

There are approximately 47 species in this genus (Baldwin and Mundo-Ocampo, 1991). They are *H. acnidae, H. arenaria, H. aucklandica, H. avenae* (Cereal Cyst Nematode), *H. betae, H. bifenestra, H. cacti, H. carotae* (Carrot Cyst Nematode), *H. ciceri, H. cruciferae, H. cyperi, H. daverti, H. fici, H. filipjevi, H. glycines* (Soybean Cyst Nematode) *, H. goettingiana, H. graminis, H. graminophila, H. hordecalis, H. humuli', H.*

6

iri, H. latipons, H. lespedezae, H. leuceilyma, H. mani, H. medicaginis, H. mediterranea,

H. mothi, H. oxian, H. pratensis, H. radicicola, H. ripae, H. rosii, H. rostochiensis, H.

sacchari, H. salixophila, H. schachtii (Sugar-Beet Cyst Nematode), *H. sonchophil , H.*

tabacum, H. trifolii, H. trifolii f. beta, H. urticae, H. ustinovi, H. vigni, H. vitis, and *H. zeae*

(Corn Cyst Nematode).

The characteristics of the vulval cone of the cyst and the length of stylet, tail and

hyaline tail terminus of the second stage juvenile are important in determining the genus of

Heterodera (Taylor, 1975). The shape of the juvenile stylet knobs is an additional

character. However, hosts and other environment factors can affect those sizes (Taylor,

1975; Lax and Doucet, 2001a,b,c.; Lax and Doucet, 2002). The genus of *Heterodera* is

characterized by sexual dimorphism (Taylor, 1975; Triantaphyllou and Hirshmann, 1962).

The morphological characteristics of males and females of *H. glycines* are described by

Baldwin and Mundo-Ocampo, 1991, Graney and Miller, 1982, Lax and Doucet, 2001b,c

and Taylor, 1975. The general measurements of *Heterodera glycines* are summarized in

Taylor, 1975, and Graney and Miller, 1982. DNA-based diagnostics have been widely used

for SCN identification, especially to distinguish *H. glycines* from *H. ciceri*, *H. medicaginis*,

H. schachtii and *H. trifolii*. (Besal et al., 1988; Subbotin et al., 2000; Zheng et al., 2000).

Life cycle

The life cycle of SCN consists of five stages, Juveniles 1 through 4 (J1-J4) and

adults. The J1 occurs within the egg, J2 occurs within the soil while the third and fourth

stages (maturing stages) occur within the plant root. After the first molt within the egg,

SCN J2 hatch, move through the soil, penetrate roots and move toward the vascular

cylinder (Niblack et al., 2006; Ithal et al., 2007), select a host cell in the cortex,

endodermis, or pericycle then induce host cell fusion as part of the formation of a permanent feeding site called a syncytium (Endo, 1992). The syncytium is a large and metabolically active plant cell, with a dense granular cytoplasm and proliferation of mitochondria, endoplasmic reticulum and free ribosomes. The second molt to a J3 occurs approximately 7 days after infection. Differentiation of the nematode to become male and female occurs in J3. Male nematodes feed for several days until the end of the J3 stage then males discontinue feeding and molt into vermiform J4. After approximately 3 weeks the vermiform males burrow out of the root (Jenkins and Taylor, 1967) to fertilize adult females (Triantaphyllou and Hirshmann, 1962). Females remain sedentary after the establishment of their feeding site, expand circumferentially while undergoing J3 and J4 molts and then mature into feeding adults. By this time, the posterior region of the female has become exposed outside the root. Following fertilization, the female produces eggs. As eggs develop the females become more lemon-shaped with their posteriors protruding from the root. Fully mature females can be seen as tiny white embedded objects along the host's roots.

Each female is capable of producing up to 600 eggs (average = 150). Some eggs are deposited in a gelatinous matrix containing chitinase and polyphenol-oxidase (Niblack and Karr, 1994; Perry and Clark, 1981) which protects the eggs from predation. The eggs serve both as a reproductive unit and as survival stage. Eggs in the gelatinous matrix hatch during the current season. The remaining eggs in the cyst will hatch in following seasons. Eggs remain viable for up to 11 years (Inagaki and Tsutsumi, 1971).

When females die, they gradually darken to brown and form a protective layer around the eggs. The dead body of an SCN female is called as cyst. A new cyst may

contain 50–400 eggs (Triantaphyllou and Hirshmann, 1962). The cysts are very durable and can persist in the soil for months. Eggs are able to survive and remain viable in the cyst for 6–8 years under very harsh environmental conditions (Alston and Schmitt, 1988; Slack et al., 1972). Cyst survival is generally greater in dry soil than in very moist soil (Heatherly and Young, 1991; Slack et al., 1972). In moist soil, cysts were reported to survive up to eight years while in dried soils up to 9 years (Inagaki and Tsutsumi, 1971). Irrespective of soil moisture, survival was greatest at 20°C and below. Cysts exposed to 40°C do not retain full viability (Ali, 1988). Desiccation of cysts reduces viability, but does not completely eliminate the hatching of active larvae from a population of cysts (Slack and Hamblen, 1961).

The optimum temperature for SCN development ranges from 23–28°C (Burrows and Stone, 1985; Melton et al., 1986; Riggs, 1982; Sortland and MacDonald, 1986). Development was most rapid at 30°C. Some development occurred at 15 and 20°C but required greater time (Sortland and MacDonald, 1986). Egg hatching occurs at 20– 30°C, and is optimal at 26°C in the day and 22°C at night (Hill and Schmitt, 1989). More eggs hatched in the presence of, and greater nematode reproduction occurred, on soybeans producing pods than on those that remained vegetative. SCN reproduces at an optimum temperature around 26º C and reproduction is greater at pH 6.5 and 7.5 than at lower pH's (Anand et al., 1995; Pedersen et al., 2010). The development of J1 to J2 within the egg was linearly related to temperature between 15 and 30 C and optimal at 24°C (Alston and Schmitt, 1988). No larval emergence was observed at 10 and 41°C when cysts were subjected to these temperatures for 30 days and then transferred to 27°C. The range of diurnal soil temperature fluctuations and accumulated degree days between 5 and 30°C has

9

an impact on the rate of development of juveniles in soybean roots. Decreasing temperature appears to be more important than soybean phenology in dormancy induction of *H. glycines* (Hill and Schmitt, 1989).

The development of *H. glycines* in the field is affected by soil texture and soil moisture. Heatherly and Young (1991) reported more cysts were produced in a wet silt loam soil, compared to a dry silt loam and declined significantly in both wet and dry clay soil. *H. glycines* will not maintain populations in fine-textured clay soil (Rajan and Lal, 2005).

Almost all stages of *H. glycines* are able to survive and be transported along with roots of infected plants. Cysts are the primary way large numbers of eggs are disseminated at one time. Cysts can be spread along with soil, roots or contaminated packing material over long distances. The most important means of spreading cysts is through soil particles (peds) contained in seed lots. Once introduced into the seed lot, soil peds or clods are quite difficult to remove with conventional equipment. Cysts spread by the wind have been detected up to 55 m from a source (Andrade and Asmus, 1997). Cysts with viable juveniles have even been recovered from excreta of birds (Epps, 1971).

Pathogenesis

Soybean cyst nematodes infect and grow in the roots of both resistant and susceptible cultivars (Davis et al., 2008; Niblack et al., 2006). Nematode growth and development depends on the successful establishment and maintenance of a syncytium. The degradation of the plant cell wall during *H. glycines* invasion is a complex, highly regulated process (Gao et al., 2002; Yan et al., 1998, 2001). Cellulose-degrading enzymes (β-1,4-endoglucanases) from *H. glycines* were identified (Bird et al., 1974; de Boer, 1999;

Smant et al., 1998; Wang et al., 1999), and assumed to play a key role in *H. glycines* invasion of the root, but not in syncytium formation. These enzymes are secreted from the stylet, and detected along the migratory path (Goellner et al., 2001; Wang et al., 1999). Pectate lyases have also been identified in *H. glycines* (de Boer et al., 2002) and assumed to aid the nematode in breaking down plant cell walls during initial root invasion.

A nematode chorismate mutase (CM) in *H. glycines* (Bekal et al., 2003; and Gao et al., 2003) enzyme has been identified. This enzyme is found in the shikimate pathway, a primary metabolic route producing aromatic amino acids (phenylalanine, tyrosine, and tryptophan) and numerous secondary metabolites playing key roles in plant cell development, structure, and defense against biotic and abiotic stress. CM is a key branch point regulatory protein controlling the production of phenylalanine and tyrosine.

CM plays a role in altering the plant's shikimate pathway to assist the nematode in parasitizing the plant. The enzymatic activity of *H. glycines* CM has been confirmed and it has been suggested that CM amends the types and levels of phenolic compounds produced by the plant to alter plant cell form and function (Bekal et al., 2003). *H. glycines* CM may play a role in assisting nematode development on resistant soybeans. The Hg-cm-1 gene is postulated to act as a virulence gene by a general suppression of shikimate chorismate-derived compounds that play a role in host plant defense (Bekal et al., 2003; Lambert et al., 2005).

Virulence

Variability in nematode virulence can be determined by the ability of SCN populations to reproduce on resistant soybean lines. This variability was recognized soon after SCN was discovered in North Carolina in 1954, since the nematode was able to adapt

11

to resistant soybean cultivars (Brim and Ross, 1966; Ross, 1962a). At that time, the terms "physiological strains" or "biotypes" were used to describe this variability. By the 1970's the term "race" was used.

The first concept of race in SCN was introduced in 1970 (Golden et al., 1970) using the resistant differentials Pickett, Peking (PI 548402), PI 88788, and PI 90763 and the susceptible check Lee. Four patterns (Races 1, 2, 3, and 4) were observed using these differentials. The first four race designations were expanded in 1988 to 16 race designations (Riggs and Schmitt, 1988) as shown in Table 2.1. A new classification scheme was proposed in 2002 (Niblack et al., 2002) called the HG Type test (HG stands for *H. glycines*). The HG Type test (Table 2.2) involves seven sources of resistance that have been used in developing resistant germplasm or cultivars in the United States. In this new scheme, the resistant cultivar Pickett is no longer used, while Peking, P 88788, and PI 90763 are still needed along with four new resistant sources: PI 437654, PI 209332, PI 89772, and PI 548316 (Cloud). Instead of using the susceptible cultivar Lee, the new scheme uses Lee 74 as a susceptible check. The HG-type is determined by using a Female Index (FI).

$$FI = \frac{\text{average number of females on indicator line}}{\text{average number of females on Lee 74}} \text{ x } 100$$

Cultivars or PI's having an FI less than 10% are considered resistant. Those with FI's greater than 10% are considered to be susceptible and thus the nematode biotype will reproduce on them. The HG designation uses a 1-7 numbering scheme and each number corresponds to a specific differential (Table 2.2). For example, HG 2.5.7 refers to a biotype that reproduces on differential numbers 2, 5 and 7. This scheme is open ended (additional

soybean lines can be added) and is easily adaptable to different geographic areas. The HG

type system is useful for making cultivar recommendations (Niblack et al., 2002).

Table 2.1. Races of the soybean cyst nematode *Heterodera glycines*, according to Riggs
and Schmitt (1988).

Races	Differentials			
	Pickett	Peking	PI 88788	PI 90763
1	-	-	+	-
2	[a]+	+	+	-
3	[b]-	-	-	-
4	+	+	+	+
5	+	-	+	-
6	+	-	-	-
7	-	-	+	+
8	-	-	-	+
9	+	+	-	-
10	+	-	-	+
11	-	+	+	-
12	-	+	-	+
13	-	+	-	-
14	+	+	-	+
15	+	-	+	+
16	-	+	+	+

[a] + =the number of females produced by a *H. glycines* population on a differential is equal
to or greater than 10% of the number produced on the standard susceptible cultivar Lee.

Host resistance

SCN resistance in soybean is measured as a reduced number of females that develop compared to the number that develop on similarly inoculated susceptible standard (Colgrove and Niblack, 2008; Niblack et al., 2006) and expressed as the female index (FI). Soybean cultivars are generally classified as highly resistant to SCN if the FI is less than 10% (Schmitt and Shanon, 1992).

Table 2.2. Indicator lines for HG Type classification of genetically diverse populations of *Heterodera glycines*.

Number	Indicator line
1	PI 548402 (Peking)
2	PI 88788
3	PI 90763
4	PI 437654
5	PI 209332
6	PI 89772

Adapted from Niblack et al. (2002).

SCN resistance in soybean is partial resistance. Inheritance of resistance to SCN is quantitative and complex. It involves three to four major genes and several minor genes (Diers et al., 1997). Three recessive genes rhg_1, rhg_2 and rhg_3 have been reported in the cultivar Peking (Caldwell et al., 1960), while the forth resistance gene rhg_4 was reported by Matson and Williams (1965) in Peking and in plant introduction (PI) 437654, but not in PI

14

88788 or PI 209332 (Brucker et al., 2005; Colgrove and Niblack, 2008). A dominant gene, *rhg₅* was identified in PI 88788 (Rao-Arelli et al., 1992; Rao-Arelli, 1994).

The *rhg1* locus is frequently found in soybean germplasm and has the greatest impact on SCN resistance (Melito et al., 2010). Peking, PI 88788, PI 437654, PI 209332 and PI 90763 have this locus which provides soybean resistance to many common SCN populations (Concibido et al., 2004). However, no single locus can provide complete resistance to all races. The combinations of these major loci can result in higher levels of resistance (Concibido et al., 1995, 1997).

Genetic mapping efforts uncovered numerous locations of SCN resistance QTL's in many PIs. The linkage groups (LGs): A1, A2, B1, B2, C1, C2, D1a, D2, E, F, G, H, I, J, L, M, and N have been associated with SCN resistance. Linkage group G has the most QTL regions associated with SCN resistance with four, followed by LGs B1, C2, and D2 with three, and LGs A1, B2, D1a, E, and M with two; the rest of the LGs have one QTL (Concibido et al., 2004).

Among the sources of resistance, Peking has nine, the highest number of QTL associated with resistance, followed by eight in PI 438489B, and six in PI 437654. Peking' (PI 548402), PI 88788, and PI 437654 were shown to carry resistance loci effective against multiple nematode races and have been used sources of resistance for commercially grown soybean cultivars (Concibido et al., 1977, 2004), however, PI 88788 has been the most widely used resistance source in breeding programs (Glover et al., 2004).

Host range

Soybean cyst nematode has a wide host range with soybeans as the major economic host. Riggs (1992) reviewed published studies of alternative SCN hosts and compiled a list

of 96 genera of Fabaceae (Leguminosae) and 50 genera representing 22 families of non-legumes that have been reported as alternate hosts of SCN. Other cultivated hosts, mainly Fabaceae, are *Lespedeza* spp., *Lupinus albus, Phaseolus vulgaris, Vicia villosa, Vigna angularis* and *Vigna radiata*. SCN also has weeds hosts, such as *Cerastium holosteoides, Lamium amplexicaule*, *Stellaria media*, common chickweed (*Stellaria media* L.), common purslane (*Portulaca oleracea*), hairy vetch (*Vicia villosa*), and sweet clover (*Melilotus officinalis*) (Riggs 1992, Riggs and Hamblen 1966). Alfalfa, barley, canola, clover (red, white, and ladino), corn, forage grasses, oats, rye, sorghum, and wheat, however, are not reported as hosts of SCN.

There is a wide variation in reproduction of SCN on cultivars/ varieties/PI's within a host species. For example, Riggs and Hamblen (1962) in their extensive study of hosts in the Leguminosae showed that out of 199 plant introductions of *Pisum sativum*, five were rated as susceptible to SCN, 132 allowed some reproduction and the rest were immune. Another example is in clovers. Although the majority of clover species in *Melilotus* and *Trifolium* are nonhosts or poor hosts (Riggs and Hamblen, 1962, 1966), at least 7 plant introductions within these genera were susceptible to SCN with FI's greater than 10.

SCN and *Phaseolus vulgaris*

Phaseolus vulgaris L., common bean, has been known as a host of SCN since the 1930's (Fujita and Miura, 1934). However, research on the SCN-common bean interaction has been limited. Melton et al. (1986) reported more Juveniles stage 2 penetrated snap bean, *Phaseolus vulgaris*, L., than soybean roots in one trial but no differences were reported in another trial. SCN has been shown to reproduce on many cultivars of dry bean in the greenhouse (Melton et al., 1985; Riggs and Hamblen, 1966). In general, there are

high levels of resistance to SCN within the Middle American gene pool while the greatest susceptibility is found in the Andean gene pool (Melton et al., 1985; Smith and Young, 2003). SCN reproduction on kidney bean was reported by Abawi and Jacobsen (1984) to be affected by the initial egg density in the soil, and environmental factors.

Prior to the study in this dissertation, there were no reports about the effects of SCN on dry bean yield under field conditions. Abawi and Jacobsen (1984) reported no significant reduction in growth of kidney bean in a 35 day greenhouse test in SCN infested soil. However, Becker and Ferraz (2004) reported a 15% reduction in yield and a 40.8 % reduction in root dry weight associated with 5,600 and 12,600 eggs per plant, respectively, in a greenhouse test.

SCN population density and spatial distribution

Most studies of spatial distribution of SCN have been conducted in large field areas. SCN distribution in fields was reported as aggregated (Avendaño et al., 2004, Kulkarni et al., 2008). The distribution of SCN is affected by nematode survivability, movement and population dynamics. SCN survivability is affected by physical and biological factors. The major distribution of SCN is through passive movement, because SCN only actively moves a short distance. Passive movements of SCN are due to runoff or flood water, wildlife, wind, and human activities (Gavassoni et al., 2007; Koenning and Sipes, 1998; Lehman, 1994). Any factor affecting the reproduction or health of plants will impact SCN reproduction (Avendaño et al., 2004; Johnson, 1993; Workneh, 1999). Population density of SCN was positively correlated with soil pH and Mg but negatively to copper (Francl, 1993). SCN was found more abundantly in coarser soils than in finer soils (Donald et al., 2001; Dropkin et al., 1976; Koenning and Barker, 1995). The number of eggs produced

per pot in a silty loam was higher than in the clay at 30 days (Young and Heatherly, 1990).

There was a consistency in the relationship between SCN and soil texture across fields and

over time (Avendaño et al., 2004). The water status of the soil also affects SCN

populations in the field (Johnson et al., 1993). Soybean cyst nematode survival in the soil

in the absence of a host was shown to be highest at field capacity, followed by dry soil, but

lowest in flooded conditions (Slack et al., 1972).

Tillage influences nematode prevalence and population density by increasing the

amount of space available for nematode movement even in fine soils rich in clay (Jones et

al., 1969; Workneh et al., 1999). SCN aggregation was significantly greater in no- and

ridge-tillage treatments than in conventional and reduced-tillage treatments (Gavassoni et

al., 2007). More cysts developed on plants in disturbed soil cores than in undisturbed cores

(Young, 1987). Prior to cultivation, high population clusters occur in the plant rows and in

the middle furrow (Francl, 1986). There is directional spatial dependence of egg and cyst

densities along soybean rows, coincident with the direction of tillage practices (Gavassoni

et. al., 2007).

Management of Soybean Cyst Nematode

SCN management strategies are intended to improve soybean health and yield, keep

SCN numbers low, preserve the yield potential of resistant varieties, and maintain

profitable soybean yields (Niblack, 2005). SCN management can involve growing non-

host crops, controlling winter annual weeds, applying nematicides, and growing SCN-

resistant soybean varieties. However, rotation to non-host crops such as alfalfa (*Medicago

sativum* L.), maize (*Zea mays* L.), and wheat (*Triticum aestivum* L.), and planting of SCN-

resistant soybean cultivars are the most important practices (Niblack, 2005).

Planting SCN-resistant soybean cultivars has two advantages (Niblack, 2005); producing good soybean yields on SCN-infested fields, and preventing increases in SCN populations. Many different soybean breeding lines have been used to develop SCN-resistant varieties. For example, the resistant cultivar Hartwig (the name of a USDA soybean breeder) was a parent to many current resistant soybean cultivars. Yield improvement has been reported when resistant cultivars are used in SCN-infested fields, especially when SCN population density was high at planting (Chen et al., 2001; Donald et al., 2006; Koenning, 2004; Wheeler et al., 1997; Young, 1996). Planting resistant cultivars with different sources of resistance in different years is recommended to prevent buildup of SCN biotypes that can attack commonly used resistance sources (Bridge and Starr, 2007).

Several rotation crops have been shown to reduce soybean cyst nematode populations. The first crop rotation experiments directed toward managing *H. glycines* were established in 1956 in North Caolina by Ross (1962b). Populations were reduced by as much as 75% or 92% following a 1- or 2-year rotation to corn, respectively, in the southeastern US (Wrather et al., 1984). Yields on SCN infested soil were 518 kg/ha in continuous soybean, 1,258 kg/ha with a soybean-corn-soybean rotation, and 1,634 kg/ha with soybean following 2 years of corn. Rotations with monocot crops such as grain sorghum (*Sorghum bicolor*) (Rodriguez-Kabana et al., 1990), bahiagrass (*Paspalum notatum*) (Rodriguez-Kabana et al., 1989), and maize (Weaver et al., 1988) have been highly effective in increasing yield of soybean, especially for nematode-susceptible cultivars. Soybean following a sorghum-sudan grass crop (*Sorghum bicolor* (L.) Moench) yielded 111 kg/ha more than soybean following fallow and 600 kg/ha more than continuous soybean (Weaver et al., 1995).

American jointvetch (*Aeschynomene americana*), bahiagrass, cotton (*Gossypium hirsutum*), sorghum, hairy indigo (*Indigofera hirsuta*), velvetbean (*Mucuna pruriens*), and wheat used as rotation crops or as winter/summer cover crops decreased *H. glycines* population densities and in most cases increased soybean yield (Dabney et al., 1988; Dillon et al., 1997; Kabana et al., 1989; Rodriguez- and 1990; Weaver et al., 1993). In Japan, Nishizawa (1978) reported that millet (*Pennisetum glaucum*), rape (*Brassica napus*), and potato (*Solanum tuberosum*) were also effective in lowering the population density of *H. glycines*.

Nematicides have been tried for SCN control, but they are not commonly used in US soybean production areas because of cost, application difficulty and inconsistency in control. One example where nematicides provided control was reported by Wrather and Anand (1988). They found that infection of the cultivar Essex by *H. glycines* in the field was delayed 2-6 weeks, and yields were significantly increased by 29, 13, and 23 % when seed was treated with the nematicides Aldicarb, Carbofuran, and EDB, respectively.

Literature Cited

Abawi, G. S., and Jacobsen, B. J. 1984. Effect of initial inoculum densities of *Heterodera glycines* on growth of soybean and kidney bean and their efficiency as hosts under greenhouse conditions. Phytopathology 74:1470-1474.

Ali, A. H. H. 1988. Influence of some physical factors affecting emergence of juveniles from cysts of soybean cyst nematode, *Heterodera glycines*. Egyp. J. Phytopathol. 20: 159–164.

Alston, D. G. and Schmitt, D. P. 1988. Development of *Heterodera glycines* life stages as influenced by temperature. J. Nematol. 20:366-372.

Anand, S. C., Matson, K. W. and Sharma., S. B. 1995. Effect of soil temperature and pH on resistance of soybean to *Heterodera glycines*. J. Nematol. 27:478-482.

Andrade, P. J. M, and Asmus, G. L. 1997. Spread of soybean cyst nematode (*Heterodera glycines*) by wind during soil preparation. Nematologia Brasileira 21:98–100.

Avendaño, F., Pierce, F. J., Schabenberger, O., and Melakeberhan, H. 2004. The spatial distribution of soybean cyst nematode in relation to soil texture and soil map unit. Agron. J. 96:181-194.

Baldwin, J. G., and Mundo-Ocampo, M. 1991. Heteroderinae, Cyct- and Non-Cyst-Forming Nematodes. In: Manual of Agricultural Nematology (Ed. Nickle, W.), pp. 275-362. Marcel Dekker, Inc. New York-Basel-Hong Kong.

Becker, W. F., and Ferraz, S. 2004. Efeito da concentração inicial de inóculo do nematóide *Heterodera glycines* no desenvolvimento do feijoeiro comum. Revista Agropecuaria Catarinense, Florianópolis 17:89-93.

Bekal S., Niblack, T. L., and Lambert, K. N. 2003. A chorismate mutase from the soybean cyst nematode *Heterodera glycines* shows polymorphisms that correlate with virulence. Mol. Plant-Microbe Interact. 16:439-46.

Besal, E. A., Powers, T. O., Radice, A. D., and Sandall, L. J. 1988. A DNA hybridization probe for detection of soybean cyst nematode. Phytopatology 78:1136-1139.

Bird, A. F., Downton, W. S. J., and Hawker, J. S. 1974. Cellulase secretion by second stage larvae of the root-knot nematode (*Meloidogyne javanica*). Marcellia 38:165-69.

Bridge, J., and Starr, J. L. 2007. Plant Nematodes of Agricultural Importance. A Color Handbook. Academic Press. San Diego, CA, USA.

Brim, C. A., and Ross, J. P. 1966. Relative resistance of 'Pickett' soybean to various strains. Phytopathology. 56:451-454.

Brucker, E., Carlson, S., Wright, E., Niblack, T. L., and Diers, B. 2005. *Rhg1* alleles from soybean PI 437654 and PI 88788 respond differentially to isolates of *Heterodera glycines* in the greenhouse**. Theor. Appl. Genet. 111:44-49.

Burrows, P. R., and Stone, A. R. 1985. *Heterodera glycines*. CIH Descriptions of Plant-Parasitic Nematodes, no. 118. CAB International, Wallingford (GB).

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

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

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

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

Concibido, V. C., Lange, D. A., Denny, R. L., Orf, J. H., and Young, N. D. 1977. Genome mapping of soybean cyst nematode resistance genes in Peking, PI 90763, and PI 88788 using DNA markers. Crop Sci. 37:258-264.

Concibido, V. C., Denny, R. L. Lange, D. A., Danesh, D. Orf, J. H., and Young, N. D. 1995. The soybean cyst nematode resistance gene on linkage group G is common among sources of resistance. Soybean Genet. Newsl. 22:269-272.

Dabney, S. M., McGawley, E. C., Boethel, D. J., and Berger, D. A. 1988. Short-term crop rotation systems for soybean production. Agronomy J. 80:197-204.

Davis, E. L., Hussey, R. S., Mitchum, M. G., and Baum, T. J. 2008. Parasitism proteins in nematode-plant interactions. Curr. Opi. Plant Biol. 11:360-366.

de Boer, J. M., McDermott, J. P., Davis, E. L., Hussey, R. S., and Popeijus, H., Smant, G., and Baum, T. J. 2002. Cloning of a putative pectate lyase gene expressed in the subventral esophageal glands of *Heterodera glycines*. J. Nematol. 34:9-11.

de Boer, J. M., Yan, Y. T., Wang, X. H., Smant, G., Hussey, R. S, Davis., E. L., and Baum, T. J. 1999. Developmental expression of secretory beta-1,4-endoglucanases in the subventral esophageal glands of *Heterodera glycines*. Mol. Plant-Microbe Interact. 12:663-69.

Diers, B. W., Skorupska, H. T., Arelli, P. R., and Cianzio, S. R. 1997. Genetic relationships among soybean plant introductions with resistance to soybean cyst nematodes. Crop Sci. 37:1966-1972.

Dillon, C. R., Keisling, T. C., Riggs, R. D., and Oliver, L. R. 1997. The profit potential of soybean production rotation systems in Arkansas. Comm. Soil Sci. Plant Analyses 28:1693-1709.

Donald, P. A., Pierson, P. E., St.Martin, S. K., Sellers, P. R., Noel, G. R., Macguidwin, A. E., Faghihi, J., Ferris, V. R., Grau, C. R., Jardine, D. J., Melakerberhan, H., Niblack, T. L., Stienstra, W. C., Tylka, G. L., Wheeler, T. A., and Wysong, D. S. 2006. Assessing *Heterodera glycines*-resistant and susceptible cultivar yield response. J. Nematol. 38:76-82.

Donald, P. A. 2001. Mapping soybean cyst nematode field distribution. Phytopathology 91:S133.

Dropkin, V. H., Baldwin, C. H., Gaither, T., and Nace, W. 1976. Growth of *Heterodera glycines* on soybeans in the field. Plant Dis. Rep. 60:977-980.

Endo, B. Y. 1992. Cellular responses to infection. Pages 37-49 in: In Biology and Management of the Soybean Cyst Nematode. Edited by Riggs R. D, and J. A. Wrather. St Paul, MN. APS Press.

Epps, J. M., 1956. Soybean cyst nematode found in Tennessee. Plant Dis. Rep. 42:594-595.

Epps, J. M. 1971. Recovery of soybean cyst nematode *Heterodera glycines* from digestive tract of birds. J. Nematol. 3:417-419.

Francl, L. J. 1986. Spatial analyses of *Heterodera glycines* populations in field plots. J. Nematol. 18:183-189.

Francl, L. J. 1993. Multivariate analyses of selected edaphic factors and their relationship to *Heterodera glycines* population density. J. Nematol 25:270-276.

Fujita K., and Miura, O. 1934. On the parasitism of *Heterodera schachtii* Schmidt on beans. Trans. Sapporo Nat. Hist. Soc. 13:359-364.

Gao, B. L., Allen, R., Maier, T., Davis, E. L., Baum, T. J., and Hussey, R. S. 2003. The parasitome of the phytonematode *Heterodera glycines*. Mol. Plant-Microbe Interac. 16:720-726.

Gao, B., Allen, R., Maier, T., Davis, E. L., Baum, T. J., and Hussey, R. S. 2002. Identification of a new beta-1,4-endoglucanase gene expressed in the esophageal subventral gland cells of *Heterodera glycines*. J. Nematol. 34:12-15.

Gavassoni, W. L., Tylka, G. L., and Munkvold, G. P. 2007. Effects of tillage practices on dissemination and spatial patterns of *Heterodera glycines* and soybean yield. Plant Dis. 91:973-978.

Glover, K. D., Wang, D., Arelli, P. R., Carlson, S. R., Cianzio, S. R., and Diers, B. W. 2004. Near Isogenic Lines Confirm a Soybean Cyst Nematode Resistance Gene from PI 88788 on Linkage Group J. Crop Sci. 44:936-941.

Goellner, M., Wang, X. H., and Davis, E. L. 2001. Endo-beta-1,4-glucanase expression in compatible plant-nematode interactions. Plant Cell. 13:2241-55.

Golden, A. M., Epps, J. M., Riggs, R. D., Duclos, J., Fox, A., and Bernard, R. L. 1970. Terminology and identity of intraspecific forms of the soybean cyst nematode (*Heterodera glycines*). Plant Dis. Rep. 54:544-46.

Graney, L. S. O., and Miller, L. I. 1982. Comparative morphological studies of *Heterodera schachtii* and *H. glycines*. In: *Nematology in the Southern Region of the United States* (Ed. Riggs, R. D.), pp. 96-107. Southern Cooperative Series Research Bulletin *No. 276.*

Heatherly, L. G., and Young, L. D. 1991. Soybean and soybean cyst nematode response to soil water content in loam and clay soils. Crop Sci. 31:191-196.

Hill, N. S., and Schmitt, D. P. 1989. Influence of temperature and soybean phenology on dormancy induction of *Heterodera glycines*. J. Nematol. 21:361-369.

Ichinohe, M. 1952. On the soy bean nematode, *Heterodera glycines* n. sp., from Japan. Mag. Appl. Zool. 17:1-4.

Inagaki, H., and Tsutsumi, M. 1971. Survival of the soybean cyst nematode, *Heterodera glycines* under certain storing conditions. Appl. Entomology and Zoology 6:156162.

Ithal, N., Recknor, J., Nettleton, D., Hearne, L., Maier, T., Baum, T. J., Mitchum, M. G. 2007. Parallel genome-wide expression profiling of host and pathogen during soybean cyst nematode infection of soybean. Mol. Plant Microbe Interac. 20:293-305.

Jenkins, W. R., and Taylor,. D. P. 1967. Plant nematology. Reinhold, New York.

Johnson, A. B. 1993. Penetration of soybean roots by soybean cyst nematode at high soil water potentials. Agron. J. 85:416-419.

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

Jones, F. G. W. 1969. The influence of soil structure and moisture on nematodes, especially *Xiphinema, Longidorus, Trichodorus* and *Heterodera* spp. Soil Biol. Biochem. 1:153–165

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

Koenning, S. R., and Sipes, B. S. 1998. Biology. Pages 156-190 in: The cyst nematodes. S. B. Sharma (ed.). Chapman and Hall, London, UK.

Koenning, S. R., and Barker, K. R., 1995. Soybean photosynthesis and yield as influenced by *Heterodera glycines*, soil type and irrigation. J. Nematol. 27:51-62.

Kulkarni, S. S., Bajwa, S.G., Rupe, J.C., and Kirkpatrick, T. L. 2008. Spatial correlation of crop to Soybean cyst nematode (*Heterodera glycines*). Transac. ASABE. 51:1451-1459.

Lambert, K. N., Bekal, S., Domier, L. L., Niblack, T. L., Noel, G. R., and Smyth, C. A. 2005. Selection of *Heterodera glycines* chorismate mutase-1 alleles on nematode-resistant soybean. Mol. Plant-Microbe Interact. 18:593-601.

Lax, P., and Doucet, M. E. 2001a. *Heterodera glycines* Ichinohe, 1952 (Nematoda: Tylenchida) from Argentina. 1. Morphological and morphometrical characterisation of second-stage juveniles. Nematol. 3:165-170.

Lax, P., and Doucet, M. E. 2001b. *Heterodera glycines* Ichinohe, 1952 (Nematoda: Tylenchida) from Argentina. 2. Morphological and morphometrical characterisation of males. Nematol. 3:543-549.

Lax, P., and Doucet, M. E. 2001c. *Heterodera glycines* Ichinohe, 1952 (Nematoda: Tylenchida) from Argentina. 3. Morphological and morphometrical characterisation of females. Nematol. 3:699-704.

Lax, P., and Doucet, M. E. 2002. *Heterodera glycines* Ichinohe, 1952 (Nematoda: Tylenchida) from Argentina. 4. Morphological and morphometrical characterisation of cysts. Nematol. 4:783-789.

Lehman, P. S. 1994. Dissemination of phytoparasitic nematodes and the role of regulatory agencies in preventing their dispersal. Pages 1-8 in: XVIII Congresso Brasileiro de Nematologia Resumos, Campinas, S.P. 33 pp.

Magenti, A. R. 1991. Nemata: Higher Classification. In: Manual of Agricultural Nematology (Ed. Nickle, W.), pp. 147-187. Marcel Dekker, Inc. New York-Basel-Hong Kong.

Matson, A. L., Williams, L. F. 1965. Evidence of a fourth gene for resistance to the soybean cyst nematode. Crop Sci. 5:477.

Melito, S., Heuberger, A. L., Cook, D., Diers, B. W., MacGuidwin, A. E., and Bent, A. 2010. A nematode demographics assay in transgenic roots reveals no significant impacts of the *Rhg 1* locus LRR-Kinase on soybean cyst nematode resistance. BMC. Plant Biol. 10:104-117.

Melton, T. A., Jacobsen, B. J., and Noel, G. R. 1986. Effects of temperature on development of *Heterodera glycines* on *Glycine max* and *Phaseolus vulgaris*. J. Nematol. 18:468-474.

Melton, T. A., Noel, G. R., Jacobsen, B. J., and Hagedorn, D. J. 1985. Comparative host suitability of snap beans to the soybean cyst nematode (*Heterodera glycines*). Plant Dis. 69:119-122.

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

Niblack, T. L., and Karr, A. L. 1994. Source of antimicrobial activity in the gelatinous matrix of *Heterodera glycines*. J. Nematol. 26:561. (Abstr.)

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

Niblack, T. L., Lambert, K. N., Tylka, G. L. 2006. A model plant pathogen from the Kingdom Animalia: *Heterodera glycines*, the soybean cyst nematode. Ann. Rev. Phytopathology 44:283-303.

Nishizawa, T. 1978. Annual population changes of soil nematodes in the field of continuous cropping or rotation. Kasetsart J. 12:3-13.

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

Perry, R. N., and Clarke, A. J. 1981. Hatching mechanisms of nematodes. Parasitology 83:435-449.

Rajan and Lal, A. 2005. Phytosanitary importance of *Heterodera glycines* for South-east Asian countries. OEPP/EPPO, Bulletin OEPP/EPPO Bulletin 35:531-536.

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

Rao-Arelli, A. P., Wrather, J. A., and Anand, S. C. 1992. Genetic diversity among isolates of *Heterodera glycines* and sources of resistance in soybeans. Plant Dis. 76:894-896.

Riggs, R. D. 1982. Cyst nematodes in the Southern USA. Pages 77-95 in: Nematology in Southern Region of the United States. Riggs R. D. (Ed) Southern Cooperative Series Research Bulletin No. 276.

Riggs, R. D. 1992. Host Range. Pages 107–114 in R. D. Riggs and J. A. Wrather, eds. Biology and Management of the Soybean Cyst Nematode. St. Paul, MN: American Phytopathological Society.

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

Riggs R. D., Wrather J. A., eds. 1992. Biology and management of the soybean cyst nematode. American Phytopathological Press: St Paul, USA.

Riggs, R. D., and Hamblen, M. L. 1962. Soybean cyst nematode studies in the family Leguminosae. Ark. Agr.. Expt. Sta. Rep. Series 110:1-8.

Riggs, R. D., and Hamblen, M. L. 1966. Further studies on the host range of the soybean cyst nematode. Ark. Agr.. Expt. Sta. Series 110:2-19.

Rodriguez-Kabana, R., Weaver, D. B., Garcia, R., Robertson, D. G., and Carden, E. L. 1989. Bahiagrass for the management of root-knot and cyst nematodes in soybean. Nematropica 19:185-193.

Rodriguez-Kabana, R., Weaver, D. B., Robertson, D. G., King, P. S., and Carden, E. L. 1990. Sorghum in rotation with soybean for the management of cyst and root-knot nematodes. Nematropica 20:111-119.

Ross, J. P. 1962a. Physiological strains of *Heterodera glycines*. Plant Dis. Rep. 46:766-69.

Ross, J. P. 1962b. Crop rotation effects on the soybean cyst nematode population and soybean yields. Phytopathology. 52:815-818.

Sasser, J. N., and Uzzell, Jr., G. 1963. Influence of nonhost crops alone and in combination with a nematicide on the longevity of the soybean cyst nematode in soil. Phytopathology 53:625 (Abstr.).

Schmitt, D. P., and Shannon, G. 1992. Differentiating Soybean Responses to *Heterodera glycines* Races**.** Crop Sci. 32:275-277.

Slack, D. A., and Hamblen, M. L. 1961. The effect of various factors on larvae emergence from cysts of *Heterodera glycines*. Phytopathology. 51:350–355.

Slack, D. A., Riggs, R. D., and Hamblen, M. L. 1972. The effect of various factors and moisture on the survival of *Heterodera glycines* in the absence of a host. J. Nematol. 4:263-266.

Smant, G., Stokkermans, J. P. W. G., Yan, Y. T., Deboer, J. M., Baum, T. J., Wang, X., Hussey, R. S., Gommers, F. J. et al.,. 1998. Endogenous cellulases in animals: isolation of beta-1,4-endoglucanase genes from two species of plant-parasitic cyst nematodes. Proc. Nat. Acad. Sci. USA 95:4906-11.

Smith, J. R. and Young, L. D. 2003. Host suitability of diverse lines of *Phaseolus vulgaris* to multiple populations of *Heterodera glycines*. J. Nematol. 35:23-28.

Sortland, M. E, and MacDonald, D. H. 1986. Development of a population of *Heterodera glycines* race 5 at four soil temperatures in Minnesota. Plant Dis. 70:932-935.

Subbotin, S. A.,Waeyenberge, L., and Moens, M. 2000. Identification of cyst forming nematode of the genus *Heterodera* (Nematoda: Heteroderidae) based on the ribosomal DNA-RFLPs. Nematology 2:153–164.

Taylor, A. L. 1975. Identification of soybean cyst nematodes for regulatory purposes. Proce. Soil and Crop Sci. Soc. Florida 34:200-206.

Triantaphyllou, A. C., and Hirshmann, K. 1962. Oogenesis and mode of reproduction in the soybean cyst nematode, *Heterodera glycines*. Nematologica 7:235.

Wang, X. H., Meyers D., Yan Y. T., Baum, T., and Smant, G. 1999. In planta localization of a beta-1,4-endoglucanase secreted by *Heterodera glycines*. Mol. Plant-Microbe Interact. 12:64-67.

Weaver, D. B., Rodriguez-Kabana, R., and Carden., E. L. 1993. Velvetbean in rotation with soybean for management *of Heterodera glycines* and *Meloidogyne incognita.* Suppl. J. Nematol. 25:809-813.

Weaver, D. B., Rodriguez-Kaibana, R., and Carden, E. L. 1995 Comparison of crop rotation and fallow for management of *Heterodera glycines* and *Meloidogyne* spp. in soybean. Suppl. J. Nematol. 27:585-591.

Weaver, D. B., Rodriguez-Katbana, R., Robertson, D. G. Akridge, R. L., and Carden., E. L. 1988. Effect of crop rotation on soybean in a field infested with *Meloidogyne arenaria* and *Heterodera glycines.* Suppl. J. Nematol. 20:106-109.

Wheeler, T. A., Pierson, P. E., Young, C. E., Riedel, R. M., Wilson, H. R., Eisley, J. B., Schmitthenner, A. F., and Lipps, P. E. 1997. Effect of soybean cyst nematode (*Heterodera glycines*) on yield of resistant and susceptible soybean cultivars grown in Ohio. Suppl. J. Nematol. 29:703-709.

Workneh, F., Yang, X. B., and Tylka, G. L. 1999. Soybean brown stem rot, *Phytophthora sojae,* and *Heterodera glycines* by soil texture and tillage relations. Phytopathology 89:844–850.

Wrather, J. A., and Anand, S. C. 1988. Relationship between time of infection with *Heterodera glycines* and soybean field. J. Nematol. 20:439-442.

Wrather, J. A., S. C. Anand, and V. H. Dropkin. 1984. Soybean cyst nematode control. Plant Dis. 68:829-833.

Yan, Y. T., Smant, G., and Davis, E. 2001. Functional screening yields a new beta-1,4-endoglucanase gene from *Heterodera glycines* that may be the product of recent gene duplication. Mol. Plant-Microbe Interact. 14:63-71.

Yan, Y. T., Smant, G., Stokkermans, J., Qin, L., Helder, J., Baum, T., Schots, A., and Davis, E. 1998. Genomic organization of four beta-1,4-endoglucanase genes in plant-parasitic cyst nematodes and its evolutionary implications. Gene 220:61-70.

Young, L. D. 1987. Effects of soil disturbance on reproduction of *Heterodera glycines*. J. Nematol. 19:141-142

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

Young, L. D., and Heatherly., L. G. 1990. *Heterodera glycines* invasion and reproduction on soybean grown in clay 41 and silt loam soils. J. Nematol. 22:618-619.

Zheng, J., Subbotin, S. A., Waeyrnberge, L., and Moens, M. 2000. Molecular characterization of Chinese *Heterodera glycines* and *H. avenae* populations based on RFLPs and sequences of rDNA-ITS regions. Russian J. Nematol. 8:109-113.

CHAPTER 3. EVALUATION OF NORTHERN-GROWN CROPS AS HOSTS OF SOYBEAN CYST NEMATODE[1]

Abstract

Sixty two cultivars/varieties of thirteen crops grown in the northern Great Plains were evaluated for suitability as hosts of the soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe) (HG type 0) using soybean Lee 74 as the susceptible host. 'Cone-tainers' with autoclaved sand were infested with 2,000 eggs placed into a 2 cm x 1 cm hole and then a 3 day-old germinated seed was placed in the hole. 'Cone-tainers' were placed in sand in plastic pots immersed in a water bath at 27° C in the greenhouse. Plants were harvested after 30 days, and females were extracted and counted. A female index (FI = [the average number of females on the test plant divided by the average number of females on soybean Lee 74] times 100) was calculated for each cultivar to assess susceptibility to the nematode. Canola, clover, lentil, and sunflower were nonhosts (no evidence of reproduction), while borage, camelina, chickpea, crambe, cuphea, field pea, nyjer, and safflower were poor hosts for SCN with FI's less than 8. Lupines were the only susceptible host with FI's of 42 to 57. This is the first report of reproduction of SCN on chickpea, crambe, cuphea and nyjer.

Introduction

North Dakota and northern Minnesota have diverse cropping systems that differ from the corn-soybean system common in much of the North Central United States. In addition to the traditional planting of small grains, potato, sugar beet and dry bean, other crops such as soybean, canola, field pea, sunflower and lentil have been integrated into the

cropping systems in the past 30 years, and there has been a dramatic increase in hectares of those crops. North Dakota, for example, was ranked number one in production of canola (*Brassica napus* L.), field pea (*Pisum sativum* L.*),* sunflower (*Helianthus annuus* L.), and lentil (*Lens culinaris* Medik.) in the United States in 2008 with values of 250, 112, 307, and 29 million dollars, respectively (NASS, 2008). In addition, specialty crops being grown commercially or under investigation for production include borage (*Borago officinalis* L.), clover (*Trifolium* sp. and *Melilotus* sp.), camelina (*Camelina microcarpa* Andrz. ex DC.), chickpea (*Cicer arietinum* L.), crambe (*Crambe maritima* L.), cuphea (*Cuphea viscosissima* Jacq.), lupines (*Lupines albus* L.), nyjer (*Guizotia abyssinica* (L.f.) Cass), and safflower (*Carthamus tinctorius* L.).

Soybean is the principal oilseed crop in North Dakota and northern Minnesota with over 1.6 million hectares in the area. Soybean is now grown throughout the eastern half of North Dakota and throughout much of the northern part of Minnesota in the Red River Valley and areas bordering the Valley. Soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe) is a major pathogen of soybean that occurs in most soybean production areas in the United States (Niblack, 2005; Wrather and Koenning, 2006). SCN was first reported in North Dakota in 2003 (Bradley et al., 2004) and the nematode is currently found in 12 counties in eastern North Dakota (S. Markell, unpublished). SCN is also found in several Minnesota counties directly to the east of the infested counties in North Dakota. To date, only HG type 0 (Niblack et al., 2002) has been identified in infested fields in North Dakota (Bradley et al., 2004). SCN will continue to expand northward into counties where the majority of the aforementioned crops are grown.

Management of SCN includes crop rotation with nonhosts to help reduce egg population densities (Niblack, 2005). The suitability of potential rotation crops as hosts for SCN, therefore, is important to understand. Furthermore, it is necessary to know which crops might be adversely affected by SCN as there are susceptible hosts other than soybean (Poromarto and Nelson, 2009; Riggs, 1992). Not all the crops grown in North Dakota and northern Minnesota have been extensively evaluated as hosts for SCN. For example, there were no reports we could find indicating the host suitability of camelina, crambe, cuphea, or safflower to SCN.

The extensive host range studies of Riggs and Hamblen (1962, 1966) indicated an important consideration when examining a species for reproduction of SCN: within a species, cultivar/variety can be an important factor in determining the amount of reproduction on the roots. For example, Riggs and Hamblen (1966) evaluated numerous plant introductions of pea and found types that were immune, resistant and susceptible. Poromarto and Nelson (2009) tested 24 cultivars of dry bean and found a range of reaction from resistant to highly susceptible cultivars. Numerous published studies however, examining reproduction of SCN on different species of plants used only one or a few cultivars/varieties of the crops tested (Fujita and Miura, 1934; Kushida et al., 2002; Schmitt and Riggs, 1991; Sortland and Mac Donald, 1987; Venkatesh et al., 2000; Warnke et al., 2008).

The objectives of this study were to: 1) evaluate reproduction of SCN on the roots of various crop cultivars commercially grown or being tested in the North Dakota and northern Minnesota area; and 2) if new susceptible hosts were identified, to assess the ability of eggs produced on those hosts to hatch and reproduce on susceptible soybean.

31

Material and Methods

Crop genotype and planting

Sixty two cultivars/varities from thirteen crops were tested for suitability as hosts

for SCN in the greenhouse (Table 3.1). The crops were: borage, camelina, canola,

chickpea, clover (*T. hybridum* L., T. *pratense* L., *T. repens* L. and *M. officinalis* L.),

crambe, cuphea, field pea, lentil, lupines, nyjer, safflower and sunflower. The soybean

cultivar Lee 74 was used as a susceptible control in all tests (Niblack et al., 2002). Each

crop was evaluated in separate experiments.

Seeds were surface disinfested with 1.0% NaOCl for one minute, rinsed with water

and germinated on seed germination paper (Anchor Paper, St. Paul, MN) for three days.

Healthy seedlings of uniform size were transplanted into a hole (2 cm in depth and 1 cm in

diameter) in autoclaved river sand in individual plastic "Cone-tainers" Type SC10 Super

Cell (3.8 cm dia; 21 cm depth; volume 164 ml; Stuewe & Sons, Inc., Corvallis, OR, USA).

"Cone-tainers" were placed in autoclaved sand in 30.5 cm dia x 30.5 cm depth plastic pots

(Cambro, Huntington Beach, CA) immersed in a water bath at 27 ± 3ºC in the greenhouse.

Plants were grown for 30 days under natural and supplemental light using high pressure

sodium lamps (1,000 $\mu E^. m^{-2.}s^{-1}$) for 16 h/day. Plants were watered daily as needed to

maintain the sand at field capacity. At 14 and 21 days after planting, plants were fertilized

with three ml of a solution of Peters Hydro-Sol 5-11-26 (W.R. Grace & Co.-Conn.,

Fogelsville, PA; at the rate of 20 ml of Peters in 980 ml of water).

SCN source, inoculation, and evaluation

Soil naturally infested with SCN was collected from a soybean field in Richland

Co., North Dakota. The population of SCN was identified as HG type 0 following the

methods of Niblack et al. (2002). The indicator lines and female index (FI) for the HG type classification were as follows: PI 548402, FI 0.1; PI 88788, FI 0.6; PI 90763, FI 0.1; PI 437654, FI 0.2; PI 209332, FI 0.1; PI89772, FI 0.1; PI548316, FI 6.5; Lee 74 FI 100 (Average female number/plant of Lee 74 = 570).

The general methods of Niblack et al. (2002) to inoculate plants were followed with some modifications. The egg source was directly from Lee 74 soybean inoculated with eggs from the soil and grown in the greenhouse under the same conditions as previously described. Cysts were extracted from soil or roots with an 18-mesh (1 mm) sieve (VWR Scientific, West Chester, PA) nested over a 60-mesh (250 µm) sieve. Cysts were crushed with a Wheaten Potter Elvehjen Tissue Grinder (55 ml capacity) (VWR Scientific, West Chester, PA) and eggs were collected on a 200-mesh (75 µm) nested over a 500-mesh (25 µm) sieve. A suspension of eggs in distilled water was prepared and adjusted to 1,000 eggs/ml. As the seedling was transplanted into the sand, a suspension of 2,000 eggs was placed in the planting hole and the seedling was covered with sand. Watchdog 450 Data loggers with soil temperature sensors (Spectrum Technologies, Inc., Plainfield, IL) were used to monitor the temperature of the sand in the "Cone-tainers". Temperatures in the sand among experiments averaged 27± 1º C, but temperature variations of 3 to 4º C were recorded almost daily.

SCN females were collected from the roots of individual 30-day-old plants. Plants were extracted from the "Cone-tainers" and the root-sand masses were soaked in water. The females were washed off the roots and sieved from the water/sand mix of the root soakings using the previously described sieves. Females from each plant were counted

with a dissecting microscope. Roots were also examined with the dissecting microscope to insure mature females were removed.

Infecting soybean with eggs from crambe and purple borage

Eggs of SCN produced on crambe and purple borage were inoculated onto soybean to determine if they would result in the same number of females per plant as eggs produced on soybean. Females produced on soybean Lee 74, the five crambe cultivars and purple borage were collected and the eggs extracted as previously described. Lee 74 plants were then immediately inoculated with 2,000 eggs per plant from each of the five crambe cultivars, purple borage and Lee 74 and grown as previously described. After 30 days, females on Lee 74 were extracted and counted. In addition, the average number of eggs per female produced on the crambe cultivars, purple borage and Lee 74 was determined. Females from each plant were crushed and the eggs collected as previously described. Egg numbers were counted with an American Optical One-Ten microscope (Buffalo, NY) and the average number of eggs per female was calculated.

Experimental design and analyses

The data from the reproduction on the various crops were not analyzed for most crops since the primary purpose was only to detect reproduction and in most cases there was no or little reproduction. However, a female index (FI) (12) (FI = [the average number of females on the test plant divided by the average number of females on the susceptible soybean Lee 74] times 100) was calculated to assess susceptibility.

For all experiments, the design was a randomized complete block with 4 replications (one plant per replication) and all experiments were repeated once. In the experiments with crambe and those infecting soybean with eggs from crambe and purple

borage, the data from individual experiments were analyzed separately by analyses of variance (ANOVA) with SAS (SAS Institute, Cary, NC) and variances were compared between repeated experiments. The data were then combined over experiments and analyzed by ANOVA. Least significant differences (Fisher's protected F test, α=0.05) were calculated following significant ($P \leq 0.05$) F tests.

Results and Discussion

With soybean, an FI of greater than 10 is considered a suitable host (Niblack et al., 2002), therefore that standard was used in this research to separate a poor host from a susceptible host. Canola, the four clovers, lentil, and sunflower were nonhosts of SCN in this study since there was no evidence of reproduction on roots in experiments where the susceptible soybean had high numbers of females (Table 3.1). Field pea cultivars could also be considered a non-host since only one cultivar in one experiment had an average of 1 female on the roots. Borage, camelina, chickpea, crambe, cuphea, nyjer and safflower were poor hosts for SCN with FI's less than 8 (Table 3.1). This is the first report we are aware of where chickpea, crambe, nyjer, and safflower were inoculated with eggs of SCN. White lupine was the only crop tested that was a susceptible host with an FI >10.

Canola, oilseed rape and sunflower were previously tested as hosts of SCN. Venkatesh et al. (2000) tested one canola cultivar against three races of SCN by direct inoculation and found no reproduction. Warnke et al. (2008) evaluated one cultivar of oilseed rape and found that penetration of roots by J2 (second stage juveniles) occurred but there was no development of mature females. They also reported that the roots of rape stimulated egg hatch. Sortland and MacDonald (1987) directly inoculated a sunflower cultivar with SCN race 5 and reported no females were formed. Both canola and sunflower

were evaluated in greenhouse and field tests as rotation crops for SCN management and there was no evidence that populations of SCN were increased by these crops (Miller et al., 2006; Sortland and Mac Donald, 1987; Warnke et al., 2006). Miller et al., 2006) in field studies at three locations in Minnesota reported those two crops reduced SCN egg population density during the season compared with a susceptible soybean. However, Jackson et al. (2005) in Missouri reported that canola increased the number of SCN eggs when used as a rotation crop in one year but not a second year of a field study. Wong and Tylka (1994) reported that wild sunflower was a nonhost of SCN and reduced SCN egg numbers in infested soil in greenhouse and field experiments.

Clover has been tested for SCN host suitability in numerous studies. Riggs and Hamblen (1966) reported that red and white clovers were nonhosts, but a few females developed on several sweet clover plant introductions. No females were observed on sweet clover or the other three clover species in our study. Although the majority of clover species in *Melilotus* and *Trifolium* are nonhosts or poor hosts (Riggs and Hamblen, 1962; Riggs and Hamblen, 1966), Riggs and Hamblen (1966) reported there were plant introductions of at least seven clover species within those genera that were susceptible to SCN with an FI greater than 10. Riggs (1987) reported that the white clover cultivar Ladino and other species of clover such as Rose clover (*Trifolium hirtum* All.) were penetrated by J2, but there was no further development of the nematode. In contrast, other species of clover, such as *Trifolium vesiculosum* Savi and Crimson clover, *T. incarnatum* L., were not penetrated by J2 (Riggs, 1987). Red clover is penetrated by J2, but the nematode does not mature into adults (Kushida et al., 2002; Schmitt and Riggs, 1991). Red clover appears to have various effects on SCN from stimulating hatching of eggs (Aiba and Mitsui, 1985;

Jackson et al., 2005; Kushida et al., 2002; Riga et al., 2001; Schmitt and Rigs, 1991) to reducing population densities in soil (Jackson et al., 2005; Kushida et al., 2002; Miller et al., 2006).

The genus *Lens,* which includes lentils, has not been adequately tested as a host for SCN. Riggs and Hamblen (1966) evaluated one plant introduction of lentil and reported no reproduction of SCN. That is the only previous report on lentil. Chickpea also has not previously been evaluated as a host, but our study indicates it is a poor host. Although all six cultivars of lentil were nonhosts in our study and a few females developed on chickpea, it is noteworthy to mention that lentil and chickpea are hosts of *H. ciceri* which occurs in the Mediterranean Basin (Castillo et al., 2008; De Waele and Elson, 2007).

In most studies where pea was evaluated as a host, it has been primarily with one or two cultivars and there was no evidence that those cultivars were susceptible to SCN (Fujita and Miura, 1934, Miller et al., 2006; Riggs, R. D., 1987; Sortland and Mac Donald, 1987; Warnke et al., 2006). However, Riggs and Hamblen (1962) in their extensive study of hosts in the Leguminosae showed that out of 199 plant introductions of *P. sativum*, five were rated as susceptible to SCN, 132 allowed some reproduction and the rest were immune. Riggs (1987) demonstrated SCN J2 will not penetrate some pea cultivars while penetration will occur in others, but the nematode will not continue development. In our study, six pea cultivars were evaluated and SCN only formed mature females on one, Majorete, but the number of females was less than 1% of those on the susceptible soybean Lee 74.

Borage was reported as a susceptible host (FI 19) by Riggs and Hamblen (1966) but only one type was evaluated. The average FI's for purple and white borage in our study

were only 5 and 0.5, respectively. Apparently, within the species there are resistant and susceptible borage types. Since there was limited information on borage as a host of SCN and there was a report that some types are susceptible hosts, the effectiveness of eggs produced on purple borage as inoculum for soybean was investigated. The number of females on the Lee 74 plants that were inoculated with eggs produced on purple borage plants was significantly lower ($P > 0.05$) than the number of females on Lee 74 plants inoculated with eggs produced on Lee 74 (Table 3.2). The mean number of SCN females per Lee 74 plant was 211 and 367 when eggs originated from purple borage and Lee 74, respectively. Apparently J2 from females produced on purple borage are less capable of infecting soybean or the eggs have reduced hatching compared to eggs formed on soybean. The average number of eggs per female produced on purple borage was not significantly ($P < 0.05$) different from the number produced on Lee 74 (Table 3.2).

All six cultivars of camelina and the three varieties of nyjer were poor hosts for SCN with FI's averaging less than 1. One cuphea type and three cultivars of safflower were evaluated and they were also poor hosts, with FI's < 2.We found no previous reports of the direct inoculation of camelina, cuphea, nyjer or safflower with eggs of SCN. Warnke et al. (2006), however, grew camelina in SCN-infested soil for evaluation as a rotation crop in a greenhouse study and there was no evidence of an increase in egg population density, indicating there was no reproduction. Safflower is reported to contain nematicidal polyacetylenes (Kogiso et al., 1976).

All white lupine cultivars were highly susceptible, with FI's ranging from 42 to 57. Although lupines have been reported as a host for SCN (Riggs and Hamblen, 1966), the cultivars tested here had not previously been evaluated. Riggs and Hamblen (1966)

reported that from 38 species or cultivars of *Lupinus*, 17 were immune, 13 were resistant,

and 8 were susceptible. Riggs (1987) reported that several white lupine cultivars were

susceptible with FI's greater than 10.

Table 3.1. Reproduction of soybean cyst nematode on crops grown in North Dakota and
northern Minnesota[a].

Crops/Cultivars	Experiment 1	Experiment 2	Average
Borage (*Borago officinalis* L.)			
1. Purple Borage	36/552	25/658	31/605
2. White Borage	0/552	5/658	3/605
Camelina (*Camelina microcarpa* Andrz. ex DC.)			
1. Blaine Creek	1/438	2/515	2/477
2. Boha	1/438	1/515	1/477
3. Calena	1/438	3/515	2/477
4. Celine	2/438	2/515	2/477
5. Ligena	1/438	1/515	1/477
6. Suneson	3/438	5/515	4/477
Canola (*Brassica napus* L.)			
1. Crusher	0/327	0/791	0/559
2. Gladiator	0/327	0/791	0/559
3. Hudson	0/327	0/791	0/559
4. HyClass 601	0/327	0/791	0/559
5. Hylite	0/327	0/791	0/559
6. Marksman	0/327	0/791	0/559
7. Patriot	0/327	0/791	0/559

Table 3.1. (Continue).

Crops/Cultivars	Experiment 1	Experiment 2	Average
8. Proseed 2013	0/327	0/791	0/559
9. Rider	0/327	0/791	0/559
10. Skyhawk	0/327	0/791	0/559
Chickpea (*Cicer arietinum* L.)			
1. Anna	0/105	6/579	3/342
2. Siera	0/105	0/579	0/342
Clover (*Trifolium* sp. and *Melilotus* sp.)			
1. Aliske Clover (*T. hybridum* L.)	0/552	0/458	0/505
2. Red Clover (*T. pratense* L.)	0/552	0/458	0/505
3. Sweet Clover (*M. officinalis* L.)	0/552	0/458	0/505
4. White Clover (*T. repens* L.)	0/552	0/458	0/505
Crambe (*Crambe maritima* L.)[b]			
1. Belann	52/610	18/342	35/476
2. Carmen	27/610	13/342	20/476
3. Indy	22/610	19/342	20/476
4. Meyer	55/610	17/342	36/476
5. Prophet	39/610	16/342	28/476
Cuphea (*Cuphea viscosissima* Jacq.)	0/552	18/658	9/605
Field pea (*Pisum sativum* L.)			
1. Admiral	0/105	0/579	0/342

Table 3.1. (Continue).

Crops/Cultivars	Experiment 1	Experiment 2	Average
2. Eclipse	0/105	0/579	0/342
3. Majoret	0/105	1/579	0/342
4. Miami	0/105	0/579	0/342
5. Mozart	0/105	0/579	0/342
6. Striker	0/105	0/579	0/342
Lentil (*Lens culinaris M*edik.*)			
1. Crimson	0/105	0/579	0/342
2. Merrit	0/105	0/579	0/342
3. Pennell	0/105	0/579	0/342
4. Redberry	0/105	0/579	0/342
5. Rich Lea	0/105	0/579	0/342
6. Sovereign	0/105	0/579	0/342
Lupines (*Lupines albus* L.*)			
1. 10018-98-1	131/458	210/331	171/395
2. 8145-94-1	204/458	246/331	225/395
3. Lupro 2085	144/458	212/331	178/395
4. 8130-94-1	130/458	202/331	166/395
5. LU206	140/458	257/331	199/395
Nyjer (*Guizotia abyssinica* (L.f.) Cass.)			
1. Early bird	0/552	0/658	0/605
2. Early bird-50	0/552	0/658	0/605
3. Unknown	1/552	2/658	2/605

41

Table 3.1. (Continue).

Crops/Cultivars	Experiment 1	Experiment 2	Average
Safflower (*Carthamus tinctorius* L.)			
1. Fincl	1/438	1/515	1/472
2. Montola 2003	4/438	3/515	4/472
3. Nutrasaft	2/438	1/515	2/472
Sunflower (*Helianthus annuus* L.)			
1. H 288	0/533	0/542	0/536
2. Proseed 9130	0/533	0/542	0/536
3. Myc 7350	0/533	0/542	0/536
4. Myc 8c841	0/533	0/542	0/536
5. Car 270	0/533	0/542	0/536
6. 8031	0/533	0/542	0/536
7. P 386	0/533	0/542	0/536
8. DM-2	0/533	0/542	0/536
9. S-37	0/533	0/542	0/536

[a] Plants inoculated with *Heterodera glycines* HG type 0 and incubated at 27° C for 30 days. The data presented are the number of females produced on the test crop over the number produced on Lee 74, the susceptible soybean check. The right column is the average of the two experiments. There were four replications in each experiment.

[b] All crambe cultivars had significantly ($P \leq 0.001$) fewer females than Lee 74.

This is the first report of reproduction of SCN on crambe. All crambe cultivars had significantly ($P \leq 0.001$) fewer females than Lee 74, and the average FI's ranged from 4 to 8 (Table 3.1). These five crambe cultivars, therefore, would be considered resistant compared to Lee 74. There were no significant differences ($P > 0.05$) in numbers of females among the five crambe cultivars in the combined data (Table 3.1). There were also no significant ($P > 0.05$) differences in the average number of eggs/female produced on the

five crambe cultivars and Lee 74 (Table 3.2). As with borage, the eggs produced on crambe were tested as inoculum on soybean. There were no significant ($P > 0.05$) differences in the number of females on the Lee 74 plants that were inoculated with eggs produced on crambe compared to Lee 74 plants inoculated with eggs produced on Lee 74 (Table 3.2). Crambe is known to contain glucosinolate compounds that are thought to play a role in defense against pests (Tsao et al., 2002; Vaughn and Berhow, 1998).

Table 3.2. Number of eggs per soybean cyst nematode female produced on crambe and purple borage and number of females formed on Lee 74 soybean inoculated with eggs produced on crambe and purple borage[a].

Cultivar[b]	Eggs/female[c]	Females on Lee 74[d]
Lee 74 (soybean)	292	367a
Belann (crambe)	284	357a
Carmen (crambe)	274	313a
Prophet (crambe)	282	320a
Meyer (crambe)	284	338a
Indy (crambe)	276	320a
Purple Borage	276	211b

[a] Plants inoculated with *Heterodera glycines* HG type 0 and incubated at 27 C° for 30 days. Data represent means from two experiments each with four replications combined for analyses.

[b] Lee 74 is the susceptible soybean for comparison.

[c] Analyses of variance indicated no significant ($P = 0.05$) difference in numbers of eggs per female.

[d] Lee 74 was inoculated with 2,000 eggs/plant produced on the respective cultivars in the left hand column. Means followed by the same letter are not significantly different (Fisher's least significant difference, $\alpha = 0.05$).

Although seven of the crops evaluated were poor hosts, if they were grown in areas with SCN, especially in rotation with soybean, reproduction of SCN on these crops under

field conditions should be evaluated. Until such data are available, the potential role these crops might play in crop rotations and SCN biology will be in question. In addition, there may be cultivars/varieties within those crops, especially crambe, that are better hosts for SCN. Fortunately, with the exception of dry bean (Poromarto and Nelson, 2009) and white lupines, all the traditional crops and apparently most of the specialty crops grown or being considered for production in North Dakota and northern Minnesota are poor hosts for SCN.

Literature Cited

Aiba, S., and Mitsui, Y. 1995. Effects of some legumes on hatching of soybean cyst nematode (*Heterodera glycines*). Ann. Rept. Plant Prot. North Japan 46:197-199.

Bradley, C. A., Biller, C. R., and Nelson, B. D. 2004. First report of soybean cyst nematode (*Heterodera glycines*) on soybean in North Dakota. Plant Dis. 88:1287.

Castillo, P., Navas-Cortés, J. A., Landa, B. B., Jiménez-Díaz, R. M., and Vovlas, N. 2008. Plant-parasitic nematodes attacking chickpea and their in planta interactions with Rhizobia and phytopathogenic fungi. Plant Dis. 92:840-853.

DeWaele, D., and Elsen, A. 2007. Challenges in tropical plant nematology. Annu. Rev. Phytopathol. 45:457-85.

Fujita, K., and Miura, O. 1934. On the parasitism of *Heterodera schachtii* Schmidt on beans. Trans. Sapp. Nat. Hist. Soc. 13:359-364.

Jackson, T. A., Smith, G. S., and Niblack, T. L. 2005. *Heterodera glycines* infectivity and egg viability following nonhost crops and during overwintering. J. Nematol. 37:259-264.

Kogiso, S., Wada, K., and Munakata, K. 1976. Isolation of nematicidal polyacetylenes from *Carthamus tinctorius* L. Agr. Biol. Chem. 40:2085-2089.

Kushida, A., Uehara, T., and Momota, Y. 2002. Effect of red clover on hatching and population density of *Heterodera glycines* (Tylenchida: Heteroderidae). Jpn. J. Nematol. 32:69-76.

Miller, D. R., Chen, S. Y., Porter, P. M., Johnson, G. A., Wyse, D. L., Stetina, S. R., Klossner, L. D., and Nelson, G. A. 2006. Rotation crop evaluation for management of the soybean cyst nematode in Minnesota. Agron. J. 98:569-578.

National Agricultural Statistics Service. 2008. Agricultural Statistics Database. United States Department of Agriculture.

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

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

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

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

Riggs, R. D. 1987. Nonhost root penetration by soybean cyst nematode. J. Nematol. 19:251-254.

Riggs, R. D. 1992. Host Range. Pages 107–114 in R. D. Riggs and J. A. Wrather, eds. Biology and Management of the Soybean Cyst Nematode. St. Paul, MN: American Phytopathological Society.

Riggs, R. D., and Hamblen, M. L. 1962. Soybean cyst nematode studies in the family Leguminosae. Ark. Agr.. Expt. Sta. Rep. Series 110:1-8.

Riggs, R. D., and Hamblen, M. L. 1966. Further studies on the host range of the soybean cyst nematode. Ark. Agr.. Expt. Sta. Series 110 2-19.

Schmitt, D. P., and Riggs, R. D. 1991. Influence of selected plant species on hatching of eggs and development of juveniles of *Heterodera glycines*. J. Nematol. 23:1-6.

Sortland, M. E., and Mac Donald, D. H. 1987. Effect of crop and weed species on development of a Minnesota population of *Heterodera glycines* race 5 after one to three growing periods. Plant Dis. 71:23-27.

Tsao, R., Peterson, C. J., and Coats, J. R. 2002. Glucosinolate breakdown products as insect fumigants and their effect on carbon dioxide emission of insects. BMC Ecology 2:5.

Vaughn, S., and Berhow, M. 1998. 1-cyano-2-hydroxy-3-butene, a phytotoxin from crambe (*Crambe abyssinica*) seedmeal. J. Chemical Ecology 24:1117-1126.

Venkatesh, R., Harrison, S. K., and Riedel, R. M. 2000. Weed hosts of soybean cyst nematode (*Heterodera glycines*) in Ohio. Weed Technol. 14:156-160.

Warnke, S. A., Chen, S. Y., Wyse, D. L., Johnson, G. A., and Porter, P. M. 2006. Effect of rotation crops on *Heterodera glycines* population density in a greenhouse screening study. J. Nematol. 38:391-398.

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

Wong, A. T. S., and Tylka, G. L. 1994. Eight nonhost weed species of *Heterodera glycines* in Iowa. Plant Dis. 78:365-367.

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

CHAPTER 4. REPRODUCTION OF SOYBEAN CYST NEMATODE ON DRY BEAN CULTIVARS ADAPTED TO NORTH DAKOTA AND NORTHERN MINNESOTA[2]

Abstract

Dry bean (*Phaseolus vulgaris*) is a host of the soybean cyst nematode (SCN; *Heterodera glycines*). Twenty-four cultivars of dry bean representing pinto, navy, black and kidney bean classes were evaluated for host suitability for SCN HG type 0 in the greenhouse. Females of SCN developed normally on all dry bean cultivars in 30 days. Eggs collected from roots of dry beans were as effective as inoculum for soybean as eggs collected from roots of soybean. Averaged over experiments, the number of SCN females per plant was significantly lower ($P \leq 0.001$) on pinto, navy and black beans than on the susceptible soybean Lee 74. No difference in the number of females between kidney beans and soybean occured. Numbers of females per plant differed ($P \leq 0.001$) among navy cultivars, but not among cultivars in the other three bean classes. A female index (FI = [the average number of females on the test plant divided by the average number of females on the susceptible soybean Lee 74] times 100) was calculated for each cultivar to evaluate resistance to SCN. FI's varied from 5 to 117 indicating a range of susceptibility in the crop. Kidney beans averaged the highest FI at 110 followed by navy, pinto and black at FI's 41, 39, and 16, respectively. SCN is a potential threat to dry bean in the northern production area of North Dakota and northern Minnesota.

Introduction

North Dakota and northern Minnesota are major dry edible bean (*Phaseolus vulgaris* L.) production areas with 340,000 hectares and a production value of 304 million dollars in 2007 (Anonymous, 2008). North Dakota was ranked number one in dry bean production in the United States in 2008. Navy and pinto beans comprise the major commercial production, but black turtle, cranberry, pink, red kidney, and small red bean classes are also grown in the region. Soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe) is a major pathogen of soybean that occurs in most soybean production areas in the United States (Niblack, 2005; Wrather and Koenning, 2006). SCN was first reported in North Dakota in 2003 (Bradley et al., 2004) and the nematode is currently found in 12 counties in eastern North Dakota (S. Markell, unpublished). To date, only HG type 0 (Niblack et al., 2002) has been identified in infested fields (Bradley et al., 2004). SCN also occurs in the three Minnesota counties immediately to the east, Clay, Norman, and Wilkin. Dry bean has been known as a host of SCN since it was first reported on *P. vulgaris* in Japan in the 1930's (Fujita and Miura, 1934). However, studies on the interaction of SCN and *P. vulgaris* are limited.

The first report of SCN in commercial production of bean in the United States was on snap bean in Illinois where the nematode was found on chlorotic and stunted plants in the field in 1981 (Noel et al., 1982). Due to that report, snap bean lines were evaluated for susceptibility to SCN and the effect of temperature on nematode development in snap bean roots was studied (Melton at al., 1985, 1986). There are few studies on pinto, navy and other dry bean classes commonly grown in the North Dakota-northern Minnesota area. Smith and Young (2003) studied 23 bean genotypes and found significant differences in

susceptibility to SCN. Becker and Ferraz (2004) examined the effect of SCN on yield of one dry bean cultivar and reported a yield reduction in greenhouse tests. Abawi and Jacobsen (1984) examined the effect of egg numbers on the growth of kidney bean in greenhouse studies, but reported growth was not adversely affected. Several of these studies demonstrated that genotype of the dry bean is a major factor in reproduction of SCN on *P. vulgaris* (Melton et al., 1985; Smith and Young, 2003).

The objectives of this study were to evaluate reproduction of SCN on the roots of various dry bean cultivars commercially grown or being tested in the North Dakota - northern Minnesota area and determine if cultivars were resistant or susceptible to SCN. In addition, eggs produced by SCN females on dry bean were evaluated as inoculum on soybean as there was only one report that such experiments had been conducted (Fujita and Miura, 1934). Preliminary reports of this work have been published (Poromarto and Nelson, 2007a, b).

Material and Methods

Dry bean genotypes

Twenty four cultivars of dry bean representing the pinto, navy, black and kidney bean classes were evaluated. Pinto bean cultivars were Buster, Maverick, Rally, Remington, Othello, GTS-900, Topaz, and Winchester; navy bean cultivars were Cirrus, Ensign, Mayflower, Navigator, Norstar, Premiere, Seahawk, and Vista; black bean cultivars were Condor, Eclipse, Jaguar, and T-39; and kidney bean cultivars were Cal Early, Chinook 2000, Montcalm, and Red Hawk. The soybean cultivar Lee 74 was used as a susceptible control in all testing (Niblack et al., 2003). Each bean class was evaluated in separate experiments.

Planting, inoculation, and cyst production

Seeds were surface disinfected with 1% NaOCl for one minute, rinsed with water and germinated on seed germination paper (Anchor Paper, St. Paul, MN) for three days. Healthy seedlings of uniform size were transplanted into a 1 x 2.5 cm hole in autoclaved river sand in individual plastic "Cone-tainers" Type SC10 Super Cell (3.8 cm dia; 21 cm depth; volume 164 ml; Stuewe & Sons, Inc., Corvallis, OR, USA). "Cone-tainers" were placed in autoclaved sand in 30.5 cm dia x 30.5 cm depth plastic pots (Cambro, Huntington Beach, CA) immersed in a water bath at 27 ± 3ºC in the greenhouse. Plants were grown for 30 days under natural and supplemental light using high pressure sodium lamps (1,000 $\mu E \cdot m^{-2 \cdot} s^{-1}$) for 16 h/day. Plants were watered daily as needed to maintain the sand at field capacity. At 14 and 21 days after planting, plants were fertilized with three ml of a solution of Peters Hydro-Sol 5-11-26 (W.R. Grace & Co.-Conn., Fogelsville, PA; at the rate of 20 ml of Peters in 980 ml of water).

Soil naturally infested with SCN was collected from a soybean field in Richland Co., North Dakota. The population of SCN was identified as HG type 0 following the methods of Niblack et al. (2003). The indicator lines and female index (FI) for the HG type classification were as follows: PI 548402, FI 0.1; PI 88788, FI 0.6; PI 90763, FI 0.1; PI 437654, FI 0.2; PI 209332, FI 0.1; PI89772, FI 0.1; PI548316, FI 6.5; Lee 74 FI 100 (Average female number/plant of Lee 74 = 570).

The general methods of Niblack et al. (2003) to inoculate plants were followed with some modifications. Cysts were extracted from the field soil with an 18-mesh (1 mm) sieve (VWR Scientific, West Chester, PA) nested over a 60-mesh (250 µm) sieve. Cysts were crushed with a Wheaten Potter Elvehjen Tissue Grinder (55 ml capacity) (VWR Scientific,

West Chester, PA) and eggs were collected on a 200-mesh (75 µm) nested over a 500-mesh (25 µm) sieve. A suspension of eggs in distilled water was prepared and adjusted to 1,000 eggs/ml. As the seedling was transplanted into the sand, a suspension of 2,000 eggs was placed in the planting hole and the seedling was covered with sand. WatchDog 450 Data loggers with soil temperature sensors (Spectrum Technologies, Inc., Plainfield, IL) were used to monitor the temperature of the sand in the "Cone-tainers". Temperatures in the sand among experiments averaged $27\pm 1°$ C, but temperature variations of 3 to $4°$ C were recorded almost daily.

Females were harvested from the roots of individual 30-day-old plants. Plants were extracted from the "Cone-tainers" and the root-sand masses were soaked in water. The females were washed off the roots and sieved from the water/sand mix of the root soakings using an 18-mesh nested over a 60-mesh sieve. Females from each plant were counted with a dissecting microscope. Roots were also examined with the dissecting microscope to insure mature females were removed.

Infecting soybean with eggs from dry bean

Eggs of SCN were produced on dry bean and then inoculated onto soybean to determine if they would result in the same number of females per plant as eggs produced on soybean. Females were produced on soybean Lee 74 and two dry bean cultivars from each of the four bean classes: GTS-900 and Remington (pinto); Vista and Navigator (navy); Montcalm and Chinook (kidney); and Condor and T-39 (black). Plants were inoculated as previously described and females harvested after 30 days. Lee 74 plants were then immediately inoculated with 2,000 eggs per plant from each of the eight bean cultivars and Lee 74. After 30 days, females were extracted and counted. In addition, the average

number of eggs per female produced on the eight dry bean cultivars and Lee 74 was determined. Females from all plants of a cultivar were bulked and a random sample of females was collected, counted, crushed and the egg numbers were counted twice with an American Optical One-Ten microscope (Buffalo, NY).

Experimental design and analyses

The experimental design was a randomized complete block with 4 replications (one plant per replication) and all experiments were repeated once. All data were log10 transformed and a comparison of the residuals between transformed and non-transformed data was performed. Transforming the data did not improve the pattern of the residuals, thus the non-transformed data were used in the analyses. The data from individual experiments were analyzed separately by analyses of variance (ANOVA) with SAS (SAS Institute, Cary, NC) and variances were compared between repeated experiments. The data were then combined over experiments for each bean class and analyzed by ANOVA. Least significant differences (Fisher's protected F test, α=0.05) were calculated following significant ($P \geq 0.05$) F tests. A female index (FI) (13) (FI = [the average number of females on the test plant divided by the average number of females on the susceptible soybean Lee 74] times 100) was calculated for each bean cultivar. By definition, the FI of Lee 74 is always 100. In experiments examining infection of soybean with eggs from dry bean, all bean cultivars were tested together in the same experiment. The data were analyzed with ANOVA as previously described.

Results

SCN females developed on all 24 dry bean cultivars in the four bean classes. In 30 days, females of SCN were readily observed on bean roots with the naked eye. Some of

the females had already formed brown cysts and were detached from the roots. Averaged over experiments, the number of SCN females per plant on pinto and navy beans was significantly less ($P \leq 0.001$) than the 195 and 513 females per plant, respectively, on the susceptible soybean Lee 74 (Figure 4.1A, 1B). No significant ($P > 0.05$) differences in number of SCN female occured among the pinto bean cultivars. Average number of females per pinto bean cultivar ranged from 53 to 100 per plant. Significant differences ($P \leq 0.001$) in numbers of females occurred among the navy bean cultivars (Figure 4.1B). Vista, for example, averaged 338 females per plant while Premiere averaged 117, the lowest among the navy cultivars. A significant ($P < 0.01$) experiment x cultivar interaction occurred with navy beans due to different rankings of the cultivars in the two experiments.

Reproduction on kidney bean cultivars was not significantly ($P > 0.05$) different from Lee 74 (Figure 4.1C). The average number of females on kidney bean plants was 787 compared to 715 on Lee 74. All black bean cultivars had significantly ($P \leq 0.001$) fewer females than Lee 74 (Figure 4.1D). However, no significant differences ($P > 0.05$) in numbers of females among the four black bean cultivars occurred in the combined data. A significant ($P < 0.001$) experiment x cultivar interaction was observed due to differences among cultivars between the first and second experiment. A significant ($P < 0.001$) difference in number of females occurred between Condor and Jaguar in the second, but not the first experiment. A female index (FI) was calculated to compare the reproduction of SCN among the dry bean classes (Figure 4.2). Averaged over experiments, kidney beans had the largest FI at 110, while black beans had the lowest at 16. Pinto and navy beans had average FI's of 39 and 41, respectively.

No significant ($P > 0.05$) differences in the number of females on the Lee 74 plants that were inoculated with eggs produced on dry bean plants occurred when compared to plants inoculated with eggs produced on Lee 74 (Table 4.1). The mean number of SCN females per plant ranged from 766 to 958. The average number of eggs per female produced on the eight bean cultivars ranged from 148 to 202, while the average on Lee 74 was 215 (Table 4.1).

Table 4.1. Number of females of soybean cyst nematode formed on Lee 74 soybean inoculated with eggs produced on dry bean and number of eggs per female produced on dry bean[a].

Cultivar (bean class)[b]	Females/Lee 74 plant[c]	Eggs/Female[d]
Lee 74 (soybean)	913	215
Condor (black)	781	181
T-39 (black)	783	165
Montcalm (kidney)	858	202
Chinook (kidney)	958	194
Vista (navy)	917	183
Navigator (navy)	766	148
GTS-900 (pinto)	862	186
Remington (pinto)	853	200

[a] Plants inoculated with *Heterodera glycines* HG type 0 and incubated at 27 C for 30 days. Data represent means from two experiments each with four replications combined for analyses.

[b] Lee 74 is the susceptible soybean for comparison.

[c] Lee 74 was inoculated with 2,000 eggs/plant produced on the respective cultivars in the left hand column. No significant differences (Fisher's least significant difference, $\alpha = 0.05$) among cultivars were measured.

[d] No statistical comparison was made on egg numbers.
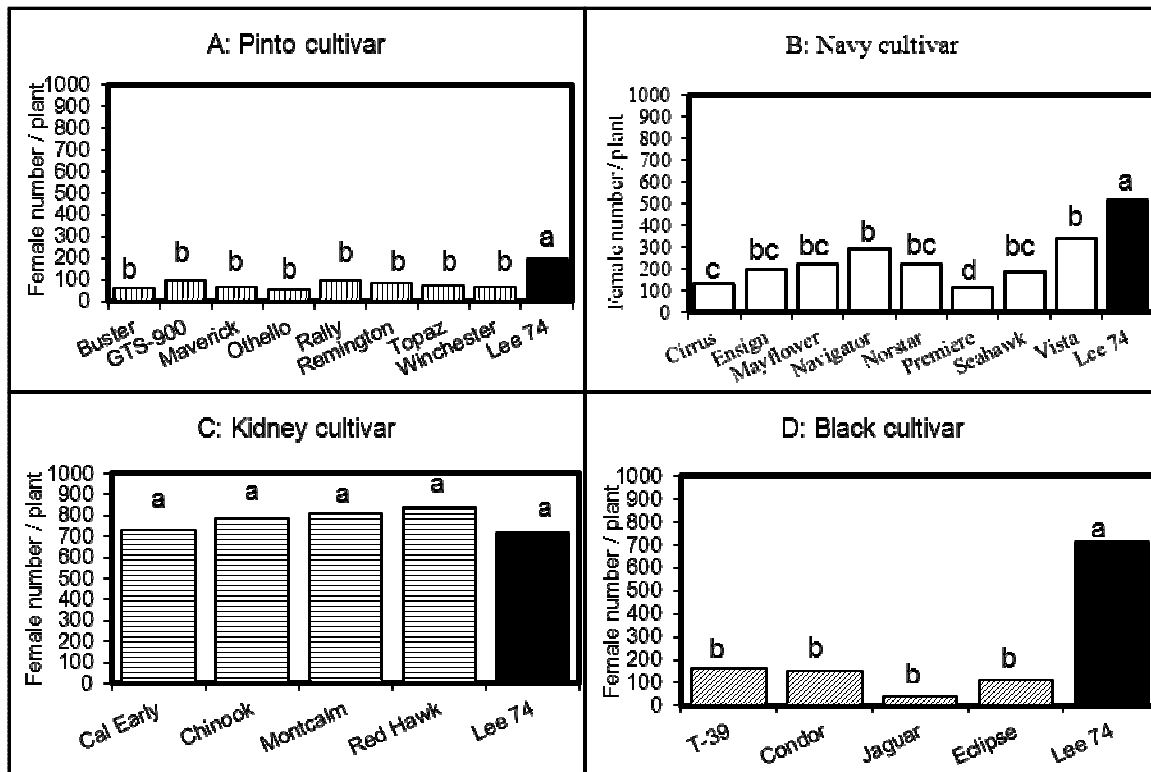
Figure 4.1. Reproduction of soybean cyst nematode HG type 0 on roots of pinto (A), navy (B), kidney (C) and black (D) bean cultivars. Females were counted 30 days after inoculation of four-day old-seedlings with 2,000 eggs per seedling in sand and incubated in a water bath at 27° C. Data are mean number of females per plant determined from two experiments each with four replications combined for analyses. Lee 74 is a susceptible soybean cultivar. Bars with the same letter are not significantly different (Fisher's least significant difference, α = 0.05).

Discussion

SCN reproduced and developed normally on all 24 cultivars of dry bean plants. Differences in numbers of SCN females reproduced on the different dry bean classes grown in the North Dakota – northern Minnesota region occurred, and differences among the navy bean cultivars tested. The results are consistent with those of Smith and Young (2003) and Melton et al. (1985).   The SCN eggs that developed on the different dry bean cultivars were as effective of inoculum on soybean as eggs from soybean. These results

have implications for management of SCN in this northern area especially where both

crops are in a crop rotation. Dry bean could increase SCN populations in infested fields and

growers might be less likely to notice infections on dry bean since SCN is a new disease in

the region and not known to be a problem on dry bean.
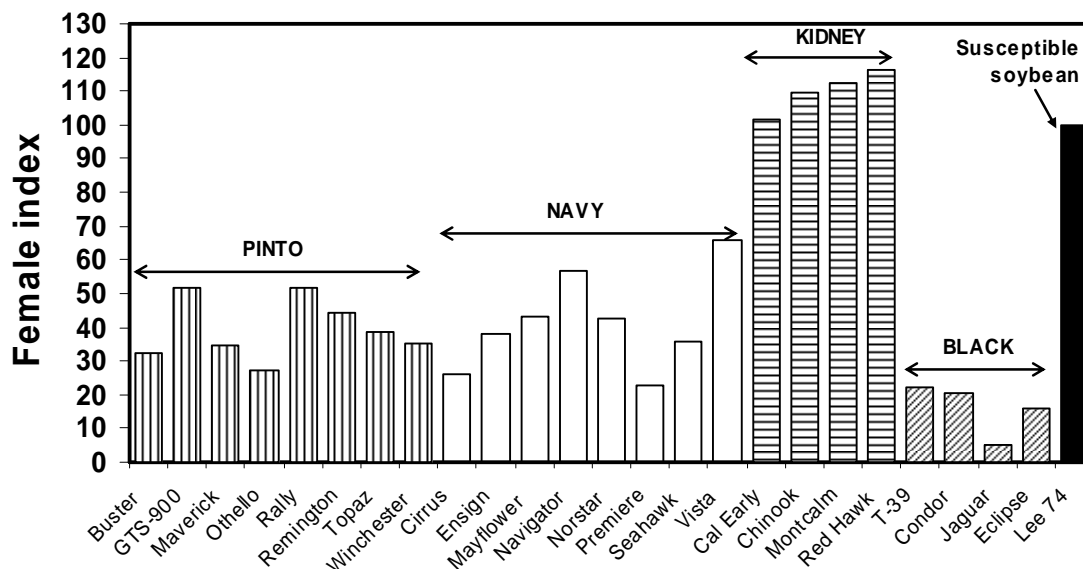
Figure 4.2. Female index (FI) for reproduction of soybean cyst nematode HG type 0 on pinto, navy, kidney and black bean cultivars. FI is the average number of females on the test plant divided by the average number of females on Lee 74 soybean times 100. Lee 74 is the susceptible soybean (FI =100) used as a standard for comparison.

No widely method rating dry bean cultivars for resistance or susceptibility to SCN

were accepted. Female index is widely used as the basis for evaluating resistance of

soybean cultivars to SCN (Niblack, 2005); the lower the FI, the greater the resistance.

Since no genes for resistance to SCN have been identified in dry bean, an argument can be

made that the term resistance should not be used until such genes or quantitative trait loci

have been identified. However, a practical reason to use a resistance scale should be

mentioned since it can provide growers with information needed to make management

decisions for the control of SCN and furthermore it assists plant scientists in planning for

future research on this potential problem Therefore, We are adopting the FI method of

Schmitt and Shannon (Schmitt and Shannon, 1992) developed for soybean to classify the

reaction of the dry bean cultivars tested. Smith and Young (2003) also referred to this

method when attempting to rate bean cultivars for resistance to SCN, thus there is

precedent for using a scale based on FI.   The Schmitt and Shannon scale categorizes

soybean reactions to SCN as the following: FI <10 resistant; FI 10-30 moderately resistant;

FI 31-60 moderately susceptible; and FI >60 susceptible. Whether resistance scales

(Niblack, 2005) used to classify SCN resistance in soybean can be directly applied to dry

bean cultivars remains to be determined. However, reproduction on dry bean compared to a

standard susceptible soybean is a reasonable approach until further information is available.

The relationship of FI to potential yield loss in dry bean will not be completely understood

until additional field research on the effect of SCN on dry beans is available.

    Based on the FI's, all four kidney bean cultivars would be considered susceptible

according to the Schmitt and Shannon scale (1992). Kidney bean shows a high

susceptibility to SCN compared to the other three bean classes. Abawi and Jacobsen (1984)

also compared a kidney bean cultivar to a susceptible soybean and reported that SCN

reproduction on the kidney bean was no different from that on soybean. Pinto bean

cultivars ranged from moderately resistant to moderately susceptible (FI's 27-52) based on

the Schmitt and Shannon scale (1992), while navy beans ranged from moderately resistant

to susceptible (FI's 23-66). Three of the black bean cultivars were moderately resistant

(FI's 16-22), while Jaguar (FI 5) was resistant. Although the black bean cultivars showed

resistance to SCN, numerous developing females observed on the roots could indicate that

over an entire season of growth, SCN might cause more damage than is indicated by a low FI determined in a 30 day test.

The data from this research indicate the kidney bean Montcalm was susceptible, but Smith and Young (2003) reported Montcalm as resistant to moderately resistant to SCN. The average FI calculated from their data for resistance to SCN race two was 10, while the FI in this current research was 113 Smith and Young (2003) used races 2, 3, 5 and 14 in their testing, while in the present study we used HG type 0 which would be equivalent to race 3 (Niblack et al., 2002). The only other dry bean cultivar besides Montcalm that has been evaluated in another study is the pinto bean Maverick. The other 22 cultivars of dry bean had previously never been evaluated for SCN reproduction nor have most cultivars adapted to the northern United States dry bean production areas. Smith and Young (2003) reported Maverick varied between moderately resistant to moderately susceptible. The average FI for Maverick calculated from their data (using SCN race two) was 33, which is similar to the FI 34 found in this current study.

Two major gene pools of *P. vulgaris*, Middle American and Andean were reported (Singh et al., 1991). Kidney and snap bean are from the Andean gene pool, while pinto, navy and black beans are from the Middle American gene pool. This current study and the report by Smith and Young (2003) indicate that within the Middle American gene pool there are high levels of resistance to SCN. A search for resistance to SCN in dry bean should, therefore, focus on that gene pool. In contrast, the Andean gene pool appears to show the greatest susceptibility based on this study and others (Melton et al., 1985; Smith and Young, 2003).

No confirmed report of SCN in dry bean fields in the region has been reported. However, SCN was recently found in counties with major dry bean hectares. The results from this study indicate that SCN can effectively reproduce on, and could reduce future dry bean production in this region. The effect of SCN on growth and yield loss of dry bean has received limited study but, recent reports indicate SCN can reduce yield of dry bean. In Brazil, Becker and Ferraz (2004) evaluated various egg levels of SCN on growth of the dry bean cultivar Ouro (Middle American gene pool) in greenhouse experiments. They reported a 14.9 percent reduction in yield and a 40.8 percent reduction in root dry weight associated with 5,600 and 12,600 eggs per plant, respectively. Poromarto and Nelson (2008) conducted a field study in North Dakota with the pinto bean GTS900 and soil infested with 0, 5,000 or 10,000 eggs/100 cm^3 soil of HG type 0. Plant height, pod number, seed weight, and total dry weight of the above-ground plant were significantly less in plants grown in soil infested with SCN compared to those grown in non-infested soil. Total seed weight from infected plants was only 44% of that in the control.

Based on studies of SCN interacting with root pathogens of soybean, another potential impact of SCN on dry bean could be an increase in severity of root rots. SCN is known to increase severity of two soil borne fungal diseases of soybean, sudden death syndrome caused by *Fusarium virguliforme* (McLean and Lawrence, 1993) and brown stem rot caused by *Phialophora gregata* (Tabor et al., 2003). SCN also can cause greater severity of seedling disease caused by *Phytophthora sojae* in soybean (Adeniji et al., 1975). Dry bean root rots are a serious problem in the area (Knodel et al., 2007) and Fusarium root rot caused by *Fusarium solani* f. sp. *phaseoli* is one of the major pathogens (Bilgi et al., 2008; Xing and Westphal, 2006).

North Dakota and northern Minnesota comprise the most northerly soybean and dry bean production area in the United States, with about 5 million acres of these crops. The most northerly occurrence of SCN is also in this area. The cold soil temperatures in the tillage layer in this region, which average -1°C over 6 months of the year (data from the North Dakota agricultural weather network: http://ndawn.ndsu.nodak.edu/), favor SCN survival since microbial degradation of eggs is minimized. Studies on SCN reproduction on soybean and survival in soil in North Dakota show that SCN reproduces at high levels on susceptible soybeans and egg populations survive well during crop rotations to non-hosts (Berlin Nelson, unpublished). SCN is spreading north and may eventually become a widespread and dominant soybean disease as it has in other states. The dry bean industry should be aware of the potential threat to dry bean production. Research on the biology of SCN on dry bean and specifically on finding sources of resistance in *P. vulgaris*, such as Jaguar black bean, should be the focus of additional investigation into this potential threat.

Literature Cited

Abawi, G. S., and Jacobsen, B. J. 1984. Effect of initial inoculum densities of *Heterodera glycines* on growth of soybean and kidney bean and their efficiency as hosts under greenhouse conditions. Phytopathology 74:1470-1474.

Adeniji, M. O., Edwards, D. I., Sinclair, J. B., and Malek, R. B. 1975. Interrelationship of *Heterodera glycines* and *Phytophthora megasperma* var *sojae* in soybeans. Phytopathology 65:722-725.

Anonymous. 2008. Agricultural Statistics Database. National Agricultural Statistics Service. United States Department of Agriculture. Online publication.

Becker, W. F., and Ferraz, S. 2004. Efeito da concentração inicial de inóculo do nematóide *Heterodera glycines* no desenvolvimento do feijoeiro comum. Revista Agropecuaria Catarinense, Florianópolis 17:89-93.

Bilgi, V. N., Bradley, C. A., Khot, S. D., Grafton, K. F., and Rasmussen, J. B. 2008. Response of dry bean genotypes to Fusarium root rot caused by *Fusarium solani* f. sp. *phaseoli,* under field and greenhouse conditions. Plant Dis. 92:1197-1200.

Bradley, C. D., Biller, C. R., and Nelson, B. D. 2004. First report of soybean cyst nematodes (*Heterodera glycines*) on soybean in North Dakota. Plant Dis. 88:1287.

Fujita, K., and Miura, O. 1934. On the parasitism of *Heterodera schachtii* Schmidt on beans. Trans. Sapporo Nat. Hist. Soc. 13:359-364.

Knodel, J. J., Bradley, C. A., Luecke, J. L., and Mars, G. A. 2007. 2004 and 2005 dry bean grower survey. N.D. State Univ. Ext. Rep. No. PP-1265.

McLean, K. S., and Lawrence, G. W. 1993. Interrelationship of *Heterodera glycines* and *Fusarium solani* in Sudden Death Syndrome of Soybean. J. Nematol. 25:434-439.

Melton, T. A, Jacobsen, B. J., and Noel, G. R. 1986. Effects of temperature on development of *Heterodera glycines* on *Glycine max* and *Phaseolus vulgaris*. J. Nematol. 18:468-474.

Melton, T. A., Noel, G. R., Jacobsen, B. J., and Hagedorn, D. J. 1985. Comparative host suitabilities of snap beans to the soybean cyst nematode (*Heterodera glycines*). Plant Dis. 69:119-122.

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

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

Noel, G. R., Jacobsen, B. J., and Leeper, C. D. 1982. Soybean cyst nematode in commercial snap bean. Plant Dis. 66:520-522.

Poromarto, S., and Nelson, B. 2007a. Soybean cyst nematode reproduction on navy, kidney, and black bean. (Abstr.) Phytopathology 97:S94.

Poromarto, S., and Nelson, B. 2007b. Soybean cyst nematode reproduction on pinto bean. (Abstr.) Phytopathology 97:S163.

Poromarto, S., and Nelson, B. 2008. Effects of soybean cyst nematode on growth of pinto bean. (Abstr.) Phytopathology 98:S126.

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

Singh, S. P., Gepts, P., and Debouck, D. G. 1991. Races of common bean (*Phaseolus vulgaris*, Fabaceae). Econ. Botany 45:379-396.

Smith, J. R., and Young, L. D. 2003. Host suitability of diverse lines of *Phaseolus vulgaris* to multiple populations of *Heterodera glycines*. J. Nematol. 35:23-28.

Tabor, G. M., Tylka, G. L., Behm, J. E., and Bronson, C. R. 2003. *Heterodera glycines* infection increases incidence and severity of brown stem rot in both resistant and susceptible soybean. Plant Dis. 87:655-661.

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

Xing**,** L., and Westphal, A. 2006. Interaction of *Fusarium solani* f. sp. *glycines* and *Heterodera glycines* in sudden death syndrome of soybean. Phytopathology 96:763-770.

CHAPER 5. EFFECT OF SOYBEAN CYST NEMATODE ON GROWTH OF DRY BEAN IN THE FIELD[3]

Abstract

Phaseolus vulgaris is a host of soybean cyst nematode (SCN; *Heterodera glycines*), but the effects of SCN on growth of dry bean plants are poorly understood. To study the effects of SCN (HG type 0) on dry bean, the cultivars GTS-900 (pinto bean), Montcalm (kidney bean) and Mayflower (navy bean) were evaluated in eight field experiments at four locations between 2007 and 2009. Plants were grown in a pasteurized Arveson loam soil that was infested with SCN eggs at densities ranging from 0 to 10,000 eggs/100 cm^3 soil. Soil was placed in 14.6 L plastic pots that were buried in the field with the bottoms removed. SCN reproduced on all three dry bean cultivars with reproduction factors (RF= the number of eggs in the soil at harvest divided by the number of eggs at planting) ranging from 6.1 to 1.2. RF's were higher for dry bean plants growing at lower egg densities compared to higher densities. Pod number (PN), pod weight (PW), seed number (SN), and seed weight (SW) of GTS-900 were significantly less at 5,000 and 10,000 eggs/100 cm^3 soil compared with the control. Averaged over those two egg densities, PN, PW, SN, and SW were reduced by 44 to 56% over the two years compared with the control. For Montcalm, significant reductions of 31 to 35% in PW, SN, SW, and total dry weight (TDW) in treatments of 2,500 and 5,000 eggs/100 cm^3 soils were recorded in 2009, but not in 2008. For Mayflower, significant reductions of 27 to 41% in PH, PW, SN, SW, and TDW in treatments of 2,500 and 5,000 eggs/100 cm^3 soil compared with the control were recorded in one out of two experiments in 2009. The reproduction of SCN on roots and the

reduction in plant growth and seed yield on three different bean classes under field conditions indicates SCN is a potential threat to the large dry bean industry in the North Dakota-northern Minnesota region.

Introduction

`   Soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe (Tylenchida: Heteroderidae), is the most serious pathogen of soybean (*Glycine max* (L.) Merr.) in the United States and suppresses yield more than any other pathogen (Hartman et al., 1999). SCN reduces yield by feeding on plant nutrients, retarding root growth, reducing water and nutrient uptake and transport from roots to shoots, and inhibiting rhizobial nodulation (Riggs and Schmitt, 1987; Williamson and Hussey, 1986). Yield losses can exceed 40% depending on many factors such as SCN population density, soil texture and fertility, rainfall, and the presence of susceptible soybean genotypes (Koenning, 2004; Koenning and Barker, 1995; Niblack et al., 1992). The typical above-ground symptoms caused by SCN are stunting, yellowing, and wilting (Hartman et al., 1999), but yield loss occurs without obvious above ground symptoms (Wang et al., 2003).   In the United States, this nematode was responsible for losses of approximately $460 to $818 million per year between 2003 and 2005 (Wrather and Koenning, 2006).

SCN was first observed in China and Japan in the 1880s (Hartman et al., 1999). In 1938 the nematode was reported from Manchuria and then from several other parts of Asia, including the Amur District in Russia. In the USA, SCN was first reported in 1954 in North Carolina (Winstead et al., 1955) and subsequently spread to 30 states and into Canada with the extension and intensification of soybean cultivation. SCN was first reported in Minnesota in 1978 (MacDonald et al., 1980) and is now detected in 55 counties

in southern and central Minnesota. In1995, SCN (race 3) was reported in South Dakota (Smolik et al., 1996) and in 2003 the nematode (HG type 0) was discovered in Richland Co., North Dakota, in the southeast corner of the state in the Red River Valley (Bradley et al., 2004).

SCN is spread readily from field to field in soil particles on farm machinery (Lal and Lal, 2006). Unfortunately, the agricultural practices in the Red River Valley, specifically in the production, harvesting and transportation of sugar beets and potatoes which result in considerable movement of soil, will exacerbate the dissemination of SCN in this area. Based on recent measurements of egg populations in infested soils in North Dakota, the nematode reproduces extremely well. In infested fields in Cass and Richland, Co., egg counts from 4,000 to greater than 20,000 eggs/100 cm^3 of soil have been detected in areas of infested fields following a susceptible soybean crop (B. Nelson, unpublished). In some of these infested fields after four years cropping to non-host crops, SCN egg densities greater than 1,000 eggs/100 cm^3 have been measured, indicating good survival between susceptible crops. These observations on SCN strongly suggest the nematode will become a widespread and serious pathogen of soybean and other susceptible crops in North Dakota.

North Dakota and northern Minnesota is a major dry edible bean (*Phaseolus vulgaris* L.) production area with 307,500 hectares (760,000 acres) and a production value of 309 million dollars in 2009 (Anonymous, 2010). North Dakota was ranked the number one producer of dry bean in the United States in 2009 with at least 22 varieties of dry beans grown in the area. Dry bean is a host of SCN (Fujita and Miura, 1934; Melton et al., 1986) and the nematode can reproduce on dry bean cultivars grown in the region (Poromarto and

Nelson, 2009). These facts suggest SCN could be a threat to dry bean production. There is limited research on the biology of SCN on dry bean and no research on the effects of SCN on dry bean production under field conditions. Indeed, we found only two papers that reported effects of SCN on dry bean growth. Becker and Ferraz (2004) in Brazil reported that SCN reduced yield and root dry weight in greenhouse experiments. In other greenhouse experiments, Abawi and Jacobsen (1984) reported that up to 108 eggs or Juvenile 2 of SCN/cm^3 of soil did not significantly reduce growth of California Red Kidney bean after 35 days growth in the infested soil. To understand the potential threat of SCN to dry bean production in the United States, information on the effects of the nematode on plant growth under field conditions is needed. The objectives of our study were to determine the effect of SCN on dry bean growth and measure reproduction of SCN on the roots under field conditions using different dry bean types adapted to this region. A preliminary report of the research has been published (Poromarto and Nelson 2008).

Material and Methods

Cultivars and field sites

The study was conducted in Richland and Cass counties of North Dakota between 2007 and 2009 using artificially infested soil in plastic pots buried in different field sites. The dry bean cultivars tested were GTS-900 (pinto bean), Montcalm (kidney bean), and Mayflower (navy bean). These cultivars were chosen because they had previously been evaluated for reproduction of SCN and showed a high level of reproduction within their respective bean class (Poromarto and Nelson, 2008). Eight experiments were conducted in the following four locations with each cultivar a separate experiment: in 2007 in the Freese field (Arveson loam, pH 7.4) in Richland County with GTS-900; in 2008 in the Ward field

66

(Mantador-Delamere-Wyndmere fine sandy loam, pH 8.0) in Richland, Co., with GTS-900

and Montcalm; in 2008 in the Fargo field site 1 (Fargo silty clay, pH 6.8) in Cass, Co., with

Montcalm, and Mayflower; in 2009 in Fargo field site 1 with Montcalm and Mayflower;

and in 2009 in Fargo field site 2 (Fargo silty clay) with Mayflower. Planting and

harvesting dates were the following: 13 June and 29 September, 2007; 16 June and 29

September, 2008; and 25 June and 5 October, 2009.

Soil from the Freese field was used in the pots in all experiments. This soil was

chosen for the experiments because SCN had reproduced to high levels on soybean in this

field. Soil from the tillage layer was collected, pasteurized for 3 hours at temperatures >72

C and then placed in 14.6 L plastic pots (model 2000EG, 28 cm dia at top x 27.5 cm

height, Nursery Supplies Inc, Portland, OR). SCN eggs were then thoroughly mixed into

the soil in each pot. Dry bean seeds were surface sterilized with 1.0% NaOCl for one

minute, rinsed with water, then germinated on seed germination paper for 3 days. Healthy

germinating seeds of uniform size were planted 2.5 cm deep at one per pot in 2007 and

2008 and two per pot for kidney and three per pot for navy bean in 2009. Plants were

grown in the pots in the greenhouse for 15 days to establish root systems and then moved

to the field where the bottom of each pot was removed as the pots were buried 24 cm deep

in the field soil.

Source of SCN and soil infestation

Eggs of SCN were obtained from the Freese site which was naturally infested with

SCN from previous soybean cultivations. The population of SCN was identified as HG

type 0 (Poromarto and Nelson, 2008) following the methods of Niblack et al. (2002). Cysts

and eggs were extracted according to methods described previously (Poromarto and

Nelson, 2008). A suspension of eggs in distilled water was prepared and adjusted to 5,000

eggs/ml and immediately added to the soil and the soil mixed for several minutes in a 141.6

lt. electric cement mixer. In May, 2007, cysts and eggs were extracted directly from the

Freese soil and then mixed into the pasteurized soil in the pots. In 2008 and 2009, eggs

from the Freese site were inoculated onto Lee 74 or Barnes soybean, two equally

susceptible soybean cultivars, and cysts were produced on plants in the greenhouse

(Poromarto and Nelson, 2008), then eggs were extracted and used to infest soil in the pots.

Experimental design and analyses

In 2007, the treatments were 0, 5,000 or 10,000 SCN eggs/100 cm^3 soil for GTS-

900. In 2008, treatments were 0, 2,500, 5,000 or 10,000 SCN eggs/100 cm^3 soil for GTS-

900, and 0, 1,000, 2,500, and 5,000 eggs/100cm^3 for Montcalm. In 2009, treatments were

0, 2,500, and 5,000 SCN eggs/100cm^3 for Montcalm and Mayflower. Each cultivar was a

separate experiment. The study was conducted in a randomized complete block design with

six replications in 2007 and four in 2008 and 2009. Plant height was recorded on 70 day

old plants. Plants were hand harvested and dried at 35 C for 5 days. The dry weight of the

above ground plant and number and weight of pods and seeds were determined. All data

were recorded as the mean measurement per plant. Following harvest, the pots were

removed from the field, the infested soil from inside the pots was air dried on a greenhouse

bench, mixed thoroughly in the cement mixer for several minutes and the average number

of cysts and eggs /100 cm^3 soil was determined from three 100 ml subsamples per

replication (Poromarto and Nelson, 2008). The data were analyzed by analyses of variance

with SAS (SAS Institute, Cary, NC) and least significant differences (Fisher's protected F

test, α =0.05) were calculated following significant ($P \leq 0.05$) F tests. Transforming the

data with Log_{10} did not improve the pattern of the residuals; thus, the non-transformed data were used in the analyses. Data from similar experiments were combined where appropriate.

Results

SCN reproduction

SCN reproduced on all three dry bean cultivars. In all experiments, the number of cysts and eggs in the soil at harvest for treatments where SCN was added was significantly ($P < 0.01$) higher than in the controls (Table 5.1). With the exception of the experiment with Mayflower at the Fargo field site 1 in 2009 there were significant ($P \leq 0.05$) differences among treatments in cyst and egg numbers at harvest with numbers always greater when larger numbers of eggs were added to the soil in the other experiments (Table 5.1). There was some contamination of the soil in the controls by SCN and reproduction occurred on plants in the controls.

GTS-900 pinto bean

In 2007, plant height (PH), pod number (PN), pod weight PW), seed number (SN), seed weight (SW), and total dry weight (TDW) of the above-ground-plant of GTS-900 pinto bean were significantly ($P < 0.05$) less in the two SCN soil infestation treatments compared with the control (Figure 5.1A). However, there were no significant differences in those variables between the two soil infestation treatments. Averaged over the two SCN treatments, PH, PN, SW, and TDW were reduced by 42, 47, 56 and 54% compared with the control, respectively, and plants contained only 44% of the seeds compared with the control. Infected pinto bean plants, especially at the higher egg density, were stunted, had

fewer branches and a less robust appearance compared with the plants in the non-infested

soil (Figure 5.2).

Table 5.1. Numbers of cysts and eggs of soybean cyst nematode produced on dry bean cultivars grown in infested soil at different field sites.

Treatment[b] Eggs/100 cm^3 soil	GTS-900[a]		Montcalm		Mayflower	
	2007	2008	2008	2009	2009	2009
	Freese	Ward & Fargo	Ward & Fargo	Fargo (Field 1)	Fargo (Field 1)	Fargo (Field 2)
	Cysts/100 cm^3 soil[d]					
Control	21	4	8	33	26	18
1,000			45			
2,500		37	61	65	77	95
5,000	78	60	80	112	94	125
10,000	95	75				
LSD[c]	**14**	**14**	**6**	**25**	**39**	**24**
	Eggs/100 cm^3 soil[d]					
Control	2,547	553	1,023	2,788	2,246	1,762
1,000			6,119			
2,500		5,787	8,348	5,725	6,921	8,546
5,000	9,567	8,986	11,061	9,797	7,346	10,783
10,000	11,694	12,562				
LSD[c]	**1,458**	**2,153**	**850**	**2,075**	**2,463**	**2,117**

[a] GTS-900, Montcalm, and Mayflower are cultivars of pinto, kidney, and navy bean, respectively.

In 2008, the research was conducted at two field sites and the data were combined for analyses. The PN, PW, SN, and SW of GTS-900 were significantly ($P < 0.05$) less in the two highest SCN soil infestation levels (5,000 and 10,000 eggs/100 cm^3 soil) compared with the control, but there were no significant differences among those two levels (Figure 5.1B). However, PH and TDW of above ground plants were significantly less than the control only when plants were infested with 10,000 eggs/100 cm^3 soils. The PH and TDW of plants growing in soil with 10,000 eggs/100 cm^3 soil were reduced by 38 and 45% compared with the control, respectively. Averaged over the two highest SCN treatments, the PN, PW, SN, and SW were reduced by 50, 40, 51, and 51%, respectively. SCN infestation at 2,500 eggs/100 cm^3 soil did not significantly ($P < 0.05$) reduce growth measurements compared with the control (Figure 5.1B).

Montcalm kidney bean

For both field experiments in 2008, none of the plant growth variables were significantly ($P < 0.05$) reduced in the infested soils compared with the control (Figure 5.1C). The results were the same whether data from each experiment were analyzed separately or when the data were combined. In contrast, in 2009, there were significant ($P < 0.05$) reductions of PN, PW, SN, SW, and TDW in infested soil compared with the control (Figure 5.1 D), but there were no significant differences between the two egg levels. The average reduction of PW, SN, SW, and TDW in the two treatments was 32, 32, 31, and 35%, respectively (Figure 5.1D). PN was significantly reduced (by 33%) only at 5,000 eggs/100 cm^3 soil compared with control.

Mayflower navy bean

Although experiments were conducted at two field locations in 2009, the data from each experiment were analyzed separately because plant growth at Fargo field 2 was considerably slower and plants did not develop as well or appear as robust as in the other Fargo site. In Fargo field 1, there were significant ($P < 0.05$) reductions of PH, PW, SN, SW, and TDW of Mayflower when grown in soil infested with 2,500 or 5,000 eggs/100 cm^3 soil compared with the control, however there were no significant differences among the two treatments (Figure 5.1 E). Averaged over the two treatments, PH, PW, SN, SW, and TDW were reduced by 27, 41, 36, 37, and 36% compared with the control, respectively. PN was significantly ($P < 0.05$) reduced (by 31%) in the 5,000 eggs/100 cm^3 soil treatment compared with the control, but not at 2,500 eggs/100 cm^3 soil (Figure 5.1E). In contrast, in Fargo field 2, there were no significant reductions of any growth variables (Figure 5.1 F).

<div align="center">

Discussion

</div>

These are the first field studies quantifying SCN reproduction on dry bean and documenting the effect of SCN on growth and yield of this important crop. SCN reproduced on all three dry bean cultivars under field conditions and the pattern of reproduction was a function of the initial egg density in the soil and host and environmental factors (McSorley, 1988; Seinhorst, 1986; Seinhorst, 1970; Steele, 1970). In soybean, the nematode at low population densities is capable of large population increases whereas at higher egg densities the rate of population increase declines, most likely due to greater competition for feeding sites in root tissue (Alston and Schmitt, 1987; Francl and Dropkin, 1986). In these experiments, the reproduction factors (RF = the number of eggs at harvest

time divided by the number of eggs at planting time) of SCN were higher in dry bean plants growing at lower egg densities compared with higher densities. In GTS-900 pinto bean, the RF's were 2.3, 1.9, and 1.2 for initial egg densities of 2,500, 5,000, and 10,000 eggs/100 cm^3 soil, respectively. For Montcalm kidney bean, the RF's were 6.1, 2.8, and 2.1 for 1,000, 2,500, and 5,000 eggs/100 cm^3 soil, respectively, and for Mayflower navy bean, the RF's were 3.1 and 1.8 for egg densities of 2,500 and 5,000 eggs/100 cm^3 soil, respectively.

The controls in all experiments became infested with SCN sometime during plant growth. Because the Ward and Freese sites were naturally infested with SCN at levels exceeding 3,000 eggs/100 cm^3 soil, the pasteurized soil in the pots at those sites mostly likely became infested with eggs carried in rain splashed or wind driven soil from the area around the pots. In the Fargo sites, however, the soil in the plot areas was not infested with SCN, therefore, it appears that controls may have been infested with SCN from the pots where eggs had been added to the soil. The different egg densities detected in the control treatments at harvest was probably due to contamination with eggs at different times during plant growth, an earlier contamination resulting in more cysts and eggs at harvest. Although we pasteurized soil for 3 hours it is also possible that some eggs might have survived the heat treatment. This natural contamination of the potted soil by either eggs from the surrounding field or from those not killed by pasteurization most likely contributed to the overall reproduction on dry bean roots, but we believe the level of contamination was not sufficient to result in a measurable effect on plant growth.
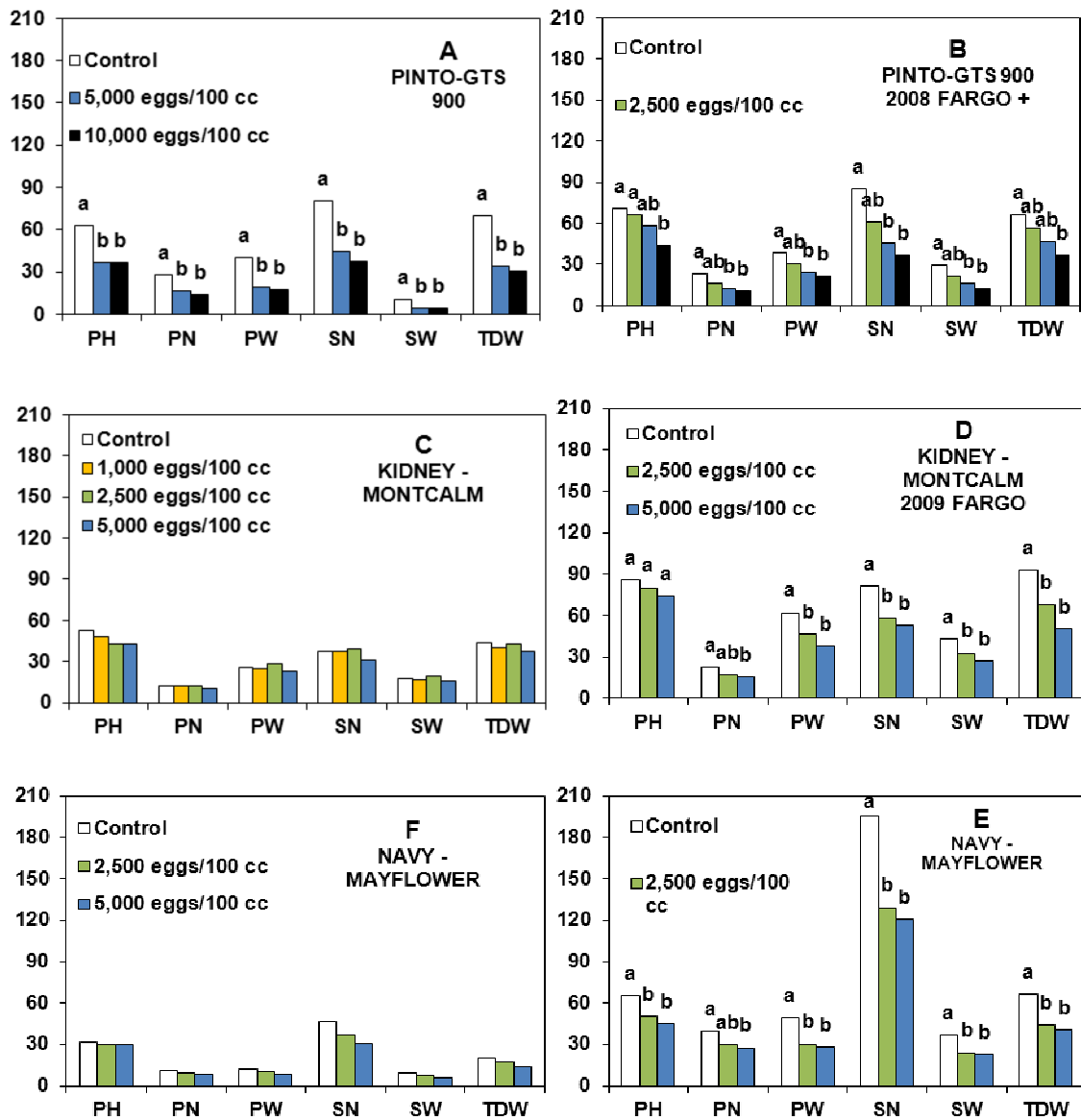
Figure 5.1. Effect of soybean cyst nematode on growth of dry bean. A and B are GTS-900 pinto bean. C and D are Montcalm kidney bean. E and F are Mayflower navy bean. PH = plant height (cm), PN = pod number, PW = pod weight (g), SN = seed number, SW = seed weight (g), TDW = total dry weight (g). Bars labeled with the same letter are not significantly different (Fisher's protected F test, α= 0.05).

Figure 5.2. Effect of soybean cyst nematode on growth of pinto bean in 2007. Plant in middle (B) is the control growing in soil without eggs added to soil. The plants on the right (C) and left (A) are growing in soil with 10,000 and 5,000 eggs/100 cm^3 of soil, respectively. Notice the more robust plant growth of the control.

When we initiated these experiments in 2007, there were no prior research data available on egg densities in soil that might affect the growth of dry bean under field conditions. Therefore, experiments were initiated with relatively high egg densities of 5,000 and 10,000 eggs/100 cm^3 soil using a pinto bean cultivar, the most important bean class grown in the North Dakota-Minnesota dry bean production region. Because plant growth was affected by 5,000 and 10,000 eggs/100 cm^3 soil in 2007, but there were no significant differences between the two treatments, in following years egg densities of 5,000 eggs/100 cm^3 soil or less were evaluated. In previous studies comparing the susceptibility of dry bean cultivars to a susceptible soybean cultivar, kidney bean cultivars were as susceptible as soybean, but navy and pinto bean cultivars were less susceptible (Poromarto and Nelson, 2009). Those were also reasons for using the high egg densities in

2007 with pinto bean and the lower egg density of 1,000 eggs/100 cm^3 soil with kidney bean in 2008.

The reductions in growth parameters of pinto bean over two years of trials point out that SCN can cause significant yield losses in that bean class with seed number reductions of 45 to 50% at the higher egg densities. Although in some experiments there were visual differences in infected plants compared with the controls, such as stunting and less robust growth, in others with some treatments no obvious visual differences were apparent, but there was a reduction in yield. Significant yield losses due to SCN without obvious symptoms of disease are well known in soybean (Niblack et al., 1992; Noel, 1992; Wang et al., 2003; Young, 1996). One potential reason why plant height and total dry weight of plants at 5,000 eggs/100 cm^3 soil were significantly reduced in 2007, but not in 2008, may have been due to drier soil conditions in 2007 which added more stress to infected plants compared with plants growing in the higher rainfall year of 2008.

The results with Montcalm kidney bean were perplexing. In greenhouse testing, Montcalm was as susceptible to SCN as soybean based on female index while GTS-900 was less susceptible than Montcalm (Poromarto and Nelson, 2009). The fact that there was no reduction in growth of Montcalm at the two sites in 2008 was surprising because 5,000 eggs/100 cm^3 soil were used and growth of GTS-900 pinto bean was reduced by SCN at the same two sites. In addition, the egg densities at harvest indicated that SCN reproduced well on Montcalm in 2008. SCN did cause a reduction in growth of Montcalm in 2009. As observed in GTS-900, SCN caused a reduction in growth of Montcalm without any obvious above ground symptoms. Montcalm may have the ability to support high reproduction of SCN and still yield well under certain conditions, but further studies are needed to verify if

this occurs. Similar to Montcalm, there was a reduction in growth of Mayflower navy bean in one experiment, but not the other. The Mayflower plants in Fargo field 2 grew slowly, were never as robust as in field 1, and yields were very low. The cause of the poor growth was not determined but, there were no obvious symptoms of an identifiable disease. Surprisingly, SCN reproduced as well on the Mayflower plants in Fargo field 2 as in field 1, but yet no differences in yield were measured in field 2.

Plant responses to parasitism by nematodes are related to physiological changes that affect the photosynthetic process (Hussey and Williamson, 1998). SCN is reported to reduce photosynthetic activity on susceptible soybean varieties by lowering the amount of nutrients, particularly nitrogen, either absorbed or translocated by the infected roots (Koenning and Barker, 1995). Suppression of nodulation of nitrogen-fixing-bacteria by SCN has also been demonstrated by Ko et al. (1984) and Ross (1969). Asmus and Ferraz (2002) noticed a correlation between reduction in yield and a reduction in the duration of leaf area caused by SCN. Ross (1969) reported that besides reducing root nodulation and N fixation, SCN causes soybean yield reductions by inciting deleterious host responses that increase with N deficiency. Physiological changes affecting photosynthetic processes, such as decreased chlorophyll content (Nagesh and Dhawan, 1988; Nehra et al., 2001), photochemical limitations (Schans and Arntzen, 1991), nutrient imbalance (Wallace, 1974) and interference of the synthesis and translocation of photosynthesis regulating factors produced in the roots (Loveys and Bird, 1973) have been reported to occur during plant response to nematode parasitism (Hussey and Williamson, 1998).

Cyst forming nematodes are known to affect the growth of crops such as broad bean, chickpea, lentil pea and vetch (Di Vito et al., 1978; Greco et al., 1988; Greco et al.,

1993; Greco et al., 1991) and *H. glycines* causes substantial damage in soybean (Asmus and Ferraz, 2002; Koenning and Barker, 1995; MacGuidwin et al., 1995; Postuka et al., 1986; Wheeler et al., 1997; Young 1996). Yield loss in soybean due to SCN is greatly affected by environmental factors such as temperature and moisture and the level of stress on the crop, plus plant variety, soil type, availability of nutrients and other conditions (Donald et al., 2006; Koenning, 2004). A linear relationship between initial SCN egg number and yield of soybean was reported by Niblack et al. (1992) in a two year study, but the slope of the predictive line was different for each year even though initial nematode number and cultivar were constant. Similar results were reported by Koenning and Barker (1995) where they found different slopes for each year, for irrigated vs. non-irrigated plots and for different soil textures.

This is the first report that SCN can cause a yield loss in dry bean under field conditions and that SCN can reproduce to high levels on dry bean in the field. In five of the eight experiments in this research, SCN caused a reduction in growth of dry bean. The results indicate that SCN poses a potential threat to the dry bean industry in the North Dakota-northern Minnesota growing region. Although SCN has not yet infested the areas where the majority of the dry bean production occurs, it is only a matter of time before SCN is introduced into those areas. In northern Minnesota, SCN was recently found in Clay and Norman counties, adjacent to North Dakota, also near dry bean production areas. The reproduction of SCN on dry bean would suggest that once SCN is introduced into dry bean fields, SCN egg densities will likely increase rather quickly in the lighter, sandy soils during warm growing seasons which are conditions favorable to the nematode. Since dry bean growers in this region are not familiar with this pathogen and yield losses may occur

78

without obvious above ground symptoms, populations of SCN may build up to high levels and large yield losses could occur before this is recognized as a serious pathogen of dry bean.

The impact that SCN could have on dry bean production in this northern region is unknown. There is limited information on SCN effects on dry bean cultivars in the field and growers have not yet reported a problem with SCN. Further research therefore, on the SCN-dry bean interaction is warranted, especially on the effects of SCN on dry beans of all classes under field conditions. However, a prudent approach to prepare for the potential management of SCN is to search for resistance to SCN within the *P. vulgaris* germplasm and initiate an educational campaign to inform dry bean growers of this potential threat. Resistance is the principal management of SCN in soybean. Because resistance genes are well known in soybean and there are regions of synteny between the genomes of soybean and dry bean (McClean et al., 2010), there is a strong possibility of finding resistance genes in the dry bean germplasm. A program to screen germplasm of the various dry bean classes for resistance to SCN has been initiated at North Dakota State University.

Literature Cited

Abawi, G. S., and Jacobsen, B. J. 1984. Effect of initial inoculum densities of *Heterodera glycines* on growth of soybean and kidney bean and their efficiency as hosts under greenhouse conditions. Phytopathology 74:1470-1474.

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

Anonymous. 2010. Agricultural Statistics Database. National Agricultural Statistics Service. United States Department of Agriculture. Online publication.

Asmus, G. L., and Ferraz, L. C. C. B. 2002 Effect of population densities of *Heterodera glycines* race 3 on leaf area, photosynthesis and yield of soybean. Fitopatol. Brasileira 27:273-278.

Becker, W. F., and Ferraz, S. 2004. Efeito da concentração inicial de inóculo do nematóide *Heterodera glycines* no desenvolvimento do feijoeiro comum. Revista Agropecuaria Catarinense, Florianópolis 17:89-93.

Bradley, C. A., Biller, C. R., and Nelson, B. D. 2004. First report of soybean cyst nematode (*Heterodera glycines*) on soybean in North Dakota. Plant Dis. 88:1287.

Di Vito, M., Lamberti, F., and Inserra, R. N. 1978. Effects of *Heterodera goettingiana* on weight of foliage of pea plants in pots. Nematologica 24:209-212.

Donald, P. A., Pierson, P. E., St.Martin, S. K., Sellers, P. R., Noel, G. R., Macguidwin, A. E., Faghihi, J., Ferris, V. R., Grau, C. R., Jardine, D. J., Melakerberhan, H., Niblack, T. L., Stienstra, W. C., Tylka, G. L., Wheeler, T. A., and Wysong, D. S. 2006. Assessing *Heterodera glycines*-resistant and susceptible cultivar yield response. J. Nematol. 38:76-82.

Francl, L. J., and Dropkin, V. H. 1986. *Heterodera glycines* population dynamics and relation of initial population to soybean yield. Plant Dis. 70:791-795.

Fujita K., and Miura, O. 1934. On the parasitism of *Heterodera schachtii* Schmidt on beans. Trans. Sapporo Nat. Hist. Soc. 13:359-364.

Greco, N., Di Vito, M., Saxena, M. C., and Reddy, M. V. 1988. Effect of *Heterodera ciceri* on yield and lentil and development of this nematode on chickpea in Syria. Nematologica 34:98-114.

Greco, N., Di Vito, M., Singh, K. B., and Saxena, M. C. 1993. Effect of *Heterodera ciceri* on the growth of selected lines of *Cicer species*. Nematol. Medit. 21:111-116.

Greco, N., Ferris, H., and Brandonisio. 1991. Effect of *Heterodera goettingiana* population densities on the yield of pea, broad bean and vetch. Rev. Nematol. 14:619-624.

Hartman, G. L., Sinclair, J. B., and Rupe, J. C. (Eds.). 1999. Compendium of soybean diseases. American Phytopathological Society, St. Paul, MN. 100 pp.

Hussey, R. S., and Williamson, V. M. 1998. Physiological and molecular aspects of nematode parasitism. Pages 87-108 in: K.R. Barker, G. A. Pederson, and G. L. Windham (Eds.) Plant and nematode interactions. Madison, Wisconsin. American Society of Agronomy.

Ko, M. P., Barker, K. R., and Huang, J. S. 1984. Nodulation of soybeans as affected by half-root infection with *Heterodera glycines*. J. Nematol. 16:97-105.

Koenning, S. R. 2004. Population biology. Pages 73-88 *in* D. P. Schmitt, J. A. Wrather, and R. D. Riggs, (Eds.) Biology and management of soybean cyst nematode, 2nd ed. Marceline, MO: Schmitt and Associates of Marceline.

Koenning, S. R., and Barker, K. R. 1995. Soybean photosynthesis and yield as influenced by *Heterodera glycines,* soil type and irrigation. J. Nematol. 27:51-62.

Lal, R., and Lal, A. 2006. Plant parasitic nematodes intercepted from seeds, soil clods and packing material under import quarantine. J. New Seeds 8:49-60.

Loveys, B. R., and Bird, A. 1973. The influence of nematodes on photosynthesis in tomato plants. Physiol. Plant Pathol. 3:525-529.

MacDonald, D. H., Noel, G. R., and Lueschen, W. E. 1980. Soybean cyst nematode, *Heterodera glycines*, in Minnesota. Plant Dis. 64:319–321.

MacGuidwin, A. E., Grau, C. R., and Oplinger, E. S. 1995. Impact of planting 'Bell', a soybean cultivar resistant to *Heterodera glycines*, in Wisconsin. J. Nematol. 27:78-85.

McClean P. E, Mamidi S, McConnell M, Chikara S, Lee R. 2010. Synteny mapping between common bean and soybean reveals extensive blocks of shared loci. BMC Genomics 11:184 doi:10.1186/1471-2164-11-184.

McSorley, R. 1988. Population dynamics. pages 109-133 in: Plant and nematode interactions. K. R. Barker, G. A. Pederson, and G. L. Windham (Eds.) Madison. American Society of Agronomy.

Melton, T. A, Jacobsen, B. J., and Noel, G. R. 1986. Effects of temperature on development of *Heterodera glycines* on *Glycine max* and *Phaseolus vulgaris*. J. Nematol. 18:468-474.

Nagesh, M., and Dhawan, S. C. 1988. Effect of inoculum density of *Heterodera avenae* on photosynthetic efficiency, chlorophyll, and mineral contents of wheat. Indian J. Nematol. 18:40-43

Nehra, S., Sharma, I. R., and Trivedi, P. C. 2001. Effect of different inoculum levels of nematode, *Heterodera avenae* on photosynthetic efficiency of barley (*Hordeum vulgare* L.). Asian J. Exp. Sci, 1:1-8.

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

Niblack, T. L., Baker, N. K., and Norton, D. C. 1992. Soybean yield losses due to *Heterodera glycines* in Iowa. Plant Dis. 76:943-948.

Noel, G. R. 1992. History, distribution, and economics. Pages 1-13 in: Biology and management of the soybean cyst nematode. R. D. Riggs and J. A. Wrather, eds. APS Press, St. Paul, MN.

Poromarto, S., and Nelson, B. 2008. Effects of soybean cyst nematode on growth of pinto bean. Phytopathology 98:S126 (Abstr.).

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

Postuka, J. W., Dropkin, V. H., and Nelson, C. J. 1986. Photosynthesis, photorespiration, and respiration of soybean after infection with root nematodes. Photosynthetica 20:405-410.

Riggs, R. D., and Schmitt, D. P. 1987. Nematodes. Pages 757-7788. In Soybean: Improvement, production, and uses. J. R. Wilcox, ed. 2nd ed. American Society of Agronomy, Inc., Crop Science Society of American, Inc., Soil Science Society of American, Inc., Madison.

Ross, J. P. 1969. Effect of *Heterodera glycines* on yields of nonnodulating soybeans grown at various nitrogen nevels. J. Nematol. 1:40-42.

Schans, J., and Arntzen, F. K. 1991. Photosynthesis, transpiration and plant growth characters of different potato cultivars at various densities of *Globodera pallida*. Netherland J. Plant Pathol. 97:297-310.

Seinhorst, J. W. 1970. Dynamics of populations of plant parasitic nematodes. Annu. Rev. Phytopathol 8:131-156.

Seinhorst, J. W. 1986. Effects of nematode attack on the growth and yield of crop plants. Pages 191-209 in: Cyst Nematodes. F. Lamberti, and C. E. Taylor (Eds).New York and London, Plenum Press.

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

Steele, A. E. 1974. Population dynamics of *Heterodera schachtii* on tomato and sugarbeet. J. Nematol. 7:105-111.

Wallace, H. R. 1974. The influence of root-knot nematode, *Meloidogyne javanica*, on photosynthesis and on nutrient demand by roots of tomato plants. Nematologica 20:27-33.

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

Wheeler, T. A., Pierson, P. E., Yound, C. E., Riedel, R. M., Wilson, H. R., Eisley, J. B., Schmitthenner, A. F., and Lipps, P. E. 1997. Effect of soybean cyst nematode (*Heterodera glycines*) on yield of resistant and susceptible soybean cultivars grown in Ohio. Suppl. J. Nematol. 29:703-709.

Williamson, V. M., and Hussey, R. S. 1996. Nematode pathogenesis and resistance in plants. The Plant Cell 8:1735-1745.

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

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

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

CHAPTER 6. REPRODUCTION OF SOYBEAN CYST NEMATODE ON DRY BEAN CULTIVARS OVER MULTIPLE GENERATIONS[4]

Abstract

Phaseolus vulgaris is a host of soybean cyst nematode (SCN; *Heterodera glycines*), a pathogen recently introduced into the major dry bean production area of North Dakota-northern Minnesota. The nematode reproduces less on most bean classes compared to soybean, but can reduce plant growth and seed yield. An important question is the following: will SCN adapt to dry beans and over time increase in ability to reproduce on roots? To answer this question the following experiments were conducted with cultivars from three bean classes. The cultivars Premiere and Cirrus (navy), Buster and Othello (pinto), and Eclipse and Jaguar (black) were grown in 'Conetainers' in sand in plastic pots immersed in a water bath at 27 degrees C in the greenhouse. Seedlings were inoculated with 2000 eggs per plant of SCN HG 0 and cysts were harvested and counted after 40 days. The eggs were immediately extracted from those cysts and seedlings were inoculated again and grown for 40 days using the same methods. Soybean Lee 74 was used as a control. A female index (number of cysts produced on the test plant divided by the number of cysts produced on Lee 74) was calculated for each bean cultivar after each 40 days. This procedure was repeated until 8 generations of eggs were completed and then the experiment was repeated. There was no significant ($P \leq 0.05$) change overtime in the female index on the six bean cultivars. Therefore, there was no evidence that SCN HG 0 was increasing reproduction on dry bean cultivars during two 11 month periods of continual reproduction of HG 0 on roots.

Introduction

North Dakota and northern Minnesota produce 35% of the dry edible bean (*Phaseolus vulgaris* L.) crop in the USA with a total production of 398 million Kg and a value of US $ 309 million in 2009 (NASS, 2010). Dry beans are planted in rotation with other row crops or small grains. Dry beans are a host of soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe) with different bean classes showing varying degrees of suitability for SCN reproduction (Poromarto and Nelson, 2009). SCN was reported on soybean (*Glycine max* (L.) Merr.) in North Dakota in 2003 (Bradley et al., 2004) in the southeast corner of the state and is now close to moving into the major United States dry bean production area of North Dakota which is centered in the Red River Valley. The nematode was also recently discovered in several counties close to bean production areas in northern Minnesota. At the present time, only SCN HG 0 has been found in this northern soybean production area. SCN recently was demonstrated to cause a yield loss in dry bean under field conditions (Poromarto et al., 2010). SCN, therefore, is a potential threat to the dry bean industry in this region.

Since SCN was first reported in the United States in 1955 in North Carolina (Winstead et al., 1955), this nematode has shown great ability to adapt to resistant soybean cultivars. Within a few years of using resistant cultivars, genetic variants among field isolates were discovered in many states (Miller, 1967; Riggs et al., 1968; Ross, 1962). There is substantial genetic diversity in *H. glycines*. In early research to classify SCN populations based on soybean compatibility, a race scheme was developed and numerous races identified (Dong, 1996; Riggs and Schmitt, 1988). More recently, Niblack et al. (2002) proposed the HG type-test to better describe the population variation and create a

more flexible classification system. Our knowledge of genetic variation concerning pathogenicity in SCN has come from research on soybean, not other crops.

The population dynamics of SCN have been studied extensively on soybean and shown to be a function of population densities in the field at planting and the developmental and reproductive potentials of the nematode. They are influenced by numerous biotic and abiotic factors such as temperature (Alston and Schmitt, 1988; Hamblen et al., 1972), soil characteristics such as soil type, texture and pH (Koenning et al., 1988; Todd and Perason, 1988), soil microorganisms (McLean and Lawrence, 1995), host susceptibility (Koenning, 2000; Riggs et al., 1977), population densities (Bonner and Schmitt, 1985; Miller, 1966; Todd et al., 2003) and management practices such as cropping systems, applications of pesticides and fertilizers (Bostian et al., 1986; Koenning and Barker, 1995; Koenning et al., 1993; Long and Todd, 2001; Schmitt et al., 1983; Sortland and Mac Donald, 1987).

Growing resistant soybean cultivars in the presence of an SCN population is one important factor in population dynamics. Many studies have shown that the nature of soybean resistance can influence the index of parasitism (the number of females developing on a resistant soybean cultivar expressed as a percent of those developing on a standard susceptible cultivar) of the SCN population. For example, Elliot et al. (1986) reported that continuous cropping of the resistant cultivar Bedford on an SCN population resulted in increased reproduction on Bedford relative to the susceptible cultivar Essex. Anand et al. (1983) demonstrated that the SCN population selected on PI 88788 developed better on Forrest, PI 87631-1, Cloud, PI 209332, and PI 88788 than on PI 89772 and PI 90763. On the other hand, populations selected on PI 89772 and PI 90763 developed very little on PI

88788 and PI 209332. Furthermore, Young (1984) showed the ability of four subpopulations to reproduce on four soybean lines was reversed by changing the soybean line used as a host during a second cycle of selection.

SCN is most common in soybean, but not dry bean production areas, thus there has been limited opportunity for the nematode population to interact with dry bean cultivars. Indeed, in North Dakota we have not yet found SCN infecting dry bean in a commercial production field. As SCN infests fields in dry bean production areas, the dynamics of the population may be impacted by this legume host. Compared to the susceptible soybean Lee 74, the nematode reproduces less on pinto, navy and black bean cultivars grown in this area (Poromarto and Nelson, 2009). For example, female indices for pinto, navy and black bean cultivars vary between 27-52, 23-66 and 5-22, respectively. Some cultivars, therefore, would be considered resistant to moderately resistant according to the Niblack or Schmitt-Shannon criteria (Niblack, 2005; Schmitt and Shannon, 1992) used to rate resistance in soybean. A system to rate dry bean for resistance to SCN has not yet been developed.

Since SCN can reduce dry bean yields (Poromarto et al., 2010), we are preparing for the potential impact of SCN on dry bean production. An important question that needs to be answered is "will there be a shift in the SCN population toward greater ability to reproduce on dry bean once there is continued cultivation of dry bean cultivars in infested fields?" Based on past research in soybean, our hypothesis was that over multiple generations, SCN would adapt toward greater reproduction on dry bean. This research was performed to determine if continuous growth of dry bean cultivars in a population of SCN HG 0 would select for biotypes more efficient in reproducing on dry beans.

Material and Methods

Dry bean cultivars

Six cultivars representing three dry bean classes were used to study the reproduction of soybean cyst nematode on dry bean cultivars over multiple generations. Premiere and Cirrus (navy), Buster and Othello (pinto), and Eclipse and Jaguar (black) were chosen because they had the lowest SCN reproduction in their bean class (Poromarto and Nelson, 2009). Soybean cultivars Lee-74 and Barnes were used as the susceptible soybean checks to insure that experiments for each generation had sufficient SCN reproduction for an adequate test, and for comparisons needed to obtain female indices of reproduction.

Planting, egg sources, and inoculation

The plant growth system and inoculation method were described previously (Poromarto and Nelson, 2009). Following surface disinfection with 1.0% NaOCl and a water rinse, seeds were germinated on seed germination paper for three days. Uniformly healthy seedlings were transplanted into individual plastic "Cone-tainers" (type SC10 Super Cell) containing autoclaved river sand then inoculated with eggs. The "Cone-tainers" were placed in autoclaved sand in 30.5 cm dia x 30.5 cm deep plastic pots (Cambro, Huntington Beach, CA) and immersed in a water bath at 27 ± 3ºC in a greenhouse. Plants received both natural and supplemental light using high pressure sodium lamps (1,000 $\mu E^{.} m^{-2.}s^{-1}$) for 16 h/day. Following planting, plants were watered daily to field capacity and fertilized at 14 and 21 days with three ml of a solution of Peters Hydro-Sol 5-11-26 (W.R. Grace & Co.-Conn., Fogelsville, PA; at the rate of 20 ml of Peters in 980 ml of water).

Soil naturally infested with SCN was collected from a soybean field in Richland Co., North Dakota in 2006. This was one of the first North Dakota fields with SCN identified in 2003. The population of SCN was identified as HG type 0 (24) following the methods of Niblack et al. (2002). The general methods of Niblack et al. (2002) to inoculate plants were followed with some modifications (Poromarto and Nelson, 2009). Immediately after the seedling was transplanted into the sand, it was inoculated with 2,000 eggs in 2 ml of distilled water then the seedling was covered with 15 mm of sand. The temperature of the sand in the "Cone-tainers" was recorded with WatchDog 450 Data loggers. Temperatures in the sand among experiments averaged $27 \pm 1^{\circ}$ C, but temperature variations of 3° C were recorded almost daily.

Females were harvested from roots 40 days following inoculation. The females were washed off the roots using pressurized tap water and collected on a 60-mesh (250-µm) sieve. Females from each plant were counted with a dissecting microscope. To insure mature females were removed, roots were examined with a dissecting microscope. The freshly produced eggs were immediately extracted from those females and a distilled water suspension of eggs at 1,000 eggs/ml was prepared and immediately used to inoculate newly germinated seeds with 2,000 eggs each. The same procedure was repeated until 8 generations of eggs were completed. To insure sufficient eggs were produced during each generation of eggs, additional plants were inoculated for each 40 day growth cycle. A female index (FI) (Niblack, 2005) (FI = [the average number of females on the test plant divided by the average number of females on the susceptible soybean Lee 74] times 100) was calculated to measure reproduction of SCN on dry bean.

Experimental design and analyses

The experiment design was a split plot with bean classes as main plots and cultivars as sub plots. The experiment used four replications and was repeated once. Transforming the data with Log_{10} did not improve the pattern of the residuals; thus, the non-transformed data were used in the analyses. Data were analyzed with analyses of variance using the Statistical Analyses System. Mean squares were equated to expected mean squares so that the proper F-tests were used for each source of variation that was tested for statistical significance.

Results

SCN reproduction on susceptible soybean

SCN reproduced well on the two susceptible soybean checks, Lee-74 and Barnes throughout all the experiments. The number of females/generation ranged between 305 to 793 and 271 to 759 with averages of 545 and 532 for Lee-74 and Barnes, respectively (Table 6.1). There were no significant differences in SCN reproduction over time for either susceptible soybean control. SCN produced significantly ($P < 0.001$) greater numbers of females on soybean than on dry bean (data not shown, but can be interpreted from FI's in Figure 6.1).

SCN reproduction on dry beans

Since the objective of the research was to determine if continuous growth of dry bean cultivars in a population of SCN would select for biotypes more efficient in reproducing on dry beans, we excluded the data of SCN reproduction on Lee-74 and Barnes soybean cultivars in the statistical analyses (Table 6.2). The FI's for SCN reproduction on dry bean cultivars over eight generations are shown in Figure 6.1. There

were no significant differences in reproduction as measured by FI between the experiments, among the three bean classes (pinto, navy and black bean), and between cultivars within bean classes (Table 6.2). Although the FI's for each cultivar did vary over time (Figure 6.1), there were no significant ($P<0.05$) differences among the eight generations for each dry bean cultivar (Table 6.2). Also, the bean class by generation interaction was not significant.

Table 6.1. The average number of soybean cyst nematode females produced on Lee 74 and Barnes soybean cultivars over eight generations[a].

Soybean Cultivars	Generation							
	1	2	3	4	5	6	7	8
Lee 74	305	793	422	534	572	731	531	474
Barnes	271	759	435	587	570	738	462	437

[a] Germinated seeds were inoculated with 2000 eggs/seedling of HG 0 and plants grown in sand at 27 ± 3ºC for 40 days. Females were washed off roots, counted, eggs were extracted and new seedlings were inoculated; this procedure was repeated eight times. Data shown are the averages of two experiments.

Discussion

I used Lee-74, a widely used susceptible soybean check (Niblack et al., 2002) and Barnes, a local susceptible soybean, as susceptible checks since there currently is no dry bean cultivar that is an accepted standard susceptible check for studies of SCN on dry bean. Lee-74 is considered a reliable soybean check for various reasons. For example, reproduction of race 4 of SCN on Lee, Pickett, and Peking soybean remained relatively constant when the nematodes were maintained on Lee soybeans (Niblack et al., 2002; Riggs et al., 1977). Triantaphyllou (1975) showed that the index of parasitism on Peking soybeans remained unchanged following propagation of a field population of SCN for

seven consecutive generations on Lee soybeans. In this current study, on neither of the

susceptible checks was there an increase in SCN reproduction over the eight generations.

Table 6.2. A summary of the analyses of variance of soybean cyst nematode reproduction over eight generations on cultivars of dry bean[a].

Source	Df.	MSE	F Value	P>F
Experiment	1	24.76	0.29	0.61
Bean classes	2	119.13	0.25	0.80
Cultivar (Bean classes)	3	305.05	3.35	0.17
Generation	7	865.46	2.21	0.16
Bean classes x Generation	14	275.72	1.49	0.23
Bean classes x Cultivar x Generation	21	109.83	0.92	0.57

[a] Plants inoculated with soybean cyst nematode HG 0 and grown in sand for 40 days at 27 ± 3ºC. Reproduction was measured as the number of females on the roots after 40 days. Females were washed off roots, counted, eggs were extracted and new seedlings were inoculated; this procedure was repeated eight times. Data analyzed were the averages of two experiments.

One surprising result of the study was that the FI's of the two black bean cultivars

were not significantly different from those of the pinto and navy bean cultivars. Averaged

over the eight generations and two experiments, the average combined FI of the two black

cultivars was 16 while the average combined FI's for pinto and navy bean were 17 and 15,

respectively. In previous evaluations of the resistance of dry bean cultivars using methods

similar to those used in this study (24), the two black bean cultivars had an average FI of

11 while the two pinto and navy cultivars had FI's of 30 and 24 respectively. The reasons

for the differences in FI between these two separate studies are unknown. The lack of a

bean class by generation interaction showed that none of the bean classes had altered the

reproduction of SCN over time.
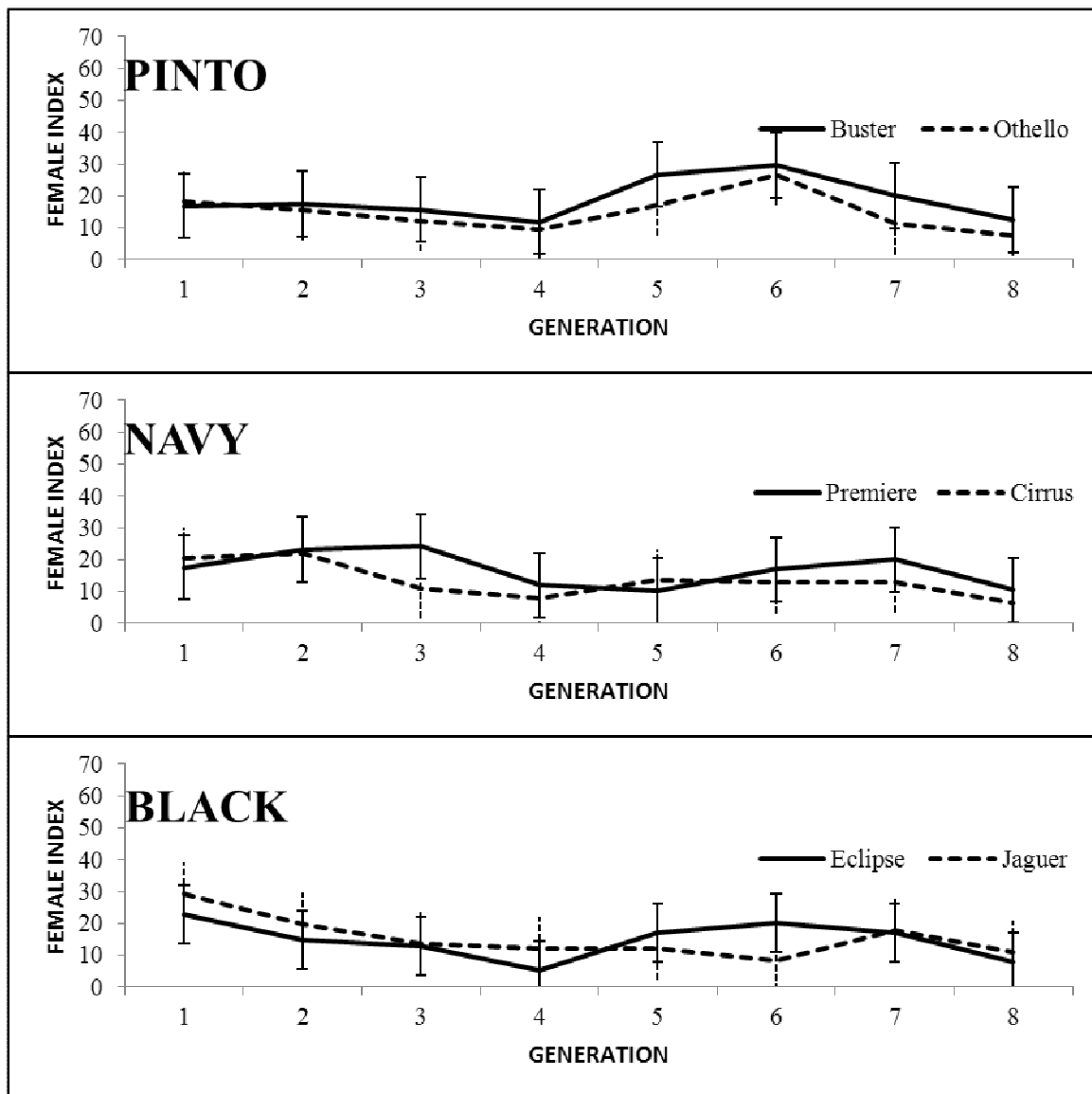
Figure 6.1. Reproduction of soybean cyst nematode on dry bean cultivars in three bean classes (pinto, navy and black) over eight generations as measured by female index (FI). FI = the average number of females on the test plant divided by the average number of females on the susceptible soybean Lee 74 times 100. Germinated seeds were inoculated with 2000 eggs/seedling of HG 0 and plants grown in sand at $27 \pm 3°C$ for 40 days. Females were washed off roots, counted, eggs were extracted and new seedlings were inoculated; this procedure was repeated eight times. Data shown are the averages of two experiments. Bar represent standard error of the mean.

Our original hypothesis, based on past research on soybean, was that SCN would

increase in ability to reproduce on resistant to moderately resistant dry bean cultivars over

consecutive plantings. An increase in the ability of SCN to reproduce following

93

continuous planting of resistant soybean cultivars has been shown in greenhouse experiments (Anand and Brar, 1983; McCann et al., 1982; Winstead et al., 1955; Trantaphyllou, 1975) and in fields infested with SCN (Francl and Wrather, 1987; Young, 1984; Young and Hartwig, 1988; Young et al., 1986). An increase in the reproduction of SCN race 3 (which is similar to HG 0) (Niblack et al., 2002) was recorded when Pickett or Peking, two resistant soybean cultivars, were the hosts for two or more consecutive inoculations, but there was no increase on the susceptible cultivar Lee when there were five continuous inoculations (Riggs et al., 1977). A similar phenomenon was also reported for SCN Races 1, 2 and 4 (Riggs et al., 1977). Triantaphyllou (1975) demonstrated an increasing index of parasitism from 2.8 to 74 for an SCN field population on the resistant Peking after seven consecutive generations. In that same study an increased index of parasitism from 16 to 85 after five generations of selection on the resistant PI 88788 soybean was also shown. The specific population of SCN can affect the outcome of such studies. Propagation of a population from a field in North Carolina for five consecutive generations on Pickett increased the index of parasitism from 22 to 73, but when that final population was tested on the resistant PI 88788, there was no change in the index of parasitism on PI 88788 (Trantaphyllou, 1975). Conversely, propagation of the same beginning population for five generations on P.I. 88788 increased the index of parasitism of the population on P.I. 88788 from 4.8 to 40, but when that final population was tested on Pickett there was no change in the index of parasitism on Pickett. This past information about increasing reproduction over time on resistant soybean cultivars by different populations of SCN was the reason for conducting this current research with dry bean.

The data from this study, however, do not support our previous hypothesis. There are at least three reasons to explain the lack of increasing female index on dry bean in this study. First, the original source of the SCN eggs was from one population taken from a 1,500 sq ft area in an infested field. The population selected may have had a narrow genetic base and lacked the ability to adapt to those dry bean cultivars. However, in general, SCN populations are heterogeneous and within a population there are genotypes that differ in their ability to reproduce on plant hosts. Miller (1971) found that progeny of single cysts from different parts of one field produced variable numbers of females on both resistant and susceptible soybeans. Another explanation is that this experiment used only HG type 0. HG 0 is the least virulent type on soybean (Niblack et al., 2002); however, virulence on soybean may or may not have any bearing on the ability to reproduce on dry bean. It is important to note that there are regions of synteny between the genomes of soybean and dry bean (McLean et al., 2010), thus there could be a similar genetic basis for resistance to SCN in the two crops. The outcome of this experiment using different HG types should, therefore, be examined. Third, the cultivars of dry beans used in this experiment have FI's greater than 10. In soybean such FI's would indicate a resistance level that is less than highly resistant. Possibly, the selection pressure needed to demonstrate an adaptation toward greater reproduction to dry bean over the time period in this study would be a highly resistant dry bean cultivar.

The most important outcome of this research was that over time there was no increase in SCN reproduction, indicating that this HG 0 population was not adapting toward increased ability to reproduce on dry bean. There are practical implications of this research if these results hold true for other populations of HG 0 in this area. It would

appear that there will not be a rapid increase in the adaptation of SCN toward increased

reproduction on some of the dry bean cultivars grown in the area. However, since this will

be a new disease for dry bean growers, a prudent approach to disease management would

be to monitor changes in the ability of field populations of SCN to reproduce on dry bean

cultivars grown in this region.

Literature Cited

Alston, D. G., and Schmitt, D. P. 1988. Development of *Heterodera glycines* life stages as influenced by temperature. J. Nematol. 20:366-372.

Anand, S. C., and Brar, G. S. 1983. Response of soybean lines to differentially selected cultures of soybean cyst nematode *Heterodera glycines* Inhinohe. J. Nematol. 15:120-123

Bonner, M. J., and Schmitt, D. P. 1985. Population dynamics of *Heterodera glycines* life stages on soybean. J. Nematol. 17:153-158.

Bostian, A. L., Schmitt, D. P., and Barker, K. R. 1986. Population changes of *Heterodera glycines* and soybean yields resulting from soil treatment with Alachlor, Fenamiphos, and Ethoprop. J. Nematol. 18:458-463.

Bradley, C. D., Biller, C. R., and Nelson, B. D. 2004. First report of soybean cyst nematode (*Heterodera glycines*) on soybean in North Dakota. Plant Dis. 88:1287.

Dong, K. 1996. Genetics and molecular aspects of *Heterodera glycines* - soybean interactions. Ph.D. diss. North Carolina State University, Raleigh

Elliot, A. P., Phipps, P. M., and Terrill, R. 1986. Effects of continuous cropping of resistant and susceptible cultivars on reproduction potentials of *Heterodera glycines* and *Globodera tabacum solanacearum.* J. Nematol. 18:375-379

Francl, L. J., and Wrather, J. A. 1987. Effect of rotating 'Forrest' and 'Bedford' soybean on yield and soybean cyst nematode population dynamics. Crop Sci. 27:565-568.

Hamblen, M. L., Slack, D. A., and Riggs, R. D. 1972. Temperature effects on penetration and reproduction of soybean-cyst nematode. Phytopathology 62:762 (Abstr.).

Koenning, S. R. 2000. Density dependent yield of *Heterodera gylicines*-resistant and susceptible cultivars. Suppl. J. Nematol. 32:502–507

Koenning, S. R., Anand, S. C., and Wrather, J. A. 1988. Effect of within-field variation in soil texture on *Heterodera glycines* and soybean yield. J. Nematol. 20:373-380.

Koening, S. R., and Barker, K. R. 1995. Soybean photosynthesis and yield as influenced by *Heterodera glycines*, soil type and irrigation. J. Nematol. 27:51-62.

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

Long, J. H., and Todd, T. C. 2001. Effect of crop rotation and cultivar resistance on seed yield and the soybean cyst nematode in full-season and double-cropped soybean. Crop Sci. 41:1137-1143.

McCann, J., Luedders, V. D., and Dropkins, V. H. 1982. Selection and reproduction of soybean cyst nematodes on resistant soybeans. Crop Sci. 22:78-80.

McClean P.E, Mamidi S, McConnell M, Chikara S, Lee R. 2010. Synteny mapping between common bean and soybean reveals extensive blocks of shared loci. BMC Genomics 11:184 doi:10.1186/1471-2164-11-184.

McLean, K. S., and Lawrence, G. W. 1995. Development of *Heterodera glycines* as affected by *Fusarium solani*, the causal agent of sudden death syndrome of soybean. J. Nematol. 27:70-77

Miller, L. I. 1966. Maturation of females of *Heterodera glycines* as influenced by inoculum level. Phytopathology 56:585.

Miller, L. I. 1967. Comparison of the pathogenicity and development of the Va. 2 isolate of *Heterodera glycines* on Pickett and Lee soybeans. Phytopathology 57:647 (Abstr.).

Miller, L. I. 1971. Physiologic variation within the Virginia-2 population of *Heterodera glycines*. J. Nematol. 3:318 (Abstr.).

National Agricultural Statistics Service. 2010. Agricultural Statistics Database. United States Department of Agriculture. Online publication. http://www.nass.usda.gov/QuickStats/index2.jsp

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

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

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

Poromarto, S., Nelson, B., and Goswami, R. S. 2010. Effect of soybean cyst nematode on growth of dry bean in the field. Plant Dis. 94:1299-1304.

Riggs, R. D., Hamblen, M. L., and Rakes, L. 1977. Development of *Heterodera glycines* pathotypes as affected by soybean cultivars. J. Nematol. 9:312-318.

Riggs, R. D., Slack, D. A., and Hamblen, M. L. 1968. New biotypes of soybean cyst nematode. Ark. Farm Res. 17:11.

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

Ross, J. P. 1962. Physiological strains of *Heterodera glycines*. Plant Dis. Rep. 46:766-769.

Schmitt, D. P., Corbin, F. T., and Nelson, L. A. 1983. Population dynamics of *Heterodera glycines* and soybean response in soil treated with selected nematicides and herbicides. J. Nematol. 15:432- 437.

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

Sortland, M. E., and Mac Donald, D. H. 1987. Effect of crop and weed species on development of a Minnesota population of *Heterodera glycines* race 5 after one to three growing periods. Plant Dis. 71:23-27.

Todd,T. C., Long, Jr. J. H., and. Oakley T. R. 2003. Density-dependent multiplication nd survival rates in. *Heterodera glycines*. J. Nematol. 35:98–103

Todd, T. C., and Pearson, C. A. S. 1988. Establishment of *Heterodera glycines* in three soil types. Ann. Appl. Nematol. 2:57-60.

Triantaphyllou, A. C. 1975. Genetic structure of races of *Heterodera glycines* and inheritance of ability to reproduce on resistant soybeans. J. Nematol. 7:356-364.

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

Young, L.D. 1984. Changes in the reproduction of *Heterodera glycines* on different lines of *Glycine max*. J. Nematol. 16:304-309.

Young, L. D., and Hartwig, E. E. 1988. Selection pressure on soybean cyst nematode from soybean cropping sequences. Crop Sci. 28:845-847

Young, L. D., Hartwig, E. E., Anand, S. C., and Widick, D. 1986. Responses of soybeans and soybean cyst nematodes to cropping sequence. Plant Dis. 70:787-791.

CHAPTER 7. SPATIAL DISTRIBUTION OF SOYBEAN CYST NEMATODE IN RESEARCH PLOTS

Abstract

Soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe (Tylenchida: Heteroderidae), is the most important pathogen of soybean (Glycine max (L.) Merr.) in the United States. The spatial distribution of SCN in ten naturally infested research sites in the Red River Valley of North Dakota was examined during 2006-2009. These sites, which ranged from 557 to 975 m^2, had been used to conduct soybean yield trials in the presence of this nematode. Egg densities were measured in plots and expressed as arithmetic means or grouped into classes using two categorical scales based on the effect of SCN populations on soybean yield. Such data were either untransformed or transformed with log10 (x+1) and used to determine spatial distribution, egg cluster sizes, minimum plot sizes and minimum replications in research size field experiments.

SCN populations varied among plots from undetected to 25,000 eggs/100 cm^3 soil, and in some sites the differences in egg densities observed between adjacent plots were as high as 6-fold. Lloyd's index of patchiness, which ranged from 1.0 to 3.3, suggested an aggregated distribution in nine of the ten sites evaluated. SCN cluster sizes were determined in five of the ten sites and ranged from 6 to 72 m^2. The optimum plot size over all sites ranged between 6 to 28 m^2 and 6 to 45 m^2 when calculated with comparable variance method (CVM) and maximum curvature technique (MCT), respectively. The minimum number of replications needed to detect specific difference among plots varied between field sites. For example, in eight of ten sites four or fewer replications were needed to detect a 15% difference of the means at the 10% confidence interval. In general,

grouping data into either of the two category groups resulted in smaller minimum plot sizes, fewer replications, and increased my ability to detect differences between plots. The spatial distribution of SCN eggs in fields can be a critical factor affecting outcomes of field experiments.

Introduction

Soybean (*Glycine max* (L.) Merr.) is one of the most important crops in United States. In 2009 the crop was planted on 31.3 million ha in the USA, producing about 89.8 million metric tons. This was about 39 % of the world's production in 2009 (Masuda and Goldsmith, 2009). In North Dakota, soybean is planted on 4.1 million acres and the state produces about 4.2 % of the national production (NASS 2010). Soybean production in North Dakota has been increasing over the past 10 years.

Soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe (Tylenchida: Heteroderidae), is the most important pathogen of soybean. The nematode has many host plants, such as soybean, dry bean, lupine, sweet clover, and chickweed, and is widely adapted to various environments and agricultural conditions (Pederson et al., 2010, Poromarto and Nelson 2009, 2010; Riggs and Hamblen 1962). SCN can reproduce wherever host plants are available and the nematode is able to survive under various environmental stresses such as low temperature (Duan et al., 2009). Since first reported in 1954 in North Carolina (Windstead 1955), the nematode has spread to 30 states and into Canada with the extension and intensification of soybean cultivation. In North Dakota, SCN was first reported in 2003 (Bradley et al., 20004). The nematodes can be spread readily from field to field in soil particles on farm machinery (Lal and Lal 2006).

Studies on the spatial distribution of SCN have been conducted primarily to gain information useful in development of disease management systems. Most studies have been conducted in large field areas and the spatial distribution has been described as aggregated (Avendaño et al., 2004, Kulkarni et al., 2008). The study of spatial distribution of SCN in research size field plots has received little attention. Francl (1986a) examined SCN distribution in a research field plot to obtain more precise sampling procedures for field experiments. He estimated that optimum plot length for minimal spatial heterogeneity in four-row mechanically tended field plots was about 6 m after trimming plot ends (Francl 1986a) and he suggested taking at least 20 soil cores to obtain an adequate sample representing the population density at the beginning of the season after plowing (Francl 1986b).

Nematode egg density and spatial distribution usually are unknown before field experiments begin, and it is not practical to create plots with uniform egg distribution. Furthermore, egg density and spatial distribution cannot be estimated based on symptoms on the previous crop. Up to 30% yield loss has been observed on susceptible compared to resistant cultivars in areas of fields heavily infested with SCN without any differences in plant height or chlorosis between the cultivars (Niblack et al., 2004). Knowledge of the spatial distribution of nematodes is important in understanding their biology, in devising sampling programs, and in understanding the results from research plot studies. In preliminary research in North Dakota when testing cultivars for resistance to SCN under field conditions, large differences in egg densities were noted between research plots. Because these differences could affect the results from research conducted under field conditions, an in depth examination of the spatial distribution of egg densities in research

size areas in naturally infested fields was conducted. The objectives of this study were 1) measure SCN egg population density in research size field plots and analyze the spatial distribution of SCN in a number of research field sites, 2) calculate minimum research plot size using various criteria and 3) determine minimal plot replications for a site. In addition to the using the biological mean, categorical data were employed to examine each of these objectives.

Material and Methods

Field sites

Ten research sites were established between 2006 and 2009 in fields naturally infested with SCN. These sites had histories of soybean production and SCN. All sites were used for evaluating soybean cultivars for yield in the presence of SCN. This study used the plots in those sites to examine spatial distribution of SCN. The research sites were established in four different 65 ha fields. Fields 1-3 were located in Richland Co., ND, near Dwight and Mooreton and field 4 was in Cass, Co., near Arthur, ND. In fields 1-3, experiments were conducted over multiple years, but each year the research sites were located in different parts of the fields. Research sites A, C, E and H were in field 1 at 46° 18' 33.84" N and 96° 50' 18.06" W; sites B, D, and F were in field 2 at 46° 20' 07.96" N and 96° 51' 32.68" W; sites G and I were in field 3 at 46° 17' 36.85" N and 96° 50' 35.71" W; and site J was in field 4 at 47° 05' 40.09" N and 97° 16' 59.30" W. The research sites ranged from 557 to 975 m^2. The soil types for these field sites were the following: Arveson loam, sites A, C, E and H; Wyndmere loam, sites B, D, and F; Galchutt-Wheatville silt loam, sites G and I; and Glyndon-Tiffany silt loam, site J.

Research in sites A and B were conducted in 2006 and the two sites contained ninety six and seventy two plots, respectively. Plots were 6.9 m^2 and contained 4 plant rows with 38 cm spacing between rows. Sites C and D, were established in 2007 and contained seventy five plots each. Plots were 9 m^2 and contained 4 rows spaced 76 cm apart. Sites E, F, and G each had 40 plots and sites H, I, and J each had 28 plots and were established in 2008 and 2009, respectively. The plot size in sites E, F, G, H, I, and J was 6.4 m^2, containing 2 rows with 76 cm spacing between rows. The plot size used in each field experiment is referred to as the basic unit.

Determining egg densities

The SCN egg density in each plot was assessed within two weeks after planting which occurred within the last two weeks of May. Oakfield soil probes were used to sample the soil to a depth of 15 cm. In sites A through D, three soil cores were taken at arbitrary locations along each row for 12 soil cores total per plot. In all the other sites, five soil cores were taken at random along the two inside rows for 10 soil cores total per plot. Soil cores from each plot were broken up by hand, air dried, and then mixed in a Twin Shell dry blender for 1 minute. The soil was then processed to determine egg densities.

To extract SCN, soil was processed as follows: (i) Soil (100 cm^3) was placed into 2,400 ml of water, and then stirred vigorously to break up any clumps and mix the cysts into a suspension, (ii) coarse soil particles were allowed to settle out for 4 seconds and the supernatant was poured over an 18 mesh sieve to filter out larger debris and the filtered liquid collected, (iii) the supernatant was then poured over a 60 mesh sieve, which retained cysts, (iv) the cysts on the 60 mesh screen were then crushed in water with a Wheaten Potter Elvehjen Tissue Grinder (55 ml capacity) and the eggs were collected by pouring the

suspension over a 200 mesh screen nested over a 500 mesh screen. The eggs on the 500

mesh sieve were collected in 50 ml of water and the eggs in two separate 1 ml aliquot were

counted with a compound microscope. The average of the two samples was calculated and

the egg density per 100 cm^3 of soil was determined for each plot at each site.

Calculating mean egg density

Four methods of expressing mean egg density were used. The first method

consisted of calculating the arithmetic mean using raw egg density numbers (UD =

untransformed mean egg density) and second, the mean was transformed with log10 (x+1)

(TD = transformed mean egg density). In the third and fourth methods, readings of raw egg

densities were grouped into 6 and 4 different categories, respectively. The median of the

data in each category was determined and transformed with log10 (x+1). The third and

fourth methods were termed GD1 (= transformed grouped mean egg density 1) and GD2 (=

transformed grouped mean egg density 2), respectively.

GD1 was established following the classification suggested by Hershman (2010),

which considers the egg density levels at which susceptible compared to resistant cultivars

would potentially be affected by SCN. GD1 categories were:

0 = Eggs not detected (0 eggs per 100 cm^3 soil)

1 = Very low (1 – 200 eggs per 100 cm^3 soil)

2 = Low (201 – 400 eggs per 100 cm^3 soil)

3 = Moderate (401-1,200 eggs per 100 cm^3 soil)

4 =High 1,201-2,000 eggs per 100 cm^3 soil)

5 =Very High (>2,001 eggs per 100 cm^3 soil)

The four categories used in the GD2 scale were based on a scale published online by the

Plant Health Initiative (http://www.planthealth.info/scn_mgmnt.htm). The rationale for the

scale was similar to the Hershman scale but with only four categories of egg densities.

GD2 categories were:

    0 = No eggs detected (0 eggs per 100 cm3 soil)

    1 = Low (1 – 2000 eggs per 100 cm3 soil)

    2 = Moderate (2001 – 12,001 eggs per 100 cm3 soil)

    3 = High (>12,001eggs per 100 cm3 soil)

Lloyd's index of patchiness (LIP)

The spatial distribution of SCN eggs in each site was characterized using three

approaches: Lloyd's index of patchiness (LIP) (Lloyd 1967), median to mean ratio (MMR),

and correlations between MMR and LIP. LIP was calculated as

$$\text{LIP} = 1 + [\frac{(s^2-m)}{m^2}]$$

where m and s^2 are the mean and the variance of the sample values, respectively. If LIP

equals one, the distribution will be considered to be at random; if LIP is greater than one,

the distribution will be considered to be aggregated; and when LIP is less than one, the

distribution will be considered to be uniform. LIP Indices were calculated using the

untransformed mean egg density (UD) and the GD1 and GD2 data that was transformed

with log10 (x+1). The median/mean ratios (MMR) for egg densities were calculated for

each site. Correlations between MMR's and LIP's were conducted using Microsoft Excel

2010.

Cluster size

Cluster sizes were calculated for sites A, B, C, D, E, F, and G by using the quadrat variance method (Campbell and Madden, 1990). The transformed mean egg density (UD) and the data grouped into two different categories (GD1 and GD2) were used for these calculations. Sites H, I, and J were not included in the calculations because they had too few data points. The variances were calculated for successively larger blocks of sample quadrat counts collected from a grid of contiguous quadrats. The stepwise increase in quadrat size was through doubling the previous plot size. The peak of maximum variance was determined to be the cluster size.

Optimum plot size

Two methods were employed to estimate optimum plot size, comparable variance methods (CVM) as suggested by Keller (1949) and maximum curvature technique (MCT), as modified by Leilah and AlBarrak (2005). The transformed mean egg density (TD) and the transformed data from categories GD1 and GD2 were used to calculate both CVM and MCT.

The formula of CVM method was:

$$V = V_x / X^2$$

where:

V = Comparable Variance.

V_x = Variance among plots.

X = Plot size in multiples of unit plots.

The optimum plot size was determined as follows: First, the variances among plots (Vx) were calculated. Second, the relative information (RI %) were determined by

106

calculating the ratios (%) between values of Vx and value of variance obtained from the smallest plot size (one basic unit). Third, the RI values were plotted against plot sizes in multiples unit plots (X). The optimum plot size was determined when RI decreased rapidly to a point where relatively no change were detected after that point

The second method used the maximum curvature technique (MCT), as modified by Leilah and AlBarrak (2005). In this technique, the coefficient of variability (CV) was plotted against the increase in plot size. The optimum plot size was determined at the point where the coefficient of variability starts to decrease slightly.

Minimum number of plot replications

The minimum number of plot replications was estimated according to the methods of Hathaway and Williams (1958). The transformed mean egg density (TD) and the transformed data from GD1 and GD2 were used for these calculations with the following formula:

$$n \geq \frac{(t^2)(s^2)}{(d^2)(\bar{x}^2)})$$

        n = Estimated sample number

        t = significant value of t in the test at 0.05 probability

        s = sample variance

        d = limit of confidence interval

        $\bar{x}$ = sample of mean

Convenience plots

Convenience plots are plots of a certain size and number of replications required to detect a difference of a specified size. Convenience plots were estimated for each site according to the methods developed by Hatheway (1961). The transformed mean egg

density (TD) and the transformed data from GD1 and GD2 were used in the following equation:

$$X^b = \frac{2 * (t_1 + t_2)^2 CV^2}{rd^2}$$

where;

X = number of basic units.

b = Smith's index of soil variability.

t1 = significant value of t in the test; degree of freedom (*df*) =30.

t2 = value of t in the table corresponding to 2(1-*P*), where P is the probability of obtaining a significant result at *P*=0.85)

CV = Coefficient of Variability.

r = number of replications.

d = percentage of the mean of difference to be detected

Smith' soil variability index (b) was calculated by:

$V_x = V / (X^b)$,

where;

V = Variance of unit plot.

V_x = Variance, on a per unit basis, of a plots formed from adjacent units.

X = Plot size in multiples of adjacent unit plots.

Results

Egg densities and spatial distribution

Egg densities of SCN varied within and among research sites (Table 7.1, Figure 7.1 and 7.2). Within research sites, egg densities ranged from undetected levels to 6,500 in site C, (Figure 7.1), 0 to 16,700 in site A (Figure 7.1), 0 to 20,750 eggs in site D (Table 7.1,

Figure 7.1), and 0 to 25,000 in site G (Table 7.1, Figure 7.2). Site E had the highest mean egg density with 7,840 eggs per 100 cm^3 soil (Table 7.1, Figure 7.1. E), while site C had the lowest overall mean density at 951 eggs per 100 cm^3 (Table 7.1, Figure 7.1. C). In all sites but J, the mean numbers of eggs (raw data = UD) was higher than the median.

The LIP's for all sites are shown in Table 7.1. Using untransformed data (UD), the LIP's for sites ranged from 1.09 to 3.34. The numerical ranking of the LIP's among the three methods of determining mean egg density were similar with site C having the highest LIP followed by site A, while site J had the lowest LIP. In this study, the sites with lowest mean egg density tended to have greater LIP's.

The mean to median ratios (=MMR) ranged between 0.98 and 2.72 in ungrouped data (UD), while in grouped data, the ratios ranged between 0.92 and 1.00 in GD1, and 0.89 and 1.05 in GD2. A strong correlation ($R^2 = 0.94$) between MMR and LIP occurred for the untransformed data (UD) which implied that the greater the MMR, the more aggregated the spatial distribution. The correlation between MMR and LIP for the grouped data (GD1 and GD2), however, was low at $R^2 = 0.41$ to 0.45.

Cluster size

SCN cluster size was determined for five of the ten sites (Table 7.2, Figure 7.3). In sites H, I and J, insufficient samples were available to determine cluster size and in site A and C cluster sizes could not be determined (Figure 7.3). Using transformed egg density data or categorical transformed data produced SCN clusters of similar sizes in sites D and E; but not in site B, F and G. Cluster sizes in sites E were 26 m^2 (4 basic units), in site D it was 72 m^2 (8 basic units). In site B cluster size was 55 m^2 (8 basic units) in transformed data (TD) and 28 m^2 (4 basic units) in the grouped data (GD1 and GD2). In site F, cluster

sizes were 52 m^2 (8 basic units) in the GD1 and GD2 and it could not be determined

(Figure 7.3) in TD. In site G, cluster sizes were 26 m^2 in TD and GD1, and 6 m^2 in GD1.

No correlation between cluster size and LIP ($r^2 = 0.04$) occured.

Optimum plot size, replications and convenience plot size

The optimum plot size over all sites ranged between 1 to 4 times and 1 to 6 times

larger than the basic plot size used in the experiment when calculated with CVM and MCT,

respectively (Table 7.2). For example, at site A using the CVM method and any of the

three data sets, the optimum plot size was 28 m^2, about four times larger than the 6.9 m^2

plots used to establish the plot. For site C, using the MCT method, optimum plot size was

estimated to be 45 m^2, five times larger than the 9 m^2 used as the plot size. All transformed

data grouped in six categories (GD1) or transformed data grouped in four categories (GD2)

in site J fell into one category. For this reason, the optimum plot sizes were determined as

6 m^2 or one basic unit.

The data set, whether categorical (GD1 and GD2) or based on transformed mean

egg count (TD), used to calculate optimum plot sizes with the CVM method did not

influence the final outcome; optimum plots were of similar size, except in site G using

GD1. All transformed data grouped in six categories (GD1) in site G fell into one

category. For this reason, the optimum plot sizes were determined as 6 m^2 or one (basic

unit). However, when using MCT method, the sizes of optimum plots were more variable.

For example, site E had an optimum plot size about twice as large using the transformed

mean egg density compared to the grouped data. On the other hand, in site I, the optimum

plot size was similar when calculated with the transformed mean egg density compared to

the grouped data.

Table 7.1. Range, median and mean of *Heterodera glycines* egg densities/100 cm^3 soil and Lloyd's Index of Patchiness for ten field research sites.

Site	Total Sample	Eggs per 100 cc soil (Range)	UD[a] Mean	Sd[d]	Median	LIP[e]	GD1[b] Mean	Sd	Median	LIP	GD2[c] Mean	Sd	Median	LIP
A	96	0-16,700	2,630	2,805	1,825	2.14	3.10	0.81	3.19	1.11	3.13	0.80	2.98	1.10
B	72	950-15,700	5,778	3,697	5,175	1.41	3.72	0.20	3.81	1.01	3.71	0.21	3.76	1.01
C	75	0-6,500	951	1,452	350	3.34	2.31	1.11	2.52	1.42	2.42	1.06	2.73	1.36
D	75	0-20,750	5,192	3,505	4,159	1.46	3.65	0.45	3.76	1.02	3.63	0.46	3.71	1.02
E	40	1,300-23,350	7,840	5,165	7,025	1.43	3.87	0.20	3.92	1.00	3.83	0.25	3.82	1.01
F	40	1,050-9,100	3,300	1,966	2,550	1.36	3.47	0.21	3.63	1.01	3.47	0.22	3.63	1.01
G	40	2,200-25,000	7,659	4,567	6,550	1.36	3.88	0.00	3.88	1.00	3.86	0.13	3.81	1.00
H	28	450-13,000	3,970	3,350	2,675	1.71	3.48	0.36	3.76	1.02	3.48	0.31	3.73	1.01
I	28	1,400-11,250	3,818	2,132	3,325	1.31	3.58	0.09	3.61	1.00	3.57	0.09	3.61	1.00
J	28	2,850-9,400	5,943	1,803	6,075	1.09	3.77	0.00	3.77	1.00	3.77	0.00	3.77	1.00

[a]UD= untransformed mean egg density

[b]GD1 = data were grouped into six categories of egg levels: 0 = eggs undetected; 1 = 1-200 eggs/100 cm3 soil; 2 = 201-400 eggs; 3=401-1,200 eggs; 4=1,201-2,000 eggs; 5>2,001 eggs. The median of the egg count for each category was transformed with log 10(x+1).

[c]GD2 = data were grouped into four categories of egg levels: 0 = eggs undetected; 1 = 1-2,000 eggs/100 cm3 soil; 2 = 2001-12,000 eggs; 3>12,001 eggs. The median of the egg count for each category was transformed with log 10(x+1).

[d]Standadr deviation of mean

[e]LIP= Loyd Index of Patchiness
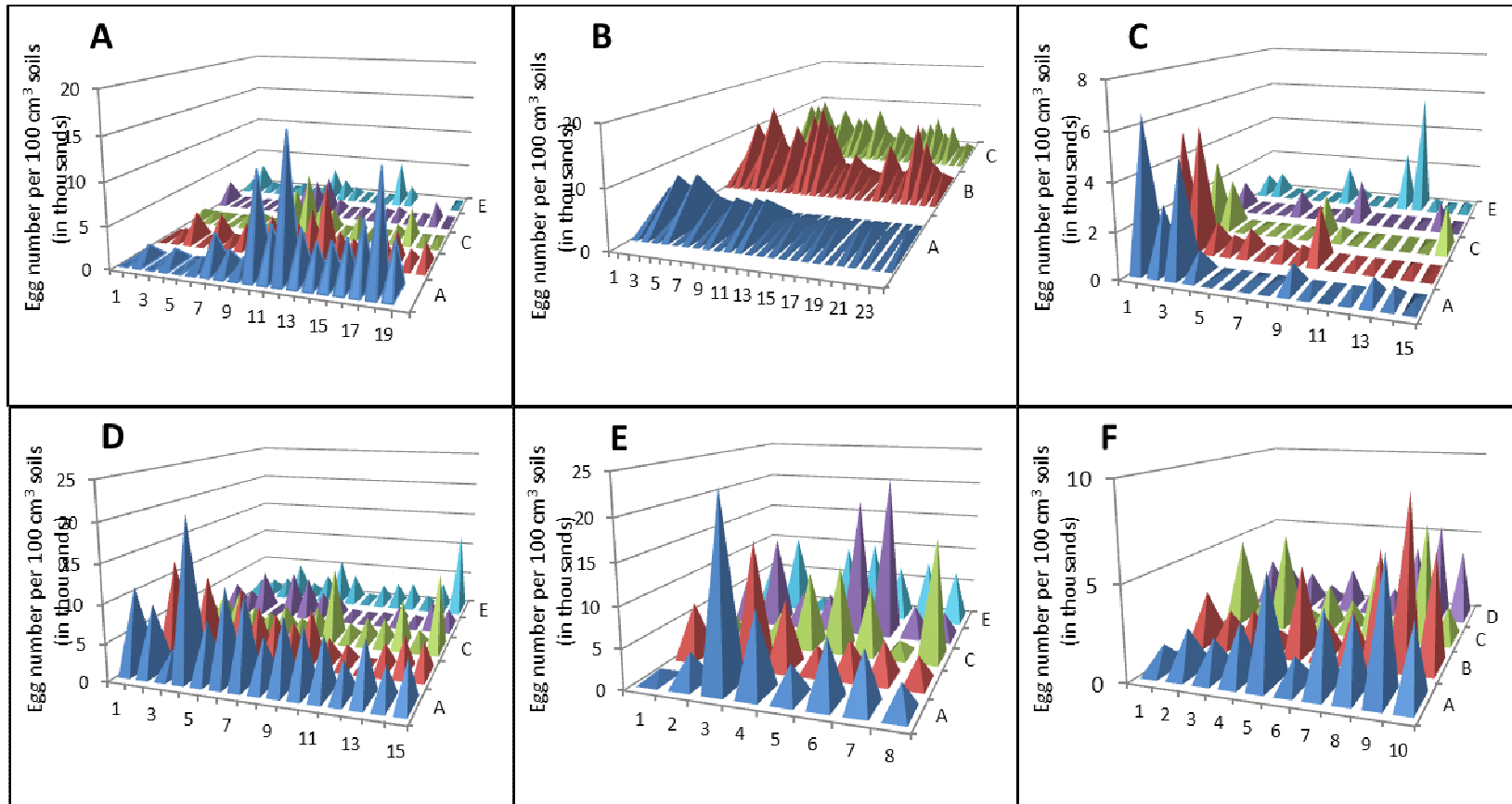
Figure 7.1. Spatial distribution of soybean cyt nematode eggs in individual research plots in different field experiments (A-F). The number and letter indicated plots and ranges, respectively.
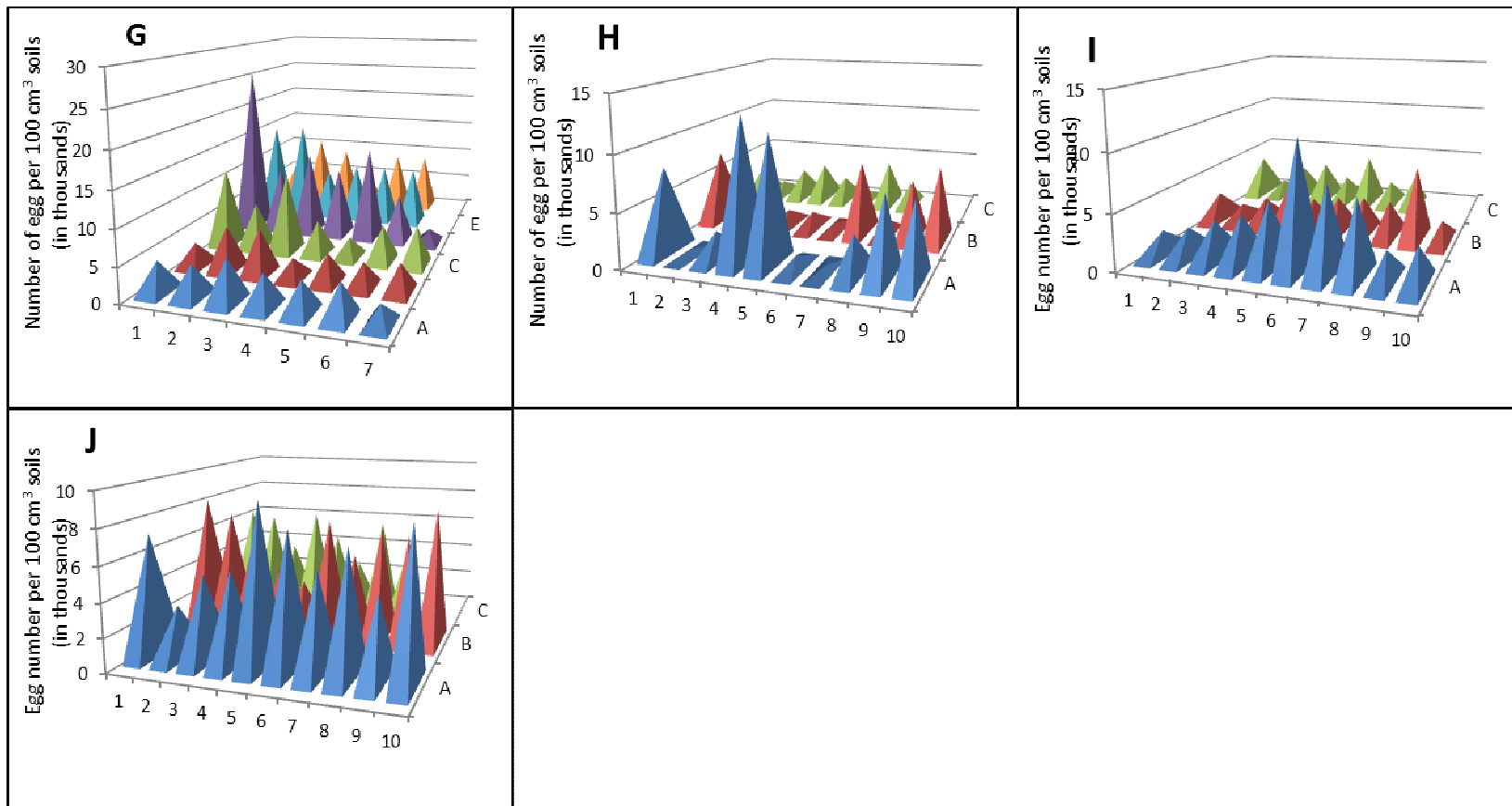
Figure 7.2. Spatial distribution of soybean cyt nematode eggs in individual research plots in different field experiments (G-J). The number and letter indicated plots and ranges, respectively.

Table 7.2. Cluster size and optimum size for plots based on *Heterodera glycines* spatial distribution of eggs in ten field sites.

Site	Data	CV[d]	b[e]	B[f]	Cluster Size	Optimum Plot Size (m²)		r[i]
						CVM[g]	MCT[h]	
A	TD[a]	27.59	0.516	0.26	ND	28	28	0.84
	GD1[b]	27.34	0.512	0.26	ND	28	28	0.82
	GD2[c]	25.93	0.582	0.29	ND	28	28	0.85
B	TD	8.20	0.538	0.27	55	21	35	0.93
	GD1	6.01	0.866	0.43	28	21	21	0.86
	GD2	6.36	0.922	0.46	28	21	21	0.91
C	TD	49.28	0.548	0.27	ND	27	45	0.78
	GD1	48.73	0.62	0.31	ND	27	45	0.80
	GD2	45.67	0.616	0.31	ND	27	45	0.84
D	TD	13.97	1.24	0.62	72	27	36	0.70
	GD1	12.74	1.78	0.89	72	27	36	0.68
	GD2	13.03	1.38	0.69	72	27	36	0.79
E	TD	7.66	3.038	1.52	26	19	38	0.52
	GD1	5.18	1.296	0.65	26	19	19	0.99
	GD2	6.49	1.764	0.88	26	19	19	0.95
F	TD	7.38	0.576	0.29	ND	13	13	0.82
	GD1	6.24	0.722	0.36	52	13	13	0.83
	GD2	6.11	0.748	0.37	52	13	13	0.84

Table 7.2. (Continue).

Site	Data	CV[d]	b[e]	B[f]	Cluster Size	Optimum Plot Size (m²)		r[i]
						CVM[g]	MCT[h]	
G	TD	6.23	0.24	0.12	26	26	6	0.67
	GD1	0.00	0	0.00	6	6	6	0.99
	GD2	3.57	0.636	0.32	26	26	6	0.77
H	TD	11.54	2.552	1.28	NA	19	26	0.79
	GD1	10.41	2.53	1.27	NA	19	26	0.43
	GD2	9.85	2.306	1.15	NA	19	26	0.71
I	TD	5.79	0.168	0.08	NA	19	6	0.23
	GD1	3.39	0.976	0.49	NA	19	6	0.71
	GD2	3.39	0.976	0.49	NA	19	6	0.71
J	TD	3.88	1.424	0.71	NA	19	19	0.71
	GD1	0.00	1	0.5	NA	6	6	0.99
	GD2	0.00	1	0.5	NA	6	6	0.99

[a] TD= transformed mean egg density (Log 10(x+1)).

[b] GD1 = data were grouped into six categories of egg levels: 0 = eggs undetected; 1 = 1-200 eggs/100 cm3 soil; 2 = 201-400 eggs; 3=401-1,200 eggs; 4=1,201-2,000 eggs; 5>2,001 eggs. The median of the egg count for each category was transformed with log 10(x+1).

[c] GD2 = data were grouped into four categories of egg levels: 0 = eggs undetected; 1 = 1-2,000 eggs/100 cm3 soil; 2 = 2001-12,000 eggs; 3>12,001 eggs. The median of the egg count for each category was transformed with log 10(x+1).

[d] CV = coefficient of variability

[e] b = estimated parameter for equations $V_x = V_1/X^b$

[f] B = estimated parameter for equations $CV_x = AX^B$

[g] CVM = comparable variance methods

^{i} r= determination coefficient

ND = Not determined

NA = Not available due to insufficient samples.

The minimum number of plots (i.e., replications) needed to compensate for the aggregated pattern of spatial distribution was lower when using categorical data, i.e. GD1 and GD2, compared to using transformed egg density (TD) (Table 7.3). Site C had the highest replications while site J had the lowest. By using 10% confidence interval, sites A and C had highest minimal plot number with a range of 15 to 86, whether data were grouped or not. In contrast, Sites G, I and J had the lowest minimum plot numbers which ranged from 2 to 3. All the data from sites G and J fit into only one category in either of the two category groups. For site I, most data points fit into one category, but there were a few that fit into different categories within both category groups.

Table 7.3. Minimum number of replications for plots based on *Heterodera glycines* spatial distribution of eggs in ten field sites.

PMD^b	Data	Field Site									
		A	B	C	D	E	F	G	H	I	J
5	TD^c	60^f	6	337	13	5	6	5	11	5	3
	GD1^d	58	5	322	11	4	5	2	9	3	2
	GD2^e	52	5	258	12	4	5	3	8	3	2
10	TD	17	4	86	5	3	3	3	5	3	3
	GD1	16	3	84	5	3	3	2	4	3	2
	GD2	15	3	74	5	3	3	3	4	3	2

Table 7.3. (Continue).

15	TD	9	3	40	4	3	3	3	4	3	3
	GD1	9	3	38	4	3	3	2	3	2	2
	GD2	8	3	31	4	3	3	2	3	2	2
20	TD	6	3	23	3	3	3	3	3	3	2
	GD1	6	3	22	3	2	3	2	3	2	2
	GD2	6	3	18	3	3	3	2	3	2	2
30	TD	4	3	12	3	2	3	2	3	2	2
	GD1	4	2	12	3	2	2	2	3	2	2
	GD2	4	2	10	3	2	2	2	3	2	2

[a] Based on one basic unit plot size. One basic unit is equal to 6.9 m2 (for site A and B), 9 m2 (for site C and D), and 6.4 m2 (for site E, F, G, H, I, and J).

[b] PMD = percentage of the mean of difference to be detected

[c] TD = transformed mean egg density (Log 10(x+1))

[d] GD1 = data were grouped into six categories of egg levels: 0 = eggs undetected; 1 = 1-200 eggs/100 cm3 soil; 2 = 201-400 eggs; 3=401-1,200 eggs; 4=1,201-2,000 eggs; 5>2,001 eggs. The median of the egg count for each category was transformed with log 10(x+1).

[e] GD2 = data were grouped into four categories of egg levels: 0 = eggs undetected; 1 = 1-2,000 eggs/100 cm3 soil; 2 = 2001-12,000 eggs; 3>12,001 eggs. The median of the egg count for each category was transformed with log 10(x+1).

[f] Number of replications

The relationship between plot sizes, number of replications and percent detectable differences for all fields are shown in Figure 7.4-12. In general, grouping data into either of the two category groups (GD1 and GD2) resulted in smaller minimum plot sizes, fewer replications, and increased my ability to detect differences between plots. For example, in site I using the transformed egg density (TD), to detect a 5% difference with the original

plot size would require five replications, however, when data were grouped into category GD1 or GD2, only three replications would be needed.

Because the plot sizes used in this research are commonly used in soybean research, I determined the convenient plot size based on spatial distribution of SCN and variability of egg density data. Using the data from Figure 7.3, the number of replications for a given percent detectable difference at one basic plot size was compared with the LIP for each site (Table 7.4, 7.5, 7.6). The most convenient plot paralleled the LIP, especially when the data were grouped (GD1 and GD2), with the most convenient plot associated with smaller LIP's (Table 7.4, 7.5, 7.6). The only exception to this was in site D and E when using data from the transformed mean egg density (TD) (Table 7.4). Site H for example, when using the transformed egg density, is relatively more convenient than D since the minimal replication needed for this site is 3 compared to 4 when I want to detect a 30% difference of the means, although site D has a smaller LIP (1.46) than site H (LIP=1.71). The higher CV of site D compared to site B might be responsible for that difference (Table 7.2).

<div align="center">

Discussion

</div>

Spatial distribution of SCN is affected by nematode movement and population dynamics (Gavassoni et al., 2007). Any means of moving soil with cysts containing eggs, such as runoff or flood water, wildlife, wind, and human activities are responsible for movement of SCN within and between fields. The genetics of the SCN population, the initial egg density, egg survival, edaphic factors (soil texture, pH, etc.), and the host (genetic status, vigor, root development) affect reproduction and development of the nematode. SCN reproduces at an optimum temperature around 26º C and reproduction is greater at pH 6.5 and 7.5 than at lower pH's (Anand et al., 1995, Pedersen et al., 2010).

More cysts develop on plants in disturbed than in undisturbed soils (Young 1987). *H. glycines* has been shown to be disseminated 6.9 m from an infestation site in conventional and reduced tillage treatments, but only 0.5 and 1.4 m for no-tillage and ridge-tillage treatments (Gavassoni 2007). Before cultivation, the distance between population clusters of this nematode can be only 1-3 meters (Francl 1986a). Directional spatial dependence of egg and cyst densities occurred along soybean rows, coincident with the direction of tillage practices (Gavassoni et al., 2007). However, egg densities commonly vary over relatively short distances within a field.

The mean egg densities in nine of the ten research sites would be considered moderate to high levels of SCN according to the Hershman (G1) or Plant Initiative scales (G2). Thus, these sites had egg densities that would be adequate for research on the soybean/SCN interaction. However, in most sites, the egg density of SCN in one plot was generally not similar to the density in an adjacent plot. The LIP's at all but one site indicated an aggregated spatial distribution of SCN when using untransformed mean egg density. Site J was the only site with a random distribution of eggs. The fact that all sites except J has mean number of eggs higher than the median also indicated egg densities were not normally distributed. The results of this study on spatial distribution in research plots are similar to what has been found in large fields, where densities vary from area to area and most fields have an aggregated spatial distribution of SCN (Avendaño et al., 2004).
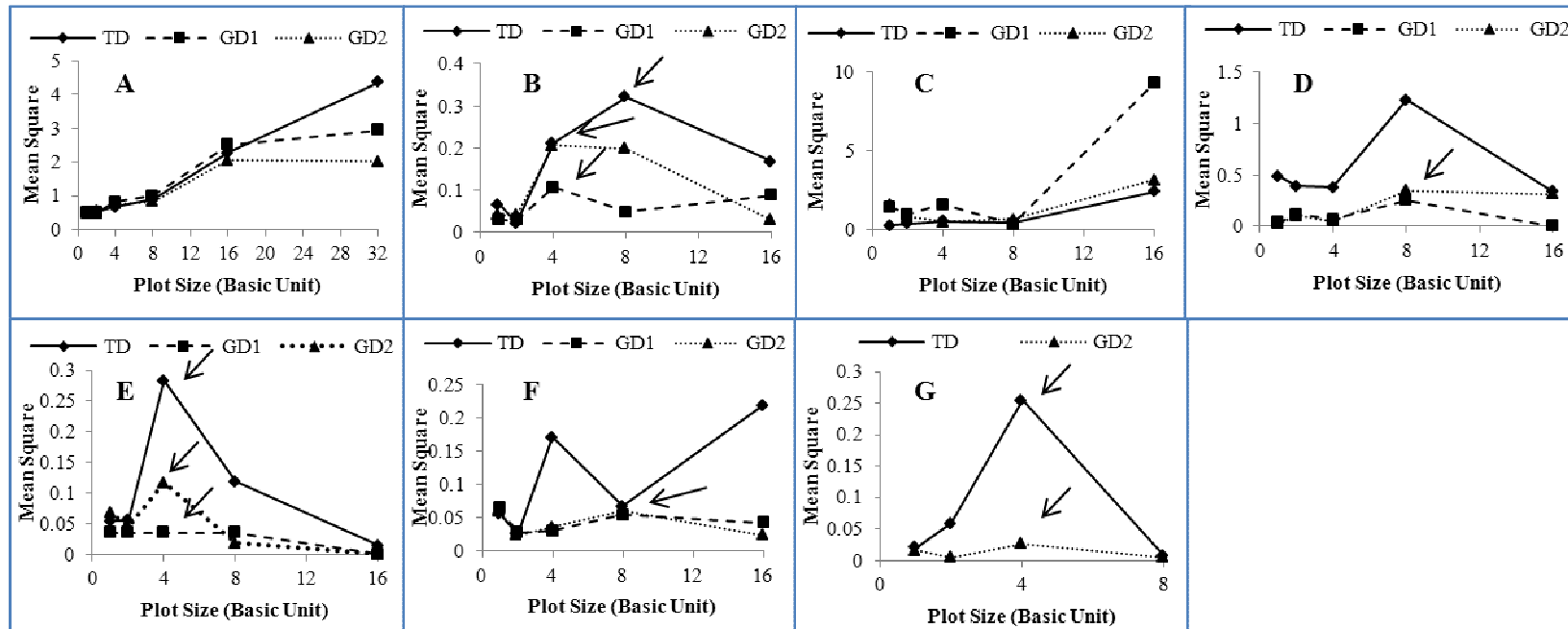
Figure 7.3. Cluster size sites for plots with eggs of soybean cyst nematodes in seven field experiments (A-G). TD= transformed mean egg density (Log 10(x+1)); GD1 = data were grouped into six categories of egg levels: 0 = eggs undetected; 1 = 1-200 eggs/100 cm3 soil; 2 = 201-400 eggs; 3=401-1,200 eggs; 4=1,201-2,000 eggs; 5>2,001 eggs. GD2 = data were grouped into four categories of egg levels: 0 = eggs undetected; 1 = 1-2,000 eggs/100 cm3 soil; 2 = 2001-12,000 eggs; 3>12,001 eggs. The median of the egg count for each category was transformed with log 10(x+1). Arrows show peaks or local maximums in the variance. The cluster sizes are the points of plot sizes right away below these peaks. One basic unit is equal to 6.9 m2 (for site A and B), 9 m2 (for site C and D), and 6.4 m2 (for site E, F, G, H, I, and J). Note: Cluster sizes in site A and C were undetected due to the peak located in the largest plot size.
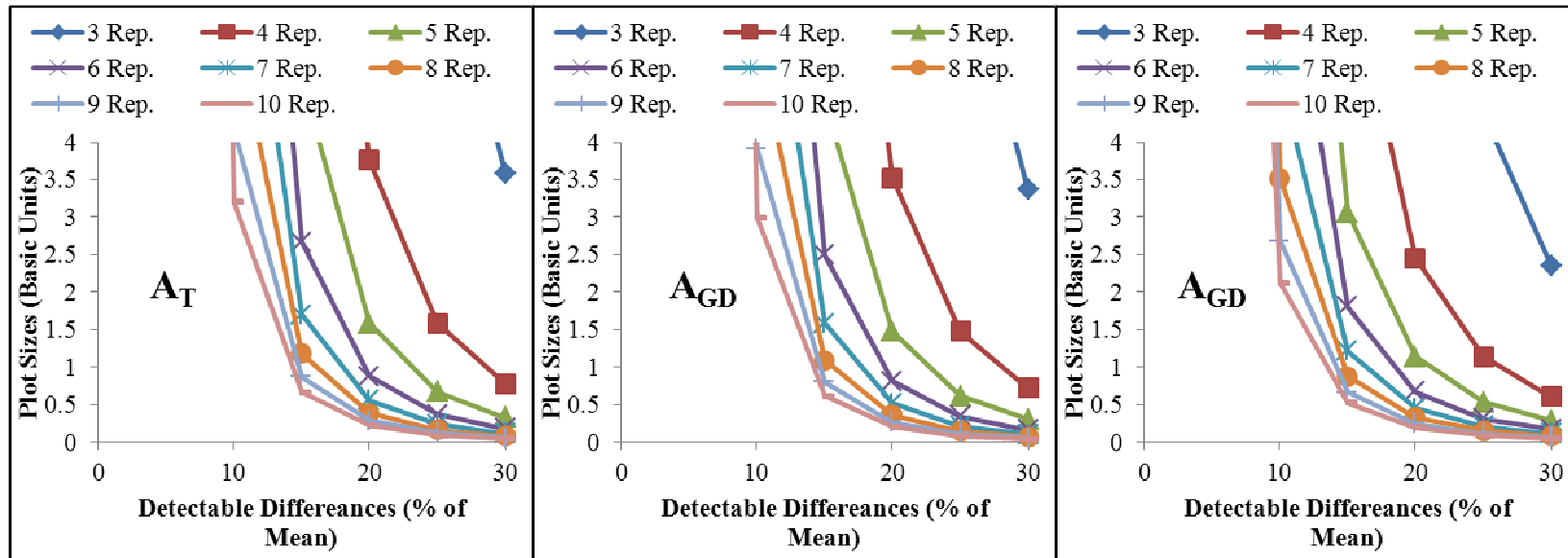
Figure 7.4. The relationship between replications and plot sizes, and detectable differences (% of means) for egg density data from field research site A. The basic unit refers to the plot size in the individual experiment. TD= transformed mean egg density (Log 10(x+1)); GD1 = data were grouped into six categories of egg levels: 0 = eggs undetected; 1 = 1-200 eggs/100 cm3 soil; 2 = 201-400 eggs; 3=401-1,200 eggs; 4=1,201-2,000 eggs; 5>2,001 eggs. GD2 = data were grouped into four categories of egg levels: 0 = eggs undetected; 1 = 1-2,000 eggs/100 cm3 soil; 2 = 2001-12,000 eggs; 3>12,001 eggs. The median of the egg count for each category was transformed with log 10(x+1). One basic = 6.9 m^2.

Figure 7.5. The relationship between replications and plot sizes, and detectable differences (% of means) for egg density data from field research site B. The basic unit refers to the plot size in the individual experiment. TD= transformed mean egg density (Log 10(x+1)); GD1 = data were grouped into six categories of egg levels: 0 = eggs undetected; 1 = 1-200 eggs/100 cm3 soil; 2 = 201-400 eggs; 3=401-1,200 eggs; 4=1,201-2,000 eggs; 5>2,001 eggs. GD2 = data were grouped into four categories of egg levels: 0 = eggs undetected; 1 = 1-2,000 eggs/100 cm3 soil; 2 = 2001-12,000 eggs; 3>12,001 eggs. The median of the egg count for each category was transformed with log 10(x+1). One basic = 6.9 m^2. Notice, in general, grouping data into either of the two category groups (GD1 and GD2) resulted in a reduction of minimum plot size, fewer replications, or smaller detectable differences. Based on one unit size plot, at least 4 replications are needed for TD when I want to detect 10% difference of the mean, while using GD1 and GD2 only 3 replications are needed.
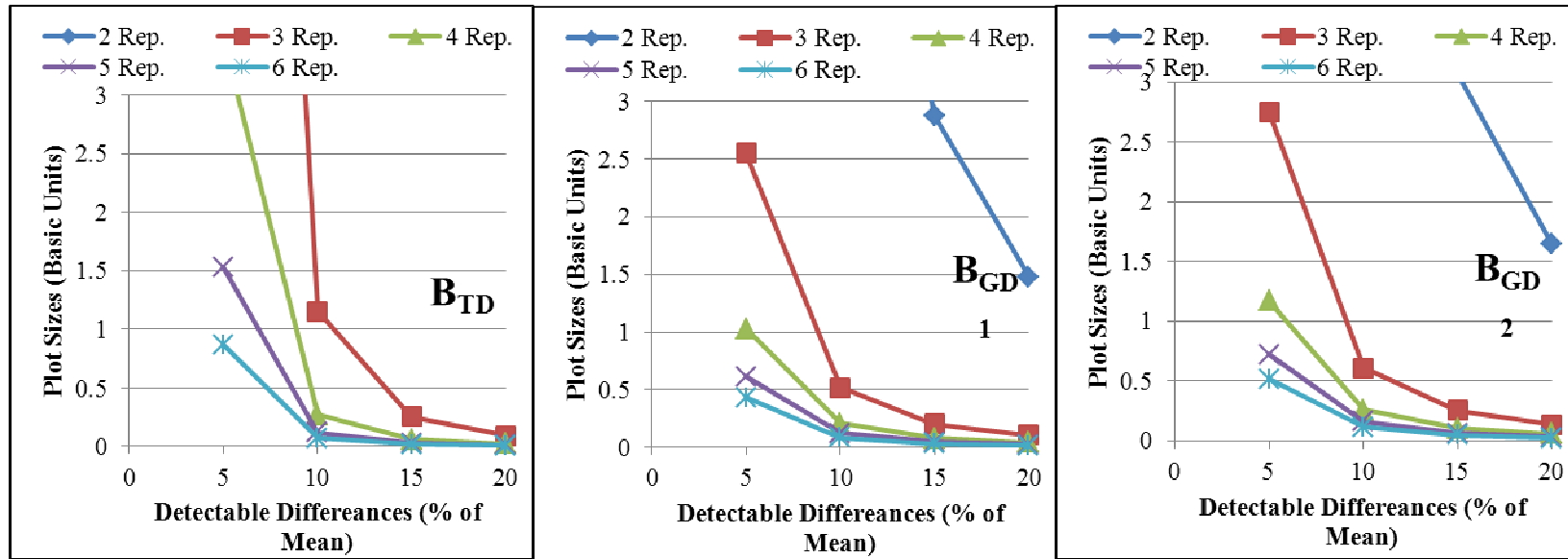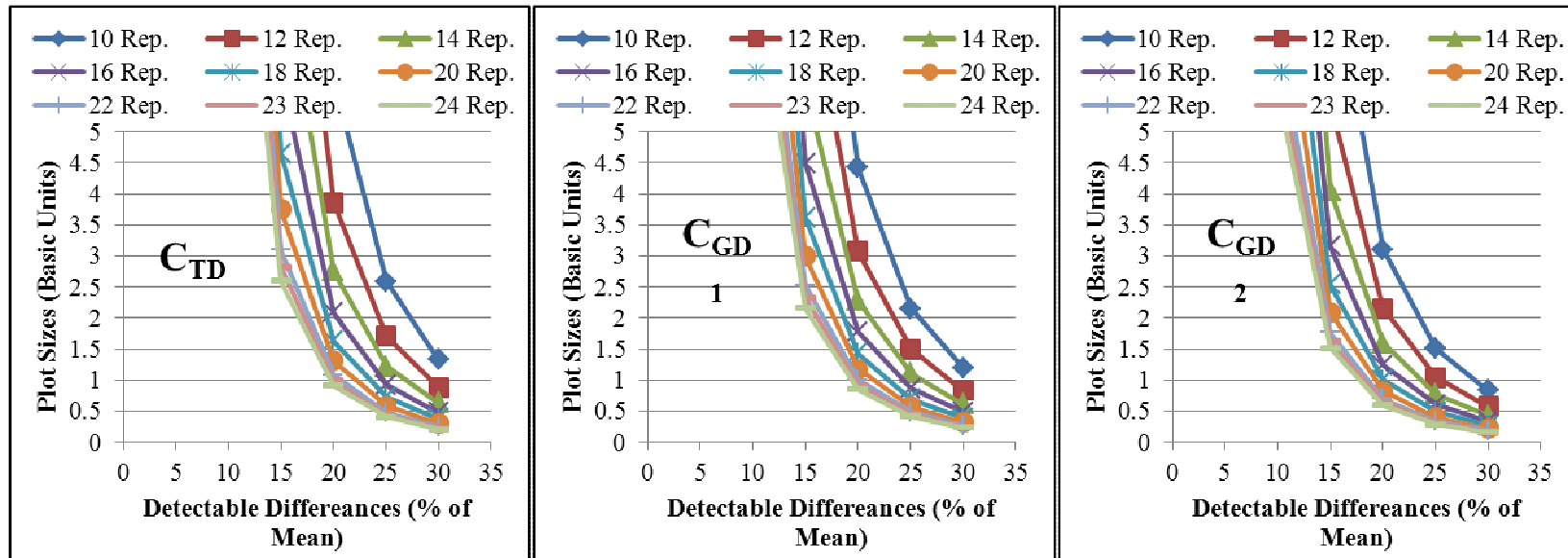
Figure 7.6. The relationship between replications and plot sizes, and detectable differences (% of means) for egg density data from field research site C. The basic unit refers to the plot size in the individual experiment. TD= transformed mean egg density (Log $10(x+1)$); GD1 = data were grouped into six categories of egg levels: 0 = eggs undetected; 1 = 1-200 eggs/100 cm3 soil; 2 = 201-400 eggs; 3=401-1,200 eggs; 4=1,201-2,000 eggs; 5>2,001 eggs. GD2 = data were grouped into four categories of egg levels: 0 = eggs undetected; 1 = 1-2,000 eggs/100 cm3 soil; 2 = 2001-12,000 eggs; 3>12,001 eggs. The median of the egg count for each category was transformed with log $10(x+1)$. One basic unit = 9 m^2. Notice, in general, grouping data into either of the two category groups (GD1 and GD2) resulted in a reduction of minimum plot size, fewer replications, or smaller detectable differences. Based on three unit size plots, the minimal replication needed for TD is 16 compared to 14 and 12 for GD1 and GD2, respectively, when I want to detect a 25% difference of the means.

Figure 7.7. The relationship between replications and plot sizes, and detectable differences (% of means) for egg density data from field research site D. The basic unit refers to the plot size in the individual experiment. TD= transformed mean egg density (Log $10(x+1)$); GD1 = data were grouped into six categories of egg levels: 0 = eggs undetected; 1 = 1-200 eggs/100 cm3 soil; 2 = 201-400 eggs; 3=401-1,200 eggs; 4=1,201-2,000 eggs; 5>2,001 eggs. GD2 = data were grouped into four categories of egg levels: 0 = eggs undetected; 1 = 1-2,000 eggs/100 cm3 soil; 2 = 2001-12,000 eggs; 3>12,001 eggs. The median of the egg count for each category was transformed with log $10(x+1)$. One basic unit = 9 m^2.
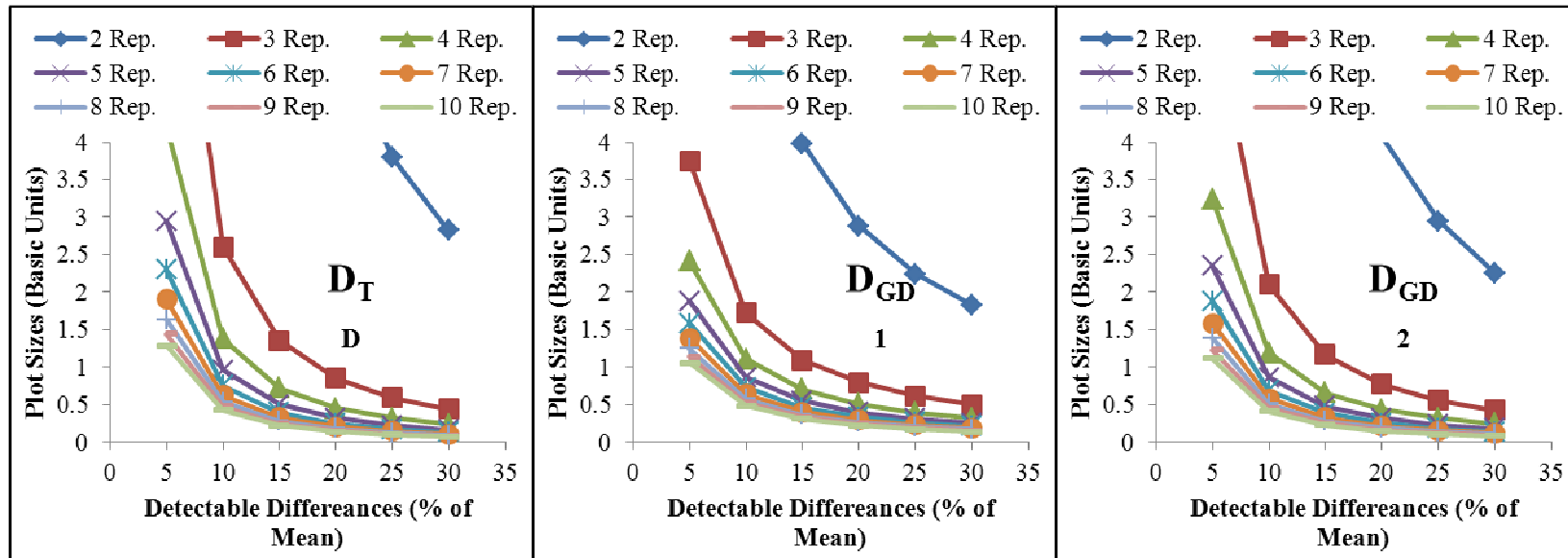
Figure 7.8. The relationship between replications and plot sizes, and detectable differences (% of means) for egg density data from field research site E. The basic unit refers to the plot size in the individual experiment. TD= transformed mean egg density (Log $10(x+1)$); GD1 = data were grouped into six categories of egg levels: 0 = eggs undetected; 1 = 1-200 eggs/100 cm3 soil; 2 = 201-400 eggs; 3=401-1,200 eggs; 4=1,201-2,000 eggs; 5>2,001 eggs. GD2 = data were grouped into four categories of egg levels: 0 = eggs undetected; 1 = 1-2,000 eggs/100 cm3 soil; 2 = 2001-12,000 eggs; 3>12,001 eggs. The median of the egg count for each category was transformed with log $10(x+1)$. One basic unit = 6.4 m^2. Notice, in general, grouping data into either of the two category groups (GD1 and GD2) resulted in a reduction of minimum plot size, fewer replications, or smaller detectable differences. Based on one unit size plot, the minimal replication needed for TD is 5 compared to 4 for GD1 and GD2, when Iwant to detect a 5% difference of the means.
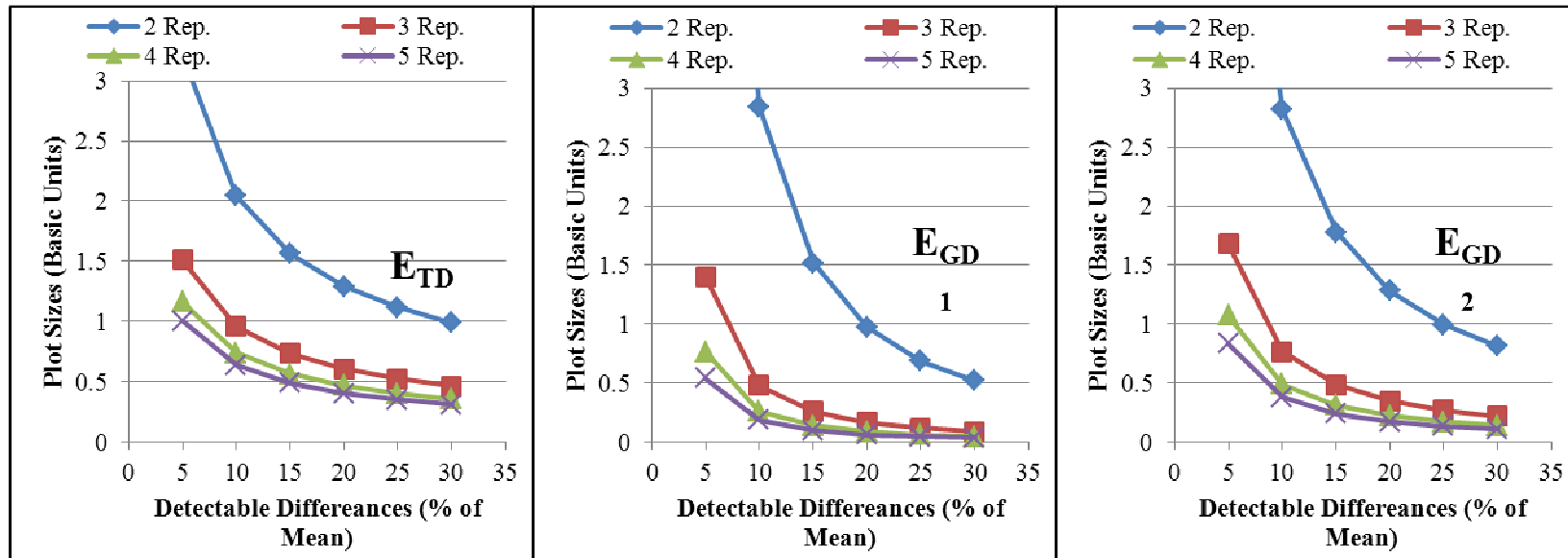
Figure 7.9. The relationship between replications and plot sizes, and detectable differences (% of means) for egg density data from field research site F. The basic unit refers to the plot size in the individual experiment. TD= transformed mean egg density (Log 10(x+1)); GD1 = data were grouped into six categories of egg levels: 0 = eggs undetected; 1 = 1-200 eggs/100 cm3 soil; 2 = 201-400 eggs; 3=401-1,200 eggs; 4=1,201-2,000 eggs; 5>2,001 eggs. GD2 = data were grouped into four categories of egg levels: 0 = eggs undetected; 1 = 1-2,000 eggs/100 cm3 soil; 2 = 2001-12,000 eggs; 3>12,001 eggs. The median of the egg count for each category was transformed with log 10(x+1). One basic unit = 6.4 m^2. Notice, in general, grouping data into either of the two category groups (GD1 and GD2) resulted in a reduction of minimum plot size, fewer replications, or smaller detectable differences. Based on one unit size plot, the minimal replication needed for TD is 6 compared to 5 for GD1 and GD2, when I want to detect a 5% difference of the means.
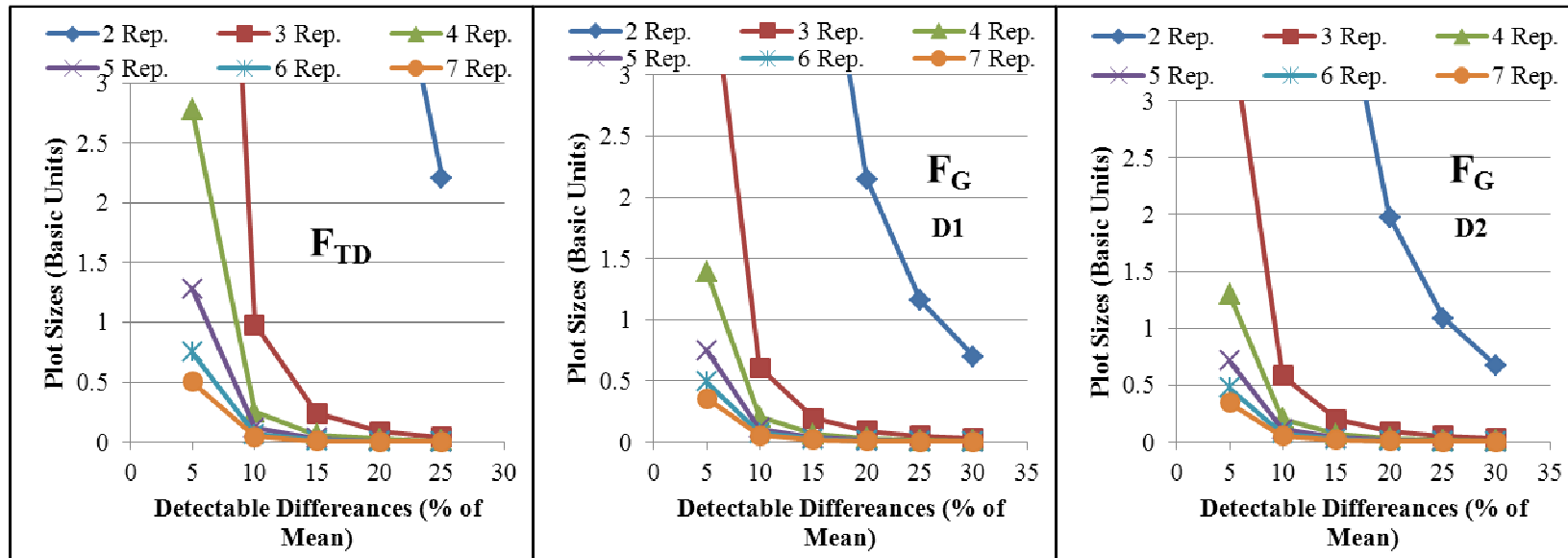
Figure 7.10. The relationship between replications and plot sizes, and detectable differences (% of means) for egg density data from field research sites G and J. The basic unit refers to the plot size in the individual experiment. TD= transformed mean egg density (Log 10(x+1)); GD2 = data were grouped into four categories of egg levels: 0 = eggs undetected; 1 = 1-2,000 eggs/100 cm3 soil; 2 = 2001-12,000 eggs; 3>12,001 eggs. The median of the egg count for each category was transformed with log 10(x+1). One basic unit = 6.4 m^2. Notice, in general, grouping data into either of the two category groups (GD1 and GD2) resulted in a reduction of minimum plot size, fewer replications, or smaller detectable differences. Based on one plot size, minimal 5 replications are needed to detect 5 % difference of the mean in TD, compared to 3 replications in GD2. Also notice in Site J, using one plot size, only 3 replications are needed to detect 5% of differences.
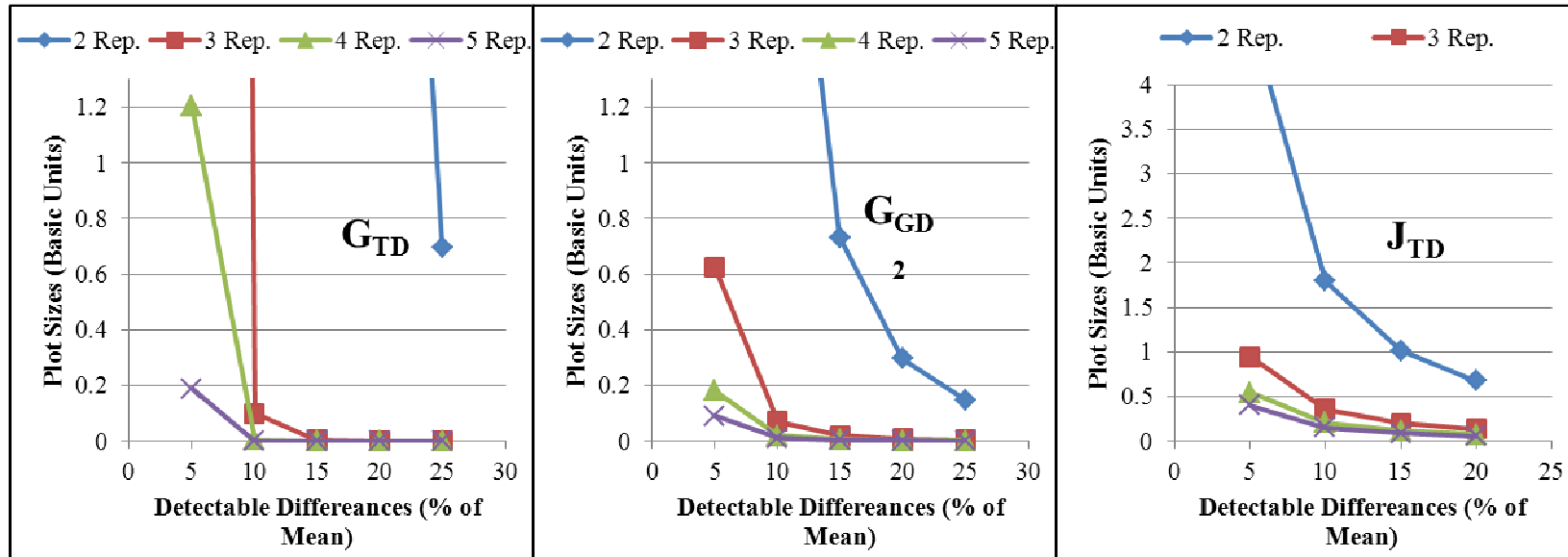
Figure 7.11. The relationship between replications and plot sizes, and detectable differences (% of means) for egg density data from field research site H. The basic unit refers to the plot size in the individual experiment. TD= transformed mean egg density (Log $10(x+1)$); GD1 = data were grouped into six categories of egg levels: 0 = eggs undetected; 1 = 1-200 eggs/100 cm3 soil; 2 = 201-400 eggs; 3=401-1,200 eggs; 4=1,201-2,000 eggs; 5>2,001 eggs. GD2 = data were grouped into four categories of egg levels: 0 = eggs undetected; 1 = 1-2,000 eggs/100 cm3 soil; 2 = 2001-12,000 eggs; 3>12,001 eggs. The median of the egg count for each category was transformed with log $10(x+1)$. One basic unit = 6.4 m^2. Notice, in general, grouping data into either of the two category groups (GD1 and GD2) resulted in a reduction of minimum plot size, fewer replications, or smaller detectable differences. Based on one unit size plot, the minimal replication needed for TD is 4 compared to 3 for GD1 and GD2, when I want to detect a 15% difference of the means.
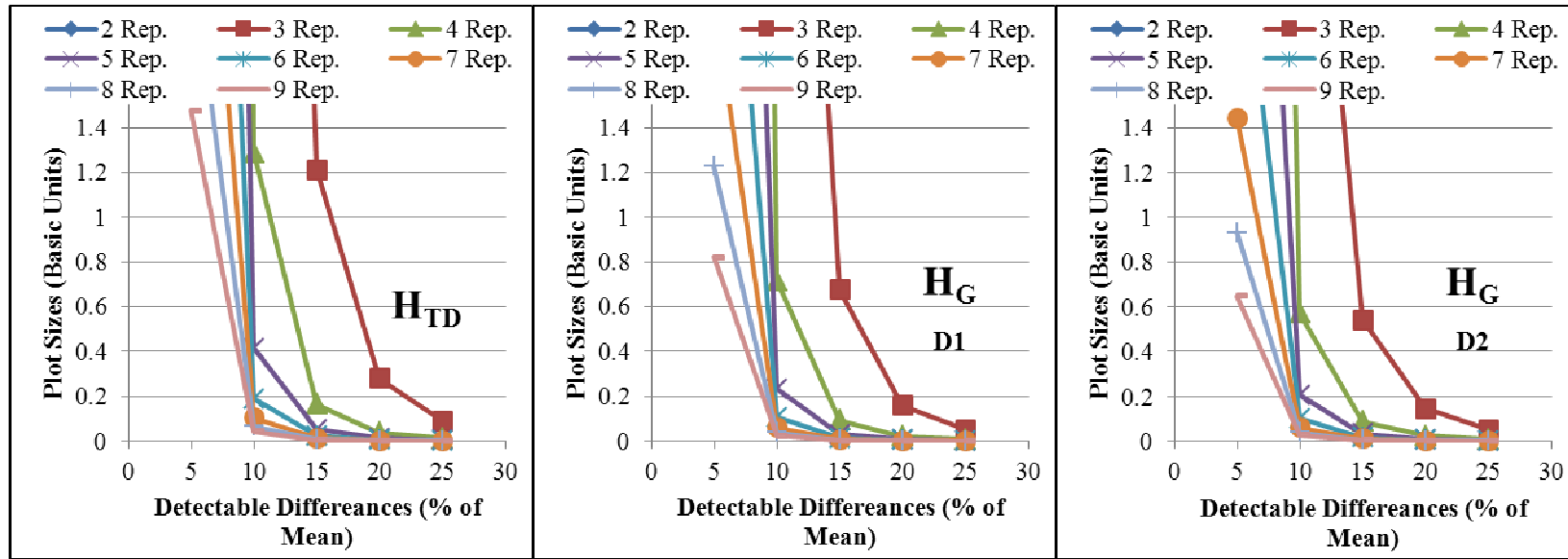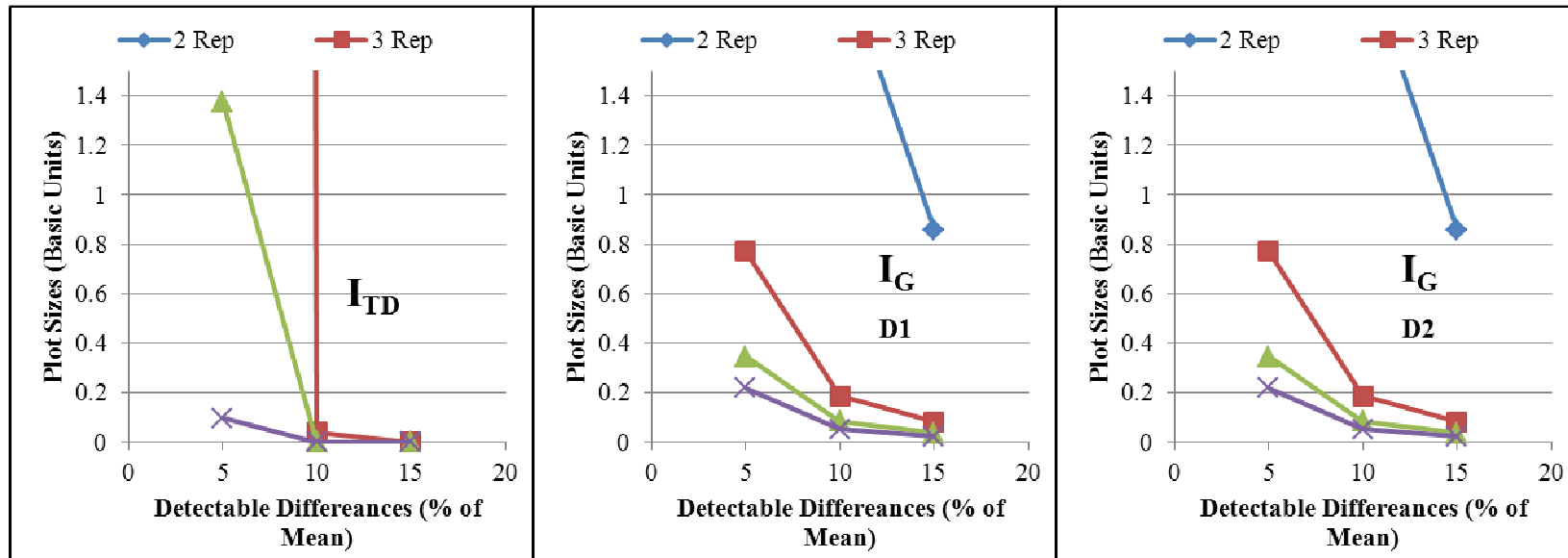
Figure 7.12. The relationship between replications and plot sizes, and detectable differences (% of means) for egg density data from field research site I. The basic unit refers to the plot size in the individual experiment. TD= transformed mean egg density (Log 10(x+1)); GD1 = data were grouped into six categories of egg levels: 0 = eggs undetected; 1 = 1-200 eggs/100 cm3 soil; 2 = 201-400 eggs; 3=401-1,200 eggs; 4=1,201-2,000 eggs; 5>2,001 eggs. GD2 = data were grouped into four categories of egg levels: 0 = eggs undetected; 1 = 1-2,000 eggs/100 cm3 soil; 2 = 2001-12,000 eggs; 3>12,001 eggs. The median of the egg count for each category was transformed with log 10(x+1). One basic unit = 6.4 m^2. Notice, in general, grouping data into either of the two category groups (GD1 and GD2) resulted in a reduction of minimum plot size, fewer replications, or smaller detectable differences. Based on one unit size plot, the minimal replication needed for TD is 5 compared to 3 for GD1 and GD2, when I want to detect a 5% difference of the means.

Table 7.4. The relationship between Lloyd's Index of Patchiness and convenience plot based on one basic plot[a] size with probability = 0.85 for soybean cyst nematode egg density data from ten infested field using TD[b].

Site	Rank	LIP[c]	Convenient Plots				
			[d]5	10	15	20	30
J	1	1.09	-	3[e]	2	2	2
I	2	1.31	-	-	3	2	2
G	3	1.36	-	-	4	2	2
E	4	1.43	-	-	4	3	2
F	5	1.36	-	-	-	3	2
B	6	1.41	-	-	-	4	2
H	7	1.71	-	-	-	-	3
D	8	1.46	-	-	-	-	4
A	9	2.14	-	-	-	-	-
C	10	3.34	-	-	-	-	-

[a]One basic unit equal to 6.9 m^2 (for site A and B), 9 m^2 (for site C and D), and 6.4 m^2 (for site E, F, G, H, I, and J).

[b]TD = Transformed Data (Log 10(x+1)).

[c]LIP = LIP= Loyd Index of Patchiness

[d]Percentage of the mean of difference to be detected

[e]Number of replications (Note, - means more than 5 replications)

The wide range of egg densities found between plots in some of these sites suggests that experimental results could be affected by the differences in egg densities and their spatial distribution within a site. For example in site E, there were two plots adjacent to each other where one plot had 20,750 and the other had 3,150 eggs per 100 cm^3 soils. That is greater than a 6 fold difference in egg density. Depending on what type of

experimentation was being conducted, the differences in SCN spatial distribution could have a major effect on the outcome of the experiments. For example, when comparing cultivars for yield on SCN infested sites, a large difference in egg density between plots could result in comparisons that are invalid because one or more cultivars were on plots with high egg densities while others were on plots with low densities. In sites such as A, C, and D where very large differences existed between plots, such a situation would more likely occur. In contrast, in site J which had a random distribution of eggs, that situation would be less likely to occur.

Table 7.5. The relationship between Lloyd's Index of Patchiness and convenience plot based on one basic plot size[a] with probability = 0.85 for soybean cyst nematode egg density data from ten infested field using GD1[b].

Site	Rank	LIP[c]	Convenient Plots				
			5[d]	10	15	20	30
J	1/2	1.00	2[e]	2	2	2	2
G	1/2	1.00	2	2	2	2	2
I	3	1.00	-	3	2	2	2
E	4	1.00	-	-	3	2	2
B	5	1.01	-	-	4	2	2
F	6	1.01	-	-	4	2	2
H	7	1.02	-	-	-	-	3
D	8	1.02	-	-	-	-	4
A	9	1.11	-	-	-	-	-
C	10	1.42	-	-	-	-	-

[a]One basic unit equal to 6.9 m^2 (for site A and B), 9 m^2 (for site C and D), and 6.4 m^2 (for site E, F, G, H, I, and J).

Table 7.6. The relationship between Lloyd's Index of Patchiness and convenience plot based on one basic plot size[a] with probability = 0.85 for soybean cyst nematode egg density data from ten infested field using GD2[b].

Site	Rank	LIP[c]	Convenient Plots				
			5[d]	10	15	20	30
J	1	1.00	2[e]	2	2	2	2
I	2	1.00	-	3	2	2	2
G	3	1.00	-	3	2	2	2
F	4	1.01	-	-	3	2	2
E	5	1.01	-	-	4	2	2
B	6	1.01	-	-	4	2	2
H	7	1.01	-	-	-	-	3
D	8	1.02	-	-	-	-	4
A	9	1.10	-	-	-	-	-
C	10	1.36	-	-	-	-	-

To illustrate the point that differences in spatial distribution of eggs could affect the outcome of experiments, consider an evaluation of soybean cultivars in site B (Figure 7.2) for yield response in the presence of SCN. Notice in this site there were three ranges of 24 plots each. Consider two hypothetical soybean cultivars 1 and 2, each with three replications, one in each range of plots. Table 7.7 shows the results of what the egg densities would have been for each cultivar for each replication after conducting three separate randomizations to determine the location of each of the cultivars in the ranges. In randomization 1, a difference of 3,117 eggs in the average egg density occurred in plots between cultivar 1 and cultivar 2, while in randomization 2 the difference was only 433 eggs. However, in randomization 3, the difference was 7,300 eggs, with cultivar 2 located on plots with a much higher egg density.

Knowing cluster size of an important factor in a field experiment is important for determining the sampling technique and choosing the experimental design (Madden and Hughes, 1999). It has been demonstrated that knowing cluster size can increase efficiency and precision of results (Smith et al., 1995, Ojiambo and Scherm 2010, Hau et al., 1982). In these experiments, in five of the sites, the cluster sizes were up to eight times the size of an individual plot. Consider site B where cluster size was determined to be 55 m^2 (8 basic units) when analyzing transformed mean egg density (Table 7.2). For a more efficient use of this site for testing cultivars for reaction to SCN, it would be necessary to include each cultivar in a cluster of plots. On the other hand, the design could be changed from a randomized complete block (with three blocks) to a split plot.

In this research, the optimum plot size and plot number (replications) for each site were calculated using data from three different sources: from the direct egg counts and

from data generated by classifying egg counts into two different category groups. The purpose of this exercise was to design plots where differences in egg density would be minimal and find the least number of replications required to conduct an experiment at the site. Because of the marked aggregated distribution of SCN at sites A, and C, using one standard plot size and 4 or 5 replications, a difference as much as 35 % of the means would likely not be detected. In other sites such as site D, differences as much as 10% of the means of two treatments will only be detected with at least 5 replications. In contrast, in site J a difference as low as 5% of the means would be detected with only 3 replications.

The two category groups by Hershman (2010) and Plant Health Initiative were used because they attempted to group egg densities into no risk, low, moderate or high risk for soybean yield. When data were grouped into the two category groups, (GD1 and GD2), all LIP's were reduced, with most slightly over 1. The use of biological meaningful categories therefore, reduced the perceived level of aggregation of SCN and the differences among and within sites became less dramatic. The use of categorical data also reduced the number of replications, but not the optimum plot size. The use of these types of categories could assist in the design of field experiments with SCN. Since most plot work with soybean and SCN would most likely be measuring yield as one criterion to measure the effects of SCN, these categories may have utility in deciding number of replications. Using the two categories however, depends on the accuracy of the egg thresholds within these categories to result in damage to soybean yield.

No region-wide predictive equation was available for yield loss based on initial nematode populations in the soil (Donald et al., 2006; Niblack et al., 1992; and Young 1998). Actual yields attained at specific SCN levels may vary due to the occurrence of

other stresses, such as pests, drought, temperature, herbicide injury, etc. There has,

however, been considerable research on the effect of SCN on yield loss in soybean, but the

results vary from state to state, and year to year. Egg densities of around 1,000 or greater

eggs per 100 cm^3 soil are reported to have considerable impact on soybean yield. An initial

population of 1,200 juveniles of *H. glycines* per pot caused a severe effect on the top dry

weight and grain yield of soybean (Asmus and Ferraz, 2002). Yield reductions of 52% and

19% were measured in Iowa when susceptible cultivars were inoculated with 1,250 eggs

per 100 cm^3 of soil in 1986 and 1987, respectively (Niblack et al., 1992). Resistant

cultivars in Minnesota produced greater yield by 23 % and 28% compared to susceptible

cultivars when egg density at planting was greater than 700 and 5,000 eggs per 100 cm^3

soil, respectively (Chen et al., 2001).

Table 7.7. Soybean cyst nematode egg densities in research plots in field A following
random assignment of two soybean cultivars to plots.

Replication	Randomization 1		Randomization 2		Randomization 3	
	CV1[a]	CV2	CV1	CV2	CV1	CV2
1	1,900[b]	2,900	3,900	1,450	1450	8,850
2	3,950	2,650	9,950	13,500	2,650	8,750
3	2,000	11,650	5,600	3,200	2,000	10,400
Average	2,617	5,733	6,483	6,050	2,033	9,333

[a] CV = cultivar

[b] Eggs/100 cc soil

Determining the egg threshold for a yield reduction has been more difficult. In

micro plot field experiments, Niblack et al. (1992) showed that the damage threshold of

susceptible cultivars was as low as 10 to 50 eggs per 100 cm^3 soil. Other research,

however, reported no difference in yield was observed between resistant and susceptible cultivars at sites where egg density at planting was lower than 500 eggs per 100 cm^3 soil (Chen et al., 2001). Furthermore, healthy crops are capable of compensating for some SCN damage thus, at low SCN egg densities there may be no or minor yield loss (Hershman 2010).

The effect of the spatial distribution of SCN on field experiments would likely be less of a concern if resistant cultivars compared to susceptible cultivars were being used. Since resistant cultivars could tolerate a range of egg densities without damage, differences in egg densities between plots may result in little effect on the experiment outcome. However, when using susceptible cultivars, differences in egg densities between plots could be important factors in the results. When the susceptibility of cultivars is known, grouping data into the two category groups may be an appropriate method of examining data for minimum plot size and number of replications that meet the objective of the research.

The results of this study show that spatial distribution of eggs in SCN research sites can vary greatly within sites and among sites. Since egg density is known to affect the soybean/SCN interaction, the spatial distribution of eggs should be considered an important factor when conducting field experiments. Researchers tend to use small plots for field experiments because inputs and cost are reduced compared to larger plots. However, the data in this study suggest the plot sizes used were too small to minimize the differences in spatial distribution. In some sites it might have been impractical to conduct field experiments with SCN and soybean using the plot sizes suggested by the analyses. Not

only would the individual plots be extremely large, but the area required for a field site could be exceedingly large depending on how many treatments were evaluated.

Most researchers who evaluate cultivars for yield on SCN infested sites conduct multiple field trials in different locations, thus reducing the potential impact of differences in spatial distribution among plots on the outcome of the evaluation. However, in other types of experimentation where it might be less likely to have multiple locations, such as chemical control experiments, examining row spacing or seeding rates using susceptible or moderately susceptible cultivars on soil infested with SCN, the differences in egg densities between plots could have a major effect on the outcome of the experiments. It may also be impractical for many researchers to sample each plot to determine egg densities when conducting experiments on SCN infested soil. However, knowledge of the large differences in SCN egg densities that can occur between plots may help researchers deal with this potential problem in field experiments. Increasing replications and conducting experiments on multiple sites are likely the most practical methods to reduce the variability due to differences in egg densities.

Literature Cited

Anand, S. C., Matson, K. W., and Sharma, S. B. 1995. Effect of soil temperature and pH on resistance of soybean to *Heterodera glycines*. J. Nematol. 27:478-482.

Asmus, G. L., and Ferraz, L. C. C. B. 2002. Effect of population densities of *Heterodera glycines* race 3 on leaf area, photosynthesis and yield of soybean. Fitopatol. Brasileira 27:273-278.

Avendaño, F., Pierce, F., and Melakeberhan, H. 2004. Spatial analyses of soybean yield in relation to soil texture. Nematol. 6:527-545.

Bradley, C. A., Biller, C. R., and Nelson, B. D. 2004. First report of soybean cyst nematode (*Heterodera glycines*) on soybean in North Dakota. Plant Dis. 88:1287.

Campbell, C. L., and Madden, L. V. 1990. Introduction to Plant Disease Epidemiology. New York: Wiley and Sons. ISBN 0471832367.

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

Donald, P. A., P.E. Pierson, S. K. St. Martin, P. R. Sellers, G.R. Noel, A. E. MacGuidwin, J. Faghihi, V.R. Ferris, C. R. Grau, D.J. Jardine, H. Melakeberhan, T.L. Niblack, W.C. Stienstra, G.L. Tylka, T. A. Wheeler, and D.S. Wysong. 2006. Assessing *Heterodera glycines*-resistant and susceptible cultivar yield response. J. Nematol. 38:76-82.

Duan, Y. X., Zheng, Y. N., Chen, L. J., Zhou, X. M., Wang, Y. Y., and Sun, J. S. 2009. Effects of Abiotic Environmental Factors on Soybean Cyst Nematode. Agricultural Sciences in China 8:317-325.

Francl, L. J. 1986a. Spatial analyses of *Heterodera glycines* populations in field plots. J. Nematol. 18:183-189.

Francl. L. J. 1986b. Improving the accuracy of sampling field plots for plant-parasitic nematodes. J. Nematol. 18:190-195.

Galal, H.E. and Abou El-Fittouh, H.A. 1971. Estimation of optimum plot size and shape for Egyptian cotton yield trials . Alexandria J. Agric. Res. 19:233-239.

Gavassoni, W. L., Tylka, G. L., and Munkvold, G. P. 2007. Effects of tillage practices on dissemination and spatial patterns of *Heterodera glycines* and soybean yield. Plant Dis. 91:973-978.

Hathaway, W. H. and E. J. Williams. 1958. Efficient estimation of the relationship between plot size and variability of crop yields. Biometrics 14:207-222.

Hathaway, W. H. 1961. Convenient plot size. Agron J. 53:279-280.

Hau, F. C., Campbell, C. L., and beute, M. K. 1982. Inoculum distribution and sampling methods for *Cylindrocladium crotalariae* in a peanut field. Plant Dis. 66:568-571.

Hershman, D. E. 2010. 2010 Soybean Cyst Nematode (SCN) Management Recommendations for Kentucky. Plant Pathology Fact She UK Cooperative Extension Service. PPFS-AG-S-24.

Karandinos, M. G. 1976. Optimum sample size and comments on some published formulae. Bull. Entomol. Soc. Am. 22:417-421.

Keller, K.R. 1949. Uniformity trial on Hops (*Humulus lupulus*, L.) for increasing the precision of field experiments . Agron. J. 4:389-392.

Kulkarni, S.S., S. G. Bajwa, S.G., Rupe, J.C., and Kirkpatrick, T.L. 2008. Spatial correlation of crop to Soybean cyst nematode (*Heterodera glycines*). Transactions of the ASABE: 51:1451-1459.

Lal, R., and Lal, A. 2006. Plant parasitic nematodes intercepted from seeds, soil clods and packing material under import quarantine. J. New Seeds 8:49-60.

Leilah, A. A., and Al-Barrak, K. M. 2005. Estimation of optimum field plot size and shape and number of replicates in sorghum yield trials. J. King Saud Univ. 17:101-116.

Lessman, K.J. and Atkins, R.E. 1963. Optimum plot size and relative efficiency of lattice designs for grain sorghum yield tests. Crop Sci. 3:477-481

Lloyd, M. 1967. Mean crowding. J. Animal Ecol. 36:1-30.

Madden, L. V., and Hughes, G. 1999. Sampling for plant disease incidence. Phytopathology 89:1088-1103.

Masuda, T. and Goldsmith, P. D. 2009. World soybean production: area harvested, yield, and long-term projections. Inter. Food Agrib. Manag. Rev. 12:143-162.

Meier, V.D. and Lessman, K.J. 1971. Estimation of optimum field plot shape and size for testing yield in *Crambe abyssinica* Hochst. Crop Sci. 11:648-650.

National Agricultural Statistics Service. 2010. Agricultural Statistics Database. United States Department of Agriculture. Online publication. http://www.nass.usda.gov/QuickStats/index2.jsp.

Niblack, T. L., Baker, N. K., and Norton, D. C. 1992. Soybean yield losses due to *Heterodera glycines* in Iowa. Plant Dis. 76:943-948.

Ojiambo, P. S., and Scherm, H. 2010. Efficiency of adaptive cluster sampling for estimating plant disease incidence. Phytopathology 100:663-670.

Pedersen, P., Tylka, G. L., Mallarino, A., Macguidwin, A. E., Koval, N. C., and Grau, C. R. 2010. Correlation between soil pH, *Heterodera glycines* population densities, and soybean yield. Crop Sci. 50:1458–1464.

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

Poromarto, S. H., and Nelson, B. D. 2010. Evaluation of northern-grown crops as hosts of soybean cyst nematode. Online. Plant Health Progress doi:10.1094/PHP-2010-0315-02-RS.

Riggs, R. D., and Hamblen, M. L. 1962. Soybean cyst nematode studies in the family Leguminosae. Ark. Agr.. Expt. Sta. Rep. Series 110:1-8.

Smith, H. F. 1938. An empirical law describing the heterogeneity in the yields of agricultural crops. J. Agric. Sci. 28:1-23.

Smith, D. R., Conroy, M. J., and Brakhage, D. H. 1995. Efficiency of adaptive cluster sampling for estimating density of winter waterfowl. Biometrics 51:777-788.

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

Young, L. D. 1987. Effects of soil disturbance on reproduction of *Heterodera glycines*. J. Nematol. 19:141-142.

Young, L. D. 1998. Influence of soybean cropping sequences on seed yield and female index of the soybean cyst nematode. Plant Dis. 82:615-619.

CHAPTER 8. GENERAL CONCLUSIONS

Soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe (Tylenchida: Heteroderidae), is the most damaging pathogen of soybean (Glycine max) and is responsible for losses of about $1.3 billion yearly between 2006-2009 (Koenning and Wrather, 2010). The pathogen is distributed worldwide (EPPO, 2009; Riggs, 2004), including Northern Minnesota, South Dakota and North Dakota (Bradley et al., 2004; MacDonald et al., 1980; Smolik et al., 1996). SCN has a broad host range (McSorley, 1988; Noel et al., 1982; Riggs, 1992; Riggs and Hamblen, 1962, 1966; Smith and Young, 2003). This nematode is a threat to soybean, dry bean, and potentially other crops produced in North Dakota and northern Minnesota since this region is a major crop production area (NASS, 2011; Warnke et al. 2006). Studies on the biology of SCN in this northern crop production region were conducted to better understand and manage this plant parasitic nematode. The general conclusions of this research were:

1. SCN is a potential threat to Lupinus and dry bean in the northern production area of North Dakota and northern Minnesota.

2. With the exception of white lupines and dry bean, all the traditional crops and most of the specialty crops grown or being considered for production in North Dakota and northern Minnesota, such as borage, camelina, chickpea, crambe, cuphea, field pea, nyjer, and safflower, are poor hosts or non-hosts for SCN. SCN reproduces well on Lupines (Female Index (FI) of 42 to 57) and dry bean (FI's of 5 to 117).

3. SCN significantly reduced pod number (PN), pod weight (PW), seed number (SN), and seed weight (SW) of pinto bean GTS-900 at 5,000 and 10,000 eggs/100 cm3 soil by 44 to 56% averaged over the two years compared to the control. For Montcalm,

significant reductions of 31 to 35% in PW, SN, SW, and total dry weight (TDW) in treatments of 2,500 and 5,000 eggs/100 cm3 soils were recorded in 2009, but not in 2008. For Mayflower, significant reductions of 27 to 41% in PH, PW, SN, SW, and TDW in treatments of 2,500 and 5,000 eggs/100 cm3 soil compared with the control were recorded in one out of two experiments in 2009. These results suggest SCN will become a serious disease for dry bean growers in the future.

4. There was no evidence that SCN HG 0 increased in reproduction on six dry bean cultivars during two 11 month periods of continual reproduction of HG 0 on roots. This suggests that a rapid adaptation of SCN to dry bean resulting in higher rates of reproduction in the field is unlikely.

5. Studies on spatial distribution of SCN in ten naturally infested sites in North Dakota over three years showed that spatial distribution of eggs in SCN research sites can vary greatly within sites and among sites. SCN populations varied among plots from undetected to 25,000 eggs/100 cm^3 soil, and in some sites the differences in egg densities observed between adjacent plots were as high as 6-fold. Lloyd's index of patchiness ranged from 1.1 to 3.3. Spatial distribution of SCN can be an important factor affecting the results of field experiments.

Literature Cited

Bradley, C. A., Biller, C. R., and Nelson, B. D. 2004. First report of soybean cyst nematode (*Heterodera glycines*) on soybean in North Dakota. Plant Dis. 88:1287.

EPPO. 2009. *Heterodera glycines*. Bulletin OEPP/EPPO 39:59-61.

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

MacDonald, D. H., Noel, G. R., and Lueschen, W. E. 1980. Soybean cyst nematode, *Heterodera glycines*, in Minnesota. Plant Dis. 64:319-321.

McSorley, R. 1988. Population dynamics. pages 109-133 in: Plant and nematode interactions. K. R. Barker, G. A. Pederson, and G. L. Windham (Ed.) Madison. American Society of Agronomy.

NASS. 2011. Agricultural Statistics Database. United States Department of Agriculture. Online publication. http://www.nass.usda.gov/QuickStats/index2.jsp.

Noel, G. R., Jacobsen, B. J., and Leeper, C. D. 1982. Soybean cyst nematode in commercial snap bean. Plant Dis. 66:520-522.

Riggs, R. D. 2004. History and distribution. Pages 9-39 in D. P. Schmitt, J. A. Wrather, and R. D. Riggs, eds. Biology and Management of the Soybean Cyst Nematode. St. Paul, MN: American Phytopathological Society.

Riggs, R. D. 1992. Host Range. Pages 107–114 in R. D. Riggs and J. A. Wrather, eds. Biology and Management of the Soybean Cyst Nematode. St. Paul, MN: American Phytopathological Society.

Riggs, R. D., and Hamblen, M. L. 1962. Soybean cyst nematode studies in the family Leguminosae. Ark. Agr.. Expt. Sta. Rep. Series 110:1-8.

Riggs, R. D., and Hamblen, M. L. 1966. Further studies on the host range of the soybean cyst nematode. Ark. Agr.. Expt. Sta. Series 110:2-19.

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

Smith, J. R., and Young, L. D. 2003. Host suitability of diverse lines of Phaseolus vulgaris to multiple populations of *Heterodera glycines*. J. Nematol. 35:23-28

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