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MULTIFACTORIAL ANALYSIS OF MORTALITY OF SOYBEAN CYST NEMATODE (*Heterodera glycines* Ichinohe) POPULATIONS IN SOYBEAN AND IN SOYBEAN FIELDS ANNUALLY ROTATED TO CORN IN NEBRASKA

Oscar Perez-Hernandez
University of Nebraska-Lincoln

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by

Oscar Pérez-Hernández

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MULTIFACTORIAL ANALYSIS OF MORTALITY OF SOYBEAN CYST NEMATODE (*Heterodera glycines* Ichinohe) POPULATIONS IN SOYBEAN AND IN SOYBEAN FIELDS ANNUALLY ROTATED TO CORN IN NEBRASKA

Oscar Pérez-Hernández, Ph.D.

University of Nebraska, 2013

Advisor: Loren J. Giesler

The soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe) is the most economically important pathogen of soybean in the U.S. The effect of annual corn rotation, soil properties, weather, and agronomic factors on SCN population densities was quantified in 45 fields in Nebraska over three years. SCN population densities (eggs/100 cm^3 of soil) in each field were determined before (Pi) and after (Pf) annual corn rotation. Average SCN population density reduction was 50.62%. Multivariate analysis was used to describe the relationship of soil texture (% of sand, silt, and clay), Pi, and Pf. Two principal components explained 92% of the variability in the data set. The first component was represented by texture and accounted for 60.5% and the second was represented by Pi and Pf and explained 31.5%. Cluster analysis identified two groups of fields: one group with predominantly sandy soil (57 to 95%) and the other with predominantly silty soil (23 to 61%). SCN Pi was significantly higher in the sandy group than in the silty group ($F = 271.19$, $P<0.0001$).

The SCN Pf was modeled using an initial set of eight predictors. A negative binomial regression model with the log link function was applied to a 35-field training data set and a final model was selected. This model was used to estimate the nematode population density after annual corn rotation in the training data set and its prediction

power was 82.1%. This predicting capability was confirmed in a validation data set in which the model's predicting capability was 79.6%.

Intra and interplot spatial variability of SCN population densities was analyzed in three experimental areas and its relationship with soybean yield was examined. SCN population densities had an aggregated pattern, showing spatial dependence with those of adjacent plots. The β-binomial distribution adequately described data of incidence and suggested that SCN population density aggregation also occurred within plots. The SCN reproduction factor was not related to the number of SCN-positive cores per plot nor was it related to soybean yield in two soybean varieties assessed, one resistant and one susceptible.

DEDICATION

To Amy, my wife.

To my children.

To my parents: Felicitas and Miguel.

To my brother Nahúm.

To all my other relatives and friends to whom a link of love, trust, support, and caring keeps me joined.

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CHAPTER I. GENERAL INTRODUCTION

Dissertation organization

This dissertation is organized into six chapters. The first chapter is a general introduction, which includes three sections: a description of the dissertation organization, a brief introduction that describes the economic importance of soybean and soybean cyst nematode (SCN) in the U.S. and in Nebraska, and a list of the literature cited in this introduction.

The second chapter is a literature review. The first three major topics that are included in this section are aimed at providing general information on the three components of the soybean-soybean cyst nematode pathosystem, namely: soybean (*Glycine max*), the nematode *Heterodera glycines*, and the soil, being the latter the habitat and ecological niche of *H. glycines* when it is not inside the host tissue. The fourth and last major topic of this literature review is a description of the control and management strategies and tactics that have been employed to mitigate the losses caused by this pathogen. Most topics within each section are presented in chronological order. The purpose is to put into perspective the current biological and ecological knowledge on the SCN components and how that understanding has helped plant pathologists to manage this pathogen.

The third to fifth chapters are three separate research studies. The first study is a description of the field mortality of SCN populations in three years of assessment and a multivariate analysis of the relationship of soil texture and SCN population densities before and after corn rotation. The second study presents the development of a negative binomial regression model to characterize SCN population density reduction after annual

rotation with corn in Nebraska. The last study describes the intra and interplot spatial variability of SCN population densities in standard soybean variety evaluations; emphasis is on the description of the relationship of spatial characteristics of SCN population densities with soybean response to SCN and yield estimation. The last chapter corresponds to the general conclusions of the studies carried out for this dissertation.

Introduction

Soybean [*Glycine max* L. (Merr.)] is the most important oilseed crop in the world today (ASA, 2012; FAO, 2013). Introduced in North America in 1765, soybean is now cultivated in at least 30 states of the U.S., extending over 31.1 million hectares and placing the U.S. as the major producer of soybean in the world (FAO, 2013; NASS, 2013). In 2012, the U.S. produced about 82 million soybean tonnes and the total crop value was estimated $43.2 billon (ASA, 2012; NASS, 2013). The revenues generated by soybean product exports make soybean the second largest crop in cash sales and the number one crop in terms of the value of exports (ASA, 2012). The top five U.S. soybean producing states are Iowa, Illinois, Minnessota, Missouri and Indiana. Nebraska ranked seventh among producing states in 2012 with a total planted area of 2 million hectares (ASA, 2012).

Soybean is vulnerable to numerous plant pathogens that adversely affect production and yield (Sinclair and Hartman, 1999). The most economically important pathogen is the soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe, 1952) (Niblack, 2005; Wrather and Koenning, 2006; Wrather, 2008). This pathogen was first reported in the U.S. in North Carolina in 1955 (Winstead et al., 1955). Soon after its

detection, it was reported in several other states, and at present, SCN infestations are found in almost every soybean-producing state of the U.S. In Nebraska, SCN was first found in 1986 (Powers et al., 1989) and now it is reported as far as west Red Willow County (Wilson and Giesler, 2013).

Soybean yield reduction due to SCN in North America, as shown from the most recent assessment, was estimated at close to 34.7 million tonnes during 2006 (Wrather, 2008). In Nebraska, economic losses owing to SCN in 2012 were estimated at 40 million U.S. dollars (Wilson and Giesler, 2013).

Despite abundant basic and applied research on SCN carried out worldwide, to date no single molecule or gene (s) providing effective control of this nematode has been discovered. Virtually, SCN field infestations have been managed through growing SCN resistant soybean varieties and rotating with non-host crops for one, two or more years (Niblack, 2005; Noel, 2008). Use of resistant varieties and crop rotation have been demostrated to significantly reduce SCN population densities (Ross, 1962; Niblack, 2005; Tylka, 2008; Giesler et al., 2012; Tylka et al., 2012). These two practices have been the most effective strategies to manage SCN and will continue to be the most economical and sustainable practices to mitigate the losses caused by SCN.

The present dissertation work addressed these two management strategies from a field multifactorial perspective and from the standpoint of spatial variability of SCN population densities at the plot scale normally used in soybean variety evaluations. The first goal was to elucidate some of the relationships between edapho-climatic, agronomic, and weather variables and SCN field mortality. The second goal was to characterize the intra and interplot spatial variability of SCN population densities and how that relates to

yield estimation and response of soybean varieties to SCN. The overall goal of the research described in this dissertation was to understand relationships between several factors and SCN population densities that could improve SCN management decisions.

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CHAPTER II. LITERATURE REVIEW

Overview of the components of the soybean cyst nematode pathosystem

The soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe, 1952) is a soilborne root pathogen that affects several species of plants (Riggs, 1992), with soybean (*Glycine max* (L.) Merr.) the host of primary economic importance. The spatial and temporal dynamics of SCN infestations take place in the soil environment and depend on the interactions of abiotic and biotic factors and on soil changes imposed by agronomic practices applied during and across growing seasons. With reference to the disease triangle (Francl, 2001), the three major components of the SCN pathosystem are the cultivated soybean, the cyst nematode *H. glycines* and the soil (Fig. 1). The amount of SCN infestation in a field is the result of the interplay of these three components and their subcomponents through diverse and complex interactions.

In the host vertex, host resistance and maturity are the primary factors responsible for SCN development (Todd, 1993; Riggs et al., 2000; Kim et al., 2010). Soybean root system plasticity, though relatively understudied in relation to SCN development, is reported to affect the dynamics of other soybean root pathogens (English and Mitchell, 1994; Gongora-Canul et al., 2012). In particular, root architecture and growth rate determine the windows of opportunity for pathogen infection and host-pathogen interactions (English and Mitchell, 1994; Gongora-Canul, 2010). Concerning the pathogen component, SCN life cycle, dispersal mechanisms, and the ability of SCN populations to differentially reproduce in different cultivars (termed HG types), largely determine resulting SCN population densities. In the environment vertex, soil and above-

ground factors along with agronomic practices imposed by growers affect the dynamics of SCN populations in the soil throughout a growing season (Fig. 1).

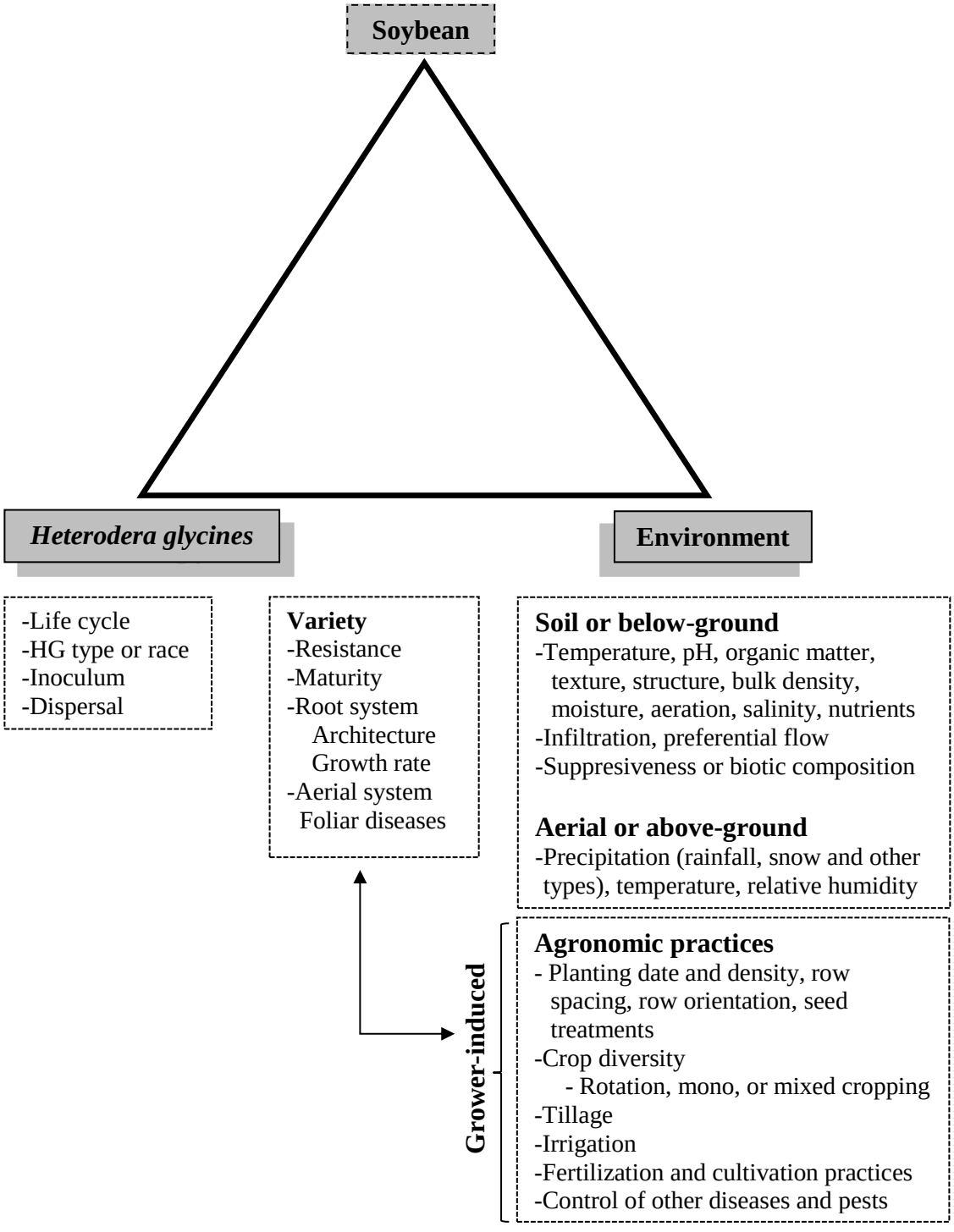

Figure 1. Major components and subcomponents of the soybean-soybean cyst nematode pathosystem and factors that can affect SCN development.

The host: soybean (*Glycine max*)

Origin, botanical description and taxonomy

Soybean is an annual leguminous plant native to northern and central China (Qiu and Chang, 2010). The soybean plant has a taproot system integrating three distinct morphological elements: a main root, lateral or secondary roots, and tertiary roots (Lertsen and Carlson, 2004). All commercial soybean cultivars are pubescent, with varying trichome densities on stems, leaves, sepals and pods. The stem is herbaceous and undergoes ramification as the plant develops. Four types of leaves are present in a developed plant: seed or cotyledon leaves, unifoliate leaves, trifoliolate leaves and prophylls (Kumudini, 2010). Leaflets of trifoliolate leaves are broadly elliptic. Flowers are hermaphroditic and originate from axillary buds on the main stem and branches. Pods are dehiscent and usually contain two to four seeds. Mature seeds are oval and contain a large embryo consisting of two cotyledons or embryonic leaves (Kumudini, 2010).

Taxonomically, soybean is classified within the following hierarchies (ITIS, 2013):

Kingdom	Plantae
Division	Magnoliophyta (flowering plants)
Class	Magnoliopsida (dicotyledoneous)
Order	Fabales
Family	Fabaceae (Papilionoidae)
Genus	*Glycine* Willd.
Species	*Glycine max* (L.) Merr.
Synonyms	*Dolichos soja* L., *Glycine hispida* (Moench) Maxim., *G.soja*).

The genus *Glycine* Willd. is divided into the subgenera *Glycine* and *Soja*. The subgenus *Soja* (Moench) Herman includes the cultivated soybean, *Glycine max* (L.) Merr., and its ancestor, the wild soybean, *Glycine soja* Sieb. & Zucc (Wang et al., 2011). The subgenus *Glycine* encompasses at least 16 wild perennial species, among them *G. canescens and G. tomentella*.

Growth and development

Development of soybean is divided into several vegetative and reproductive stages (Fehr and Caviness, 1977). The duration and developmental rate of each stage is significantly influenced by cultivar, temperature, photoperiod, and water availability (Pedersen, 2004). Temperature is the major factor that determines the early vegetative development of soybeans; higher temperatures hasten emergence and vegetative growth. Photoperiod promotes floral induction; short days (long nights or dark periods) induce flowering.

A special synchrony exists between the above and belowground phenology of a soybean plant (Torrion et al., 2012). This phenomenon is mainly affected by the geotropic potential of the root system, soil physical and chemical properties, and soil-affecting agronomic practices (Mitchell and Russell, 1971). Depending on conditions in the agroecosystem, varying rates of soybean root development, root depth and rooting patterns can occur during soybean growth (Tsutsumi et al., 2003; Torrion et al., 2012). Most soybean roots concentrate in the upper 70 cm of the soil profile (Mitchell and Russell, 1971).

Soybean adapts well to tropical and temperate regions and can grow in different soil types. Optimum soil pH range for plant development and productivity is from 6.3 to 7.4 (Schulte and Walsh, 1995; Sawyer et al., 2002). Varieties of soybean are classified into 13 maturity groups (000 to X) on the basis of their response to photoperiod. In the U.S., maturity groups grown are 00 to IX, each adapted to certain latitudinal ranges (Pedersen, 2004). Group 00 corresponds to the earliest maturity group and group IX to the latest. Based on the growth pattern, soybeans are generally classified into two groups: indeterminate and determinate. Varieties of indeterminate growth habit continue their vegetative growth after the initiation of flowering (Pedersen, 2004). Most of the varieties within the maturity group 00 to IV exhibit an indeterminate growth habit and are typically grown in north central regions. In soybean varieties with determinate growth, the vegetative growth ceases before flowering begins. Soybean varieties with this growth habit are cultivated in the southern U.S., Mexico, and some regions of South America. Regardless of the growth habit, cultivated soybeans are generally annual plants. The length of the growing cycle of soybean crops varies according to the cultivar and maturity group, but usually ranges from four to six months.

Genetics and genome

Cultivated soybean and other members of the genus *Glycines* have 20 pairs (2n=40) of small and morphologically homogeneous chromosomes (Walling et al., 2006). This number is twice as great as that of the closest soybean phaseoloid relatives. The presence of 20 chromosome pairs in soybean is believed to have originated from at least two rounds of large genomic duplications/polyploidizations that occurred 59 and 13

million years ago (Schmutz et al., 2010). Thus, the ancestor of soybean and all members of the genus *Glycine* are considered to be polyploids. Polyploidization has evolutionary importance in higher organisms and is often proceeded by a diploidization event for restoration of diploidy (Walling et al., 2006). Recent analysis on centromeric satellite repeats using computational and cytogenetic approaches confirmed the hypothesis that the most recent polyploidization event in the soybean genome was allopolyploidy (Gill et al., 2009). This means that the duplication of the genome combined the genomes of two species, as contrasting with autopolyploidization, where duplications originate from a single species (Gill et al., 2009). The two species whose cross gave rise to the most recent polyploidizaiton event in the soybean genome are theorized to be extinct and to have had 2n=20 chromosomes. Since the soybean genome duplications occurred millions of years ago, soybean is also referred to as a palaeopolyploid species. Functional gene redundancy in palaeopolyploids is controlled by rapid silencing and/or lost from the duplicated genomes. Throughout evolution, most paleopolyploids have lost their polyploidy stage via diploidization and are currently considered as diploids.

The first assembled reference genome of a cultivated soybean (*Glycine max* var. William 82) was established in 2010 through the whole-genome shotgun approach (Schmutz et al., 2010). The whole genome sequence comprised 950 megabases, which represented about 85% of the predicted 1.1 gigabase genome. About 36,215 protein-coding genes from a total of 46,430 predicted genes in the soybean genome are located in the chromosome ends and are responsible for most genetic recombination. The soybean genome is highly duplicated, with almost 75% of genes occurring in multiple copies (Schmutz et al., 2010). Most homologous genes occur in blocks of two, three or four

chromosomes. The soybean genome also contains a significant amount of transposable elements, predominantly long terminal repeat retrotransposons (Class I) and to a lesser extent, DNA transposons.

Growing areas in the U.S. and economic importance

Soybean is a crop of global importance. Among all cultivated crops in the world soybean has the highest protein and oil content; its uses include human and animal food and industrial applications (Kumudini, 2010). Introduced to North America in 1765, soybean is now cultivated in at least 30 states of the U.S. (Fig. 2), extending over 31.1 million hectares (ASA, 2012). The top five soybean producing states are Iowa, Illionois, Minnessota, Missouri and Indiana. Nebraska ranked 7th among soybean producing states in 2012 with a total planted area of 2 million hectares (ASA, 2012).

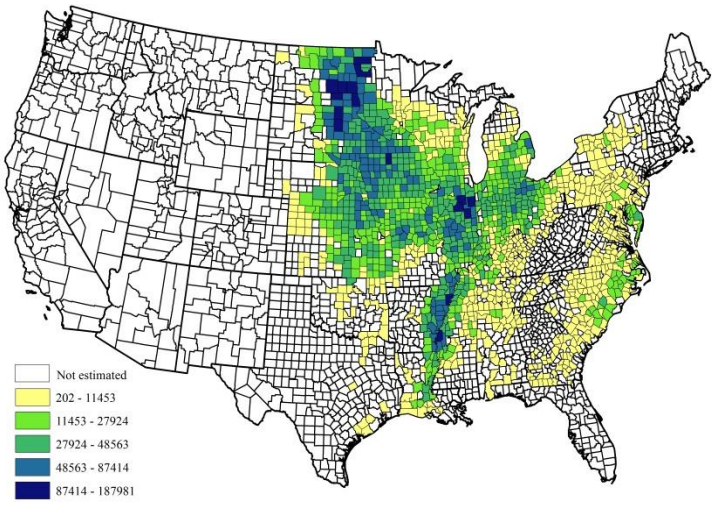

Figure 2. Soybean-planted hectares by county for selected U.S. states, 2012. Map generated using data from the National Agricultural Statistical Service, USDA.

The U.S. remains the major soybean producer in the world. In 2012, it produced about 82 million tonnes and the total crop value was estimated at $43.2 billon (ASA, 2012; NASS, 2013). Total exports of soy products surpassed $21.5 billion (ASA, 2012). The revenues generated by soybean product exports make soybean the second largest crop in cash sales and the number one crop in terms of the value of exports (ASA, 2012).

The pathogen: *Heterodera glycines*

Probable origin and global spread

Although it is difficult to determine the exact geographic region where SCN originated, it is generally accepted that SCN likely evolved with soybean in China. This hypothesis seems plausible because soybean and existing sources of SCN resistance are native to China (Yu, 2011). SCN damage on soybean could have been reported from northeastern China as early as 1880 (Noel, 1992; Liu et al., 1997). The first published report of SCN dates back to 1915 in Japan (Hori, 1915). The nematode was reported in Korea in 1936 and in Northeast China (formerly known as Manchuria) in 1938. In 1954, SCN was found in the U.S. (Winstead et al., 1955) and in 1968 it was reported in Egypt (Riggs, 1975). SCN has also been detected in South America: in Colombia, in the early 1980s, in Brazil in 1992 (Mendes and Dickson, 1993) and in Argentina in 1997 (Dias et al., 1998). To date, SCN is reported to also occur in Canada, Italy and Iran (Riggs, 2004; Yu, 2011).

Occurrence and distribution in the U.S. and Nebraska

Following its discovery in North Carolina 1954 (Winstead, 1955), SCN was found in Missouri and Tennessee in 1956, in Arkansas, Kentucky and Mississippi in 1957, and in Virginia in 1958 (Riggs, 1975). At present, SCN infestations are found in almost every soybean-producing state of the U.S. In Nebraska, SCN was first found in 1986 in the southeast area of the state (Powers et al., 1989). A survey of 552 fields conducted from 1986 to 1988 confirmed the presence of the nematode in 35 fields of 20 eastern counties of the state. As of 2012, SCN is found in all major soybean producing counties of Nebraska, extending as far west as Red Willow County (Giesler, unpublished data).

Morphology and taxonomy

The morphological account of each SCN stage presented below is largely based on the description provided by Turner and Rowe (2006). **SCN eggs** are oval with a length and breadth ranging from 86-134 μm and 36-52 μm, respectively. The length to width ratio is 2.1 to 2.6 (Turner and Rowe, 2006). The eggshell surface is smooth and without ornamentations. **Second-state** j**uveniles (J2)** are vermiform, with an offset head bearing three annules. The stylet is 23 ± 0.1 μm long with anteriorly curved basal knobs (Turner and Rowe, 2006). Body length averages 471 ± 30.0 μm and width 18.0 ± 0.5 μm. The anus is located at 45 ± 3.0 μm from the posterior terminus. In the middle of the body region, four lateral incisures are present. The pharynx of developed juveniles, as in most tylenchids, is divided into a nonmuscular procorpus, a muscular metacorpus and a posterior glandular region (Hussey, 1989). The pharyngeal glands consist of two

subventral and one dorsal cell that are connected to the pharyngeal lumen through small valves that control the release of secretions (Hussey, 1989).

Adult females and males are sexually dimorphic. Males are vermiform, robust and possess a hemispherical head with 5-6 annules. The male stylet is approximately 26.9 μm long with massive and rounded knobs. The length and width of the body are 1328 ± 51 μm and 28.6 ± 2.5 μm, respectively. The bidentate spicules average 34.3 ± 1.2 μm in length, with a simple gubernaculum 11.4 ± 2.0 μm long. Adult males also have four incisures in the lateral field.

Females are white and turn yellow towards maturity. Their body is about 470 μm long and 210 μm wide near maturity, lemon-shaped and tapers at the posterior region. The vulva is terminal and ambifenestrate. The stylet is weak with slight basal knobs. Stylet length is 27.5 ± 1.5 μm. The pharyngeal bulb is spherical and very prominent. The pharyngeal glands overlap the intestine lateroventrally. Ovaries are paired and fill the female body. Cysts (dead females) are dark brown with rugose lines on the surface and contain approximately 500 eggs. The anterior part of the cyst corresponds to the neck of the dead female and posterior part to the vulval cone or perineal area. Two thin-walled areas or fenestrae are present on the vulval cone, making the cysts ambifenestrate with two semifenestrae. In young cysts the fenestral area is membranous but later decays and leaves a pair of openings in the wall of the cyst (Turner and Rowe, 2006). Fenestrae are about 40 ± 4.0 μm long and 38 ± 3.9 μm wide. The semifenestral length and width average 19 ± 3.6 μm and 16.05 ± 3.6 μm, respectively. A vulval bridge approximately 4.0 ± 0.5 μm wide is present, and the vulval slit is about 40 ± 5.2 μm in length. The underbridge is strong and has a large plate-like growth in the center. The length of the

underbridge is approximately 100 μm. Bullae are heavy and crowd towards the top of the cone (Tuner and Rowe, 2006).

While the classification and taxonomy of nematodes is in a state of flux, in the most recent molecular-based nematode classification, SCN is classified within the class Chromadorea Inglis, 1932, order Rhabditida Chitwood, 1933, suborder Tylenchina Thorne, 1949, and family Hoplolaimidae Filipjev, 1984 (Decraemer and Hunt, 2006). Based on the classical or morphological scheme of classification (Siddiqi, 2000), *H. glycines* is placed in the order Tylenchida Thorne, 1949, family Heteroderidae Filipjev, 1941 (Skarbilovich, 1947) and subfamily Heteroderinae Filipjev and Schuurmang-Stekhoven, 1941.

Biology

Life cycle and infection process

The SCN life cycle comprises three major stages: egg, juvenile, and adult. Cysts that result from numerous infections in the plant throughout the growing season and that remain in the soil normally contain hundreds of viable eggs. A first-stage juvenile (J1) develops within each of these eggs. Then, through a first molt and while still within the eggshell, J1s develop into second-stage juveniles or J2s. At this stage the nematode possesses a well-developed stylet that gives it the ability to penetrate the root epidermis and infect a suitable host (Lauritis et al., 1983). Induced by temperature or stimulated by substances that are released from the roots of the host plant, infectious juveniles hatch from the eggshells and then emerge from the cysts (Niblack, 2005). Juveniles locate a root, presumably by chemolocation (Perry, 1996), and use their stylet to penetrate one of

the cells of the epidermal tissue. Once inside, juveniles migrate towards the central cylinder of the root, without establishing a permanent feeding site. The nematode chooses a first cell for feeding in or near the vascular tissue. Through the stylet, the nematode injects a variety of secretions into this initial feeding cell; such secretions affect the nucleus and other cellular organelles, resulting in significant morphological and metabolic changes in the cell. The cell walls of adjacent cells dissolve, turning into a large joined cytoplasm known as syncytium, which is a system of feeding cells or metabolic sink that provides all the nutrients necessary for the development of the nematode (Davis et al., 2004). The syncytium is maintained as long as the nematode is present and actively feeding. After an initial period of feeding, the J2 grows into a sausage-like stage and three to four days after infection it molts to the next stage (J3) and continues to swell. Six to seven days after infection, the J3 undergoes another molt and becomes a fourth-stage juvenile (J4). At this developmental stage, some of the juveniles begin a period of elongation within the cuticle; these juveniles become males. The J4 undergoes a final molt 8 to 9 days after infection. The adult males regain a vermiform shape and then emerge from the interior of the root, but do not feed on the root. In contrast, juveniles that develop into females continue to feed and to swell until they are too large to be contained within the root and finally break through the surface, with the head and neck remaining embedded in the root tissue. Fully developed females acquire a characteristic lemon shape and remain attached to their source of nourishment throughout their lives.

Adult females release a sexual attractant to which the males respond. After fertilization, over the course of several weeks hundreds of viable eggs may develop.

Afterwards, the female releases a portion of the eggs and retains the others. Finally the female dies and its body develops into a cyst. If soil temperature and moisture are favorable, the nematode can complete several generations per year, depending also on weed species present, soil type, and soybean variety planted (Niblack and Tylka, 2010). The SCN life cycle lasts from 21 to 22 days under optimal conditions of temperature and moisture (Lauritis et al., 1983; Niblack et al., 2006).

Molecular, genetic and biochemical events of parasitism

Most research and understanding in SCN parasitism is based on the *Arabidopsis thaliana — H. schachtii* model system (Davis et al., 2004; Mitchum and Baum, 2008). Because *A. thaliana* is successfully infected by *H. schachtii* (but not by SCN) it provides a broad access to molecular, genetic, and biochemical resources that allow the search for homologs to SCN (Baum et al., 2000; Davis, 2008). Moreover, experimentation with soybean roots is difficult and largely hindered by the time required to regenerate plants and the limited reverse genetic capabilities (Davis, 2008).

As with most described sedentary endoparasitic nematodes, *H. glycines* has developed a highly specialized form of parasitism and relationship with its hosts. Successful parasitism, referred to as a compatible interaction (Mitchum and Baum, 2008), is a sophisticated and dynamic process that involves hatching, attraction and penetration of the root tissue, recognition of cells and tissues suitable for feeding-site formation, modification of host tissue and induction of a syncytium, and an active response from the host (Hussey, 1989). All of these processes, along with additional morphological and physiological modifications induced in the hosts, are mediated, at least partially, through

secretions that are produced in the nematode's esopharyngeal glands and delivered through the stylet (Hewezi and Baum, 2013). Both the pharyngeal secretory glands and the nematode stylet are considered as evolved adaptations to parasitism for plant nematodes (Hussey, 1989; Hewezi and Baum, 2013). The two subventral and the single dorsal pharyngeal glands are involved in the secretory activity during the several stages of infection. However, while both gland cell types are involved, the subventral glands appear to be more active during the early stages of infection of root penetration, migration and feeding site initiation (Hewezi and Baum, 2013). When the juveniles start feeding and beging the actual sedentary stage, secretions from the dorsal gland cell are more active.

As products of genes predominantly expressed in the pharyngeal glands, the secretions released through the stylet are regarded as the genetic key to nematode parasitism. The genes coding for these proteinaceous secretions have been called parasitism genes (Hussey, 1989; Davis et al., 2004) and more recently, also referred to as effector genes. Hewezi and Baum (2013) used the term effector to refer to nematode secretions that facilitate nematode parasitism and those that are associated with triggering plant defense response and immunity. Until the early 1990s, little was known about the function and purpose of the pharyngeal secretions (Hussey, 1989). At present, although knowledge of the full process still remains elusive, several mechanisms have been uncovered. These range from mediating susceptibility of host plants to triggering plant defense responses (Hewezi and Baum, 2013). Effector proteins discovered so far include plant cell wall modifying enzymes (Smant et al., 1998), proteins that negate host defense responses (Davis et al., 2008), numerous regulators of host cell metabolism and cycle

(Bekal et al., 2003; Lee et al., 2011), proteins that facilitate nuclear localization (Elling et al., 2007), mimics of plant molecules (Olsen and Skriver, 2003), regulators of stress signaling (Patel et al., 2010), and activators of the hypersensitive response (Davis et al., 2008; Hewezi and Baum, 2013).

Races and HG types

The term "race" when originally proposed in the context of SCN followed the definition of "physiological race" used for fungi in plant pathology: a strain of a single pathogen species that attacks a specific cultivar of a particular host (Agrios, 2005). Four races of SCN were first proposed in 1970 (Golden et al., 1970) and the number of races was expanded to 16 in 1988 to account for additional identified differential soybean lines (Riggs and Schmitt, 1988). Until 2002, the system was misapplied as a means of studying *H. glycines* genotypes (Niblack et al., 2002), since it is not possible to apply infective juveniles to two hosts at the same time (Niblack et al., 2006). In an attempt to correct this misuse of the race scheme, a new classification method, designated HG typing (HG letters representing the first letters of the Latin binomial *H. glycines*) was proposed in 2002 (Niblack et al, 2002). This scheme is based on the use of seven soybean lines referred to as differentials, which are original sources of resistance archived in the USDA soybean germoplasm collection. More importantly, these are the sources of resistance from which most U.S. cultivars have been developed and used in the field (Niblack et al., 2002).

In the HG type scheme, determination of the HG type of a population is determined through a bioassay conducted according to a standardized set of experimental

requirements. Soil in adequate containers, where a replicated set of soybean indicator lines and the standard susceptible Lee 74 are transplanted, is infested with a suspension of equal numbers of *H. glycines* eggs and J2s; after 30 days a female index is estimated (Niblack et al., 2002). The female index is calculated as the quotient of the mean number of females on a test soybean line and the mean number of females on the standard susceptible multiplied by 100. The standard susceptible cultivar Lee 74 is used because this cultivar has not exhibited variation in response to SCN reproduction as have the cultivars Lee or Lee 68, used in previous SCN race schemes. The criterion for determination of host compatibility in the HG type system is set at 10%, that is to say, a soybean indicator line with a female index ≥ 10 is considered as a suitable host of the tested SCN population (Niblack et al., 2002).

Host range

The host range of *H. glycines* includes both cultivated and noncultivated plant species from several botanical families (Thorne, 1961; Riggs, 1992; Johnson et al., 2008). Riggs (1992) reported 96 genera in the family Fabaceae and 50 genera in 22 other families of non legumes as alternative hosts of SCN race 3. Although the cultivated soybean is the major SCN host of economic importance so far, cultivars of dry beans (*Phaseolus vulgaris* L.), including pinto, navy, black and kidney bean, have been shown to sustain reproduction of SCN suggesting that SCN could become a potential problem in the production of dry beans (Poromarto and Nelson, 2009). Recently, Shi and Zheng (2013) reported *H. glycines* parasitizing tobacco in natural infested fields in Central China.

Several species of winter annual weeds are also confirmed to be suitable hosts of SCN (Johnson et al., 2008; Mock et al., 2009). Some of them include purple deadnettle (*Lamium purpureum* L.), henbit (*Lamium amplexicaule* L.), field pennycress (*Thlaspi arvense* L.), shepherd's purse [*Capsella bursa-pastoris* (L.) Medik], common chickweed [*Stellaria media* (L.) Vill.], and smallflowered bittercress (*Cardamine parviflora* L.) (Venkatesh et al., 2000; Johnson et al., 2008; Mock et al., 2009).

Symptoms and damage caused by SCN

Soybean root infection by SCN results in morphological and physiological symptoms at the cellular, tissue and organ level. Cellular damage is caused during migration of the juvenile through the epidermal and cortical cells. Soybean plants infected with high SCN populations produce poor root systems that malfunction in the uptake of water and nutrients (Niblack and Tylka, 2010). Consequently, the resulting aboveground symptoms appear as stunting and chlorosis, with the latter often resembling iron, potassium or nitrogen deficiencies. Other common symptoms include a reduced number of pods per plant and seeds per pod (Mueller, 1984). In many cases, however, especially in experimental field plots in northern areas of the U.S., no obvious aboveground symptoms are induced (Wang et al., 2003). In such situations, the most commonly observed symptom is a significant reduction in yield.

Ecology, epidemiology and evolution

As a soil inhabitant, SCN interacts with both biotic and abiotic factors. Interactions of SCN with fungi, fungal-like organisms, bacteria, insects, weeds and other

nematodes have been reported (Bond and Wrather, 2004) and several soybean root diseases are exacerbated when SCN is present. Kaitany et al. (2000) reported greater incidence of *Phytophthora sojae* in areas with higher SCN population densities. Sudden death syndrome of soybean is reported to be more severe in soils infested with *H. glycines* (McLean and Lawrence, 1993; Xing and Westphal., 2006). Interactions of SCN with nodulating bacteria (Huang et al., 1984) and phytopathogenic bacteria have also been reported (Appel et al., 1984).

Dispersal of SCN occurs through the action of water, wind and agricultural practices (Lehman, 1994). Generally, any means causing displacement of SCN-infested soil and plant tissues can move SCN propagules from one place to another, either over short intrafield distances or over longer distances from field to field. Epps (1971) reported recovery of SCN cysts and infective juveniles from the digestive tracts and excrement of blackbirds.

The evolutionary relationships of members of the family Heteroderidae with other already-described nematode species of the Phylum Nematoda have been studied through the analysis of small subunit ribosomal (18S) DNA sequences. The most recently constructed phylogenetic tree is that proposed by Van Megen and collaborators (2009) and is based on 1215 small subunit ribosomal DNA sequences (*ca* 1700 bp each). According to that tree, *H. glycines* is placed in major Clade 12B (Van Megen et al., 2009). *H. glycines* is reported to belong to a monophyletic group of Tylenchida (Van Megen et al., 2009).

The environment: the soil

Soil phases and influence in nematode biology

Soil is a natural system that originates from the fragmentation, decomposition, and reintegration of rock material caused by physical, chemical and biological processes (Hillel, 2004). As a system, soil is polyphasic, particulate, heterogeneous, disperse and porous. It integrates a solid phase or soil matrix; a liquid phase or soil solution; and a gaseous phase or soil atmosphere. The solid phase is the permanent component of the soil and consists of mineral particles of varying shapes and sizes to which organic matter and hydrated iron oxides attach. The liquid phase is represented by soil water, which ubiquituously contains dissolved particles; and the gaseous phase is the air within the soil (Hillel, 2004).

As a soil inhabitant, SCN is affected directly or indirectly by the conditions prevailing in these soil phases. For example, root development and rooting patterns are affected by the soil conditions (Mitchell and Russel, 1971) and the profuse growth of roots through the soil could favor encounters between the roots and SCN propagules thereby influencing SCN population densities (Alston and Schmitt, 1987).

Properties known to affect soybean cyst nematode

Texture

A stable property of the soil defined as the proportion of the minerals sand, silt and clay, texture is a widely studied physical property influencing SCN (Slack et al., 1972; Heatherly et al., 1982; Koenning et al., 1988; Heatherly and Young, 1991). Published field studies on the influence of texture on SCN populations have assessed

such interaction during the soybean growing season, generally through a spring, fall, or spring and fall sampling. Studies considering SCN-texture relationship with attention to the non-host-crop growing season, however, are sparse. Texture affects many physical, chemical and biological processes, such as adsorption, ion exchange, hydration, expansion, floculation, and microbial activity in the soil (Hillel, 2004).

In a regional survey of 1,462 soybean fields sampled in the U.S., Workneh et al. (1999b) found that soil texture and tillage significantly affected SCN population densities and that clay content was negatively correlated with SCN densities in no-till fields. Within two individual fields planted to soybean, Avendaño et al. (2004) determined that SCN cyst densities were higher in loamy sand than in sandy clay loam areas. Heatherly and Young (1991), in a greenhouse study found that the number of SCN cysts in soybean plants increased in silt loam soil texture and decreased in clay texture. Texture significantly determines soil moisture, which is reported to affect nematode movement and egg hatching (Wallace, 1955), SCN development (Heatherly et al., 1982), and survival (Alston and Schmitt, 1988).

Within a single field varying in sand content from 72 to 87%, Koenning et al. (1988) investigated the soybean yield in the presence of SCN over two years. Their findings indicated that soybean yield was significantly reduced with increasing sand; the final SCN population densities were lowest in plots with the greatest sand content. However, the number of cysts observed 30 days after planting was not associated with texture in the two years studied.

pH

The association of soil pH with severity of diseases induced by soilborne plant pathogens has been previously documented for several crops (Trolldenier, 1981; Schmitthenner and Canady, 1983; Sanogo and Yang, 2001). Although the physical and chemical mechanisms responsible for this pH effect on pathogens are unknown, the general and common observation has been that increased soil acidification reduces disease severity (Trolldenier, 1981; Kurtzweil and Grau, 2001; Sanogo and Yang, 2001). For plant-parasitic nematodes, Norton et al. (1971) reported both positive and negative correlations between pH and population densities of several species of nematodes. Burns (1971) reported that at soil pH between 6.0 and 8.0, the root lesion nematode *Pratylenchus alleni* exhibited greater colonization of soybean roots than at pH 4.0.

In a survey conducted in Scotland, Duggan (1963) found positive correlations between pH and the population densities of the cyst nematode *Heterodera avenae*. Tefft et al. (1982) observed optimal hatching rates of encysted eggs of *H. glycines* at pH 6.0. Hatching was 50% higher at pH 6.0 than at pH 5.4 (Tefft et al., 1982). In a factor analysis of the relationship of 12 edaphic factors and *H. glycines* population densities, Francl (1993) determined that pH and magnesium levels factored together and both were positively associated with cyst densities of *H. glycines* across two years. More recently, Pedersen et al. (2010) reported a consistent positive correlation between pH and SCN population densities in two locations over two years of study. In their experiments, a negative correlation between yield and both pH and SCN population densities was also observed.

Temperature

Since SCN is a poikilothermic organism, its rates of development and survival are significantly affected by temperature (Ross, 1964; Lauritis et al., 1983; Alston and Schmitt, 1988). Ross (1964) reported significant effect of soil temperature on development, male to female ratio, and frequency of *H. glycines* juvenile degeneration in susceptible soybean roots. In his experiment, no development of SCN occurred at 10°C, and above 31°C male to female ratio and larval degeneration was more frequent than between 17 and 28°C. Egg hatching was observed to follow a linear trend with incubation temperature during a 2-week period test (Tefft et al., 1982). Slack and Hamblen (1961) reported optimum emergence of SCN juveniles from cysts incubated in Syracuse glasses at temperature of 24°C. In hot-water treatments of encysted juveniles and eggs, free eggs, and free juveniles, Endo (1962) found a liner response of the tested stages to temperature in the range 43 to 63°C. However, maximum survival of free juveniles was lower than that of encysted juveniles and eggs. In other study, Slack et al. (1972) reported that between 16 to 36°C, survival of SCN juveniles was negatively correlated with temperature increase. At constant temperature of 25°C, *H. glycines* completed its life cycle in 21 days in soybean root explants grown in gnotobiotic conditions (Lauritis et al., 1983). In a laboratory experiment, Alston and Schmitt studied the effect of temperature on SCN rates of development. The estimated basal temperature for egg development and thermal optimum for hatching were estimated to be 5 and 24°C, respectively (Alston and Schmitt, 1988). The same authors reported that a temperature range of 24 to 30°C was optimal for embryogenesis and hatch. In field experiments, Hill and Schmitt (1989)

observed the greatest SCN hatching in August and September when mean soil temperatures were between 25 and 29°C.

Control and management strategies

Biological and chemical control

At present, control of SCN field infestations is difficult to accomplish through biological and chemical methods. Several groups of microorganisms have been identified as antagonistic to SCN populations and a few commercial formulations are available; a few nematicides are also registered for soil application, and more recently, for seed treatments (Giesler and Wilson, 2011). Implementation of biological and chemical control methods, costs, and efficacy on a large field-scale, however, make these two methods of control unfeasible and a non-viable option to control SCN. Chemical methods also have environmental and personal health concerns rendering nematicides a risky option for control of this pathogen.

In some fields, SCN populations are naturally regulated by soil macro and microorganisms through predation, parasitism and intra or interspecific competition (Chen, 2004). Fungi and bacteria have been the subject of the bulk of research attention in the search for antagonistic organisms to SCN populations. Some species of fungi reported as biological control agents of SCN include *Cylindrocarpon heteronema*, *Hirsutella minnesotensis*, *Hirsutella rhossiliensis*, *Paecilomyces lilacinus* and *Lecanicillium lecanii* (Chen, 2004). In Minnesota, the fungi *Hirsutella minnesotensis* and/or *H. rhossiliensis* are found to cause high parasitism of second-stage juveniles of SCN in some fields (Chen and Reese, 1999). Only a few species of bacteria are reported

as biological control agents of SCN (Chen, 2004). Nour et al. (2003) found at least 30 bacterial phylotypes, predominantly in the genera *Lysobacter* and *Variovorax,* associated to the exterior surface, interior, and within a polymer plug region of *H. glycines* cysts. At the time of preparation of this review, Syngenta® announced the release of a seed treatment formulation with the bacterium *Pasteuria nishizawae* as the active ingredient (http://www.syngentacropprotection.com/news_releases/news.aspx?id =174360). This formulation is claimed to confer effective protection against SCN activity.

Cultural control

Despite abundant fundamental and field-applied research on SCN carried out worldwide to date, no single molecule or gene (s) providing effective control of this nematode has been discovered. Fundamentally, SCN field infestations have been managed through growing SCN resistant soybean varieties and rotating with nonhost crops for one, two or more years (Niblack, 2005; Noel, 2008). Use of resistant varieties and crop rotation are demonstrated to significantly reduce SCN populations (Koenning et al., 1993; Niblack, 2005). These two practices have been the most effective strategies to manage SCN and will continue to be the most promising long-term practices to mitigate the losses caused by SCN. From an integrated crop management standpoint, timing of SCN field scouting and additional cultural practices aimed at maintaining the nutritional state of the crop (irrigation, weed control, fertilization, drainage, etc.), combined with genetic, biological and chemical control, can all contribute to mitigate the yield losses induced by SCN infestations.

Resistant varieties

The use of SCN-resistant soybean varieties has been an effective strategy to manage SCN (Niblack, 2005; Tylka, 2008). At present, numerous high yielding, SCN-resistant soybean varieties are available to farmers for incorporation into their crop management plan. Resistant varieties prevent SCN juveniles from completing the establishment of a syncytium, either by slowing development or hastening deterioration of the feeding site, and causing starvation of the juveniles within the root tissue (Tylka, 2008). Consistently, resistant varieties yield more than susceptible in field evaluations. Use of resistant varieties has prevented significant reduction of yield and thereby increases in crop profits. For instance, estimates of yield losses indicate that use of the resistant cultivar 'Forrest' alone increased profits by $405 million from 1973 to 1982 (Bradley and Duffy, 1982).

Origin, sources and genetic basis of resistance

Soon after the initial detection of SCN in North Carolina in 1954 (Winstead et al., 1955), resistance to SCN was sought as a potential method for control of this pathogen. Ross and Brim (1957) were the first to identify soybean resistance to SCN in the U.S. Later, after several years of planting resistant cultivars, Ross (1961) reported genetic variation of SCN populations, which was also confirmed by subsequent research (Miller, 1969; Riggs et al., 1981). A large number of plant accessions, referred to as plant introductions (PI), were brought into the U.S. mainly from China to enrich the gene pool for future breeding programs. At least 118 soybean plant introductions (PIs) with SCN resistance have been identified from the USDA-ARS soybean germplasm collection as of

2013 (Rao-Arelli et al., 2000; Vincent et al., 2009; Kim et al., 2010). Nevertheless, despite the large number of sources of resistance, only two PIs (PI 88788 as number one and PI 548402 or Peking) are the predominant sources for the majority of SCN-resistant cultivars used in the northern region of the U.S., so the genetic base of host resistance remains narrow (Concibido et al., 2004). From the two aforementioned plant introductions, one resistant gene is known to be carried by the varieties derived from PI 88788 and four resistance genes from PI 548402.

The genetic basis of SCN resistance was first elucidated through crosses of resistant and susceptible soybeans (Caldwell et al., 1960). These researchers reported that the observed resistance from PI 548402 ('Peking') was the result of three independently inherited recessive quantitative trait loci (QTLs), at the time referred to as resistance genes, and were designated as *rhg1*, *rhg2*, and *rhg3*. A fourth resistance QTL from 'Peking', designated as *Rhg4* and conferring dominant resistance, was reported in a later study (Matson and Williams, 1965). Rao-Arelli et al. (1992) identified an additional dominant resistance gene in PI 88788, designated as *Rhg5*.

The understanding of resistance to SCN has been aided by genetic marker technology and QTL mapping. QTLs are stretches of DNA that include or are closely linked to the genes that underlie a quantitative trait, thus QTLs may not necessarily be the genes themselves (Vincent and Matthews, 2009). A quantitative trait is a phenotypic characteristic that varies in degree and is attributed to the effect of two or more genes in conjunction with the environment. A minimum of 61 resistance QTLs have been mapped on more than 18 soybean linkage groups from eight different sources of resistance (Concibido et al., 2004; Kim et al., 2010). Mapping of these QTLs has improved the

understanding of quantitative disease resistance to SCN in soybeans. Nonetheless, because of the narrow genetic base, SCN has adapted and overcome resistance in soybean cultivars due to shifts to virulent HG types.

Cloning of the *rhg1* and *Rhg4* candidate resistance genes was reported in early 2000 (Hauge et al., 2001; Lightfoot and Meksem, 2002). The *rhg1* gene, from Peking, encodes a leucine-rich repeat receptor-like kinase that is similar to the rice Xa21 leucine-rich repeat receptor kinase (Ruben et al., 2006; Kim et al., 2010). Kim et al. (2010) reported fine-scale mapping of the *rhg1-b* allele derived from the PI 88788. The *rhg1-b* allele is placed to a 67-kb region of the 'Williams 82' genome sequence. In their analysis, this group showed that the receptor-like kinase gene, previously identified as a candidate for the 'Peking'-derived SCN resistant *rhg1* gene, is adjacent to -but outside of- the *rhg1-b* interval defined in their study.

More recently, Liu et al (2012) reported the map-based cloning of a gene at the *Rhg*4 locus. The gene was identified to encode a serine hydroxymethyltransferase, an enzyme structurally conserved across biological kingdoms and that is responsible for interconversion of serine and glycine and is essential for cellular one-carbon metabolism.

Significant research on mapping and cloning resistance genes, and breeding efforts to develop SCN-resistant soybean varieties has been carried out thus far. However, given the complexity of its genetic basis, SCN resistance, resistance is still incompletely understood. A current barrier to the understanding of the genetic basis of resistance of soybean to SCN is the nature of the resistance itself in this pathosystem. Resistance to SCN is quantitative, which means it occurs in a genetically variable (segregating) population of individuals that does not fit typical Mendelian segregation

ratios (Concibido et al., 2004). This also means that as a quantitative trait, SCN resistance is multigenic, controlled by a few to many genes that can interact with each other and with the environment, and possibly affecting more than one trait. Because of this, quantitative resistance is not as well understood as qualitative or monogenic resistance and has consequently not been extensively used by soybean breeders.

Crop rotation

Rotation with nonhost crops has been a recommended management practice for reducing SCN populations since the 1960s (Ross, 1960; Thorne, 1961). In the U.S., the earliest studies on the effects of crop rotation on SCN populations and soybean yield are those conducted by Ross in North Carolina (Ross, 1960; 1962) and Epps and Chambers in Tennessee (Epps, 1960; Epps and Chambers, 1965). Ross reported that during the second consecutive season of nonhost cropping (corn, cotton and cowpea) SCN populations were significantly reduced, in some cases to undetectable levels. Epps and Chambers (1965) reported that no SCN individuals were recovered after two consecutive years of growing cotton in field bin experiments designed to control possible SCN reinfestation caused by water movement. Moreover, their results suggested that in the climate and conditions where the experiment was carried out, SCN could have survived four years but not five years. Noteworthy is that in Ross's and Epps and Chamber's studies, SCN population density was measured as the number of second stage juveniles that emerged from cyst extracted from soil samples.

Numerous cultivated plants have been evaluated for their suitability and potential for use as rotational crops in SCN management (Riggs, 1992; Miller et al., 2006; Warnke

et al., 2006). Warnke et al., (2006) tested the effect of growth and decomposition of 46 cultivated plants on *H. glycines* population densities in the greenhouse. Their findings suggested that with leguminous plants, as a group, egg populations were lower than with monocots, including corn, wheat, barley and oats among others. In that study, sunn hemp (*Crotalaria juncea*) was the plant showing the lowest SCN eggs and cyst numbers. In an assessment of 16 different rotation crops in northern U.S., Miller et al. (2006) found that one year of rotation with the tested crops was not sufficient for effective management of SCN.

In the North Central region of the U.S., corn is by far the most widely used crop in rotation with soybean (Niblack, 2005; Chen, 2007; Noel, 2008; Giesler and Wilson, 2011). A considerable amount of research confirms that rotation with corn in annual, biannual, or in more than two years, consistently results in lower SCN population densities as compared with susceptible soybeans (Young and Hartwig, 1992; Noel and Edwards, 1996; Noel, 2008). The mechanisms responsible for reduction of SCN populations by crop rotation are not fully understood. Nevertheless, recently, Warnke et al. (2008) compared the effect of sunn hemp, Illinois bundleflower, oilseed rape, perennial ryegrass, red clover, corn, and *H. glycines*-susceptible soybean in their effects on *H. glycines* hatch, viability and development in greenhouse and laboratory experiments. Their findings suggested that stimulating hatch of juveniles of second stage and impeding the subsequent successful infection establishment was the main mechanism involved in reducing the *H. glycines* population density.

Soybean yield is normally higher when soybean is grown following corn rotation (Noel, 2008). A combination of corn rotation with nematicides applied as soil fumigants

showed enhanced control of SCN in the field (Sasser and Grover, 1991). Young and Hartwig (1992) reported that in experimental plots, soybean yield was higher in corn-SCN resistant soybean variety rotation than in continuous monocropping with a resistant variety for 11 years. Use of bio-covers has also been tested to control SCN in combination with crop rotation. Wight et al. (2011) found that bio-covers of winter wheat, *Vicia faba* and poultry manure did not differ significantly in the egg population density of SCN. A study in Minnesota (Porter et al., 2001) indicated that up to five years of consecutive planting of an SCN nonhost crop were not enough to deter SCN population densities from infested land.

Tillage and irrigation

Tillage systems are classified into several classes based on the amount of surface residue that is left after harvest (Anonymous, 1997; CTIC, 2001). Conservation tillage refers to operations that maintain 30% or more residue after planting; reduced-till, or minimum till, practices maintain 15 to 30%; and conventional tillage practices maintain less than 15% residue cover (Anonymous, 1997).

The influence of various tillage regimes on SCN population densities has been widely investigated, but contrasting results have been observed among studies (Noel, 2008; Westphal et al., 2009). The sole inference that can be made from such inconsistent results is that tillage effects are site- or region-specific (Noel and Wax, 2003; Niblack and Chen, 2004). In a regional survey of 1,462 fields, Workneh et al. (1999a) found greater SCN prevalence and population densities in tilled than in non-tilled fields. Noel and Wax (2003) reported that SCN reproduction, measured as the ratio of egg count at harvest to

egg count at planting, was higher in no-tillage than in conventional tillage in a field with silty clay loam texture.

Although apparently inconsistent, tillage effects on SCN populations might depend on the timing of tillage operations in the growing season. For instance, LaMondia (2008) found that in tobacco, prompt tillage after harvest consistently resulted in lower tobacco cyst nematode populations. The effect of tillage in the dispersal of *H. glycines* is confirmed from plot experiments (Gavassoni et al., 2007) and appears to be associated with regional spread (Workneh et al., 1999a). A recent study on tillage intensity on *H. glycines* population densities (Westphal et al., 2009) showed that reduced tillage intensity resulted in lower SCN population densities in rotational experimental plots.

The effect of irrigation on SCN populations has not been directly investigated. However, in irrigated fields the water provided to the crop presumably creates singular soil conditions that affect soil moisture, which is reported to affect SCN development (Heatherly et al., 1982).

SCN management challenges

The effectiveness of management options for SCN is strongly influenced by the biological and ecological characteristics of this pathogen (Niblack, 2005). The sophisticated mechanisms of parasitism that SCN has evolved to sustain an endoparasitic and sedentary habit in its hosts are mediated by an arsenal of parasitism proteins that are expressed in the pharyngeal gland cells (Davis et al., 2004). The soybean yield reductions associated with significant increases in SCN populations during a growing season suggest that as a soil-dweller and parasite of soybean roots, SCN is dominant over other

microbial populations in the soil surrounding the host roots. Prominent biological characteristics that influence management of SCN include: the presence of a cyst stage as a survival mechanism, high reproductive capacity, existence of diverse virulence phenotypes (Niblack et al., 2002), and a high level of specialization as a plant parasite (Niblack et al., 2006).

The ability to form or become a cyst enhances SCN survival and persistence in an infested field for a long period of time in the absence of a suitable host. Egg viability within the cyst is preserved and in consequence, crop rotation even for several consecutive years does not result in total mortality of SCN populations (Warnke et al., 2008). Two additional biological features of cyst nematodes that can influence the effects of the nonhost rotation management practice are the presence of dormancy (diapause and quiescence) as a survival "strategy" and the unsynchronized hatching of juveniles (Yen et al., 1995). These two mechanisms hinder estimations of initial population densities and thus, their relationships to epidemic progress. The aforementioned adaptations for survival allow SCN to persist and spread within and between fields and to other diverse habitats.

The high reproductive capacity of SCN (completion of its life cycle in a relatively short period of time and production of a high number of eggs per female) helps compensate for the death of individuals occurring during the nonhost rotation, due to predation, parasitism, or competition with other nematode species for a common ecological niche (Agrios, 2005). In an evolutionary framework, this is an attribute that guarantees the perpetuation of this species. A cyst nematode-infested field with low populations may increase its numbers by several orders of magnitude in a single growing

season (Noel, 2008), provided favorable weather conditions and a suitable or susceptible host. This is because SCN is a multivoltine species and might produce up to 4 to 6 generations in a single growing season or a single year.

Resistant varieties lower cyst populations (Niblack, 2005), but since resistance is not complete, some individuals are still capable of reproducing in any given variety. Throughout a growing season, under favorable conditions for nematode development, individuals that are able to reproduce in a resistant variety can reinfect the host and complete several reproductive cycles. This prompts the possibility of emergence of a resistant population in just one growing season. Understanding the nature of resistance and cyst nematode population virulence helps make decisions on variety selection in a management plan.

Field populations of SCN vary in their ability to infect and reproduce in different soybean cultivars (Ross, 1961; Golden et al., 1970, Niblack et al., 2002). The ability to reproduce on a resistant host is termed virulence. Such ability may suggest both genetic diversity among cyst nematode field populations and similar diversity within populations. The variation in parasitism among populations of SCN, as measured by their reproductive capacity, is a crucial management issue. The existence of different virulence phenotypes and the ability of the nematode to complete several generations per year provides a wider window of opportunity for increasing disease pressure thus overcoming host more rapidly. In practice, the variability of virulence in phenotypes has to be considered carefully when recommending a resistant variety in a management program (Niblack, 2005). In order to minimize or delay the development of resistant-breaking SCN

populations, it is important to know the source of resistance from which a planted variety has been derived and to rotate sources of resistance.

SCN exhibits very specialized parasitic behavior, including a relatively narrow suitable host range, variation in virulence, synchronization between its life cycle and host availability, evolution of strategies for survival, and the hatching mechanism. In the latter mechanism, J2s do not hatch unless stimulated by host root diffusates, at least in some cases (Tefft and Bone, 1985). Hatching can also be temperature or age mediated (Niblack, 2005). This variation in hatching timing makes difficult the estimations of SCN inoculum potential thereby complicating understanding of how SCN populations affect yield. In addition to the biological characteristics described above, SCN populations seem to also possess ecological features that make them successful in the complex interrelationship with other soil inhabitants. At least in monocrop settings it appears that SCN is dominant over other competitors in the soil and capable of inducing significant yield reductions.

The biological and ecological characteristics of SCN, combined with other factors outlined in this chapter, are the reasons for many challenges with the management of this pathogen.

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CHAPTER III. RELATIVE CHANGE OF SOYBEAN CYST NEMATODE POPULATION DENSITY AFTER ANNUAL CORN ROTATION IN NEBRASKA AND DESCRIPTION OF THE RELATIONSHIP OF SOIL TEXTURE AND POPULATION DENSITY REDUCTION USING MULTIVARIATE METHODS

ABSTRACT

The field mortality of soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe) populations was studied in 2009, 2010 and 2011 in 45 soybean production fields annually rotated to corn in Nebraska. The SCN population density (eggs/100 cm^3 of soil) for each field was determined before and after the corn rotation year from ten 3 x 3 m sampling grids systematically selected and georeferenced for site-specific resampling. Summary statistics and correlation analysis were used to describe the SCN field mortality. Multivariate analysis was used to characterize the relationship of soil texture and SCN population density reduction during the rotation year. The SCN population density before and after rotation were highly correlated ($r = 0.90$, $P < 0.0001$) in most fields surveyed. The population density of the nematode after the rotation year exhibited an average decrease of 42% in 2009, 60% in 2010 and 56% in 2011, with an overall average of 50.62% over the three years. Two principal components explained 92% of the total variability in the data set. The first component represented soil texture and the second corresponded to SCN population densities before and after rotation. Cluster analysis identified two groups of fields: one group with predominantly sandy soil (57 to 95%) and the other with predominantly silty soil (23 to 61%). SCN Pi was significantly higher in the sandy group than in the silty group ($F = 271.19$, $P<0.0001$).

INTRODUCTION

The soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe, 1952) is a significant and widespread pathogen of soybean in the major soybean producing countries of the world (Noel et al., 1994; Liu et al., 1997; Riggs, 2004; Doucet et al., 2008; Yu, 2011). In the U.S., SCN has consistently remained the number one pathogen limiting soybean yield for the last 15 to 20 years (Doupnik, 1993; Niblack, 2005; Wrather and Koenning, 2006; Wrather, 2008). Soybean yield reduction due to SCN, as shown from the most recent assessment, was estimated at close to 34.7 million metric tons during 2006 (Wrather, 2008). In Nebraska, soybean was grown on about 2.0 million hectares in 2012 (NASS, 2013) and economic losses owing to SCN were estimated at 40 million U.S. dollars (Wilson and Giesler, 2013). Yield losses in experimental plots have been observed to be as high as 40% (Giesler and Wilson, 2011).

Current recommendations for management of SCN in Nebraska and other SCN-infested regions include rotating non-host crops (mainly corn) for one or two years and growing SCN resistant soybean varieties (Niblack, 2005; Giesler and Wilson, 2011). While the effects and benefits of these two practices in reducing SCN population densities are well documented (Koenning et al., 1993; Schmitt, 2004; Niblack, 2005), it is commonly suggested that crop rotation might not be as effective in reducing SCN population densities in northern as in southern U.S. regions. In the southern U.S. for example, two years of rotation with a non-host crop were sufficient to reduce SCN population densities to almost undetectable levels (Ross, 1962; Schmitt, 1991; Koenning et al., 1993). In contrast, in the northern U.S., up to five years of rotation with a non-host crop were not enough to eliminate SCN problems (Porter et al., 2001). In Nebraska, corn

is exclusively the major SCN non-host crop and is grown predominantly in annual rotation with soybean. More than 2.1 million hectares of corn are under irrigation, which represents over 60% of the total corn cultivated area in the state (NASS, 2013). In irrigated fields, the water provided to the crop modifies soil conditions, notably the soil moisture, which affects not only the development of plant roots, but also that of soil macro and microorganisms (Hillel, 2004). Soil moisture has been largely associated with the hatching behavior of cyst nematodes, including SCN (Wallace, 1955; Slack et al., 1972; Clarke et al., 1978). Its properties and relation with SCN population densities depend substantially on soil texture (Heatherly and Young, 1991). The influence of soil texture in SCN population densities is reported in several studies (Slack et al., 1972; Heatherly et al., 1982; Koenning et al., 1988; Heatherly and Young, 1991). In a regional survey of 1,462 soybean fields in U.S., Workneh et al. (1999) found that soil texture and tillage significantly affected SCN population densities and that clay content was negatively correlated with SCN densities in no-till fields. Within two individual soybean fields, Avendaño et al. (2004) determined that SCN cyst densities were higher in loamy sand than in sandy clay loam areas. In a greenhouse study, Heatherly and Young (1991) found that the number of SCN cysts in soybean plants increased in silt loam soil and decreased in clay soil.

Most of the field studies on the influence of texture on SCN population densities have assessed such a relationship in the soybean growing season, generally through a spring, fall, or spring and fall sampling. However, few studies have considered SCN-texture relationship with attention to the non-host crop growing season. Because most of the corn grown in Nebraska is under irrigation and precipitation levels in the state are

lower than those in southern U.S. regions, Nebraska possess a set of unique agroecological and agroclimatic conditions that could allow a distinctive assessment of the effect of non-host rotation on SCN population densities. This could provide significant insights not only for SCN management but also for comparison of specific management components for SCN in field production settings. Information on SCN population densities in commercial-size production fields, and population density changes after rotation with corn in Nebraska does not exist.

This research study was designed to (i) determine the percent change of SCN population densities in soybean commercial fields annually rotated to corn in Nebraska, and (ii) describe the relationship of soil texture and SCN population density reduction after annual corn rotation using multivariate methods.

MATERIALS AND METHODS

Field selection

Average-size commercial production fields (37 to 55 ha) with a history of SCN and in a soybean-corn rotation regime were identified in the major soybean producing areas of Nebraska in 2009, 2010, and 2011. All identified fields had been tested positive for SCN from at least one composite sample diagnosed in a nematology laboratory of the University of Nebraska-Lincoln. Only fields with a report of at least 250 SCN eggs/100 cm^3 of soil were preliminarily selected for the study. A set of 29 fields were selected in 2009, a second set of 30 fields in 2010, and a last set of 20 fields in 2011 (Fig. 1). These groups of fields are herein referred to as 2009-field set, 2010-field set, and 2011-field set,

respectively. The three sets of fields included both rain-fed and central-pivot irrigated fields as well as tilled and no-till fields.

Sampling

Fields following one year of soybean production were sampled. In each of the selected fields, ten 3 x 3 m sampling grids were selected systematically in a zig-zag pattern and the center of each grid was georeferenced with a GeoXT handheld GPS unit (GeoExplorer 2008 series Trimble®, Westminster, CO) for site-specific resampling. From each grid, twenty 2.5-cm-diameter, 15-to-20-cm deep soil cores, spaced at 60 cm, were collected and mixed in a bucket to obtain a single composite sample. Samples were collected in plastic bags, placed in a cooler, and stored at 4ºC in a cold room until they were processed. Sampling of each field set was conducted from late April to the end of May (preceding corn planting) and from late April to the end of May of the following year (preceding soybean planting).

In order to enhance a zig-zag pattern of the sampling grids within each field, the boundaries of all selected fields were mapped and added to the mobile field software that was used in this study (ArcPad 7.0®, ESRI®, Redlands, CA). This helped visualize and improve the separation distance and positioning of each sampling grid at the time of sampling. Attempt was made to avoid sampling areas in the field that could represent unusual observations, such as entry ways or low areas. Also, for sampling consistency, the actual collection of the soil cores in each sampling grid was done in an east-west direction at all times.

Sample processing and determination of SCN population density

In the laboratory, each composite soil sample was crushed and thoroughly mixed to obtain a homogenous sample. Then, a 100 cm^3 subsample, measured by volumetric displacement, was processed to determine the number of SCN eggs/100 cm^3 of soil using standard sieving techniques for cyst nematodes: decanting the sample content on the 710-µm-pore sieve stacked over the 250-µm-pore sieve to collect the cysts and then on the 125-µm-pore sieve stacked over the 25-µm-pore sieve to extract the eggs. The extracted eggs from each sample were collected in water in a 100-ml beaker keeping the water volume in the beaker at < 20 ml for all the samples. One ml of acid fuchsin was added to each sample and the sample was microwaved for 1.2 to 1.4 min (700 Watts power microwave) to stain the eggs. After the sample was raised to a standard volume of 20 ml, one ml from each sample was collected and placed in a counting dish, observed under a dissecting microscope at x35, and the number of eggs was recorded. Three counts were performed for each sample to calculate an average representing one field sampling grid.

Percent change of SCN population density after rotation

To meet the field selection criteria sought at the inception of the study, fields whose average SCN population density before rotation was below 250 eggs/100 cm^3 of soil and that had four or more SCN-negative sampling grids were excluded from analyses. Out of the 79 fields that were initially sampled in the three years, 45 were used in the final analyses. An SCN population density average before (Pi) and after corn rotation (Pf) was obtained for each field from the eggs counts of the 10 sampling grids.

The percentage of SCN population density change after the corn rotation year in each field was calculated with the formula:

$$\text{SCN population density change} = \frac{\text{Pf - Pi}}{\text{Pi}} x\ 100$$

where:

Pf = the final SCN population density (eggs/100cm^3 of soil) or population density after annual corn rotation.

Pi = the initial SCN population density or population density before corn rotation.

Summary statistics of percent change of SCN population density after rotation: mean, median, range, and the standard deviation were obtained for each field set.

Correlation analysis for rotation effects

The Pearson's product-moment correlation coefficient was used to describe the association between SCN egg densities before (Pi) and after corn rotation (Pf) in all fields of the three sets. A moderate to high correlation is expected between Pi and Pf if an association between corn rotation and SCN population density reduction is verified. In other words, a significant correlation would be suggestive that the SCN population density reduction in the studied fields is the result, at least partially, of the corn rotation effect.

Relationship of soil texture and SCN population density reduction after rotation

The 45 fields used in the previous evaluation were also used to investigate the relationship among soil texture (% of sand, silt, and clay), Pi, and Pf using multivariate analysis. A single composite sample for each field was obtained from the six to ten

samples having similar texture and being SCN-positive. Categorization of the soil texture similarity among samples of each field was achieved visually and by tact based on the protocol proposed by Thien (Thien, 1979). The texture of the single composite sample representing each field was determined by a commercial soil testing laboratory (Wards laboratories, Inc., Kearney, NE). The average SCN population density for each field was obtained based on the number of samples used in each case.

Principal component (PCA) and cluster analyses were used to describe the association of soil texture and SCN population reduction in all fields of the three sets. Both Pi and Pf were transformed to the logarithmic scale for use in PCA and clustering. The PCA was applied on the correlation matrix of the variables using the PRINCOMP procedure of SAS (SAS Institute, Cary, NC). PCA was mainly used as a screening method of the data set and for verification of clustering results. Selection of the appropriate number of principal components was based on the percentage of variance explained. This was predetermined at $\geq 90\%$ and was ascertained from the eigenvalues of each of the selected components. A scree plot was also used to aid in deciding the number of principal components to choose. Selected principal components were also used to evaluate multivariate assumption in the data through normality tests for pairs of components and normal probability plots. In addition, scatterplots of paired and three components were used for checking the data set for possible outliers or other abnormalities.

The analysis of clusters was used in our study to investigate whether fields with similar texture tended to exhibit similar SCN population density reduction. The values of the five measures of association (sand, silt, clay, Pi and Pf) were standardized to give

each variable an equitable weight in the analysis. The standardization consisted in dividing the data values of each variable by the variable's range (Milligan and Cooper, 1988), as shown by the following formula:

$$Z_{ij} = \frac{x_{ij}}{(Max(X_i) - Min(X_i))}$$

where:

Z_{ij} = the transformed value of each *jth* datum of the X_i variable, *i=1,2,…p, and j=1,2,…n*

x_{ij} = *jth* datum of the X_i variable

Max (X_i) = the maximum value of the X_i variable

Min (X_i) = the minimum value of the X_i variable

After standardization, data were analyzed with the CLUSTER procedure of SAS®. The average distance method was used as the method of clustering and the cubic clustering criterion (CCC), Hotelling's pseudo test statistic (T^2) and tree diagrams were used to help determine the appropriate number of clusters in the data set. The complete linkage and the Ward's minimum variance clustering methods were used to verify the clustering results of the average method.

As a final assessment to the cluster analysis results, the uncertainty in the clustering produced by the average linkage method was evaluated with a multiscale bootstrap resampling method (Efron et al., 1996; Shimodaira, 2004) using the Pvclust package of R software (Suzuki and Shimodaira, 2006). The multiscale bootstrap analysis provides two kinds of *P* values that indicate how strong the cluster is supported by the data. One is the approximately unbiased (AU) *P* value and the other the bootstrap

probability value (BP). For a cluster with AU *P* value > 0.95, the hypothesis that the cluster does not exist is rejected with significance level 0.05.

RESULTS

Percent change of SCN population density after rotation

The highest SCN population densities before rotation (Pi) were observed in fields of the 2010-field set (Fig. 2). The largest population density variability was also observed in this field set, with SCN egg densities reaching a maximum field average of 15,259 eggs/100 cm^3 of soil and up to 57,640 eggs/100 cm^3 in individual sampling grids.

SCN population densities exhibited an average change of -42% in the 2009-field set with a range of decline of 0 to 77% (Fig. 3). In four fields, or about 19% of the 21 fields that were examined, SCN population density declined between 45 and 67%, and in ten fields (about 48%), the population density declined between 23 and 45%. In the remaining eight fields, the percentage of SCN population density decline was below 23 in seven fields and above 67 in one field (Fig. 4).

In the 2010-field set, average population density change was -60% with a decline range of 1 to 94% (Fig. 3). Six fields, or about 33% of the 18 fields that were examined, showed an SCN population density decline between 45 and 67%, and seven fields (about 39%) showed a SCN population density decline between 23 and 45%. In the remaining five fields, the percentage of SCN population density decline was below 23 in four fields and above 67 in one field (Fig. 5).

In the 2011-field set the average SCN population density change was -56% with a range of decline of 5 to 86% (Fig. 3). Four fields out of the five that were examined showed a population density decline between 51 and 86%, and one field, a decline of 5% (Fig. 6).

Overall, the highest SCN population density decline occurred in the 2010-field set (Fig. 3). In this rotation year, there were also relatively more fields with a decline between 45 and 67% (Fig. 5).

Correlation analysis for rotation effects

Within fields, SCN egg densities in individual sampling grids before (Pi) and after rotation (Pf) were positively correlated in most fields. Observed correlations were significant ($P \leq 0.05$) in most fields of the three field sets. The average correlation was 0.66, 0.61 and 0.51 for the 2009, 2010 and 2011 field sets, respectively. Using the Fisher's z transformation, correlation estimate between Pi and Pf of the 220 individual samples of the 2009-field set was 0.76 ($P<0.0001$, confidence interval 0.70 to 0.81) whereas for the 170 individual samples of the 2010-field set, correlation was 0.76 ($P<0.0001$, confidence interval 0.68 to 0.81).

Correlation between Pi and Pf for field averages was also significant and positive for the 2009 and 2010-field sets. In the former, the correlation coefficient was 0.92 ($P<0.0001$) and in the latter, correlation was 0.89 ($P<0.0001$). For the 2011-field set, observed correlation was 0.64 with a low level of significance ($P=0.25$).

Relationship of soil texture and SCN population density reduction after rotation

Pairwise scatterplots of sand, silt, clay, Pi and Pf suggested positive correlations between silt and clay and Pi and Pf (Fig. 7). Eigenvalues of the correlation matrix indicated that the first two principal components explained approximately 92% of the total variability in the data set (Table 1, Fig. 8). The first eigenvalue accounted for 60.5% of the total variability whereas the second accounted for 31.5% (Table 1). This implies that the five measured variables nearly fell within a two-dimensional subspace of the sample space. A scree plot of the eigenvalues against each principal component also showed that at the third principal component the eigenvalues tended to level off, suggesting two principal components were enough to describe the data set. The eigenvalues of the third, fourth and fifth principal component were close enough to zero and therefore were ignored (Fig. 9).

Overall, the first two principal components had a univariate normal distribution. Yet, a scatterplot of the first three principal components displayed at least three observations out of an elliptical region, suggesting such observations as potential outliers (Fig. 10 A-B). Further exploration of those observations using the multivariate outlier detection method of Filzmoser et al. (2005) identified the same observations outside a 95% theoretical quantile of a Chi-square distribution (α=0.05) with 3 d.f. (Fig. 11).

The two selected principal components were interpretable, as based on their eigenvectors, the correlation coefficients of each of the variables with each component, and the significance level of the correlations (Tables 2 and 3). The eigenvectors of the first principal component represented a contrast of sand, Pi, and Pf vs. silt and clay. Essentially, based on the values of the eigenvectors (Table 2) and their respective

correlations (Table 3), this principal component was a measure of soil texture itself. The second principal component was also a contrast between sand and the rest of the variables. This component represented Pi and Pf.

The Cubic Clustering Criterion (CCC) suggested the existence of two major clusters, as shown by the occurrence of a small peak at two clusters in the graphs of CCC vs. number of clusters (Fig. 12). Another peak was apparent at four clusters, suggesting that four minor clusters or subclusters were also likely in the data set (Fig. 12). The plot of the pseudo T^2 statistics suggested that clustering occured at seven, four, and two clusters. This is interpreted by looking at the plot from right to left until a first value that is markedly larger than the previous value is found, then moving back to the right in the plot by one step in the cluster history (Fig 12). From both the CCC and T^2 it was concluded that the number of clusters in these data was likely to be two or four, with the CCC suggesting two or four and T^2 suggesting two, four or seven. The hierarchical tree diagram revealed the existence of two major clusters or at least four subclusters (Fig. 13). This clustering result seemed plausible based on the plot of the scores of the first two principal components when two and four clusters are used for inspection of the clusters. The clustering results of both the complete linkage and Ward's method also suggested two major groups or at least four subgroups in the data set (Fig. 14 and 15).

The multiscale bootstrap analysis showed that the two clusters previously discerned were statistically supported by the data at α=94%; AU values for both clusters were of 96 and 94 (Fig. 16) and standard errors of the *P* values were below 0.05 in most of the clusters. With all the evidence gathered, it was concluded that natural clustering indeed existed in this data set.

Following the clustering results, we tested the hypothesis that the means of all variables were the same for both groups. Using the first two principal components as inputs, the Hotelling-Lawley Trace test indicated that there was a significant difference between the groups considering all the variables at once (Hotelling-Lawley Trace=164.31, $P<0.001$). Univariate tests showed significant difference for the first (F=208, $P<0.0001$) and the second principal component (F=4.37, $P<0.04$).

DISCUSSION

The information attained in this study constitutes the first description of SCN population density reduction in soybean fields annually rotated to corn in Nebraska. It is the first SCN mortality assessment in the North Central region of the U.S. in a zone with a relatively large hectarage of irrigated corn and soybean. The main focus of the research was to quantify the percent change of SCN population density after annual corn rotation and to describe the relationship between soil texture and SCN population density reduction after rotation. Seventy nine production fields using site specific GPS-based sampling methodology were initially surveyed in this study in an attempt to answer these research questions. Nevertheless, only 45 were used in final analysis based on specific, objective-driven field selection criteria sought at the inception of the study. These two criteria were the minimum number of SCN eggs densities detected with our method (40 eggs) and a minimum SCN initial population density (250 eggs/100 cm^3 of soil) for a justifiable quantification of population density change over the rotation year.

Corn rotation is well documented to reduce SCN population densities both in experimental and observational research (Koenning et al., 1993; Porter et al., 2001). Furthermore, in the present research, correlation analysis was used to verify that there was an association between SCN Pi and Pf between samples of the same field and between field averages, as a result of corn rotation. Observed significant positive correlations between counts of samples of most fields and between field averages confirmed that SCN population density change was associated with the corn rotation year. For instance, in the 2009 and 2010-field sets, significant positive correlation above 0.89 were observed. In the 2011-field set, although positive correlation between field averages was also noticed, it was associated at a low level of significance. In that field set, the low correlation is mainly attributed to the low number of fields that were included for the analysis, as only six fields were used.

On average, SCN population densities after annual rotation with corn declined from 42 to 60% in the 45 fields surveyed throughout three years of study. While no analogous field studies exist for other regions for an equivalent comparison, this population decline is higher than expected and falls within the range of average reductions reported by Porter et al. (2001) for end-of-season SCN egg densities in experimental plots. In their work, annual alternation of corn and soybean in two locations varied from 222 to 164 eggs/cm^3 soil (26%) in one location and from 1,216 to 448 eggs/cm^3 soil (63%) in another location in 1998.

Texture is the most stable component of the soil matrix over time. It governs many physical, chemical and hydrological soil properties (Hillel, 2004). In the present study, soil texture in the ten grids that were sampled in each field typically belonged to

only a particular textural type. However, in some fields, the soil texture in two or three sampling grids was observed to be remarkably contrasting. For example, in a given field, eight samples could have belonged to a sand-predominant textural class while two samples belonging to a silt-predominant textural class. In order to account for such variability in our assessment, only samples of similar soil texture were used to represent the texture of a field. Our data set encompassed a broad spectrum of different textural classes representing nine of the 12 classes of the USDA texture triangle (Fig. 17) and at least two main contrasting textural components. In other reports of SCN-texture relationship, less than seven textural types have been circumstantially assessed (Workneh et al., 1999; Avendaño et al., 2004).

Texture effect on SCN population densities has been described in previous research in soybean, either in greenhouse or field experiments (Koenning et al., 1988; Workneh et al., 1999). In the present study, the relationship between soil texture and SCN population densities is described with emphasis on the non-host crop growing season. Multivariate analysis (PCA and Cluster Analysis) with sand, silt, clay, Pi and Pf as measures of association was employed to investigate the relationship between soil texture and SCN population density reduction after annual rotation with corn. Both PCA and cluster analysis have been applied in a wide range of research studies dealing with data sets with numerous variables. PCA has the property of disclosing important relationships among variables that ordinarily are not revealed by univariate methods (Johnson and Wichern, 2002). For instance, Francl (1993) used PCA to study the relationship of selected edaphic factors and SCN population density in a soybean field and found that pH and magnesium factored together and both were positively correlated with SCN

population densities. In the present research, PCA applied on the correlation matrix produced two indices that were uncorrelated in order of their importance and that described most of the variation in the data. The resulting principal components were interpretable, with the first component suggesting that 60.5% of the variation in the data set was essentially related to soil texture, with sand increasing while silt and clay decreased and vice versa. Moreover, this component also implied that compared with silt and clay, sand is the textural constituent that had a stronger association with SCN Pi or Pf in the corn rotation year (eigenvector values for Pi and Pf of 0.33 and 0.23, respectively and correlation coefficients of 0.57 for Pi and 0.41 for Pf at $P<0.05$). The second principal component revealed that Pi and Pf explained 31.5% of total variation in the data, with both measures increasing or decreasing jointly. Altogether, the results of the principal components in the context of this analysis: i) advise consideration of the simultaneous correlations that may exist between certain proportions of the three soil textural constituents, especially when using them as inputs in analysis involving texture and SCN population relationships; ii) suggest that a positive correlation Pi and Pf would be expected after rotation with corn in the textural ranges included in this data set.

The analysis of clusters was used in our study to investigate whether fields with similar texture tended to exhibit similar SCN population density reduction. Since the clustering algorithm takes into account the values of Pi and Pf, the resulting clustering indirectly reflects the SCN population density change in each field. The five measuring variables used were standardized with a method involving the range of the variables as a divisor (Milligan and Cooper, 1988). This standardization method is reported to provide superior recovery of underlying cluster structures as compared with other commonly used

methods. The method showed to be more effective than the traditional z-score standardization in data sets including outliers and purposely-induced error perturbed distances (Milligan and Cooper, 1988). Since two variable types were included in our data set (texture and SCN egg counts) and they had different units of measurement, failure to standardize them would mean that the variables with higher variance would tend to predominate in the analysis. Standardization guarantees that each variable is given a relatively similar weight in the analysis.

The average linkage (average distance) method was used as the main clustering algorithm. This method is a single-link, hierarchical agglomerative algorithm that considers the distance between two clusters as the average distance between all pair of objects with one member of a pair belonging to each group (Johnson and Wichern, 2002). Single-link clustering approaches are the most efficient of the hierarchical approaches of clustering (Johnson, 1998). Two well defined clusters or at least four subclusters were identified with the average linkage method as guided by the cubic clustering criterion, the Hotelling's pseudo test statistic, and a dendogram. In particular, the latter revealed a discernible structure that allowed a straightforward identification of the groups. Because different clustering algorithms can produce different clustering, in cluster analysis it is generally advisable to compare the results of several methods and almost mandatory to verify and adjust the results of clustering programs by looking at multivariate plots (Jonhson, 1998). Here, almost identical grouping to the average linkage method was also obtained with the complete linkage and Ward's minimum variance methods. Likewise, a plot of the first two principal components with selection of two or four groups also displayed a similar grouping with the tree diagrams (Fig. 10A).

Finally, with the multiscale bootstrap resampling method, using 1000 bootstrap replications and the Euclidian-based dissimilarity matrix, the analysis showed that the two clusters previously discerned were statistically supported by the data at an alpha relatively close to 95%; AU for both clusters was 96 and 94 (Fig. 16) and *P* values with standard errors were below 0.05 in most of the clusters. Implementation of this method has been limited to clustering in phylogenetic analysis. Yet, since it is a bootstrap-based approach, it can be applied to a wide range of statistical situations (Susuki and Shimodaira, 2006).

With all the evidence gathered, it was concluded that natural clustering indeed existed in this data set. Both of the identified groups were significantly different from each other when considering all the variables simultaneously, as shown by the results of multivariate analysis of variance (Hotelling-Lawey Trace =164.3, $P<0.001$). Examination of the two major clusters revealed that fields with predominant silt and clay (silt loam, silty clay loam, silty clay, loam, clay loam and clay) were placed in one group while fields with predominant sand (sand, loamy sand and sandy loam) were placed together in another group. It follows that SCN population density change during the annual corn rotation was similar in soils with a silt range of 23 to 61%, clay 6 to 44%, and sand 13 to 37%. By the same token, SCN population density change was also similar in soils with a sand range of 57 to 95%, silt between 1 and 28% and clay between 7 to 20%. The mean percent difference for each textural component between the two groups was 61 for sand, 38 for silt and 22 for clay (Table 4). Mean percent population decline during the annual rotation was 44 in the silt-predominant group and 56 in the sand-predominant group (Table 4).

The next logical question or comparison dictated by the clustering was whether the observed SCN population density change was significantly different between the two identified groups. Using Pf as a response and log of Pi as a covariate, a Generalized Linear Model with assumed Poisson distribution for Pf was first fitted to the data. The Pi within-field variability was included in the model as a random effect and since both groups included fields from different rotation years, year effect was also accounted for by incorporating it as a fixed effect. The field by year interaction was added to the model as a random effect. The maximum likelihood was chosen as the estimation method of the parameters with Laplace's likelihood approximation (Glimmix procedure of SAS®). Upon fitting the model to the data, the Pearson χ^2/d.f. fit statistics and the large F value suggested overdispersion in the data (Pearson χ^2/d.f. = 287.90; F=21.47). This problem was countered with use of the negative binomial as an alternative model to fit the data. All sources of variation and effects were kept the same as in the Poisson model. Optimization of nonlinear parameters was performed with a maximum number of iterations = 50 with the Dual-Quasi-Newton technique. The denominator degrees of freedom approximation for the *F* test of fixed effects were approximated by the Kenward-Roger's method. The overdispersion problem was eliminated (Pearson χ^2/d.f. = 1.0) and the analysis indicated significant differences for the covariate Pi (F=271.19, $P < 0.0001$). However, no significant differences in Pf between the two groups was observed (F=0.84, P=0.36).

Multiple arbitrary comparisons across different textural classes and fields could have been made at the beginning of this study. Nonetheless, arbitrary comparisons are often influenced by researcher's biases that inevitably results in erroneous or invalid

conclusions. The comparison suggested here from the clustering analysis was based on a quantitative examination of the data structure, considering simultaneously the relationship between soil texture, Pi and Pf. Since our data set included textural classes with high sand content (up to 95%), but no textural classes with high silt or clay (highest silt and clay contents of 62 and 44%, respectively), our data are insufficient to allow conclusions on the effect of the three major textural components on SCN population density reduction during corn rotation. Research including contrasting data of silt, clay and sand contents will be helpful to fill some gaps in the relationship texture-SCN population density reduction, either during non-host cropping or during the soybean cropping season.

In summary, the outcome of this research indicates that SCN population density decline after one-year corn rotation in Nebraska was 50.62% in the three years of study and that soil texture was associated with SCN population density change. SCN population density reduction during corn rotation seemed to be higher in fields with predominant sand texture than in fields with predominant silt-and-clay texture, but such a difference was not statistically significant with the data in this study. Other factors such as amount of rainfall in the season, soil temperature, tillage, and soil pH, are necessary for a more precise assessment and for an improved understanding of SCN field population density changes during corn rotation. Analysis of additional factors that could affect SCN field mortality or SCN population density declines during rotation will be a key piece to understand management, control practices, and their effects on SCN.

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Table 1. Eigenvalues of the correlation matrix, difference, proportion, and cumulative proportion of the total variability accounted for by each principal component (PC) in a data set consisting of 45 fields and five variables or association measures: sand, silt, clay, Pi, and Pf.

PC	Eigenvalue	Difference	Proportion	Cumulative
1	3.02319	1.44970	0.6046	**0.6046**
2	1.57349	1.34553	0.3147	**0.9193**
3	0.22796	0.05267	0.0456	0.9649
4	0.17528	0.17523	0.0351	1.0000
5	0.00005		0.0000	1.0000

Pi=SCN population density (eggs/100 cm^3 of soil) before corn rotation
Pf=SCN population density (eggs/100 cm^3 of soil) after annual corn rotation

Table 2. Eigenvectors of the three principal components (PC1 to PC3) for five variables measured in each of 45 fields of the three selected field sets in this study.

	PC1	PC2	PC3
Sand	0.55	-0.22	0.13
Silt	-0.54	0.16	-0.52
Clay	-0.50	0.29	0.55
Pi[a]	0.33	0.60	-0.51
Pf[b]	0.23	0.69	0.38

[a] Pi=SCN population density (eggs/100 cm^3 of soil) before corn rotation
[b] Pf=SCN population density (eggs/100 cm^3 of soil) after annual corn rotation

Table 3. Pearson correlation coefficients and associated significance between the first three principal components (PC) and the five variables used as association measures in the analysis.

Variable	PC1	PC2	PC3
Sand	0.96	-0.28	0.06
	<0.0001	0.06	0.68
Silt	-0.93	0.21	-0.25
	<0.0001	0.17	0.09
Clay	-0.87	0.36	0.26
	<0.0001	0.01	0.07
Pi[a]	0.57	0.76	-0.24
	<0.0001	<0.0001	0.10
Pf[b]	0.41	0.86	-0.18
	0.005	<0.0001	0.23

[a] Pi=SCN population density (eggs/100 cm^3 of soil) before corn rotation
[b] Pf=SCN population density (eggs/100 cm^3 of soil) after annual corn rotation

Table 4. Summary statistics of the five variables for each of the two major groups emerged from the cluster analysis in this study.

	N	Variable	Mean	Stdev	Min	Max
Group 1	24	Sand	22	8.2	13	37
		Silt	49	9.3	23	61
		Clay	29	6.6	19	44
		Pi	2397	2451	363	9307
		Pf	1221	1288	138	5101
		PR	44	25	1	95
Group 2	21	Sand	83	10	57	95
		Silt	10	7	1	28
		Clay	7	4	1	20
		Pi	3861	4339	651	16954
		Pf	1420	1428	208	6461
		PR	56	27	-9	86

Group 1 corresponds to the groups with silt-and-clay predominant texture

Group 2 corresponds to the group with sand predominant texture

N=number of observations in each group

Pi=SCN population density before rotation

Pf=SCN population density after one year of corn rotation

PR= relative SCN population density change [(Pf-Pi/Pi) x 100] during the rotation year

Stdev=standard deviation

Min and Max=minimum and maximum value

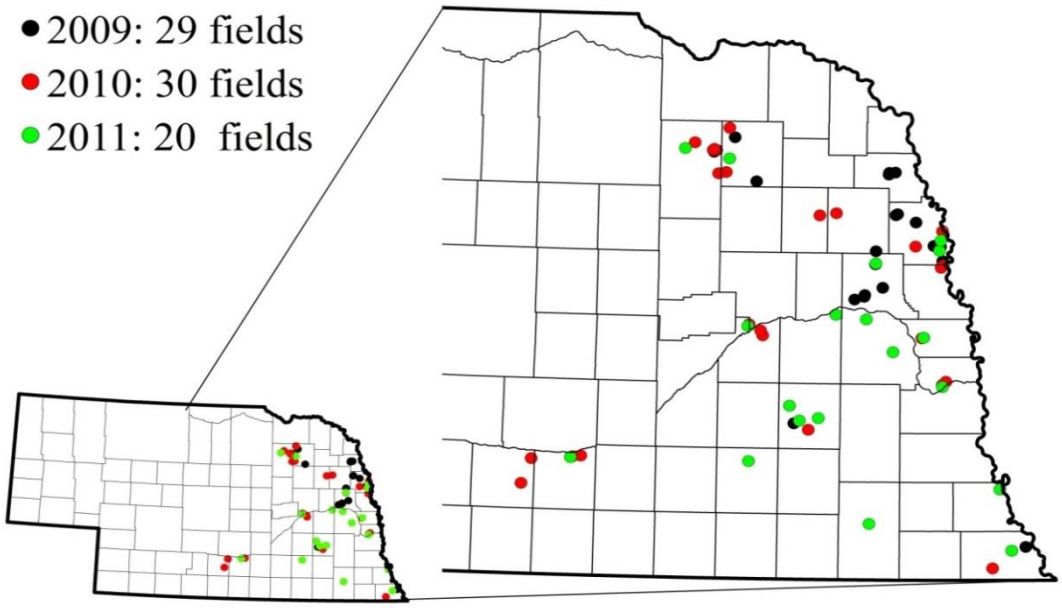


Figure 1. Location of the fields that were selected in 2009, 2010, and 2011 in Nebraska for this study.

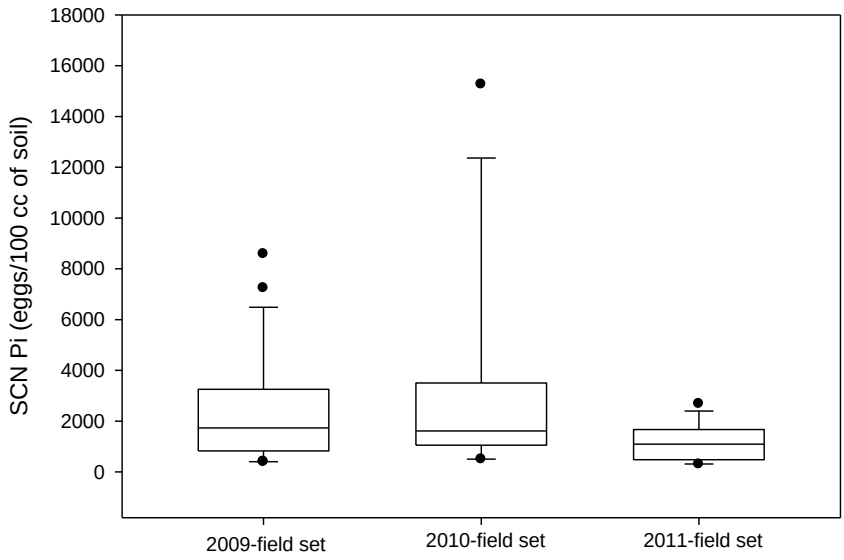


Figure 2. Summary of SCN population densities before rotation (Pi, eggs/100 cm^3 of soil) in the 2009, 2010 and 2011-field set selected in this study.

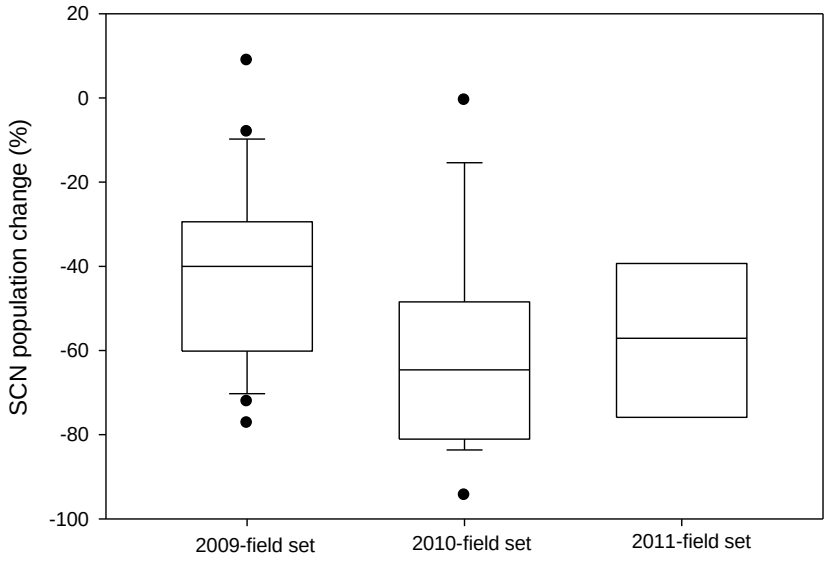


Figure 3. Summary of SCN population density change (%) after annual corn rotation in the 2009, 2010 and 2011-field set selected in this study.

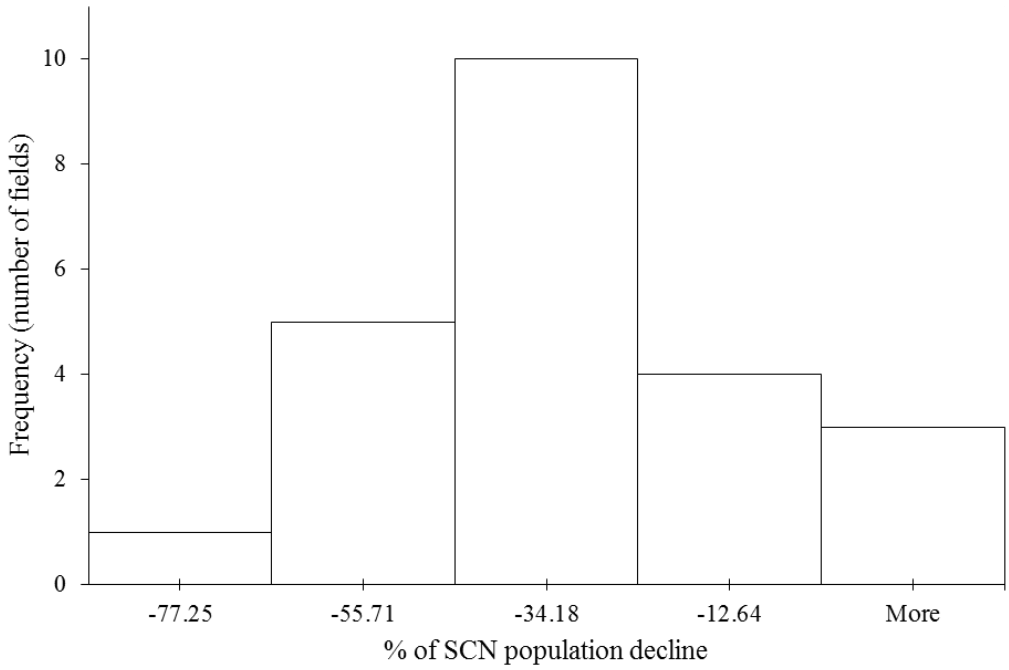


Figure 4. Frequency distribution (number of fields) of SCN population density decline after annual corn rotation in the 22 fields of the 2009-field set.

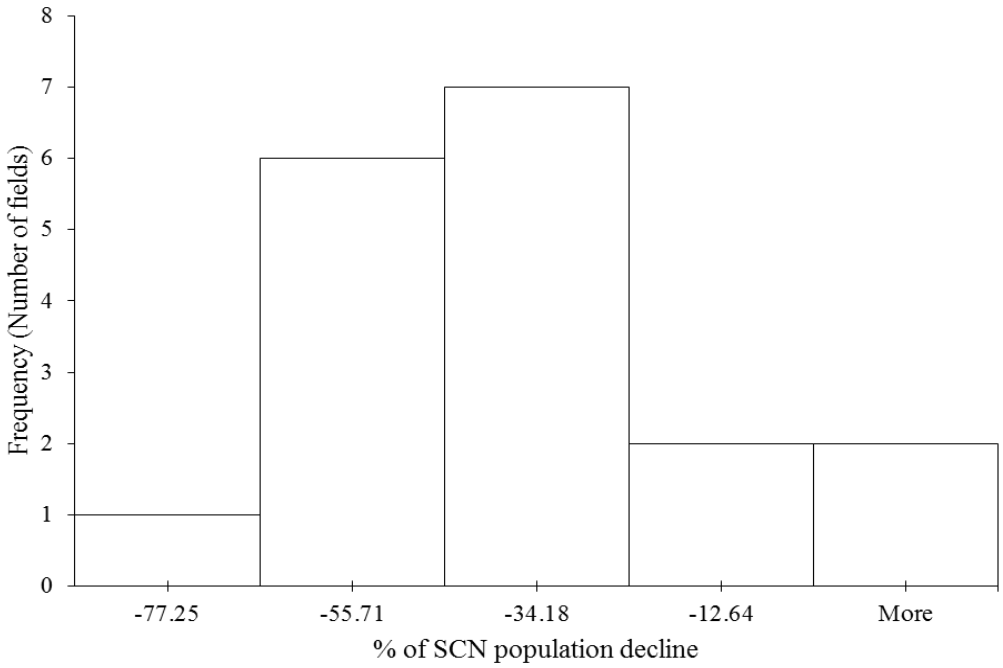


Figure 5. Frequency distribution (number of fields) of SCN population density decline after annual corn rotation in the 18 fields of the 2010-field set.

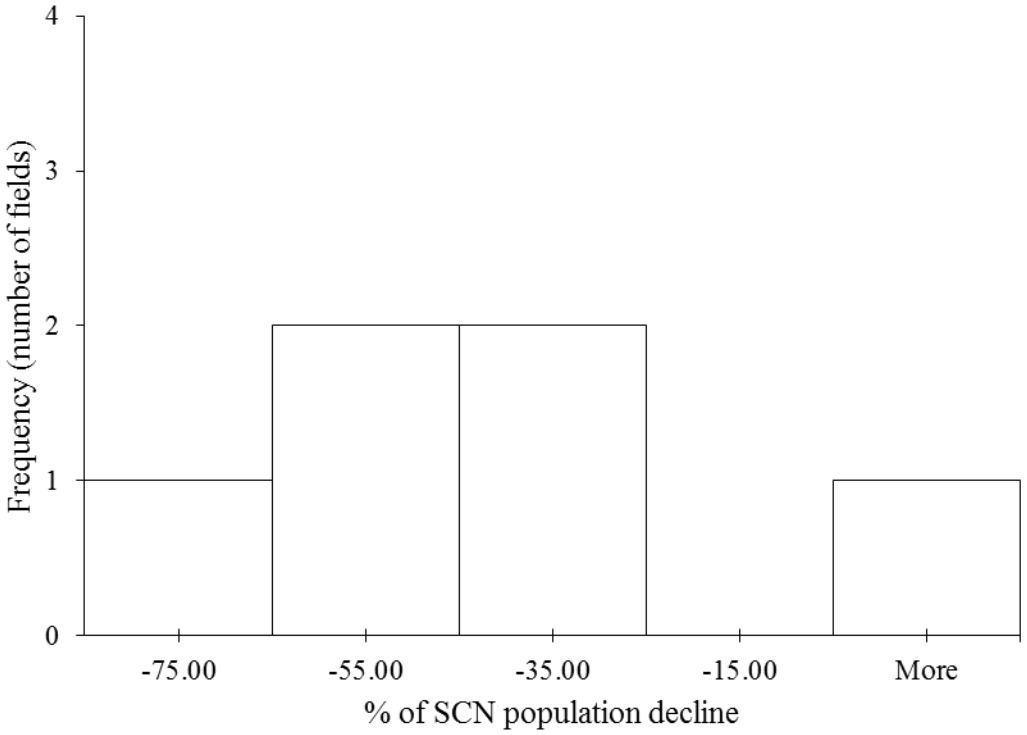


Figure 6. Frequency distribution (number of fields) of SCN population density decline after annual corn rotation in the six fields of the 2011-field set.

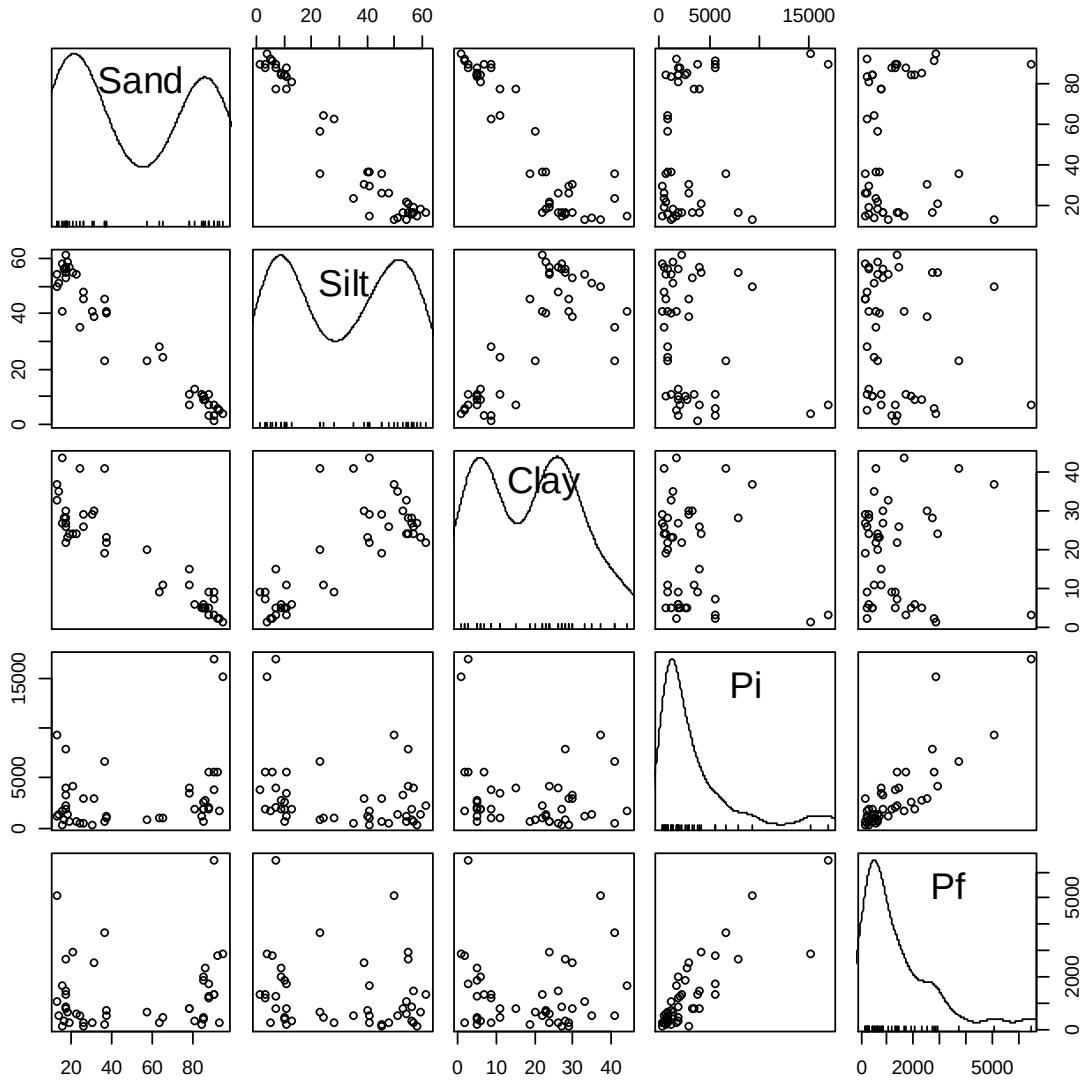


Figure 7. Scatterplot matrix displaying the relationship of the five variables: sand, silt, clay, SCN population density before (Pi) and after (Pf) annual corn rotation. The diagonal panel represents the distribution of each variable. Data from 45 fields.

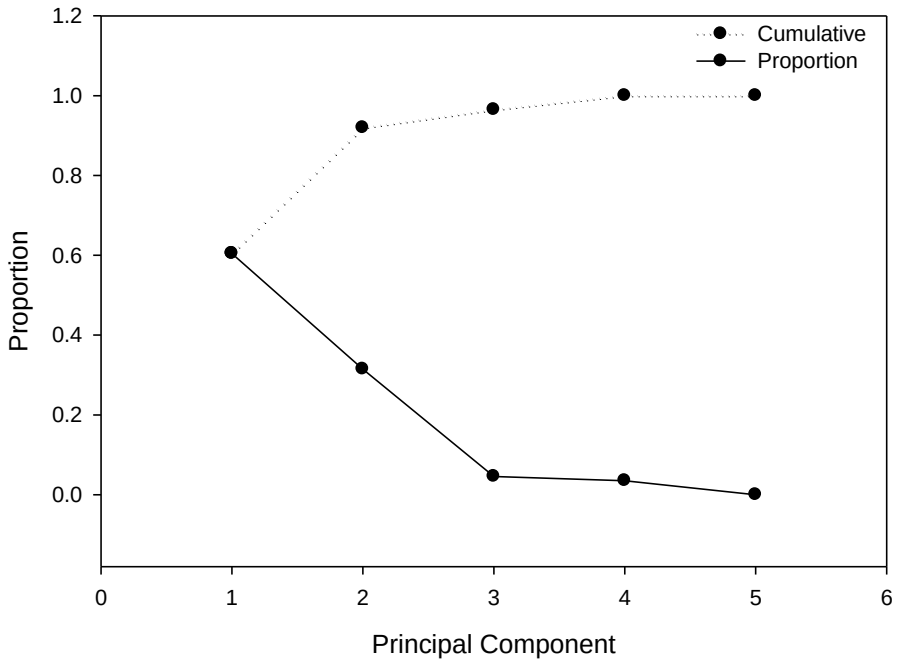


Figure 8. Proportion and cumulative proportion (% of explained variation) by each of the five principal components derived from the data set in this study.

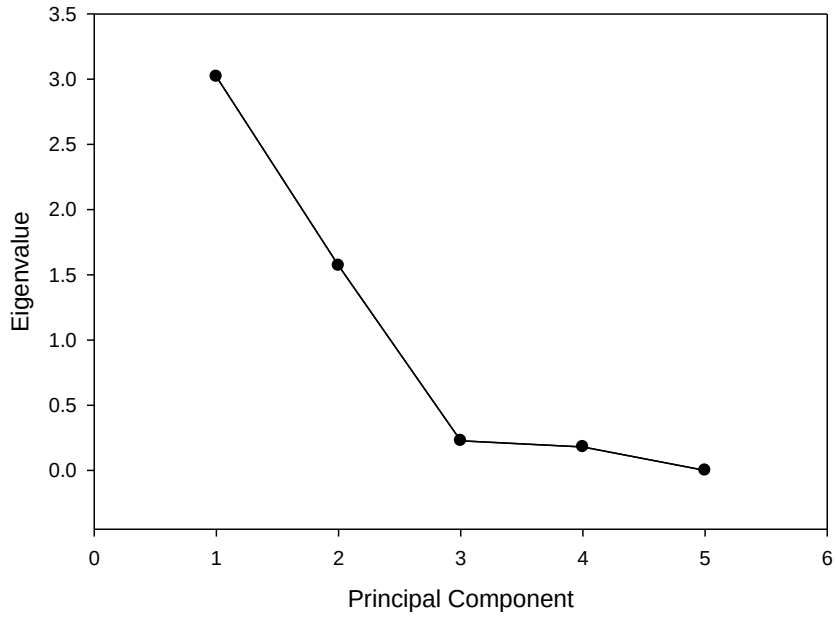
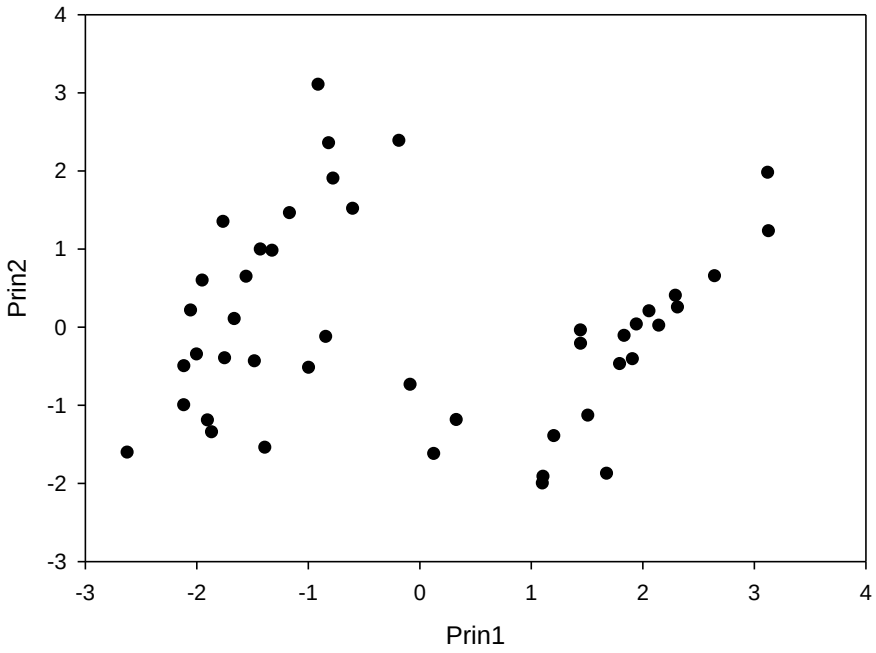


Figure 9. Scree plot of the eigenvalues of the principal components from a data set consisting of 45 fields and five variables: sand, silt, clay, Pi, and Pf. Pi=SCN initial population density or population density before rotation, and Pf=SCN final population density or population density after the one-year corn rotation.

A



B

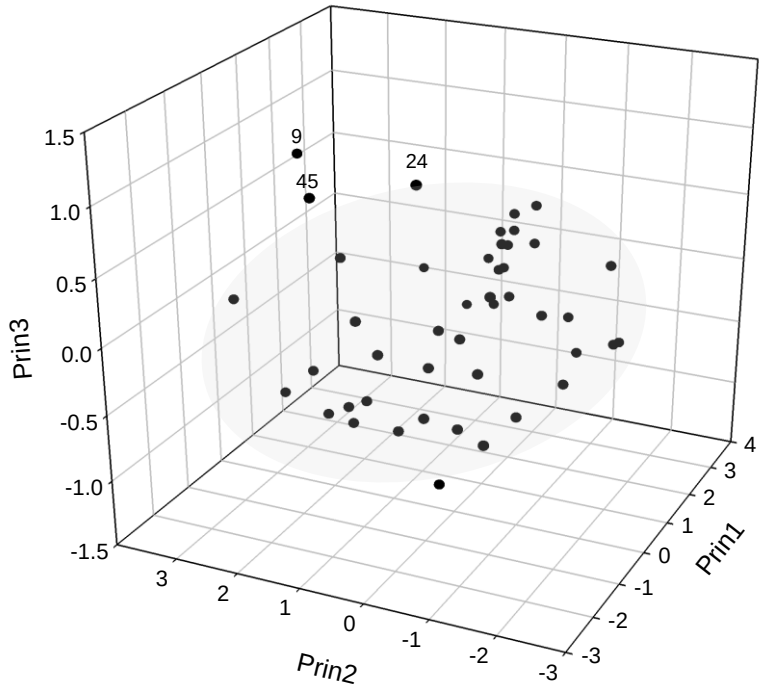


Figure 10. A. Scatterplot of the first two principal components (Prin1 and Prin2) chosen to represent most of the variation in the data set in this study, and B. Three-dimensional scatterplot of the first three principal components (Prin1, Prin2 and Prin3) showing the presence of three observations (9, 24 and 45) outside an elliptical shape used commonly as a graphical aid to assess multivariate normality.

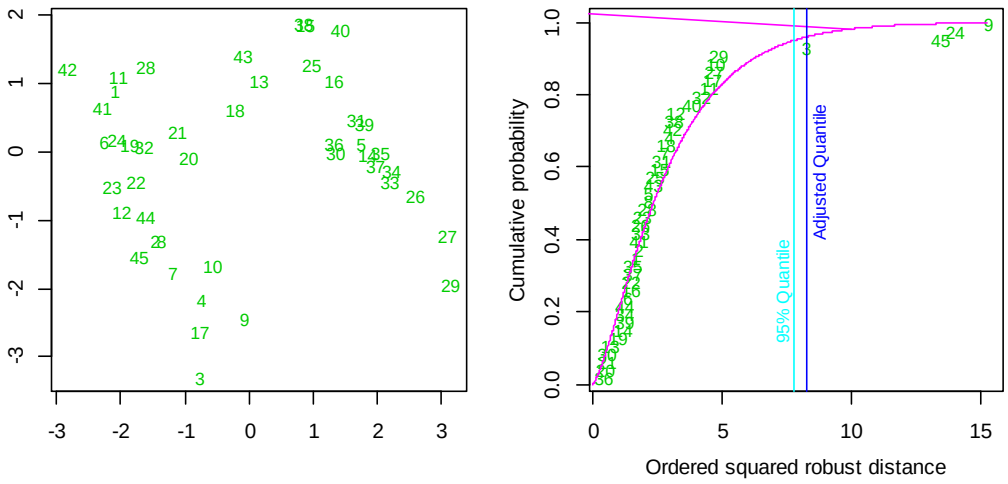


Figure 11. Outlier detection using the ordered square robust distance. Left is a plot of the data and right represents the cumulative probability based on a 95% theoretical quantile. Observations that appear to the right of the vertical 95% quantile line are identified as outliers.

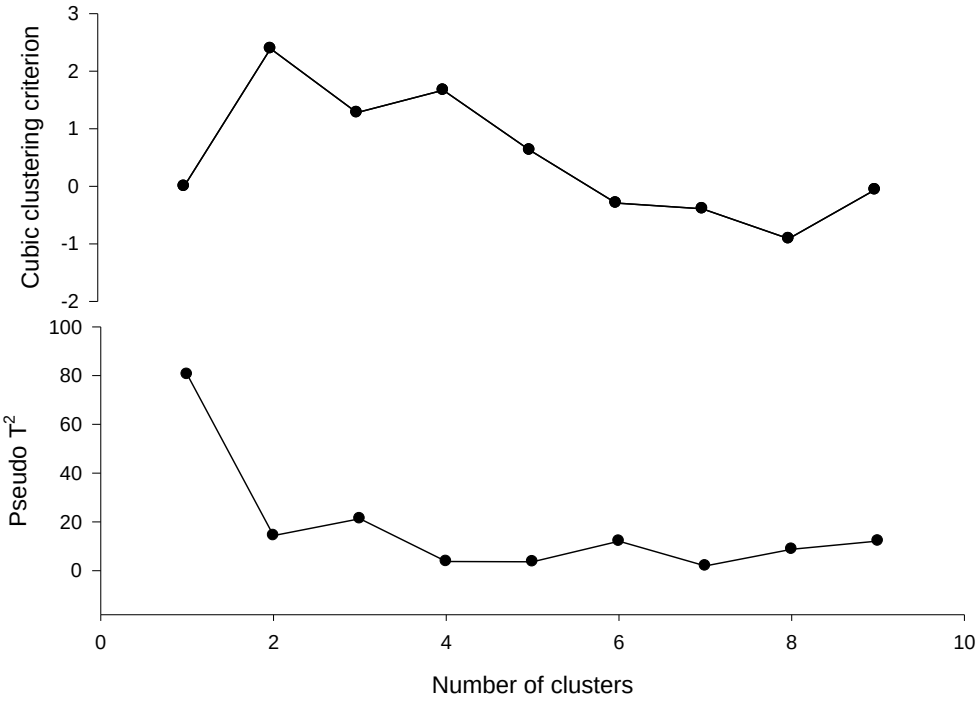


Figure 12. Cubic clustering criterion (CCC) and pseudo T^2 statistic vs. number of clusters used to decide on the number of clusters in the data set. A peak in the CCC and a valley in the latter are indicative of the number of clusters to choose.

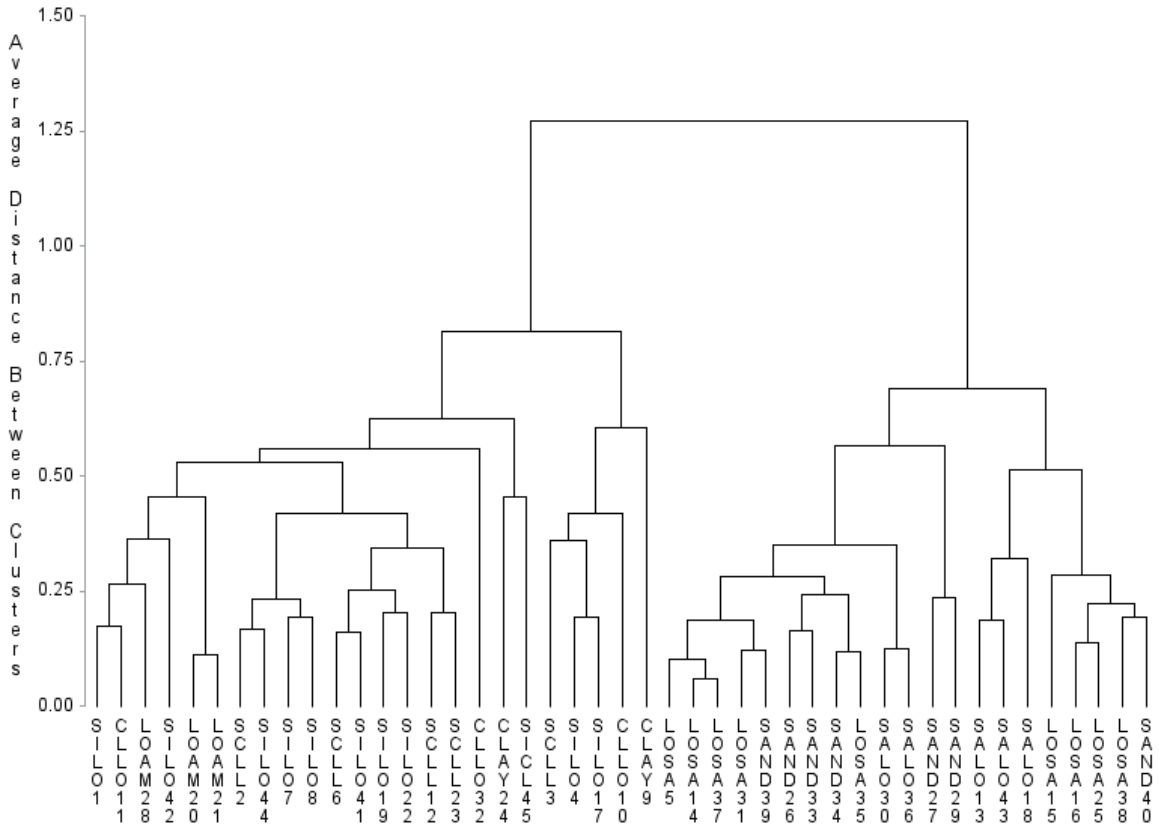


Figure 13. Hierarchical tree diagram produced by the average distance clustering method. The leaves of the three are represented by each of the nine soil textural classes determined in the fields sampled in this study along with the field ID. SILO=silt loam, CLLO=clay loam, LOAM=loam, SICL=silty clay, CLAY=clay, SCLL=silty clay loam, LOSA=loamy sand, SAND=sand, and SALO=sandy loam.

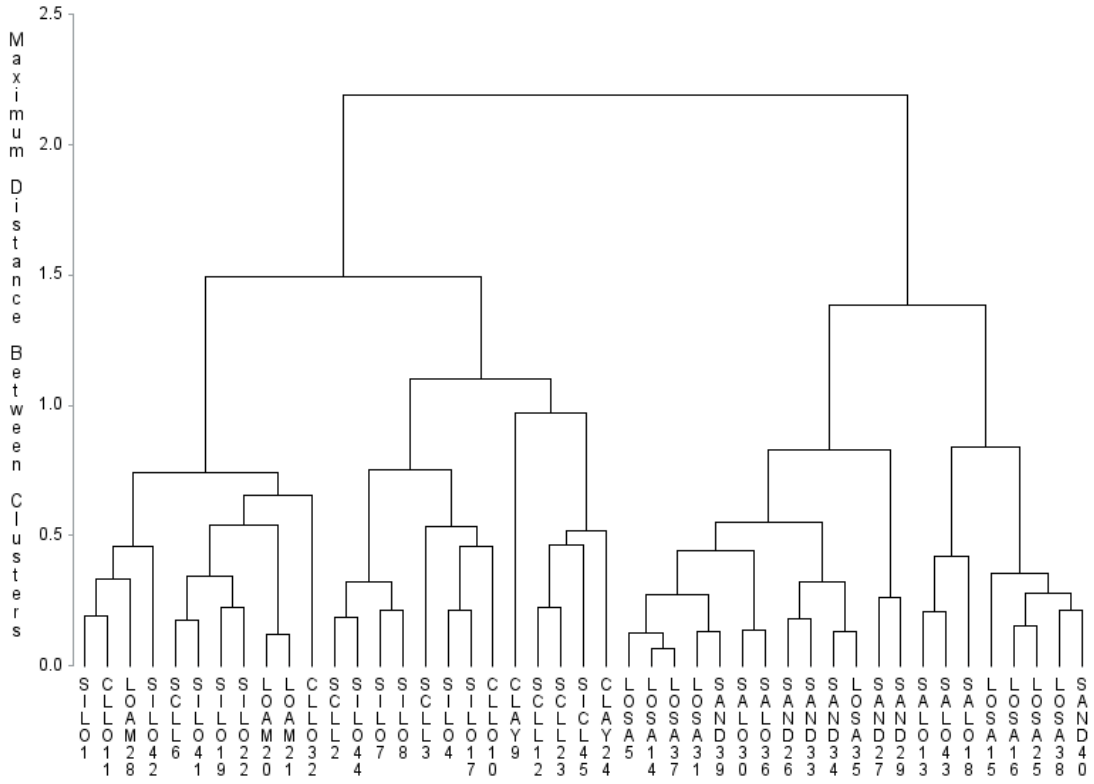


Figure 14. Hierarchical tree diagram or dendogram from the complete linkage method. The leaves of the three are represented by each of the nine soil textural classes determined in the fields sampled in this study along with the field ID. SILO=silt loam, CLLO=clay loam, LOAM=loam, SICL=silty clay, CLAY=clay, SCLL=silty clay loam, LOSA=loamy sand, SAND=sand, and SALO=sandy loam.

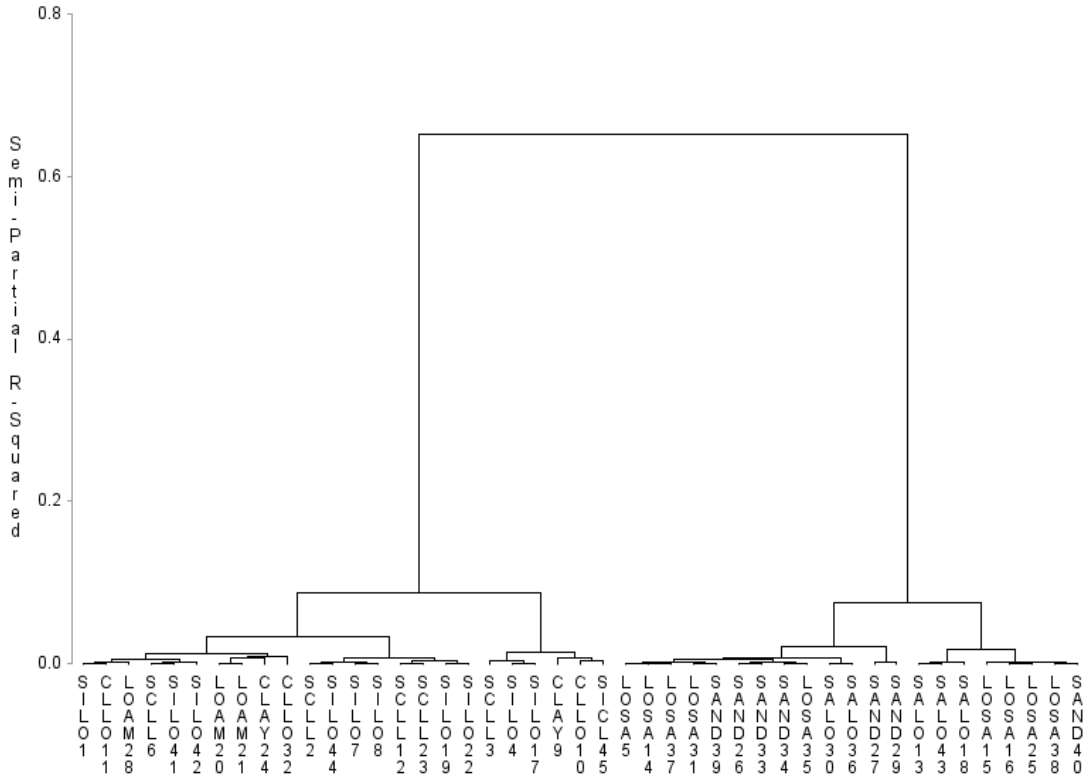


Figure 15. Hierarchical tree diagram using the Ward's minimum variance method for the data set (45 fields and seven measuring variables). The leaves of the three are represented by each of the nine soil textural classes determined in the fields sampled in this study along with the field ID. SILO=silt loam, CLLO=clay loam, LOAM=loam, SICL=silty clay, CLAY=clay, SCLL=silty clay loam, LOSA=loamy sand, SAND=sand, and SALO=sandy loam.

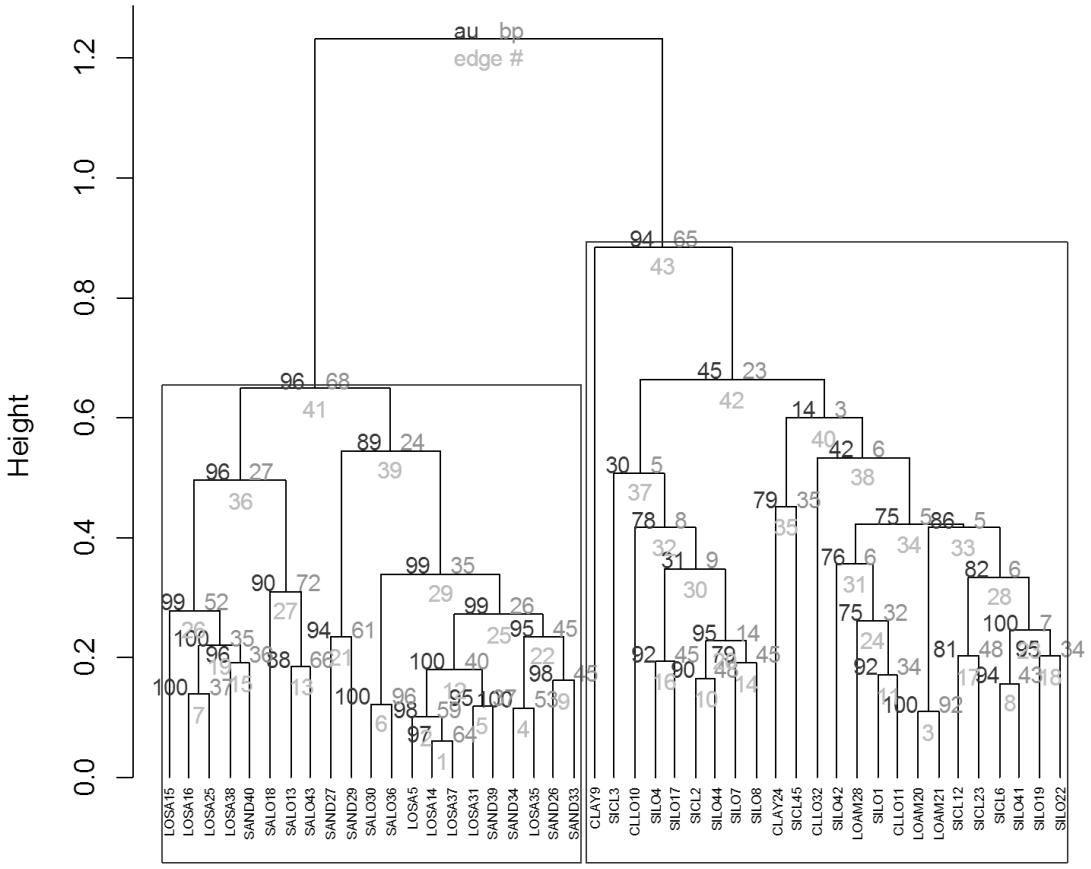


Figure 16. Hierarchical clustering of 45 fields and five variables (sand, silt, clay, P_i, and P_f). Values at branches are: the approximately unbiased (AU) *P* values (left), bootstrap probability (BP) *P* values (right), and cluster labels (bottom). Clusters with AU≥94 are indicated by the rectangles. The first rectangle from left to right is a cluster labeled 41 with AU = 0.96 and BP= 0.68.

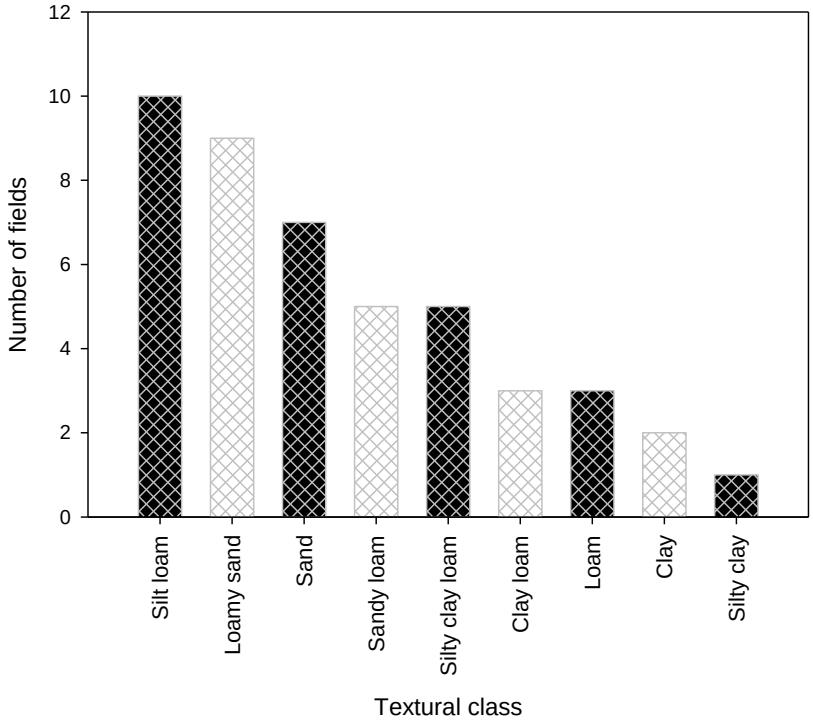


Figure 17. Soil textural classes and corresponding number of fields with each texture that were represented in this study from 45 sampled fields.

CHAPTER IV. MODELING OF SOYBEAN CYST NEMATODE FIELD POPULATION DENSITY AFTER ANNUAL CORN ROTATION IN NEBRASKA

ABSTRACT

SCN nematode population density after annual corn rotation (Pf) in Nebraska was modeled as a count response (number of eggs/100cm^3 of soil) using SCN initial population density (Pi), soil, weather, and agronomic variables as inputs. Stepwise regression with a negative binomial model (NB2) using the log link function was applied to a randomly selected training data set of 35 fields and a final model with soil pH and Pi as predictors was developed. The model indicated that for every one-unit increase in soil pH, SCN Pf is expected to increase by 52.73% holding Pi constant. A ten percent change in Pi will result in 8.3% change in Pf while holding soil pH constant. In the context of SCN population density reduction after annual corn rotation, this can be generally interpreted as: in fields with soils having higher pH (within the range 5.5 and 7.9, which was the pH range on which the model was built), the annual SCN population density reduction will be lower with constant Pi. Likewise, the higher the SCN population densities before rotation (range 363 to 16,954 eggs/100cm3 of soil) the SCN population density reduction will be lower with constant pH. This model was used to estimate the nematode population density after annual corn rotation in the training data set and it showed a high predicting power (82.1%). This predicting capability was confirmed in a validation sample data set, where the model showed a predicting power of 79.7%. No trend for over or underestimation was observed.

INTRODUCTION

The soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe, 1952) has remained the cause of the highest economic losses in soybean production in the United States (Wrather and Koenning, 2006). In Nebraska, economic losses due to SCN in 2012 were estimated at $40 U.S. million dollars (Wilson and Giesler, 2013). Management of SCN in Nebraska is primarily based on growing SCN resistant soybean varieties and rotating to non-host crops (Giesler and Wilson, 2011). The effectiveness of resistant varieties and non-host rotation in reducing SCN population densities has been consistently demonstrated in numerous research studies (Ross, 1962; Young and Hartwig, 1992; Koenning et al., 1993; Tylka, 2008; Warnke et al., 2008; Giesler and Wilson, 2011).

Cultivated in more than 3.9 million hectares in Nebraska (NASS, 2013), corn is exclusively the major non-host crop used for management of SCN (Giesler and Wilson, 2011). About 2.1 million ha of the cultivated corn (60%) in Nebraska are irrigated (NASS, 2013) and the crop is predominantly grown in annual rotation with soybean. Consistently, SCN population densities are reported to decrease at the end of a corn growing season (Ross, 1962; Porter et al., 2001; Noel, 2008). However, to inform soybean farmers and field managers on SCN population densities before soybean is planted, there is a need to better understand the level of annual SCN population density reduction in non-host crops.

Annual SCN population density reduction with non-host rotation occurs, conceptually, during two periods: i) the on-season or growing season of the non-host crop, and ii) the off-season or period from the time the non-host crop is harvested until

the next crop is to be planted. Within the framework of annual corn rotation, the on-season is commonly from May to October (NASS, 1997) and the off-season from November to April of the subsequent year. Both in the on and the off-season, SCN population densities are affected by the interaction of soil physical, chemical and biological properties, agronomic practices imposed on the crop, as well as weather variables affecting the phenology of corn, the growth of alternative hosts, and the soil conditions. Soil texture and pH are the most widely studied properties in relation to SCN population densities, but most research has assessed such a relationship in soybean (Heatherly et al., 1982; Koenning et al., 1988; Heatherly and Young, 1991; Francl, 1993). For instance, Heatherly and Young (1991) found that the number of SCN cysts in potted soybean plants increased in silt loam soil texture and decreased in clay texture. Workneh et al. (1999) found that soil texture and tillage significantly affected SCN population densities and that clay content was negatively correlated with SCN densities in no-till fields. Soil texture affects soil moisture (Hillel, 2004) and the latter has been largely associated with the hatching behavior of cyst nematodes (Wallace, 1955; Slack et al., 1972; Clarke et al., 1978).

Soil pH is a chemical property that is also reported to affect severity of soilborne pathogens (Duggan, 1963; Norton et al., 1971; Francl, 1993). In a factor analysis of the relationship of 12 edaphic factors and *H. glycines* population densities, Francl (1993) determined that pH and magnesium levels factored together and both were positively associated with cyst densities of *H. glycines* across two years. More recently, Pedersen et al. (2010) reported a consistent positive correlation between pH and SCN population

densities in two locations over two years of study. In their experiments, a negative correlation between yield and both pH and SCN population densities was also observed.

Since SCN is a poikilothermic organism, its rates of development and survival are significantly affected by temperature (Ross, 1964; Lauritis et al., 1983; Alston and Schmitt, 1988). Ross (1964) reported a significant effect of soil temperature on development, male to female ratio, and frequency of *H. glycines* juvenile degeneration in susceptible soybean roots. In his experiment, no development of SCN occurred at 10°C, and above 31°C the male to female ratio and larval degeneration was more frequent than between 17 and 28°C. Slack and Hamblen (1961) reported optimum emergence of SCN juveniles from cysts incubated in Syracuse glasses at temperature of 24°C. In another study, Slack et al. (1972) reported that between 16 and 36°C, survival of SCN juveniles was negatively correlated with temperature increase. At constant temperature of 25°C, *H. glycines* completed its life cycle in 21 days in soybean root explants grown in gnotobiotic conditions (Lauritis et al., 1983). In a laboratory experiment, Alston and Schmitt studied the effect of temperature on SCN rates of development. The estimated basal temperature for egg development and thermal optimum for hatching were estimated to be 5 and 24°C, respectively (Alston and Schmitt, 1988). The same authors reported that a temperature range of 24 to 30°C was optimal for embryogenesis and hatch. In field experiments, Hill and Schmitt (1989) observed the greatest SCN hatching in August and September when mean soil temperatures were between 25 and 29°C.

Tillage influence on SCN has been investigated, but contrasting results have been observed among studies (Noel and Wax, 2003; Niblack and Chen, 2004; Noel, 2008; Westhpal, 2010). More recently, LaMondia (2008) reported that in tobacco, prompt

tillage after harvest consistently resulted in lower tobacco cyst nematode populations. Irrigation effect on SCN population densities has not been directly investigated. However, in irrigated fields the water provided to the crop creates singular soil conditions that affect soil moisture and consequently, SCN population development (Heatherly et al., 1982).

While no modeling studies of factors affecting SCN field population density reduction after corn-rotation have been conducted, a few modeling attempts have successfully described the population growth of cyst nematodes in small field plots in susceptible hosts (Jones, 1955; Jones and Perry, 1978). Jones and Perry (1978) reported that a modification of the logistic model for population growth described adequately the cyst nematode initial and final population densities during one growing season in susceptible host crops. Also, as previously described, research has successfully documented the relationship of temperature, pH and texture with SCN population densities (Alston and Schmitt, 1988; Heatherly and Young, 1991; Workneh et al., 1999; Pederson et al., 2010).

Modeling of SCN field population density after annual corn rotation, with consideration of soil factors and weather in the on and off-season, could allow understanding of intricate relationships among these factors and SCN population densities, thereby improving management decisions. The objective of this research was to develop a model to characterize and predict the annual SCN population density reduction after one corn-growing season in Nebraska.

MATERIALS AND METHODS

Data collection and preparation

Data from and for 45 fields were used for development of a model to describe the annual SCN field population density reduction after one-season corn rotation in Nebraska. Field selection, sampling methodology and determination of SCN population densities in each field (represented by the number of eggs per 100 cm^3 of soil) have been previously described in detail (Pérez-Hernández and Giesler, 2013). Briefly, commercial production fields (37 to 55 ha) with a history of SCN and in a soybean-corn rotation regime were selected in 2009, 2010, and 2011 in the major soybean growing areas of Nebraska. In each field, ten 3 x 3 m grids were sampled systematically in a zig-zag pattern collecting twenty 2.5-cm-diam., 15-to-20-cm deep soil cores from each grid. Each field was sampled twice: in mid-spring, before corn planting, and in mid-spring of the following year, preceding planting of the next crop (soybean in most cases). The SCN population density estimated before rotation is herein referred to as SCN initial population density (Pi) and the population density estimated after the annual rotation is referred to as SCN final population density (Pf).

The 45 selected fields had an average SCN Pi $\geq$ 250 eggs/100 cm^3 estimated from at least six SCN-positive sampling grids having similar soil texture. Determination of SCN population density on each sampling grid was done in the laboratory from a 100 cm^3 subsample measured by volumetric displacement and processed with standard sieving techniques for SCN (Khan, 2008). Three counts of SCN eggs on each subsample were performed under the microscope to obtain an average egg density per sampling grid and then per field.

Input variables

The response variable was the SCN Pf, which is expressed as the egg density (number of eggs/100 cm^3 of soil) recorded at the end of the rotation year following one season of corn.

The selected set of predictors included variables reported to affect SCN population densities in previous studies as well as variables identified as potential predictors. The predictor set included eight variables: 1) SCN Pi, expressed as egg density or egg number/100 cm^3 of soil, 2) soil pH, 3) % of soil organic matter (OM), 4) a soil texture index (TI), 5) accumulated rainfall in the growing season in mm (ACR), 6) number of days with soil temperature below freezing (STF), and the categorical predictors 7) tillage, and 8) irrigation. Soil pH, OM, and percentage of sand, silt and clay used to obtain the TI, were determined by a commercial soil testing laboratory (Wards laboratories, Inc., Kearney, NE) from a single composite sample representing each field. The TI was derived from a principal component analysis applied on the sand, silt and clay proportions. In the analysis, the three textural constituents were shown to be highly collinear and they were reduced to the first principal component, which explained 93% of the total variability in the data set.

Variables ACR and STF were obtained for each field from weather stations of the National Weather Service and automated weather stations of the High Plains Regional Climate Center via CLIMOD access. The ACR, complemented with rainfall data of the Nebraska-Rain database, was estimated for the growing season (May to October) whereas STF was estimated for the off-season (November to April). ACR was estimated from daily precipitation data by inverse distance weighting (IDW) spatial interpolation from

weather stations located within a 25-km radius from each target field. Distance from the weather station of interest to each target field was calculated from the latitude and longitude of both the station and the target field using the Haversine formula (Sinnott, 1984) in a routine written in Python 3.0 programing language. The procedure for the IDW is given by $\hat{x} = \sum_1^n x_i w_i \Big/ \sum_{i=1}^n w_i$, *w*here: $\hat{x}$ is the IDW estimate, x_i is the particular measurement at the *ith* surrounding station, and the weight function w_i is derived from the inverse of the distance between the target field and the *i*th surrounding station. Data of STF were estimated based on daily average soil temperature of the closest automated weather station to a target field (within a maximum radius of 35 km). This was obtained differently than the ACR because soil temperature is only available from automated weather stations, and occasionally, only one automated weather station was available for a target field. Information on the categorical variables tillage and irrigation was obtained while conducting the sampling and from agronomic questionnaires sent to the cooperating growers.

Data preparation and preliminary checks

Data preparation and preliminary checks started with exploratory analysis and preliminary diagnostics for the response and predictor variables, including the marginal distribution of the response. The analysis consisted of examination of the mean and variance relationship of Pf for the entire data set and for each categorical predictor variable, and description of mean and variance and additional summary statistics for the predictors. Also, the quantitative explanatory variables were evaluated for their association with the response Pf (PROC CORR of SAS, SAS Institute Inc., Cary, NC).

Model construction

The original sample data set was randomly split into a model building or training data set and a validation data set (Proc SURVEYSELECT of SAS). Approximately 80% of the observations (35 fields) were assigned to the training data set and 20% (10 fields) to the validation set. A Poisson regression model was not pursued because true, yet severe overdispersion was confirmed in the data. Stepwise regression with a negative binomial model (NB2) using the log link function was implemented. The NB2 model with mean μ and variance function $\mu + \alpha\mu^2$ has density

$$f(y_i|\mu,\alpha) = \frac{\Gamma(y+\alpha^{-1})}{\Gamma(y+1)\Gamma(\alpha^{-1})}\left(\frac{\alpha^{-1}}{\alpha^{-1}+\mu}\right)^{\alpha^{-1}}\left(\frac{\mu}{\alpha^{-1}+\mu}\right)^{y},$$

$\alpha \geq 0,$ $y = 0,1,2,\ldots.$ $\Gamma(.)$ is the gamma function

(Cameron and Trivedi, 2013).

A full first order model with the eight explanatory variables, Pi transformed to the natural logarithmic scale, was fitted to the training data set followed by an assessment of the proper functional form of the predictor variables with partial regression plots. Partial regression plots were obtained with the *avPlots* function in the **car** package of the R program. Partial regression plots are scatterplots of residuals, where the residuals in the vertical axis are obtained from regressing the response on all the regressors, but the regressor x_i. Therefore, these residuals are the part of the response variable that is not explained by all the regressors except x_i (Fox and Weisberg, 2011). Correspondingly, the residuals in the horizontal axis are obtained by regressing x_i on the other regressors. These residuals represent the part of x_i that is not explained by the other regressors or that

remains when the other regressors are conditioned. Following this assessment, the predictors with explanatory power showing a significance level ≤ 0.30 from fitting the full first order model were retained and a full second order model with these predictors was fitted to the data. Stepwise backward elimination was applied to select a candidate model based on the Akaike's information criterion (AIC) and the Bayesian Information criterion (BIC) (*Step* function of the **MASS** package of R). The backward elimination processes iteratively fits a model from a starting base model and removes the least significant variable in each step; the process ends when the corresponding AIC and BIC stopped getting smaller. Fitting a full second order model with all the eight predictors was not considered because with only 35 observations not all coefficients could be estimated.

Model refinement and selection

The goodness-of-fit and refinement of the candidate model selected by backward elimination was done through residual analysis, determination of influential observations, and standard goodness-of-fit tests. Residual analysis included Pearson and deviance residuals. Presence of influential observations was determined through difference in fitted values (DFFITS), Cook's Distance, and DFBETAS methods, implemented in R. Also, the leverage of identified unusual observations was assessed using the hat-values as a leverage measure. This was implemented with the *influenceIndexPlot* function of the **car** package of R (Fox and Weisberg, 2011), following the process described by Paula (Paula, 2013). Observations are said to have a high leverage if while being relatively far from the center of the regressor space and considering the correlation pattern among the

regressors, still have an influence in the estimation of the regression coefficients (Fox and Wiesberg, 2011). Standard goodness-of-fit tests included deviance, likelihood ratio tests, as well as information criterion statistics AIC and BIC (glm function of R and GENMOD and GLIMMIX procedures of SAS).

Model validation

The predicting capability of the final model was first assessed by regressing the predicted values of the response variable SCN Pf (obtained with the negative regression model fitted to the training data set) against the observed values in the training data set. The final selected model was also fitted to the validation data set and the relative change in the regression coefficients was evaluated. Predicted vs. observed values for the validation sample data set were also assessed with simple linear regression.

RESULTS

Distribution of the response, summary statistics and correlations

The count response Pf showed a skewed distribution (Fig. 1) and extremely different values for the unconditional mean and variance. Graphically, the mean and variance showed a nonlinear relationship, suggesting overdispersion of Pf (Fig. 2). This was confirmed by the dispersion parameter observed in a Poisson regression model that was fitted to the data before the actual model construction process. The mean and variance of SCN Pf varied for each level of tillage and irrigation, suggesting both of these variables as good predictor candidates of Pf (Table 1). Significant positive correlations (P

≤ 0.05) were observed with the response variable Pf and Pi (0.84), pH (0.27), and STF (0.29) (Table 2, Fig. 3). The variable Pi was positively correlated with STF (0.29) and negatively correlated with TI. Positive correlations were also observed between pH and OM (0.42) and pH and TI (0.41).

Negative binomial regression model

The full first order negative binomial regression model fitted to the eight variables indicated significant predictor capability for Pi, pH, and tillage (Table 3). Variables OM, TI, ACR, STF and irrigation were not significant at the 0.05 level (Table 3). The partial regression plots suggested that Pi had a linear relationship with Pf given the rest of the variables were in the model (Fig. 4). Variable pH was also linearly related to Pf given that the other variables were present in the model. Tillage and TI showed a weak linear relationship with Pf, but neither a quadratic nor a cubic functional form was suggested by the partial regression scatter plot (Fig. 4). Plots of OM, irrigation, ACR and STF showed a random scattering of points suggesting no relationship with the response variable Pf given the other variables were in the model (Fig. 4). In all the plots, observations 24 and 28 stood out as potential outliers. This issue was approached more carefully in the model refinement step.

Variables retained for fit of the full second order model, based on the $P \leq 0.30$ criterion, were: Pi, pH, TI, Tillage and Irrigation. Backward elimination on this full model, based on the AIC, BIC, and Deviance, selected a model with Pi, pH, TI, tillage, irrigation, and interactions of tillage with both TI and irrigation (Table 4). None of the quadratic forms included in the full second order model appeared to be significant. This

model is written as:

$$\log(\text{Pf}) = -\beta 0 + \beta 1 LogPi + \beta 2\, pH - \beta 3 TI - \beta 4 Tillage - \beta 5 Irrigation - \beta 6(TI)(Tillage) + \beta 7(Tillage)(Irrigation)$$

and is henceforth referred to as the candidate model.

Model refinement and selection

Residual plots

Pearson and deviance residual plots showed no systematic features or patterns, indicating overall a good fit of the candidate model (Figs. 5 and 6). The lack-of-fit test computed for each numerical predictor in this model indicated no lack-of-fit (t values of 0.036, 0.025 and 0.013 for LogPi, pH and Ti, respectively, with associated *P* values of 0.85, 0.87, and 0.91). However, the residual value of observations 28 and 31 appeared isolated from the rest, suggesting these observations could be extreme and, likely, influential in the model fit (Figs. 5 and 6).

Influential observations

The difference in fitted values method, DFFITS, suggested observations 20, 31 and 28 as influential on single fitted values according to the DFFITS guidelines for influential observations for small to medium sized data sets (Fig. 7). Cook's Distance method identified observations 24, 28 and 20 as having the greatest Cook's distance (Fig. 8A), yet none of these observations were influential on all predicted values based on the Cooks' Distance (D) guidelines for influential observations: if D > $F(0.50, p, n-p)$, where p is the number of predictors and n is the number of observations in the data set, then observation is influential. The DFBETAS method, which measures the influence of the

i*th* observation on each estimated regression coefficient, consistently identified observation 24 as influential (Fig. 9). This observation was confirmed to have a high leverage both in the estimators of the regression coefficients and the level of significance associated with those estimators in the candidate model (Table 5, Figs. 8A and 8B). With removal of observation 24, the values of the coefficient estimates changed from 1.0 up to 100%, being more remarkable for tillage and the interaction terms (Table 5). Although no change in the coefficient sign occurred, change in the level of significance was drastically affected (Table 5). Without observation 24, the fit of the candidate model resulted in a model in which only the log transformed SCN initial population density and pH showed explanatory power.

Follow up assessment of observations 28 and 31 showed that the coefficients estimates and significance level associated with them in the candidate model did not change. This suggested that both of these observations were not influential. The final model obtained is then a model having SCN initial population density and pH:

$$\log(Pf) = \beta 0 + \beta 1 LogPi + \beta 2\, pH$$

Goodness-of-fit tests

The Akaike's and Bayesian information criteria of the final model were 510.82 and 516.93, respectively whereas for the intercept-only model, these information criteria were 568.7 and 571.8. The Pearson χ^2 was 1.04 for the final model and 1.16 for intercept-only model. The goodness-of-fit tests (log-likelihood ratio, Pseudo R^2 and Deviance) indicated good fit of the model. Log-likelihood ratio was 61.87 ($P < 0.0001$); Pseudo R^2 was 0.1096 and the Chi-square test for the Deviance was 34.89 (P=0.2878).

Model validation

Based on all the above remedial measures and model refinement, the final model selected for prediction of SCN population density was the model with SCN initial population density and pH in it. With the actual values of the coefficients, the model is expressed as: Log(Pf) = -2.2617 + 0.8402(LogPi) +0.4233pH, or $Pf = e^{-2.2617 + 0.8402(LogPi) + 0.4233pH}$

This model suggests that the SCN population density reduction after annual corn rotation depended greatly on the SCN population density before rotation and on soil pH. With lower soil pH, the reduction in population density after rotation will be lower. Prediction capability of this model was high. The R^2 value from the regression between the observed and estimated SCN final population density values in the training data set was 0.821. The slope of the regression line was not significantly different from 1(P = 0.99, Fig. 10). Likewise, in the validation data set, R^2 was 0.797, and the slope was not significantly different from 1 (P = 0.97, Fig. 11). The relative change in the regression coefficients was below 12% for the intercept and 7% for $\beta 1$ and $\beta 2$ (Table 6).

Based on the model, for every one-unit increase in soil pH, SCN Pf is expected to increase by 52.73% holding Pi constant. A ten percent change in Pi will result in a 8.3% change in Pf while holding soil pH constant. In the context of SCN population density reduction after annual corn rotation, this can be generally interpreted as: in fields with soils having higher pH (within the range 5.5 and 7.9, which was the pH range on which the model was built in), the annual SCN population density reduction will be lower with constant Pi. Likewise, the higher the SCN population densities before rotation (range 363

to 16,954 eggs/100cm3 of soil) the SCN population density reduction will be lower with constant pH.

DISCUSSION

The SCN population density after annual corn rotation, Pf, was modeled as a count response (number of SCN eggs/cm^3 of soil) employing a set of screened potential predictors. The analysis was approached through a cycle of model specification, estimation, testing and validation. A base Poisson regression equation was the starting model fitted to a pool of eight regressors whose predicting capability was envisaged from previous knowledge on their effects on SCN population densities and from the ecology of the nematode *Heterodera glycines*. The Poisson regression model is the simplest and standard model for count data (Cameron and Trivedi, 2013). The model is derived from the Poisson distribution where the intensity parameter μ is allowed to be a function of covariates.

Several assumptions in a Poisson regression model, analogous to those in the ordinary least squares, have to be satisfied for efficient estimators and valid statistical inference. Implicit in all the Poisson regression properties is the equidispersion restriction or stipulation of equality of the conditional mean and variance (Hilbe, 2011; Stroup, 2013). The equidispersion condition is comparable to the condition of homoskedasticity in the linear model in which heteroskedastic errors are conducive to wrong parameter estimations and thereby erroneous conclusions.

The unconditional distribution of the response variable followed a Poisson distribution. Nevertheless, fitting of the Poisson regression model resulted in a large violation to the Poisson equidispersion condition. In practice, count data are frequently overdispersed and such a problem causes considerable overinflation of parameter estimates and overly optimistic t statistics. The first step when overdispersion is identified is to determine if it is true or apparent overdispersion (Hilbe, 2011). True overdispersion was confirmed to exist in this data through several diagnostic tests. Therefore, the negative binomial regression model (NB2) with log link function was used to fit the data.

Eight initial predictor variables, including SCN population densities measured before corn rotation, soil pH, organic matter content (%), a texture index, accumulated rainfall in the growing season, number of days with soil temperature below freezing, along with two categorical predictors (tillage and irrigation) were used for model development. A final model with soil pH and SCN population densities before rotation as predictors was developed. The model had high explanatory power and appeared not to over or underestimate predictions of SCN population densities after annual corn rotation. The results suggests that in soybean fields annually rotated to corn in Nebraska, soil pH and the SCN population levels before corn rotation are the major determinants for the resulting population density reductions after annual corn rotation. The relationship between pH and SCN population densities reflected from the model applies to the corn season. Yet, this is in agreement with previous studies in which, though examined for the soybean growing season, an association of soil pH with SCN population densities has been consistently observed (Norton et al., 1971; Francl, 1993; Tylka et al., 1998;

Rogovska et al., 2009; Pedersen et al., 2010). The observed relationship is that higher soil pH is associated with higher SCN population densities.

The final model developed in the present study also included the SCN population density before corn rotation as an input predictor. Because SCN field population oscillations are negligibly affected by immigration and emigration, use of initial population density as a predictor has both ecological and mathematical sustenance. Jones and Perry (1978) modeled successfully the population growth of cyst nematodes during one growing season on a susceptible host using initial population densities (at plating) and final population densities (at harvest).

While only pH and SCN population level before rotation showed the strongest predicting capability for final population densities, other variables and interactions were relatively near a marginal significance level in the model construction process. For instance, in the candidate model selected by backward elimination, after removal of the influential observation, tillage and texture index showed a level of significance of 0.14 and 0.21 (Table 5).

Fitting a negative binomial regression model (data not shown) to the data set, including main effects, quadratic forms, and interactions based on the significance level observed in the full first order model, observed correlations between predictors, and known ecological relationships between SCN and soil, weather, and agronomic variables, showed that the number of days with soil temperature below freezing and texture index could be good candidate predictors. Because the data set on which the model was built is relatively small, a parsimonious model, yet with high predicting capability offers better approximations for prediction of the response variable. If future enhancements are to be

done on the selected model in this study, soil texture and soil temperature are suggested as additional predictors that should be considered with a larger data set.

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Table 1. Mean and variance of the response variable (SCN population density after corn rotation (Pf)) by level of the categorical predictors tillage and irrigation used as input variables in the model development. N is the number of fields in each variable level of in the data set.

	N	Mean	Variance
Tillage			
No till	21	1,160	993,578
Till	24	1,448	2,546,405
Irrigation			
Non-irrigated	6	2,180	3,162,180
Irrigated	39	1,180	1,535,881

Table 2. Pearson correlation coefficients and associated significance, between the response variable (SCN population density after annual corn rotation (Pf)) and the selected quantitative predictor variables: SCN population density before rotation (Pi), soil pH, soil organic matter (OM), texture index (TI), accumulated rainfall (ACR), and number of days with soil temperature below freezing (STF).

	Pf[i]	Pi[i]	pH	OM[ii]	TI[iii]	ACR[iv]	STF[v]
Pf	1.00	0.84[a]	0.27[c]	- 0.03[d]	- 0.12[d]	-0.10[d]	0.29[b]
Pi		1.00	0.05[d]	- 0.18[d]	- 0.26[c]	-0.07[d]	0.29[b]
pH			1.00	0.42[b]	0.41[b]	-0.11[d]	-0.05[d]
OM				1.00	0.95[a]	0.01[d]	-0.25[d]
TI					1.00	-0.11[d]	-0.22[d]
ACR						1.00	0.17[d]
STF							1.00

[a] Significant at $P \leq 0.0001$
[b] Significant at $P \leq 0.05$
[c] Marginally significant, P between 0.06 and 0.09
[d] Not significant, $P \geq 0.10$

[i] Pf and Pi are field average SCN egg densities (eggs/100 cm^3 of soil)
[ii] OM = soil organic matter
[iii] TI was obtained from principal component analysis of sand, silt, and clay
[iv] ACR in mm in the corn growing season (May to October) for corresponding year
[v] STF is number of days with soil temperature below freezing from November to April of the corresponding rotation year

Table 3. Type III tests of fixed effects from fitting the full first order negative binomial regression model with eight predictor variables to the training data set.

Effect or predictor variable	Num DF	Den DF	F Value	Pr > F
LogPi[a]	1	26	45.46	<0.0001
pH[b]	1	26	12.86	0.0014
OM[c]	1	26	0.91	0.3495
TI[d]	1	26	2.79	0.1069
ACR[e]	1	26	0.45	0.5094
STF[f]	1	26	0.17	0.6815
Tillage	1	26	4.16	0.0517
Irrigation	1	26	1.35	0.2550

[a] LogPi = Natural logarithm of SCN population density (eggs/100 cm^3 of soil) before corn rotation
[b] pH = soil pH
[c] OM = soil organic matter
[d] TI = texture index obtained from principal component analysis of sand, silt and clay
[e] ACR = accumulated rainfall in mm in the growing season
[f] STF = number of days with soil temperatures below freezing

Table 4. Starting full second order model and final candidate model selected by automated backward elimination from fitting of the negative binomial regression model to the model construction data set. Model selection was based on Akaike's information criteria (AIC), Bayesian Information Criterion (BIC), and Deviance (DVC).

Model	AIC	BIC	DVC
Starting model: full second order[a]			
Pf [b]~ LogPi + pH + Tillage + TI + Irrigation + $LogPi^2$ + pH^2 + TI^2 + LogPi*pH + LogPi*Tillage + LogPi*TI + LogPi*Irrigation + pH*Tillage + pH*TI + pH*Irrigation + Tillage*TI + Tillage*Irrigation	541.16	572.27	2.22
Elimination beginning…			
Final candidate selected model			
Pf ~ LogPi+pH+Tillage+TI+Irrigation+Tillage*TI+ Tillage*Irrigation	527.64	541.64	1.32
Elimination end.			

[a] Starting full second order model was based on the predictors: natural logarithm of SCN population density before rotation (LogPi), pH, Tillage, Texture Index (TI), and Irrigation, retained after fitting the full first order model. Asterisks indicate interaction and the "plus" sign between the predictors does not indicate the actual sign of the regression coefficients.

[b] Pf = SCN population density after annual rotation with corn

Table 5. Relative change (%) in the estimates of the regression coefficients and values of standard errors and significance level after fit of the candidate negative binomial regression model before and after removal of observation 24.

Coefficient	With observation 24			Without observation 24			
	Estimate	Std Error	Pr > χ^2	Estimate	Std Error	Pr > χ^2	Relative change
Intercept	-1.7088	0.92	0.06	-1.8912	0.94	0.04	10.7%
LogPi[a]	0.7675	0.09	<0.0001	0.7874	0.09	<0.0001	2.6%
pH[b]	0.4433	0.12	0.0003	0.4477	0.12	0.0003	0.99%
TI[c]	-0.1056	0.08	0.1821	-0.0993	0.07	0.2142	-6.0%
Tillage	-1.3159	0.25	<0.0001	-0.8735	0.60	0.1462	-33.6%
Irrigation	-0.0303	0.33	0.9267	-0.0636	0.33	0.8487	109.9%
Tillage*TI	-0.5116	0.15	0.0005	-0.2779	0.35	0.3886	-45.7%
Till*Irrig	2.2864	0.57	<0.0001	1.4773	1.15	0.1981	-35.4%

[a] LogPi = Natural logarithm of SCN population density (eggs/100 cm^3 of soil) before corn rotation

[b] pH = soil pH

[c] TI = texture index obtained from principal component analysis of sand, silt and clay

Table 6. Relative change (%) in the estimates of the regression coefficients with the final negative binomial regression model for SCN population density after annual corn rotation applied to the training data set and to the validation data set. Model included LogPi and pH as predictors.

Term	Training data set	Validation data set	Relative change (%)
Intercept	-2.2617	-2.5455	12
LogPi[a]	0.8402	0.9022	7
pH[b]	0.4233	0.3921	7

[a] LogPi = Natural logarithm of SCN population density (eggs/100 cm^3 of soil) before corn rotation

[b] pH = soil pH

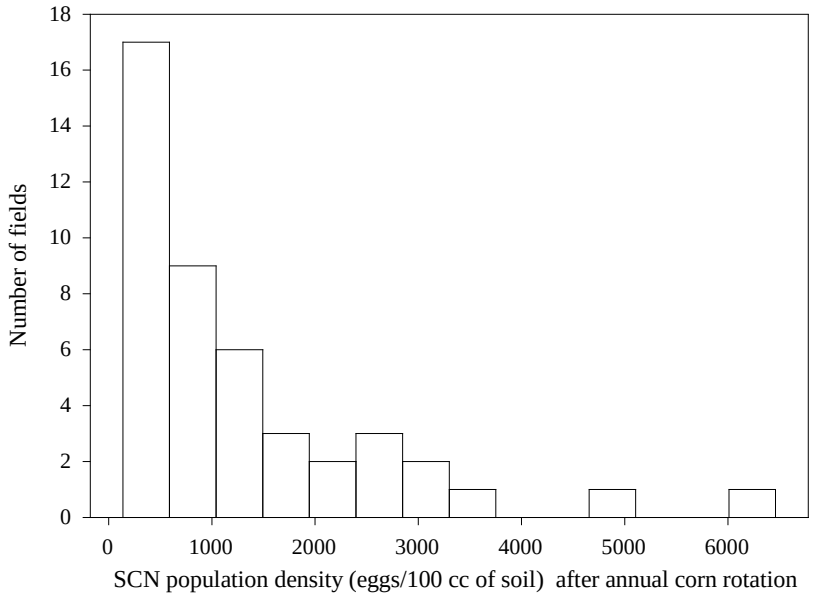

Figure 1. Frequency distribution of the response variable (SCN population density after annual corn rotation (Pf)) in a sample of 45 fields annually rotated to corn in Nebraska.

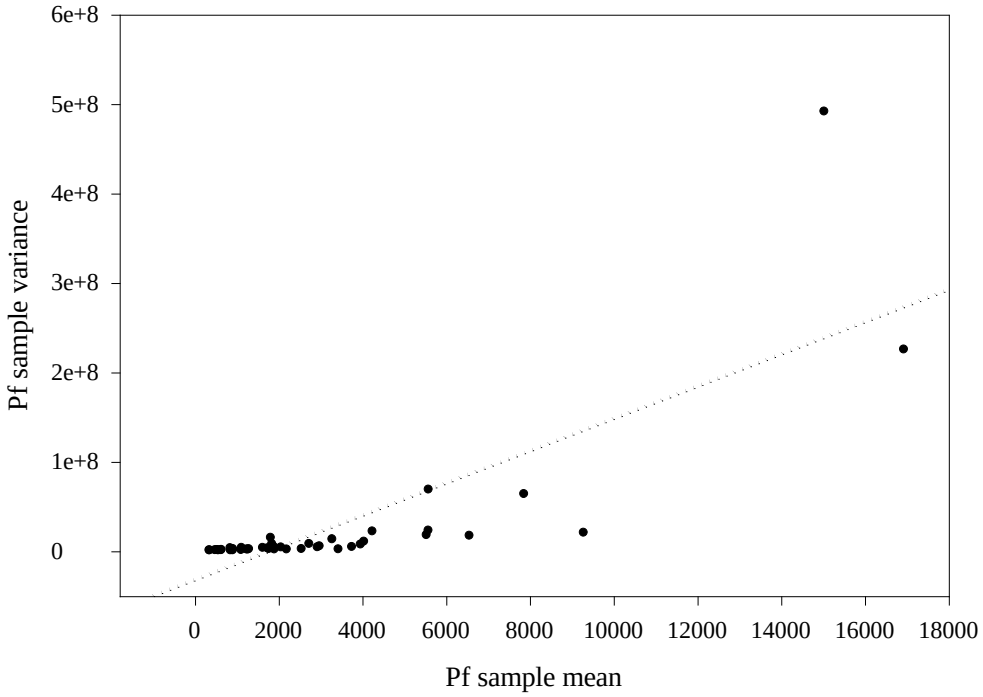

Figure 2. Unconditional mean and variance of the response variable SCN population density after annual corn rotation (Pf). Mean and variance were calculated from the SCN egg counts in the sampling grids in each of the 45 fields included in the study data set.

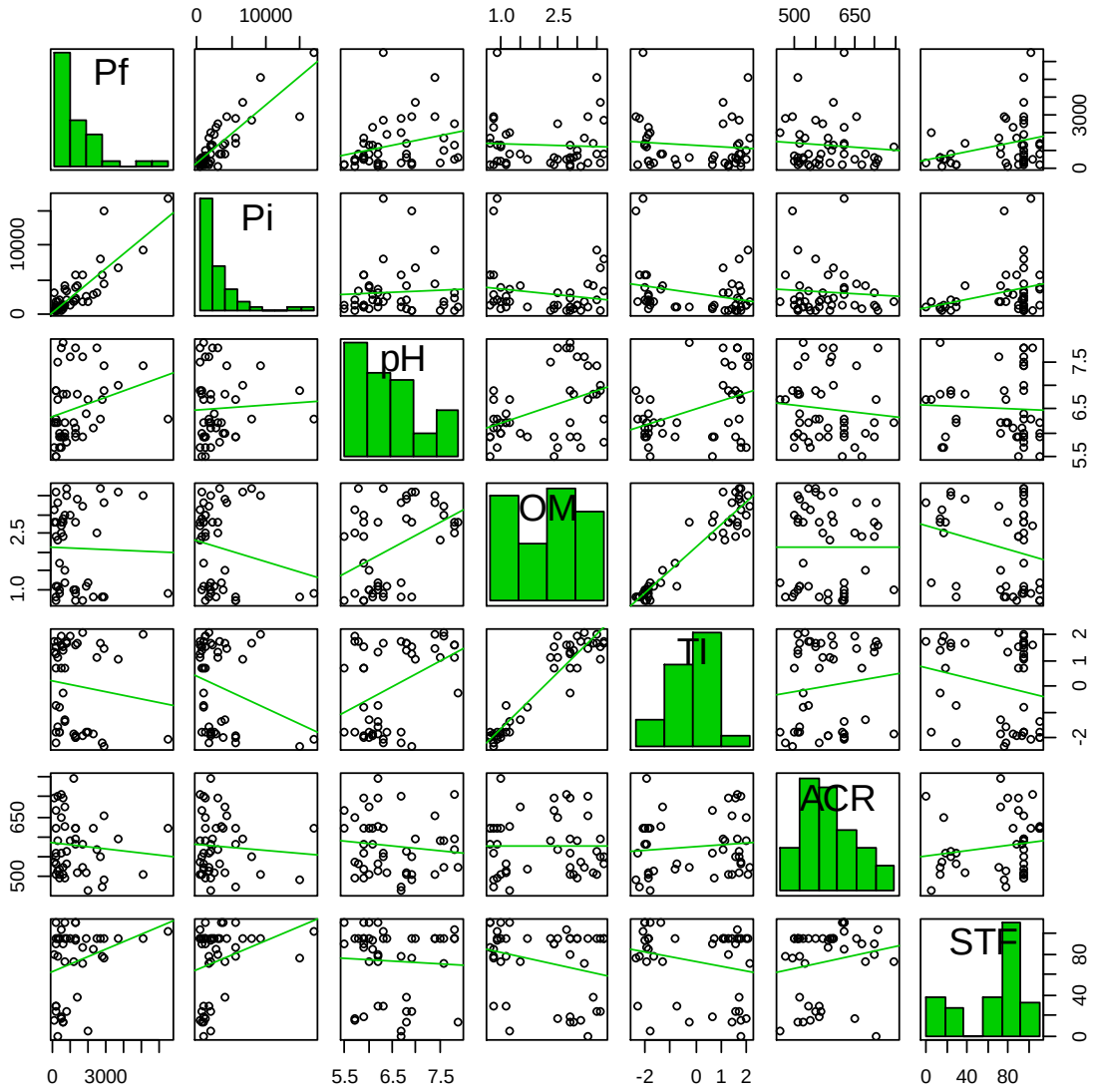

Figure 3. Scatterplot matrix of the response variable SCN population density after annual corn rotation (Pf) and the selected quantitative predictor variables SCN initial population density (Pi), pH, organic matter (OM), texture index (TI), accumulated rainfall (ACR), and number of days with soil temperature below freezing (STF). Line plotted in each panel corresponds to the least-squares line. Sample data set of 45 included fields.

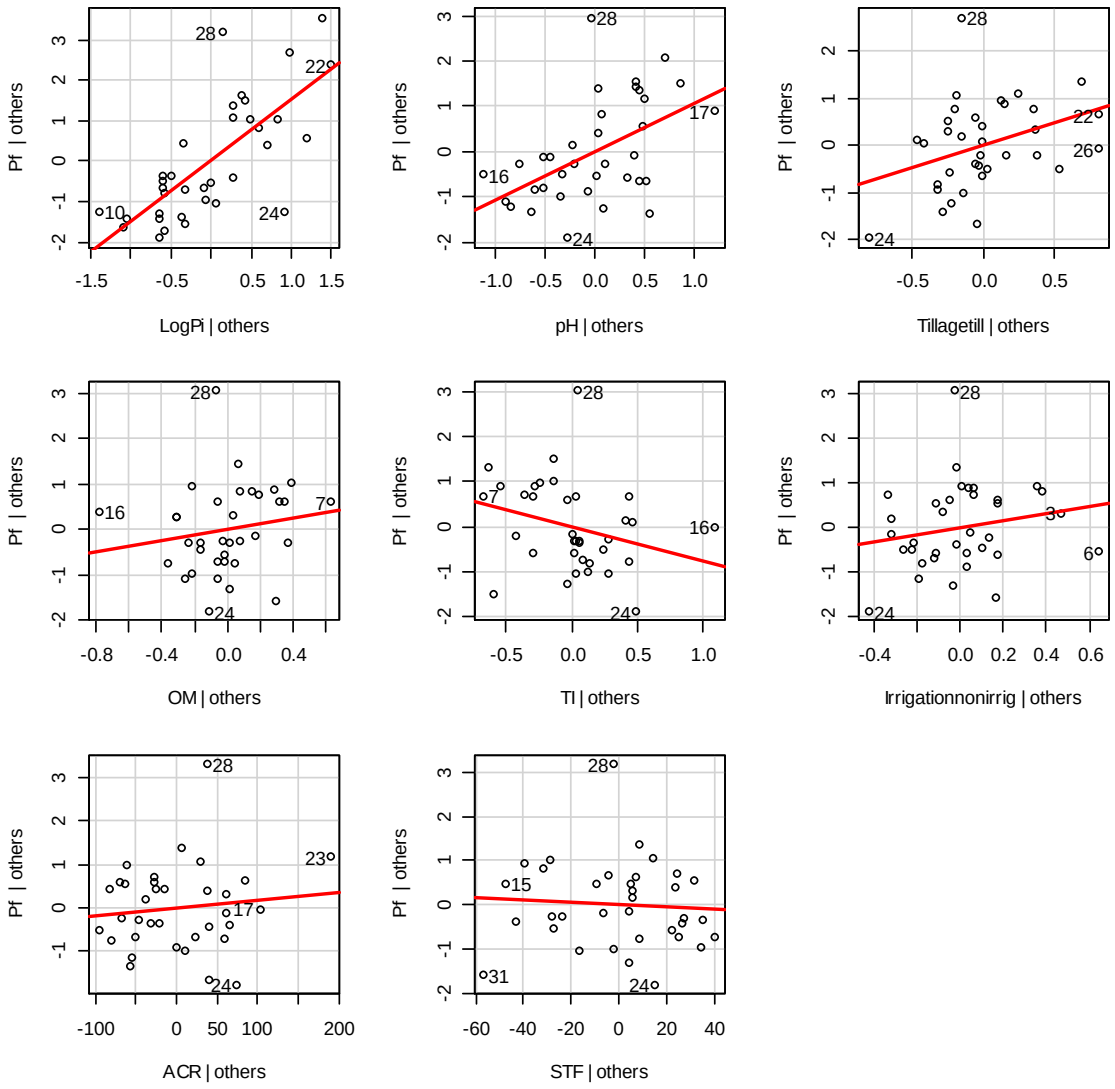

Figure 4. Partial regression plots of SCN population density after annual corn rotation (Pf) on SCN population density before rotation transformed to the natural log (LogPi), pH, Tillage, organic matter (OM), texture index (TI), Irrigation, accumulated rainfall (ACR), and number of days with soil temperature below freezing (STF) in the training data set.

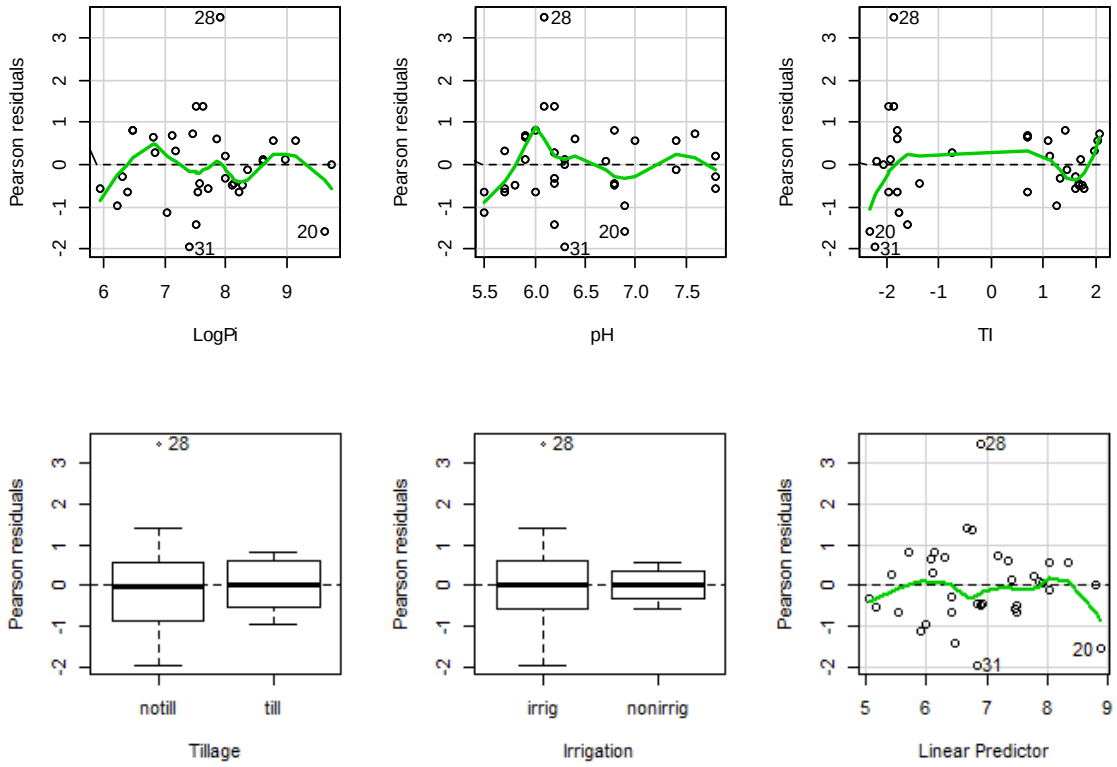

Figure 5. Pearson residual plots for the negative binomial regression model fit to the training data set with predictors: SCN initial population density transformed to the natural log (LogPi), pH, texture index (TI), tillage, and irrigation.

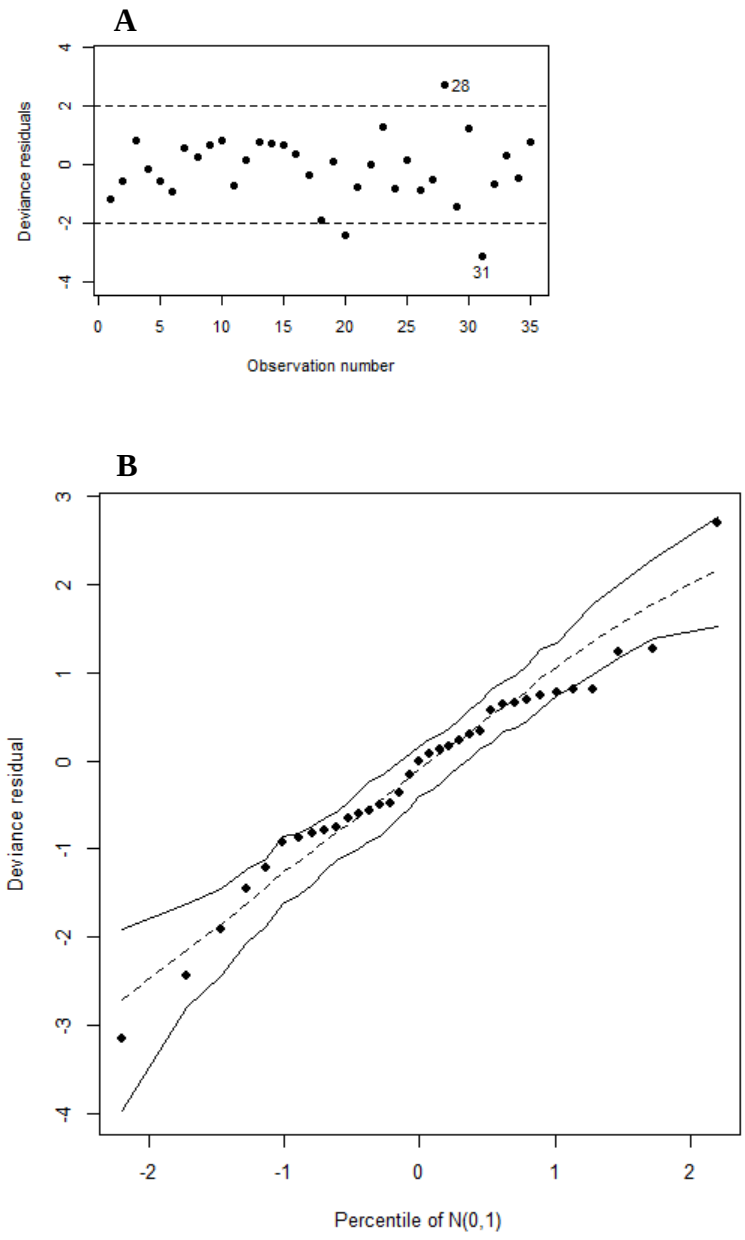

Figure 6. Deviance residuals plots, A: against the observation number and B: against the percentile of a normal distribution (with generated envelopes) for the negative binomial regression model fit to the training data set with predictors: SCN initial population density transformed to the natural log (LogPi), pH, texture index (TI), tillage, and irrigation.

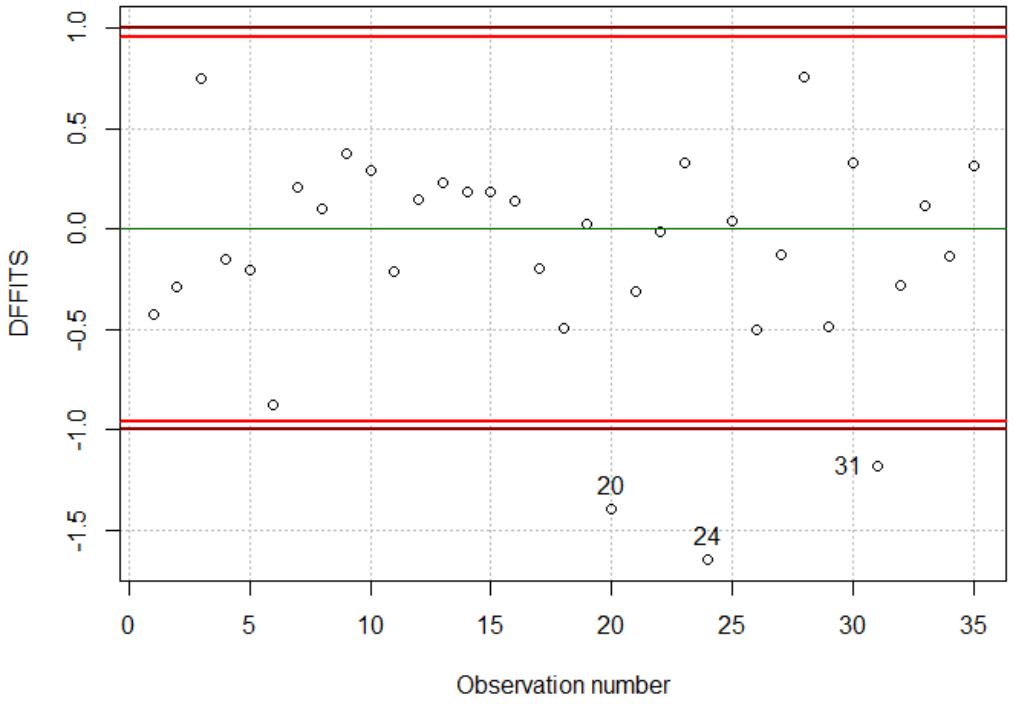

Figure 7. Difference in fitted values (DFITTS) for the negative binomial regression model fit to the training data set with predictors: SCN population density before corn rotation, transformed to the natural log (LogPi), pH, texture index (TI), Tillage, Irrigation, and the interaction Tillage*TI and Tillage*Irrigation. Influential observations on single fitted values are shown outside the dark red border line according to the DFFITS guidelines for influential observations for small to medium sized data sets. If DFFITS > |1| then the observations is regarded as influential.

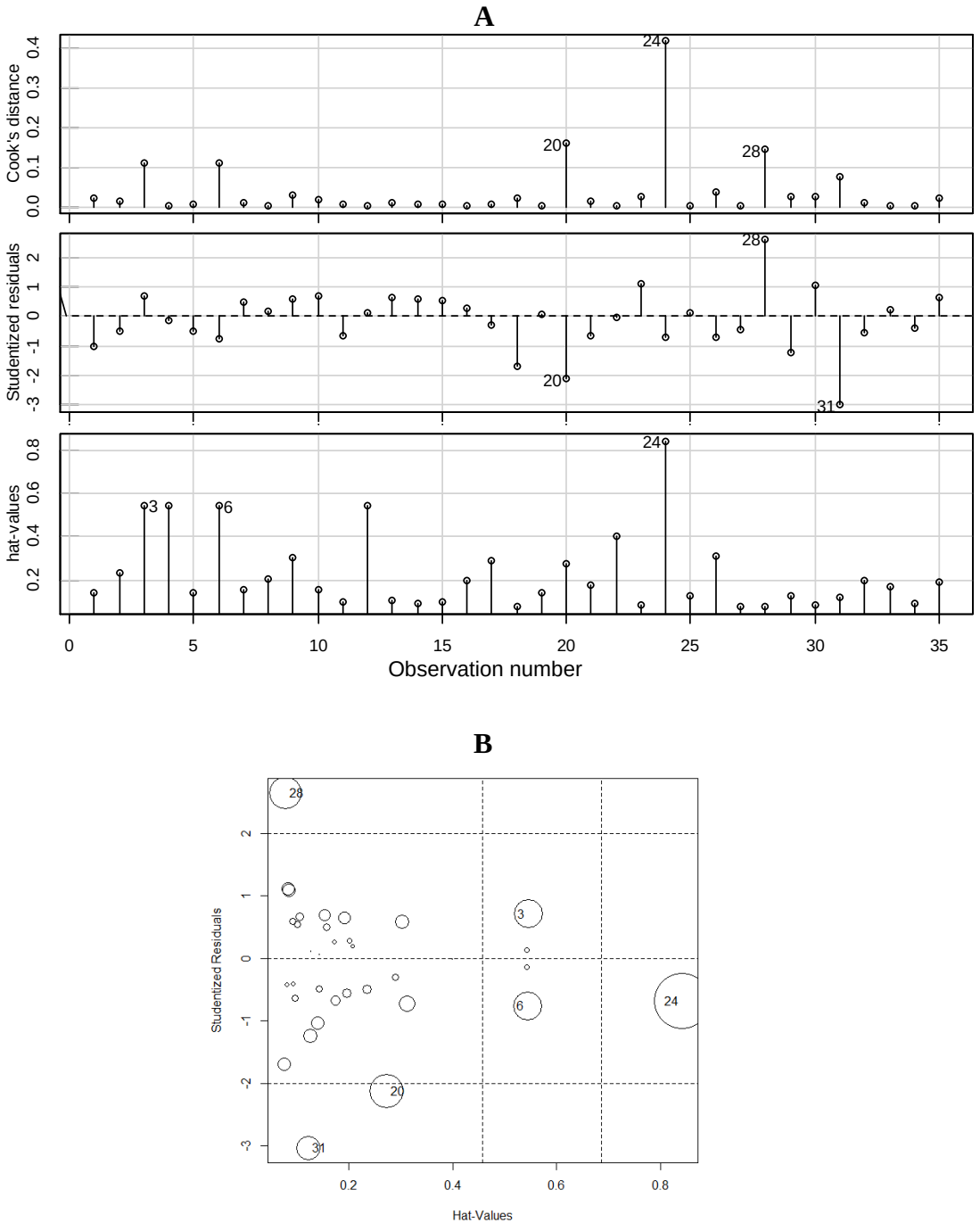

Figure 8. A-Index diagnostics plots, including Cook's Distance, Studentized residuals and hat-values for negative binomial regression of SCN population density after annual corn rotation vs. predictors: SCN initial population (LogPi), pH, texture index, tillage, irrigation and interactions between tillage-texture index and tillage-irrigation. B- Plot of hat-values, Studentized residuals, and Cook's distances for negative binomial regression. Size of the circle is proportional to Cook's Distance. Observations with high hat values have high leverage.

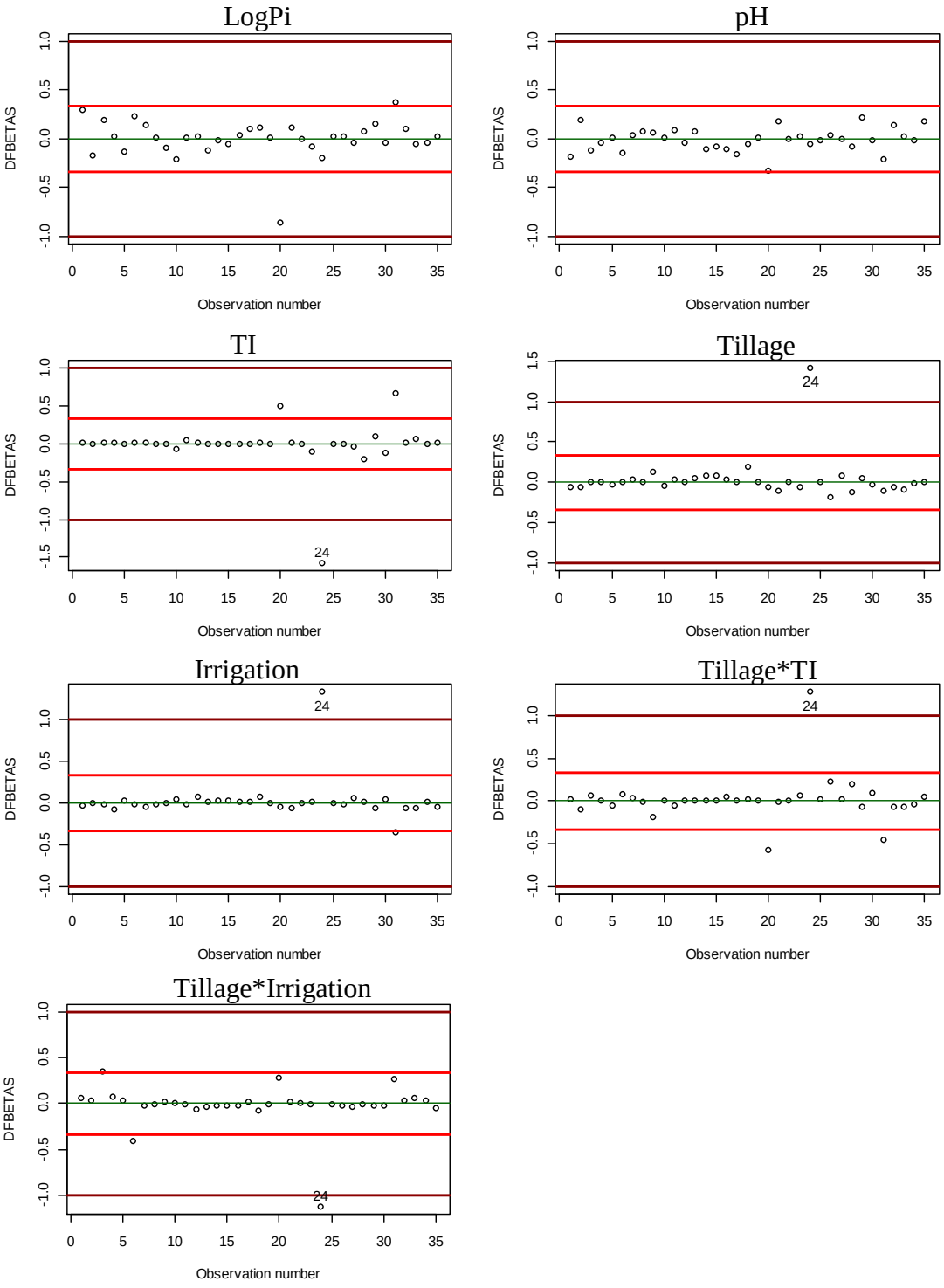

Figure 9. DFBETAS values for the negative binomial regression model fit to the training data set with predictors: SCN initial population density transformed to the natural log (LogPi), pH, texture index (TI), Tillage, Irrigation, and the interaction Tillage*TI and Tillage*Irrigation. Influential observations on the estimated coefficients are those whose absolute value is >1 (shown outside the dark red border lines in each plot), according to the DFBETAS guidelines for influential observation for small to medium sized data sets.

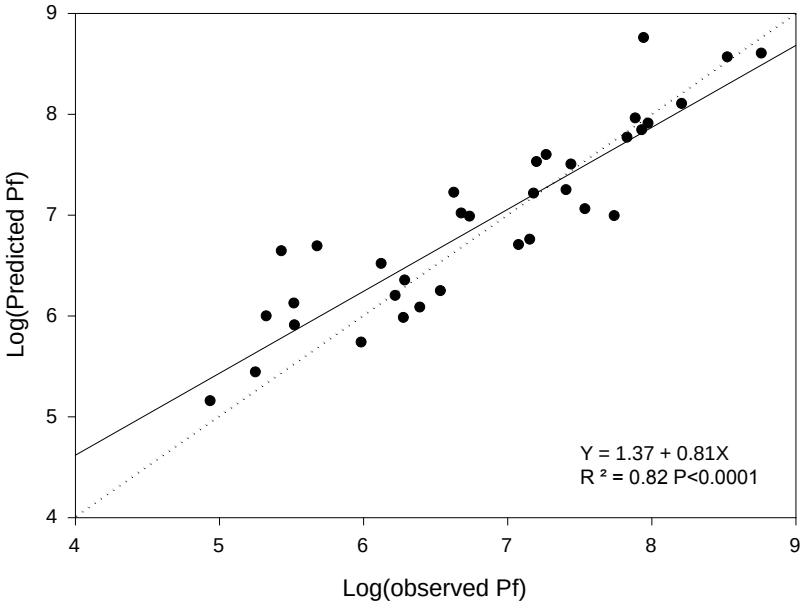

Figure 10. Regression of the predicted and the observed SCN population density (in the natural logarithmic scale) after annual corn rotation in Nebraska based on a negative binomial regression model fitted to the training data set. Model included SCN initial population density and soil pH as predictors. Solid line is the regression line and dotted line is the line with slope = 1, passing through the origin. Regression equation is shown in the graphic. R^2 rounded to nearest two decimals.

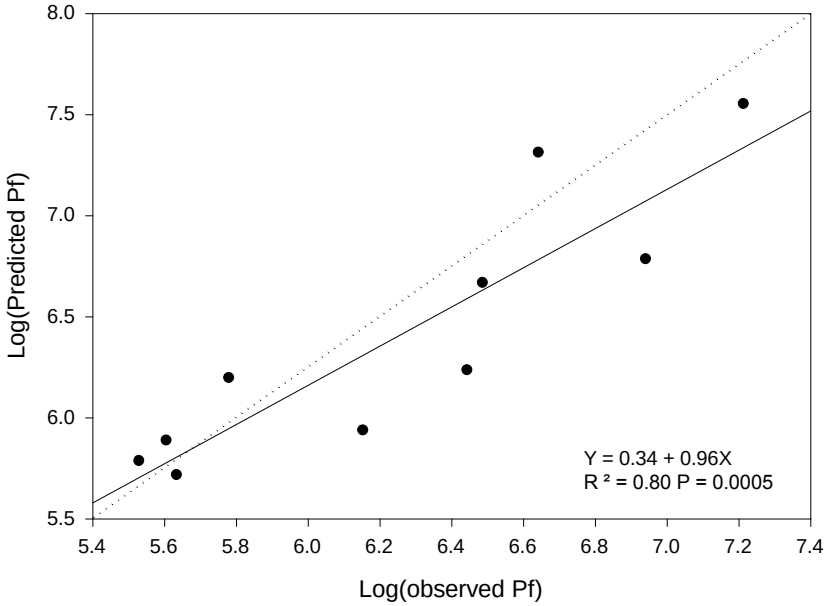

Figure 11. Regression of the predicted and observed SCN population density (in the natural logarithmic scale) after annual corn rotation in Nebraska based on a negative binomial regression model fitted to the validation sample data set. Model included SCN initial population density and soil pH as predictors. Solid line is the regression line and dotted line is the line with slope = 1, passing through the origin. Regression equation is shown in the graphic. R^2 rounded to nearest two decimals.

CHAPTER V. INTRA AND INTERPLOT SPATIAL VARIABILITY OF SOYBEAN CYST NEMATODE POPULATION DENSITIES: A LOOK AT ITS RELATION WITH YIELD ESTIMATION IN STANDARD SOYBEAN VARIETY EVALUATIONS

ABSTRACT

The intra and interplot spatial variability of SCN population densities was analyzed in three experimental areas through a composite and an intensive single-core sampling. The relationship between the observed SCN population density variability and soybean yield of two varieties, tested under a randomized complete block design, was also examined. Spatial autocorrelation determined that SCN population densities occurred in an aggregated pattern in the experimental areas, with population densities showing spatial dependence with those of adjacent plots. The β-binomial distribution adequately described data of incidence (number of SCN-positive soil cores per plot) and suggested that SCN population density aggregation also occurred within plots (index of aggregation $\theta > 0$). Correlogram analysis of thirty six transects of 28 to 35-m showed that SCN population densities were spatially correlated with population densities located up to 15 m. SCN reproduction factor was not related to the number of SCN-positive cores per plot nor was it related to soybean yield in the two soybean varieties assessed (one resistant and one susceptible). Consideration of plant density in the estimation and comparison of yield of the two varieties consistently showed significant yield differences that were not evident when plant density was ignored.

INTRODUCTION

The soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe, 1952) has remained the most economically important pathogen of soybean in the U.S. (Niblack, 2005; Wrather and Koenning, 2006). In Nebraska, economic losses due to this nematode were estimated at $40 U.S. million dollars in 2012 (Wilson and Giesler, 2013). Management of SCN in Nebraska has relied on crop rotation and the use of SCN resistant soybean varieties (Giesler and Wilson, 2011). Use of SCN resistant soybean varieties and crop rotation are the most economical and sustainable strategies to mitigate the impact of this pathogen (Niblack, 2005; Tylka, 2008).

More than 100 sources of SCN resistance have been identified and are now available to breeders (Rao-Arelli et al., 1992; Rao-Arelli et al., 2000). The importance of gene identification for development of resistant varieties is well recognized (Kim et al., 2010). Of more practical relevance, however, is how to better assess resistance in relation to cultivar response to SCN in field experiments. Every year, hundreds of soybean varieties are evaluated for agronomic performance and response to SCN in the U.S. (Giesler and Wilson, 2011; Tylka et al., 2012). The effectiveness of resistant varieties in suppressing SCN population densities has been assessed according to an index of nematode reproduction, referred to as the SCN reproduction factor (Rf) (Niblack et al., 2002; Niblack, 2005). The Rf sustained by a given variety depends entirely upon the SCN population density at planting (Pi or initial population) and the population density at harvest (Pf or final population) estimated in an experimental plot. Estimation of SCN population densities (Pi and Pf) in experimental plots is affected not only by the detection limit of the processing method used, but also by the spatial variability of SCN population

densities in a plot. The spatial variability of SCN population densities in a field plot determines largely the probabilities of recovering SCN cysts and eggs from soil cores during sampling. Soil cores are typically collected only along the two center rows of a four-row experimental plot (Niblack, 2005; Tylka et al., 2012).

The analysis of spatial variability through spatial pattern has been an important tool in the understanding and interpretation of pathogen populations and disease dynamics (Noe and Campbell, 1985; Campbell and Madden, 1990; Gottwald et al., 1992). Field variation, optimum plot size and shape, and relationship with soil factors are among the practical information that spatial pattern analysis provides (Modjeska and Rawlings, 1983). Francl (1986) reported intensive sampling for SCN in field plots and the analysis of spatial variability suggested that 6 m was the minimal plot length to account for SCN spatial heterogeneity. Relationship of SCN spatial heterogeneity with soybean yield was not the focus of his analysis (Francl, 1986).

Reliable and accurate assessment of response of resistant varieties to SCN are fundamental to the selection of improved cultivars and to enable better cultivar recommendations to soybean growers. The objectives of this research were to (i) determine the spatial pattern of SCN population densities in standard variety evaluation field plots; (ii) describe the SCN field spatial variability commonly encountered in standard-size variety evaluation experimental plots using geostatistics; (iii) determine the relationship between SCN population densities and mean incidence intraplot and interplot; and (iv) characterize the information obtained from objectives i to iii in relation to soybean yield.

MATERIALS AND METHODS

Field plot establishment

Three experimental areas (one per location) were established in fields with a history of SCN near Bellwood, Plattsmouth and Waterloo, Nebraska in 2012. The Bellwood field had a loamy sand soil texture and was under central-pivot irrigation. The Plattsmouth field had a silty clay loam texture type and was non-irrigated, and the Waterloo field had a sandy loam soil type and was under central-pivot irrigation. Bellwood and Plattsmouth fields were conventional-till fields and Waterloo was a no-till field.

The experimental area in each location consisted of an array of 6 x 6 plots, each plot being 5.2-m long and 3-m wide. In each location, the experimental area was stratified into six blocks and each of two glyphosate-resistant soybean varieties, one registered as SCN-resistant (Pioneer 93Y15, source of resistance PI 88788) and the other as SCN-susceptible (Pioneer 93M11), was randomly assigned to three plots in ach block. Varieties were planted at a density of 140,000 seeds per acre. The four soybean rows of each plot were spaced at 0.76 m, and 0.60 m alleys were left between plots. Planting was done on May 22 in Bellwood and on May 23 in Plattsmouth and Waterloo. Agronomic management of the plots followed the recommended practices for high yield soybean production in the region.

Sampling for SCN

The first sampling for SCN was executed immediately after planting in each location and consisted of a composite or bulk sampling followed by a single-core

sampling. Before collecting the soil samples, a 4-m inner segment of the two center rows of each plot was delimited and marked for future reference. Five soil cores, one at every 1.0 m interval, were collected along each of the center rows. The 10 cores obtained were mixed to a composite sample. Cores were 2.5-cm-diameter and 15 to 20-cm-deep. Following the collection of the composite samples in the plots, 10 additional soil cores were collected along the same center rows of each plot, in a similar manner as before. However, in this sampling each single core constituted an individual sample. Each single core was extracted within a radius of 10 cm from where the cores for the composite samples were removed, attempting to obtain cores with similar soil amounts. Core extraction was repeated if a core appeared to have less soil amount than that predefined for a regular rich-soil core sample. Soil probes were washed and rinsed in a bucket between each core extraction to avoid SCN contamination between individual samples and between plots.

A second sampling of composite samples was conducted at harvest in each location. These samples were used for estimation of SCN population density at harvest, which was used in the calculation of the SCN reproductive factor in each of the two varieties assessed. In order to sample near the areas of the two center rows where soil cores were removed in previous samplings, probing was done within an approximate radius of 10 to 15 cm from where first composite sampling cores were removed. This was aided by the plots markers that were placed in the center rows at the beginning of the study. In addition to SCN sampling, soil composite samples were collected in each location for analysis of soil texture, fertility, pH, organic matter and soluble salts. These were done by a commercial soil laboratory (Wards Laboratories, Inc., Kearney, NE).

Sample processing and determination of SCN population density

In the laboratory, each composite and single-core soil sample was thoroughly mixed to obtain a homogenous sample. For the composite samples a 100 cm^3 subsample, measured by volumetric displacement, was processed to determine the number of SCN eggs/100 cm^3 of soil using standard sieving techniques for cyst nematodes. The rest of the methodology has been previously described (Pérez-Hernández and Giesler, 2013). For the single-core samples, the soil samples were processed in the same manner as the composites. Weight and volumetric displacement of each sample was determined before processing and the obtained SCN population density was adjusted to the standard 100 cm^3 of soil.

Spatial pattern of SCN population densities in the experimental areas

The spatial pattern of SCN population densities in the three experimental areas was determined from egg densities of the first composite sampling using spatial autocorrelation analysis with the LCOR2 software (Gottwald et al., 1992). The analysis is based on the Modjeska and Rawlings model for spatial correlation analysis of uniformity data (Modjeska and Rawlings, 1983). The model assumes symmetry of the correlations and that all pairs of observations that have the same spatial relationship have the same correlation. In the analysis, all counts or quadrats values are compared with all others in the matrix of quadrats.

In the 6 x 6 plot matrix of each experimental area in the present study, each plot was considered as a quadrat, so the spatial position of each individual quadrat was identified by the row number and the column (position) of the quadrat within the row (a

row corresponded to the block of the two soybean center rows of each plot where SCN was sampled). In the data matrix, the spatial location was identified by subscripts ***i*** and ***j***, such that ***i*** = 1,2,…, and ***j*** = 1,2,… designate row and column numbers respectively. The correlations at different distances are given by $\rho(l, k)$, where $l = i' - i$ and $k = j' - j$ define lags in two dimensions or number of quadrats from quadrat (i, j) across rows and columns, respectively, to quadrat (i', j'). The mathematical basis of the correlation model is given in Modjeska and Rawling (1983).

In addition to the spatial autocorrelation analysis, geostatistical maps of SCN egg densities were generated with data of the first composite sampling to visually inspect the SCN spatial pattern in experimental area. Maps were generated using the nearest neighbor spatial interpolation method in SURFER software (Golden Surfer 6.04, Inc., Golden, CO).

Intraplot SCN population density and relationship with incidence

The relationship between the mean SCN population density in each plot (obtained from the first composite sampling) and the incidence observed within each plot (obtained from the single-core sampling) was examined with correlation analysis and a discrete probability distribution. Incidence was operationally defined as the number of soil cores that tested positive to SCN out of the 10 cores that were collected in each plot. Frequency distribution of incidence was determined for each experimental area. In the statistical literature (Snedecor and Cochran, 1989), sampling in which incidence data are collected from groups of neighboring sampling units is referred to as cluster sampling. In the present study, every plot in each experimental area was considered as a sampling unit,

thus there were N = 36 sampling units in the Bellwood and Plattsmouth experimental area (32 in Waterloo) and n = 10 individuals observed in each sampling unit. The number of plots testing positive to SCN in the entire experimental area was represented by Y and the number of "diseased" individuals (SCN-positive cores) in the *ith* sampling unit by Y_i; i = 1,2,3,…,N. The proportion of diseased individuals in the *ith* sampling unit was represented by y_i. The mean proportion, which is an estimate of the probability of a soil core containing SCN countable units (cyst with eggs), was then estimated as

$$\bar{y} = \frac{\sum yi}{N} = \frac{\sum (Yi / n)}{N} = \frac{\sum Yi}{n.N}$$

and the unbiased estimate of the variance of the y_i values is:

$$s_y^2 = \frac{\sum (Y_i - \bar{y})^2}{N-1}$$

Since incidence here is a discrete variable with a natural denominator, a β-binomial distribution was used to describe the data. The Goodness of fit of the distribution was assessed with the moment estimates, p and θ. The respective estimates for these parameters are $\bar{y}$ for p and the θ parameter is estimated as:

$$\hat{\theta} = \frac{s_y^2 - \bar{y}(1-\bar{y})/n}{\bar{y}(1-\bar{y}) - s_y^2}$$ In this case, p is interpreted as the average probability of a soil core being SCN-positive and θ is an index of aggregation.

Interplot spatial variability of SCN population density

The interplot spatial variability of SCN population densities was determined with geostatistical analysis using the egg density data obtained from the single-core sampling

of the rows of each block. Twelve transects (two in each block) were formed and examined in each experimental area, with values for alleys assigned by running average interpolation. The length of the transects was 35-m in Bellwood and Plattsmouth experimental areas and 28 and 32-m in the Waterloo experimental area. Transect lengths in Waterloo were different because in a few blocks soil conditions made some plots unusable for sampling. Spatial variability of SCN along the transects was described with correlograms created in Sigma plot (Sigma Plot, Systat Software, Inc. San Jose, CA). The correlogram (Dobermann et al., 1997) in the analysis measures the similarity between adjacent SCN egg densities. In our data, the set of n=28, 32 or 35 egg density values in the transects was denoted by $z(u_\alpha)$, $\alpha = 1,2,\ldots n$, where u_α is the vector of spatial coordinates of the αth observation. The similarity between adjacent egg density values is depicted by plotting each observation $z(u_\alpha)$ versus the one measured 1m away, $z(u_\alpha + h)$ with $|h| = 1$m. Scattergrams, plots of pair of measurements at points separated by a given distance $|h|$, were generated to obtain tables of the correlation coefficients and distance. The graphical representation of distance vs. the Pearson correlation coefficient is called a correlogram.

Soybean plant density and yield assessment

The number of soybean plants in the 4-m segment originally delimited in the two center rows of each plot was determined six weeks after planting and again within 1 to 5 days before harvest. This number was converted or adjusted to number of plants per acre. Prior to harvest, plots were end-trimmed to exactly the 4-m segment. Harvest of the plots

was done on September 28 at Plattsmouth, October 8 at Bellwood and October 11 at Waterloo. Yield was adjusted to 13% moisture at harvest and converted to bushels/acre.

SCN reproduction factor estimation and comparison of variety yield

The SCN reproduction factor for each variety was calculated with the formula: Rf = Pf/Pi, where Pf is the final SCN population density or population at harvest and Pi is the initial SCN population density or population at planting. Descriptive statistics of the variables Pi, Pf, Rf, plant density (PD), and yield were obtained for each variety, by location. A multivariate analysis of variance (MANOVA) was performed to test for differences between the varieties considering all variables at once (Pi and Pf log transformed) (GLM Procedure of SAS, SAS Institute, Cary, NC). Next, focus was on the comparison of the two soybean varieties for differences in PD and yield. Yield of the two varieties was compared without considering plant density or number of plants per acre and with consideration of plant density (GLIMMIX procedure of SAS). These two comparisons were made by location.

RESULTS

Spatial pattern of SCN population densities in the experimental areas

In the Bellwoood experimental area, SCN egg densities in the plots were positively correlated ($P = 0.01$) with densities of adjacent plots within block and across blocks (Fig. 1, Table 1). No skewness of the spatial pattern was detected here in relation

to the sides of the experimental area, as interpreted from the values of the diagonal lag correlations of the LCOR2 output (Fig. 1-A).

In the Plattsmouth field plot, SCN population densities in the plots were also positively correlated ($P = 0.05$) with egg densities of adjacent plots within block and across block. In addition, significant correlations ($P = 0.05$) occurred at two plots away in a diagonal direction (Fig. 1, Table 1). As in Bellwood, no spatial pattern skewness was detected in relation to the sides of the experimental area.

In the Waterloo field plot, SCN population densities in the plots were positively correlated ($P = 0.05$) with population densities at two plots away within block, but not across block (Table 1). No skewness of the spatial pattern was detected here in relation to the sides of the field plot. The surface prediction maps showed higher SCN population densities in the entire experimental area of Bellwood compared with Plattsmouth and Waterloo locations (Figs. 2 to 4). However, in the Plattsmouth experimental area, the SCN population densities appeared to be more evenly distributed (Fig. 3).

Intraplot SCN population density and relationship with incidence

SCN population densities varied across locations. Mean population densities from the first composite sampling was 2,081, 406, and 47 SCN eggs/100 cm^3 of soil in Bellwood, Plattsmouth and Waterloo experimental areas, respectively. Overall, SCN egg densities in the plots, estimated from the first composite sampling, were positively correlated with the number of SCN-positive soil cores in each plot (correlation coefficients of 0.34, 0.54 and 0.63 for Bellwood, Plattsmouth, and Waterloo, respectively; $P < 0.05$; Fig. 10). The highest frequencies of SCN-positive soil cores per

plot were observed in Bellwood, with most plots having 8 to 10 positive cores (Fig. 11). The lowest frequency of positive soil cores per plot was observed in the Waterloo experimental area (Fig. 11). The observed mean proportion of SCN-positive soil cores and the associated empirical variance was 0.94 and 0.0054 in Bellwood, 0.81 and 0.0441 in Plattsmouth, and 0.41 and 0.0435 in Waterloo, respectively. The β-binomial distribution described the incidence data adequately with aggregation index (θ) of 0.0035, 0.2694 and 0.0972 in Bellwood, Plattsmouth, and Waterloo experimental areas, respectively (higher aggregation in Plattsmouth). Based on this distribution, the average probability of a soil core being SCN-positive was 94% in the Bellwood field plot, 81% in the Plattsmouth field plot and 41% in the Waterloo field plot.

Interplot spatial variability of SCN population density

Variability of SCN egg densities with distance was more remarkable in the transects of the Bellwood experimental area (Fig. 5) than those of Plattsmouth and Waterloo (Fig. 6 and 7). The change of SCN population levels with distance suggested the existence of a dispersal gradient of SCN along the transects examined. The dispersal gradient varied even between rows of the same block, but this variability was more evident in Bellwood than in the other two locations. The distances at which correlations became negligible varied across locations and transects (Tables 2 to 4. Figure 8). The distances with high correlations are interpreted as the range distance beyond which two SCN population density values can be considered as statistically independent. The average range was 9.0, 8.0, and 4.0 m in Bellwood, Plattsmouth and Waterloo,

respectively (Fig. 8, Tables 2 to 4). A scattergram representing transect 2 of the Bellwood field area is displayed in Fig. 9.

SCN reproduction factor estimation and variety yield comparisons

The observed value of the SCN reproduction factor, Rf, was consistently higher in the susceptible than in the resistant variety in Bellwood and Waterloo, but the opposite occurred in Plattsmouth (Table 5). In Bellwood, SCN Rf was 1.6 and 47 in the resistant and susceptible variety, respectively. These values, though apparently different, were only marginally significant ($P = 0.09$), as suggested by the statistical comparison using the 18 replications per variety. In Plattsmouth, SCN Rf between the resistant and susceptible variety was not significantly different (Rf = 406 in the resistant and 203 in the susceptible; $P = 0.55$). In Waterloo, SCN Rf was significantly different between the varieties (Rf=6.5 and 42 in the resistant and susceptible variety, respectively; $P = 0.02$).

The overall multivariate analysis of variance (data from all locations together) indicated significant differences between the varieties considering SCN Pi, Pf, Rf, plant density and yield (Hotelling-Lawley Trace = 14.34, $P<0.0001$). Further, comparisons of the varieties for individual variables showed significant differences for Pf, plant density and yield, but not for Pi and Rf. In the MANOVA by location (Table 6), SCN Pf, plant density, and yield were significantly different between the varieties ($P < 0.0001$) in Bellwood. In Plattsmouth, only SCN Pf and plant density were significantly different between the varieties ($P < 0.0001$). In the Waterloo field plot, only Rf and plant density were significantly different between the two varieties ($P < 0.05$), but initial and final population density, and yield were not significantly different.

Yield of the resistant variety was significantly greater than that of the susceptible in the Bellwood location with and without adjustment to plant density. Noteworthily, with adjustment to plant density, the estimated yield difference between the varieties was 24.62 bushels/acre whereas without adjustment, the difference was 14.04 bushels/acre. Subtracting these two estimates gives a difference of 10.58 bushel/acre, which represents about 75.3 % of not-accounted yield when plant density was overlooked. In Plattsmouth and Waterloo, yield of the resistant and susceptible variety was not significantly different when plant density was ignored ($P > 0.20$ in locations). However, when the number of plants per acre was taken into account (used as a covariate in the ANOVA model), a significant difference in yield between the varieties became apparent in the two locations ($P = 0.002$ and 0.004, for Plattsmouth and Waterloo, respectively). In Plattsmouth, the estimated yield was 40.51 and 40.04 bushels/acre for the resistant and susceptible variety, respectively, when plant density was not taken into account. Those estimates changed to 43 and 37 bushels/ acre when yield was adjusted to number of soybean plants per acre. In Waterloo, estimates were 38.86 and 50.37 bushels/acre without considering plant density and 60.02 and 29.20 with consideration of plant density for both the resistant and susceptible variety.

DISCUSSION

The main focus of this study was to determine the spatial structure, density and variability of SCN egg numbers at the intra and interplot scale in evaluations of soybean varieties for response to SCN. The experiment was motivated by analysis of current

standard methodology used in variety evaluations and the need for reliable and accurate assessments. The variety evaluation methodology is described in detail in previous research (Niblack, 2005; Giesler and Wilson, 2011; Tylka, et al., 2012), but typically, at the end of the assessments, selection of "best" varieties is based on yield performance and response to SCN. Yield comparisons are made through standard ANOVA tests while SCN response is judged by a reproduction factor. The Rf depends entirely on SCN Pi and Pf, and within the framework of variety evaluations, estimations of Pi and Pf are affected by the recovery of SCN population densities from single-cores. This is affected by the spatial variability of population densities in a given plot and by the distance at which the single cores are collected while sampling along the rows of interest.

In this study, the spatial pattern of SCN population densities was first determined in three experimental areas using spatial autocorrelation. Albeit not widely used to study nematode population densities, analysis of spatial pattern has been used to study the dynamics of other plant pathogens and diseases (Campbell and Madden, 1990; Gottwald et al., 1992). The few previous studies on spatial pattern of SCN and other nematodes have provided valuable information on practical subjects, such as plot size determination and characterization of areas used in continuous experimentation (Noe and Campbell, 1985; Francl, 1986). In past studies, however, little emphasis has been placed on the relationship of patterns with variety testing and sampling. Rather than testing the null hypothesis of non-randomness and infer on the SCN spatial on other plots, the focus of the present research was to examine the structure of SCN population densities in standard plot size and its relationship with soybean yield. Autocorrelation analysis showed that in the studied areas, SCN population densities in the plots were spatially dependent with

those in adjacent plots, or with plots located up to two positions away. Such a dependence had directionality (was anisotropic) in one location. The spatial dependence of a biological characteristic, ―presumably of physical and chemical properties as well, measured in an experimental area can provide both ecological and practical information on/for that experimental area. For instance, in this study, the SCN pattern observed in each area is not only a reflection of the structure of SCN population densities in the plots alone, but also a reflection of the soil heterogeneity, the outcome of the SCN dispersal, or even, a reflection of the length of time the field has been infested. Within a physical and ecological framework, the observed pattern may also discern soil conditions and biological, chemical, and physical interactions affecting *H. glycines*, and presumably, soybean growth and development, thereby yield. Thus, plots in which low or medium levels of SCN population densities were detected may suggest that a factor or combination of factors could be having a negative effect on SCN population densities. This would be revealed in a spatial analysis through positive correlations between plots where such factors occur.

From a practical perspective and a strict statistical point of view, if varieties are to be compared for their response to SCN, then correlations between experimental units should be taken into account. Presence of spatial dependence violates the independence assumption of standard ANOVA tests that routinely are used to make such comparisons. Severe violations of independence and/or of other theoretical model assumptions can result in erroneous or less precise estimates (severe inflations or deflations of parameter estimates). For example, in the Bellwood experimental area in the study, the adjacent autocorrelations observed would dictate that only plots at distances free of spatial

dependence (not spatially autocorrelated or skipping adjacent plots along and across blocks) would be included in the comparison of yield between the varieties (as long as SCN population densities are being related to yield).

Through soil core extraction at every meter interval within a plot, this study examined the association of the intraplot SCN population density to the probability of capturing or recovering SCN countable units in single-core samples. While *H. glycines* population densities may vary at scales from mm to a few cm (Francl, 1986), the sampling distances employed in this study represent distances at which SCN sampling within a plot is normally done in practice in variety evaluations. The general hypothesis inciting the comparison between SCN population densities (from composite samples) and incidence data (from single-core samples) is that the number of recovered SCN-positive cores in the two center soybean rows of a plot is expected to vary. Yet, more importantly is that such a variation may arise or be dominated by incidence and SCN counts from one of the two center soybean rows. For instance, if SCN occurred only along one of the two soybean rows of a plot and it was successfully recovered from such row, then the soybean plants along that row would be exposed to a higher pathogen pressure than plants in the other row of the plot. Because the SCN reproduction factor is calculated from the final and initial SCN population densities estimated from both rows, this raises the question whether a Rf calculated for a soybean variety in situations like the hypothesized above indeed represents a measure of the variety in limiting the reproduction of SCN. Our data showed that the intraplot density is related to the number of SCN-positive cores found. That relationship appeared to be less strong in the experimental area with the highest

mean SCN population density, but in that experimental area the frequency of SCN-positive cores was 8 to 10 cores most of the time.

Distribution theory has been widely used in biology and ecology to study relationships between diseased entities (Smith, 1983; Madden and Hughes, 1994; 1995). Here, a probability distribution model was fitted to incidence data (number of SCN-positive soil cores per plot out of 10 collected) to characterize the relation of incidence within each sampling unit (plot) relative to each other. A β-binomial distribution with incidence as a discrete variable adequately fitted the data and indicated that in SCN-infested experimental areas with a maximum and mean density of 330 and 50 eggs/100cm^3 of soil, respectively, the average probability of finding one SCN-infested soil core was 41%. The observed index of aggregation suggested that SCN population are also aggregated within plots.

Geostatistics includes statistical tools to characterize the spatial variability of a diverse number of phenomena (Cressie and Hawkins, 1980). Central to geostatistics is the use of the correlogram and semivariogram. In our study, intense mapping of SCN was carried out to refine the information on intraplot SCN spatial variability. Correlograms provided information on the SCN dispersal distance over 35-m transects. For example, based on the range of the significant correlations in the Bellwood experimental area, the estimated mean distance at which SCN points are no longer autocorrelated was 9 m. The 36 transects examined indicated gradients of dispersal and existence of SCN infestation foci along the transects. Considering transects along the same block, it was noticeable that shape of the gradients varied greatly even within the same block. Generally, the

largest range was observed in the Bellwood field plot (Table 5), which had a mean population density of 2,081 eggs/cm^3 of soil.

The SCN reproduction factor, Rf, sustained by a soybean variety in a single growing season has been an important measure of soybean variety response to SCN. In this study, intense and point-precise sampling within plots was conducted for estimation of the Rf. The difference in the Rf between the resistant and the susceptible was marginally significant in Bellwood (P = 0.09), not significant in Plattsmouth (P =0.55) and significant in Waterloo (P = 0.02). This result is in partial agreement with the characterization of the *Heterodera glycines* (HG) typing of the SCN populations in the three locations of this study (Giesler et al., 2012). HG type 2.7. occurred in Bellwood, and according to the HG type test scheme, this "race" is capable of reproducing greater than 10% in varieties having source of resistance from PI 88788 (Niblack et al., 2002), which is the source of the resistant variety used in this study. HG type 0 occurred in Plattsmouth, which according to the HG type test is not expected to attack any of the seven sources of resistance listed in the HG type scheme (Niblack et al., 2002). Nonetheless, this population reproduced successfully in the resistant variety in the Plattsmouth location. The observed Rf was 406.4, which was in fact the highest Rf observed in the entire experiment. HG type 7 occurred in Waterloo. In this location, the Rf on the resistant variety was 6.4 and 42.2 in the susceptible. The discordance between the Rf observed and the observed HG type suggests the hypothesis SCN Rf and female index may differ in field conditions. The calculated SCN Rf appeared not to be related to yield neither in the resistance nor in the susceptible variety in any of the locations.

Yield is the major parameter for judging the selection of varieties in agriculture. Main yield components include number of plants per plot (plant density), number of pods per plant, number of seeds per pod, and weight of seeds (Wilcox, 1994). In the present study, plot plant density (converted to number of plants per acre) was examined for its relationship with yield. Plant density varied for variety and location, but it was consistently different between the varieties. Comparisons of yield of the varieties without regarding the number of plants per acre indicated that the resistant variety yielded more than the susceptible in Bellwood, but not in Plattsmouth and Waterloo (Table 5). The difference in number of plants per acre between the resistant and susceptible variety in Plattsmouth was of 7,389 whereas in Waterloo the difference was 18,178 plants. When stand counts are incorporated to adjust yield to number of plants, significant differences between the varieties became apparent in Plattsmouth and Bellwood. Soybean is well known to compensate for yield. However, thresholds or ranges of number of plants for such compensation is not known. In the present analysis, data were estimated based on 18 replications per variety (include table of estimates, p values and standard errors). The results suggest that the number of plants should be considered when assessing yield and SCN Rf.

In summary, this study demonstrated that at the scale of standard size plots used in variety evaluations SCN egg densities tend to be aggregated, at least with population densities of adjacent plots. This spatial dependence, coupled with information obtained from a wider sample of experimental plots, could be incorporated into analyses to determine the number of replications needed to detect differences among varieties for their response to SCN.

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Table 1. Autocorrelation coefficients (**ρ**) and associated degrees of freedom (df) for SCN egg density data in Bellwood, Plattsmouth, and Waterloo experimental areas, obtained with LCOR2 software.

	Axes	0		1		2		3	
		ρ	df	**ρ**	df	**ρ**	df	**ρ**	df
Bellwood	0	1.00**	34	0.56**	28	0.22	22	0.06	16
	1	0.59**	28	0.36	23	0.22	18	-0.09	13
	2	0.36	22	0.38	18	0.18	14	0.15	10
	3	-0.01	16	0.12	13	-0.20	10	0.15	7
Plattsmouth	0	1.00**	34	0.46*	28	0.33	22	0.32	16
	1	0.43*	28	0.34	23	0.49*	18	0.49	13
	2	0.10	22	0.14	18	0.50	14	0.41	10
	3	-0.05	16	0.06	13	0.23	10	0.25	7
Waterloo	0	1.00**	34	0.17	28	-0.19	22	-0.03	16
	1	0.40*	28	0.21	23	-0.15	18	-0.16	13
	2	0.42*	22	0.11	18	-0.18	14	-0.19	10
	3	0.45	16	0.19	13	-0.07	10	-0.15	7

* and ** indicate significant autocorrelations at $P = 0.05$ and 0.01, respectively

Table 2. Correlation coefficients (**ρ)** associated to the distances at which SCN population densities (eggs/100 cm^3 of soil) are considered to be dependent or independent in each of twelve 35-m long transects (T_1 to T_{12}) sampled in the Bellwood experimental area. Correlations up to only 15-m are shown.

	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}
D[a]	Correlation coefficients (**ρ**)											
1	0.84	0.97	0.81	0.67	0.69	0.90	0.74	0.93	0.85	0.77	0.92	0.87
2	0.57	0.89	0.48	0.20	0.12	0.71	0.27	0.81	0.56	0.51	0.82	0.63
3	0.36	0.80	0.18	-0.29	-0.43	0.55	0.23	0.67	0.27	0.28	0.68	0.45
4	0.26	0.72	0.05	-0.27	-0.64	0.43	0.33	0.56	0.10	0.15	0.52	0.37
5	0.23	0.69	0.01	-0.30	-0.47	0.29	0.23	0.49	0.03	0.08	0.34	0.42
6	0.23	0.70	0.03	-0.20	0.04	0.15	0.03	0.53	-0.03	-0.03	0.17	0.51
7	0.20	0.69	0.14	-0.11	0.41	0.05	0.00	0.59	-0.09	-0.12	0.04	0.55
8	0.17	0.77	0.31	-0.05	0.44	-0.02	-0.25	0.67	-0.13	-0.18	-0.06	0.44
9	0.09	0.85	0.55	0.02	0.14	-0.09	-0.37	0.74	-0.17	-0.23	-0.15	0.29
10	0.07	0.91	0.72	0.14	-0.34	-0.12	-0.44	0.69	-0.27	-0.27	-0.21	0.19
11	0.15	0.89	0.69	0.15	-0.53	-0.13	-0.48	0.55	-0.39	-0.30	-0.23	0.24
12	0.39	0.81	0.40	0.08	-0.35	-0.15	-0.41	0.41	-0.50	-0.32	-0.26	0.49
13	0.60	0.74	0.09	-0.11	0.06	-0.18	-0.36	0.25	-0.53	-0.32	-0.29	0.65
14	0.76	0.66	-0.09	-0.32	0.39	-0.18	-0.22	0.12	-0.50	-0.28	-0.26	0.63
15	0.75	0.78	-0.15	-0.36	0.41	-0.15	-0.07	0.22	-0.46	-0.33	-0.20	0.51

[a] D = distance in meters

Shaded portions of the columns indicate the range or distance beyond which SCN population densities in a transect are considered to be independent.

Table 3. Correlation coefficients (**ρ)** associated to the distances at which SCN population densities (eggs/100 cm^3 of soil) are considered to be dependent or independent in each of twelve 35-m long transects (T_1 to T_{12}) sampled in the Plattsmouth experimental area. Correlations up to only 15-m are shown.

	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}
D[a]	Correlation Coefficient (**ρ**)											
1	0.92	0.86	0.91	0.81	0.86	0.86	0.89	0.84	0.91	0.82	0.94	0.90
2	0.77	0.64	0.76	0.51	0.63	0.68	0.69	0.73	0.84	0.48	0.86	0.75
3	0.60	0.42	0.66	0.39	0.54	0.53	0.53	0.62	0.73	0.24	0.80	0.66
4	0.45	0.27	0.60	0.37	0.64	0.45	0.46	0.65	0.70	0.18	0.81	0.65
5	0.30	0.20	0.52	0.31	0.71	0.42	0.46	0.67	0.68	0.28	0.83	0.68
6	0.17	0.21	0.39	0.20	0.65	0.59	0.51	0.64	0.67	0.40	0.82	0.65
7	0.15	0.46	0.27	0.05	0.52	0.21	0.59	0.54	0.59	0.40	0.72	0.52
8	0.20	0.71	0.16	-0.06	0.46	0.08	0.71	0.43	0.51	0.32	0.60	0.26
9	0.18	0.80	0.08	-0.12	0.42	-0.01	0.81	0.38	0.37	0.14	0.46	0.05
10	0.13	0.59	-0.02	-0.17	0.44	-0.04	0.78	0.35	0.20	-0.02	0.37	0.15
11	0.10	0.27	-0.16	-0.23	0.42	-0.12	0.64	0.28	-0.02	-0.03	0.24	0.17
12	-0.04	0.00	-0.30	-0.23	0.29	-0.25	0.51	-0.04	-0.22	-0.07	0.17	-0.04
13	-0.25	-0.16	-0.34	-0.20	0.12	-0.39	0.50	-0.32	-0.38	0.00	0.09	-0.30
14	-0.40	-0.13	-0.37	-0.20	-0.03	-0.47	0.65	-0.42	-0.45	-0.19	0.07	-0.31
15	-0.36	0.07	-0.42	-0.37	-0.07	-0.48	0.81	-0.31	-0.42	-0.32	0.08	-0.31

[a] D = distance in meters

Shaded portions of the columns indicate the range or distance beyond which SCN population densities in a transect are considered to be independent.

Table 4. Correlation coefficients (**ρ)** associated to the distances at which SCN population densities (eggs/100 cm^3 of soil) are considered to be dependent or independent in each of twelve 28 and 32-m long transects (T_1 to T_{12}) sampled in the Waterloo experimental area. Correlations up to only 15-m are shown.

	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}
D[a]	Correlation Coefficient (**ρ**)											
1	0.48	0.55	0.33	0.29	0.70	0.37	0.67	0.70	0.80	0.80	0.84	0.79
2	-0.15	-0.08	-0.10	-0.13	0.19	0.02	0.09	0.40	0.39	0.42	0.56	0.39
3	-0.36	-0.23	0.12	-0.16	-0.08	0.32	-0.21	0.22	-0.03	0.05	0.31	0.09
4	-0.34	-0.20	0.36	-0.23	-0.13	0.42	-0.21	0.14	-0.30	-0.12	0.14	-0.07
5	-0.26	0.34	0.01	-0.32	-0.09	0.10	0.04	0.09	-0.42	-0.05	0.20	-0.23
6	0.03	0.42	0.02	-0.18	-0.03	-0.18	0.28	0.02	-0.44	0.13	0.48	-0.38
7	0.30	0.19	0.35	0.09	-0.02	0.22	0.08	0.09	-0.41	0.23	0.59	-0.34
8	0.09	0.30	0.24	0.22	-0.07	0.43	-0.16	0.05	-0.38	0.29	0.47	-0.17
9	-0.27	-0.20	-0.06	0.19	-0.14	0.11	-0.20	-0.09	-0.32	0.25	0.33	0.21
10	-0.21	-0.39	-0.14	0.27	-0.15	-0.18	-0.08	-0.10	-0.27	0.17	0.23	0.60
11	0.07	-0.37	-0.13	0.11	-0.14	0.05	-0.07	-0.20	-0.21	0.14	0.32	0.75
12	0.22	-0.20	-0.14	-0.17	-0.05	0.53	-0.28	-0.42	-0.14	0.22	0.55	0.59
13	0.13	0.12	-0.15	-0.07	-0.08	-0.04	-0.35	-0.49	-0.01	0.43	0.64	0.34
14	-0.44	0.45	-0.15	0.02	-0.15	-0.58	-0.08	-0.33	0.19	0.57	0.44	0.25
15	-0.47	0.15	-0.11	-0.36	-0.21	-0.32	0.37	0.06	0.51	0.54	0.15	0.28

[a] D = distance in meters

Shaded portions of the columns indicate the range or distance beyond which SCN population densities in a transect are considered to be independent.

Table 5. Descriptive statistics (mean, standard deviation, min. and max. values) of SCN initial and final population densities (Pi and Pf), reproduction factor (Rf), soybean plant density per acre (PD), and yield in one SCN resistant and one susceptible soybean variety in the three locations in Nebraska.

Location	Variety	N	Variable	Mean	Std deviation	Min	Max
Bellwood	Resistant	18	Pi	2,406	2,472	147	9,107
			Pf	1,918	1,780	293	7,787
			Rf	1.64	2.0	0.06	8.5
			PD	28,172	5,399	15,628	36,577
			Yield	52	5	43	61
	Susceptible	18	Pi	1,756	2,113	80	8,000
			Pf	20,966	18,314	2,107	67,573
			Rf	47	112	2.8	484
			PD	49,996	3,671	38,905	51,540
			Yield	38	3.7	30	44
Plattsmouth	Resistant	18	Pi	362	352	0	1,200
			Pf	3,598	2,440	747	9,853
			Rf	406	1,397	1.24	5,893
			PD	44,077	3,768	36,910	50,875
			Yield	41	4	32	45
	Susceptible	18	Pi	450	321	0	1,280
			Pf	11,874	6,167	1,253	25,107
			Rf	203	592	2.41	2,533
			PD	51,466	3,321	46,220	57,526
			Yield	40	4	33	47
Waterloo	Resistant	6	Pi	158	138	13	333
			Pf	331	271	13	693
			Rf	6.5	11.5	0.17	30
			PD	12,247	4,266	6,318	16,958
			Yield	39	14	15	50
	Susceptible	6	Pi	49	44	0	120
			Pf	1,138	1,077	67	2,800
			Rf	42	28	2.33	70
			PD	30,425	7,041	16,293	34,915
			Yield	50	16	22	64

N = number of plots (replications) on which descriptive measures and analysis were done
Pi = SCN population density at planting (eggs/100 cm^3 of soil)
Pf = SCN population density at harvest (eggs/100 cm^3 of soil)
Rf calculated as Pf/Pi

PD = soybean plant density or number of soybean plants per acre
Resistant variety =Pioneer 93Y15, resistance source PI 88788
Susceptible variety =Pioneer 93M11
Yield in bushels per acre at 13% moisture

Table. 8. Statistical significance of the comparison of Pi, Pf (both log transformed), SCN reproduction factor (Rf), soybean plant density per acre (PD) and yield, of the SCN resistant and susceptible soybean variety after multivariate analysis of variance in three locations: Bellwood, Plattsmouth, and Waterloo.

	Bellwood	Plattsmouth	Waterloo
Variable	*P* value	*P* value	*P* value
LogPi	0.2030	0.4164	0.1762
LogPf	0.0001	0.0001	0.1451
Rf	0.0850	0.5544	0.0241
PD	0.0001	0.0001	0.0005
Yield	0.0001	0.7348	0.2244

Resistant variety: Pioneer MY913 with resistance source from PI 88788
Susceptible variety: Pioneer MY321
Yield (bushels/acre) adjusted to 13% moisture

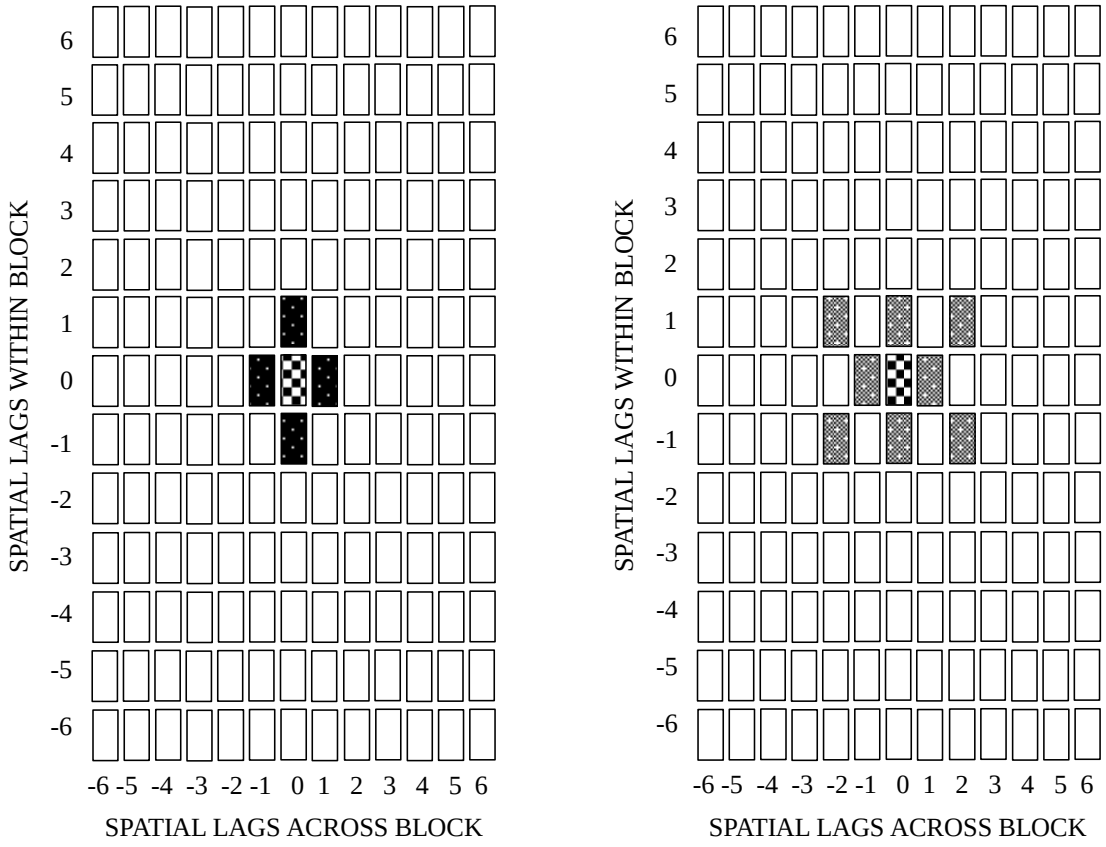

Figure 1. Proximity pattern of SCN egg densities derived from the autocorrelation coefficients output after analysis with LCOR2. **A**. Bellwood experimental area data. **B**. Plattsmouth. Black, gray and white boxes represent correlation coefficients significant at *P*=0.01, 0.05, and non-significant, respectively. The location (0,0), shown as the chess center box, represents the quadrat of origin.

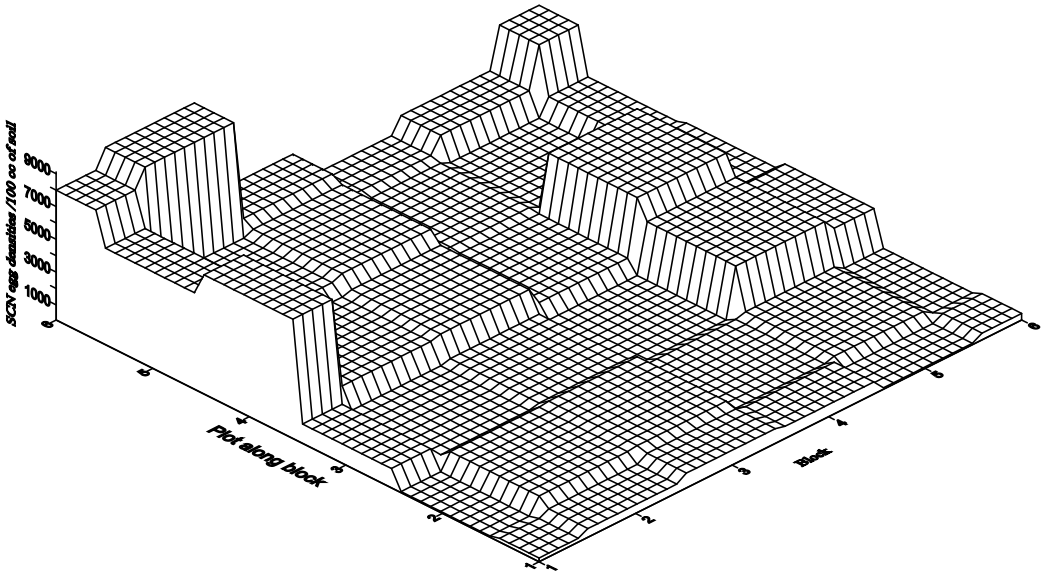

Figure 2. Surface prediction map of SCN egg densities (eggs/100 $cm^3$of soil) from first composite sampling, using nearest neighbor spatial interpolation. Bellwood experimental area.

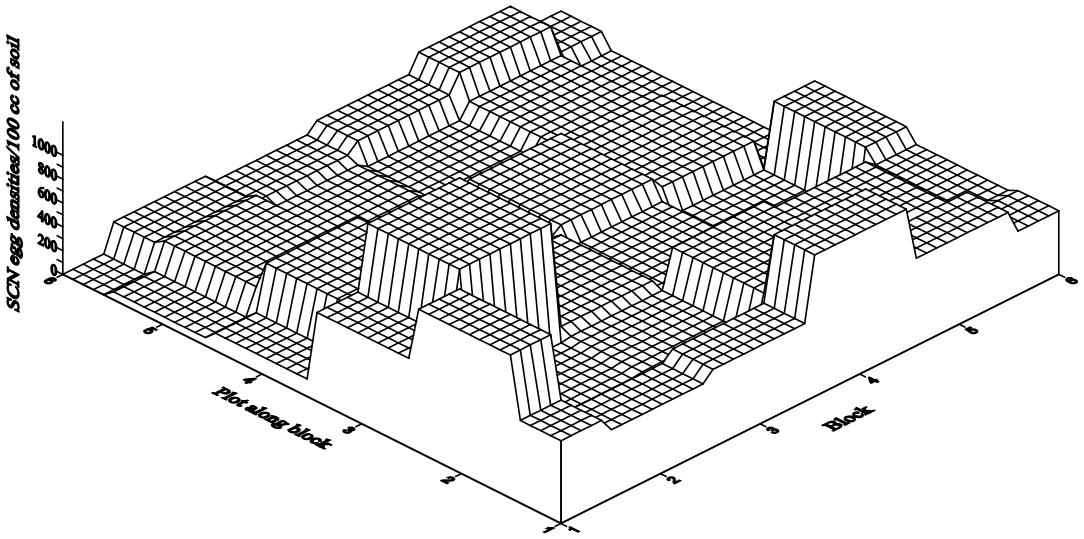

Figure 3. Surface prediction map of SCN egg densities (eggs/100 cm^3 of soil) from first composite sampling, using nearest neighbor spatial interpolation. Plattsmouth experimental area.

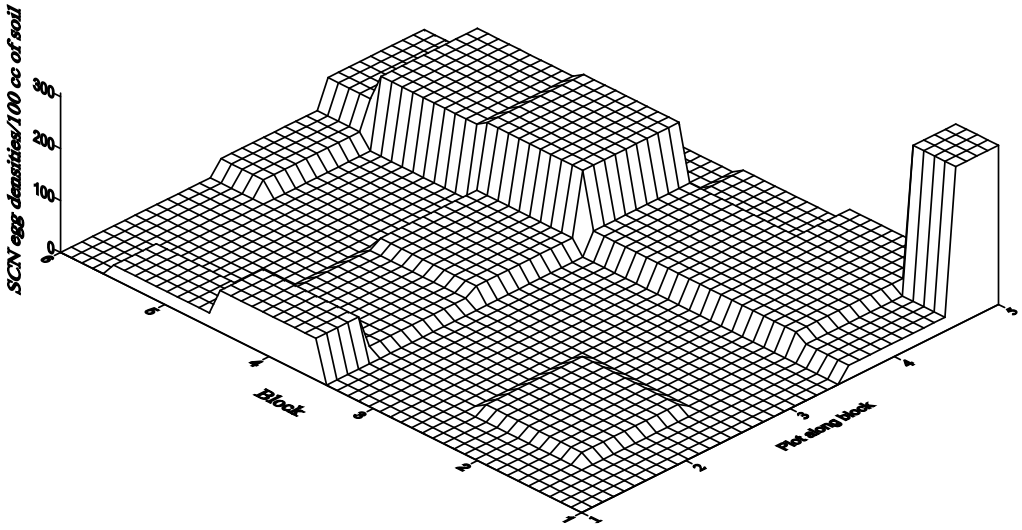

Figure 4. Surface prediction map of SCN egg densities (eggs/100 cm^3 of soil) from first composite sampling, using nearest neighbor spatial interpolation. Waterloo experimental area.

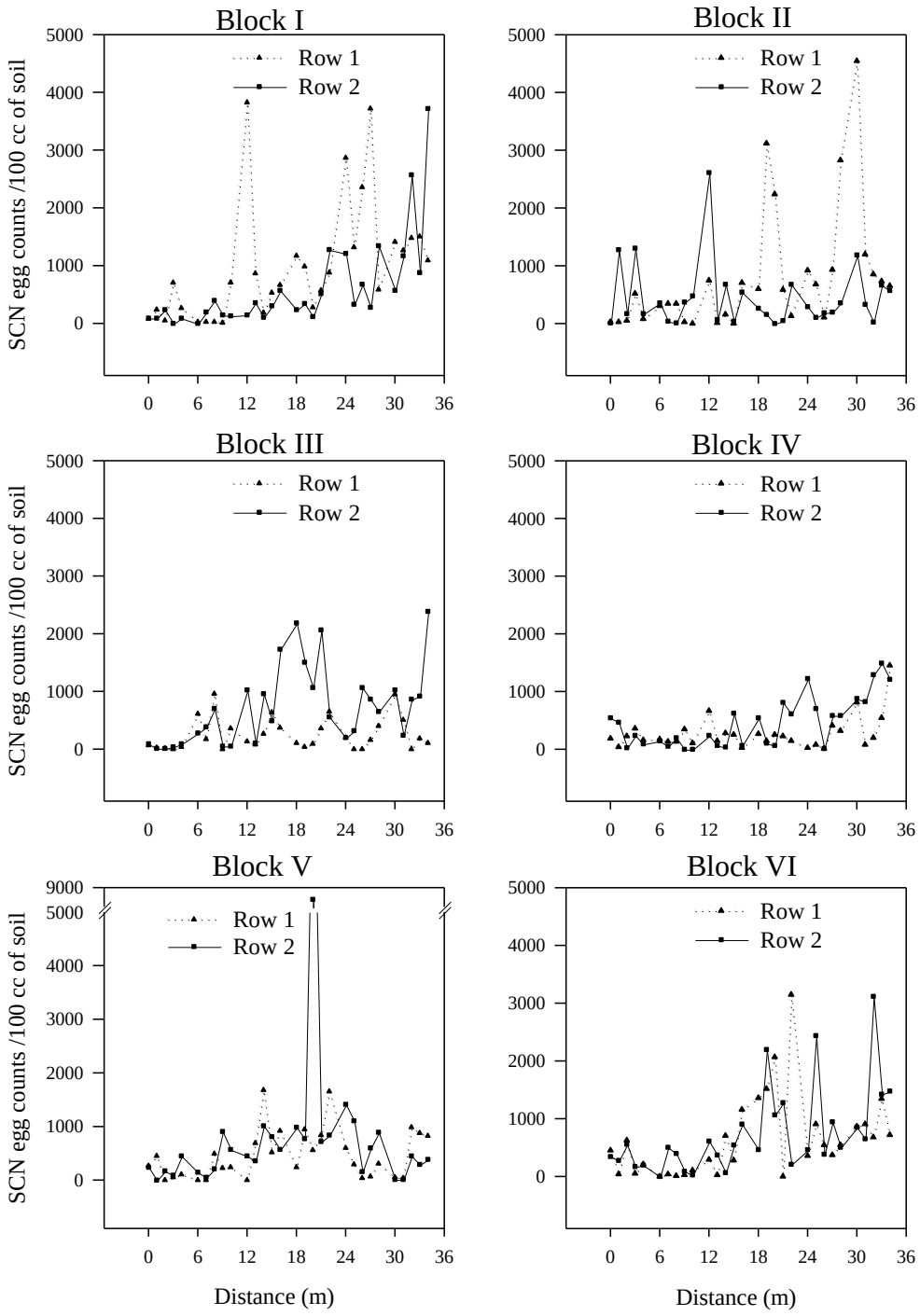

Figure 5. Variation of SCN population densities (eggs/100 cm^3 of soil) with distance (m) in 12 transects of 34 m formed from the six blocks in the Bellwood experimental area. Two transects were formed along each block. Population densities were estimated from single cores.

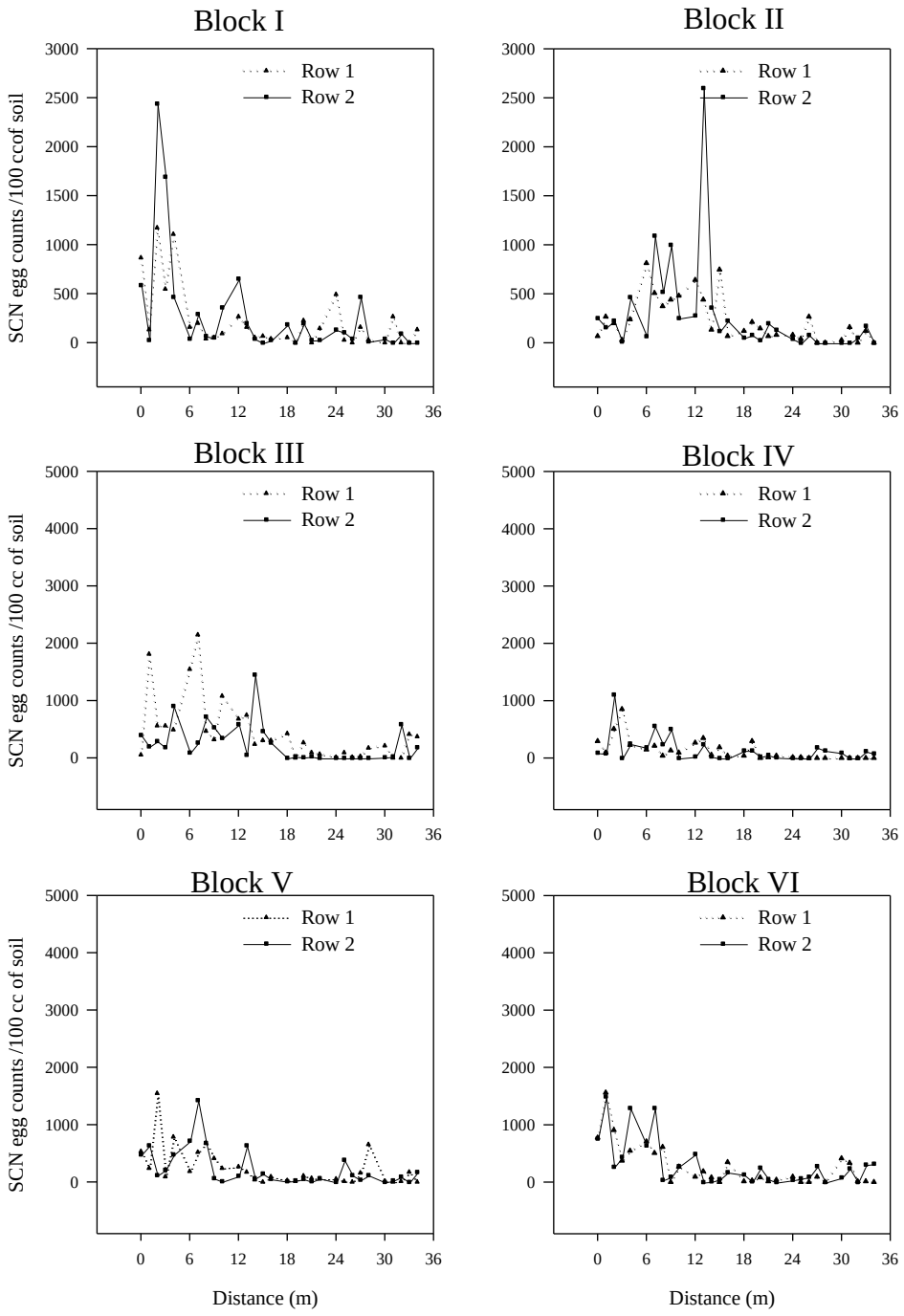

Figure 6. Variation of SCN population densities (eggs/100 cm^3 of soil) with distance (m) in 12 transects of 34 m formed from the six blocks in the Plattsmouth experimental area. Two transects were formed along each block. Population densities were estimated from single cores.

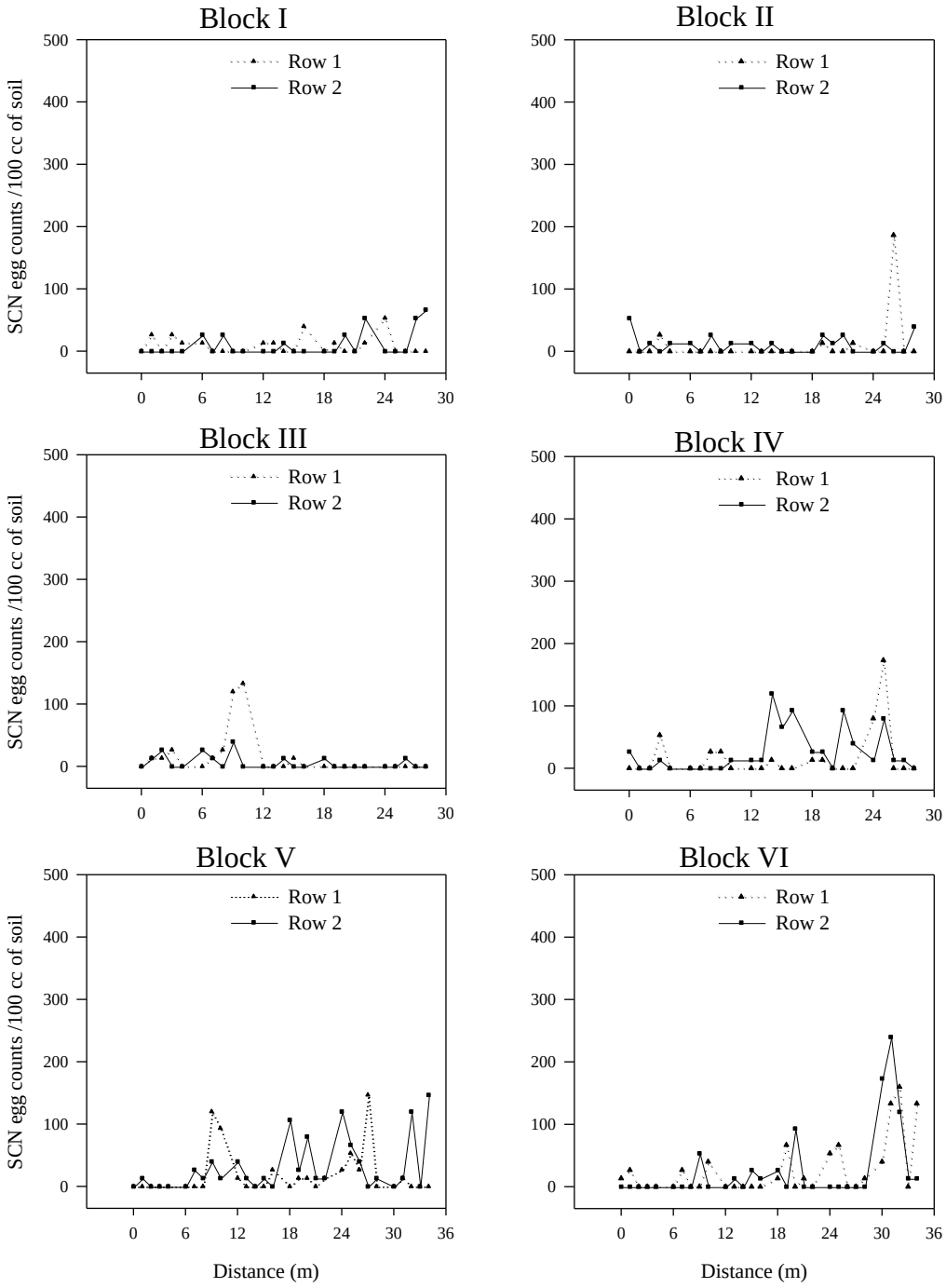

Figure 7. Variation of SCN population densities (eggs/100 cm^3 of soil) with distance (m) in 12 transects of 28 m formed from the six blocks in the Waterloo experimental area. Two transects were formed along each block. Population densities were estimated from single cores.

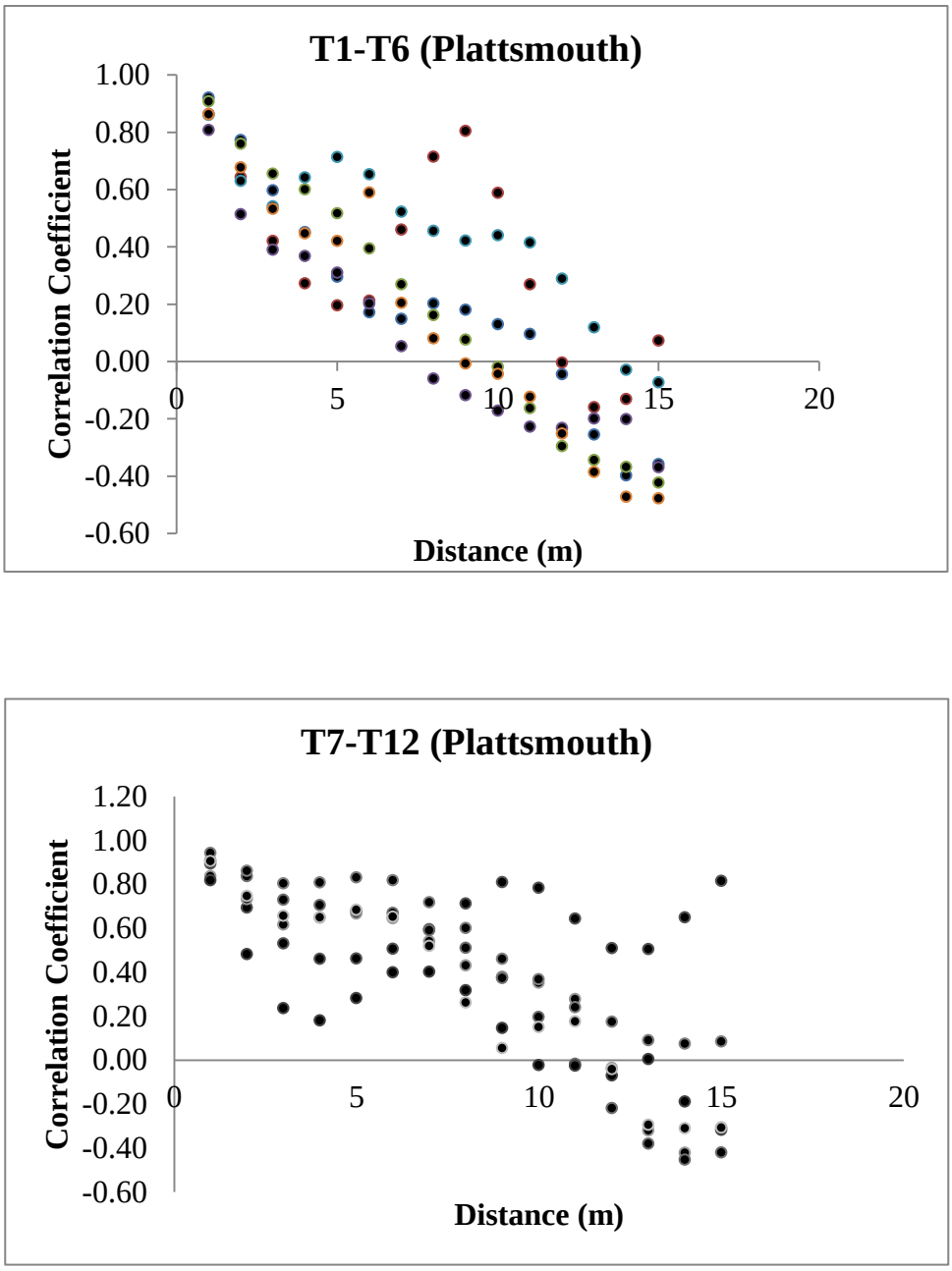

Figure 8. Correlograms (correlation coefficient between SCN population density values vs. distance) for the 12 transects (T1-T12) of the Plattsmouth field plot. Upper plot shows transects 1 to 6 and lower plot transects 7 to 12.

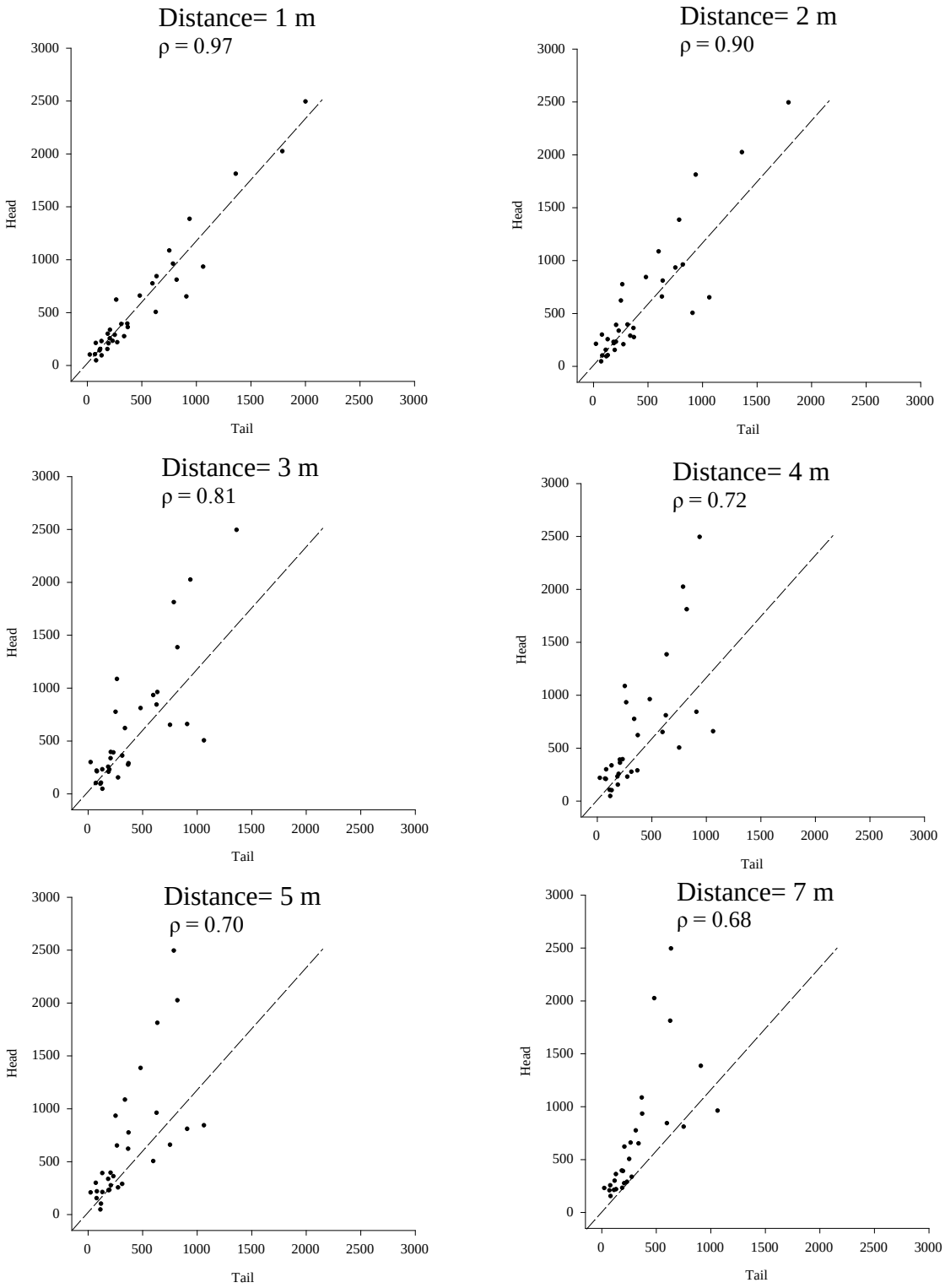

Figure 9. Scattergrams between SCN egg density values separated by a distance of 1m, 2 m, 3m, 4m, 5m and 7m. Data for transect 2 from the Bellwood experimental area. The Tail and Head correspond to the value at the start and end of the vector, respectively.

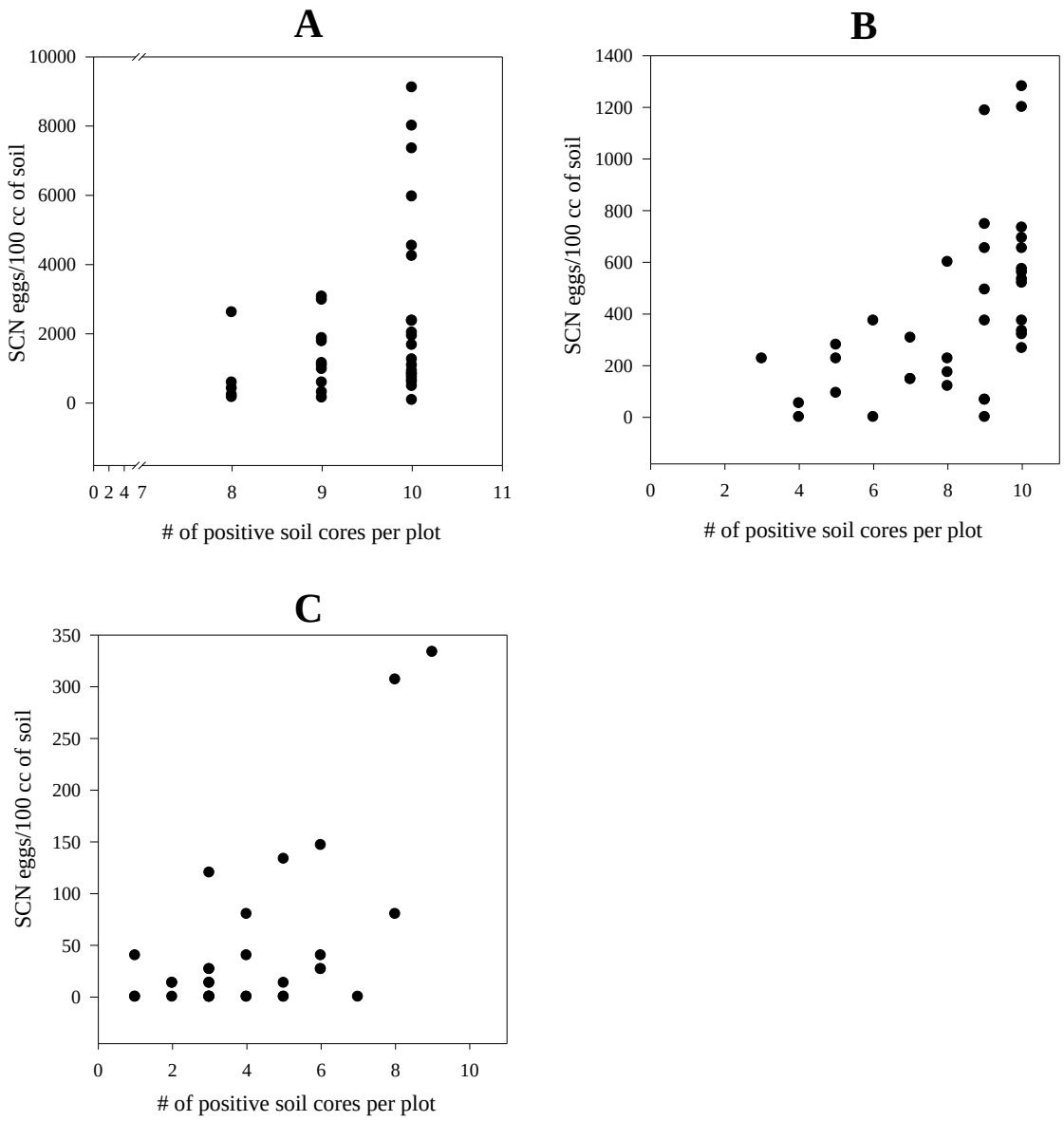

Figure 10. SCN egg densities from first composite sampling and number of SCN-positive soil cores from single-core sampling in each plot in A. Bellwood, B. Plattsmouth, and C. Waterloo experimental area.

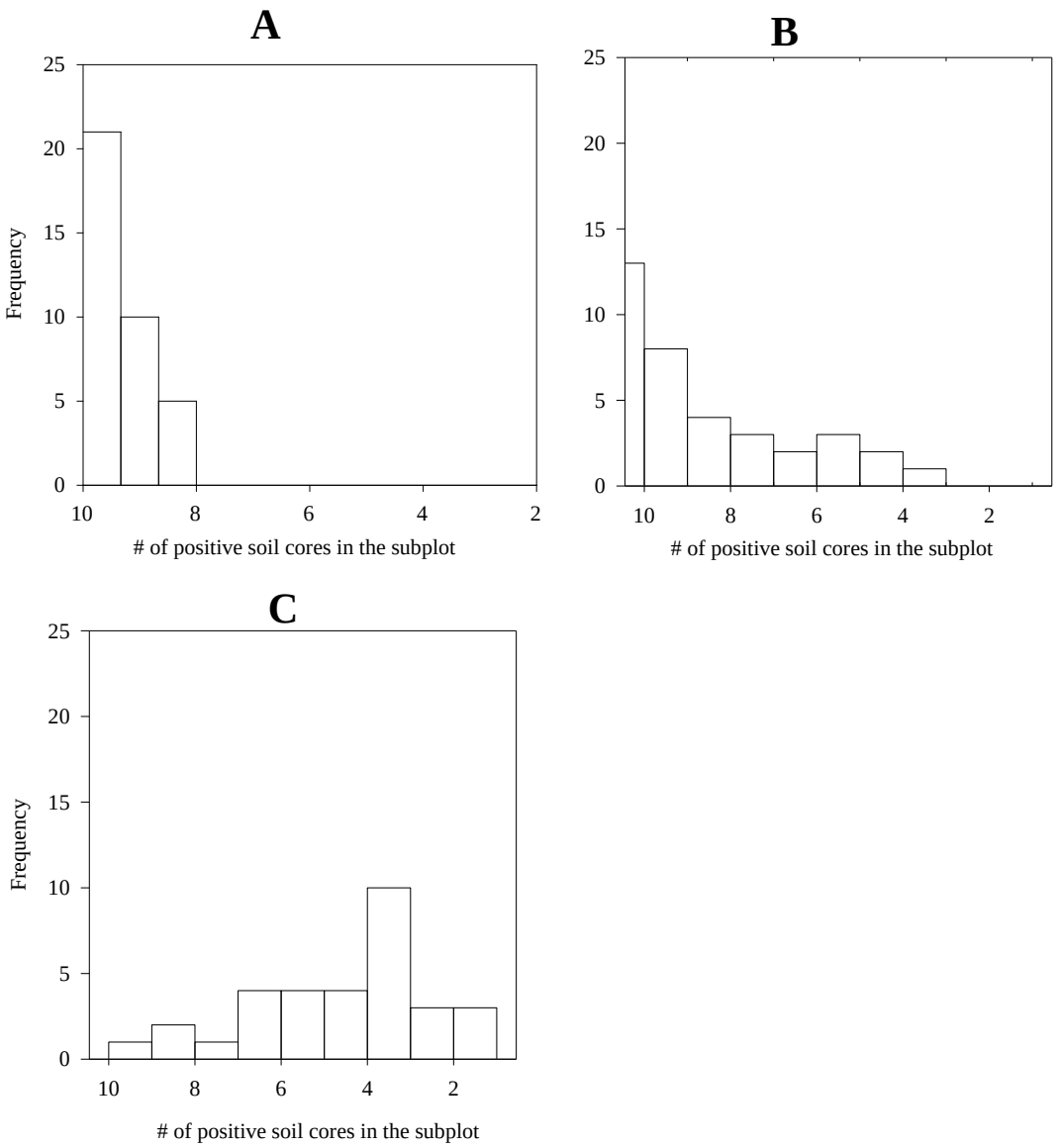

Figure 11. Frequency distribution of number of SCN-positive soil cores out of 10 collected in each plot in A. Bellwood, B. Plattsmouth, and C. Waterloo experimental area.

CHAPTER VI. GENERAL CONCLUSIONS

Rotation with nonhost crops and growing soybean cyst nematode (SCN) resistant soybean varieties are effective practices to reduce SCN population densities and remain the most economical and sustainable strategies to mitigate the impact of this pathogen.

In three consecutive years of assessment, the percent change in SCN population density after annual corn rotation in Nebraska was found to be 50.62%. Soil texture (% of sand, silt and clay) was related to SCN population densities before (Pi) and after corn rotation (Pf), but such a relationship depended on the proportional ranges of the textural constituents. Soil texture alone was found to be a dominant component over the Pi and Pf variability observed in commercial soybean fields annually rotated with corn. Consistently, higher population densities before rotation were found in fields having soils with an increased sand content than in fields with predominant silt or clay content. Specifically, higher SCN population densities occurred in soils with sand content range between 57 and 95% (1 to 28% silt and 7 to 20% clay) than in soils with silt and clay content range between 23 to 61% and 6 to 44%, respectively (sand content between 13 and 37%). Although SCN Pi showed a strong relationship with soil texture, SCN Pf with consideration of the Pi appeared to have an insignificant association with texture. As the soil is a polyphasic system where complex biological, chemical and physical interactions take place, it is reasonable to hypothesize that SCN population densities can also be affected by other soil components such as soil structure, compaction, pH, organic matter, soil temperature, and soil moisture.

Modeling of the SCN population density after annual corn rotation found that soil pH and the SCN population densities before rotation are the major determinants of the

resulting SCN population densities after rotation in commercial production fields. In Nebraska, prediction of such SCN population density can be made with the model that was developed in this study. The model showed a high predicting capability (79.6%) and the only inputs needed were data of SCN population densities before rotation and soil pH from samples representing a field. While only two input variables showed a high explanatory power, additional predictors such as tillage, texture and soil temperature are potential candidates to research and these should be evaluated in larger data sets.

In variety evaluation trials, even in experimental areas with mean SCN egg densities as low as 47 eggs/cm^3 of soil, spatial autocorrelation among SCN population densities in adjacent plots was identified. Consequently, comparison of varieties for response to SCN should take into account such correlations to ensure valid estimates with the methods of analysis currently used. The SCN reproduction factor, often used to assess the response of a soybean variety to SCN, was not related to soybean yield in field conditions neither in a resistant nor in a susceptible variety in three locations. This claim is supported by analysis on four times greater number of replications than what is normally done in standard variety evaluation trials. Parameters more accurately revealing the relationship between variety agronomic performance and response to SCN are therefore needed to improve variety selection and enhance SCN management decisions.

Number of soybean plants per acre is one major component of soybean yield and should be considered in yield estimations, especially if the yield response of a given variety is being related to the SCN population density and not necessarily to the reproduction factor. More accurate and realistic soybean yield estimations were obtained when plant density was taken into account. This should be considered cautiously, since,

though it is suggested that soybean has a compensatory yield response for number of plants, a threshold still needs to be determined. Research on soybean root growth patterns in different soil types and conditions and phenological synchrony with above-ground growth should be coupled with refined characterization of SCN population densities and their spatial variability. This will provide insights into the dynamics of SCN development and on how this minute organism is capable of inducing significant yield reductions in such an important crop.