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CHARACTERIZATION OF THE SPATIAL DISTRIBUTION OF *Heterodera glycines* Ichinohe 1955 (NEMATODA), SOYBEAN CYST NEMATODE IN TWO MICHIGAN FIELDS

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By

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ABSTRACT

CHARACTERIZATION OF THE SPATIAL DISTRIBUTION IN TWO MICHIGAN FIELDS OF *Heterodera glycines* Ichinohe 1955 (NEMATODA), SOYBEAN CYST NEMATODE

By

Maria Felicitas Avendaño

Heterodera glycines Ichinohe 1955 (NEMATODA) (soybean cyst nematode, SCN) is recognized as the major pest limiting soybean [*Glycine max* (L.) Merr.] production, accounting for approximately 1.67 x 10^9 U.S. dollars of soybean yield loss annually in the United States. Despite current management efforts, SCN continues to spread throughout soybean producing areas worldwide. The goal of this project was to understand SCN spatial distribution in soybean fields as the first step towards developing site-specific management (SSM) strategies for SCN. If SCN is to be managed site-specifically its spatial distribution should be structured and relatively time invariant, and it has to be related to yield-limiting factors easier to monitor and manage. The literature suggests that SCN may meet these requirements for SSM. Geostatistical tools and classical statistics were applied to test the hypotheses that SCN's spatial distribution within a field is sufficiently structured and time invariant; that SCN spatial distribution and population densities are related to soil properties; and that the relations among SCN population density, soil properties and soybean yield are sufficient in magnitude to aid in the management decision-making process. A nested survey sampling design was applied on two SCN-infested fields in MI and soil and soybean root samples were collected at monthly intervals during the growing seasons of 1999 and 2000. Soil samples were

analyzed for SCN population density, soil fertility and soil texture. The SCN population in the roots was also quantified. To assess host response, soybean leaf samples were collected twice in 2000 for tissue analysis, and soybean yield was recorded in 1999 and in 2000.

The within field variability in cysts, eggs per cyst, and eggs was large in both fields. The spatial structure in SCN population varied with sampling times, but a periodic pattern in semivariograms appeared consistently from planting to harvest in both fields. The difficulty in adequately fitting wave models to the empirical semivariograms underestimated in some cases the spatial structure in SCN population. Soil texture, pH, and calcium concentration in the soil were strongly correlated and cross- correlated with SCN density in the soil, and to a lesser extent in the roots. Correlations were maintained consistently over time. The nutritional status of the crop reflected the interactions of soil fertility, soil texture, and SCN population density. Bean yield was also strongly correlated with soil texture, soil pH and calcium concentration, and SCN population density in the soil.

The results contribute significantly towards the understanding of SCN spatial distribution in soybean fields, provide evidence of the underlying factors involved in determining grain yield, and lay the base for further research on cause and effect relations to advance understanding SCN biology, ecology, and management opportunities. The spatial variability in yield correlated to the combined effect of SCN density and unfavorable soil conditions observed in this work provided support for the notion of management zone delineation. Thus, the hypotheses tested were demonstrated true.

To Gustavo and Lupecita

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KEY TO SYMBOLS AND ABBREVIATIONS

C_0	Nugget effect
cdf	Cumulative distribution function
DAP	Days after planting
GPS	Global positioning system
h	Lag distance
r	Pearson's correlation coefficient
SCN	Soybean cyst nematode
sd	Standard deviation
$\mathbf{s}_i$, $\mathbf{s}_j$	Locations of observations separated by the lag h
SSM	Site-Specific Management
$Z(\mathbf{s})$	Realization of a mean-constant spatial process
$\hat{\gamma}(h)$	The semivariance at a given lag distance

CHAPTER ONE

INTRODUCTION AND REVIEW OF LITERATURE

The phylum Nematoda (Nemata) Cobb, 1919 (position reviewed by Chitwood and Chitwood, 1950 and Maggenti, 1991) is comprised of organisms defined as roundworm invertebrates with a body cavity and complete digestive tract, which are nonsegmented, appendageless, and have a bilateral symmetry (Hirschmann, 1971; Poinar 1983). The digestive tract of nematodes includes a stoma (mouth), alimentary canal (esophagus and intestine), and anus. Nematodes have a complex nervous system, a secretory-excretory system, a reproductive system, and musculature system (longitudinal muscles), but have no specialized respiratory or circulatory systems. The body is completely covered by a flexible and permeable cuticle. Traditionally, nematodes have been placed in two classes within the phylum: the Adenophorea and the Secernentea based largely on morphological and physiological differences (Maggenti, 1991). More recently, embryology and molecular techniques such as the use of small subunit ribosomal DNA sequences are being used to redefine the phylogenetic tree and taxonomy of the phylum Nematoda (Thomas et al., 1997, Blaxter et al., 1998, Voronov et al., 1998).

Nematodes are found in all habitats and ecosystems of the biosphere. They occur in soil, decaying organic matter, all forms of plant life and most animals, including domesticated and wild species (Norton, 1978). To date, some 20,000 species of nematodes have been described, and estimates of the actual number range from 40,000 to 10 million (Blaxter, 1998). If the known nematode species are grouped according to simple habitat categories, then of the total, 50% are marine nematodes, 25% are soil

inhabiting nematodes (microbivorous and predators), 15% are animal-parasitic nematodes and 10% are plant-parasitic nematodes (Viglierchio, 1991). Although many nematodes parasitize plants or animals, others are beneficial, as they contribute to nutrient cycling or serve as biological control agents of plant pests (Stirling, 1991; Yeates, 1996; Niles and Freckman, 1998).

Nematode diversity is often high in the soil environment and it is important for the long-term stability of soil structure and function (Ettema, 1998). The use of feeding groups classifications has provided significant advances for investigating the role of nematodes in soil ecosystem processes (Wardle et al., 1995). Major trophic groups of nematodes include plant-feeding nematodes (parasites), plant-associated nematodes (herbivores), hyphal-feeding nematodes, bacterial-feeding nematodes, predators, animal parasites, and omnivores (Yeates, et al., 1993). Relationships between nematode functional groups and ecological processes have been found in field experiments (Yeates, 1999). The coexistence of species depends largely on the stability of the environment and the relationships in the abundance of species and trophic groups shifts with different levels of disturbance (Bongers, 1990). For example, agroecosystems, particularly those under monoculture management, are less diverse in nematode species than less disturbed environments such as grasslands (Yeates and Bongers, 1999). The proportion of plant-parasitic nematodes in the soil ecosystem is relatively small when compared to bacterial feeders, for example. Nonetheless, populations of plant parasites can increase because of many environmental stressors (Wasilewska, 1995). Fertilization, and in turn increased nutrient uptake by higher plants, seems to shift the composition of plant-feeding

nematodes, so that plant-associated nematodes are replaced by plant-parasitic nematodes (Bongers et al., 1997).

Agronomists recognize that plant-parasitic nematodes often constrain crop growth. The annual worldwide losses caused by nematodes on life-sustaining crops are estimated to be about 11 percent, adding to an estimate of 100×10^9 U.S. dollars yield loss annually (Sasser and Freckman, 1987). Severe infestations of fields with nematodes such as *Meloidogyne* spp. or *H. glycines* Ichinohe 1952 (soybean cyst nematode, SCN) often result in annual yield losses of 10 to 50 % (McSorley, 1987; Sasser and Freckman, 1987; Wrather et al., 2001a).

Plant-parasitic nematodes are usually invisible to the naked eye (0.5 to 5 mm long, and 50 to 250 μ wide). All known plant-parasitic nematodes possess a buccal stylet with which they puncture and feed upon the cells of their hosts (Hirschmann, 1971). Based on their feeding behavior, nematodes can be classified into three groups: destructive (host cells killed), adaptive (cells modified), and neoplastic (cells modify and undergo new growth). Root-lesion (*Pratylenchus* spp.), cyst (*Heterodera* and *Globodera* spp.), and root-knot (*Meloidogyne* spp.) nematodes are representatives of the three feeding behaviors respectively (Dropkin, 1980). All three genera are the most wide spread nematodes in almost all of the life sustaining crops (Sasser and Freckman, 1987).

Plant-parasitic nematodes can traverse limited distances by their own active movements (Wallace, 1959; Prot and Netscher, 1979). Although distances are short (less than a meter) active movement can be important if it enables nematodes to be disseminated by other agents such as wind, water, vehicles and animals. Nematodes may

be moved on agricultural equipment from farm to farm, or with soil, seeds, plant material, animals and humans to different locations around the world (Lehman, 1994).

Although plant-parasitic nematodes occur in most cultivated soils, associated damage usually results from high population densities, rather than from mere occurrence. Expected economic losses for annual crops generally are inversely correlated with the level of infestation at the time the crop is planted. In any cultivated field, estimates of current population density and future increase are, therefore, critical in anticipating crop losses, and fundamental in making nematode management decisions (Duncan and Noling, 1998). The fact that initial numbers of nematodes can be related to the yield of annual crops has enabled nematologists to develop functional advisory programs (Barker and Nusbaum, 1971) focusing on maintaining phytopathogenic nematode populations densities below economic thresholds (Ferris, 1978; Heald, 1987). If the predicted crop loss is less than the economic decision interval, management is unnecessary; if it is above the economic decision interval, management is justified. If the predicted crop loss is within the decision interval, a subjective decision must be made based upon the grower's economic status or risk aversion/risk acceptance level (Ferris, 1984). Yet, the development of reliable estimates for economic losses caused by nematodes has proven to be very difficult (Ferris, 1993; Roberts, 1993). This situation results from a number of factors, including the impact of environment on the activity of detrimental and beneficial nematodes (Yeates et al., 1993), as well as crop plants and the frequent involvement of nematode and disease complexes, and concomitant plant nematode species.

Variability in nematode population density is a serious problem in the analysis and interpretation of experimental data (Noe and Campbell, 1985). Plant-parasitic

nematodes are not uniformly distributed through cultivated soils, but occur in clusters (Goodell and Ferris, 1981; Alby et al., 1983; McSorley et al., 1985; Webster and Boag, 1992; Robertson and Freckman, 1995), with frequency distributions typically describing negative binomial functions (Taylor et al., 1979; Seinhorst, 1982). This aggregation adds a substantial degree of uncertainty to most estimates of population size and adds significantly to the effort required for comprehensive measurement (McSorley and Parrado, 1982).

Estimates of nematode field populations require accurate and affordable sampling procedures. The recommended practice for estimating population density is to sample an area of two hectares or less, taking composite sample units (Barker, 1978). Soil cores in each sample unit are combined and mixed thoroughly so that a representative portion (usually 100 cm^3) can be processed to extract the nematodes (Dropkin, 1980). The degree of precision necessary in sampling and the structure of the sampling plan itself, depend on the purpose for which the sample is taken (Barker and Campbell, 1981).

Studies of nematode population dynamics seek to understand and predict nematode population growth, and to use this knowledge to improve nematode management (Seinhorst, 1970; Nusbaum and Barker, 1971; Duncan and McSorley, 1987; Ferris and Noling, 1987; McSorley and Philips, 1993; McSorley, 1998; Donald et al, 1999). Many agricultural practices are implemented with the intent of lowering nematode population densities and improving plant growth.

Management practices designed to limit crop losses to plant-parasitic nematodes involve one or more tactics that focus on the strategies to reduce the initial inoculum, and/or limit the rate of nematode population density increase (Roberts, 1993). The

prevention of spread of nematodes, general land management, and cultural practices, such as crop rotation, physical treatment of infested plant material or media, and the use of chemical treatments are among the traditional management tactics used in different crop-nematode systems. Other promising treatments are biological controls (Stirling, 1991) and the development of host resistance through genetic engineering (Atkinson et al., 1994; Opperman et al., 1994; Cai et al., 1997).

Crop rotation is a management practice of primary importance for limiting losses due to plant-parasitic nematodes (Noe, 1998). The emphasis in crop rotation studies has been to reduce the population levels of plant-parasitic nematodes to below-threshold levels. The effectiveness of crop rotations to suppress nematode populations is variable depending on the nematode species, the host range of the nematode of interest and the interactions between nematode species, and among pathogenic races of the same species (Noe, 1998; Hirunsalee et al., 1995). The main limitation to the use of crop rotations is the lack of a suitable or non-profitable nonhost crop for some plant-parasitic nematode systems.

Planting resistant cultivars and using rotations are practical methods of suppressing nematode damage to crops of low economic value (Young, 1998). Resistant cultivars without nematicide treatment often yield as much as high-yielding susceptible cultivars treated with nematicides (Epps et al., 1981). Use of resistant cultivars has the following advantages: suppresses nematode reproduction, reduces need for toxic chemicals, shortens length of rotations, does not require use of specialized equipment, maintains cost of seed generally equal to that of susceptible cultivars (Boerma and Hussey, 1992), and may limit disease complexes associated with nematodes (Mai and

Abawi, 1987). There are some limitations to the use of resistant cultivars, however. The quality, yield, and economic return of some resistant cultivars may be lower than the use of higher quality susceptible cultivars with nematicide treatments (Johnson, 1990). Infestations with multiple nematode species can pose difficulties for effective use of resistant cultivars, since resistance is usually effective against a single nematode race, species or genus (Young, 1998). Also, planting highly resistant cultivars places selection pressure on the nematode population for biotypes that can reproduce on the resistant cultivar (Hartwig, 1981; Young, 1990). Shifts in pest species also limit the effectiveness of nematode-resistant cultivars (Barker, 1989; Johnson, 1989).

The soybean cyst nematode is recognized today as the major pest limiting soybean production, accounting for approximately 54% (approximately 1.67×10^9 U.S. dollars) of the soybean yield loss annually attributed to disease causing agents in the United States (Wrather et al., 2001a, 2001 b). Soybean [*Glycine max* (L.) Merr.] is one of the four major crops in the world, along with maize, wheat, and rice (FAO, 2002), and is probably the leading source of protein and vegetable oil (Riggs and Niblack, 1993). Over 100 species of nematodes other than SCN parasitize soybeans (Schmitt and Noel, 1984).

The soybean cyst nematode has a narrow host range, primarily in the Leguminosae, soybean being the most economically important host (Riggs and Wrather, 1992). The life cycle of SCN can be described as follows (Ichinohe, 1955). The first-stage juvenile develops within the egg where it molts, emerging as the infective-stage juvenile (J2). The J2 moves through soil, invades a root, and establishes a feeding site disrupting vascular tissue. Root penetration occurs by slitting of plant cell walls from thrusts of the robust stylet (Endo, 1978). In susceptible plants, cells in contact with the

initially stimulated cell coalesce into a multinucleated syncytium via the dissolution of adjacent cell walls. The induction of a syncytium is essential for SCN development and survival (Endo, 1992). After feeding begins, the J2 becomes sedentary, and molts three more times, as it enlarges. The fourth-stage male juvenile ceases feeding about 9 days after infection (Endo, 1992) and reverts to an elongate form. Unlike the sedentary female, the adult male is mobile and leaves the root after mating. The lemon-shaped adult female changes color from white, to yellow, to brown as it matures, ceases feeding about 21 days after infection (Endo, 1992), and becomes a cyst. The female may produce 200-600 eggs (Young, 1992); some are deposited in a gelatinous matrix outside the vulva, but most of them are retained within the body. Duration of the life cycle varies from 3 to 4 weeks (Ichinohe, 1955). The most common dispersal mechanism of SCN is movement of soil (Lehman, 1994). Strong winds, farming equipment, animals and flooding may disperse eggs and cysts in a field or move them to new locations.

The feeding stages J2, J3, J4, and adults destroy plant roots, interfere with nutrient uptake, and serve as vehicles for other pests like fungi and bacteria (Dropkin, 1980; Melakeberhan et al., 1985, 1987, 1988, 1990; Blevins et al., 1995). Producers associate nematode damage with severe stunting and chlorosis of plants. However, the aboveground disease symptoms are non-specific and thus non-diagnostic. Noel (1992) measured 20 % to 30 % yield losses in fields infested with the nematode when there was an absence of severe stunting and chlorosis. The universal symptom of SCN infection of a susceptible soybean cultivar is reduction of yield (Riggs and Niblack, 1993). Detection of the nematode involves examining roots for presence of cysts or white females followed by soil sampling.

The three major steps in managing SCN according to Young (1998) are: periodic sampling for nematode infestations, identification of the race present, and selection of appropriate control measures. Planting resistant cultivars is the most widely used management practice. Triantaphyllou (1975) correctly pointed out field populations are not pure races but have virulence genes in different frequencies. The term "race" or H-G type is used to distinguish intra-specific forms that show physiological variation, such as differences in host preference. SCN races are distinguished by reproduction on a set of soybean genotypes called differentials (Riggs and Schmitt, 1988; Niblack, 2002). The selection pressure of growing a resistant cultivar may change the race classification of a population (Anand et al., 1994). Since resistance genes fail to remain effective for many years, practices that extend the durability of the genes have been proposed. Rotation of resistant and susceptible cultivars, often in combination with non-host crops, and rotation of cultivars with different sources of resistance (Young, 1982; Leudders and Dropkin, 1983) have been suggested to extend the time that resistance genes are effective. Other cultural practices like late date of planting (Hussey and Boerma, 1983; Koenning and Anand, 1991); tillage management (Wrather et al., 1992; Gavassoni et al., 2001); irrigation (Barker and Koenning, 1989); herbicide application (Kraus et al., 1982; Bostian et al., 1984), and flooding (Stover, 1979) are also used to control SCN population and reduce soybean yield losses to SCN. Melakeberhan (1997) discussed the role of plant-nematode-nutrient interactions in nematode management. Increased nutrition generally increases plant growth and nutrient accumulations in plant tissue with or without an effect on nematode population densities (Oteifa, 1952; Oteifa and Elgindi, 1976; Trudgill, 1980 and 1987; Melakeberhan et al., 1987; Melakeberhan, 1997b; Melakeberhan et al.,

1997). If nutrition benefits the host in the presence of nematodes, it is logical to suggest that healthy plant-based nutrient recommendations may not be adequate under nematode infested conditions, and that nutrition can be used as a tool for nematode management (Melakeberhan, 1997).

Despite current management efforts, SCN continues to spread through out soybean producing areas worldwide (Yokoo, 1936; Hung, 1958; Norton, et al., 1983; Nishizawa, 1984; Anderson et al., 1988; Noel, 1992; Doucet, 1999). In the United States, it is currently found in 26 of 28 soybean-producing states (Schmitt and Riggs, 1989), a rapid spread since it was first reported in 1954 in North Carolina (Winstead et al., 1955). In Michigan, SCN first report was in 1987 in Gratiot County (Bird et al., 1988), and in 1994, SCN was found in 12 of 16 counties surveyed (Warner et al., 1994).

The potential for site-specific management (SSM) of plant-parasitic nematodes has been explored for a variety of crop-plant-parasitic nematode systems. In potato production for example, SSM strategies are being explored to reduce use of pesticides and increase economic return to farmers in nematode-infested fields (Evans et al., 2002; Morgan et al., 2002). The works of Workneh et al. (1999), and Donald et al. (2001) in soybean systems, and of Wyse-Pester et al. (2002) in irrigated cornfields are further examples of exploratory analysis towards the development of SSM for plant-parasitic nematodes.

The goal of SSM is to manage each parcel of agricultural land for maximum crop production, while protecting or improving natural resources. In practice, SSM involves applying the right management, at the right time, at the right place, in the right way (Pierce et al., 1994). In order to apply SSM strategies for the control of nematode

populations, it is necessary to know its spatial distribution, and how it changes through time.

While it is difficult to compare over a range of experimental conditions, variability in the spatial distribution of the cyst nematodes has been clearly demonstrated. A study on local variance of SCN using geostatistics showed that the scale of heterogeneity in the distribution of eggs between-rows was similar to that within-row (Francl, 1986a, 1986b). Levels of *H. avenae* and *Globodera rostochiensis* in soil are strongly autocorrelated at a normal working scale (Webster and Boag, 1992). In a 4-year field study the spatial dependence in SCN egg population varied both within season and from season to season (Donald et al., 1999). A better understanding of the spatial and temporal dynamics of the incidence of SCN would enable its more effective management by farmers. While previous studies clearly show the spatial dependence of SCN population density, more details are needed on the nature of the spatial dependence and how it varies in adjacent fields and over time.

The goal of this dissertation was to understand SCN spatial distribution in soybean fields as the first step towards the long-term goal of developing SSM strategies for SCN.

For SCN to be managed site-specifically, its spatial distribution has to be sufficiently structured and time invariant, and it has to be related to yield limiting factors that are easier to monitor and manage. The literature reviewed suggests that SCN may meet the requirements for SSM. SCN can cause severe yield loss, and there is evidence that it has an aggregated spatial distribution. In addition, correlations between SCN population density and soil attributes (soil physical and chemical properties) have been

found from experimental studies. The spatial relations between SCN population dynamics and soil properties within a given field, and the combined effect of the two on soybean yield are key elements to accurate assess the potential for SSM of SCN, however, a within field spatial analysis of all the variables combined has not been carried out yet.

The working hypotheses of this Dissertation were that SCN's spatial distribution within a field is sufficiently structured and time invariant; that SCN spatial distribution and population densities are related to soil properties; and that the relations among SCN population density, soil properties and soybean yield are sufficient in magnitude to aid in the management decision-making process.

In Chapter Three, the objective was to assess the magnitude, structure, and persistence in time of the spatial distribution patterns of SCN cysts, eggs, and eggs per cyst under field conditions using geostatistical tools. In Chapter Four, the relationship between soil texture and SCN population density variability were characterized addressing the following objectives: i- to assess the spatial structure of texture within fields of known SCN population density in Michigan and its relationship to published soil survey maps; ii- to determine the extent to which the spatial variability in SCN cyst population density relate to soil texture; iii- to quantify the relationship between soil separates (sand, silt, and clay) to SCN population density; and iv- to assess the extent to which this relationship holds between fields with similar soil types but different SCN populations. Population dynamics of SCN were studied in Chapter Five. Specific objectives were: i- to characterize SCN population dynamics in soybean roots and the surrounding soil in two fields in Michigan over two growing seasons; ii- to investigate

the extent of the correlation of soil texture with SCN population in the roots and in the soil, and with eggs per cyst; and iii- to analyze SCN population dynamics spatially in relation to soil texture. The purpose of Chapter Six was to answer the question of whether soil fertility affects SCN distribution and root infection, and is this reflected in tissue analysis? The following objectives were addressed: i- to characterize soil fertility variability and its relationship with soil texture and tissue analysis in two fields of known SCN infection in Michigan; and ii- to analyze the relation of SCN in the soil and in soybean roots with soil fertility and tissue analysis. Finally, in Chapter Seven soybean yield was analyzed as an integrator of the effect of the variables analyzed in previous chapters. The extent to which yield was related to soil texture, soil fertility, and SCN population densities was investigated by correlating spatial information on these variables.

CHAPTER TWO

GENERAL MATERIALS AND METHODS

Selection of Experimental Sites

In fall of 1998, an exploratory soil sampling for SCN was performed in nine commercial farms in Shiawassee and Saginaw counties, MI, to locate two fields with a range of SCN infection levels from high to undetectable. In each field, soil samples were collected every 30 to 40 m following a Z pattern. The location of each sample was marked with a flag and the geographic position was determined with a Global Positioning System (GPS). A soil sample consisted of ten soil cores collected with a cone auger at a depth of 20 cm within a 30 cm radius of each flag. The number of samples collected per field varied with the dimensions of the fields. Cysts were extracted from the soil by passing a suspension of 100 cm^3 of soil in water through an 850-μm pore sieve (mesh #20) nested on a 75-μ pore sieve (mesh #200). Cysts retained in the #200 sieve were further separated from soil particles following the sugar flotation-centrifugation method (Dunn, 1969), and counted under a stereoscopic microscope. Georeferenced cyst counts were plotted within maps of the corresponding field sampled to identify general patterns in SCN spatial distribution. Fields with very low or undetected SCN population densities (less than 10 cysts 100 cm^{-3} of soil), as well as fields where all samples collected had high SCN levels (more than 30 cysts 100 cm^{-3} of soil), were not considered further.

Two fields (Field A and Field B) located 3.2 km apart in Shiawassee County, MI, were chosen based on the range in SCN population levels, management history, and accessibility, and the research was conducted from spring 1999 until fall 2000. Field

information such as management, soil series, soybean cultivars planted, and planting and harvesting dates are provided in Materials and Methods section in Chapter Three.

Soil and soybean root sampling for SCN and soil characterization

Soil samples were collected following a geostatistical sampling design applied in each field to study the spatial distributions of SCN population and soil attributes. The sampling design and SCN extraction procedures are explained in Chapter Three. The efficiency of the extraction procedure was tested for different soil types and SCN cyst densities, and alongside other extraction methods (Appendix A). Soil texture analysis is presented in Chapter Four, and soil fertility details are presented in Chapter Six.

To study spatial dynamics in SCN spatial distribution, soybean root samples were collected at approximately monthly intervals in 1999 and 2000. The procedures followed, sampling dates, and nematode staining techniques are described in Chapter Five.

Soybean tissue analysis and seed yield

Soybean leaf samples were collected from Field A and B to evaluate the nutritional status of the plants. Related procedures are described in Chapter Six. The cooperating farmer using a yield monitoring system mounted on the combine and connected to a GPS receiver recorded soybean yield. More information is provided in Chapter Seven.

Statistical Analysis

Classical statistical methods applied are described in each chapter. The spatial analysis of SCN, soil attributes, grain yield, and spatial relations among these variables were analyzed with geostatistical tools. Geostatistics have been used in nematology to

study the spatial distribution of a variety of plant-parasitic nematodes (Wallace and Hawkins, 1994; Webster and Boag, 1992; Donald et al., 1999).

Software Used

Classical statistics analyses were performed with SAS® System Release 8 (SAS Institute, Cary, NC). Geostatistical analyses such as variography and mapping were performed with the Surfer 7.02 software package (Golden Software, 1999), and cross-correlograms with the Variowin 2.21 software package (Panatier, 1998). A review of the software selected for geostatistical analyses is presented in Appendix B.

CHAPTER THREE

GEOSTATISTICAL ANALYSIS OF FIELD SPATIAL DISTRIBUTION PATTERNS OF SOYBEAN CYST NEMATODE.

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GEOSTATISTICAL ANALYSIS OF FIELD SPATIAL DISTRIBUTION PATTERNS OF SOYBEAN CYST NEMATODE.

ABSTRACT

Site-specific management of SCN is plausible if its spatial and temporal dynamics are adequately known and structured. The hypothesis that variation in the spatial distribution of SCN is sufficient in magnitude and structure and sufficiently time-invariant to support the use of site-specific management in SCN infested fields was tested. A nested survey sampling design with distances reduced by geometric progression was applied on two fields in Michigan. Cysts were extracted from single-core soil samples collected before planting in 1999 and 2000, the number of eggs per cyst was estimated and the number of eggs per sample was obtained by multiplying eggs per cyst by the number of cysts. The distribution of the three variables was characterized using geostatistical tools including semivariograms, kriging, and cross-correlograms on log-transformed values of the original data. Mean cyst population density ranged from 6 to 33 cysts 100 cm^{-3} of soil in the two fields. Although the spatial structure of SCN population was insufficient for SSM and varied between fields, and SCN population density varied between years, the location of areas of high or low cyst density could be identified repeatedly. The reasons why nematodes exhibited an aggregated distribution are not well understood. The evaluation of factors associated with the determination of SCN spatial distribution is part of an ongoing project towards the development of SCN site-specific management.

Key words: *Heterodera glycines*, nested design, kriging, semivariogram, site-specific management.

The soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is a major pest of soybean [*Glycine max* (L.) Merr.] worldwide, accounting for approximately 54% (Approximately 1.67 x 10^9 U.S. dollars) of the soybean yield loss annually attributed to disease-causing agents in the United States (Wrather et al., 2001a, 2001 b). Conducive cropping systems, highly adaptive behavior, and limited sources of resistance (Young, 1992; Young and Hartwig, 1992; Diers et al., 1999; Wang et al., 2000) are among the reasons SCN continues to be an economic threat. Eradication has been unsuccessful and the repeated planting of resistant cultivars in the field results in the selection of a nematode population that can overcome plant resistance, reducing the longevity of the cultivar (Young, 1998). Thus, a realistic strategy for managing SCN appears to be through cultural-based nematode population suppression practices (Bridge, 1996). An important consideration in managing SCN is that its aggregated distribution varies in space and time (Francl, 1986a, 1986b; Donald et al., 1999). The spatial-temporal variability in SCN is often overlooked in commonly used nematode sampling strategies that may miss field population clusters and in existing management thresholds based on whole-field sampling procedures. Thus, current SCN management practices involve treating whole fields rather than nematode-infected areas only, and with uniform rather than condition-specific inputs. Advances in precision agriculture suggest that site-specific management of SCN is plausible, but only if the spatial and temporal dynamics of SCN are adequately known and structured (Pierce and Nowak, 1999).

It is known that plant-parasitic nematodes have aggregated spatial distribution with frequency distributions generally described by the negative binomial function (Anscombe, 1950; Seinhorst, 1982). Taylor's Power Law (Taylor, 1984) has been used to

describe the distribution and to devise sampling strategies for nematodes (Ferris et al., 1990; McSorley and Dickson, 1991). Unfortunately, while recommendations call for systematic soil sampling to obtain samples that represent the entire area sampled (Ferris et al., 1990; McSorley and Dickson, 1991), samples are composited to form a single sample representative of the site average. Such spatially non-explicit sampling is not conducive to examination of the possibility of site-specific management practices. The high cost of obtaining and analyzing nematode samples makes it imperative that methods of assessing spatial variability of SCN are highly effective and efficient. Efficiencies can be achieved through robust sampling designs or by relating SCN populations to more easily measured properties such as soil pH or soil texture, that are known to have a somewhat structured spatial variation (Pierce and Nowak, 1999).

Geostatistics provides tools for describing spatial variation of soil properties and for local interpolation (kriging) to predict and map values at unsampled locations. Geostatistical methods can be applied to describe spatial autocorrelation of nematode distribution, soil properties, and host response to nematode infestation (Boag, 1998). While it is difficult to compare over a range of experimental conditions, variability in the spatial distribution of the cyst nematodes has been clearly demonstrated. A study on local variance of SCN using geostatistics showed that the scale of heterogeneity in the distribution of eggs between-rows was similar to that within-row (Francl, 1986a, 1986b). Webster and Boag (1992) showed that the levels of *Heterodera avenae* and *Globodera rostochiensis* in soil are strongly autocorrelated at a normal working scale. Donald et al. (1999) found in a 4-year field study that the spatial dependence in SCN egg population varied both within season and from season to season. In the field studied, the direction of

spatial variation was related to the direction of tillage but not to other factors such as soil type or weed distribution (Donald et al., 1999). A good understanding of the spatial and temporal dynamics of the incidence of SCN would enable its more effective management by farmers. While previous studies clearly show the spatial dependence of SCN population density, more details are needed on the nature of spatial dependence and how it varies in adjacent fields and over time.

The objective of this work was to assess the magnitude, structure, and persistence in time of the spatial distribution patterns of SCN cysts, eggs, and eggs per cyst under field conditions using geostatistical tools. We postulate that the spatial distribution of SCN is sufficiently structured and time-invariant to support the use of site-specific management in SCN infested fields.

MATERIALS AND METHODS

Experimental Sites and Site Management

The study was conducted in Shiawassee County, MI in 1999 and 2000 on two fields (Field A and Field B) located 3.2 km apart and maintained by the cooperating farmer. Field A was 24 ha, managed under no-tillage since 1996, and planted to soybean in 1996 and 1997, and to corn in 1995 and 1998. Field B was 13 ha, conventionally tilled in 1995 and in 1998, and managed under no-tillage in between and thereafter. Field B was planted to corn in 1995, soybean in 1996 and in 1997, and wheat in 1998. In 1999, a SCN -susceptible soybean variety (Asgrow 1901), Roundup-Ready, was grown in both fields. Soybean was planted in 19.1-cm rows at a rate of 519 000 viable seeds ha^{-1}. Row orientation was North-South in Field A and East-West in Field B. Fields A and B were

planted within the same week in mid-May in 1999 and in early June in 2000; planting delayed by wet soil conditions. Weed control was maintained using Roundup at the recommended rate with one preplant application in Field A in 1999 and 2000, and one application postemergence in 1999. In Field B, there was one preplant application in 2000, and one postemergence application in 1999.

Soil series in Field A were Belding sandy loam (Coarse-loamy, mixed, frigid Argic Endoaquods), Breckenridge sandy loam (Coarse-loamy, mixed, nonacid, frigid Mollic Endoaquepts), Brookston loam (Fine-loamy, mixed, superactive, mesic Typic Argiaquolls), Conover loam (Fine-loamy, mixed, active, mesic Udollic Endoaqualfs), and Newaygo sandy loam (Fine-loamy over sandy or sandy-skeletal, mixed, frigid Alfic Haplorthods) (Figure 3.1.a). Soil series in Field B were Brookston loam, Newaygo sandy loam, and Berville loam (Fine-loamy, mixed, mesic Typic Argiaquolls) (Soil Survey Division-NRCS-USDA, 2001). Soil series maps were digitized from Threlkeld and Feenstra (1974) (Figure 3.1.b).

Soil Sampling Design

1999:

A geostatistical sampling design was established in an 8 ha area and a 5.25 ha area in the center of Fields A and B, respectively (Figure 3.1.a and b). A grid of 50 x 50 m cells was marked on the area sampled. A nested survey sampling design with geometric progression reduced distances (adapted from Webster and Boag, 1992) was applied to both fields within alternate cells of the grid. In each selected cell, a first pair of single-

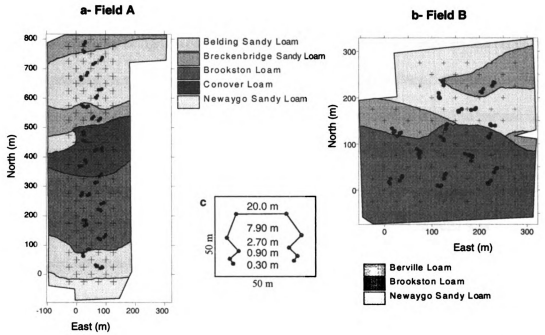

Figure 3.1. Soil series maps and location of the soil samples collected from a- Field A and b- Field B in Shiawasee Co., MI in 1999 and 2000. c- Nested sampling design with geometric progression reduced distances. Distances between pairs of samples are indicated; the direction in which two consecutive samples were oriented was randomly selected. This design was applied within 50 x 50 m cells of a grid centered in each field. Black circles in figures a and b indicate sets of sample locations in selected cells for both 1999 and 2000. The crosses in figures a and b indicate the locations of the additional samples collected in 2000 from alternate nodes of a 25 x 25 m grid. Soil series maps were digitized from Threlkeld and Feenstra (1974).

core samples was collected 20 m apart so that the segment connecting them passed through the center of the cell. A new point 7.9 m away from each sampled location was chosen in a random direction and also sampled. The procedure was repeated at 2.7, 0.9, and 0.3 m (Figure 3.1.c). The angles for each new location were randomly selected. Each angle was expressed in north and east coordinates to facilitate the location of the sampling sites in the field. This arrangement produced ten sampling locations per cell that were flagged and geo-referenced using GPS (Figure 3.1.a-b, black circles). The advantage of the nested survey design is to provide pairs of observations at various short-

and long-range separation distances. This design guards against the potential pitfalls of regular grid sampling designs when the shortest separation distance exceeds the range of the spatial process, in which case description of the spatial dependency structure of the spatial process would not be possible. Sampling locations were staked before harvest in 1999 to mark them for sampling in 2000.

2000:

In addition to the 1999 sampling design, a grid with 25 x 25 m cells was superimposed on each field in 2000. The additional grid was added to expand the level of sampling detail because the nested design applied in 1999 provided very few pairs of samples for separation distances between 20 and 60 m. Soil samples were obtained from alternate nodes of the new grid, providing 119 and 77 additional samples from Fields A and B, respectively (Figure 3.1.a, b, crosses).

Soil Sampling for SCN

At each flag location, single-core soil samples were obtained within a week before planting using an 8-cm diameter by 23-cm depth bucket auger (Riverside Augers, Eijkelkamp, Giesbeek, The Netherlands). A total of 160 and 279 samples was collected from Field A and 110 and 187 samples were collected from Field B in 1999 and 2000, respectively. Soil cores were placed in individual plastic bags and, upon arrival at the lab, were stored in 10-gal Rubbermaid containers at 4°C until they were processed (within 30 days).

Cyst Extraction

Cysts were extracted by adding 100 ml of soil in a beaker containing 400 ml of tap water. A semi-automatic elutriator (Research Services Instrument Shop, The

University of Georgia, Athens, GA) was used for the extraction following standard procedures (Byrd, et al. 1976) with 60% extraction efficiency (Appendix A). The last step in the elutriation process consists of the collection of nematodes and small soil particles suspended in water in a bowl. The bowl drains through 15 Tygon tubes into a 75-μ aperture sieve (#200 mesh) where cysts are retained. The volume of water and the amount of soil particles flowing from 15 tubes overflowed the sieve, so cysts were collected from seven tubes instead. Cysts collected in the sieve were further separated from soil particles following the sugar flotation-centrifugation method (Dunn, 1969). Cysts were then counted under a stereomicroscope and counts were adjusted to estimate the total number of cysts per sample if all 15 tubes had been used. Three cysts were randomly selected from each sample and crushed. All eggs and second-stage juveniles contained in a cyst were counted. The average was used to determine the eggs per cyst for each sample containing at least one cyst. Egg numbers were estimated by multiplying the average number of eggs per cyst by the total number of cysts in each sample.

Statistical Analysis

SCN population in Fields A and B were analyzed separately, and no statistical comparison was made between fields. For reasons beyond experimental control, a few samples were not collected from Fields A and B in 1999 or 2000. In order to compare populations between years, incomplete pairs (1999 or 2000 sample missing for a specific location) and additional data collected in 2000 were omitted only for the descriptive statistical analysis. Descriptive statistics for cysts, eggs per cyst, and eggs were calculated with the SAS® System Release 8 (SAS Institute, Cary, NC). Histograms and cumulative distribution functions (cdf) were plotted to compare the frequency distribution of cysts,

eggs per cyst, and egg counts between years in each field. Logarithmic transformation of the data was performed whenever frequency distributions were highly skewed. Cumulative distribution functions for cysts [$\log_{10}$ (cysts+1)], eggs [$\log_{10}$ (eggs+1)], and eggs per cyst [$\log_{10}$ (eggs per cyst +1)] were calculated and tested for log-normality with the Kolmogorov-Smirnoff test, used to compare probability distributions to a specific function. Logarithmic transformed means and sample variances were compared between years within fields with a paired t-test and an F-test, respectively (α=0.05).

We used Taylor's Power Law to provide a measure of aggregation in SCN population density (Taylor, 1961; 1984; Ferris et al., 1990). Means and variances were computed from pairs of samples of cysts, eggs, and eggs per cyst, the samples being combined in different ways to give a range of spatial separations. The slope of the regression of log variance on log mean (parameter b') was estimated by simple linear regression. Parameter b' is an index of aggregation, varying continuously from zero for a regular distribution, through one for a random distribution, to ∞ for a highly contagious distribution.

Geostatistical Analysis

The semivariogram is a structural tool for depicting the spatial dependency in a realization of a mean-constant spatial process Z(**s**). Attributes of interest for which semivariogram analysis was performed were the numbers of cysts, eggs per cyst, and egg population densities. Various estimators of the semivariogram are used in practice; Schabenberger and Pierce (2002) summarize several of these. Here, the classical Matheron estimator

$$\hat{\gamma}(h) = \frac{1}{2|N(h)|} \sum_{\|\mathbf{s}_i - \mathbf{s}_j\| = h} \{Z(\mathbf{s}_i) - Z(\mathbf{s}_j)\}^2$$

was used (Matheron 1963). The semivariance $\hat{\gamma}(h)$ at a given lag distance h is estimated as one half the average squared difference between all observations at locations $\mathbf{s}_i$, $\mathbf{s}_j$ that are separated by the lag h. The semivariogram for a given direction is displayed as a plot of $\hat{\gamma}(h)$ versus distance. Depending on the data and sampling interval used, the shape of the experimental semivariogram may take many forms. In general, the semivariance increases with increasing distance between sample locations, rising to a more or less constant value (the sill) at a given separation distance called the range of spatial dependence. Samples separated by distances closer than the range are spatially related. Those separated by distances greater than the range are no longer spatially autocorrelated. Semivariances may also increase continuously without showing a defined range and sill, thus preventing definition of a spatial variance, indicating that the range is greater than the largest lag (h), or the presence of a trend effect and/or nonstationarity (Webster and Burguess, 1980). Stationarity means that the random field sampled looks similar everywhere. A random field is second-order stationary if the mean of the random field is constant and does not depend on locations, and the covariance between two observations is only a function of their spatial separation (Schabenberger and Pierce, 2002). Whenever semivariograms showed nonstationarity, the data were detrended by carrying out a polynomial least squares regression and semivariogram analysis was performed on the residuals. Other semivariograms show complete absence of spatial structure, implying that the value observed at one location carries no information about values at other locations. The nugget effect (C_0) is a discontinuity of the semivariance near the origin. It consists of measurement error variability and/or the sill of a micro-scale spatial process. The error variance is a measure of repeatability of the data measurements whereas micro-

scale variance is a measure of variation that occurs at separation distances less than the smallest sample spacing (Cressie, 1993).

To reduce the influence of extreme values and to achieve greater symmetry, log-transformed data were used for the geostatistical analysis. Experimental omnidirectional semivariograms of cysts [$\log_{10}$ (cysts + 1)], eggs per cyst [$\log_{10}$ (eggs per cyst + 1)], and eggs [$\log_{10}$ (eggs + 1)] were calculated for each field and year for lags ranging from 1 to 30 m, with a lag tolerance of one half of the lag used ($h/2$). The minimum number of pairs required for each lag was 30. The reduced number of samples in the E-W direction in Field A, and the predominant SW-NE arrangement of the samples in Field B prevented the calculation of reliable directional semivariograms. Variography was carried out with the Surfer 7.02 software package (Golden Software, 1999).

Kriging is the best linear unbiased prediction of regionalized variables at unsampled locations using the structural properties of the semivariogram and the sampled values at observed locations. Soil properties often exhibit lognormal probability distributions, in which case log-Gaussian kriging is employed. It involves computation of semivariograms and kriging on log-transformed values of the original data using the same procedures as for simple linear kriging (Cressie, 1993). When a drift or trend (non-stationarity of the mean) existed within the area of interest, universal kriging was used; otherwise, ordinary kriging was the method of choice. Universal kriging takes the drift into account provided the form of the drift and the semivariogram are known (Journel and Huijbregts 1978). The distributions of cysts, eggs, and eggs per cyst were mapped separately for each field and year with ordinary or universal log-kriging predicting values

at the nodes of a 1x1 m cell grid using all the data points in each sample and the parameters from the models fitted to the empirical semivariograms.

The cross-correlogram is used to describe the spatial continuity between measurements of different attributes or of the same attribute measured at different times. The cross-correlation function given by Goovaerts (1997) was used here to calculate cross-correlograms for logarithmic transformed cysts, eggs per cyst, and eggs between years; and for logarithmic transformed eggs per cyst and eggs in 1999 with cysts in 2000. Only the data points from locations sampled in both 1999 and 2000 were used for this analysis.

RESULTS

Population Densities of SCN

Cyst and egg population densities of SCN, as well as eggs per cyst, varied significantly between fields and years. Cyst population density for entire fields ranged from as low as 6 cysts 100 cm^{-3} of soil in Field A in 1999 to as high as 33 cysts 100 cm^{-3} of soil in Field B in 2000. The lowest and the highest egg population densities were also found in Field A in 1999 (87 eggs 100 cm^{-3} of soil) and in Field B in 2000 (4939 eggs per 100 cm^{-3} of soil), respectively (Table 3.1).

Field A

While mean cyst density in Field A remained similar and low in 1999 and in 2000, with positively skewed frequency distribution (Table 3.1, Figure 3.2.a), the sample variance of cyst density increased significantly in 2000 (Table 3.1). Taylor's Power Law index of aggregation b' indicated a moderately aggregated distribution of cysts in 1999 and 2000. Mean egg density and sample variance were significantly higher in 2000 than

in 1999 as a result of the increased production of eggs per cyst in 2000 (Table 3.1, Figure 3.2.b, c). The degree of aggregation in the population of eggs was also greater in 2000 than in 1999, whereas the opposite was true for the number of eggs per cyst (Table 3.1). The proportion of cysts without eggs decreased from 36% in 1999 to 23% in 2000, indicating that more cysts had eggs at planting and, therefore, that there was a greater infection potential in 2000 (Figure 3.2.b). The presence of empty cysts was proof that SCN was present in the field.

Field B

SCN population density in Field B was moderate in 1999 and high in 2000. The mean number of cysts found in 2000 was 2.3 times greater than in 1999, with greater variability as well (Table 3.1). The distribution of relative frequencies was positively skewed in both years (Figure 3.2.d). In 1999, cysts were not detected in 19.3% of the samples whereas in 2000 this proportion decreased to 13.6% (Figure 3.2. d). The same moderate degree of aggregation in cyst population was observed in 1999 and 2000 (Table 3.1). The number of eggs found in 2000 was significantly greater and more variable than in 1999 (Table 3.1, Figure 3.2.f). In contrast to Field A, the production of eggs per cyst was reduced by 25% from 1999 to 2000 (Table 3.1), and 13.7% of the cysts were without eggs in 2000 compared to only 2.3% in 1999 (Figure 3.2.e). Therefore, the increase in egg density in 2000 was due to the greater number of cysts and not to increased egg production. Taylor's index of aggregation indicated aggregation in egg population, and insufficient evidence of aggregation in the number of eggs per cyst in 1999 and 2000 (Table 3.1).

Table 3.1. SCN (soybean cyst nematode) cyst and egg population densities, and eggs per cyst from two fields in Shiawassee Co., MI before planting in 1999 and 2000. Summary statistics.

	Cysts		Eggs †		Eggs per cyst ‡	
	1999	2000	1999	2000	1999	2000
Field A	Counts 100 cm^{-3} of soil					
Arithmetic mean §	6.4 NS	8.4 NS	86.6 *	851.5 *	13.0 *	48.9 *
Standard deviation ¶	6.56 *	10.8 *	129.2 *	1571.4 *	20.6 *	48.2 *
CV, %	102	129	149	184	158	98
Min-Max	0-41	0-69	0-656	0-8369	0-270	0-168
Sample size #	157	157	115	115	115	115
b' value (se) ††	1.4 (0.03)	1.5 (0.03)	1.6 (0.02)	2.0 (0.03)	1.8 (0.02)	1.4 (0.05)
Field B						
Arithmetic mean §	14.5 *	32.9 *	2442.6 *	4938.7 *	116.5 *	87.9 *
Standard deviation ¶	22.1 *	58.2 *	3531.5 *	8975 *	58.8 NS	69.7 NS
CV, %	152	176	144	182	50	79
Min-Max	0-121	0-484	0-17 368	0-68 284	0-298	0-285
Sample size #	109	109	79	79	79	79
b' value (se) ††	1.7 (0.03)	1.7 (0.03)	2.1 (0.04)	1.8 (0.02)	1.1 (0.13)	0.9 (0.06)

†Egg density in each sample was estimated by multiplying the average number of eggs per cyst by the total number of cysts found in the sample.

‡ The number of eggs per cyst is the average number of eggs counted after crushing three randomly selected cysts in each sample.

§ Arithmetic means of logarithmic transformed data were compared between years using the Paired t-Test. Arithmetic means of original data are shown for clarity.

¶ Sample variances of logarithmic transformed data were compared between years using the F-test. Standard deviations of original data are shown for clarity.

Sample size for eggs and eggs per cyst analyses include only samples with at least one cyst.

†† Taylor's Power Law index of aggregation b' and standard error (se).

* Significance at the 0.05 probability level.

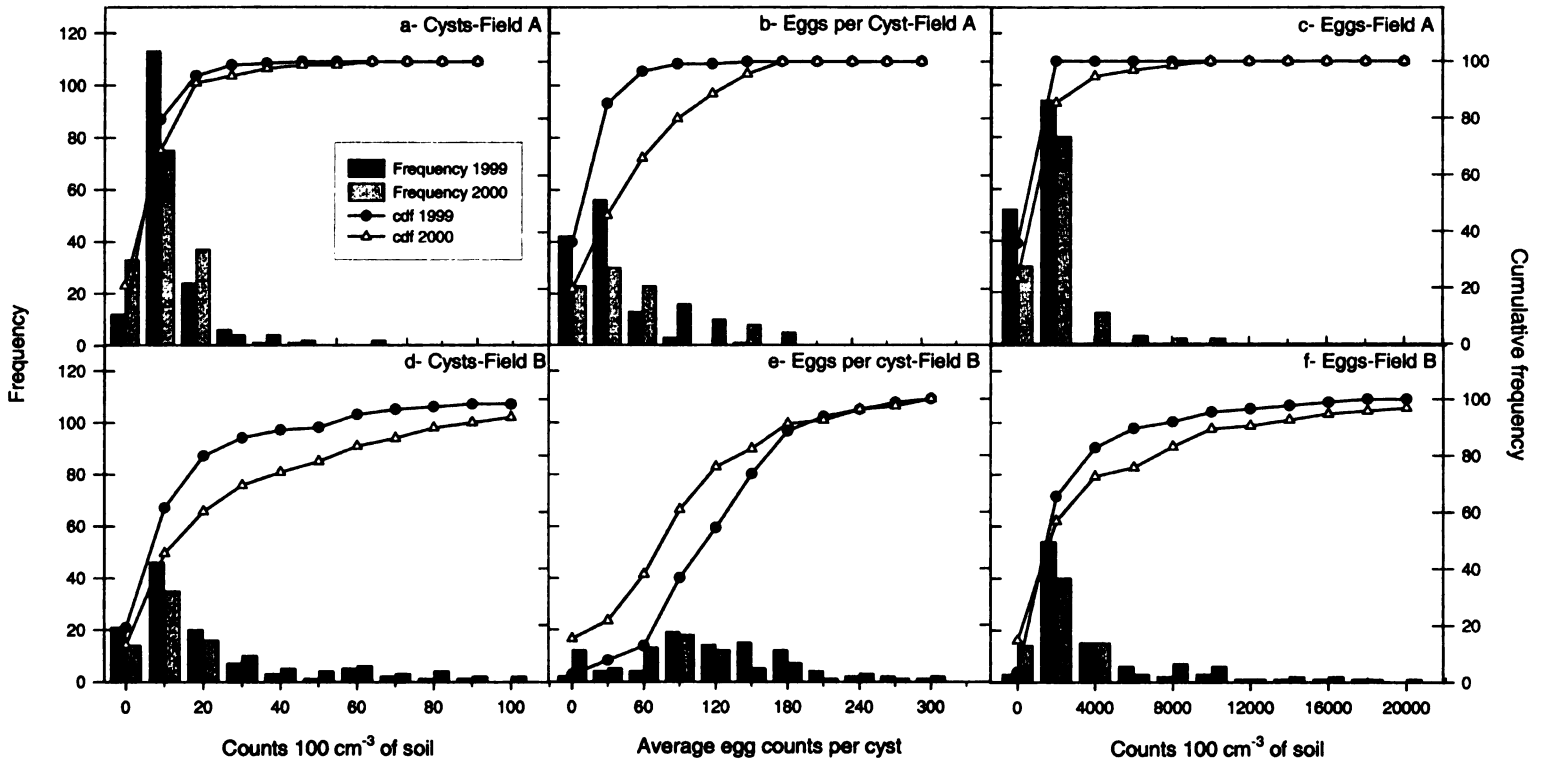

Figure 3.2. Frequency distribution and cumulative distribution function (cdf) of a- cysts 100 cm^{-3} of soil, b- eggs per cyst, and c- eggs 100 cm^{-3} of soil from Field A; and d- cysts 100 cm^{-3} of soil, e- eggs per cyst, and f- eggs 100 cm^{-3} of soil from Field B. Samples were collected before planting in 1999 and 2000. The number of eggs per cyst was estimated as the average number of eggs found after crushing three randomly selected cysts in each sample.

Eggs per cyst relative frequency distributions appeared normally distributed in both years with medians (109 eggs cyst^{-1} in 1999 and 75 eggs cyst^{-1} in 2000) close to the means (113 eggs cyst^{-1} in 1999 and 86 eggs cyst^{-1} in 2000) (Figure 3.2.e).

Geostatistical Analysis

Although the spatial structure of SCN population density varied between fields and years, the spatial autocorrelation in cyst and egg population densities, as well as eggs per cyst, was structured to a lesser degree in 2000 than in 1999. Empirical semivariograms were calculated and graphed for lags ranging from 3 to 30 m, but only those calculated for a lag of 10 m were selected for modeling because they represented more clearly the structure of the spatial variability in these fields. The models fitted, and their corresponding parameter estimates are given in Table 3.2.

Field A

The data exhibited non-stationarity in mean for logarithmic transformed cyst data in 1999 (data not shown). Therefore, the semivariogram was calculated with the residuals after a polynomial trend was removed. The experimental semivariograms of cyst density in 2000, and egg density and eggs per cyst in both years showed stationarity in the distribution (Figure 3.3. b-f). Empirical semivariograms clearly showed periodicity in the spatial structure of the distribution of cysts, eggs, and eggs per cyst (Figure 3.3. a-f). However, because of the large nugget effect in some cases, a wave or hole-effect model could not be fitted (Figure 3.3.b, c, f). The empirical semivariogram of cysts in 1999 revealed two scales of spatial structure. One described by a wave effect model with a short range indicating small clusters of cysts, and the second described by a spherical model with a much greater range describing the spatial autocorrelation of those

Table 3.2.

† Models were fitted by least squares based on empirical semivariograms calculated for lags (h) ranging from 1 to 25 m, with a lag tolerance of h/2. The minimum number of pairs required for each lag was 30.

‡ Whenever semivariograms showed nonstationarity, the data were detrended carrying out a simple polynomial least squares regression and semivariogram analysis was performed on the residuals. The polynomial order of the trend is indicated when a drift or trend was removed.

§ C_0 is the nugget effect or a discontinuity in semivariance at the origin due to microscale variability or sampling error.

¶ C is the partial sill defined for spherical models.

Observations that are spatially separated by more than the range are uncorrelated.

†† $C_0/(C+C_0)$ is an indicator of the degree of spatial structure, the lower the number the stronger the spatial autocorrelation.

‡‡ Semivariograms for cysts were calculated for $\log_{10}$ (cysts 100 cm^{-3} of soil + 1). Cysts were extracted from a 100 cm^3 soil subsample with a semiautomatic elutriator and counted.

§§ Semivariograms of eggs per cyst were calculated for $\log_{10}$ (eggs per cyst + 1). The number of eggs per cyst was estimated as the average number of eggs counted after crushing three randomly selected cysts in each sample.

¶¶ Semivariograms for eggs were calculated for $\log_{10}$ (eggs 100 cm^{-3} of soil + 1). Egg density was determined for each sample by multiplying the average number of eggs per cyst by the number of cysts in the sample.

Table 3.2. Parameters of the theoretical semivariogram models of SCN (soybean cyst nematode) population density in two fields in Shiawassee Co., MI before planting in 1999 and 2000. †

	Drift ‡	Model function	C_0 §	C ¶	Range #	$\frac{C_0}{C+C_0}$ ††
Field A 1999					m	
Cysts ‡‡	Linear	Nugget	0.08			0.50
		Wave		0.04	3.6	
		Spherical		0.04	819.3	
Eggs per cyst §§	No	Nugget	0.34			0.77
		Spherical		0.1	168.3	
Eggs ¶¶	No	Nugget	0.8			0.77
		Wave		0.24	4.5	
Field A 2000						
Cyst	No	Nugget	0.18			0.69
		Spherical		0.08	122	
Eggs per cyst	No	Nugget	0.33			0.44
		Wave		0.41	7.13	
Eggs	No	Nugget	1.32			
Field B 1999						
Cysts	Linear	Nugget	0.07			0.35
		Wave		0.13	12.16	
Eggs per cyst	No	Nugget	0.08			0.44
		Wave		0.1	11.93	
Eggs	No	Nugget	0.25			0.43
		Wave		0.33	10.22	
Field B 2000						
Cyst	Linear	Nugget	0.09			0.37
		Wave		0.08	2.62	
		Spherical		0.07	268	
Eggs per cyst	No	Nugget	0.44			
Eggs	No	Nugget	1.1			0.86
		Wave		0.18	4.24	

smaller clusters (Figure 3.3.a). A similar pattern was observed in 2000, although the periodicity could not be modeled in this case (Figure 3.3.b). The semivariance of eggs per cyst increased with increasing lag distance up to a range of almost 170 m in 1999 (Figure 3.3. c). In 2000, a wave effect model with a range of 7 m described the periodicity in the distribution of eggs per cyst (Figure 3.3.d). The semivariance for eggs fluctuated about the sample variance with increasing separation distance between data pairs in 1999 and 2000, indicating a clustered distribution of eggs within a very short range, even though a wave effect model could not be fitted in 2000 because of the large nugget (first lag represented by 131 pairs) (Figure 3.3.e-f).

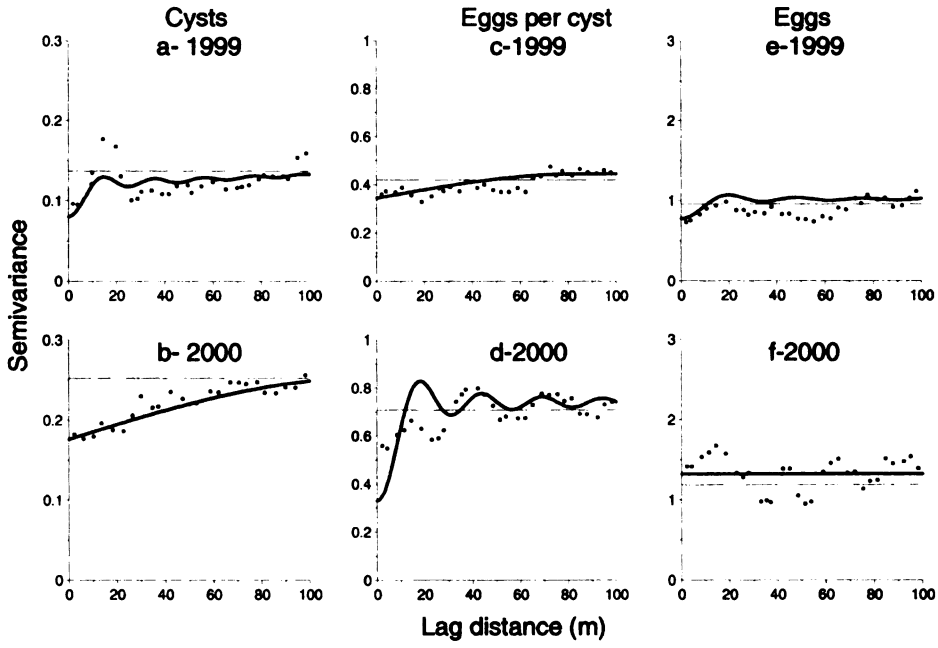

Figure 3.3. Semivariograms of a, b- cysts [$\log_{10}$(cysts 100 cm^{-3} of soil +1)], c, d- eggs per cyst [$\log_{10}$ (eggs per cyst +1)] and e, f- eggs [$\log_{10}$ (eggs 100 cm^{-3} of soil +1)] from Field A in a, c, e- 1999 and b, d, f- 2000. Black circles indicate omnidirectional empirical semivariogram, the solid line indicates the theoretical model fitted by means of least squares and the dashed line is the sample variance.

Field B

The data exhibited non-stationarity in mean for logarithmic transformed cyst data in both years (data not shown). Therefore, semivariograms were calculated from residuals after removal of a polynomial trend. In 1999, the distribution of cysts showed strong spatial autocorrelation described by a wave effect model with a range of 12 m (Figure 3.4.a). In 2000, the structure of the empirical semivariogram was similar to the one observed for cysts in Field A in 1999. A wave effect model and a spherical model described the short and the long-range structures, respectively (Figure 3.4. b).

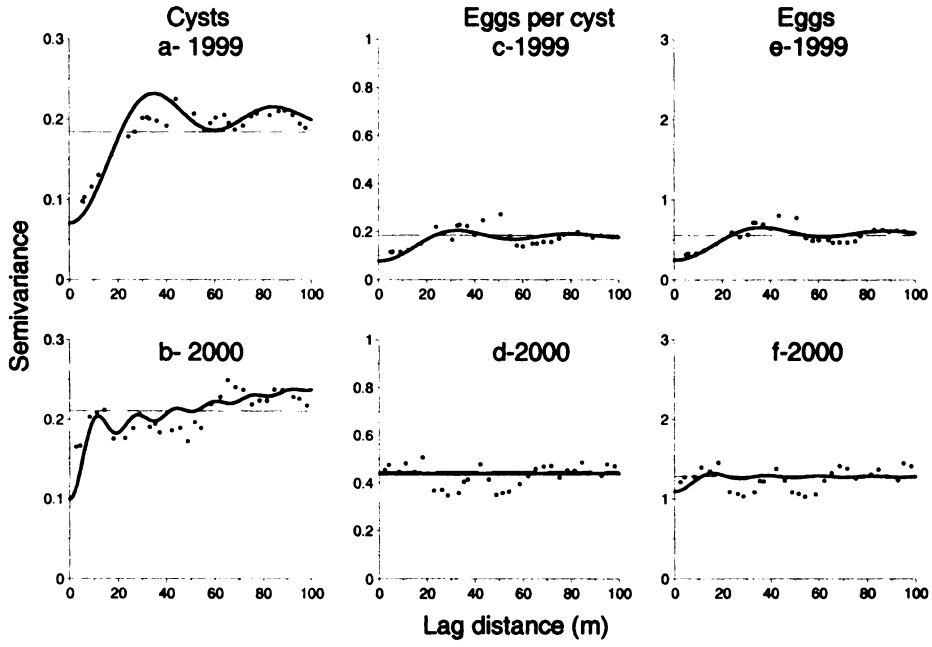

Figure 3.4. Semivariograms of a, b- cysts [$\log_{10}$(cysts 100 cm^{-3} of soil +1)], c, d- eggs per cyst [$\log_{10}$ (eggs per cyst +1)] and e, f- eggs [$\log_{10}$ (eggs 100 cm^{-3} of soil +1)] from Field B in a, c, e- 1999 and b, d, f- 2000. Black circles indicate omnidirectional empirical semivariogram, the solid line indicates the theoretical model fitted by means of least squares and the dashed line is the sample variance.

The periodicity observed in the empirical semivariograms of eggs per cyst and eggs could be described by wave effect models, except for eggs per cyst in 2000 where the nugget effect was too large (174 pairs in the first lag) (Figure 3.4.c- f). These semivariograms indicate clustered distribution of eggs and eggs per cyst.

Kriging

The distributions of cysts, eggs, and eggs per cyst were mapped separately for each field and year with ordinary or universal log-kriging using the parameters from the models fitted to the experimental semivariograms (Table 3.2).

The distribution of cysts in Field A varied slightly from 1999 to 2000. In 1999, small clusters of cysts were aggregated in larger patches. Cyst density was lower in the center of the area sampled and in the north end. The highest cyst density was found in the south portion of the field and in a band of approximately 100 m wide located between 550 and 650 m north (Figure 3.5. a). In 2000, cyst density increased throughout the field, and the distribution pattern changed in some portions of the field when compared to the pattern observed in 1999 (Figure 3.5. b). Even though the grouping in small clusters disappeared, cyst density remained low in the center and the northwest corner of the field. The most significant change in the distribution was observed in the southeast corner of the field where cyst density decreased markedly in 2000. Despite of the similarities in cyst distribution between years, the cross-correlogram indicated weak correlation between cysts in 1999 and 2000 for very short lags, and no cross-correlation beyond a separation distance of 20 m (Figure 3.6.a). The poor spatial structure and the low number of eggs per cyst in 1999 generated a distribution of kriged values rather uniform throughout the field (Figure 3.5.c).

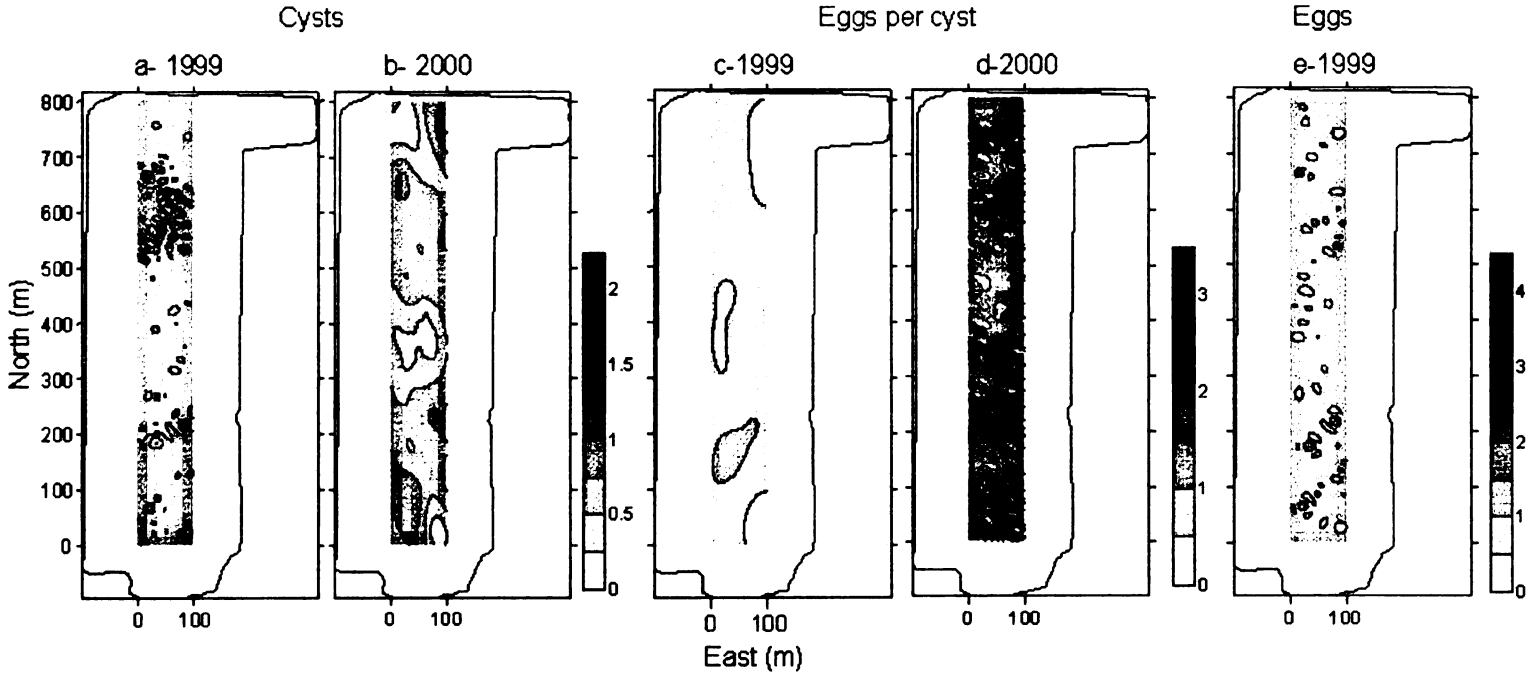

Fig. 3.5. Log-kriged maps represent the distribution of cysts in the area sampled within field A in a- 1999 and b- 2000; the distribution of eggs per cyst in c- 1999 and d- 2000, and the distribution of eggs in e- 1999. The shading scale indicates levels of cysts [$\log_{10}$ (cysts 100 cm^{-3} of soil +1)], eggs per cyst [$\log_{10}$ (eggs per cyst +1)], and eggs [$\log_{10}$ (eggs 100 cm^{-3} of soil +1)]. A solid line delineates the field boundaries.

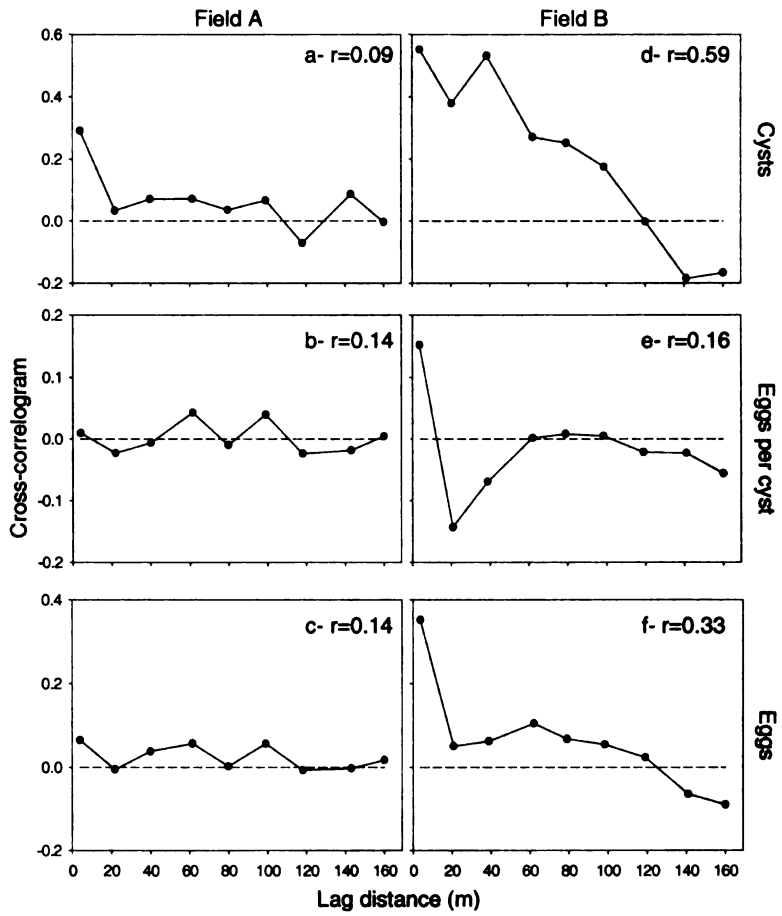

Fig. 3.6. Cross-correlograms between a, d- cysts [$\log_{10}$ (cysts 100 cm^{-3} of soil +1)], b, e- eggs per cyst [$\log_{10}$ (eggs per cyst +1)], and c, f- eggs [$\log_{10}$ (eggs 100 cm^{-3} of soil +1)] in 1999 and 2000 in Field A (a, b, c) and Field B (d, e, f). r = linear correlation coefficient.

The increased number of eggs per cyst and cysts with eggs in 2000 may have contributed to a better-defined spatial structure. Kriged values for eggs per cyst in 2000 showed small clusters of high and low values distributed randomly throughout the field (Figure 3.5.d). The distribution of high and low egg density areas resembled that of cysts in 1999 (Figure 3.5.e). Because of the nature of the semivariogram model (pure nugget effect), the map of egg distribution in 2000 represented the egg mean density throughout the field (not shown). The distribution of eggs per cyst and eggs were uncorrelated between 1999

and 2000 (Figure 3.6.b, c). Cyst population density at planting in 2000 was poorly correlated with eggs per cyst or eggs at planting the previous year (Figure 3.7. a, b).

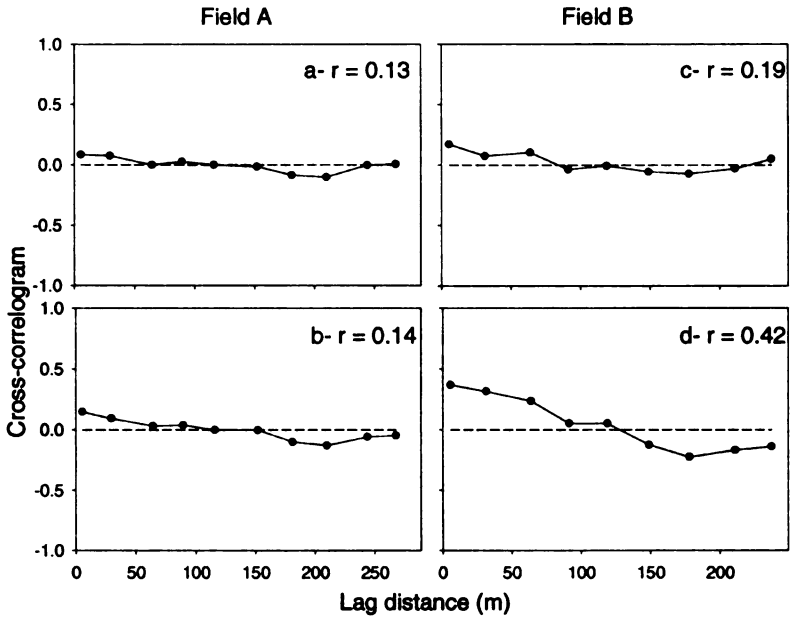

Fig. 3.7. Cross-correlograms between a, c- eggs per cyst [$\log_{10}$ (eggs per cyst +1)] in 1999 and cysts [$\log_{10}$ (cysts 100 cm^{-3} of soil +1)] in 2000; and b, d- eggs [$\log_{10}$ (eggs 100 cm^{-3} of soil +1)] in 1999 and cysts in 2000, in Field A (a, b) and Field B (c, d). r = linear correlation coefficient.

A well defined area with high cyst density was found in the northeast corner and an area with low cyst density in the southwest corner of the sampled site in Field B in 1999. Clusters of high and low cyst density were mixed in between these extreme locations (Figure 3.8. a). The pattern was maintained in 2000 with a relatively high linear correlation coefficient (Figure 3.6.d), but the infected area was larger, extended towards the south and without the inclusion of low-density clusters (Figure 3.8. b). The cross-correlogram for cysts between years showed a decrease in correlation as the separation distance between samples increased (Figure 3.6.d). Patches of eggs per cyst density slightly higher than the mean were distributed throughout the field in 1999, with the

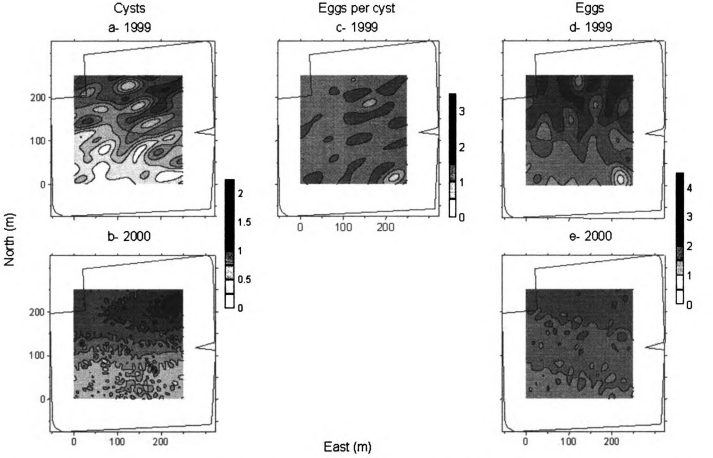

Fig. 3.8. Log-kriged maps represent the distribution of cysts in the area sampled within field B in a- 1999 and b- 2000; the distribution of eggs per cyst in c- 1999; and the distribution of eggs in d- 1999 and e- 2000. The shading scale indicates levels of cysts [$\log_{10}$ (cysts 100 cm^{-3} of soil +1)], eggs per cyst [$\log_{10}$ (eggs per cyst +1)], and eggs [$\log_{10}$ (eggs 100 cm^{-3} of soil +1)]. A solid line delineates the field boundaries.

lowest numbers located on the southeast corner of the site (Figure 3.8. c). In 2000, the distribution of eggs produced per cyst was represented by the mean throughout the field because of the nature of the semivariogram model (not shown). The distribution of eggs matched well that of cysts in 1999 and 2000 as expected, since the number of eggs is a linear function of cysts and eggs per cyst and the CV of cysts was much greater than the CV of eggs per cyst (Figure 3.8. d, e, Table 3.1). In 1999, it was possible to identify clusters of spatial autocorrelation in the distribution of eggs, whereas in 2000 the clusters appear to merge into more homogeneous bands. The correlation in the distribution of eggs per cyst between years was poor at short lags, becoming negative with increasing separation distance, and non-existent beyond 60 m (Figure 3.6.e). The distribution of eggs was moderately correlated between years at very short lags and uncorrelated beyond 20 m (Figure 3.6.f). Cyst population density in 2000 was poorly correlated with the number of eggs per cyst in 1999, but relatively well correlated with egg density at planting in 1999 (Figure 3.7.c, d).

DISCUSSION

The objective of this work was to assess the magnitude, structure, and persistence in time of the spatial distribution patterns of SCN cysts, eggs and eggs per cyst under field conditions using geostatistical tools. Geostatistics has been used to study the spatial distribution of soil inhabiting plant pathogens such as fungus-vectored viruses (Workneh et al., 2001), soil nematode community structure (Robertson and Freckman, 1995), and cyst nematodes under semi-controlled and field conditions (Francl, 1986a, 1986b; Webster and Boag, 1992; Evans et al., 1998; Donald et al., 1999; Donald et al., 2001). In

almost all cases, spatial patterns have been demonstrated. However, the precision of defining the spatial structure seems to vary with the type of sampling design used. The sampling design selected for this study allowed for the construction and analysis of detailed semivariograms for short separation distances, thus contributing to the understanding of SCN spatial distribution patterns. A regular grid may provide more uniform coverage of the area to be sampled, but the scale of spatial autocorrelation may be missed if the distance between nodes is larger than or equal to the range of the semivariogram. For exploratory spatial analysis, the nested sampling design of Webster and Boag (1992) has an advantage over regular grids in that it provides information for a variety of short separation distances (lags), thus enabling the analysis of the structure of the semivariogram when the scale of spatial variability is unknown. Taylor's Power Law has been used to determine the degree of aggregation of many nematode species (Boag and Topham, 1984; Duncan et al., 1989; Ferris et al., 1990; McSorley and Dickson, 1991; Webster and Boag, 1992). While the index of aggregation b' was not affected by sample size (Boag and Topham, 1984), it differed by species and by the separation distance between samples (Boag and Topham, 1984; Webster and Boag, 1992). In Fields A and B, Taylor's index of aggregation did not correspond with the information obtained from the semivariograms on the spatial structure in SCN population. This might be due to the periodicity encountered in SCN population, with positive and negative correlation between samples as the separation distance increased. A plot of Taylor's index b' versus separation distance between samples would probably have reflected the fluctuations observed in the empirical semivariograms.

The spatial distribution of SCN cysts, eggs, and eggs per cyst exhibited varying degrees of aggregation and structure, and the spatial structure varied between years. While the exact causes of varied SCN spatial distribution are poorly understood, nematode multiplication and population density equilibrium are influenced by host-nematode interactions and the prevailing environmental conditions (Seinhorst, 1967). For example, SCN reproduction and subsequent survival could be influenced by non-host plants or the presence of a resistant cultivar (Riggs, 1987). These, in turn, may lead to a less structured and more random distribution of the surviving cysts in soils. Hence, the low number of SCN cysts observed in Field A before planting in 1999 appears to be the outcome of corn, a non-host crop, grown the previous year and thereby possibly explaining the poorly structured spatial distribution observed for cysts in Field A in 1999. When infective juveniles hatch in the presence of a susceptible soybean variety, SCN can complete several generations during a growing season and population density can increase sharply if conditions are adequate (Lauritis et al., 1983; Bonner and Schmitt, 1985). SCN population densities that were below the detection limit at planting in 1999 may have increased to detectable levels in 2000 after susceptible soybean, generating changes in the spatial structure poorly correlated between years. The cysts and eggs distribution maps in Field B showed that the areas with more eggs in 1999 resulted in increased cyst density in 2000 and areas with fewer eggs in 1999 resulted in relatively fewer cysts in 2000 (Figure 3.8), indicating a positive correlation based on spatial location between eggs at planting and cyst density the following spring. This correlation was corroborated by the cross-correlogram (Figure 3.7.d). Over time, and under adequate

conditions, the spatial distribution of cysts in this field, and possibly in Field A too, may become more structured.

Plant-parasitic nematodes act as sinks of photoassimilates and nutrients and their ability to function as such will vary considerably depending on their physiological age (Bird and Loveys, 1975). SCN females are more likely to reach maturity in primary rather than in secondary or tertiary roots, thus, maturity may be related to the distance between the infection site (the sink) and the shoot (Melton et al., 1986). Plants with large root biomass offer more feeding sites to nematodes, favoring infection but not necessarily improved egg production. In Field B, we observed that the soybean plants located where cyst and egg densities were high grew less well than plants in areas with lower nematode densities. At the same time, cysts located in the northeast corner of the field contained similar numbers of eggs in 2000 as cysts found in areas with healthier plants (pure nugget effect semivariogram). Soil properties are very likely also involved in this relationship (Koenning et al., 1988; Todd and Pearson, 1988; Koenning and Barker, 1995). Among other factors, poor growth could be a function of soil moisture, where generally more, deeper, and less evenly distributed roots develop under drought stress, offering more feeding sites to nematodes (Huck et al., 1986). The portion of the field where plants grew poorly was well drained (Newaygo sandy loam), suggesting that drought stress may have been an adverse factor for plant growth in addition to the abundance of SCN. These observations correspond to those of Koenning and Barker (1995) where plant growth was adversely affected in coarse soils with poor water holding capacity even when there was a continuous water supply. A combination of causal factors could result in patches of cyst

density and variation of egg production, thereby generating spatial autocorrelation over time and in the presence of a suitable host.

Although strong winds, farming equipment, animals and flooding may disperse eggs and cysts in a field or move them to new locations, the most common dispersal mechanism of SCN is movement of soil (Lehman, 1994). Even though both fields were in no-tillage management between 1999 and 2000 with a residue coverage protecting the soil, some SCN movement is possible. For example, soil peds containing eggs and cysts adhered to the residue may have been moved by the wind and by planting and harvesting operations. In addition, the surface run-off in the spring of 2000 may have altered previously existent spatial structure towards a more uniform pattern.

Earlier reports indicated a strong relationship between the spatial distribution of SCN and soil texture (Workneh et al., 1999; Donald et al., 2001). In this study, variations in soil properties are likely to have had the most influence on SCN spatial structures and trends observed, especially in Field B. The influences may have been direct on the nematode population density, indirectly mediated through the host, or a combination of both.

The site-specific management of SCN is plausible because SCN studies have shown that it is not uniformly distributed within fields and because the cost and performance of eradication practices suggest that management offers the most viable control option for SCN. To make site-specific SCN management successful, the spatial distribution of SCN must be highly structured and temporally stable within a given field. In the fields evaluated in this study, the within-field variability in cysts and eggs per cyst was large (CV > 100%), but spatial variability was weakly structured as evidenced by

high nugget variances and poor fit of semivariogram models. Poor spatial structure and the high cost of sampling nematodes make the success of site-specific management in these fields unlikely. However, although there was poor correlation in cyst density between years in Field A, the temporal variability in SCN distribution was small. There were areas within each field in which SCN was not detected or occurred at only low density, and the areas of high or low SCN population density remained approximately at the same locations between years. We also know from remote sensing and yield maps (not presented here) that soybean performance was correlated with SCN infection. Therefore, the notion of SCN management zones, areas in which different SCN management practices would be applied, could be a viable option if appropriate criteria for the delineation of effective management zones were determined. Deriving and evaluating SCN management zone delineation criteria is a continuing goal of this research effort.

CHAPTER FOUR

THE SPATIAL DISTRIBUTION OF SOYBEAN CYST NEMATODE IN RELATION TO SOIL TEXTURE AND SOIL MAP UNIT

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THE SPATIAL DISTRIBUTION OF SOYBEAN CYST NEMATODE IN RELATION TO SOIL TEXTURE AND SOIL MAP UNIT

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THE SPATIAL DISTRIBUTION OF SOYBEAN CYST NEMATODE IN RELATION TO SOIL TEXTURE AND SOIL MAP UNIT

ABSTRACT

Evidence suggests that variation in soil texture may be a key variable to explain the variability of soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, population density within infested fields and be important to the delineation of SCN management zones. The purpose of this work was to assess the spatial structure of soil texture in two fields of known SCN population density and its relationship to published soil survey maps; and to quantify the relationship between sand, silt, and clay with SCN population density variability across fields and over time. Cysts were extracted by elutriation from single-core soil samples collected in a geostatistical sampling design. Soil texture analysis was performed for a subset of samples from each field using a modified hydrometer method. Classical and geostatistical analyses were employed to characterize and map soil texture for each field and analyze the effect of sand, silt, and clay on SCN population. Cyst population density was consistently higher in loamy sand than in sandy clay loam. The proportions of sand, clay, and silt in the soil were spatially structured and affected SCN population density strongly and consistently over time. The number of eggs per cysts was not related to soil type or texture ($\alpha = 0.05$). This study demonstrates the value of soil survey maps as indicators of where SCN can be expected in an infested field and how the addition of site-specific texture data can improve the spatial prediction of SCN. In addition to providing the basis for future experimentation to define soil texture tolerance limits for SCN, this study lays a foundation for new and integrated approaches to site-specific management (SSM) of SCN.

Soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is a major economic pest in soybean with wide geographic distribution in the major soybean growing areas of the U.S. (Wrather et al., 2001b). The resilience of SCN makes management and not eradication the most viable option for minimizing its impacts on soybean production (Bridge, 1996). Site-specific management of SCN is of interest because population densities vary spatially within fields (Donald et al., 1999; Avendaño et al., 2003- Chapter Three), and with variable management in response to SCN population density soybean growers might increase the efficacy and reduce the costs of SCN management practices. To be of value, SSM requires that the spatial variability be highly structured to ensure that spatial prediction and corresponding management maps are accurate (Pierce and Nowak, 1999). However, based on geostatistical sampling of SCN populations, Avendaño et al. (2003, Chapter Three) found that the spatial variability of SCN in two Michigan fields was poorly structured leading them to conclude that the success of SSM of SCN in these fields is unlikely, particularly given the high cost of sampling nematodes. In areas within these same fields, however, there were repeated occurrences of non-detectable or low densities of SCN and the authors reported correspondence between SCN infection and remote sensed imagery and yield maps. This observation suggested that other criteria might be available to delineate management zones for SSM of SCN in these fields (Avendaño et al., 2003, Chapter Three). This notion is supported by evidence that environmental conditions created by the interaction of weather, soil, landscape, and plant factors assist in the dispersion of eggs, determine SCN survivability, or limit its growth potential and thereby regulate the spatial dynamics

of SCN (Lehman, 1994; Koenning and Sipes, 1998; Donald et al., 1999; Workneh et al., 1999; Donald et al., 2001).

The determination of spatio-temporal dynamics of yield-limiting factors and the identification of cause-and-effect relationships among limiting factors are also critical components of SSM of nematodes (Evans et al., 2002; Melakeberhan, 2002; Wyse-Pester et al., 2002). Within the two fields studied by Avendaño et al. (2003, Chapter Three), SCN population densities appeared to vary by soil mapping unit, which were differentiated primarily on soil texture differences. This would suggest that soil texture or some combination of individual soil separates (sand, silt, and clay), are related directly or through the soil properties and processes they influence to SCN population densities and may be useful in delineating SCN management zones. The literature supports two important points in this regard: that nematode population dynamics are related to soil texture (particle size composition), structure (spatial arrangement and continuity of the soil pores between and within the particles), and related soil hydraulic properties and that these soil properties vary spatially and often with strong spatial structure.

Soil texture and structure strongly affect crop production and ecosystem health, including the nematode community (McKeague and Wand, 1982; Heal and Dighton, 1985; Gupta, 1994; Topp et al., 1997; Workneh et al., 1999). While there seems to be some variation among nematodes, generally coarse sandy soils favor nematode population growth by providing more space for nematode movement than do poorly structured soils containing finer particles which cannot form stable compound aggregates and so pack closer and diminish total porosity (Jones et al., 1969). Population densities of *Pratilenchus penetrans*, *Aglenchus agricola*, *Tylenchorhynchus* spp., *H. trifolii*, and

Paratylenchus spp. were significantly correlated with sand or silt particle size classes (Wallace et al., 1993). However, this relationship may be reversed for other nematodes. For example, higher densities of *Meloidogyne incognita* were associated with higher levels of clay in a loamy sand soil (Noe and Barker, 1985). Tillage influences nematode prevalence and population density by increasing the amount of space available for nematode movement even in fine soils rich in clay (Jones et al., 1969; Workneh et al., 1999; Donald et al., 2001). In fields under tillage management, repeated soil disruption during land preparation and cultivation may have alleviated oxygen deficiencies arising from water saturation due to high clay content, thus favoring the nematode population (Young, 1987; Workneh et al., 1999). In a field study on silt loam, the response of field-grown soybean to SCN varied depending upon the water status of the soil and SCN level (Johnson et al., 1993). In this case, the increase in SCN penetration of the soybean root system corresponded positively to the increase in soil oxygen diffusion rate and corresponding decrease in water potential. Water holding capacity was found to be the most important soil factor affecting the success of the oat crop at various levels of *H. avenae* (Fidler and Bevan, 1963). A positive correlation was also found between soil moisture and SCN survival (Slack et al., 1972), although Barker and Koenning (1989) noticed that numbers of SCN eggs, infective juveniles, and cysts were affected by soil texture, but not by soil moisture. Soybean plants respond to moisture stress by increasing root biomass, which would favor reproduction of SCN thus increasing population density under drought conditions (Koenning et al., 1988; Barker and Koenning, 1989; Koenning and Barker, 1995). Thus, the correlation found by Slack et al. (1972) could have been an indirect effect on the nematode population of soil moisture on plants. Koenning and

Barker (1995) also found that although SCN can increase to damaging levels in fine textured soils, the low rate of increase in these soils limits the damage potential of this nematode to soybean, as does the fact that damage is less severe per unit increase in population density. The low reproductive rate in soils with high clay content results in a longer time being necessary for the nematodes to attain damaging levels in fine textured soils. When the crop is damaged, nematode population equilibrium will decline to levels at which soybean yield suppression in subsequent years may not be perceptible.

Given that SCN population dynamics are regulated at least in part by soil properties and associated processes, the delineation of site-specific SCN management zones would appear feasible if soil properties are spatially structured and if quantitative relationships between SCN and these soil properties occur and are known. Considerable evidence supports the spatial variability of soil properties and that this variability can have spatial structure adequate for SSM (Robertson et al., 1993; Pierce and Nowak, 1999; Kravchenko and Bullock, 2000 and 2002, Cassel et al., 2000, Basso et al., 2001; Gaston et al., 2001; Mueller et al., 2001). We conclude that there is sufficient evidence to suggest a significant relationship between SCN populations and soil texture and that soil texture varies spatially. However, quantitative relationships on the spatial co-variance of SCN with soil texture and/or predictive relationships between soil properties and SCN needed for management zone delineation are not available. We therefore hypothesize that the variation of SCN population density between soil mapping units observed in the fields studied by Avendaño et al. (2003, Chapter Three) suggest that soil texture may be a key variable to explain the variability of SCN population density within these fields and be important to the delineation of SCN management zones.

The purpose of this work was to characterize the relationship between soil texture and the variability observed in SCN population density for the MI fields reported by Avendaño et al. (2003, Chapter Three). Specific objectives were to: assess the spatial structure of texture within fields of known SCN population density in Michigan and its relationship to published soil survey maps; determine the extent to which the spatial variability in SCN cyst population density relate to soil texture; quantify the relationship between soil separates (sand, silt, and clay) to SCN population density; and assess the extent to which this relationship holds between fields with similar soil types but different SCN populations.

MATERIALS AND METHODS

Research Site, Sampling Design, and Soil Sample Collection

The experimental design of the initial phase of this study to assess the spatial variability of SCN population was reported by Avendaño et al. (2003, Chapter Three). Briefly, the study was conducted in Shiawassee County, MI in 1999 and 2000 on two fields, (Field A and Field B) maintained by the cooperating farmer. Field A was 24 ha, managed under no-tillage since 1996, and planted to corn in 1998. Field B was 13 ha, conventionally tilled after wheat in 1998, and managed under no-tillage thereafter. In 1999, an SCN-susceptible soybean variety (Asgrow 1901), and in 2000, an SCN-resistant variety (Asgrow 2201), both Roundup-ready, were grown in both fields. Soybean was planted in 19-cm rows at a rate of 519 000 viable seeds ha^{-1} in 1999, and 494 000 viable seeds ha^{-1} in 2000. Rows orientation was north-south in Field A and east-west in Field B.

Soil series in Field A were Belding sandy loam (Coarse-loamy, mixed, frigid Argic Endoaquods), Breckenridge sandy loam (Coarse-loamy, mixed, nonacid, frigid Mollic Endoaquepts), Brookston loam (Fine-loamy, mixed, superactive, mesic Typic Argiaquolls), Conover loam (Fine-loamy, mixed, active, mesic Udollic Endoaqualfs), and Newaygo sandy loam (Fine-loamy over sandy or sandy-skeletal, mixed, frigid Alfic Haplorthods). Soil series in Field B were Brookston loam, Newaygo sandy loam, and Berville loam (Fine-loamy, mixed, mesic Typic Argiaquolls) (Soil Survey Division-NRCS-USDA, 2001). Soil series maps were digitized from Threlkeld and Feenstra (1974) (Figure 4.1.b and 4.2.b).

The spatial sampling for SCN population density consisted of a geostatistical sampling design applied within 8 and 5.25 ha in the center of both fields, as shown in Figure 4.1.a and described by Avendaño et al. (2003, Chapter Three). A bucket auger (8 cm diameter by 23 cm depth; Riverside Augers, Eijkelkamp, Giesbeek, The Netherlands) was used to collect 160 single-core soil samples from Field A and 110 from Field B, within one week before planting and within three days after harvest in 1999 and in 2000. Soil cores were placed in individual plastic bags and, upon arrival at the lab, were stored in 10-gal Rubbermaid containers at 4°C until they were processed (within 30 days).

SCN analysis

Cysts were extracted from 100 cm^3 sub-samples using a semi-automatic elutriator (Research Services Instrument Shop, The University of Georgia, Athens, GA; Byrd et al., 1976). The system had an extraction efficiency of 60%. Cysts were further separated from soil particles following the sugar flotation-centrifugation method (Dunn, 1969) and then counted under a stereo-microscope. Three cysts per sample were randomly selected,

crushed, and eggs and second-stage juveniles were counted, with the average used to determine the eggs per cyst for each sample containing at least one cyst.

Soil texture analysis

A subset of the 1999 sampling locations was selected to evaluate the relationship between soil texture and SCN population densities. Sample sites were chosen to include all soil series described for each field (Threlkeld and Feenstra, 1974) and to include areas of high as well as undetectable cyst density. Particle size analysis was conducted in duplicate on a sub-sample of soil from each of the 25 and 24 selected samples from Field A and Field B, respectively, using a modified hydrometer method (Gee and Bauder, 1986). Oven-dried soil was lightly crushed on a tray using a rolling pin to break up soil structure until the sample passed through a 2-mm aperture sieve (mesh #10). Forty grams of the < 2mm sieved soil was pre-treated in 100 ml deionized water (DI-water) with 30% hydrogen peroxide to oxidize the organic matter. Samples were then soaked in 100 ml of calgon solution (sodium metaphosphate 50 g l^{-1}) added to 200 ml of soil-DI-water suspension for a minimum of 7 hours. Suspensions were then mixed for 5 minutes at medium speed with an electric mixer to complete the dispersion of soil particles, and transferred to a 1000 ml sedimentation cylinder. Volume was adjusted to 1000 ml with DI-water. After thorough mixing of the soil suspension, the cylinder was left undisturbed for exactly eight hours; the suspension density was then measured with a hydrometer (ASTM 152H Bouyoucos style) reading the upper edge of the meniscus. Next, the contents of the cylinder were poured through a 45-μ aperture sieve (mesh #325) to retain sand particles. The sand retained in the sieve was carefully rinsed with DI-water, transferred to previously tarred and labeled aluminum weighing dishes, oven-dried

overnight at 105°C, and weighed upon cooling immediately after removal from the oven. The hydrometer reading was corrected for a blank cylinder containing 100 ml calgon solution in 900 ml DI-water. The proportion of sand in each sample was calculated as net sand weight divided by the initial weight of the sample (40 g) multiplied by 100. The clay fraction was calculated dividing the difference between the hydrometer reading of a sample and the blank reading by the initial sample weight, and multiplying this number by 100. Silt fraction was calculated subtracting the percentages of sand and clay from the initial sample weight. Soil type was determined for each sample based on the percentage contributed by each soil fraction as defined in the texture triangle recommended by USDA (Soil Survey Division Staff, 1993).

Statistical Analysis

Descriptive statistics were applied to characterize soil particle size distribution for each field. The soil separates sand, and clay were subjected to geostatistical analysis to determine empirical omnidirectional semivariograms (Matheron, 1963). The parameters of theoretical semivariogram models fit to the empirical semivariograms were estimated by (nonlinear) least squares. The spatial distributions of sand, and clay were mapped by predicting values at the nodes of a 1 x 1 m grid with universal or ordinary kriging using the structural properties of the estimated theoretical semivariogram and the sampled values at observed locations. The predicted values for the spatial distribution of silt were determined as 100-(predicted sand value + predicted clay value) at each node of the 1 x 1 m grid.

Cyst population densities and numbers of eggs per cyst at planting and harvest are shown as box-plots in Figure 3. Cyst density and number of eggs per cyst means were

compared across sampling times within fields with Fisher's (protected) LSD test (α= 0,05) using logarithmic transformed data [$\log_{10}$ (cysts 100cm-3 soil + 1), $\log_{10}$ (eggs per cyst + 1)] to increase symmetry and to stabilize the variance.

Each SCN observation was associated with a kriging-predicted value for the proportion of sand, clay, and silt, matched by location. A soil type classification was assigned to each SCN observation based on the predicted proportion contributed by each soil separate as defined by the texture triangle recommended by USDA (Soil Survey Division Staff, 1993). Cysts and eggs per cyst were then compared among soil types by sampling time within fields using Fisher's (protected) LSD test for means (α= 0.05). The effects of each of the soil texture fractions on transformed cysts and eggs per cyst were analyzed with analysis of variance (ANOVA). Regression coefficients were determined on means by simple linear regression analysis. Regression models were compared between fields, and between sampling times within fields for parallelism when appropriate (α = 0.05) (SAS®, Release 8; SAS Institute, Cary, NC).

The cross-correlogram is a geostatistical tool used to describe the spatial continuity between measurements of different attributes or of the same attribute measured at different times. The cross-correlation function given by Goovaerts (1997) was used here to calculate cross-correlograms for logarithmic transformed cysts and eggs per cyst with sand and clay percentage in the soil. Only the data points from locations sampled for SCN and soil texture in both 1999 and 2000 were used in this analysis.

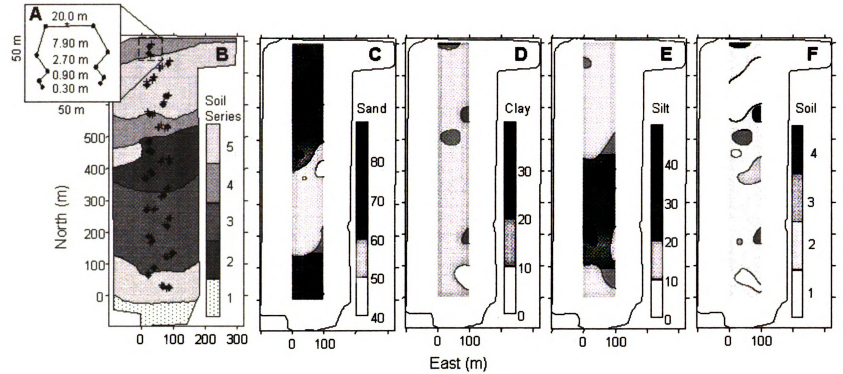

Figure 4.1. Field A. a- Nested design for the collection of soil samples. Samples were collected at the indicated distances in random directions within grid cells of 50 x 50 m. b- Location of sampled sites and soil series present in Field A. Crosses indicate the location of samples collected for the determination of SCN population density; black circles indicate which of those samples were also used for soil particle size analysis. Soil series are 1- Newaygo sandy loam, 2- Conover loam, 3- Brookston loam, 4- Breckenridge sandy loam, and 5- Belding sandy loam. Soil series map was digitized from Threlkeld and Feenstra (1974). c-Spatial distribution of sand, d- clay, and e- silt percentages in the soil as interpolated by ordinary kriging within the area sampled. The shadings in c, d, and e represent percentage ranges. f- Soil type delineation determined based on the proportion of sand, silt, and clay. Soil types are 1- Loamy sand, 2- Sandy loam, 3- loam, and 4- sandy clay loam.

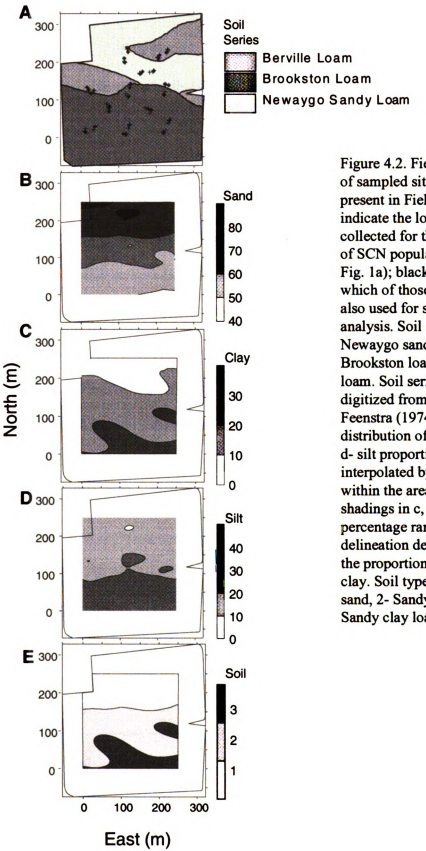

Figure 4.2. Field B. a- Location of sampled sites and soil series present in Field B. Crosses indicate the location of samples collected for the determination of SCN population density (see Fig. 1a); black circles indicate which of those samples were also used for soil particle size analysis. Soil series are 1- Newaygo sandy loam, 2- Brookston loam, and 3- Berville loam. Soil series map was digitized from Threlkeld and Feenstra (1974). b-Spatial distribution of sand, c- clay, and d- silt proportion in the soil as interpolated by universal kriging within the area sampled. The shadings in c, d, and e represent percentage ranges. e- Soil type delineation determined based on the proportion of sand, silt, and clay. Soil types are 1- Loamy sand, 2- Sandy loam, and 3- Sandy clay loam.

RESULTS

Soil Particle Size Distribution and Spatial Analysis

Soil particle size distribution of the surface layer in Field A and Field B correspond to an overall surface texture of sandy loam (Table 4.1). Even though on average soil particle size composition was similar, there were differences between Field A and Field B in the range of variability observed for each fraction, and considerable differences were observed when the data was analyzed spatially. Variation in the sand separate was similar in both fields but clay varied more in Field B while Field A varied more in the silt fraction.

Semivariogram models showed considerable spatial structure in the distribution of sand and clay, with similar ranges of autocorrelation for each separate in Field A and Field B (Table 4.2). The values predicted by kriging were used to generate contour maps of the levels of sand, clay, and silt (Table 4.2, Figures 4.1.c-e and 4.2.c-e). In Field A, the proportion of sand was the highest in the areas delineated for Belding sandy loam, and lowest in the area of Brookston loam (Figure 4.1.b, c).

Table 4.1. Soil texture in Field A and Field B as determined using a modified Bouyoucos hydrometer method (Gee and Bauder, 1986).

	Fraction	Sample size	Mean	Std. Dev†.	Min.‡	Max.§	CV
Field A			%		%	%	
	Sand	25	63.9	8.81	44.7	74.1	13.78
	Clay	25	15.6	3.00	10.0	20.0	19.17
	Silt	25	20.5	8.47	10.6	42.8	41.50
Field B							
	Sand	24	67.4	10.23	49.9	85.3	15.15
	Clay	24	14.0	5.33	6.2	22.5	38.34
	Silt	24	18.6	5.29	8.4	28.8	28.48

† Standard deviation, ‡ Minimum, § Maximum.

Table 4.2. Parameters of the theoretical semivariogram models of sand, silt, and clay fractions in Field A and Field B before planting in 1999. †

		Drift‡	Model function	C_0§	C¶	Range#	$\frac{C_0}{C+C_0}$ ††
Field A						m	
	Sand‡‡	None	Nugget	~ 0			0
			Exponential		87.0	70	
	Clay‡‡	None	Nugget	3.9			
			Gaussian		6.9	130	0.4
Field B							
	Sand‡‡	Linear	Nugget	~ 0			
			Spherical		28.8	66	0
	Clay‡‡	Linear	Nugget	~ 0			
			Spherical		11.1	129	0

† Models were fitted by least squares based on empirical semivariograms calculated for lags ranging from 30 to 90 m (h), with a lag tolerance of h/2. The minimum number of pairs required for each lag was 30

‡ Whenever semivariograms showed nonstationarity, the data were detrended carrying out a simple polynomial least squares regression and semivariogram analysis was performed on the residuals. The polynomial order of the trend is indicated when a drift or trend was removed.

§ C_0 is the nugget effect or a discontinuity in semivariance at the origin due to microscale variability or sampling error.

¶ C is the partial sill defined for spherical, exponential and gaussian models.

Observations that are spatially separated by more than the range are uncorrelated. The range is indicated for spherical models, and the practical range is indicated for exponential and gaussian models.

†† $C_0/(C+C_0)$ is an indicator of the degree of spatial structure, the lower the number the stronger the spatial autocorrelation.

‡‡ Semivariograms were calculated for the percentage of sand and clay present in soil samples, quantified using a modified Bouyoucos hydrometer method (Gee and Bauder, 1986)

The proportion of clay in the soil in Field A was mostly between 10- 20 % except for a few small patches where the proportion reached values slightly higher than 20 % in the area of Breckenridge sandy loam (Figure 4.1.b, d). The highest level of silt was located within the area of Conover loam (Figure 4.1.b, e). In Field B, the lowest level of sand, and the highest of clay and silt corresponded with the delineation for Brookston loam, whereas the highest level of sand and the lowest of clay and silt were located in the area of Newaygo sandy loam and Berville loam. Intermediate levels were found in the

transition zone between Brookston loam and the other two soil types (Figure 4.2.a-d). Overlaying the soil separate maps, we mapped the spatial distribution of the resulting soil map units in each field based on the proportion contributed by each separate (Figures 4.1.f and 4.2.e). Sand content seemed to dictate soil map units delineation in Field A (Figure 4.1.b, f), while clay content dictated soil map units delineation in Field B (Figure 4.2.c, e). Field B graded from sandy clay loam in the south to loamy sand in the north, while Field A was predominately sandy loam and loamy sand with patches of sandy clay loam and loam (Figures 4.1.f and 4.2.e). The soil maps obtained this way were useful to locate areas of very distinct soil map units located within the delineations reported by Threlkeld and Feenstra (1974) in each field.

SCN Population Density

The within fields and between years variability in preplant cyst and eggs per cyst for 1999 and 2000 have been reported in Avendaño et al. (2003, Chapter Three). Briefly, the number of cysts 100 cm^{-3} soil at planting and at harvest was similar in 1999 and in 2000 in Field A (Figure 4.3.a); whereas in Field B, there were more cysts in 2000 than in 1999 and more so at harvest than at planting (Figure 4.3.b). A section of Field B containing 26% of the samples was ponded with water at harvest in 2000. The localized flooding reduced the number of samples characterized by low cyst density observed in previous samplings influencing the results obtained for this particular sampling time as evidenced by a sample mean greater than expected (Figure 4.3.b). Generally, there were more eggs per cyst in Field B than in Field A in both years and sampling times, with the greatest number of eggs per cyst observed at harvest in 1999 (Figure 4.3.c, d).

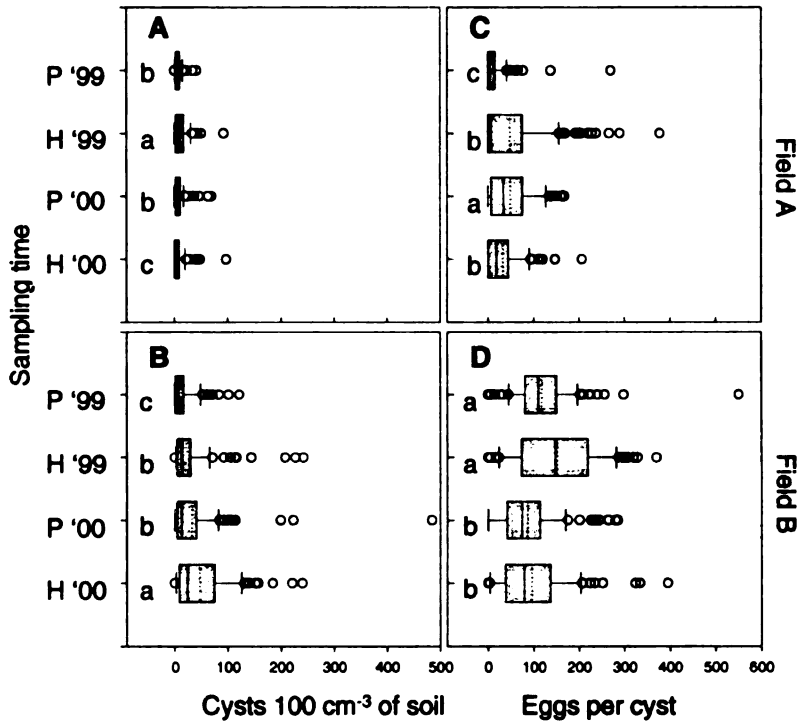

Figure 4.3. Boxplots of SCN (a) cyst population density and (c) eggs per cyst in Field A; and (b) cyst population density and (d) eggs per cyst in Field B. The y-axis indicates the sampling time: P'99: Planting 1999, H'99: Harvest 1999, P'00: Planting 2000 and H'00: Harvest 2000. The sample mean is indicated with a dashed vertical line and the median with a solid vertical line in each box. Significant differences among the means at different sampling times within each field are indicated with lower case letters. Mean comparisons were performed on logarithmic transformed data; original data are shown for clarity. Means with the same letter were not significantly different (protected LSD, 5 % significance level).

Relationship between Soil Map Units and SCN Population Density

Cyst population density varied by soil map unit. In Field A, cyst density was consistently higher in loamy sand and sandy loam than in the other two soil map units, although differences in cyst density among soil map units were statistically significant only at harvest in 1999 and planting in 2000 (Figure 4.4.a-d). In Field B, the number of cysts per 100 cm^3 of soil was the highest in loamy sand and lowest in sandy clay loam in

both years and sampling times (Figure 4.4.a-d). The number of eggs per cyst was not significantly different among soil map units in either field or sampling time (Table 4.3).

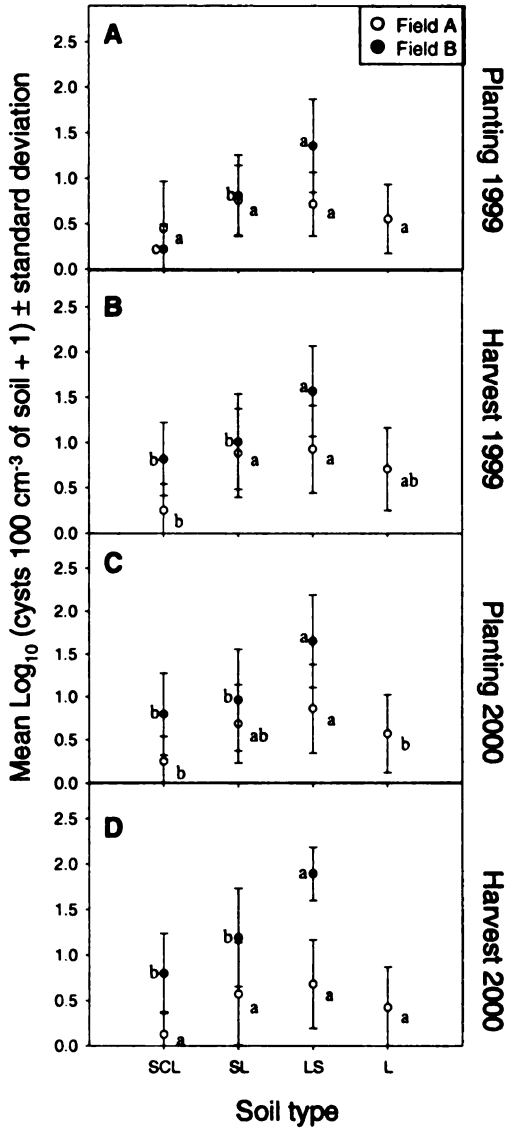

Figure 4.4. SCN mean cyst population density [$\log_{10}$ (cysts 100 cm^{-3} of soil + 1)] ± standard deviation by soil type in Field A (open circles) and in Field B (black circles) at a- planting 1999, b harvest 1999, c planting 2000 and d harvest 2000. Soil types are (SCL) sandy clay loam, (SL) sandy loam, (LS) loamy sand and (L) loam. Means with the same letter within field are not significantly different (protected LSD, 5 % significance level).

Table 4.3. Mean SCN eggs per cyst by soil type in Field A and Field B in 1999 and in 2000.

	Field A				Field B			
	1999		2000		1999		2000	
Soil type†	Planting	Harvest	Planting	Harvest	Planting	Harvest	Planting	Harvest
	Mean eggs per cyst ± se				Mean eggs per cyst ± se			
SCL	12±10.6	84±84.5	44±18.0	0	96±23.3	163±19.8	87±14.6	126±42.9
SL	14±3.8	48±8.7	49±5.9	32±6.2	113±7.8	136±13.9	86±12.4	86±11.1
LS	9±2.2	57±11	53±8.3	26±4.9	133±17.9	168±16.9	84±8.9	96±14.3
L	29±9.2	19±13.4	35±10.3	52±15.2				
LSD 0.05‡	NS	NS	NS	NS	NS	NS	NS	NS

† Soil types were (SCL) sandy clay loam, (SL) sandy loam, (LS) loamy sand, and (L) loam as defined by the texture triangle recommended by USDA (Soil Survey Division Staff, 1993).

‡ Logarithmic transformed means [$\log_{10}$ (eggs per cyst 100 cm^{-3} of soil +1)] within sampling times were compared among soil types using Fisher's (protected) LSD test at the 0.05 probability level. Untransformed data are shown for clarity.

Relationship between Soil Particle Size and SCN Population Density

SCN population density was strongly affected by the proportion of sand, clay, and silt in the soil (Figure 4.5, Table 4.4). The effect of sand was characterized by a positive slope, not significantly different across sampling times in Field B (Table 4.4, Figure 45.a-d). The regression model at harvest 1999 in Field A had the same slope and intercept (α = 0.05) as the model for Field B at harvest 1999 and at planting in 2000 (Figure 4.5.b). Cyst density decreased with increasing clay proportion in the soil at all sampling times in Field B and at harvest both years in Field A, with the same slope (α = 0.05) across sampling times and between fields (Table 4.4, Figure 4.5.e-h). Silt proportion in the soil also had a negative effect on SCN; cyst population density decreased linearly with increasing percentage of silt at all sampling times in Field B (Table 4.4, Figure 4.5.e-h). Regression models were also parallel for silt across sampling times. The relationship between silt and cysts was not significant in Field A.

Unlike the cyst population, the relationship between eggs per cyst and soil particle size was highly variable (Table 4.5, Figure 4.6). The proportion of sand in the soil had a negative effect on eggs per cyst at planting in Field A, but a positive effect at harvest the same year (Figure 4.6.a, b). Clay percentage had a negative effect on eggs per cyst at harvest in Field A in 2000 (Figure 4.6.h), and silt percentage had a negative effect on eggs per cyst at planting in 1999 in Field B (Figure 4.6.i). Otherwise, sand, clay, or silt had no significant effect on eggs per cyst.

Table 4.4. Regression models and coefficients of determination of the relationship between cyst population density and sand, clay, or silt proportion in the soil in Field A and Field B in 1999 and in 2000.

		Sand		Clay		Silt	
	Sampling time	Model†‡	r^2	Model†‡	r^2	Model†‡	r^2
Field A							
	Planting 1999	NS		NS		NS	
	Harvest 1999	y=-0.09+0.015x	0.16	y=1.89-0.065x	0.64	NS	
	Planting 2000	NS		NS		NS	
	Harvest 2000	NS		y=2.01-0.089x	0.75	NS	
Field B							
	Planting 1999	y=-2.05+0.045x	0.77	y=2.21-0.088x	0.76	y=2.53-0.084x	0.81
	Harvest 1999	y=-0.89+0.032x	0.54	y=2.14-0.066x	0.89	y=2.59-0.072x	0.66
	Planting 2000	y=-1.58+0.042x	0.76	y=2.53-0.091x	0.63	y=2.76-0.081x	0.76
	Harvest 2000	y=-1.69+0.048x	0.67	y=2.69-0.090x	0.79	y=2.83-0.069x	0.70

† Models within each soil separate (sand, clay, silt) were tested for parallelism across sampling times. None of the slopes were significantly different (α= 0.05).

‡ y = $\log_{10}$ (cysts 100 cm^{-3} of soil +1), x = % sand, %clay, or % silt.

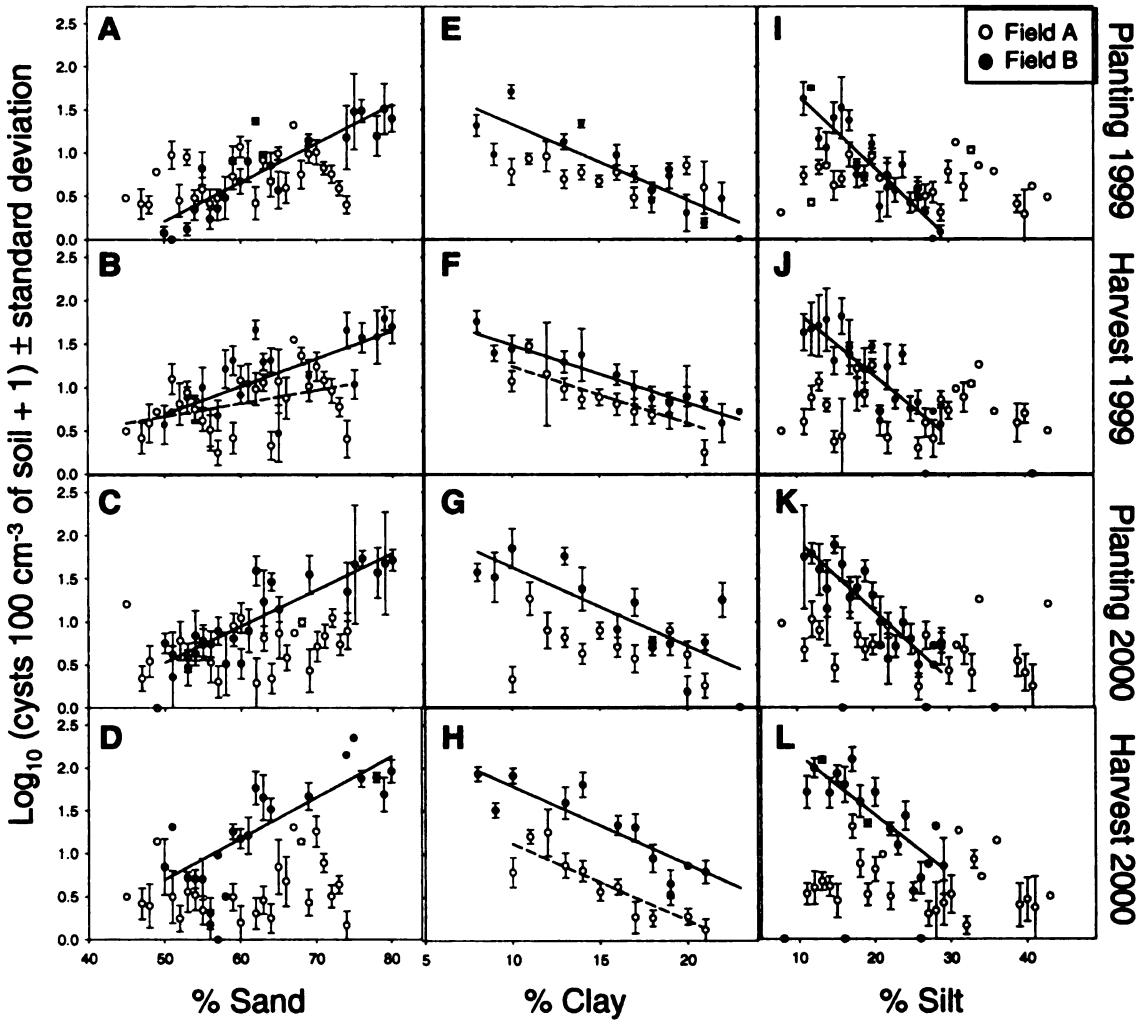

Figure 4.5. Relationship between SCN cyst population density [$\log_{10}$ (cysts 100 cm^{-3} of soil + 1)] and the proportion of each soil fraction in the sample. Means ± standard deviation for Field A (open circles) and for Field B (black circles) are indicated. The left column (a, b, c, d) corresponds to the percentage of sand, the central column (e, f, g, h) corresponds to clay percentage and the right column (i, j, k, l) corresponds to silt percentage; at (a, e, i) planting 1999, (b, f, j) harvest 1999, (c, g, k) planting 2000, and (d, h, l) harvest 2000. Regression curves fitted are indicated as solid lines (Field B) and dashed lines (Field A) where significant (5 % significance level). Regression coefficients are shown in Table 4.4.

Table 4.5. Regression models and coefficients of determination of the relationship between eggs per cyst and sand, clay, or silt proportion in the soil in Field A and Field B in 1999 and in 2000.

	Sand		Clay		Silt	
Sampling time	Model†‡	r^2	Model‡	r^2	Model‡	r^2
Field A						
Planting 1999	y=1.85-0.019x	0.27	NS		NS	
Harvest 1999	y=-1.06+0.032x	0.20	NS		NS	
Planting 2000	NS		NS		NS	
Harvest 2000	NS		y=2.54-0.095x	0.53	NS	
Field B						
Planting 1999	NS		NS		y=2.86-0.051x	0.32
Harvest 1999	NS		NS		NS	
Planting 2000	NS		NS		NS	
Harvest 2000	NS		NS		NS	

† Models within each soil separate (sand, clay, silt) were tested for parallelism across sampling times. Slopes were not significantly different (α= 0.05).

‡ y = $\log_{10}$ (eggs per cyst 100 cm^{-3} of soil +1), x = % sand, %clay, or % silt.

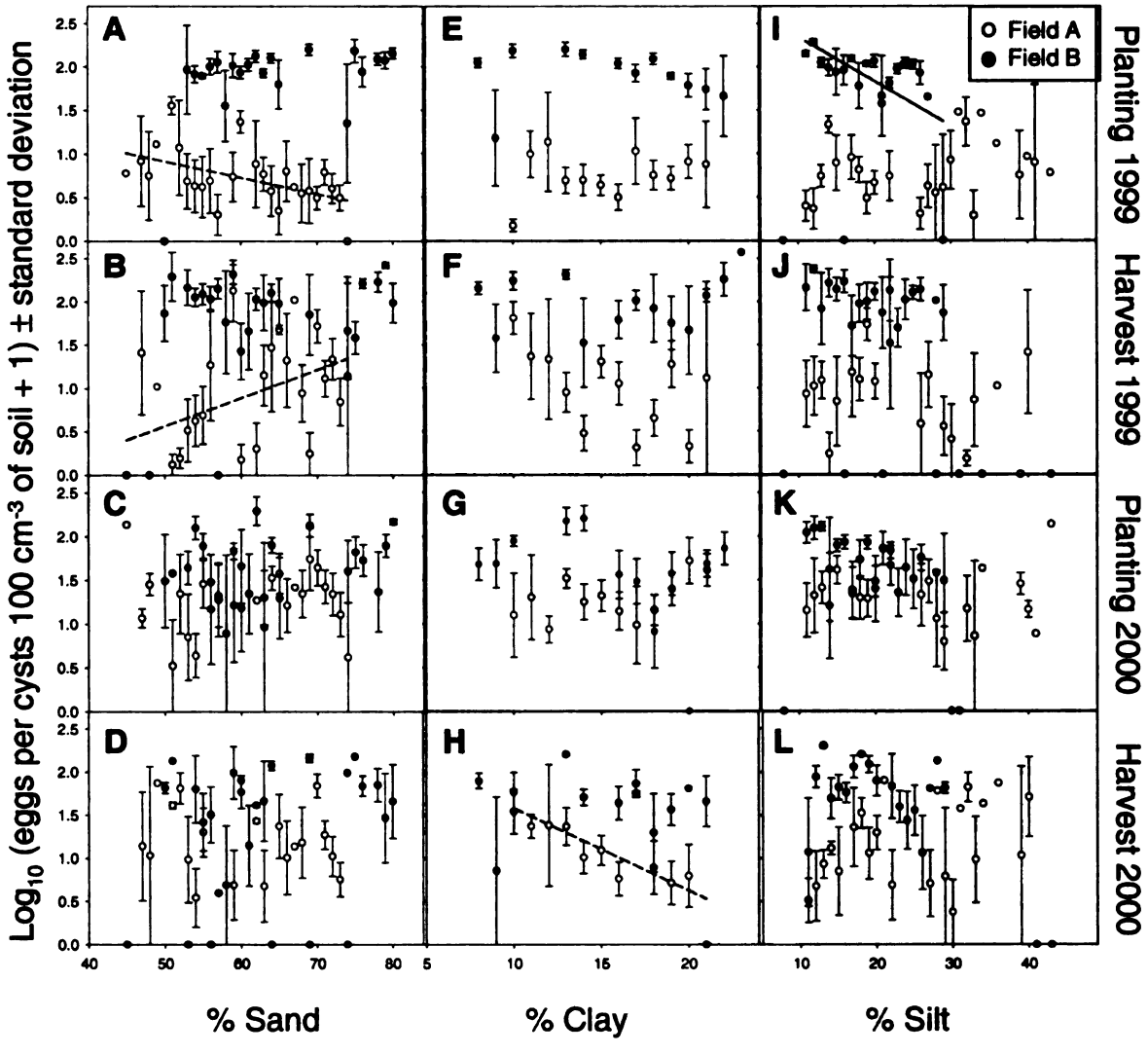

Figure 4.6. Relationship between SCN eggs per cyst [$\log_{10}$ (eggs per cyst 100 cm^{-3} of soil + 1)] and the proportion of each soil fraction in the sample. Means ± standard deviation in Field A (open circles) and in Field B (black circles) are indicated. The left column (a, b, c, d) corresponds to sand percentage, the central column (e, f, g, h) corresponds to clay percentage and the right column (i, j, k, l) corresponds to silt percentage; at (a, e, i) planting 1999, (b, f, j) harvest 1999, (c, g, k) planting 2000, and (d, h, l) harvest 2000. Regression curves fitted are indicated as solid lines (Field B) and dashed lines (Field A) where significant (5 % significance level). Regression coefficients are shown in Table 4.5.

Linear correlation coefficients for cysts with sand were very low in Field A (Figure 4.7.a-d). The low correlation decreased even more with increasing separation distance between samples. Cysts were better correlated with clay at harvest than at planting, and more so in 2000 (Figure 4.7.b, d). The separation distance at which the cross-correlation cysts-sand or cysts-clay reached zero was the same (approximately 110 m) at harvest in 2000 (Figure 4.7.d).

Cyst population density was highly correlated with sand and clay at all times in Field B, and the correlation decreased in absolute value at the same rate for both separates, reaching zero at the same separation distance between samples (Figure 4.7.e-h). The distance at which cross-correlations were zero increased from approximately 115 m to 130 m from planting in 1999 to harvest in 2000. The symmetry in the cross-correlograms indicated that the effect of sand on cyst population was equal in magnitude to the effect of clay, but with opposite sign.

The number of eggs per cyst was only correlated with clay at harvest in 2000 in Field A; otherwise, correlations were very low as indicated by the linear correlation coefficients (Fig 4.8.d). Cross correlograms showed fluctuations in correlation between eggs per cyst and sand or clay as separation distance between samples increased. In Field B, the symmetry between sand and clay cross correlograms observed for cysts was also evident for eggs per cyst (Figure 4.8.e-h).

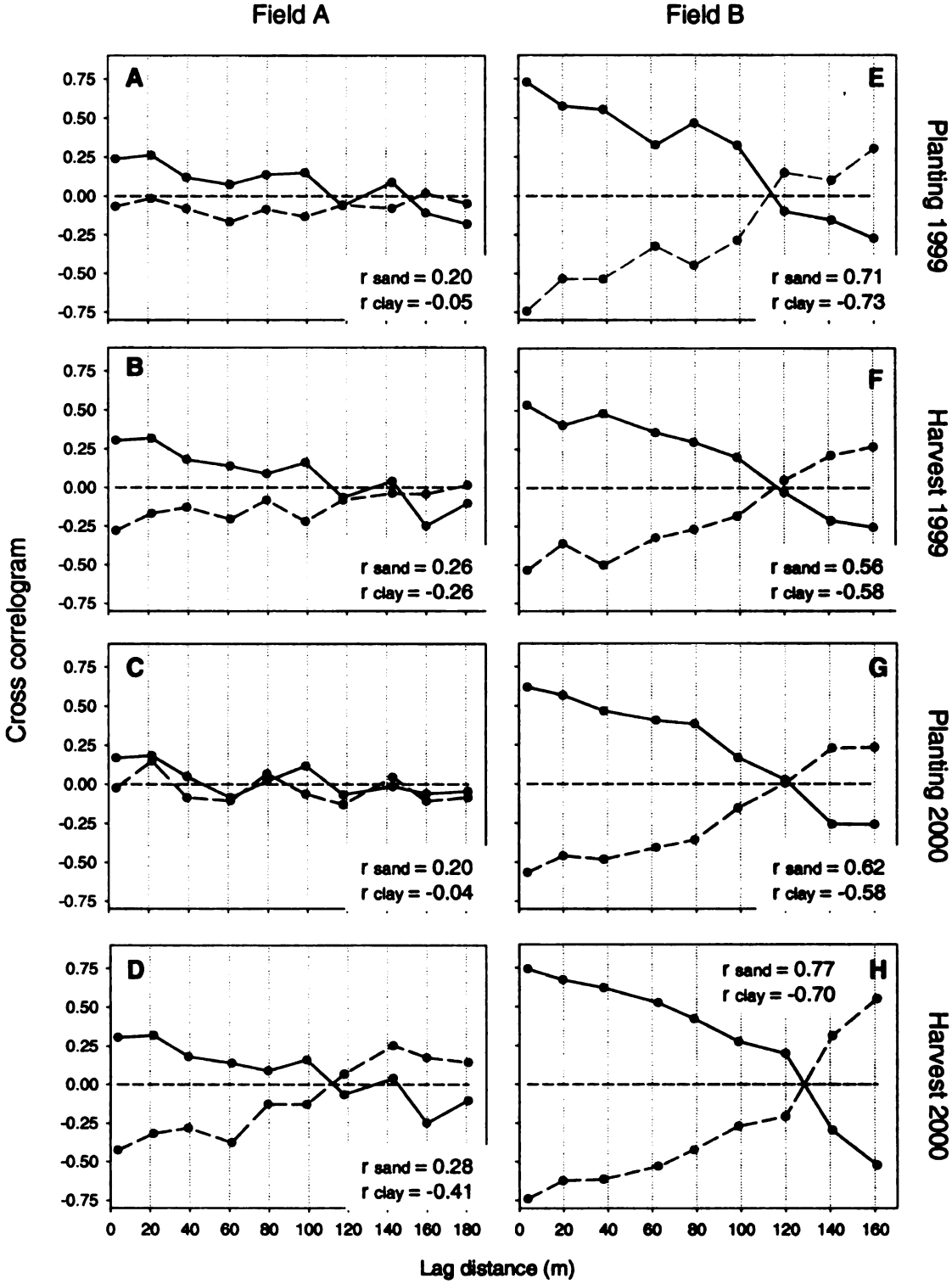

Figure 4.7. Cross-correlograms of SCN cyst population density and percent sand (solid line) or clay (dashed line) in the soil in Field A (a-d) and Field B (e-h). a, e- Planting 1999; b, f- Harvest 1999; c, g- Planting 2000; d, h- Harvest 2000. Linear correlation coefficients for cyst density with sand and clay are indicated.

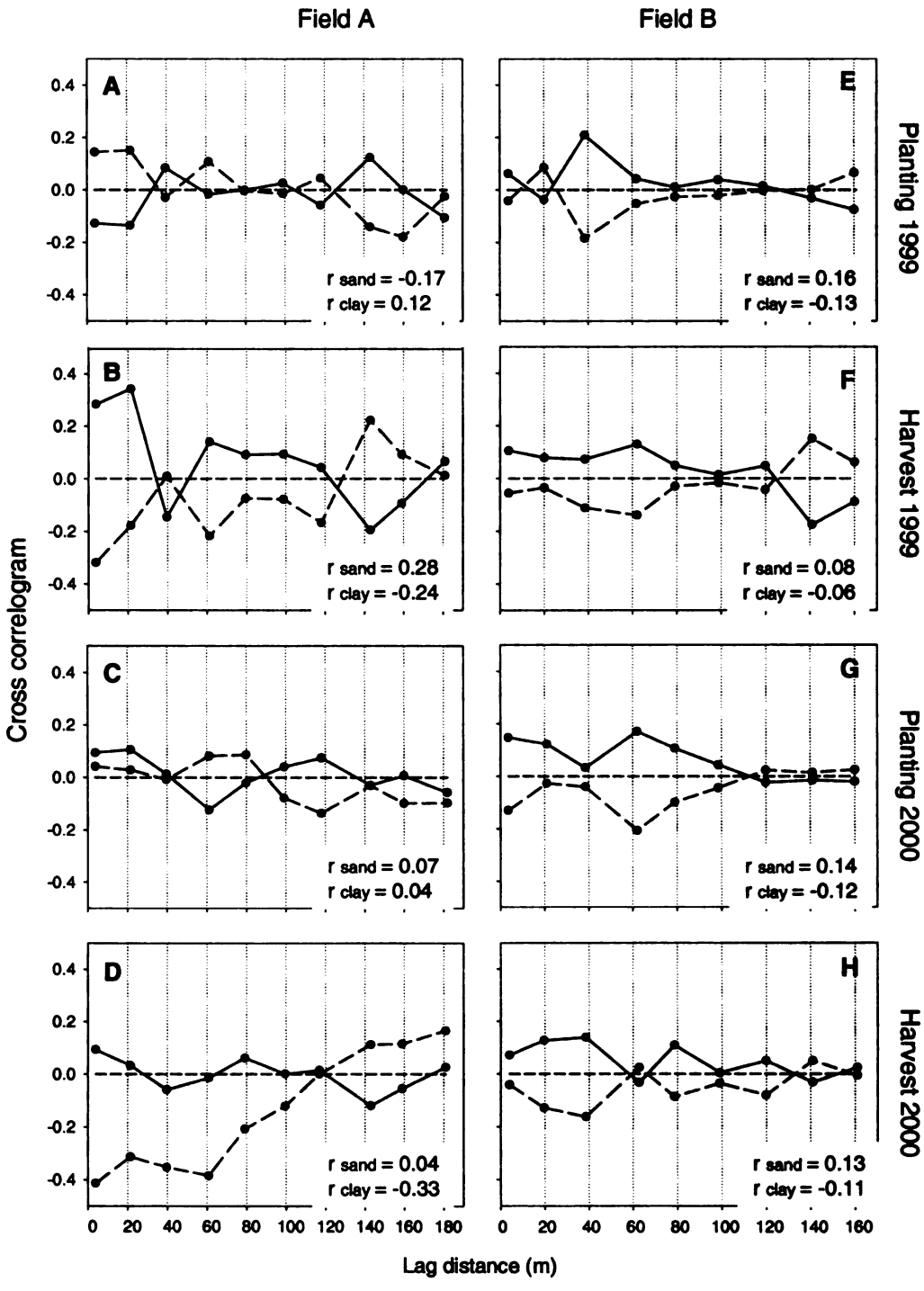

Figure 4.8. Cross-correlograms of SCN eggs per cyst and percent sand (solid line) or clay (dashed line) in the soil in Field A (a-d) and Field B (e-h). a, e- Planting 1999; b, f- Harvest 1999; c, g- Planting 2000; d, h- Harvest 2000. Linear correlation coefficients for eggs per cyst with sand and clay are indicated.

DISCUSSION

This study was conducted as part of a project designed to investigate the potential application of SSM of SCN in Michigan soybean production systems (Avendaño et al., 2003, Chapter Three). The work presented here provides further insights into the potential of SSM for SCN by describing detailed relationships between SCN population density and soil texture.

While it is not known when SCN was introduced into the fields and what undetermined factors may have contributed to the difference in SCN population density, Field A and Field B presented us with the opportunity to study the relationship between SCN population and soil texture under two different conditions frequently encountered by soybean growers. Cyst population density was high in Field B and it increased during the study whereas in Field A, cyst density was much lower. While soil survey maps suggested that Field A and Field B had very similar soil types, geostatistical sampling and analysis provided a different perspective into the relationship between soil texture and SCN population dynamics. The spatial distribution of the soil separates was highly structured in both fields, allowing the construction of reliable maps for the distribution of sand, silt, and clay in each field. The arrangement of soil map units obtained from superimposing these maps corresponded in general terms with the soil survey maps reported by Threlkeld and Feenstra (1974).

The relationship between soil map units and SCN population density was consistent between the two fields, although differences were attenuated in Fields A, where cyst population remained low at all times. Cyst population density was consistently

higher in loamy sand than in the other soils; it was lowest in sandy clay loam (Figure 4.4). While this observation is consistent with previous studies, where SCN was found more abundantly in coarser soils than in finer soils (Dropkin et al., 1976; Koenning and Barker, 1995; Donald et al., 1999), the relationship between soil types and ranges of textural composition needs careful consideration. Koenning and Barker (1995) reported highest SCN egg density in Fuquay sand (91% sand: 6%silt: 3% clay), Norfolk sandy loam (84:12:4) and Portsmouth loamy sand (72:18:10) when compared with Cecil sandy clay loam (53:18:29) and Cecil sandy clay (48:13:39). The lowest nematode density was found in soils with more than 25% clay. We propose that SCN can sustain high population density levels (above 20 cysts per 100 cm^3 of soil) only in soils composed of more than 60% sand, less than 20% silt and less than 20% clay. However, the soil texture defined for this composition corresponds to sandy loam, loamy sand and sand. The difference in texture between sandy loam and sandy clay loam in our research fields was the result of reduced sand content (~60%) combined with increased clay (>20%). It is important to note that only a portion of the area defined for sandy loam in the texture triangle is favorable for SCN. Sandy loam with more than 20% silt was associated with low levels of cysts in our study. The particle size composition of sandy clay loam and loam are beyond the 60:20:20 limits, accordingly with our proposition, these soil types had significantly less cysts than loamy sand (Figure 4.4). Therefore, it might be best that references to any relationship between SCN population and soil include soil texture in addition to soil classification.

The number of eggs per cysts was not related to soil map units or texture in our study, with a few exceptions. The data suggest that soil texture affects SCN population at

the mobile stages during root finding and penetration, and perhaps development in the roots, rather than the reproductive potential, fecundity, or hatching. This phenomenon was previously reported by Todd and Pearson (1988) when they recovered more SCN females and cysts from newly infested roots in sandy loam (60:30:10) than in silty loams (30:46:24 and 14:60:26). Nevertheless, Young and Heatherly (1990) attributed the lower rate in SCN reproduction to soil type in a study using Sharkey clay (8.5:34:57.5) and Dubbs silt loam (23:60.5:16.5). This indicates that a high proportion of clay and very low sand content are necessary to interfere with SCN reproductive potential. However, the effect of this kind of soil on root development should also be taken into consideration (Russell, 1977), since the effect of soil texture on cyst fecundity may be strongly influenced by plant conditions (Koenning and Barker, 1995).

It is difficult to discriminate which of the separates: sand, silt or clay, has the greatest influence on cyst density. The analysis of cyst density by soil separate indicated that sand had the opposite effect of clay or silt. These observations indicate that the combination of the separates, that is, the resulting soil texture has a greater influence on SCN population than a specific separate by itself. This is the first report to document consistency in the relationship between SCN and soil texture across fields and over time. Wyse-Pester et al., (2002) explored the possibility of using correlation between soil attributes (soil texture) and nematode density to reduce the cost of sampling in an effort to map nematode distribution for SSM. Although the spatial dependency indicated a potential for mapping *Helicotylenchus* spp., *Tylenchorhynchus capitatus*, and *Pratylenchus neglectus* infestations, the small variation in soil texture in their research fields resulted in inconsistent and weak correlations with nematode density.

Site-specific management of soybean yield-limiting factors requires an understanding of the spatio-temporal dynamics of the prevailing conditions. In addition to providing the basis for future experimentation to define soil texture tolerance limits for SCN, this study lays a foundation for new and integrated approaches to SSM of SCN and other yield-limiting factors. The co-variation of SCN with other factors in soils is helpful to assess spatial variability of SCN populations within fields if SSM is to be a plausible strategy to manage SCN in soybean production. We conclude that soil survey maps can be useful in predicting expected levels of SCN in an already infested field but they should be used with caution. Soil map unit delineations based on the texture triangle may not be sufficient when referring to SCN population and the proportion of each soil fraction should be used in addition. We have shown in this study the benefit of texture-based analysis over soil type-based analysis therefore, a soil survey map can be used to identify high-risk sectors, and then a texture analysis of soil from these zones may help delineating soil map units of expected high cyst density.

CHAPTER FIVE

RELATIONSHIP BETWEEN SOIL TEXTURE AND SOYBEAN CYST NEMATODE POPULATION DYNAMICS IN SOIL AND IN SOYBEAN ROOTS

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RELATIONSHIP BETWEEN SOIL TEXTURE AND SOYBEAN CYST NEMATODE POPULATION DYNAMICS IN SOIL AND IN SOYBEAN ROOTS

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RELATIONSHIP BETWEEN SOIL TEXTURE AND SOYBEAN CYST NEMATODE POPULATION DYNAMICS IN SOIL AND IN SOYBEAN ROOTS

ABSTRACT

The purpose of this work was to identify i) the extent to which soil texture affects *Heterodera glycines*, soybean cyst nematode (SCN) population dynamics and ii) to assess the potential for management zone delineation based on soil map units for designing an alternative SCN management practice. Traditional statistics and geostatistics were applied to analyze SCN population dynamics and spatial distribution in the soil and in soybean roots in relation to soil texture in two Michigan fields. Soil texture had a strong and consistent effect on SCN cyst population density and spatial distribution in one field, but not in the other. Coarser soils supported higher SCN population density than finer soils delineating areas of high or low population density within the field. The effect of soil texture was weaker on the third and fourth larval stages and immature females, and not significant on infective larvae in the host roots. It is not clear from this work whether the effect of soil texture on the number of eggs per cyst was on fecundity (egg production) or on hatching. The stability in the relationship between SCN spatial population dynamics and soil properties indicates the potential for delineation of management zones to reduce the economic loss due to SCN in infested fields.

Soybean cyst nematode's (SCN, *Heterodera glycines* Ichinohe) ability to adapt to a wide range of environmental conditions seems to be one of the reasons for its widespread distribution and economic significance in soybean [*Glycine max* (L.) Merr.] production worldwide (Winstead et al., 1955; Wrather et al., 2001a, b). The broad distribution over a range of production systems and soil types in the USA demonstrates the limitations of current SCN management tactics (Bradley et al., 1996; Workneh et al., 1999; CTIC, 2000). Avendaño et al. (2003, Chapter Three) investigated SCN spatial distribution within fields to assess the potential for SCN site-specific management (SSM). Even though the spatial structure of SCN population density was poor, and varied between fields and years, it was possible to identify areas within a field where SCN population densities remained high or low, over time. Wyse-Pester et al., (2002) explored the possibility of using correlation between soil attributes (soil texture) and nematode density to reduce the cost of sampling in an effort to map nematode distribution for SSM. Although the spatial dependency indicated a potential for mapping *Helicotylenchus* spp., *Tylenchorhynchus capitatus*, and *Pratylenchus neglectus* infestations, small variation in soil texture resulted in inconsistent and weak correlations with nematode density. However, SCN has been found correlated with soil texture consistently over time (Chapter Four), with higher equilibrium density in coarse soil than in finer soils (Dropkin et al., 1976; Koenning and Barker, 1995; Workneh et al., 1999). In addition, SCN population density was spatially structured to a greater degree in fields with diverse soil types than in more homogeneous fields (Avendaño et al., 2003-Chapter Three; Donald et al., 2001). The temporally stable location of hot spots, or areas of high SCN population density reported in Chapter Four, consistently correlated with soil texture makes the

delineation of SCN management zones based on soil map units an attractive alternative (Doerge, 1998).

The relationship between SCN development and the soil environment is poorly understood. SCN development is certainly affected by the genetic characteristics of the host plant. There is disagreement on whether exudates from susceptible or resistant soybean plants stimulate more SCN hatching (Sikora and Noel, 1996, Schmitt and Riggs, 1991; Tefft and Bone, 1985). Nevertheless, it is clear that development is most successful on SCN-susceptible soybean varieties than on SCN-resistant varieties (Wallace et al., 1995), especially during the vegetative phase (Sikora and Noel, 1996; Hill and Schmitt, 1989; Tefft and Bone, 1985). The average number of eggs per cyst, however, was not correlated with soil texture (Chapter Four), and infective juveniles were equally successful infecting roots in a clay soil (8.5% sand, 34% silt, 57.5% clay) and a silt loam (23% sand, 60.5% silt, 16.5% clay) in a greenhouse test (Young and Heatherly, 1990). Hence, we postulate that soil texture affects SCN survival in the soil and development rather than reproduction and root invasion.

Measuring the extent to which soil texture affects SCN population dynamics and particular life stages is a necessary further consideration to the possibility of management zone delineation based on soil map units as a plausible SCN management alternative. The objectives of this work were to: i- characterize SCN population dynamics in soybean roots and the surrounding soil in two fields in Michigan over two growing seasons; ii- investigate the extent of the correlation of soil texture with SCN population in the roots and in the soil, and with eggs per cyst; and iii- analyze SCN population dynamics spatially in relation to soil texture.

MATERIALS AND METHODS

Research Site and Sampling Design

The study was conducted in Shiawassee County, MI in 1999 and 2000 on two fields (Field A and Field B) maintained by the cooperating farmer. Field A was 24 ha, managed under no-tillage since 1996, and was planted to corn before this study in 1998. Field B was 13 ha, conventionally tilled after wheat in 1998, and was managed under no-tillage thereafter. In 1999, a SCN -susceptible soybean variety (Asgrow 1901), and in 2000, an SCN-resistant variety (Asgrow 2201), both Roundup-ready, were grown in both fields. Soybean was planted in 19-cm rows at a rate of 519 000 viable seeds ha^{-1} in 1999, and 494 000 viable seeds ha^{-1} in 2000. Fields A and B were planted 5/22/99 and 5/16/99, respectively, and 6/9/00.

The experimental design was developed at the initial phases of this study to assess the spatial variability of SCN population and the effect of soil texture on SCN cyst population density at harvest and at planting reported by Avendaño et al. (2003, Chapter Three and Chapter Four). The spatial sampling for SCN population density consisted of a nested survey sampling design with distances reduced by geometric progression (adapted from Webster and Boag, 1992) applied within 8 and 5.25 ha in the center of Fields A and B, respectively (Figure 3.1.a,b). Pairs of single-core samples were collected 20, 7.9, 2.7, 0.9, and 0.3 m apart (Figure 3.1.c). Each sampled location was chosen in a random direction. The angles for each new location were expressed in north and east coordinates to facilitate the location of the sampling sites in the field. This arrangement produced 160 and 110 sampling locations that were flagged and geo-referenced using GPS in Field A and Field B, respectively.

Soil and Soybean Root Sampling for SCN.

Soil samples for SCN cyst quantification and soybean root samples for SCN developmental stages were collected from Field A and Field B at about 30 day intervals from planting to harvest in 1999 and 2000 (Table 5.1). Adverse weather conditions prevented sample collection after 76 DAP in 2000, and delayed harvest until November, when roots were at an advanced state of decomposition so only soil samples were collected at this time. Sampling dates were selected based on the length of SCN life cycle (3-4 weeks). In 2000, the first set of samples was collected before 20 days after planting to detect the early stages of the infection.

A single core of soil was obtained at each flag location using an 8 cm diameter by 23 cm deep bucket auger (Riverside Augers, Eijkelkamp, Giesbeek, The Netherlands). The position of the auger was rotated around the flag in successive samplings. Soil cores were placed in individual plastic bags and stored at 4°C upon arrival at the lab until processed. Cysts were extracted from a 100 ml subsample of soil measured by water displacement from each sampled location. A semi-automatic elutriator (Research Services Instrument Shop, The University of Georgia, Athens, GA) was used for cyst extraction following standard procedures (Byrd, et al. 1976) with 60% extraction efficiency. Cysts collected in 75-μ aperture sieves (#200 mesh) were further separated from soil particles following the sugar flotation-centrifugation method (Dunn, 1969). Cysts were then counted under a stereo-microscope. Three cysts per sample were randomly selected, crushed, and eggs and second-stage juveniles were counted, with the average used to determine the eggs per cyst for each sample containing at least one cyst.

Table 5.1. Sampling dates for soil and soybean root samples collected from Fields A and B in 1999 and in 2000.

Sample collected	Field A		Field B	
	Date	DAP†	Date	DAP†
	1999			
Soil	May 13	-8	May 20	4
Soil and roots	June 22	31	June 29	44
Soil and roots	July 20	59	July 28	73
Soil and roots	August 16	86	August 24	100
Soil and roots	September 25	126 (harvest)‡	September 18	125 (harvest)‡
	2000			
Soil	June 5	-4	June 12	3
Soil and roots	June 26	17	June 28	19
Soil and roots	July 24	45	July 25	46
Soil and roots	August 21	73	August 25	77
Soil	November 3	147 (harvest)‡	November 11	155 (harvest)‡

† Days after planting. A negative sign indicates days before sampling.
‡ Samples were collected within a week before or after soybean was harvested.

Three soybean plants were dug out at each flag location using a small shovel, and the soil adhered to the roots was gently shaken off before cutting off the stem of the plant at its base and placing the roots in a plastic bag. At the lab, roots were gently washed with tap water to remove soil, cut into approximately 4-cm sections, and mixed. Two grams of root fragments were stored at 4°C until stained within 24 hours and the rest was discarded. SCN inside roots were stained with a NaOCl-acid fuchsin technique (Byrd et al., 1983) modified as follows. The treatment time and the proportion of chlorine bleach (5.25% NaOCl) used to partially break down plant tissue and facilitate the penetration of the dye were adjusted depending on the age and thickness of the roots. Roots were left in 1:3 bleach: water solution (1.31% NaOCl) for 3 minutes at 17 - 19 DAP, and for 5 minutes at 44 - 46 DAP; older roots were left in 1:1 bleach: water solution (2.62% NaOCl) for 5 minutes at 73 DAP, for 6 minutes at 100 DAP, and for 7-10 minutes at 125

DAP. Nematodes were then stained with acid-fuchsin following the procedure described by Byrd et al. (1983). Stained samples were kept at 4° C until counted.

Roots preserved in acidified glycerol were spread forming a single layer and pressed between clear plastic plates to facilitate visualization of nematodes under a stereo-microscope. SCN developmental stages were determined as illustrated in Agrios (1997) and counted in four categories as follows. Vermiform infective juveniles (J2) were counted separate from J3 and J4 males and females (J3/J4) identified as short, stout, well-stained nematodes; females without eggs were classified as immature females; and whenever eggs were visible inside the females or inside gelatinous matrix, they were counted as mature (gravid) females.

Soil Texture

Maps of sand, clay, and silt proportion in the soil in Field A and Field B from the previous chapter (Chapter Four) (Figure 4.1.c-e, and 4.2.b-d) were used to associate percentage values of sand, clay, and silt predicted by kriging to each SCN observation based on location in the field. In Field A, percent sand in the soil ranged from 45% to 74%, percent clay ranged from 10% to 21%, and percent silt ranged from 8% to 43% (Chapter Four). Soil series in Field A were Belding sandy loam (Coarse-loamy, mixed, frigid Argic Endoaquods), Breckenridge sandy loam (Coarse-loamy, mixed, nonacid, frigid Mollic Endoaquepts), Brookston loam (Fine-loamy, mixed, superactive, mesic Typic Argiaquolls), Conover loam (Fine-loamy, mixed, active, mesic Udollic Endoaqualfs), and Newaygo sandy loam (Fine-loamy over sandy or sandy-skeletal, mixed, frigid Alfic Haplorthods) (Threlkeld and Feenstra, 1974). In Field B, percent sand ranged from 50% to 80%, percent clay ranged from 8% to 23%, and percent silt ranged

from 11% to 29% (Chapter Four), and soil series were Brookston loam, Newaygo sandy loam, and Berville loam (Fine-loamy, mixed, mesic Typic Argiaquolls) (Threlkeld and Feenstra, 1974).

Statistical Analysis

Descriptive statistics were applied to characterize the population of developmental stages in the roots, cysts in the soil, and eggs per cyst at each sampling date for each field. The data was logarithmic transformed to increase symmetry and to stabilize the variance. Cysts, developmental stages in roots, and eggs per cyst means were compared between sampling dates within fields, with Fisher's (protected) LSD test for means (α= 0.05). The effects of each of the soil texture fractions (sand, clay, silt) on transformed cysts, eggs per cyst, and developmental stages in the roots were analyzed with analysis of variance (ANOVA). Only data from samples collected at 31 and 44 DAP in 1999, and 17 and 19 DAP in 2000 were used for analysis of the relationship between SCN and soil texture because SCN counts at later samplings were too low for the analyses to be significant. The regression coefficients were determined on means by simple linear regression analysis. Significant regression models were tested for parallelism by soil fraction across developmental stages, sampling times, and fields. Models were compared by pairs with a sum of squares reduction test on dummy variables (α = 0.05) (SAS®, Release 8; SAS Institute, Cary, NC).

The spatial variability in cyst population was quantified by describing the spatial dependence in the distribution at each sampling time with empirical omnidirectional semivariograms. The semivariogram is a structural tool for depicting the spatial

dependency in a realization of a mean-constant spatial process Z(**s**). Here, the classical Matheron estimator

$$\hat{\gamma}(h) = \frac{1}{2|N(h)|} \sum_{\|\mathbf{s}_i - \mathbf{s}_j\| = h} \{Z(\mathbf{s}_i) - Z(\mathbf{s}_j)\}^2$$

was used (Matheron 1963). The semivariance $\hat{\gamma}(h)$ at a given lag distance h is estimated as one half the average squared difference between all observations at locations $\mathbf{s}_i$, $\mathbf{s}_j$ that are separated by the lag h. Depending on the data and sampling interval used, the shape of the experimental semivariogram may take many forms. In general, the semivariance increases with increasing distance between sample locations, rising to a more or less constant value (the sill) at a given separation distance called the range of spatial dependence. Samples separated by distances closer than the range are spatially related. Those separated by distances greater than the range are no longer spatially autocorrelated. Semivariances may also increase continuously without showing a defined range and sill, thus preventing definition of a spatial variance, indicating that the range is greater than the largest lag (h), or the presence of a trend effect and/or nonstationarity (Webster and Burguess, 1980). Stationarity means that the random field sampled looks similar everywhere. A random field is second-order stationary if the mean of the random field is constant and does not depend on locations, and the covariance between two observations is only a function of their spatial separation (Schabenberger and Pierce, 2002). Whenever semivariograms showed nonstationarity, the data were detrended by carrying out a polynomial least squares regression and semivariogram analysis was performed on the residuals. Other semivariograms show complete absence of spatial structure, implying that the value observed at one location carries no information about values at other locations. Nugget effect (C_0) is a discontinuity of the semivariance near the origin (lag

h=0). It consists of measurement error variability and/or the sill of a micro-scale spatial process. The error variance is a measure of repeatability of the data measurements, whereas micro-scale variance is a measure of variation that occurs at separation distances less than the smallest sample spacing (Cressie, 1993).

Empirical omnidirectional semivariograms of cysts [$\log_{10}$ (cysts 100 cm^{-3} of soil+ 1)] at planting for both fields were obtained from Avendaño et al. (2003, Chapter Three), and for all other sampling times were calculated for lags ranging from 1 to 30 m, with a lag tolerance of one half of the lag used (h/2). The minimum number of pairs required for each lag was 30. The reduced number of samples in the E-W direction in Field A, and the predominant SW-NE arrangement of the samples in Field B prevented the calculation of reliable directional semivariograms. The parameters of the semivariograms were estimated by least squares fitting of theoretical semivariogram models with the Surfer 7.02 software package (Golden Software, 1999).

Kriging is the best linear unbiased prediction of regionalized variables at unsampled locations using the structural properties of the semivariogram and the sampled values at observed locations. When a drift or trend (non-stationarity of the mean) existed within the area of interest, universal kriging was used; otherwise, ordinary kriging was the method of choice. Universal kriging takes the drift into account provided the form of the drift and the semivariogram are known (Journel and Huijbregts 1978). The distributions of cysts, were mapped separately for each sampling time and field with ordinary or universal kriging predicting values at the nodes of a 1x1 m cell grid using all the data points in each sample and the parameters from the models fitted to the empirical

semivariograms. Maps of cyst distribution at planting in both fields were obtained from Avendaño et al. (2003, Chapter Three).

RESULTS

SCN Population in the Soil

Cyst density in the soil was relatively low in Field A and Field B at the beginning of the study (Tables 5.2 and 5.3). In Field A, cyst population density remained low throughout 1999, decreased slightly overwinter and over the following season, reaching an average of 7 cysts 100 cm^{-3} of soil at harvest in 2000 (Table 5.2). In Field B, cyst population tripled its density 44 DAP, then decreased significantly at 73 DAP and remained stable until harvest in 1999. Cyst densities declined over the 2000-growing season but reached almost 47 cysts 100 cm^{-3} of soil at harvest, density comparable to that observed at 44 DAP in 1999 (Table 5.3).

The number of eggs per cyst varied between fields, sampling times and years (Tables 5.2 and 5.3). In Field A, the number of eggs per cyst was lower in 1999 than in 2000 with the lowest number found at 59 DAP in 1999; and the highest at 17 DAP in 2000 (Table 5.2). In Field B, the number of eggs per cyst varied greatly in 1999, but stayed constant at a moderate level in 2000 (Table 5.3).

SCN Population in Soybean Roots

SCN population in the roots behaved similarly in Fields A and B in 1999, but differed greatly in 2000 (Tables 5.2 and 5.3). All stages, including a few mature females, were detected in the roots collected at 31 and 44 DAP from Fields A and B, respectively. Very few nematodes were found in the roots collected later in the 1999 season.

Table 5.2. SCN mean population density in soybean roots and in the soil in Field A in 1999 and in 2000.

		Soil		Soybean roots†			
	Sampling event‡	Cysts§	Eggs per cyst¶	J2	J3/J4	Immature females	Mature Females
	DAP	Counts 100 cm^{-3} of soil ± std	Counts ± std	Counts 2 g^{-1} of soybean root ± std			
1999	Planting	6.4 ± 6.6 c	12.7 ± 28.0 c	-	-	-	-
	31	10.6 ± 13.0 b	16.4 ± 27.7 c	19.8 ± 28.8 a	38.4 ± 51.4 a	18.8 ± 28.0 a	0.8 ± 2.0 a
	59	9.4 ± 20.6 c	11.8 ± 26. 0 c	0.8 ± 1.7 c	0.7 ± 1.7 c	0 ± 0.2 c	0.1 ± 0.5 c
	86	14.9 ± 15.8 a	37.1 ± 51.4 b	0.3 ± 0.9 d	0.2 ± 0.5 d	0 ± 0.2 c	0.1 ± 0.3 cd
	126	11.4 ± 12.9 b	47.8 ± 72.9 b	0 d	0 d	0 c	0 d
2000	Planting	8.2 ± 10.8 c	48.4 ± 48.6 b	-	-	-	-
	17	7.5 ± 10.9 cd	53.8 ± 48.0 a	8.9 ± 21.4 b	51.2 ± 113.2 b	0.8 ± 4.5 b	0 d
	45	4.0 ± 6.2 e	32.7 ± 52.8 b	0 d	0.1 ± 0.5 d	0 ± 0.1 c	0.4 ± 1.1 b
	73	6.1 ± 8.1 cd	18.9 ± 38.7 c	0 d	0 ± 0.1 d	0 ± 0.5 c	0 ± 0.3 cd
	147	6.9 ± 11.6 de	32.7 ± 40.8 b	-	-	-	-

Means within a column followed by the same letter are not significantly different, Fisher's (protected) LSD test (alpha=0.05) on logarithmic transformed data, original data are shown for clarity.

† SCN developmental stages counted in stained soybean roots were vermiform, infective juveniles (J2), male and female third and fourth stage larvae identified as stout, brightly stained nematodes (J3/J4), cyst-shaped females without visible eggs (immature females), and females with eggs inside or in gelatinous matrix (mature females).

‡ Samples were collected at the number of days after planting indicated (DAP).

§ Cysts were extracted by elutriation from subsamples (100 cm^3 of soil) from single core samples collected from Field A.

¶ Average number of eggs per cyst after crushing three cysts in a sample and counting the number of eggs in each cyst.

Table 5.3. SCN mean population density in soybean roots and in the soil in Field B in 1999 and in 2000.

	Sampling event‡	Soil		Soybean roots†			
		Cysts§	Eggs per cyst¶	J2	J3/J4	Immature females	Mature Females
	DAP	Counts 100 cm^{-3} of soil ± std	Counts ± std	Counts $2g^{-1}$ of soybean root ± std			
1999	Planting	14.5 ± 22.1 e	94.8 ± 81.2 cd	-	-	-	-
	44	48.0 ± 55.2 a	25.5 ± 34.7 e	49.7±88.2 a	22.7±22.4 c	10.4±22.7 a	4.7±13.7 a
	73	28.4 ± 46.3 bc	60.3 ± 51.9 d	0.6±1.2 c	2.3±3.3 d	1.5±2.1 b	1.2±1.7 b
	100	28.8 ± 33.3 b	119.5 ± 108.1 ab	0.5±1.5 cd	0.5±1.2 e	0.1±0.4 c	0.4±0.8 c
	125	27.5 ± 43.2 bc	139.1 ± 99.7 a	0 d	0±0.1 e	0±0.1 c	0.1±0.5 cd
2000	Planting	33.0 ± 58.4 bcd	75.7 ± 70.4 d	-	-	-	-
	19	21.7 ± 31.4 bcd	61.5 ± 56.6 d	7.9±17.4 b	159.4±161.8 a	14.1±34.7 a	0±0.3 d
	46	19.0 ± 27.8 de	71.0 ±76.9 d	0.4±3.5 cd	7.5±25.7 d	6.9±24.7 b	1.8±5.7 bc
	77	20.6 ± 27.6 cd	95.1 ± 85.0 bcd	5.5±12.3 b	66.0±62.6 b	11.7±17.1 a	1.5±3.0 b
	155	46.7 ± 53.4 a	95.9 ± 82.4 abc	-	-	-	-

Means within a column followed by the same letter are not significantly different, Fisher's (protected) LSD test (alpha=0.05) on logarithmic transformed data, original data are shown for clarity.

† SCN developmental stages counted in stained soybean roots classified as described in Table 2.

‡ Samples were collected at the number of days after planting indicated (DAP).

§ Cysts were extracted by elutriation from subsamples (100 cm^3 of soil) from single core samples collected from Field B.

¶ Average number of eggs per cyst after crushing three cysts in a sample and counting the number of eggs in each cyst.

In Field A there was a large number of J2 and J3/J4 in the roots 17 DAP in 2000, but after this sampling nematode counts were practically zero (Table 5.2).

In Field B there was a very large number of J3/J4, as well as a relatively high number of immature females at 19 DAP in 2000 (Table 5.3). Although all stages except mature females were well represented in the roots, numbers fell at 46 DAP. At 77 DAP, a new root invasion was detected, with J2 counts comparable to those observed at 19 DAP. A large number of necrotic tissue marks were observed in the roots from both fields in 2000 (not quantified), even where not many nematodes were found.

SCN Population Dynamics and Soil Texture

The two fields were distinct in regards to the effect of soil texture on SCN population in the soil and in the roots. Sand, clay and silt proportion in the soil had little effect on SCN population density or eggs per cyst in Field A (Tables 5.4-6, Figures 5.1-3). The number of cysts in the soil increased with increasing percentage of sand only at 59 DAP (Table 5.4), and decreased with increasing percentage of clay at the same rate at 59, 86, and 126 DAP in 1999 and at 17 and 147 DAP in 2000 (Table 5.4). Silt had no effect on cysts in Field A (Table 5.4). The number of eggs per cyst increased at 59 and 126 DAP, and decreased at planting in 1999 with increasing sand percentage (Table 5.5). Although coefficients of determination were higher for clay, the effect was also only significant for three sampled times. The rate of decrease in eggs per cyst numbers as clay percentage in the soil increased was similar at 86 DAP in 1999, and at 76 and 150 DAP in 2000 in Field A (Table 5.5). The proportion of silt had no effect on eggs per cyst in Field A (Table 5.5). Only sand and silt proportion affected SCN population in soybean roots in Field A, and this effect was only significant on immature females in the roots at

31 DAP in 1999 (Figures 5.1.c, 5.3.c, Table 5.6). Although the number of immature females decreased with increasing clay percentage (Figure 5.2.c), the effect was not statistically significant (Table 5.6).

Table 5.4. Simple linear regression parameters for the effect of soil texture on SCN cyst population density in the soil in Fields A and B in 1999 and in 2000.

Sampling	Soil separate								
event†	Sand			Clay			Silt		
DAP	y_0‡	a§	r^2	y_0‡	a§	r^2	y_0‡	a§	r^2
				Field A 1999					
Planting	NS			NS			NS		
31	NS			NS			NS		
59	-0.56	0.02b	0.21	1.42	-0.04a	0.57	NS		
86	NS			1.83	-0.05a	0.69	NS		
126	NS			1.89	-0.06a	0.64	NS		
				Field A 2000					
Planting	NS			NS			NS		
17	NS			1.35	-0.04a	0.40	NS		
45	NS			NS			NS		
73	NS			NS			NS		
147	NS			2	-0.09a	0.75	NS		
				Field B 1999					
Planting	-2.03	0.04a	0.77	2.21	-0.09a	0.76	2.53	-0.08a	0.81
44	-1.23	0.04a	0.61	2.55	-0.06a	0.71	2.98	-0.07a	0.77
73	-1.35	0.04a	0.75	2.35	-0.07a	0.60	2.49	-0.06ab	0.73
100	-1.5	0.04a	0.75	2.42	-0.08a	0.80	2.61	-0.06ab	0.62
125	-0.89	0.03a	0.54	2.14	-0.06a	0.89	2.59	-0.07a	0.66
				Field B 2000					
Planting	-1.58	0.04a	0.76	2.53	-0.09a	0.63	2.76	-0.08a	0.76
19	-0.85	0.03a	0.65	1.77	-0.05a	0.54	2.00	-0.04b	0.50
46	-1.07	0.03a	0.52	1.82	-0.05a	0.40	1.94	-0.04b	0.41
77	NS			NS			NS		
155	-1.69	0.05a	0.67	2.69	-0.09a	0.79	2.83	-0.07a	0.70

Regression models significant at the 5% level.
† Soil samples were collected at the days after planting (DAP) indicated.
‡ Y_0 is the intercept of the regression model fitted [Log_{10} (cysts $100cm^{-3}$ of soil +1)]
§ a is the slope of the regression model fitted. Slopes followed by the same letter within each soil separate were not significantly different ($\alpha = 0.05$).

Table 5.5. Simple linear regression parameters for the effect of soil texture on the number of SCN eggs per cyst in Fields A and B in 1999 and in 2000.

Sampling event†	Soil separate								
	Sand			Clay			Silt		
DAP	y_0‡	a§	r^2	y_0‡	a§	r^2	y_0‡	a§	r^2
				Field A 1999					
Planting	1.57	-0.01b	0.18	NS			NS		
31	NS			NS			NS		
59	-0.91	0.02a	0.17	NS			NS		
86	NS			2.86	-0.12b	0.48	NS		
126	-0.92	0.03a	0.17	NS			NS		
				Field A 2000					
Planting	NS			NS			NS		
17	NS			NS			NS		
45	NS			NS			NS		
73	NS			2.43	-0.11b	0.49	NS		
147	NS			2.53	-0.09b	0.53	NS		
				Field B 1999					
Planting	NS			NS			2.86	-0.05b	0.32
44	2.09	-0.01b	0.26	0.55	0.04a	0.46	0.46	0.03a	0.34
73	NS			NS			NS		
100	0.43	0.02a	0.22	NS			2.69	-0.04b	0.41
125	NS			NS			NS		
				Field B 2000					
Planting	-0.14	0.02a	0.23	2.62	-0.08b	0.32	2.45	-0.05b	0.33
19	NS			NS			NS		
46	0.03	0.02a	0.28	2.2	-0.06b	0.34	NS		
77	NS			NS			NS		
155	NS			NS			NS		

Regression models significant at the 5% level.
† Soil samples were collected at the days after planting (DAP) indicated.
‡ Y_0 is the intercept of the regression model fitted [Log_{10} (eggs per cyst $100cm^{-3}$ of soil +1)]
§ a is the slope of the regression model fitted. Slopes followed by the same letter within each soil separate were not significantly different ($\alpha = 0.05$).

In Field B, the proportion of sand, clay, and silt had a strong effect on SCN cyst population in the soil. Cyst densities increased linearly with increasing sand percentage, with a slope similar across sampling times ($\alpha = 0.05$), and different from the model fitted

for Field A (Table 5.4). The effect of clay was also consistent across sampling times, cyst densities decreased with increasing clay percent, with a slope not significantly different from the trend fitted for Field A (Table 5.4). Cyst densities were also negatively correlated with silt, with a slope consistent across sampling times (Table 5.4). The relationship between soil texture and eggs per cyst varied greatly (Table 5.5). At 44 DAP eggs per cyst decreased with increasing sand, and increased with increasing clay and silt percentages, but the slope of the regression lines had the opposite sign of the trends fitted for the other significant sampling times and of those observed for cysts (Table 5.5). The number of J3/J4 stages and immature females in the roots was affected by sand, clay, and silt (Table 5.6, Figures 5.1.e,f – 3.e, f). The population density of J2s in the root was not affected by soil texture (Table 5.6, Figures 5.1.d – 3.d). In Field B, The number of J3/J4 and immature females increased with increasing sand percentage at the same rate in 1999 and 2000, and decreased with increasing clay percent in 1999, and increasing silt percent in 1999 and 2000 (Table 5.6, Figures 5.1.e-f – 3.e-f).

Spatial Analysis of Cyst Population

The structure in the spatial distribution of cysts was highly variable between fields and in time. Semivariogram models revealed variable degree of spatial structure, ranging from highly structured (Field A at 31 DAP in 1999) (Table 5.7, Figure 5.6.a) to complete absence of spatial dependence (Field B at 100 DAP in 1999 and 19 DAP in 2000) (Table 5.7, Figure 5.7.c, e)). Overall, the degree of spatial structure in cyst distribution was higher in Field A than in Field B (lower nugget variance to total variance ratio) (Table 5.7). A wave model, also known as hole-effect model, is applicable when the attribute of interest shows some degree of periodicity in the spatial distribution.

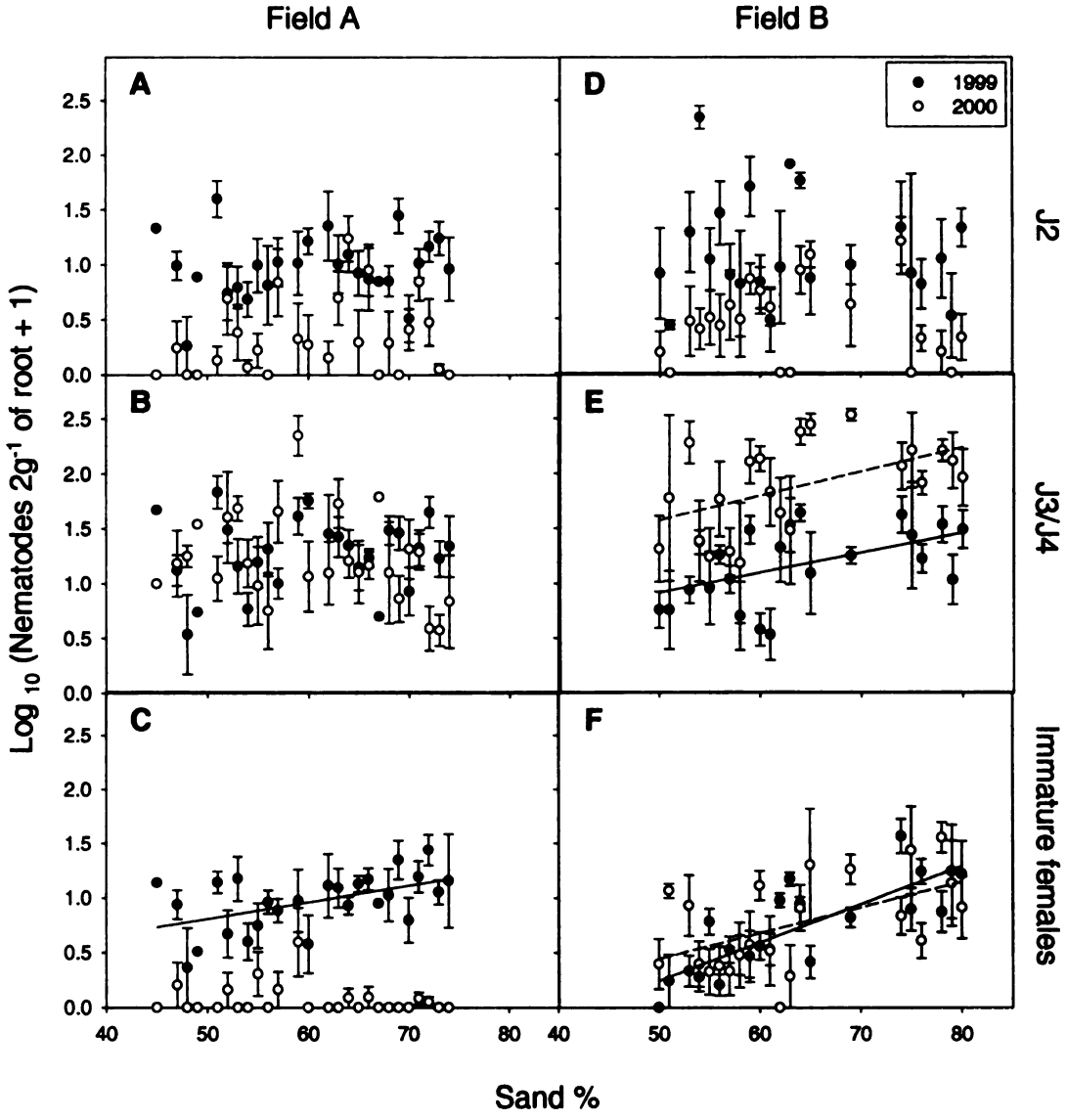

Figure 5.1. Relationship between sand percentage in the soil and SCN second- (J2), third/fourth-juveniles (J3/4), and immature females in soybean roots in Field A (A-C) and Field B (D-F). In Field A, root samples were collected at 31 and 17 days after planting (DAP), and in Field B at 44 and 19 DAP in 1999 and in 2000, respectively. Parameters of the regression lines are shown in Table 5.6.

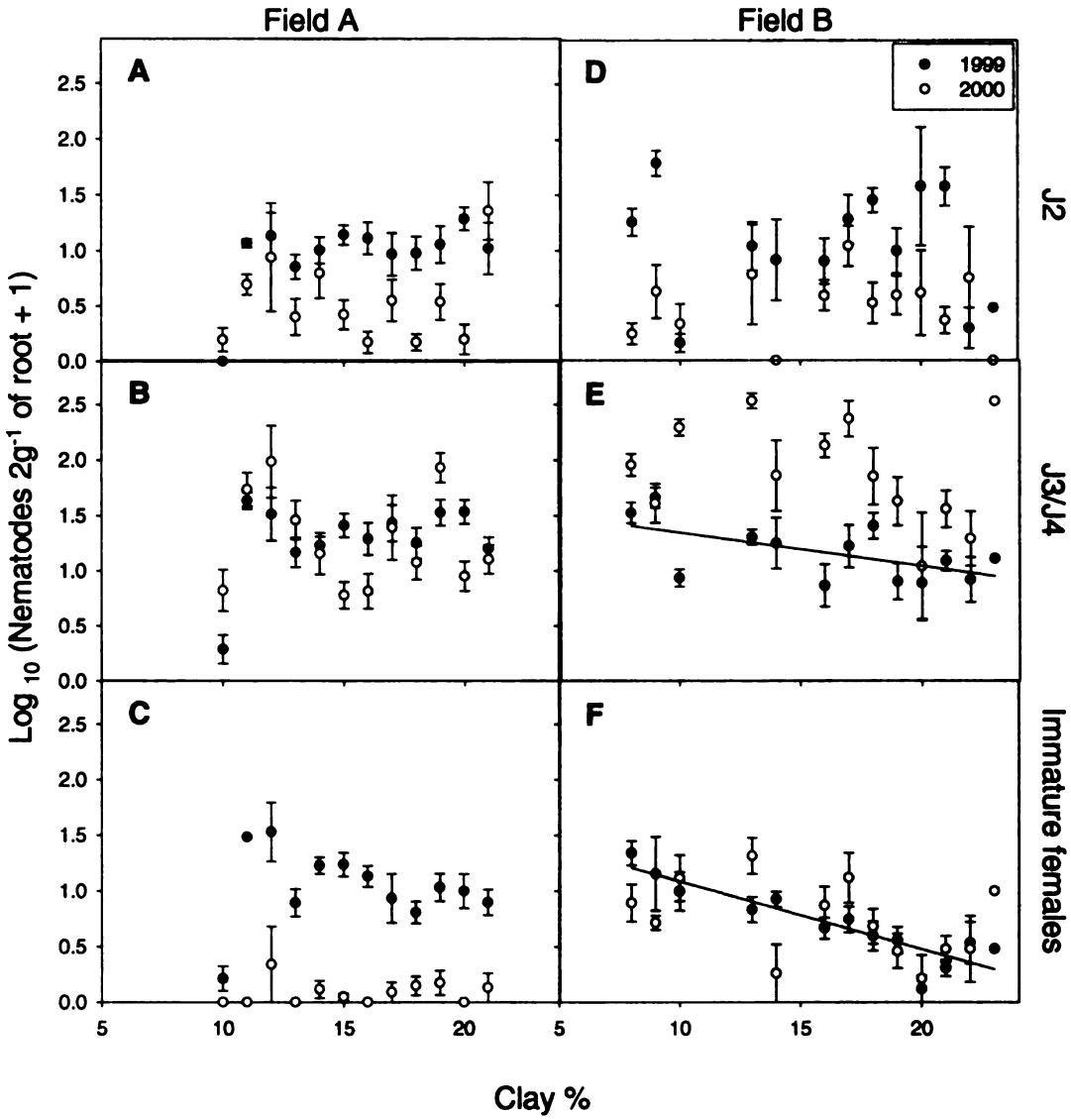

Figure 5.2. Relationship between clay percentage in the soil and SCN second- (J2), third/fourth-juveniles (J3/4), and immature females in soybean roots in Field A (A-C) and Field B (D-F). In Field A, root samples were collected at 31 and 17 days after planting (DAP), and in Field B at 44 and 19 DAP in 1999 and in 2000, respectively. Parameters of the regression lines are shown in Table 5.6.

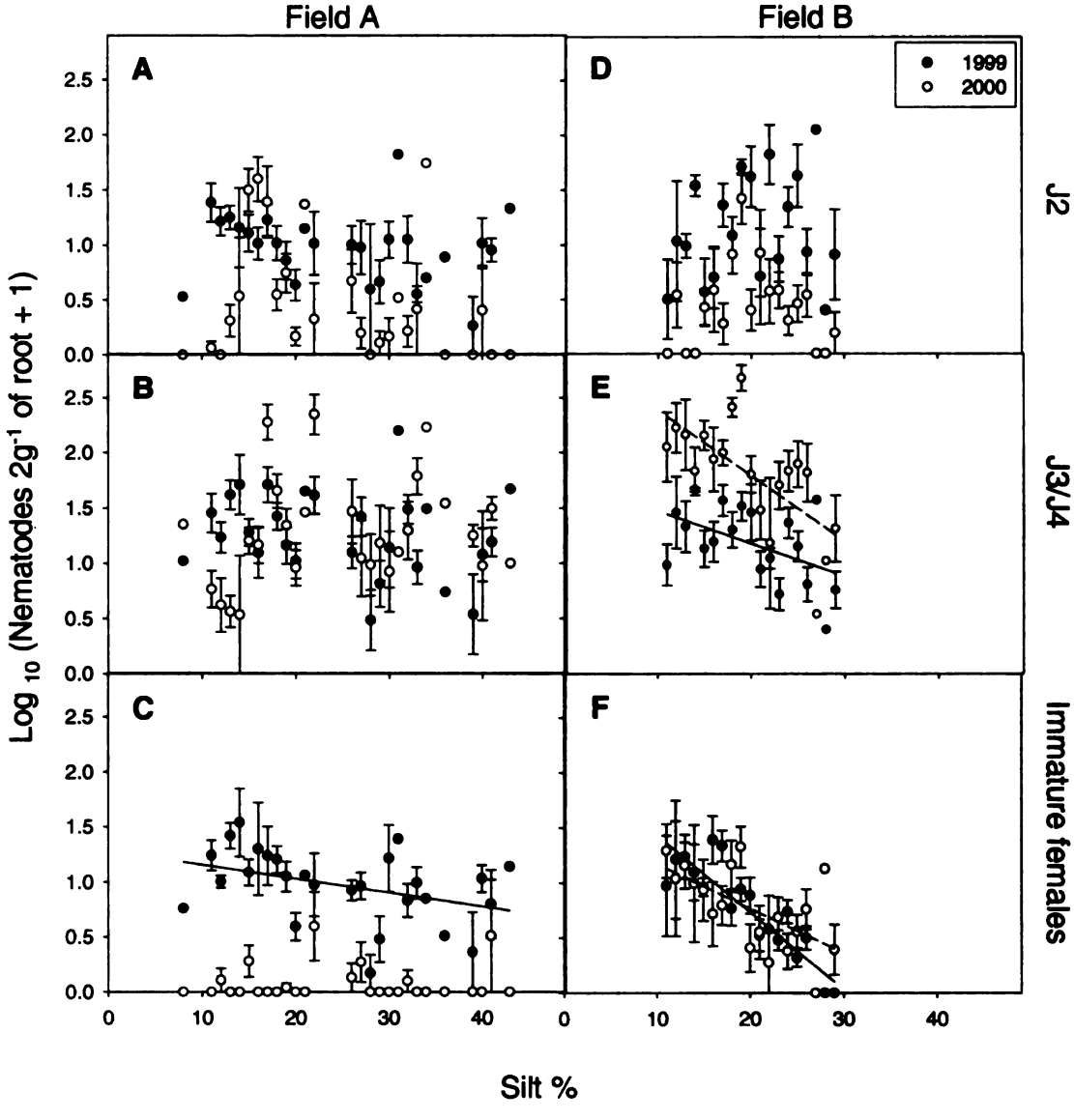

Figure 5.3. Relationship between silt percentage in the soil and SCN second- (J2), third/fourth-juveniles (J3/4), and immature females in soybean roots in Field A (A-C) and Field B (D-F). In Field A, root samples were collected at 31 and 17 days after planting (DAP), and in Field B at 44 and 19 DAP in 1999 and in 2000, respectively. Parameters of the regression lines are shown in Table 5.6.

Table 5.6. Simple linear regression parameters for the effect of soil texture on SCN population density in soybean roots in Fields A and B in 1999 and in 2000 shown in Figs. 5.1-3.

	Soil separate								
	Sand			Clay			Silt		
SCN stage†	y_0‡	a§	r^2	y_0‡	a§	r^2	y_0‡	a§	r^2
				Field A 1999¶					
J2	NS			NS			NS		
J3/J4	NS			NS			NS		
Immature females	0.05	0.01b	0.26	NS			1.28	-0.01c	0.15
				Field A 2000¶					
J2	NS			NS			NS		
J3/J4	NS			NS			NS		
Immature females	NS			NS			NS		
				Field B 1999¶					
J2	NS			NS			NS		
J3/J4	0.005	0.02a	0.25	1.65	-0.03a	0.34	1.76	-0.03b	0.23
Immature females	-1.5	0.03a	0.66	1.69	-0.06a	0.81	2.13	-0.07a	0.78
				Field B 2000¶					
J2	NS			NS			NS		
J3/J4	0.47	0.02a	0.26	NS			2.98	-0.06ab	0.44
Immature females	-0.73	0.02a	0.28	NS			1.57	-0.04a	0.37

Regression models significant at the 5% level.

† SCN developmental stages as defined in Table 2.

‡ Y_0 is the intercept of the regression model fitted [Log_{10} (nematodes $2g^{-1}$ of root +1)]

§ a is the slope of the regression model fitted. Slopes followed by the same letter within each soil separate were not significantly different ($\alpha = 0.05$).

¶ Root samples were collected at 31 and 17 DAP in 1999 and 2000, respectively from Field A, and at 44 and 19 DAP in 1999 and 2000, respectively from Field B.

Certain periodicity was detected in all empirical semivariograms of cyst distributions in our study. However, because of the large variance at short lags (nugget variance) wave models could only be fitted at 31 and 45 DAP in Field A (Table 5.7, Figures 5.4.a,f), and at 125, 46, 77, and 155 DAP in Field B (Table 5.7, Figures 5.5.d, f, g, h) and other models had to be used instead with poor fit. In some cases, the spatial dependence in cyst distribution was best described by nesting several models to describe a short range variability nested within a longer range variability. This was the case at planting and at 31 DAP in 1999 and at 147 DAP in 2000 in Field A, and at planting in 2000 in Field B (Table 5.7), when a wave model (range < 10 m) was nested within a spherical or an exponential model (range > 100 m), in addition to the nugget effect. The range of spatial autocorrelation in Field A decreased from 819 m (planting) to 125 m at harvest in 1999 and 122m at planting in 2000. However, at 17 DAP in 2000 the range increased and remained constant at approximately 290 m through out the 2000 growing season until harvest (Table 5.7). In Field B, there was great variability in the range of spatial dependence. The range for spherical semivariogram models decreased from 395 m (44 DAP) to 153 m (73 DAP) in 1999, and increased at planting in 2000 (268 m) (Table 5.7). The range for wave models also decreased in 1999 from 12 m at planting to 8 m at harvest, but varied greatly in 2000 (Table 5.7).

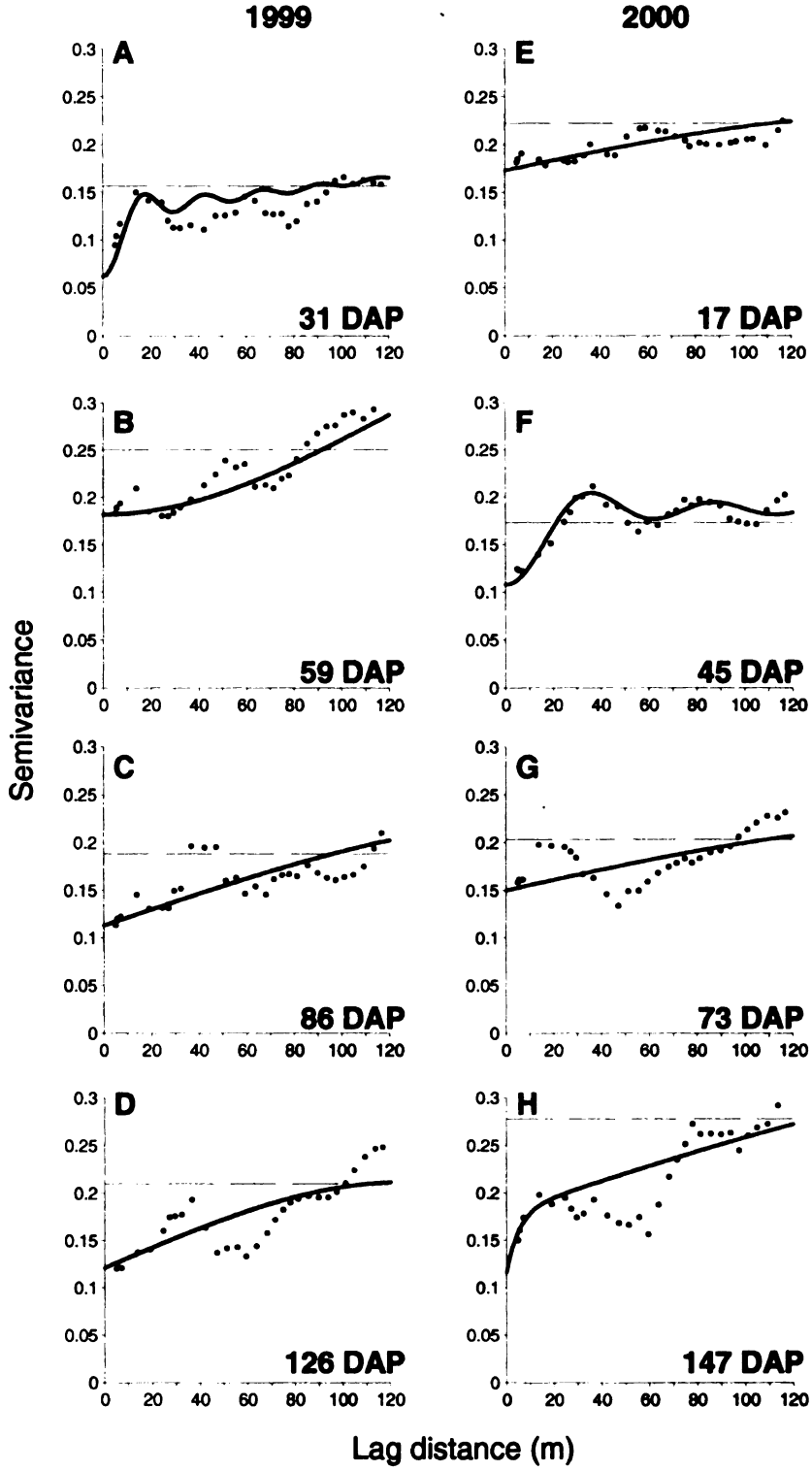

Figure 5.4. Semivariograms of cyst population density [Log_{10}(cysts 100 cm^{-3} of soil +1)] in Field A at (A) 31, (B) 59, (C) 86, and (D) 126 DAP in 1999; and at (E) 17, (F) 45, (G) 73, and (H) 147 DAP in 2000. Black circles indicate omnidirectional empirical semivariogram, the solid line is the theoretical model fitted by means of least squares, and the dashed line is the sample variance.

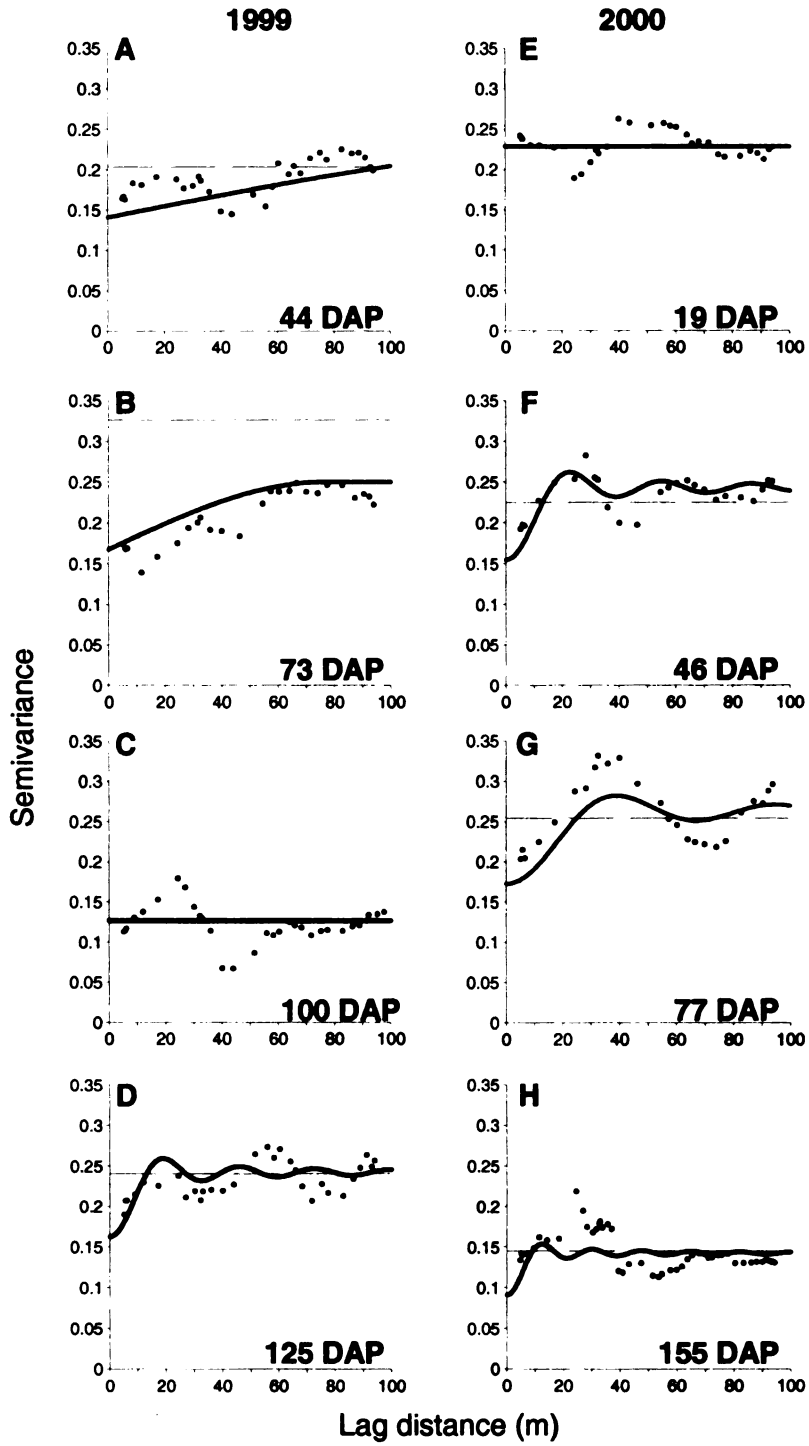

Figure 5.5. Semivariograms of cyst population density [Log_{10}(cysts 100 cm^{-3} of soil +1)] in Field B at (A)44, (B) 73, (C) 100, and (D) 125 DAP in 1999; and at (E) 19, (F) 46, (G) 77, and (H) 155 DAP in 2000. Black circles indicate omnidirectional empirical semivariogram, the solid line is the theoretical model fitted by means of least squares, and the dashed line is the sample variance.

Table 5.7. Parameters of the theoretical semivariogram models of SCN cyst population density from samples collected at monthly intervals in Fields A and B in 1999 and in 2000.†

		Drift‡	Model function	C_0§	C¶	Range#	$\frac{C_0}{C+C_0}$ ††
Field A‡‡						m	
1999	Planting§§	Linear	Nugget	0.08			0.50
			Wave		0.04	3.6	
			Spherical		0.04	819.3	
	31 DAP¶¶	None	Nugget	0.06			0.28
			Spherical		0.08	753	
			Wave		0.07	4.33	
	59 DAP	Linear	Nugget	0.18			0.43
			Gaussian		0.24	164.1	
	86 DAP	Linear	Nugget	0.11			0.5
			Spherical		0.11	316.7	
	126 DAP	Linear	Nugget	0.12			0.57
			Spherical		0.09	124.9	
2000	Planting§§	No	Nugget	0.18			0.69
			Spherical		0.08	122	
	17 DAP	Linear	Nugget	0.17			0.74
			Spherical		0.06	286.7	
	45 DAP	Linear	Nugget	0.11			0.58
			Wave		0.08	8.9	
	73 DAP	Linear	Nugget	0.15			0.68
			Spherical		0.07	285	
	147 DAP	Linear	Nugget	0.11			0.34
			Exponential		0.06	10.4	
			Spherical		0.15	295	

Table 5.7 (cont'd).

Field B							
1999	Planting§§	Linear	Nugget	0.07			0.35
			Wave		0.13	12.16	
	44 DAP	Linear	Nugget	0.14			0.61
			Spherical		0.09	395	
	73 DAP	None	Nugget	0.17			0.68
			Spherical		0.08	152.6	
	100 DAP	Linear	Nugget	0.13			1
	125 DAP	Linear	Nugget	0.16			0.67
			Wave		0.08	8.4	
2000	Planting§§	Linear	Nugget	0.09			0.37
			Wave		0.08	2.62	
			Spherical		0.07	268	
	19 DAP	Linear	Nugget	0.23			1
	46 DAP	Linear	Nugget	0.15			0.62
			Wave		0.09	6.3	
	77 DAP	Linear	Nugget	0.17			0.65
			Wave		0.09	8.7	
	155 DAP	Linear	Nugget	0.09			0.64
			Wave		0.05	2.7	

† Models were fitted by least squares on empirical semivariograms calculated for lags ranging from 1 to 25 m (h).
‡ Whenever semivariograms showed nonstationarity, the data were detrended carrying out a simple polynomial least squares regression and semivariogram analysis was performed on the residuals. The polynomial order of the trend removed is indicated.
§ C_0 is the nugget effect or a discontinuity in semivariance at the origin due to microscale variability or sampling error.
¶ C is the partial sill.
# The range of spatial autocorrelation.
†† $C_0/(C+C_0)$ is an indicator of the degree of spatial structure, the lower the number the stronger the spatial autocorrelation.
‡‡ Semivariograms were calculated for $\log_{10}$ (cysts 100 cm^{-3} of soil+ 1).
§§ Planting data from Avendaño et al. (2003a).
¶¶ Cysts were extracted from soil subsamples collected at the indicated days after planting (DAP).

Cyst population densities were distributed in more or less defined clusters in Field A (Figure 5.6) The size and shape of the clusters of higher cyst density in Field A, changed over time, but the locations of hot spots or high cyst density areas remained the same. Two relatively large areas located on the first 200 m and between 500 and 700 m from the south boundary of the area sampled had consistently more cysts at each sampling time than the rest of the field sampled (Figure 5.6). These areas corresponded with areas of high sand percentage in the soil (Figure 4.1.c). There was a trend towards a better definition of the hot spots boundaries in Field A in 1999 (Table 5.7, Figure 5.6), whereas in 2000 there was great variability in the spatial patterns, as reflected by the structure of the semivariogram models (Table 5.7, Figure 5.6). In the cases were a wave model was nested within another model with a larger range, cyst distributions appeared in very small clusters grouped into larger clusters showing two scales of spatial variability (Figure 5.4.a, b, h, Figure 5.6).

The spatial distribution of cysts graded from high density in the northeast to low density in the southwest side in Field B (Figure 5.7). As the 1999 season progressed the area of high cyst density increased in size towards the south side of the field (Figure 5.7). The lack of spatial structure in cyst population at 100 DAP (Figure 5.5.c) produced a map showing uniform distribution of cysts throughout the field with a population density equal to the mean (Figure 5.7). At harvest, the areas of highest cyst density were again concentrated on the north side of the field (Figure 5.7). At planting in 2000, cyst densities distribution was similar to that observed the year before, but with high cyst density in a larger area of the field (Figure 5.7). Cyst density decreased somewhat as the season progressed, but at harvest the soil in the north two thirds of the field had high levels of

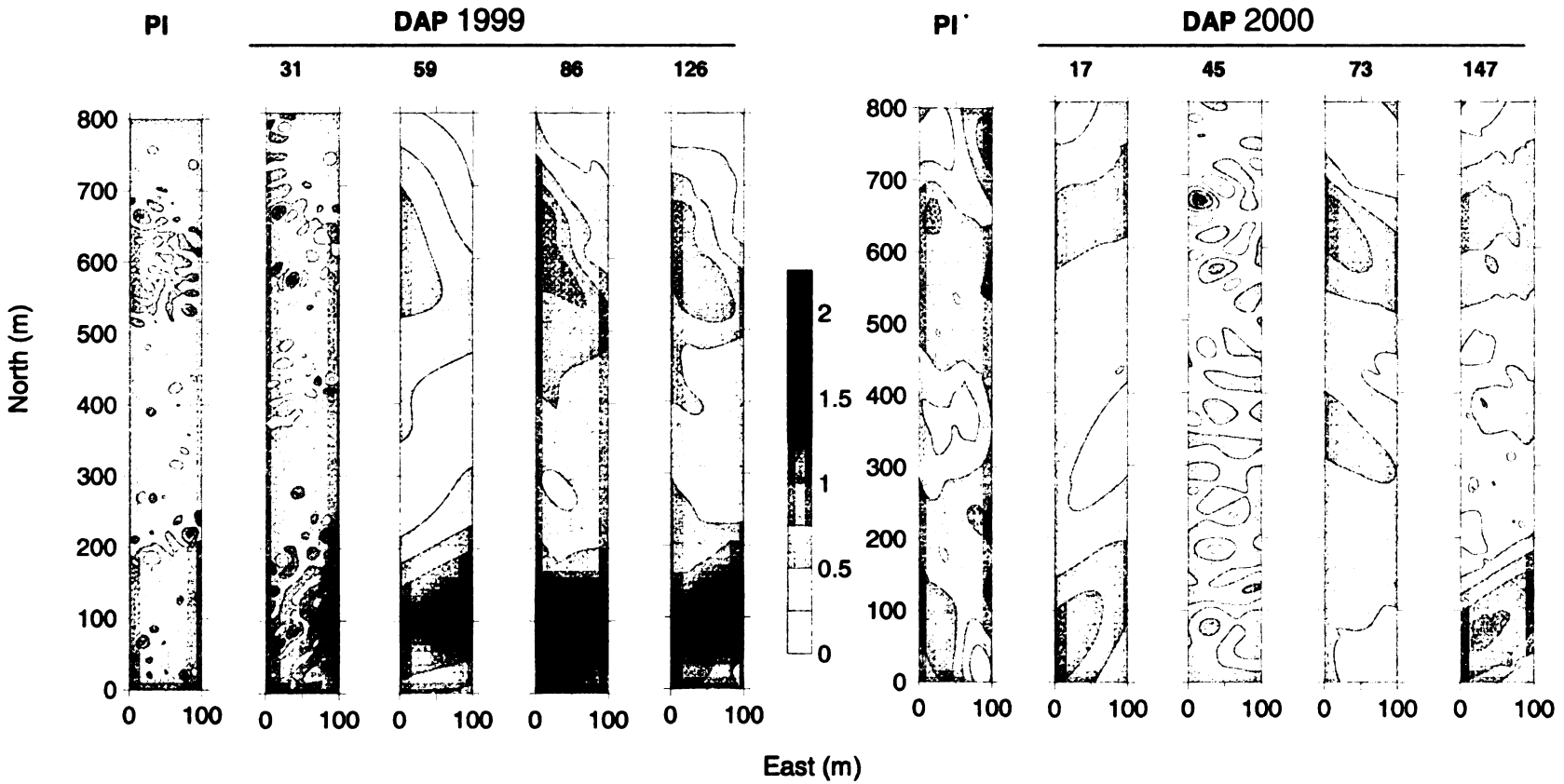

Figure 5.6. SCN cyst spatial distribution in Field A at planting (Pl) (Avendaño et al., 2003- Chapter Three), and at the days after planting (DAP) indicated, in 1999 and in 2000. Shadings scale indicate levels of $\log_{10}$ (cysts 100 cm^{-3} soil + 1). Parameters of the empirical semivariograms are shown in Table 5.7.

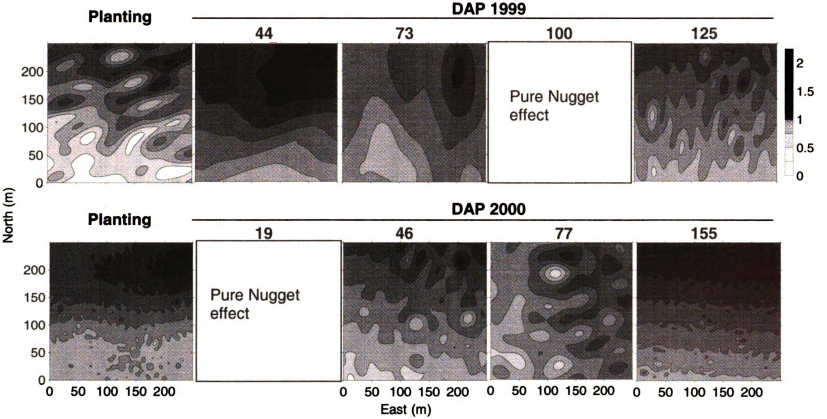

Figure 5.7. SCN cyst spatial distribution in Field B at planting (Avendaño et al., 2003- Chapter Three), and at the days after planting (DAP) indicated, in 1999 and 2000. Shadings scale indicates levels of $\log_{10}$ (cysts 100 cm^{-3} soil + 1). Parameters of the empirical semivariograms are shown in Table 5.7.

cysts while levels remained low on the south side (Figure 5.7). The spatial pattern of cysts at 73 DAP in 2000 was somewhat different of that observed at the other sampling times. At this time, there was more variability along the east-west axis than the north-south axis of the field (Figure 5.7).

DISCUSSION

The relationship between soil texture and SCN population dynamics in the soil and in soybean roots was studied as part of a project designed to investigate the potential for SSM for SCN in Michigan. The two fields selected for this research provided an interesting scenario since they differed in SCN population density and in soil physical characteristics

SCN populations in Field A and Field B were active, and infections occurred through out the season, as indicated by the presence of all developmental stages in the roots at almost all sampling times since early in the season. The rapid increase in the number of cysts in the soil and the presence of gravid females in the roots about a month after planting followed by a decline in SCN population density in roots and soil indicate that most population increase occurred in the first generation, as it seemed to be the case in Illinois (Lawn and Noel 1986). The rate of increase in plant-parasitic nematode populations is often density-dependent and driven largely by the amount of food available (Seinhorst, 1966). The optimal activity of SCN and, therefore, highest population increases normally occur during May and June when roots are primarily in or near the plant row (Alston and Schmitt, 1987; Bonner and Schmitt 1985). When the density of a population exceeds its food supply, starvation results, and the population decreases until a

new equilibrium with the food supply is reached (Seinhorst, 1966). Since root growth deep into the soil and between rows occurs later, population increase of SCN lags behind that in the row, even if population density does not exceed its food supply (Alston and Schmitt, 1987; Bonner and Schmitt 1985). Yen et al. (1995) attributed a decline in hatching in August to early dormancy induced in July-August following planting in May-June. They propose the existence of a primary generation that takes 20 to 40 days from first hatching at planting (dependent on soil temperature) followed by dormancy as an obligate condition for eggs retained within cysts of the first generation. Additional, smaller generations may be produced by non-dormant J2s hatched from eggs produced in the gelatinous matrix. Early damage to soybean roots caused by an abundance of J2s early in the season, and consequently reduced root growth may have caused the drastic population decline in the roots observed in Field A and Field B at mid season, similar to the observations of Wallace et al. (1995). In addition, the reduction in number of larval stages in the roots associated with the increase in eggs per cyst observed in August and at harvest in Field B in 1999 and 2000, and in Field A in 1999 was probably the result of reduced hatching due to induced early dormancy and a decline in the stimulatory effect of roots with plant age (Sikora and Noel, 1996; Tefft and Bone, 1995, Yen et al., 1995; Hill and Schmitt, 1989).

SCN overwinter survival was good based on the similarity in the number of cysts at harvest in 1999 and at planting in 2000. Root penetration was reported equivalent in susceptible and resistant soybeans (Acedo et al., 1984; Melton et al., 1986), but final egg density and cyst production were significantly lower for the resistant cultivar than for the susceptible cultivar (Wallace et al., 1995). Here, infective juveniles were successfully

invaded the SCN-resistant soybean variety planted in 2000, reaching the adult stage in less than 20 days after planting. Six weeks after planting, however, the number of cysts in the soil declined about 30% in Field B and 50% in Field A. At 77 DAP a second massive infection of the roots was observed in Field B. This second infection was also successful as evidenced by greater number of cysts at harvest than at planting. The numbers of eggs per cyst at harvest in 2000 were also higher or similar to those recorded at planting the same year or at harvest in 1999. Apparently, planting a resistant cultivar for one year was not an effective tactic to reduce SCN levels, particularly in Field B.

Cyst population density was positively correlated with sand, and negatively correlated with clay and silt proportion in the soil within the ranges of 45% to 80% sand, 8% to 23% clay, and 8% to 43% silt. Differences in soil type, topography and management history may have contributed to the differences in population dynamics and correlations observed between our research fields. Wallace et al. (1995) observed that SCN population dynamics differed between sites that differed in soil texture and organic carbon, primarily. SCN can maintain higher equilibrium population density in soils with higher sand content, and although it may increase to damaging levels in fine textured soils, the low reproductive rate in soils with high clay content results in a longer time being necessary for the nematode to attain damaging levels (Koenning and Barker, 1995). In our study, the lowest cyst population density and eggs per cyst numbers were observed in soils with low percent of sand; and roots of plants growing in soil with high clay content (low sand) had more nematodes early in the season, but resulted in low number of cysts later on. Soybean responds to moisture stress by increasing root biomass (Huck et al., 1986), which would favor reproduction of SCN by offering more feeding sites

(Koenning and Barker, 1995). The stress posed to the plant by the large nematode population in conjunction with possible water stress during the summer may have promoted new root growth and a second massive infection of the roots in Field B in 2000. Slow drainage (low hydraulic conductivity), associated with fine textured soils, often results in anaerobic conditions persisting for relatively long periods of time (Vrain, 1986). Soil oxygen levels may become the limiting factor for the aspects of the nematode's life cycle that require aerobic respiration, such as movement, hatch, and development. The area of Field B characterized by Brookston loam, soil rich in clay and poor drainage (Chapter Four), was also characterized by reduced number of cysts over two years, although abundant number of infective juveniles were observed in the roots at 44 DAP in 1999, and J3/J4s at 19 DAP in 2000. This section of the field remained flooded for several days after rainfalls, while sandier areas of the field dried faster, therefore it is possible that oxygen availability may have been a factor in SCN population dynamics.

A collection of semivariogram models can be used to describe a spatial process (Schabenberger and Pierce, 2002). In nematology, the spherical model seems to be the most frequently used (Donald et al., 1999, 2001; Workneh et al., 1999; Wyse-Pester et al., 2002, Webster and Boag, 1992). The short separation distance between samples in the nested design allowed us to detect a short-range spatial variability in the distribution of cysts described by a wave or hole effect semivariogram model. The wave model permits positive and negative autocorrelation as it fluctuates about the sill, with fluctuations decreasing with increasing lag distance (Cressie, 1993, Schabenberger and Pierce, 2001). Avendaño et al. (2003, Chapter Three) used the wave model to describe the spatial

structure in the distribution of cysts at planting in Field A and Field B, and we showed here that the periodicity in the spatial structure was maintained over the season. Part of the variability observed in the semivariogram models shown in Table 7 was because of the difficulty in modeling the periodicity observed in empirical semivariograms. For example, the peaks and valleys in the empirical semivariogram at 44 DAP in Field B in 1999 could not be modeled because of the large nugget. A spherical model was fitted instead to describe the long-range spatial structure (Figure 5.5.a), whereas a wave model was adequate to describe the structure of the empirical semivariogram at 46 DAP in 2000 (Figure 5.5.f). The periodicity in the spatial structure was possibly generated by SCN's biology, low mobility and slow spread. Under favorable conditions of moisture, temperature and host availability, infective juveniles hatched from eggs in a cyst infect nearby roots creating a very small infestation. Over time these small patches increase in size and merge onto nearby clusters creating the short scale spatial variability observed. These clusters are in turn arranged in larger clusters, which creates the larger scale spatial variability.

CONCLUSION

Soil texture had a strong and consistent effect on SCN cyst population density and spatial distribution in one field, but not in the other. Soils with more than 60% sand supported higher SCN population density and favor the nematode spread more than finer soils. Soil texture also had an effect on SCN developmental stages that occur inside the host, although this relationship seems to involve another factors not accounted for in this study such as the physiological status of the host. It is not clear from this work whether the effect of soil texture on the number of eggs per cyst was on fecundity (egg

production) or on hatching. This is an interesting question to address in the future under controlled conditions to advance in the understanding of the effect of soil texture on SCN population. The stability in the relationship between SCN spatial population dynamics and soil properties indicates there is potential for delineation of management zones to reduce the economic loss due to SCN in infested fields.

CHAPTER SIX

SPATIO-TEMPORAL ANALYSIS OF SOYBEAN CYST NEMATODE, SOIL FERTILITY AND PLANT NUTRIENT STATUS.

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SPATIO-TEMPORAL ANALYSIS OF SOYBEAN CYST NEMATODE, SOIL FERTILITY AND PLANT NUTRIENT STATUS.

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SPATIO-TEMPORAL ANALYSIS OF SOYBEAN CYST NEMATODE, SOIL FERTILITY AND PLANT NUTRIENT STATUS.

ABSTRACT

We studied the relationships between the spatial distribution of *Heterodera glycines* Ichinohe, the soybean cyst nematode (SCN), and soil fertility under field conditions, and between SCN and the nutritional status of the infected soybean. Soil samples collected following a geostatistical sampling design were analyzed for SCN, soil texture and soil fertility. Leaf-tissue samples were collected from selected locations for complete nutrient analysis. Geostatistical analysis was applied in conjunction with correlation and regression analysis. Soil fertility affected SCN spatial distribution, especially soil pH. The spatial distribution of SCN was affected by a combination of soil pH and soil texture consistently over time. SCN population density was also related to Ca and Mg in the soil and the nutritional status of the infected soybean, with similarities and differences between fields. The number of SCN cysts recovered at harvest was correlated with the nutritional status of soybean at mid season in Field B and not in Field A, but the number of eggs per cyst was not. This work laid the foundation for future research on the interaction between plant nutrition and SCN population dynamics.

Soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe) is distributed over a wide range of soybean production zones in the USA (Winstead et al., 1955; Wrather et al., 2001a, b). Variability in soil structure and soil properties has direct and indirect effects on SCN and other nematodes fitness. Soil texture has a strong effect on SCN cyst population density and spatial distribution in the soil, a weaker effect on SCN developmental stages inside soybean roots, and no effect on the number of infective juveniles in roots (Chapter 5; Todd and Pearson, 1988). Following are some examples of soil fertility effect on SCN. The relationships between soil fertility and soybean growth in the presence of SCN have been studied mostly in relation to K levels in the soil with diverse results depending on experimental conditions and SCN races used (Luedders et al., 1979; Hanson et al., 1988; Blevins et al., 1995; Howard et al., 1998; Melakeberhan 1999; Smith et al., 2001). Most studies were done under controlled greenhouse conditions for 30 days or less or in experimental plots by adding fertilizers. Even though K concentrations varied in the different experiments, SCN population density in the soil increased when moderate levels of K fertilizer were added, but not when none or high levels were applied (Luedders et al., 1979; Hanson et al., 1988; Howard et al., 1998; Smith et al., 2001). A positive linear correlation has been found between SCN population density and soil pH within the range of 5.5 to 8.4, but it is not clear if there is a direct relationship between soil pH and SCN population densities or if the effect is indirect and plant mediated (Tylka et al., 1998; Grau et al., 1999). Melakeberhan et al. (1997) reported that *Pratylenchus penetrans* population density was negatively affected while plant growth rate was increased when cherry rootstocks were subjected to an optimum fertilization regime as opposed to a nutrient deficient program, and Trudgill (1987) stated

that damage by potato cyst nematode (*Globodera rostochiensis* and *G. pallida*) was highest at low fertilization levels. Increasing levels of SCN in the soil affected the concentration and translocation of nutrients in plant tissue. For example, Mg and Ca concentrations in roots were increased, whereas P remained unchanged, and Mg translocation was increased with SCN treatment (Blevins et al., 1995). Melakeberhan (1997, 1999) discussed a set of hypotheses about the possible roles of soil fertility on plant growth in the presence of plant-parasitic nematodes.

Understanding the spatio-temporal variability of soil prevailing conditions might help attempts to site-specific management strategies (Donald et al., 1999; Avendaño et al., 2001) which, in turn, require that the spatial variability of the attribute of interest is highly structured to ensure that spatial prediction and corresponding management maps are accurate (Pierce and Nowak, 1999). Even though the spatial variability of SCN is poorly structured in general (Workneh et al., 1999; Donald et al., 1999; Avendaño et al., 2003-Chapter Three), the possibility of management zone delineation for SSM of SCN is supported by evidence that environmental conditions created by the interaction of weather, soil, landscape, and plant factors assist in the dispersion of eggs, determine SCN survivability, or limit its growth potential and thereby regulate the spatial dynamics of SCN (Lehman, 1994; Koenning and Sipes, 1998; Donald et al., 1999; Workneh et al., 1999; Donald et al., 2001, Avendaño et al., 2003- Chapter Three and Chapter Four). Moreover, Avendaño et al., (2003- Chapters Three, Four, and Five; Donald et al., 2001) found areas within fields with repeated occurrences of non-detectable or low densities of SCN and hot spots or areas of high density, consistently correlated with sand and clay percent in the soil composition over time.

The purpose of this work was to answer the question does soil fertility affect SCN distribution and root infection, and is this reflected in tissue analysis? Specific objectives were: i) to characterize soil fertility variability and its relationship with soil texture and tissue analysis in two fields of known SCN infection in Michigan; ii) to analyze the relation of SCN in the soil and in soybean roots with soil fertility and tissue analysis.

MATERIALS AND METHODS

Experimental Sites and Site Management

The study was conducted in 1999 and 2000 on two fields (Field A and Field B) in Shiawassee County, MI. Field A consisted of 24 ha, was managed under no-tillage since 1996, and was planted to corn prior to this study in 1998. Percent sand ranged from 45% to 74%, percent clay ranged from 1% to 21%, and percent silt ranged from 8% to 43% within Field A (Chapter Four). Field B was comprised of 13 ha, was conventionally tilled after wheat in 1998, and was managed under no-tillage thereafter. Percent sand ranged from 50% to 80%, percent clay ranged from 8% to 23%, and percent silt ranged from 11% to 29% within Field B (Chapter Four). An SCN -susceptible soybean variety (Asgrow 1901), and an SCN-resistant variety (Asgrow 2201), both Roundup-Ready, were grown in both fields in 1999 and in 2000, respectively. Soybean was planted in 19-cm rows at a rate of 519 000 viable seeds ha^{-1} in 1999, and 494 000 viable seeds ha^{-1} in 2000. Fields A and B were planted 5/22/99 and 5/16/99, respectively, and 6/9/00. Weed control was maintained using Roundup at the recommended rate. There was one preplant application in Field A in 1999 and 2000, one application postemergence in 1999, and a

midseason application in Aug. 2000. In Field B there was one preplant application in 2000, one postemergence in 1999 and a mid season application in Aug. 2000.

Sampling Design, Collection of Samples and Soil Analysis

The spatial sampling for soil samples consisted of a nested survey sampling design with distances reduced by geometric progression (adapted from Webster and Boag, 1992) applied within 8 and 5.25 ha in the center of Fields and B, respectively, as described in Chapter Three. Single-core soil samples were collected using an 8 cm diameter by 23 cm deep bucket auger (Riverside Augers, Eijkelkamp, Giesbeek, The Netherlands) from 160 and 110 locations in Field A and Field B, respectively. Each sample collected before planting in 1999 was thoroughly mixed and then divided in three sub-samples. One sub-sample was used for SCN analysis (Chapters Three, Four, and Five), the second was used for texture analysis (Chapter Four), and the third portion of the sample was analyzed for pH, P, K, Ca, and Mg (Soil and Plant Analysis Laboratory, Michigan State University).

SCN analysis

Cysts in the soil, eggs per cyst, and developmental stages in soybean roots were quantified from soil and root samples collected at planting, at harvest, and at the dates indicated in Table 5.1 (Chapters Three, Four, and Five). The maps of SCN spatial distribution in Field A and Field B mapped in Chapter Four were used in this work to relate SCN spatial distribution to soil fertility.

Soybean Leaf Tissue Nutrient Analysis.

A leaf sample consisted of the upper-most fully developed trifoliate from 20 plants randomly chosen from selected clusters including up to four neighboring locations of the nested survey sampling design (Figure 6.1). In 1999, one set of 21 leaf samples was collected from Field B 61 days after planting (DAP), approximately two weeks after plants were in full bloom (R2 stage) (Ritchie and Benson, 1994). In 2000, two sets of 24 samples were collected from Field A and two sets of 20 samples from Field B. The first set of samples was collected at 40 DAP, when only occasional flower buds were visible (V9 stage), and the second set was collected at 81 DAP, when most plants had 2 cm-long pods in the lower 2 or 3 nodes (R3 stage) (Ritchie and Benson, 1994). Sampling times were designed to cover the vegetative and reproductive growth phases of the plant. Leaves were collected in paper bags and dried at 80°C for 24 hours before the analyses. Tissue concentrations of N, P, K, Ca, Mg, Fe, Zn, Mn, Cu, and B were analyzed by the Plant and Soil Analysis Laboratory at Michigan State University in 1999, and by A & L Great Lakes Laboratory, Fort Wayne, Indiana in 2000.

The one to four SCN and soil fertility observations comprised within each cluster sampled for tissue analysis were averaged, and the mean was associated with each tissue nutrient value for the corresponding cluster. Each tissue analysis observation was also associated with a kriging-predicted value for the proportion of sand, clay, and silt matched by location.

Statistical Analysis

Descriptive statistics were applied to characterize soil pH, soil fertility, and tissue nutrients, the results are shown as boxplots. Pearson's simple linear correlation

coefficients were calculated for soil fertility and soil texture; soil fertility and tissue analysis; SCN population density in the soil and in the roots with soil pH and nutrients; and SCN population density (The SAS System Release 8; SAS Institute, Cary, NC).

Geostatistical tools were applied to quantify the spatial variability in the distribution of soil pH and soil nutrients. Omnidirectional empirical semivariograms (Matheron, 1963) were calculated for lags ranging from 4 to 40 m (h), with a lag tolerance of h/2. The minimum number of pairs required for each lag was 30. The parameters of theoretical semivariogram models fit to the empirical semivariogram were estimated by (nonlinear) least squares. The spatial distribution of soil pH and soil nutrients were mapped by ordinary or universal kriging. Interpolated values were predicted at the nodes of a 1 x 1 m grid using the structural properties of the estimated theoretical semivariogram and the sampled values at observed locations. Details on the geostatistical analysis were described by Avendaño et al., (2003- Chapter Three).

The cross-correlogram is another geostatistical tool used to describe the joint variability or spatial continuity between measurements of different attributes or of the same attribute measured at different times. The cross-correlation between two attributes at the same location (zero lag distance) equals the linear correlation coefficient for those two attributes. The cross-correlation function given by Goovaerts (1997) was used here to calculate cross-correlograms for logarithmic transformed cysts and nematodes in roots in 1999 and 2000 with pH, Ca, and Mg in the soil. Only the data points from locations sampled for both attributes were used for this analysis.

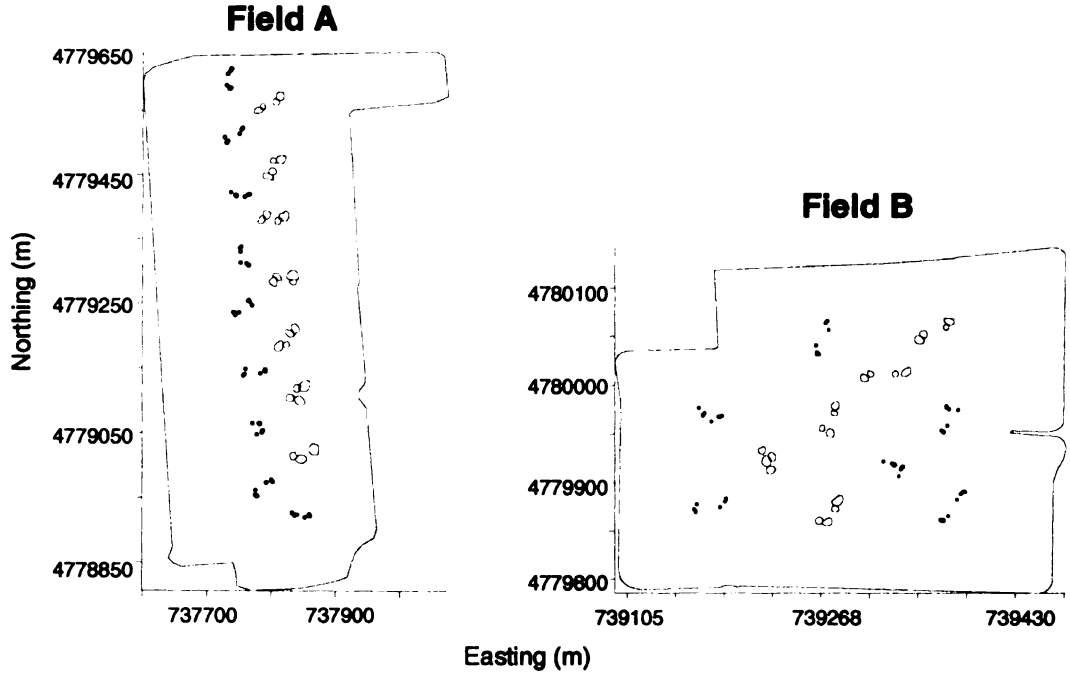

Figure 6.1. Field A and Field B, location of sampling sites. White irregular areas indicate where soybean leaf samples were collected in 1999 from Field B, and in 2000 from Fields A and B. Each circle includes from one to four sites from the nested survey sampling design where soil samples were previously collected (black circles)(Chapter Three).

RESULTS

Soil Analysis

Soil pH in Field A was lower than in Field B (Figure 6.2), with a highly structure spatial distribution within a range of 71 m (Table 6.1). Soil pH lower than 6.5 or higher than 7.5 appeared in medium to small size clusters through out the area sampled in Field A (Figure 6.3). The absence of nugget in the empirical semivariogram of pH in Field B indicated a highly structured spatial distribution (Table 6.1). Soil pH was higher on the north side of Field B than on the south side. The long range of spatial autocorrelation (106 m) showed the variability in soil pH levels in large clusters in this field (Figure 6.4).

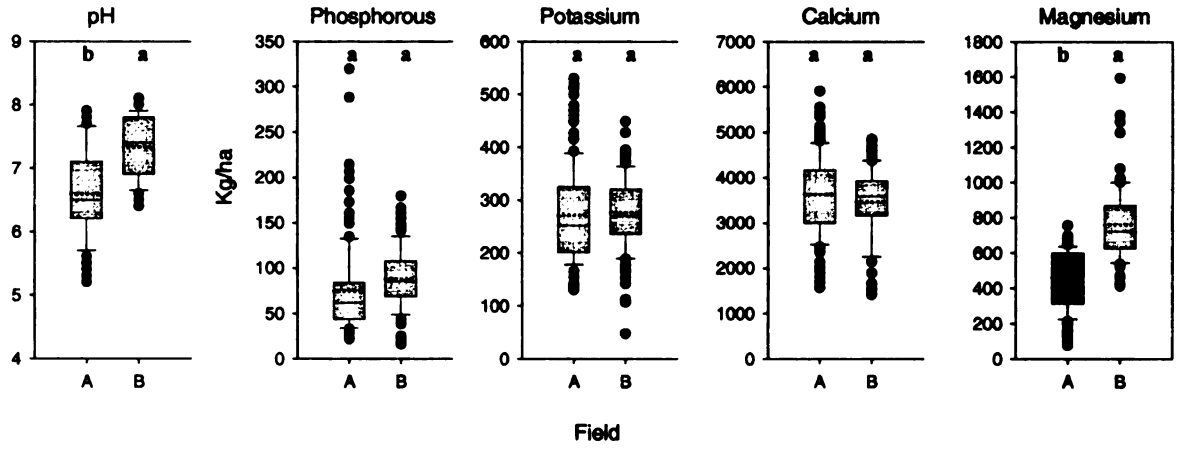

Figure 6.2. Box-plots of pH and P, K, Ca, and Mg quantified in soil samples collected at planting in 1999 from Field A and Field B. Means (dotted line) with the same lower case letter were not significantly different between fields (protected LSD, 5% significance level). Medians are indicated with a full line. Whiskers represent the 5th and 95th percentiles; outliers are indicated with black circles.

Phosphorus concentration in the soil in Field A was lower and with greater variability than in Field B (Figure 6.2). The spatial distribution of P concentration was moderately structured in field A, with a range of autocorrelation of 317 m (Table 6.1). The contour map showed rather uniform distribution of P levels with higher concentration in about one third of Field A (Figure 6.3). Phosphorus distribution in Field B was highly structured within a range of 46 m (Table 6.1). Small patches of high P concentration were identified scattered through out the field (Figure 6.4).

Potassium concentration in the soil was similar in Field A and Field B (Figure 6.2), and moderately structured spatially within a range of 81 m in Field A and 52 m in Field B (Table 6.1). Patches of below average K concentration were distributed throughout Field A in bands oriented more or less SW-NE (Figure 6.3). In Field B, two rather large areas of low K concentration extended from the north side of the field to almost the south boundary of the area sampled (Figure 6.4).

Table 6.1. Parameters of the theoretical semivariogram models of soil pH, P, K, Ca, and Mg in Fields A and B before planting in 1999.†

		Drift ‡	Model function	C_0 §	C ¶	Range #	$\frac{C_0}{C+C_0}$ ††
Field A						m	
	pH	None	Nugget	0.1			
			Spherical		0.34	71	0.23
	P	None	Nugget	1494			
			Exponential		1213	317	0.55
	K	None	Nugget	2380			
			Spherical		3420	81	0.41
	Ca	Linear	Nugget	33700			
			Exponential		350000	106	0.09
	Mg	None	Nugget	402			
			Exponential		12900	194	0.03
Field B							
	pH	None	Nugget	~ 0			
			Spherical		0.19	106	0
	P	None	Nugget	13.7			
			Spherical		855	46	0.01
	K	None	Nugget	2810			
			Exponential		1900	52	0.60
	Ca	Linear	Nugget	31300			
			Spherical		405000	292	0.07
	Mg	None	Nugget	7440			
			Spherical		44100	202	0.14

† Models were fitted by least squares based on empirical semivariograms calculated for lags ranging from 4 to 40 m (h), with a lag tolerance of h/2. The minimum number of pairs required for each lag was 30.

‡ Whenever semivariograms showed nonstationarity, the data were detrended carrying out a simple polynomial least squares regression and semivariogram analysis was performed on the residuals. The polynomial order of the trend is indicated when a drift or trend was removed.

§ C_0 is the nugget effect or a discontinuity in semivariance at the origin due to microscale variability or sampling error.

¶ C is the partial sill defined for spherical models.

Observations that are spatially separated by more than the range are uncorrelated.

†† $C_0/(C+C_0)$ is an indicator of the degree of spatial structure, the lower the number the stronger the spatial autocorrelation.

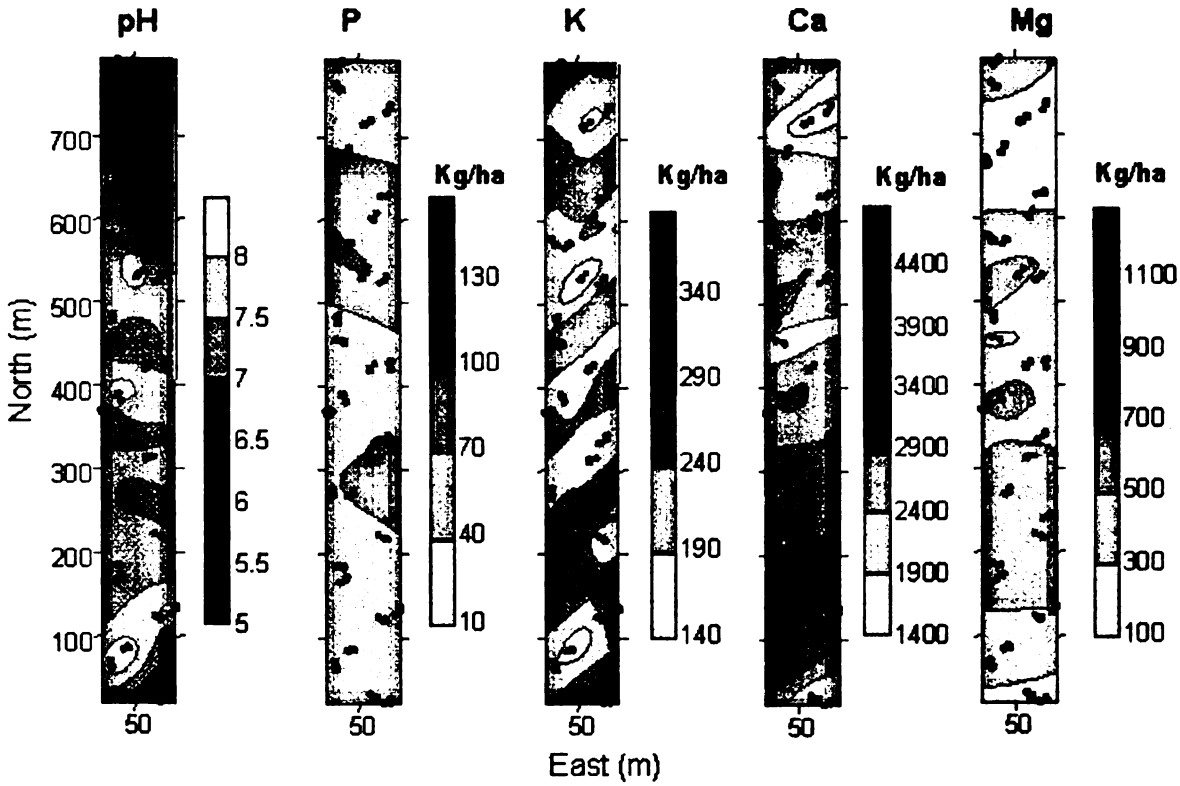

Figure 6.3. Spatial distribution of pH, and concentration of P, K, Ca, and Mg in the soil as interpolated by kriging from samples collected before planting in 1999 in Field A at the locations indicated with black circles.

Calcium concentration in the soil was similar in Field A and Field B (Figure 6.2). The spatial distribution of Ca was highly structured in both fields, but the range of spatial autocorrelation was larger in Field B (Table 6.1). Calcium concentration in the soil was higher in the south side of both fields, decreasing gradually towards the north (Figures 6.3 and 6.4). Magnesium concentration was lower in Field A than in Field B, with much lower minimum and maximum values in Field A (Figure 6.3). The spatial distribution of Mg was highly structured, with a range of about 200 m in both fields (Table 6.1). Magnesium concentration was lower on the north side of Field A (Figure 6.3). High concentration of Mg was found in a band about 100 m wide stretching from the east side of the Field B towards the center (Figure 6.4).

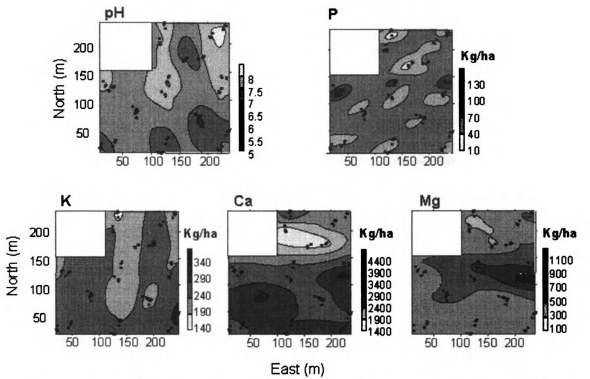

Figure 6.4. Spatial distribution of pH, and concentration of P, K, Ca, and Mg in the soil as interpolated by kriging from samples collected before planting in 1999 in Field B at the locations indicated with black circles.

The following soil fertility attributes were related to texture in Field A and in Field B. Soil pH was correlated with sand ($r = 0.58$), clay ($r = -0.48$) and silt ($r = -0.63$) percentages in the soil in Field B exclusively ($\alpha = 0.001$). Phosphorus concentration was not correlated with soil texture in either field. Potassium and soil texture were poorly correlated in Field A [r (sand) = -0.25, r (clay) = 0.25, r (silt) = 0.23, $\alpha = 0.05$], and only significantly correlated with clay percentage in Field B ($r = -0.17$, $\alpha = 0.05$). Calcium however, was strongly correlated with soil texture in both fields, especially with clay in Field B [Field A: r (sand) = -0.48, r (clay) = 0.26, r (silt) = 0.42. Field B: r (sand) = -0.75, r (clay) = 0.80, r (silt) = 0.63, $\alpha = 0.001$]. Magnesium was more strongly correlated with

soil texture in Field A [r (sand) = -0.69, r (clay) = 0.51, r (silt) = 0.54, α= 0.001] than in Field B [r (sand) = -0.22, r (clay) = 0.31, r (silt) = ns, α = 0.05].

Tissue analysis

The appearance of soybean plants in Field A was uniform throughout the field in 1999 and 2000. The canopy was completely closed at V9, and plants were homogeneous in size and color at V9 and R3. Abundant number of large nodules (not quantified), pink inside when dissected was observed on roots at V9. Soybean plants at V9 in Field B were green and homogeneous in size in approximately the south half of the field. On the north side, green, fully-grown plants were alternated with large patches of stunted plants with fewer and chlorotic leaves, many of which had necrotic edges. Green plants had abundant, large nodules pink inside; whereas, yellow plants had reduced number of nodules, small in size and most of them brown or yellow inside when dissected. Numerous cysts were easily observed with the naked eye on roots from yellow plants. At R3, differences in plant appearance were accentuated in Field B.

Descriptive statistics of macronutrients and micronutrients concentration in leaf tissue are shown as boxplots (Figures 6.5 and 6.6). In Field A, all nutrients were within soybean sufficiency ranges (Vitosh et al., 1997). The maximum values of K, Fe, Zn, and Cu concentration in leaf tissue at V9 were above normal levels, without reaching toxic concentrations. At V9, N, Ca, Mg, Fe, and Cu concentrations were higher than at R3, when the variability was greater. The concentration of B in leaf tissue was lower at V9 than at R3, whereas P, K, S, Zn, and Mn concentrations did not differ between samplings (Figure 6.5). In Field B, Cu concentration in leaf tissue samples collected at R2 stage in 1999 was below the sufficiency limit and the minimum values of a few other elements

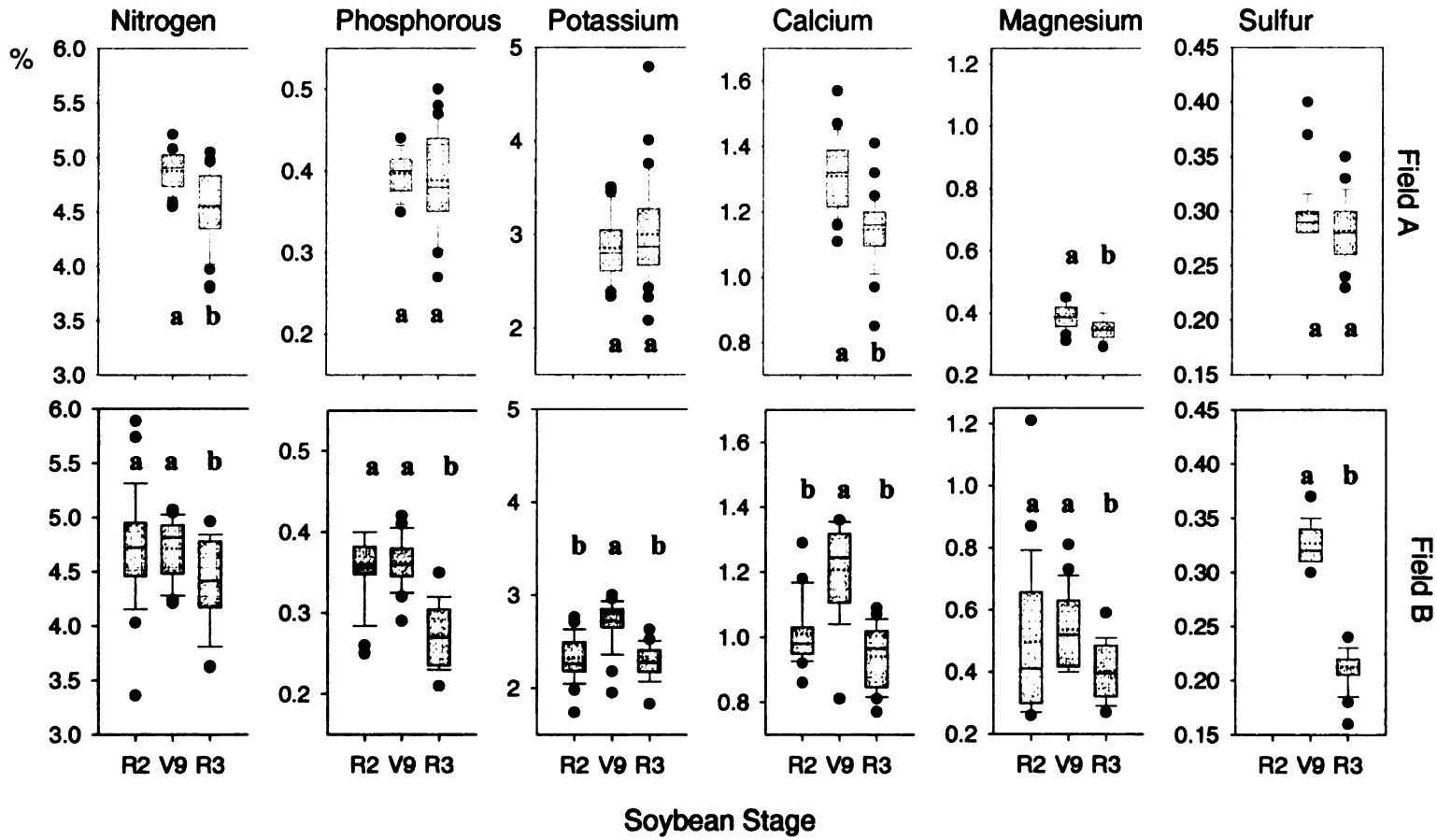

Figure 6.5. Box-plots of N, P, K, Ca, Mg, and S concentration (%) in soybean leaf tissue samples collected at R2 in 1999 from Field B, and at V9 and R3 in 2000 from fields A and B. Significant differences among the means (dotted lines) at different sampling times within each field are indicated with lower case letters. Means with the same letter were not significantly different (protected LSD, 5 % significance level). Medians are indicated with a full line. Whiskers represent the 5th and 95th percentiles, outliers are indicated with black circles.

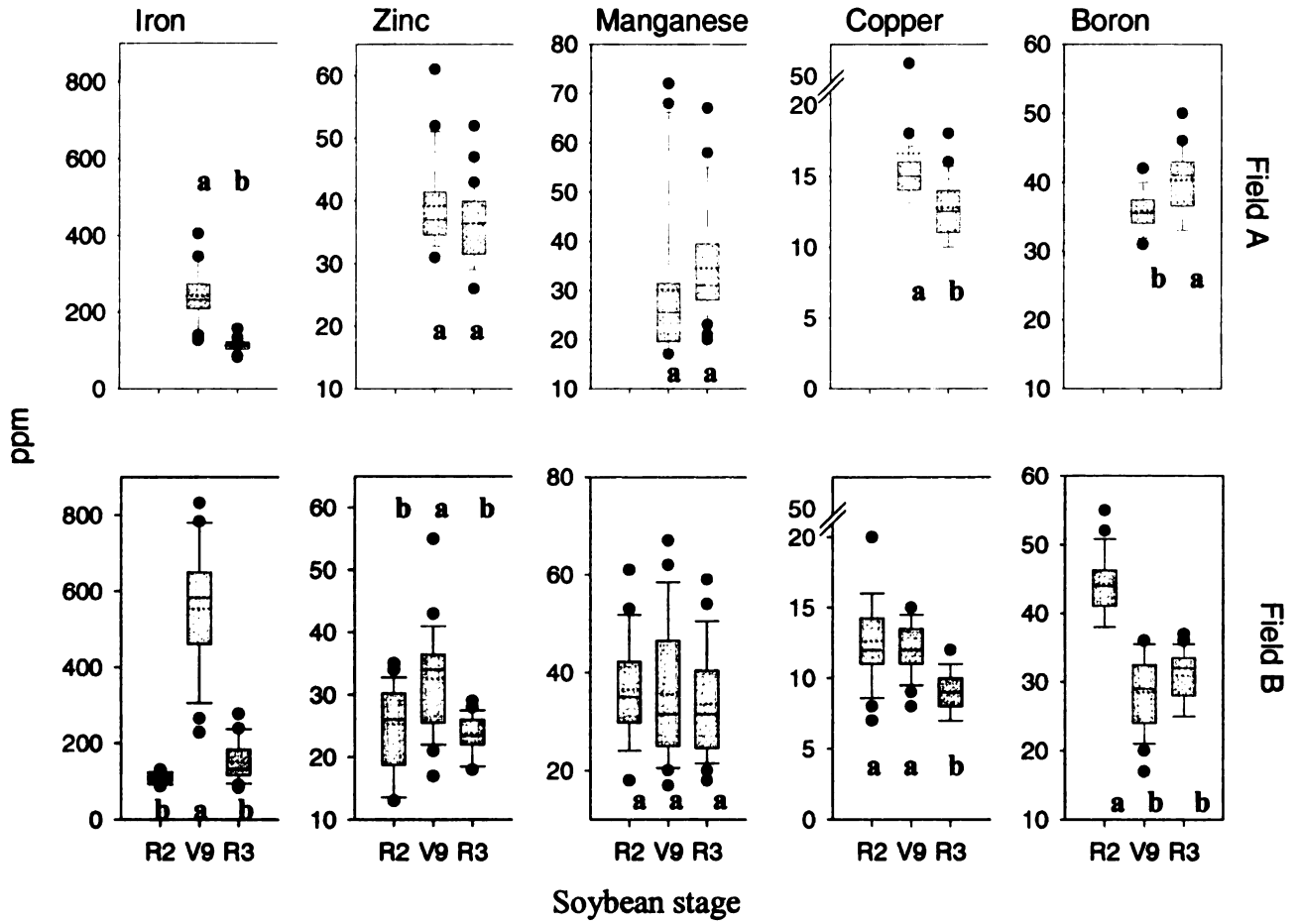

Figure 6.6. Box-plots of Fe, Zn, Mn, Cu, and B concentrations (ppm) in soybean leaf tissue samples collected at R2 in 1999 from Field B, and at V9 and R3 in 2000 from fields A and B. Significant differences among the means (dotted lines) at different sampling times within each field are indicated with lower case letters. Means with the same letter were not significantly different (protected LSD, 5 % significance level). Medians are indicated with a full line. Whiskers represent the 5th and 95th percentiles, outliers are indicated with black circles.

were also below sufficiency limits (P, Zn, and Mn). All other nutrients were within normal ranges at this growth stage (Vitosh et al., 1997). Iron concentration in the leaves at the V9 stage in 2000 was high, whereas in a few samples, Zn, Mn, Cu, and B concentrations were below sufficiency limits. At the R3 stage in 2000, N, P, K, and S, as well as Zn, Mn, and Cu concentrations were below sufficiency limits in some of the samples. Mean nutrient concentrations at R2 in 1999 were equal to or higher than at R3 in 2000, and equal to or lower than at V9, with the exception of B. Manganese was the only element which concentration remained unchanged at different sampling times (Figure 6.6).

Some of the nutrients in tissue from Field A were correlated with soil attributes (Table 6.2). Potassium, Zn, and Mn were negatively correlated with pH, Ca, and Mg in the soil; Mn was also negatively correlated with P. Calcium and Fe in tissue were not correlated with soil fertility. Correlations for other nutrients in tissue varied with the growth stage of the plant. Correlations between tissue nutrients and soil fertility varied by year and by plant growth stage in Field B (Table 6.3). In 2000, Mg and pH were the soil attributes correlated with the most tissue nutrients. High concentration of Mg in the soil was related to high levels of P, Zn, and B in leaves at V9, and low levels of the same elements at R3. Also, high pH was related to low levels of P, Zn, and B at V9, but high levels at R3. The relation of S, Mn, and Cu to soil pH and Mg at V9 remained similar at R3. Other nutrients were also related to soil fertility in Field B (Table 6.3).

Soil texture and tissue analysis were strongly correlated in Field B in 2000. Correlations were weaker and only with a few tissue nutrients in Field A (Table6.3). Off the tissue nutrients at R2 in 1999, only Mn was correlated with soil texture in Field B.

Table 6.2. Pearson's correlation coefficients for percent sand, clay and silt, soil pH, and soil macronutrients (Kg ha^{-1}) with tissue nutrients at V9 and R3 in 2000 in Field A.

Soil fertility and texture	Nutrients in soybean leaf tissue										
	N	P	K	Ca	Mg	S	Fe	Zn	Mn	Cu	B
	%						ppm				
	Field A V9, 2000										
pH	NS	NS	-0.60**	NS	0.68**	NS	NS	-0.56**	-0.63**	NS	NS
P	NS	NS	-0.22**	NS	NS	0.56**	NS	NS	-0.51*	NS	NS
K	NS	NS	NS	NS	NS	-0.42*	NS	NS	NS	NS	NS
Ca	0.41*	NS	-0.70**	NS	0.70**	NS	NS	-0.58**	-0.77**	NS	0.67**
Mg	NS	NS	-0.81**	NS	0.84**	NS	NS	-0.68**	-0.74**	NS	0.59**
Sand	NS	NS	0.49*	NS	NS	NS	NS	NS	0.41	NS	NS
Clay	NS	NS	NS	NS	0.59**	NS	NS	-0.47*	-0.48*	NS	NS
Silt	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Field A R3, 2000										
pH	NS	NS	-0.51**	NS	NS	NS	NS	-0.73**	NS	-0.55**	-0.56**
P	0.38*	NS	NS	NS	NS	0.54**	NS	NS	-0.49**	NS	NS
K	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Ca	0.55*	-0.40*	-0.67**	NS	NS	NS	NS	-0.55**	-0.57**	-0.48*	NS
Mg	0.55**	-0.41*	-0.74**	NS	0.49*	NS	NS	-0.58**	-0.63**	-0.52*	NS
Sand	NS	NS	0.51**	NS	NS	NS	NS	NS	0.52**	NS	NS
Clay	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Silt	NS	NS	-0.54**	NS	NS	NS	NS	NS	-0.43*	-0.41*	NS

*, ** Significant at the 0.05, and 0.01 probability level, respectively.

Table 6.3. Pearson's correlation coefficients for percent sand, clay, and silt, soil pH, and soil macronutrients (Kg ha^{-1}) with tissue nutrients at R2 in 1999, and at V9 and R3 in 2000 in Field B.

Soil fertility and texture	Nutrients in soybean leaf tissue										
	N	P	K	Ca	Mg	S	Fe	Zn	Mn	Cu	B
	%						ppm				
	Field B R2, 1999										
pH	-0.45*	NS	-0.63*	0.45*	0.70*	NS	NS	-0.66	NS	NS	-0.69
P	NS	0.53*	NS	NS	NS	NS	NS	NS	NS	NS	NS
K	NS	NS	0.61	-0.56	-0.48	NS	-0.53	NS	NS	NS	NS
Ca	NS	NS	NS	NS	-0.55	NS	NS	0.73	NS	0.50	0.56
Mg	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.47	
Sand	NS	NS	NS	NS	NS	NS	NS	NS	0.54*	NS	NS
Clay	NS	NS	NS	NS	NS	NS	NS	NS	-0.51*	NS	NS
Silt	NS	NS	NS	NS	NS	NS	NS	NS	-0.54*	NS	NS
	Field B V9, 2000										
pH	NS	-0.83**	NS	-0.46*	NS	0.65**	NS	-0.58**	-0.70**	0.86**	-0.82**
P	0.49*	-0.58**	NS	NS	NS	NS	NS	NS	-0.45*	NS	-0.49*
K	0.64*	NS	NS	NS	NS	-0.49*	NS	NS	NS	-0.57**	0.48*
Ca	NS	NS	NS	0.52*	NS	NS	NS	NS	NS	NS	NS
Mg	NS	0.49*	NS	NS	0.54*	-0.85**	NS	0.59**	0.45*	NS	0.46*
Sand	-0.77**	NS	NS	NS	0.84**	NS	-0.69**	-0.72**	0.74**	-0.73**	-0.85**
Clay	0.71**	NS	NS	NS	-0.86**	NS	0.73**	0.70**	-0.67**	0.69**	0.87**
Silt	0.79**	NS	NS	NS	-0.76**	NS	0.61**	0.70**	-0.79**	0.74**	0.78**

Table 6.3 (cont'd).

					Field B R3, 2000						
pH	NS	0.60**	0.70**	NS	NS	0.70**	0.46*	0.45*	-0.77**	0.64**	0.70**
P	0.49*	NS	NS	NS	0.47*	NS	NS	NS	-0.50*	NS	NS
K	0.64**	NS	NS	NS	NS	NS	NS	NS	0.49*	NS	NS
Ca	NS	NS	NS	0.54*	NS	NS	NS	NS	NS	NS	NS
Mg	NS	-0.72**	-0.67**	-0.60**	NS	-0.84**	-0.59**	-0.50*	0.45*	NS	-0.64**
Sand	0.66**	0.72**	NS	NS	0.89**	0.53*	0.55*	-0.79**	0.47*	0.65**	-0.82**
Clay	-0.72**	-0.76**	NS	NS	-0.91**	-0.57**	-0.49*	0.71**	NS	0.69**	0.81**
Silt	-0.55*	-0.62**	NS	NS	-0.81**	-0.45**	-0.59**	0.83**	-0.56**	-0.57**	0.78**

*, ** Significant at the 0.05, and 0.01 probability level, respectively.

SCN Population and Soil Fertility

Nematode population density was greater in Field B than in Field A. While cyst density did not change much over the duration of the study in Field A, in Field B there was great variability, with the number of cysts in the soil at harvest greater than at planting in 1999 and 2000 (Figure 5.6 and 5.7). Correlation coefficients were low for cyst density in relation to soil pH in both fields (Table 6.4). The spatial distribution of cysts at planting, at harvest, and in June was cross-correlated with soil pH up to a distance of 60 - 70 m in Field A and up to 120-130 m in Field B (Figure 6.7). Areas of higher cyst density were consistently observed at the locations were soil pH was also higher within the area sampled in both fields (Figure 5.6 and Figure 6.3).

Table 6.4. Pearson's linear correlation coefficients for SCN population density in the soil or in soybean roots in relation to soil pH, Ca, and Mg concentration.

		Field A 1999			Field B 1999		
Sample		pH	Ca	Mg	pH	Ca	Mg
			Kg/ha			Kg/ha	
Planting	Soil†	0.32**	0.24**	NS	0.42**	-0.61**	NS
June‡	Soil	0.49**	0.46**	0.25**	0.55**	-0.44**	NS
	Roots§	0.22**	0.19*	NS	NS	-0.24*	NS
Harvest¶	Soil	0.45**	0.27**	NS	0.29**	-0.55**	NS
		Field A 2000			Field B 2000		
Planting	Soil	0.24**	0.17*	NS	0.48**	-0.53**	NS
June	Soil	0.20**	NS	-0.18*	0.41**	-0.32**	NS
	Roots	0.38**	0.34**	0.27**	0.22*	0.19*	NS
Harvest	Soil	0.32**	NS	-0.17*	0.29**	-0.66**	-0.21*

*, ** Significant at the 0.05, and 0.01 probability level, respectively.

† Log_{10} (cysts $100cm^{-3}$ of soil + 1). Cysts were extracted from the soil by elutriation and sugar flotation.

‡ June samples were collected at 31 and 44 DAP in 1999 and at 17 and 19 DAP in 2000 from Field A and Field B, respectively.

§ Log_{10} (nematodes in 2g of root + 1). SCN developmental stages counted in 2 g of stained soybean roots.

¶ Harvest samples were collected at 125 and 126 DAP in 1999 and at 147 and 155 DAP in 2000, from Field A and Field B, respectively.

Cysts in the soil had a much larger range of cross-correlation than SCN in the roots (Figure 6.7). SCN population in the root in June was poorly correlated with pH in Field A and practically uncorrelated with pH in field B (Table 6.4, Figure 6.7). Cyst population density in the soil was correlated with P only at planting in 1999 in Field A with a very low correlation coefficient ($r = 0.16$, $\alpha = 0.05$). SCN population density was not correlated with K concentration in the soil. Cyst population was positively correlated with Ca in Field A, with low correlation coefficients and cross-correlations over a very short separation distance between samples (Table 6.4, Figure 6.4). Cysts were more strongly correlated with Ca in Field B, but correlation coefficients were negative in this field (Table 6.4). Calcium and cysts were negatively cross-correlated up to a separation distance of about 110 m, consistently over time. Beyond that range, samples became positively cross-correlated (Figure 6.8). Cross correlation between SCN in the roots and Ca was poor in both fields (Figure 6.8). SCN population density was correlated with Mg in Field A only. Cyst population density at 31DAP in 1999 and nematodes in the roots at 17 DAP in 2000 were positively correlated with Mg ($r = 0.25$ and $r = 0.27$, respectively, $\alpha = 0.01$), whereas cyst population density at 17 DAP and at harvest in 2000 were negatively correlated with Mg ($r = -0.18$ and $r = -0.17$, respectively, $\alpha = 0.05$). Cross-correlograms of cysts and Mg showed low correlation up to about 100 m before becoming zero (Figure 6.9).

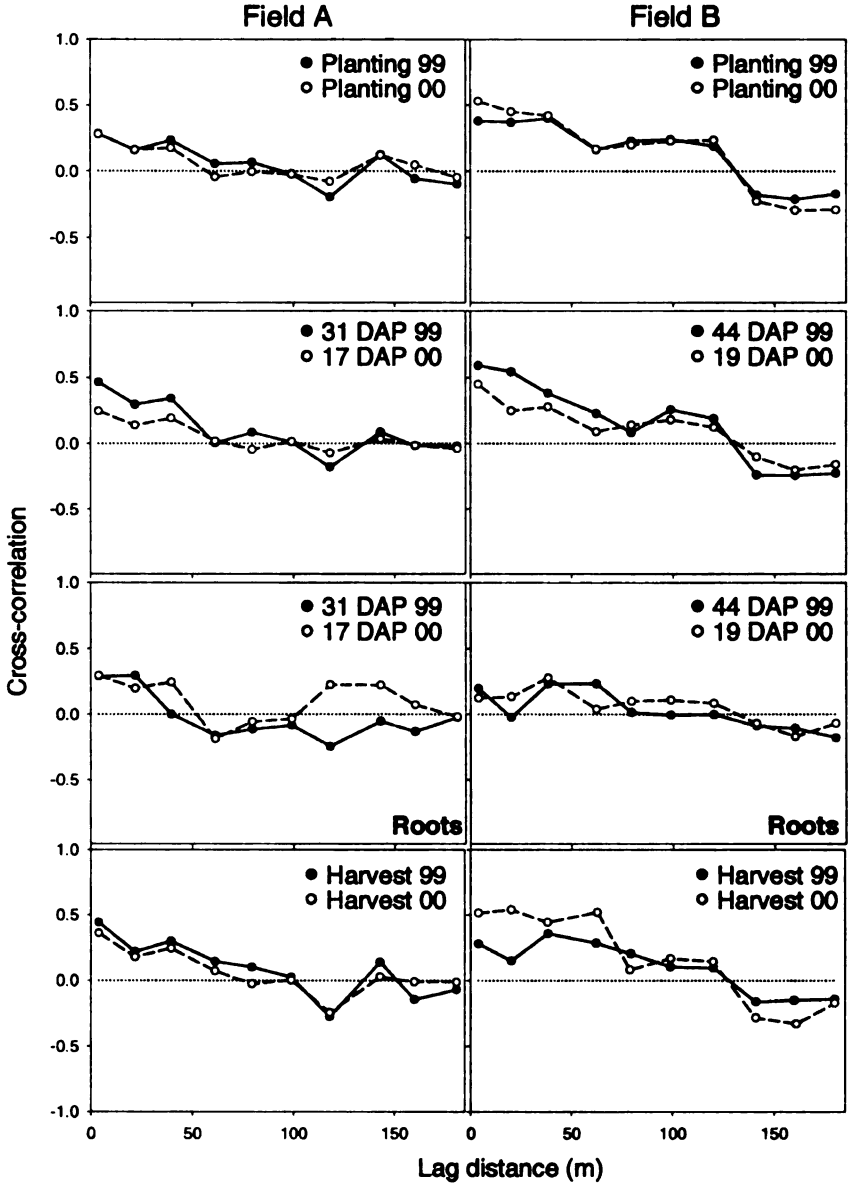

Figure 6.7. Cross-correlograms of soil pH and SCN population density in Field A and Field B. Lag distance is the separation distance between samples. SCN population density was determined in soil samples, or in root samples where indicated.

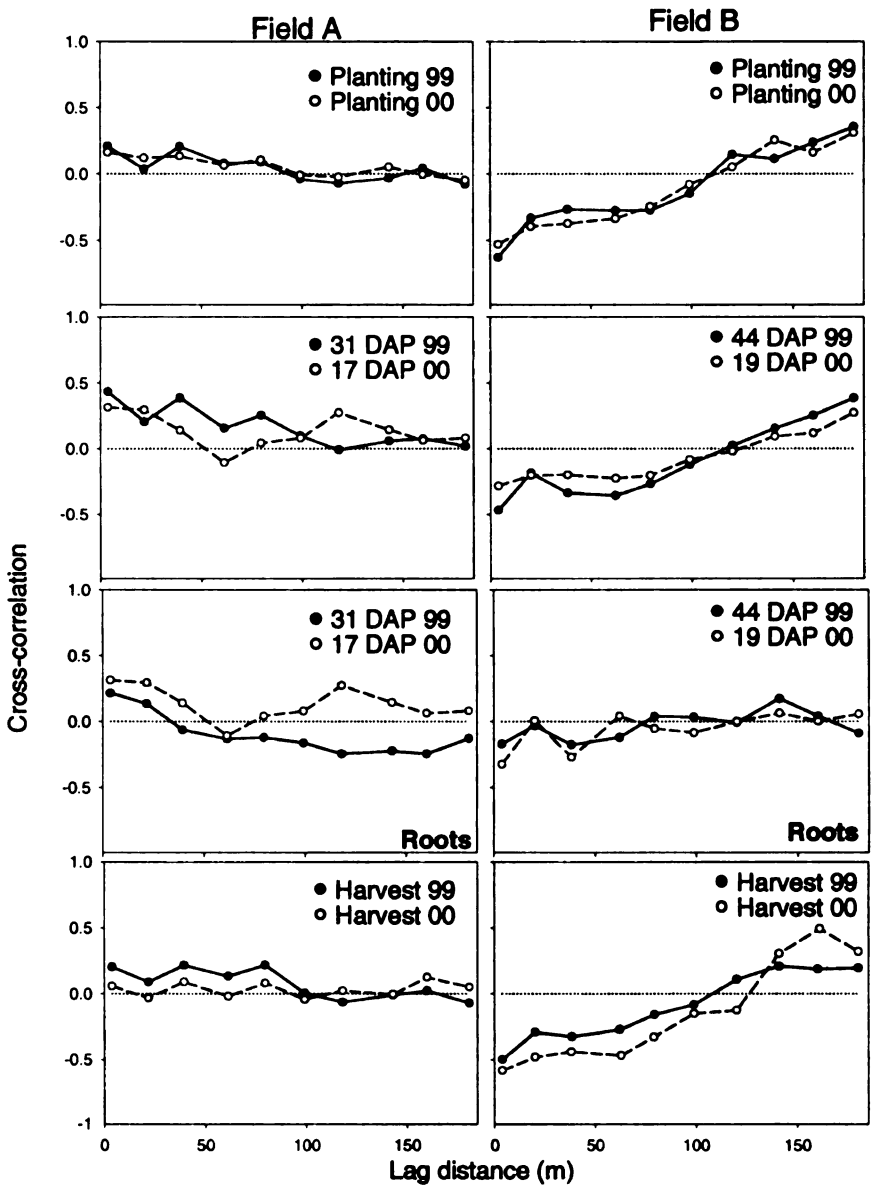

Figure 6.8. Cross-correlograms of soil Ca and SCN population density in Field A and Field B. Lag distance is the separation distance between samples. SCN population density was determined in soil samples, or in root samples where indicated.

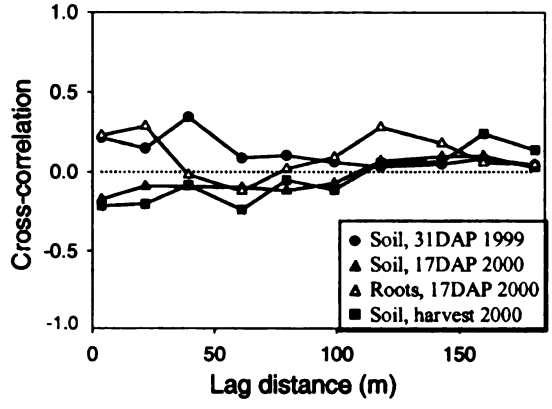

Figure 6.9. Cross-correlograms of Mg concentration in the soil and SCN population density in Field A in the cases where correlation analysis was significant. Cyst population density was quantified in the soil at 31 DAP in 1999, and at 17 DAP and harvest in 2000. SCN population in the roots was quantified at 17 DAP in 2000. Lag distance is the separation distance between samples.

SCN Population and Tissue Analysis

SCN cyst population densities at planting and at 17 DAP were not related to nutrient levels in tissue at V9 or R3 in Field A ($\alpha = 0.05$). However, SCN population in roots in samples collected at 17 DAP were negatively correlated with K and Mn at V9 [r (K) = -0.46, r (Mn) = -0.49, $\alpha = 0.05$] and R3 [r (K) = -0.45, r (Mn) = -0.40, $\alpha = 0.05$] and with Zn at R3 [r (Zn) = -0.43, $\alpha = 0.05$]; and positively correlated with Mg at V9 [r = 0.69, $\alpha = 0.01$]. Tissue nutrients at R2, V9, or R3 were related to SCN population density at planting and at 44 or 19 DAP in 1999 and 2000, respectively in Field B (Table 6.5). The sign of the correlations varied by element and with plant phenology. The effect of SCN on leaf tissue nutrients was mostly negative at V9 and mostly positive at R3 in 2000. Magnesium was the only element that was always positively correlated with cyst density, whereas the correlation with Zn was always negative. Cysts population density in soil and nematodes in root at 44 DAP were negatively correlated with B in 1999.

Table 6.5. Pearson's linear correlation coefficients for cyst population density in the soil at planting and at 19 and 44 DAP in 1999 and 2000, respectively, in relation to macro and micronutrients concentration in soybean leaves at R2 in 1999, and V9 and R3 stages of growth in Field B in 2000.

	Nutrients in soybean leaf tissue†									
SCN sample‡	N	P	K	Mg	S	Fe	Zn	Mn	Cu	B
			%					ppm		
						R2				
Planting	NS	NS	NS	0.53*	NS	NS	-0.63**	NS	NS	-0.52*
44 DAP	NS	NS	-0.49*	0.63**	NS	NS	-0.67**	NS	NS	-0.65**
						V9				
Planting	-0.70**	NS	NS	0.75**	NS	-0.69**	-0.59*	0.56*	-0.64**	-0.82**
19 DAP	-0.77**	NS	NS	NS	NS	-0.58*	-0.65*	0.70*	-0.73**	-0.72**
						R3				
Planting	0.78**	0.80**	0.48*	0.79**	0.68**	NS	-0.56*	NS	0.72**	-0.71**
19 DAP	NS	0.54*	NS	0.51*	NS	NS	-0.59*	0.64**	0.49*	-0.75**

*, ** Significant at the 0.05, and 0.01 probability level, respectively.

† Correlations with calcium were not significant in all cases, data not shown.

‡ Cysts were extracted from the soil by elutriation and sugar flotation. Correlation analysis was performed on $\log_{10}$ (cysts $100cm^{-3}$ of soil + 1).

The concentration of K in leaf tissue was affected at the reproductive stages, with opposite signs in 1999 and 2000. Manganese, N, Cu, Fe, P, and S concentrations in leaf tissue were inconsistently correlated with cysts or SCN population in roots, positively in some cases and negatively in other (Table 6). Calcium was the only element in leaf tissue that was not correlated with SCN at any stage of the plant in either field.

DISCUSSION

This work showed that SCN population density and spatial distribution were correlated with soil fertility, and that a combination of soil fertility and SCN population density affected the nutritional status of soybean. The results presented here emphasize the relevance of the interactions between SCN population and edaphic factors on the nutritional status of the host as reflected in tissue analysis.

The spatial distributions of SCN and soil pH were positively correlated consistently over time in the two fields studied, as it was observed from the experiments of Tylka et al., (1998) and Grau et al., (1999). Even though the degree of spatial structure (nugget variance/total variance ratio) and the maximum distance to which pH was auto-correlated and cross-correlated with SCN density varied by field, correlation parameters within field were consistent over time indicating that soil pH was an important factor acting directly or indirectly (plant mediated) in shaping SCN population distribution in a given field. It was reported in Chapter Four that SCN population in Field B was negatively correlated with clay percentage in the soil. This study showed clay percentage and soil pH were negatively correlated, indicating that the spatial distribution of SCN was affected by the combination of soil pH and soil texture.

SCN population density was also related to Ca and Mg concentration in the soil, with similarities and differences between fields. Calcium and SCN population density were correlated consistently over time, but with great variability between fields. In Field A high levels of Ca in the soil were associated with high levels of SCN. Conversely, in Field B the correlation was negative and the effect of Ca on the spatial distribution of SCN was more important than in Field A. Even though Ca concentrations in the soil were similar between fields, the strong association of high levels of Ca with fine textured soils generated the negative correlation with SCN population observed in Field B. Calcium and Mg uptake and translocation mechanisms are similar under normal conditions (Reinbott and Blevins, 1991), but they behaved differently in response to SCN treatment, with levels in leaves increased with high SCN density (Blevins et al., 1995; Francl, 1993). Variability in Ca and Mg concentration in tissue between fields and sampling times was observed in this study. Calcium variability however, was not correlated with SCN population density, whereas Mg in tissue was positively correlated with soil SCN population, pH, and soil Mg in both fields. The spatial distributions of Mg and SCN were inconsistently cross-correlated over time. The inconsistent crosscorrelation of Mg with SCN over time indicated that the two variables were only correlated at the same locations, and samples at neighboring locations were no longer correlated.

The effect of SCN population density on K concentration in leaves is not clear and there is some controversy on whether K fertilization is beneficial to soybean under SCN infection. Part of the controversy results from differences in soil K levels in the different studies (Luedders et al., 1979; Hanson et al., 1988; Blevins et al., 1995; Howard et al., 1998). Smith et al., (2001) have recently shown that SCN population density after

the first 30 days of infection did not affect K levels in leaves, but it increased K in petiole and stem tissue, and reduced K in root tissue. The results presented here were consistent with a three-year field research (Hanson et al., 1988) in which SCN population density was not correlated with K in the soil. Under field conditions, K in tissue at the end of the vegetative and beginning of the reproductive phases was negatively correlated with levels of SCN density early in the season in this study. Potassium concentration in the soil in Field A and Field B was similar to the highest level tested by Luedders et al. (1979), at which they observed a decrease in SCN numbers. Potassium concentration in tissue was higher in Field A than in Field B, even though there were no differences in K concentration in the soil. We also observed that K concentration in leaves was negatively correlated with pH, Ca and Mg in the soil. The higher levels of pH and Mg in the soil in Field B, and the strong positive correlation between pH and SCN population may have contributed all together to reduce K concentration in leaves.

Nitrogen in leaves was sufficient in both fields, with concentrations at R3 somewhat lower than at V9.The relation between SCN population and N concentration in leaves varied with the plant developmental stage. The maximum rate of N_2 fixation is reached at the beginning of flowering, followed by a rapid decline. The decline is most likely an expression of sink competition for photosynthates between the developing pods and the root nodules (Haystead and Sprent, 1981). Nitrogen at R3 was lower than at V9 probably because of nitrogen mobilization to the seeds and reduction in N_2 fixation rate after flowering. Nitrogen mobilization from leaves with reduced nitrogen concentration may have been comparatively less; therefore, the negative relationship between SCN and N at V9 reversed at R3. The pink coloration observed in nodules from healthy looking

plants was due to leghemoglobin, a red-colored enzyme with a central Fe atom in the porphyrin ring. The concentration of leghemoglobin is closely correlated with the N_2-fixing capacity of root nodules (Werner, et al., 1981). The grey coloration and reduced-size observed in nodule from chlorotic plants could be a sign of reduced N_2-fixation activity. Although not quantified, the number of cysts observed on roots was negatively related to the size, number and quality of the nodules in our study. SCN competes with nodules for photosynthates affecting N_2-fixation rates (Poskuta et al., 1986; Sinclair, 1994). In another study, nodules from SCN infected soybean had lower fresh weights per plant and lower specific nitrogenase activity than nodules from uninfected plants (Huang and Barker, 1983).

Phosphorous in tissue was within sufficiency ranges in Field A and in Field B except at R3. Increasing SCN infection level did not vary P concentration in leaves, but decreased the concentration in nodules (Blevins et al., 1995). In our study, P in leaves at R3 was positively correlated with nematodes in the soil and soil pH, and was below sufficiency limits in Field B. At this stage P was also negatively correlated with Mg in the soil in both fields.

Micronutrients in tissue were also affected by SCN population density at planting and early in the season. In Field B, high SCN population density at planting was associated with lower Fe concentration in leaves at V9, but noticeably, Fe concentration at this time was well above the normal sufficiency limit for soybean. In Fe-deficient leaves, contents of chlorophyll decline (Morales et al., 1990) and leaves look chlorotic. Chlorosis symptoms may also appear when plants are not using available Fe effectively, and large amounts of Fe accumulate in the leaves (Mengel and Geurtzen, 1988). This

phenomenon is usually found in plants grown in sandy, calcareous soils, when there is a large supply of P, or when different forms of nitrogen are supplied. It is possible that high levels of SCN interfered in some way with Fe utilization causing high concentration of this element in leaf tissue and the chlorotic appearance of plants in portions of Field B. Iron concentration in tissue samples from Field A was not correlated with SCN population density or soil fertility.

Manganese concentration in tissue was affected negatively by pH and P in the soil, and by SCN population density in Field A, whereas SCN population had a positive effect on Mn in tissue in Field B. Manganese was negatively correlated with Mg in the soil, and since Mg in the soil was much lower in Field A than in field B, Mn in tissue was not surprisingly lower in Field A. On the other hand, Mg in the soil was positively correlated with Mg in tissue and SCN population, and Mg and Mn in tissue were negatively correlated at V9 in Field A. High levels of Mg and SCN in the soil, correlated with high levels of Mg in tissue, resulted in lower Mn in tissue. At higher levels of soil Mg, Mg in the soil and Mn in tissue were positively correlated, and there was no relation with nematodes as observed in Field B, and no correlation between Mg and Mn in tissue either. Manganese in tissue was negatively correlated with Ca in the soil, and Ca was positively correlated with nematodes. Calcium in the soil was similar in Field A and B, but Ca had no effect on Mn in tissue in Field B, perhaps related to differences in pH and P, or differences in soil texture between fields. In its chemical behavior, Mn shows properties of alkali cations such as Mg and Ca and the heavy metals Zn and Fe. It is therefore not surprising that these ion species affect uptake and translocation of Mn in the plant although the mechanism of these effects needs still to be elucidated (Fox and

Guerinot, 1998). Nematodes can affect Mn uptake. Barley plants grown in Mn deficient soil with and without Mn supply had similar number of nematode infections, but plant growth was suppressed without Mn and not affected with Mn supplement (Wilhelm et al. 1985). Manganese concentration was lower in Field A than in Field B, and so were P in the soil and pH, therefore the levels of Mn in tissue were the result of the interaction between nematodes, pH, and P in the soil.

Zinc concentration in tissue was negatively correlated with SCN population density. Zinc in leaves was also correlated with pH, Ca, and Mg in the soil with variability by field and sampling time, similar to the observations for Mn. Zinc is involved in the same enzymatic functions as Mn and Mg (Jones et al., 1998) therefore it was probably affected by the same interactions as Mn.

Copper tissue concentration was within normal range. Calcium, Mg, and pH were negatively correlated with Cu in tissue in Field A, and positively in Field B. The movement of Cu is strongly dependent on the Cu status of the plant (Loneragan, 1981). The relation of SCN with Cu in tissue varied by field and by sampling time. In Field B, Cu in tissue at V9 was negatively related with SCN, but the relation was positive at R3. Copper at R3 was lower than at V9, probably because of translocation from vegetative parts to seeds (Caballero et al., 1996), but the more nematodes the more Cu at this stage, so nematodes may be interfering with mobilization. Francl (1993) found negative correlations between Cu and SCN.

Boron in tissue was within the sufficiency range, and while it was not affected by SCN in Field A, it was consistently and negatively correlated with SCN in Field B. The

correlation of B with other elements such as Ca, Mg and K, or soil pH varied by field and sampling time.

CONCLUSION

This study shed light on the complexity of the interactions among soil fertility and texture, SCN population density, and the nutritional status of the host. The information presented here based on observations on naturally infected fields provides the basis for designing experiments to test cause and effect relationships and advance in the understanding of the relationship between SCN and soybean for management purposes. For example, our results indicate the recommendation to growers to increase K fertilization of SCN infested fields to increase soybean yield should be carefully revised, taking into consideration not only K concentration in the soil, but also soil pH and Ca levels. In addition, this work assists with the delineation of management zones for SCN control based on soil fertility in addition to soil texture.

CHAPTER SEVEN

SPATIAL ANALYSIS OF SOYBEAN YIELD IN RELATION TO SOIL TEXTURE, SOIL FERTILITY AND SCN POPULATION DENSITY.

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SPATIAL ANALYSIS OF SOYBEAN YIELD IN RELATION TO SOIL TEXTURE, SOIL FERTILITY AND SCN POPULATION DENSITY.

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SPATIAL ANALYSIS OF SOYBEAN YIELD IN RELATION TO SOIL TEXTURE, SOIL FERTILITY AND SCN POPULATION DENSITY.

ABSTRACT

The purpose of this work was to identify the extent to which soybean yield was spatially correlated with soil texture, soil fertility, and SCN population densities in two fields in Michigan (Field A and Field B). Soybean yield was measured with a commercial yield monitoring system mounted on the combine connected to a GPS receiver. Yield of the susceptible soybean variety planted in 1999 was 20% and 30% greater than the resistant variety planted in 2000 in Field A and Field B, respectively. Spatial variability in yield was highly structured in Field B, and moderately in Field A. Correlation analysis was performed on yield and sand, clay and silt percentage in the soil, soil pH, P, K, Ca and Mg concentration in the soil, and cysts and SCN in roots population densities data available from previous work. Yield was correlated with sand ($r = -0.89$), clay ($r = 0.86$), and silt percentage ($r = 0.84$), and with soil pH ($r = -0.60$) and Ca concentration ($r = 0.60$) in Field B in 1999. In 2000, Pearson's correlation coefficients were slightly lower. These variables were strongly spatially correlated with yield, as indicated by the cross-correlograms. Yield was also negatively correlated and spatially cross-correlated with SCN population density in the soil. In Field B, correlation coefficients between yield and Pi (SCN population density at planting) were –0.48 in 1999, and –0.45 in 2000, in Field A were –0.16 in 1999, and –0.20 in 2000. Thus, providing basis for future work on delineating management zones for SCN. In fields where soil properties and SCN densities appear spatially structured, and where there is a history of yield spatial variability due to the combined effect of SCN and unfavorable soil conditions, management zone delineation could be an appropriate strategy to overcome yield losses.

The geostatistical analysis of soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe) distribution patterns (Avendaño et al., 2003, Chapter Three), the relationship between SCN and soil texture and soil map units (Chapter Four), soil texture and SCN life cycle (Chapter Five), and spatio-temporal dynamics of SCN and soil and plant nutrition (Chapter Six) were documented. From the preceding chapters, it is clear that the two fields exhibited differences and similarities. For example, the fields differed in soil type, texture, fertility, and SCN population density. SCN was correlated with pH, texture and Ca in both fields, but more in Field B. Nutrients were related with SCN but not consistently between fields or time.

Seed yield is the ultimate interest of a soybean grower, and knowing any relationship between any of the correlations described above and crop yield will be advantageous to the grower's decision-making ability. Therefore, the purpose of this work was to identify the extent to which yield was related to soil texture, soil fertility, and SCN population densities by correlating spatial information on these variables.

MATERIALS AND METHODS

The study was conducted in 1999 and 2000 on two fields (Field A and Field B) in Shiawassee County, MI. Field A consisted of 24 ha, was managed under no-tillage since 1996, and was planted to corn prior to this study in 1998. Percent sand ranged from 45% to 74%, percent clay ranged from 10% to 21%, and percent silt ranged from 8% to 43% within Field A (Chapter Four). Field B was comprised of 13 ha, was conventionally tilled after wheat in 1998, and was managed under no-tillage thereafter. Percent sand ranged from 50% to 80%, percent clay ranged from 8% to 23%, and percent silt ranged from

11% to 29% within Field B (Chapter Four). The soybean varieties planted were Asgrow 1901 in 1999 (SCN-susceptible) and Asgrow 2201 in 2000 (SCN-resistant). Both varieties were Roundup-Ready. Soybean was planted in 19.1-cm rows at a rate of 519 000 viable seeds ha^{-1} in 1999, and 494 000 viable seeds ha^{-1} in 2000. Fields A and B were planted 5/22/99 and 5/16/99, respectively, and 6/9/00. Weed control was maintained using Roundup at the recommended rate. There was one preplant application in Field A in 1999 and 2000, one application postemergence in 1999, and a midseason application in Aug. 2000. In Field B there was one preplant application in 2000, one postemergence in 1999 and a mid season application in Aug. 2000. Rows orientation was north-south in Field A and east-west in Field B.

SCN, soil texture and soil fertility data were obtained from Avendaño et al. (2003, Chapter Three), and Chapters Four, Five and Six. The spatial sampling for soil samples consisted of a nested survey sampling design with distances reduced by geometric progression (adapted from Webster and Boag, 1992) applied within 8 and 5.25 ha in the center of Fields A and B, respectively, as described in Avendaño et al. (2003, Chapter Three). Single-core soil samples were collected using an 8 cm diameter by 23 cm deep bucket auger (Riverside Augers, Eijkelkamp, Giesbeek, The Netherlands) from 160 and 110 locations in Field A and Field B, respectively. Each sample collected before planting in 1999 was thoroughly mixed and then divided in three sub-samples. One sub-sample was used for SCN analysis, the second was used for texture analysis, and the third portion of the sample was analyzed for pH, phosphorous, K, Ca, and Mg.

The collaborating farmer measured soybean yield in both fields in 1999 and 2000 with a commercial yield monitoring system mounted on the combine connected to a GPS receiver.

Statistical Analysis

Sample mean and variance were calculated for yield in each field and year. Means between years within fields were compared with the z-test for means (α= 0.05), and variances between years within fields were compared with the F-test (α= 0.05).

A subsample was extracted from each yield data set matched by the locations were soil sampled had been collected for texture, fertility, and SCN analysis to perform correlation analyses. Pearson's simple linear correlation coefficients were calculated for yield and soil texture, yield and soil fertility, and yield and SCN population density (The SAS System Release 8; SAS Institute, Cary, NC).

Geostatistical analysis was used to quantify the spatial variability in yield in Field A and Field B in 1999 and in 2000. The spatial analysis of yield was performed on a subsample consisting on the data points collected within the area sampled for soil and SCN analyses. General geostatistical methods were described in Avendaño et al. (2003, Chapter Three). Directional semivariograms were calculated to explore anisotropy in the spatial variability in yield. Geometric anisotropy can be visualized as elliptic iso-correlation contours, defined by the ratio of two orthogonal axes (radius 1 and radius 2) and an orientation angle. We define the orientation angle as the counterclockwise rotation between the positive X-axis (east direction) and radius 1. The ratio of major and minor axis is defined as the ratio of the largest and shortest range in the empirical directional semivariograms. Directional semivariograms were calculated for lags of 12 m so that at

least 30 data pairs were available for each lag in each direction with an angle tolerance of 22.5 angular degrees. The parameters of the semivariograms and the degree of anisotropy were estimated by least squares fitting of theoretical semivariogram models.

The cross-correlogram is a geostatistical tool used to define the joint spatial dependence or continuity between measurements of different attributes or of the same attribute measured at different times. In the cross-correlation function, the cross-correlation coefficient at lag equal to zero is the same as the classical correlation coefficient between two variables. The cross-correlation function given by Goovaerts (1997) was used here to calculate cross-correlograms for yield and the following attributes: sand, clay and silt percentage in the soil, soil pH, P, K, Ca and Mg concentration in the soil, and logarithmic transformed cysts and nematodes in roots population densities. Cross-correlations were calculated for each field and year in the cases were linear correlations were significant (α= 0.05). Only the data points from locations sampled for both attributes were used for these analyses.

RESULTS

Soybean yield

The SCN-susceptible soybean planted in 1999 yielded more kilograms per hectare of seeds than the SCN-resistant variety planted in 2000 in both fields (Table 7.1). Yield variance in Field B was significantly higher in 1999 than in 2000, and two times greater than in Field A in both years. The georeferenced data collected by the yield monitor was plotted and a map of seed yield in 1999 and 2000 were obtained for each field.

Table 7.1. Soybean yield within the areas sampled in Fields A and B in 1999 and in 2000.

	Soybean seed yield (Kg ha^{-1})			
	Field A		Field B	
Year	Mean†	Std. Dev.‡	Mean†	Std. Dev.‡
1999	3104.5 a	239.5 a	2940.7 a	688.2 a
2000	2498.1 b	252.3 a	1997.1 b	543.9 b

† Means within columns followed by the same letter were not significantly different at the 5% level (z-test for means).
‡ Standard deviations followed by the same letter indicate variances within columns were not significantly different at the 5%level (F-test for variance).

In Field A, seed yield was relatively uniform through out the field in both years (Figure 7.1). The lighter coloration along the south edge of the field indicated lower yields in this area slightly elevated over the rest of the field. In Field B yield maps, two distinct zones could be identified. Seed yield on a large section on the north side of the field was low and appeared patchy, whereas on the rest of the field seed yield was much higher and uniform (Figure 7.2). In 2000, the lower yield zone occupied a greater proportion of the field. On the west side of the south zone there was a sector of lower yield, corresponding to an area that was ponded for long periods from September until harvest in 2000.

The directional semivariograms of yield in Fields A and B are shown in Figure 7.3. The parameters of the corresponding semivariogram models fitted are shown in Table 7.2. The spatial structure in yield distribution within the area sampled for soil was moderate in Field A and high in Field B. In Field A, the shape of the semivariograms in 1999 and 2000 were similar, but the variability in yield distribution in 1999 was greater than in 2000 as evidenced by greater sill and nugget variances in 1999. The longest and shortest ranges of spatial autocorrelation extended beyond the longest and shortest dimensions of the field, respectively.

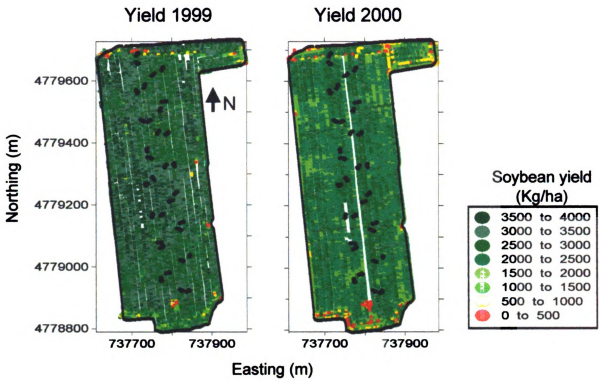

Figure 7.1. Soybean seed yield in Field A in 1999 and in 2000. Seed yield was recorded by a yield monitor system connected to a GPS unit mounted on the combine. Black circles indicate the locations where soil samples were collected for SCN, soil texture and soil fertility analyses. **This image in color in the dissertation.**

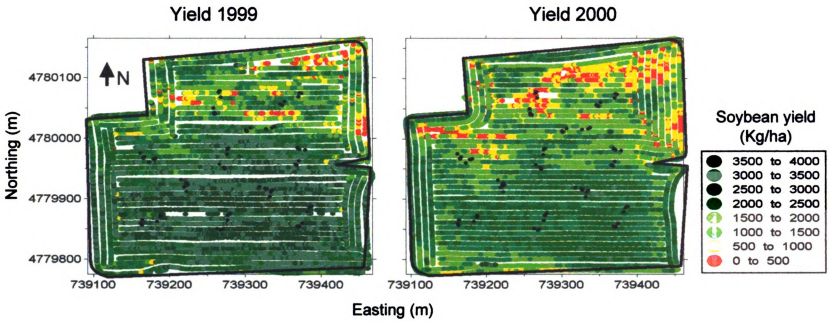

Figure 7.2. Soybean seed yield in Field B in 1999 and in 2000. Seed yield was recorded by a yield monitor system connected to a GPS unit mounted on the combine. Black circles indicate the locations where soil samples were collected for SCN, soil texture and soil fertility analyses. **This image in color in the dissertation.**

Table 7.2. Parameters of the theoretical semivariogram models of soybean yield in Fields A and B in 1999 and in 2000.†

	Drift‡	Model function	C_0 §	C¶	$\frac{C_0}{C+C_0}$ #	Range††	Anisotropy‡‡	
							Angle§§	$\frac{Radius1}{Radius2}$ ¶¶
			10^3	10^3		m		
Field A 1999	No	Nugget	239					
		Exponential		260	0.48	714	12	0.5
Field A 2000	No	Nugget	118.5					
		Exponential		109.2	0.48	956	25	0.5
Field B 1999	Linear	Nugget	79.9					
		Exponential		228	0.26	67	77	0.5
Field B 2000	Linear	Nugget	26.7					
		Exponential		221	0.11	26	86	0.5

† Models were fitted by least squares based on empirical semivariograms calculated for lags of 12 m (h), with a lag tolerance of h/2. The minimum number of pairs required for each lag was 30

‡ Whenever semivariograms showed nonstationarity, the data were detrended carrying out a simple polynomial least squares regression and semivariogram analysis was performed on the residuals. The polynomial order of the trend is indicated when a drift or trend was removed.

§ C_0 is the nugget effect or a discontinuity in semivariance at the origin due to microscale variability or sampling error.

¶ C is the partial sill.

$C_0/(C+C_0)$ is an indicator of the degree of spatial structure, the lower the number the stronger the spatial autocorrelation.

†† Observations that were spatially separated by more than the range at the orientation angle indicated (*radius 1*) were uncorrelated.

‡‡. Anisotropy can be visualized as an ellipse specified by the length of its two orthogonal axes (*radius 1* and *radius 2*) and by an orientation angle.

§§ The orientation angle is defined as the counterclockwise angle between the east direction and radius 1.

¶¶ For the exponential model, radii are defined as the largest and the shortest range in the empirical directional semivariograms.

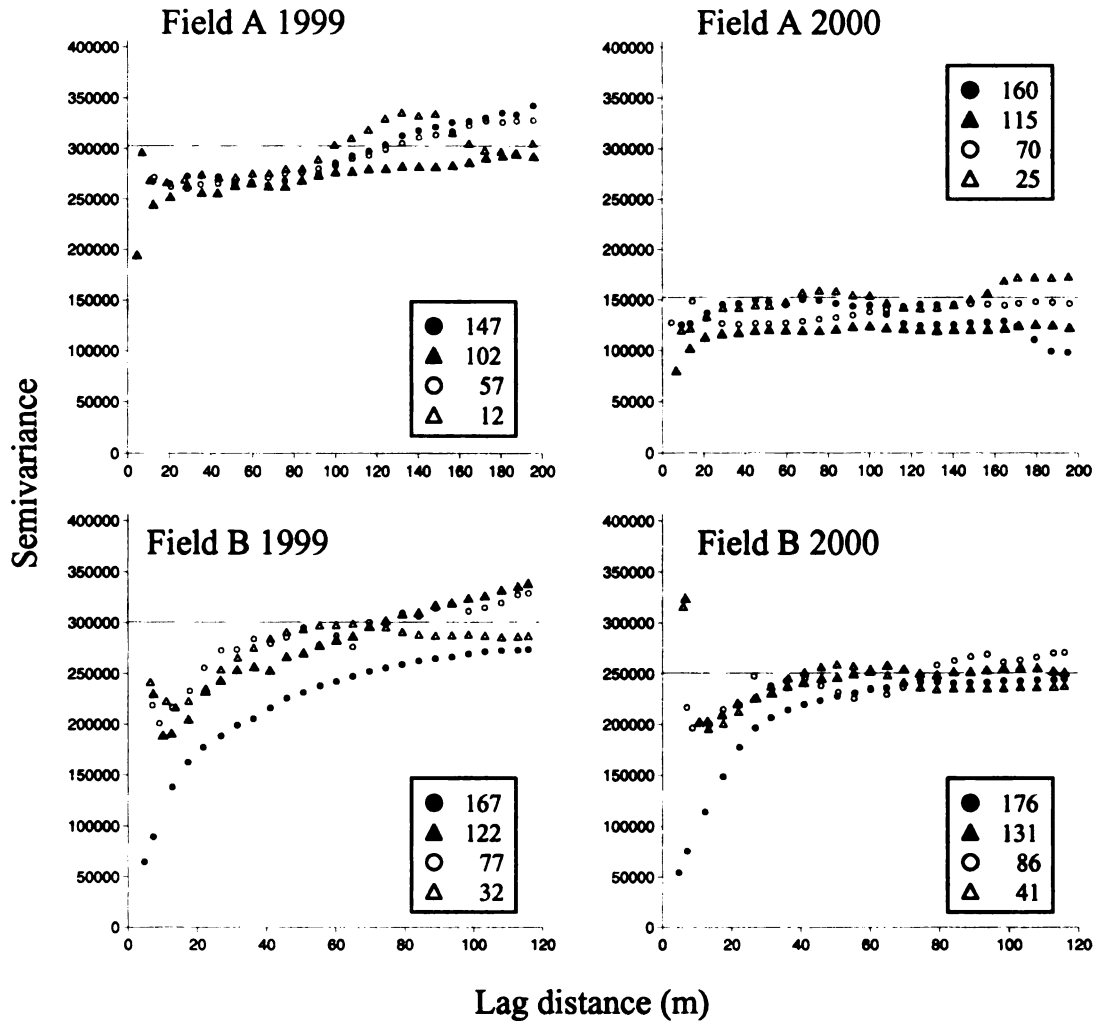

Figure 7.3. Anisotropic semivariograms of soybean yield (Kg/ha) in Fields A and B in 1999 and in 2000 in different directions in degrees from East. The dashed line is the sample variance. Parameters of the semivariogram models are shown in Table 7.2.

There was less yield variability along than across rows as indicated by the anisotropy parameters. In Field B, yield spatial variability was highly structured, and more so in 2000 than in 1999. Yield variability was greater along the north-south direction as indicated by the shorter range of spatial autocorrelation perpendicular to the row orientation both years.

Soybean Yield and Soil Attributes

Soybean yield and soil texture were strongly correlated in Field B both years, weakly in Field A in 1999, and not linearly correlated in Field A in 2000 (Table 7.3). Soils with high percentage of sand and low levels of clay and silt yielded significantly less seed than finer soils. Soybean yield and soil texture were cross-correlated over a range of 120 to 130 m, consistently across fields and over time (Figures 7.4 and 7.5). In Field A, sand was more strongly cross-correlated with yield than silt or clay (Figure 7.4), but in Field B the cross-correlograms for the three soil separates with yield were very similar in structure, with sand negatively correlated with yield while the relation with clay and silt was positive (Figure 7.5). Soil pH and yield were related in both fields in 2000 and only in Field B in 1999. High yield was associated with low pH in all cases (Table 7.3), but the range of spatial correlation between the two attributes varied between fields.

Table 7.3. Pearson's correlation coefficients for soybean seed yield with sand, clay, silt, and soil pH and concentrations of P, K, Ca and Mg (Kg ha^{-1}) in Fields A and B in 1999 and in 2000.

	Soybean seed yield			
	Field A		Field B	
Soil attributes†	1999	2000	1999	2000
Sand	-0.36**	NS	-0.89**	-0.77**
Clay	0.28**	NS	0.86**	0.73**
Silt	0.27**	NS	0.84**	0.73**
pH	NS	-0.39**	-0.60**	-0.56**
P	NS	NS	0.20*	NS
K	NS	0.32**	0.34**	0.29**
Ca	NS	-0.30**	0.60**	0.47**
Mg	0.31**	-0.19*	NS	NS

*, ** Significant at the 0.05 and 0.01 probability levels, respectively.
† Soil nutrients and soil texture were analyzed on samples collected at planting in 1999.

In Field A, pH and yield from samples separated by more than 50 m were not linearly correlated (Figure 7.4), whereas in Field B the relation was maintained for up to 140 m (Figure 7.5). Soybean yield was poorly correlated with soil fertility, with great variability between fields and years. In Field B, soils with higher levels of Ca and K produced higher seed yield in 1999 and 2000, whereas in Field A higher seed yields were obtained from soils with high levels of K and lower levels of Ca and Mg, although this was true only in 2000. In 1999, yield was only correlated with Mg concentration in the soil in Field A (Table 7.3). Yield was correlated with P only in Field B in 1999. The spatial correlation between yield and soil nutrients was poor in Field A, except for Mg in 1999. The cross-correlation yield-Mg was almost identical to the cross-correlograms for clay and silt (Figure 7.4). In Field B, Ca was cross-correlated with yield for up to 120 m approximately, whereas P and K were not spatially correlated with yield (Figure 7.5).

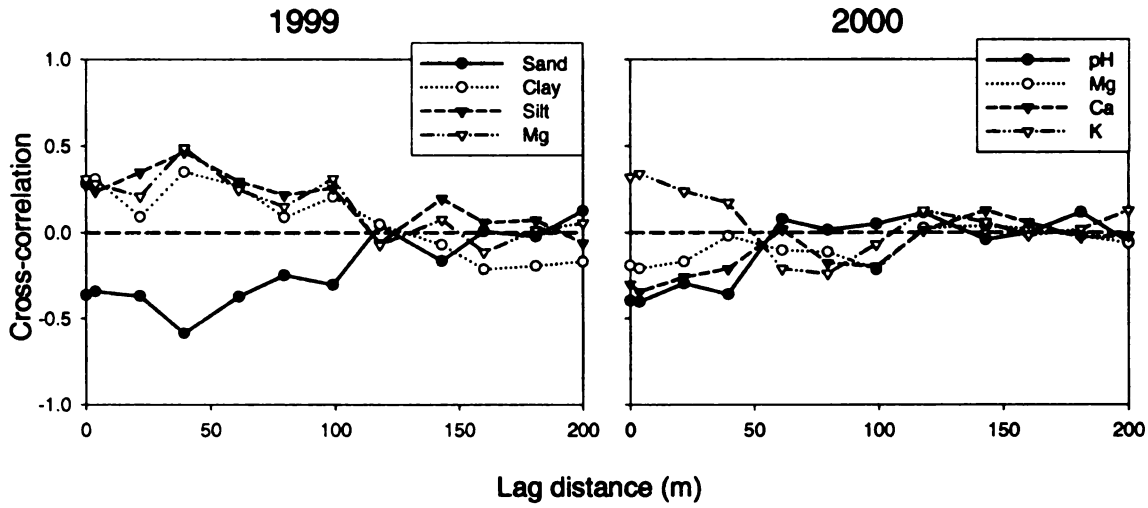

Figure 7.4. Cross-correlograms of yield and soil texture (% sand, % clay, and % silt), and soil fertility (pH, and Mg, Ca, K) in Field A in 1999 and in 2000. Cross-correlation at zero lag distance equals the linear correlation coefficient.

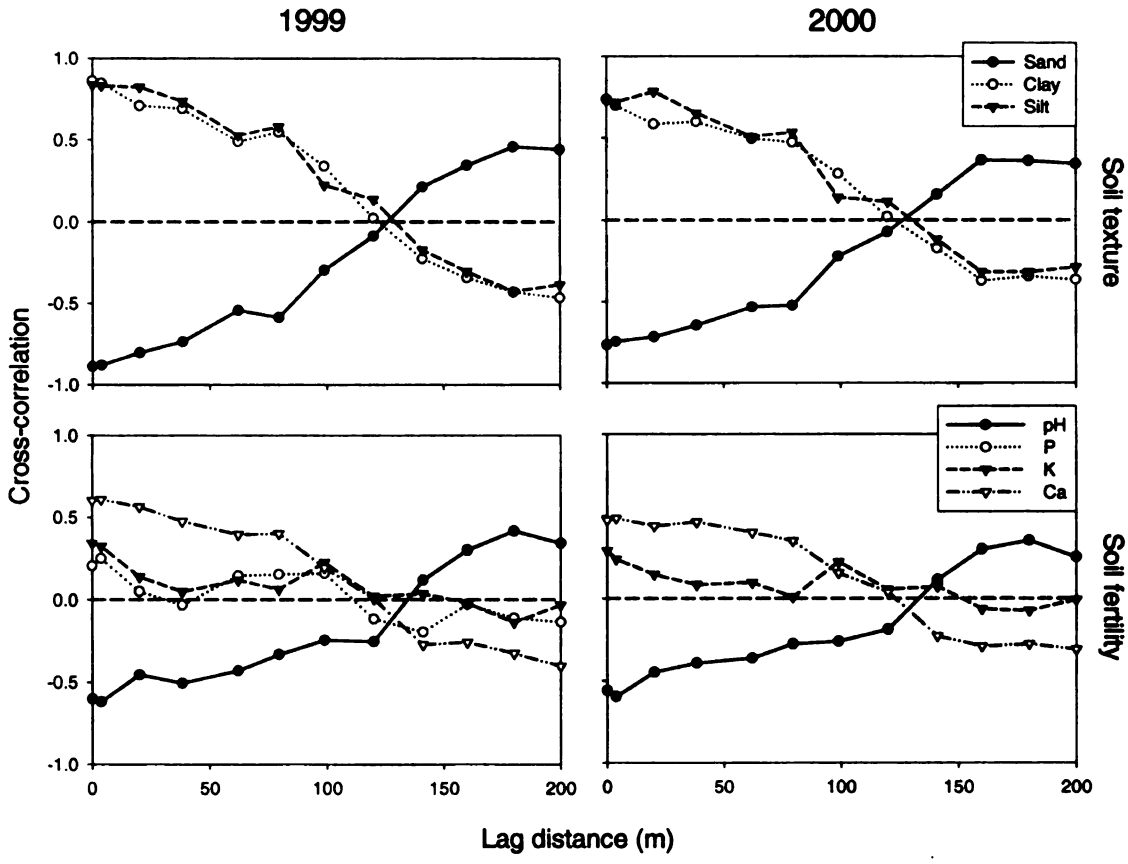

Figure 7.5. Cross-correlograms of yield and soil texture (% sand, % clay, and % silt), and soil fertility (pH, and P, K, Ca in Kg ha^{-1}) in Field B in 1999 and 2000. Cross-correlation at zero lag distance equals the linear correlation coefficient.

Soybean Yield and SCN Population

Soybean yield and SCN population density were more strongly correlated in Field B than in Field A, and cyst population in the soil was more strongly and more often related to yield than SCN infection levels in the roots. Correlations were negative in all cases (Table 7.4). The correlation between SCN and yield in Field A was very poor, and in 2000, it was only significant for the number of cysts in the soil at planting. In Field B however, SCN population densities at planting and over the season were relatively strongly correlated with 1999 and 2000 seed yields. SCN population in the roots and yield were only related in 1999. Even though the spatial correlation for yield and SCN

population density was poor in Field A, the structure of the cross-correlograms was very similar for all SCN samples, with a range slightly above 50 m (Figure 7.6). Moreover, cyst population density at planting in 2000 explained yield variability up to a separation distance of about 50 m between observations as well (Figure 7.6).

Table 7.4. Pearson's correlation coefficients for soybean seed yield and SCN population density sampled at monthly intervals in Fields A and B in 1999 and in 2000.

	Soybean seed yield			
	Cysts in the soil†		Larvae and adults in roots‡	
SCN sample	1999	2000	1999	2000
		Field A		
Planting	-0.16*	-0.20**		
June§	NS	NS	NS	NS
July	-0.25**	NS	-0.32**	NS
August	-0.18*	NS	NS	NS
Harvest	-0.23**	NS	NS	
		Field B		
Planting	-0.48**	-0.45**		
June#	-0.49**	-0.36**	-0.28**	NS
July	-0.45**	-0.37**	NS	NS
August	-0.65**	-0.19*	-0.22*	NS
Harvest	-0.45**	-0.59**	-0.19*	

*, ** Significant at the 0.05 and 0.01 probability levels, respectively.
† SCN cysts extracted by elutriation and sugar flotation from 100 cm^{-3} of soil sub-samples.
‡ SCN larval stages and mature females counted in 2 g of stained soybean roots. Root samples were not collected at harvest in 2000.
§ In 1999, Field A samples were collected at 31 (June), 59 (July), 86 (August), and 126 (harvest) days after planting (DAP). In 2000, samples were collected at 17 (June), 45 (July), 73 (August), and 147 DAP (harvest).
# In 1999, Field B samples were collected at 44 (June), 73 (July), 100 (August), and 125 DAP (harvest). In 2000, samples were collected at 19 (June), 46 (July), 77 (August), and 155 DAP (harvest).

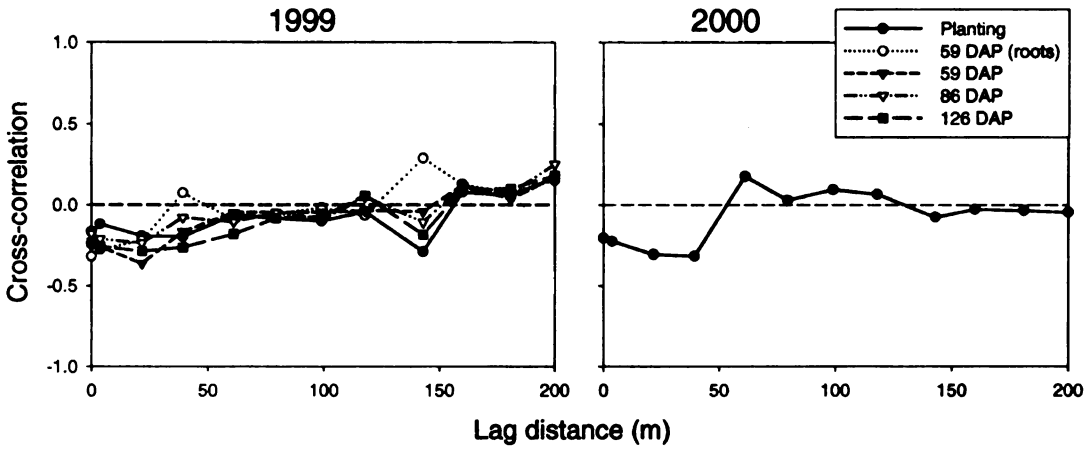

Figure 7.6. Cross-correlograms of yield and SCN population density in Field A in 1999 and in 2000. SCN population density was quantified in soil (cysts) and in soybean root samples (all developmental stages) collected at the days after planting (DAP) indicated. Cross-correlation at zero lag distance equals the linear correlation coefficient.

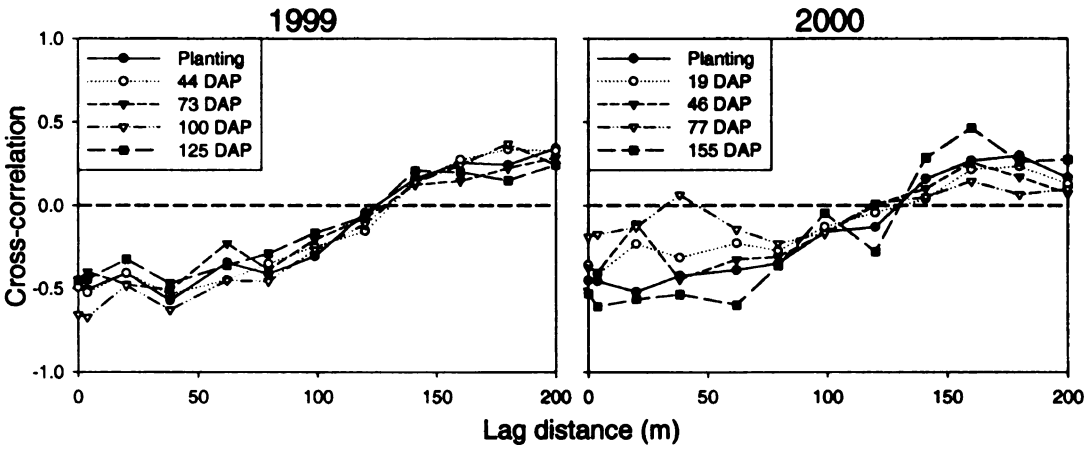

Figure 7.7. Cross-correlograms of yield and SCN cyst population density in soil samples in Field B in 1999 and in 2000. Soil samples were collected at the days after planting (DAP) indicated. Cross-correlation at zero lag distance equals the linear correlation coefficient.

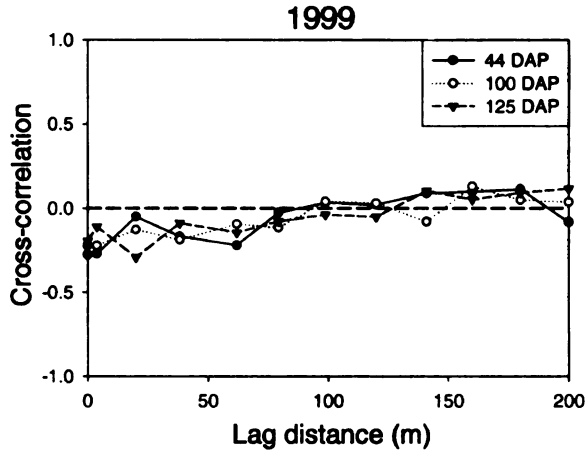

Figure 7.8. Cross-correlograms of yield and SCN population density in soybean roots in Field B in 1999. Root samples were collected at the days after planting (DAP) indicated. Cross-correlation at zero lag distance equals the linear correlation coefficient.

The range of spatial correlation between yield and cyst densities in Field B was about 130 m for all samples collected from planting to harvest in 1999. In 2000, there was great variability in the cross-correlograms, but still the range for yield and cyst densities was maintained (Figure 7.7). The level of infection, or number of SCN in the roots was practically not spatially correlated with yield in Field B in 1999 (Figure 7.8).

DISCUSSION

In addition to genetics, crop yield is a function of biotic and abiotic factors limiting plant productivity (Marschner, 1995). Often, the yield-limiting factors have spatio-temporal structure that could be helpful in management decision-making (Pierce and Sadler, 1997; Cassel et al., 2000). For example, linking spatial information of soil texture, nutrients, and pest problems can allow for diagnostic determination of the

predominant factor(s) controlling crop production. This then becomes the basis for developing precision input strategies (Sudduth, 1999).

Better yield was obtained from the SCN-susceptible soybean variety sown in 1999 than from the resistant variety sown in 2000. Soybean yield potential in the presence of SCN varies among varieties and with level of infection, field topography, soil properties, climate, and management practices (Koenning et al., 1988; Koenning et al., 1995; Tylka et al., 1998; Niblack 1999; Koennning, 2000; Kravchenko and Bullock, 2000; Chen et al., 2001; Long and Todd, 2001). The differences in yield observed between years in this study could have been a consequence of differences in weather conditions and date of planting between years, in addition to the genetic differences existent between varieties (Koenning et al., 1993; Noel and Edwards, 1996). The difference in yield between fields, however, were probably due to differences inherent to each field such as soil properties, or to other factors unique to each field such as SCN population load.

Spatial variability analysis of the relationships among yield, SCN population density, soil texture and soil fertility exhibited variable responses between the two fields and among the parameters. Of all the variables examined, soil texture, soil pH and Ca concentration had strong spatial correlation with yield. Analysis of cross-correlation between a variable and another variable that is sampled at neighboring locations with increasing distance provides insight into the spatial covariance structure (Davis, 1986). A large degree of cross-correlation was observed between yield and soil texture, pH, and Ca concentration in Field B. In general, soil texture and soil fertility were more variable and were better spatially structured in Field B than in Field A (Chapter Four, Chapter Six).

The greater spatial structure and variability in yield in Field B was therefore expected, as well as the poor spatial structure and rather uniform distribution of yield in Field A.

The strong spatial correlations in Field B generated a "good zone" on the south side of the field, area of good yield, and lower pH, and a "problem zone" on the north side, a sandier area of higher pH and Ca concentration where yield was extremely low. In addition to differences in soil factors, these two areas differed significantly in SCN population density. The cross-correlations between yield and SCN population densities extended over approximately the same range as yield and soil properties, providing evidence that SCN population in the soil contributed to the delineation of the good and poor yield areas in Field B as well.

The relationships demonstrated in this work provide the basis for future work on delineating management zones for SCN. In fields where soil properties and SCN densities appear spatially structured, and where there is a history of yield spatial variability due to the combined effect of SCN and unfavorable soil conditions, management zone delineation could be an appropriate strategy to overcome yield losses.

CHAPTER EIGHT

GENERAL DISCUSSION AND CONCLUSION

The goal of this Dissertation was to understand the spatial distribution of the SCN in soybean fields to assess the potential for developing SSM strategies for SCN. In order to apply SSM strategies, it is necessary to know SCN spatial distribution, and how it changes through time. Being able to detect the presence of the nematode before it reaches damaging levels and to predict its location in a field will reduce effort and cost of control practices while increasing their efficiency. In addition, knowing the role of soil nutrition in the relationship plant-nematode interactions may provide tools for reducing or even preventing low yield patches, therefore increasing productivity.

In Chapter Three, the magnitude, structure, and persistence in time of the spatial distribution patterns of SCN cysts, eggs, and eggs per cyst were studied under field conditions to test the hypothesis that the spatial distribution of SCN is sufficiently structured and time-invariant to support the use of SSM in SCN infested fields. The structure of SCN spatial distribution was further analyzed in Chapter Four in relation to soil texture. For that purpose, the structure in soil texture spatial distribution and its relationship to published soil survey maps were assessed within the study sites. The extent to which the spatial variability in SCN cyst population density was related to soil texture was also determined and quantified; and the extent to which this relationship held between fields with similar soil types but different SCN population levels was assessed. In Chapter Five, SCN population dynamics in soybean roots and in the surrounding soil were described and analyzed spatially in relation to soil texture. Chapter Six focused on

the relationship between soil fertility and SCN population, and how did this relationship reflected in tissue analysis to explain the differential degree of damage due to SCN observed in infected soybean fields. Soil fertility variability was characterized in relation to soil texture and tissue analysis, and SCN in the soil and in soybean roots was analyzed in relation to soil fertility and tissue analysis. The purpose of Chapter Seven was to identify the extent to which yield was related to soil texture, soil fertility, and SCN population densities by correlating spatial information on these variables. Seed yield is the ultimate interest of a soybean grower, and knowing the relationships among variables within a field with soybean yield would be advantageous to the grower's decision-making ability.

While it is not known when SCN was introduced into the fields and what undetermined factors may have contributed to the difference in SCN population density, Field A and Field B presented me with the opportunity to study the relationships where SCN population was involved under two different conditions frequently encountered by soybean growers. Cyst population density was high in Field B and it increased during the study, whereas in Field A, cyst density was much lower and remained constant over two years. The within field variability in cysts, eggs per cyst, and eggs was large in both fields, and even though the spatial structure in SCN population in general was poor, the temporal variability was small. Several infections took place over the growing seasons of 1999 and 2000, but the greatest density increase occurred during the first month after planting, followed by a decline in July- August (46 to 100 DAP). The low nematode counts in roots recorded during the summer could be a reflection of the infective behavior of SCN. Since the roots sampled in this study were collected close to the base of the

plant, an important portion of the SCN population in the roots may have been missed if new infections were occurring at the root tips, where tissue is softer, rather than on older portions of the roots. However, a decrease in SCN population density in roots accompanied by a decline in cyst densities in the soil is an indication of reduced hatching and consequently reduced new infestations, supporting the notion of early-induced dormancy proposed by Yen et al. (1995). Decrease hatching indicative of SCN dormancy in midsummer has been observed in Iowa and north Missouri (Yen et al., 1995), whereas in North Carolina, dormancy is induced at the end of the growing season (Hill and Schmitt, 1989). The resistant soybean variety planted in 2000 did not prevent SCN population density increase, cyst density at harvest was equal to or higher than at planting, and the number of eggs per cyst was comparable to those observed at harvest in 1999. The spatial structure in SCN population varied with sampling times, but the periodicity pattern in semivariograms appeared consistently from planting to harvest in both fields. The difficulty in fitting an appropriate wave model to the empirical semivariograms underestimated in some cases the spatial structure in SCN population. Nonetheless, two levels were identified in the spatial structure of SCN population. A short-range process described by the wave model, where cyst densities in the soil were arranged in small clusters, and a larger range process described by spherical or exponential models that represented the spatial arrangement of the small clusters. For management decision-making purposes, the larger range process is of interest to detect high infestation areas in a field. Once these areas are located, a more intensive sampling may be necessary to identify the extent of the infestation.

Although at present the two fields are managed similarly, differences in management histories could possibly have been a factor in the variability in SCN populations currently observed between fields. Moreover, the differences in soil texture and soil fertility between the two fields were also involved in defining SCN population density and spatial distribution, given that soil texture, pH, and calcium concentration were strongly correlated with SCN population density in the soil, but to a lesser extent with SCN in the roots. The proportion of sand, clay, and silt in the soil were strongly cross-correlated with SCN population density at different sampling times, indicating that soil texture data at any given location in a field may provide some information about SCN population density over a given separation distance. The separation distance determined by the range of the cross-correlograms in Field A and Field B was 110 to 130 m. Soybean roots from plants grown in fine textured soil had higher SCN population density, but the number of cysts in the soil was lower than in sandier soil. This indicates that even though a larger number of infective juveniles were able to penetrate soybean roots in fine soils, reproduction or survival was lower than in coarser soils. Soil texture is a soil attribute relatively invariant in time that is less expensive to analyze than SCN. Based on the consistency in time and across fields observed in the correlation between soil texture and SCN population density, the information provided by texture analysis could be used to identify SCN high-risk areas in a field. Therefore, the cost and effort of sampling for SCN can be reduced if data on soil texture are available for a particular field.

The nutritional status of the crop was correlated with soil fertility, soil texture, and with SCN population density. Tissue nutrient analysis reflected the interactions of all

of these variables combined. In a field study such as this one, the variables under study are beyond the researcher's control, and conclusions are based on observations of natural phenomena only. The results presented here on the relations between SCN and tissue nutrient analysis constitute a valuable source of information for designing controlled experiments and advance in the understanding of SCN role in plant nutrition. Despite the differences between fields, however, some consistencies were observed. For example, calcium concentration in leaf tissue was not related to SCN population density, and, whereas magnesium was directly related to SCN population density at all sampling times, zinc and boron were inversely correlated with SCN levels in both fields. Seed yield was strongly correlated with soil texture, soil pH and calcium concentration, and with SCN population density in the soil. The differences in yield observed between years could be explained in terms of soybean variety, weather conditions, planting date, or previous crop. Differences between fields however, were the result of the interactions of SCN and soil attributes among other factors not accounted for in this project, such as field topography and soil moisture.

The analyses of yield maps showed relatively homogeneous seed yield throughout Field A, and two distinct areas with good and poor yield in Field B. The two zones identified in Field B were very different with regards to the soil attributes measured. The soil in the good yield area was sandier, with higher pH and calcium concentration, whereas in the poorer yield section soil was finer, with lower pH and calcium concentration. Moreover, SCN population density was consistently higher on the poor yield area and remained low on the good yield portion of the field over the duration of the

study. In Field A, however, the correlation between different soil attributes was weaker and SCN density was low through out the field.

The working hypotheses of this Dissertation were demonstrated to be true. SCN's spatial distribution was sufficiently structured and time invariant for SSM to be an effective management strategy. In addition, SCN spatial distribution and population density were related to soil properties such as soil texture, soil pH, and calcium concentration in the soil that are easier to measure and manage. The relations among SCN population density, soil properties and soybean yield were sufficiently strong to aid in the management decision-making process. Therefore, SSM for SCN is plausible.

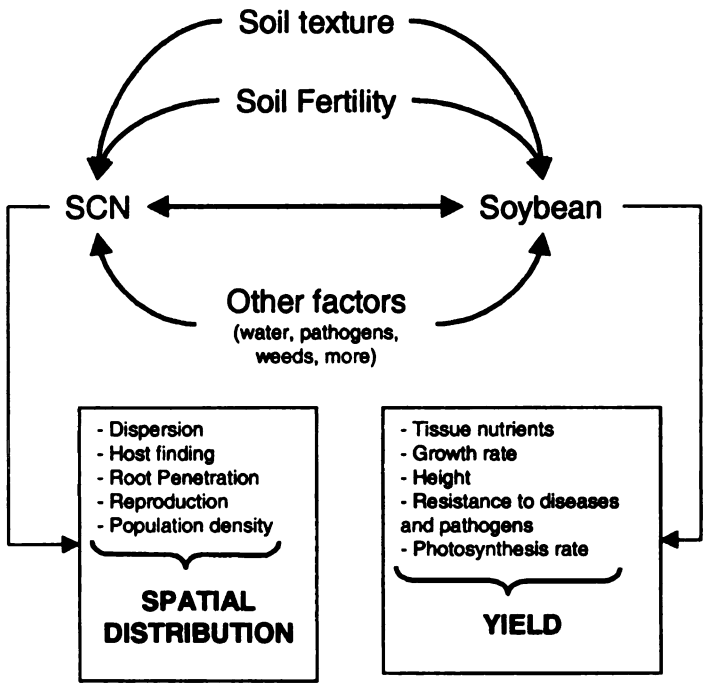

Figure 8.1. SCN-soybean system. The diagram represents some of the factors and elements involved in this system in a commercial soybean field.

The correlations and interactions observed during this study are represented in a diagram (Figure 8.1). The success of SCN population in the soil and in soybean roots

depends on the presence of a host (soybean), and conversely, soybean development, growth and yield are affected by the presence of SCN. These interactions rise from the parasitic nature of the relationship. Environmental factors can modify the host-parasite relationships positively or negatively. Among them, soil fertility and soil texture affect the nutritional status of the plant, and SCN population density and spatial distribution. Other factors not accounted for in this study, such as soil water availability, pathogens and pests, weeds, and weather have an important role in this system as well affecting the plant or SCN. The outcomes of the interaction of all the elements represented in Figure 8.1 are the spatial distribution of SCN population and soybean yield, and these will vary by field because conditions vary by field as well.

The results of this work made a significant contribution towards the understanding of SCN spatial distribution in soybean fields, providing evidence of the underlying factors involved in determining seed yield, and laying the basis for further research on cause and effect relations to advance in the understanding of SCN biology, ecology, and management opportunities. The differential degree of SCN infection and yield loss between fields, accompanied by differences in the spatial arrangement of soil attributes observed provided the foundation for developing SSM of SCN through the delineation of management zones.

APPENDIX A

ELUTRIATION PROCEDURE EFFICIENCY TO EXTRACT SCN CYSTS FROM THREE SOIL TYPES

A variety of techniques, all of which are based on the flotation and sieving principles developed by Cobb (1918), have been used to extract nematodes from soil. The semi-automatic elutriator and the wet-sieving and decanting technique are currently the most frequently used methods to extract soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, cysts from the soil (Tylka, 1998; Smolik, 2001; Donald, 1999). In both techniques, cysts are collected in a 250 µm pore sieve (mesh #60). However, SCN morphometrics indicate that cysts can be as small as 200 µm (Hirschmann, 1956). Moreover, when cysts are extracted from sandy soils, a large amount of sand particles can get retained in a #60 sieve, increasing the difficulty of separating the cysts from soil. Another difficulty in cyst extraction from soil is to obtain a good sub-sample of soil. The soil is usually mixed with water by hand to dislodge large clumps. The elutriator enhances this step by mixing the soil and water with pressurized air. In addition, it can significantly reduce the amount of work required to analyze a large number of samples and the variability due to human error in the extraction process (Byrd et al., 1976).

Because of the variability of soil texture and structure, pre-treating the soil with a soap solution to release cysts trapped in small soil aggregates has been proposed (Tylka, personal communication). However, there is no published information. Pre-treating the soil with a soap solution, removing the sieve #60 from the elutriator setup, and collecting cysts in a 75-µm pore sieve (mesh #200) could potentially increase extraction efficiency. Before I started my research, therefore, I tested efficiency of four methods of cyst extraction using a semiautomatic elutriator to extract cysts from sandy loam, muck and

clay loam soils. The contamination level for each method, and the efficiency for extracting cysts from low to high inoculum levels were also tested for each soil type.

MATERIALS AND METHODS

Soil types, SCN inoculum and Experimental Design

The experimental soils used were clay loam collected from a plowed field at MSU-Collins Road facility, muck obtained from MSU-Muck Farm Research Facility, and sterilized sandy loam obtained from MSU-Greenhouse facilities. The experimental soil was artificially inoculated with SCN cysts, mostly yellow and brown, obtained from commercial soybean fields in Shiawassee and Saginaw Counties, MI. Cysts extracted from soil were counted under a stereoscopic microscope and placed in test tubes in 10 ml tap water at the right inoculum level. Inoculum levels were 0 (control), 50, 100 and 150 cysts. Inocula were kept at 4°C until used to inoculate the experimental soils for the efficiency test within 48 hours of extraction.

Elutriator and Extraction Methods

The equipment used for this test was a semiautomatic elutriator manufactured at the University of Georgia in 1999 (Research Services Instrument Shop, The University of Georgia, Athens, GA), described by Byrd, et al. (1976) as North Carolina Elutriator (NC-EL). A semi-automatic elutriator consists of four funnels where four soil samples are simultaneously mixed with water using pressurized air (40 psi). As the funnels fill up, the overflowing soil-water suspension is poured through an 850-μm pore sieve (mesh #20) to retain large soil particles and plant debris. Sieves are positioned at an angle and showerheads spray water on the sieves to help the soil pass through and into another

funnel that directs the flow to a sample splitter. The sample splitter is a metal bowl that collects nematodes and small soil particles suspended in water and drains through 15 Tygon tubes into a sieve where cysts are retained.

Four extraction methods were tested: Method 1 consisted on placing a sieve #60 underneath the sieve #20 to collect cysts directly from the spout after mixing the soil with water. Everything that passed through the sieve #60 was discarded. Method 2 consisted on placing the sieve #20 at the spout and a sieve #200 to collect cysts from the sampler splitter. Seven aliquants were collected because the volume of water and the amount of soil particles flowing from 15 tubes overflowed the sieve. Methods 3 and 4 consisted of Methods 1 and 2, respectively, with pretreatment of the soil with a soap solution. The soap solution was prepared by dissolving one 50 oz. box of Electrasol™ dishwasher soap in 18 L of hot water (Tylka, personal communication). Soil samples were prepared for elutriation for all treatments as follows except when noted. Four one-liter plastic beakers were filled with 350 ml of tap water. For methods 3 and 4 one hundred milliliters of soap solution were placed in the beakers and then tap water was added to 350 ml. The appropriate nematode inoculum was added and the volume was adjusted to 400 ml. To measure soil volume independent of moisture or air space, soil was added into the beaker until a final volume of 500 ml was reached. The suspension was thoroughly mixed, let sit for 5 minutes, stirred again, and sit for another 5 minutes before elutriation began. After the elutriation cycle was over (approximately 4 minutes) the material collected in sieves #60 or #200 was transferred to 50 ml centrifuge tubes and spun for 5 minutes at 4000 rpm. The supernatant was discarded. The tubes were then filled with a sucrose solution (454 g sugar in 1 L DI water) and the pellet was stirred with a spatula until it was

completely dispersed. Tubes were then spun for 30 seconds at 4000 rpm. The supernatant was poured through a sieve #200 and cysts retained were transferred to test tubes after rinsing off the sucrose solution with tap water. The procedure was based on the sugar flotation centrifugation method described by Dunn (1969). Cysts were then counted under a stereoscopic microscope and counts from Methods 2 and 4 were adjusted to estimate the total number of cysts per sample if all 15 tubes had been used. The elutriator was rinsed carefully with a fine high-pressure handheld nozzle after each run to prevent contamination with cysts that may have been trapped in the apparatus.

Efficiency tests were run separately for each type of soil. The experimental design was a randomized complete block design blocked by method, with cyst inoculum levels as the treatment factor with ten replications (runs). Treatments were randomly assigned to each funnel in each run. The number of cysts recovered from the inoculum level zero was subtracted from the number of cysts recovered from the other inoculum levels in each run to account for contamination. The level of contamination was reported as the average number of cysts among all runs recovered from the inoculum level zero within each method. Efficiency of each method for each inoculum level was calculated as the percentage of cysts recovered from the inoculum.

Statistical Analysis

The hypothesis whether the four methods tested were equally efficient for the three soil types was tested with ANOVA. The effect of each inoculum level on the efficiency of each method was tested for each soil by calculating least significant differences between means at the 5 % level. The statistical analysis was performed with SAS Release 8 (SAS Institute, Cary, NC).

RESULTS

Method Efficiency by Soil Type

Clay Loam: Methods 2, 3 and 4 were equally efficient to extract cysts from clay loam, and significantly more efficient than Method 1 (Table A.1). Logistically, either method with soap worked well without complications. One person was enough to handle the whole process effectively. The two methods without soap also ran well, except for the centrifugation step after Method 1. The soil did not stick to the centrifuge tubes therefore pouring off excess water without losing sample was difficult.

Muck: Method 4 was the most efficient for muck, significantly better than either method using a sieve # 60 (Table A.1). Methods 1 and 3 required shower nozzles pointed away from the sieves and two operators tapping the sieves continuously because the soil clogged the sieve openings and the sample tended to overflow. Two centrifuge tubes were needed per sample because of the large amount of soil trapped in the sieves with the cysts. Flow was improved with the pretreatment of the soil with soap but still two operators were required tapping the sieves continuously to prevent clogging. Methods 2 and 4 required very little tapping by one person and one centrifuge tube per sample.

Sandy Loam: Either method with soap was significantly more efficient to extract cysts from sandy loam than either method without soap (Table A.1). In method 1, the sand particles clogged the sieves very easily causing them to overflow, and requiring two operators tapping the sieves to facilitate the flow. Soap improved the operation, but still some tapping was required. Both methods using the # 200 sieve presented no problems.

Table A.1. Efficiency (%) of four elutriation methods to extract SCN cysts from artificially inoculated soil.

	Extraction Method			
Soil	1- #60†	2- #200‡	3- #60 + Soap§	4- #200 + Soap
Clay Loam	43 ± 40.2 b	61 ± 65.4 a	68 ± 57.5 a	58 ± 50.4 a
Muck	15 ± 22.3 b	27 ± 35.2 b	19 ± 24.0 b	39 ± 31.4 a
Sandy Loam	22 ± 17.1 b	20 ± 17.7 b	51 ± 42.5 a	60 ± 38.7 a

Efficiency means in a row followed by the same letter were not significantly different (protected LSD, 5% level).
† Sieve #60: 250μ opening
‡ Sieve #200: 75μ opening
§ A soap solution was added to the soil suspension in Method 3 and 4.

Contamination

The level of contamination, that is the number of cysts recovered from the control, was almost zero for sandy soil, low for muck, and high for clay loam (Table A.2). For muck and clay loam, contamination levels were lower with Methods 2 and 4.

Method Efficiency by Inoculum levels, by Soil Type

Clay Loam: For low inoculum levels (50 cysts/ 100 cm^3 of soil), Methods 2 and 3 were significantly more efficient than method 1. At a higher cyst inoculum (100 cysts/ 100 cm^3 of soil) there were no significant differences in efficiency among methods. For 150 cysts/ 100 cm^3 of soil, Methods 2, 3 and 4 were all more efficient than method 1 (Figure A.1).

Table A.2. Average number of cysts recovered from the control sandy loam, muck and clay loam samples, using different elutriation methods.†

	Soil		
Extraction Method	Clay Loam	Muck	Sandy Loam
1- #60‡	37.3	4.4	0.6
2- #200§	13.1	1.4	1.0
3- #60 + Soap#	35.3	5.4	0.1
4- #200 + Soap	10.3	1.2	0.5

† The number of cysts presented was the average of ten replications (runs).
‡ Sieve #60: 250μ opening
§ Sieve #200: 75μ opening
# A soap solution was added to the soil suspension in Method 3 and 4.

Muck: At 50 cysts/ 100 cm^3 of soil there were no differences among methods. For 100 cysts/ 100 cm^3 of soil Method 4 was better than Methods 1 and 3. Method 2 was better than 1 and not different from the other two methods. For 150 cysts/ 100 cm^3 of soil Method 4 was better than Methods 1 and 3; Methods 1, 2 and 3 were equally poor in efficiency (Figure A.1).

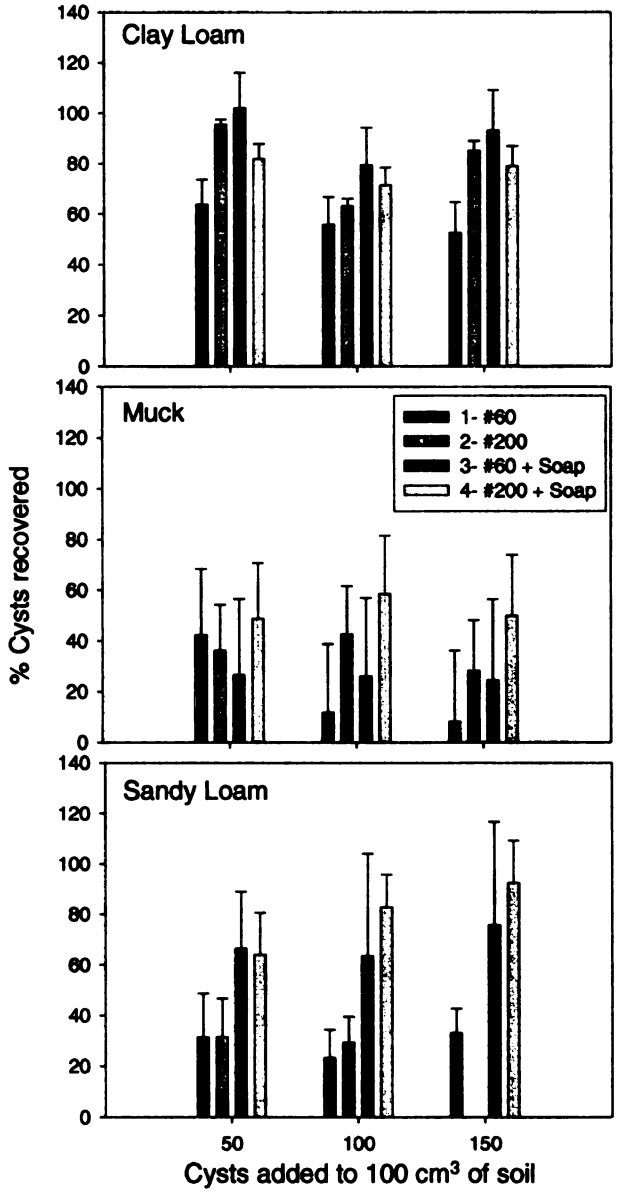

Fig. A.1. Extraction efficiency (means ± sd) of four elutriation methods to recover SCN cysts from sandy loam, muck and clay loam artificially inoculated with 50, 100 and 150 cysts 100 cm^{-3} of soil.

Sandy Loam: For 50 and 100 cysts/ 100 cm^3 of soil, Methods 3 and 4 were better than Methods 1 and 2. Method 2 was not tested with 150 cysts/ 100 cm^3 of soil, but Methods 3 and 4 were better than Method 1 (Figure A.1).

DISCUSSION

The North Carolina Elutriator (NC-EL) was adequate to extract SCN cysts from sandy loam and clay loam soils, with efficiency of 60%, whereas the procedure presented several difficulties when working with muck soil, with a great reduction in efficiency. The method using the sieve #60 without soap treatment was the least efficient for all soil types. The most efficient extraction method for the three soil types tested was to mix the soil with soap solution and collect cysts in the sieve #200. The method with soap and a sieve #60 was equally efficient regarding the percentage of cysts recovered from clay loam and sandy loam, but logistically, this method required more labor than with the sieve #200, especially with sandy loam soil.

The source of the contamination was probably cysts retained in the elutriator from one run to the next. The highest level of contamination was observed with the clay loam. However, this soil was obtained from a field that could have been infected with SCN before it was taken to the lab. Therefore, even though contamination appeared lower with soap and the #200 sieve than with the other methods, this portion of the test should be conducted again with SCN-free soil.

Extraction efficiencies from clay loam and sandy loam did not vary significantly with increasing inoculum level. There was great variability in extraction efficiencies from

muck, and the poor efficiency overall, made it difficult to draw conclusions from the comparison of inoculum levels.

SCN is usually found in soils with much less organic matter content than that found in muck. The results of this test indicate that for the soil types usually inhabited by SCN, 60% cyst extraction efficiency can be obtained by using a semi-automatic elutriator equipped with #200 sieves, and pre-treating soil samples with soap solution.

APPENDIX B

SOFTWARE FOR GEOSTATISTICS AND SPATIAL ANALYSIS

A great variety of software packages are available for spatial analysis, geostatistics, and GIS. Some of them can be expensive, while others can be downloaded from the Internet as freeware. The AI-GEOSTATS web site, a hub for geostatistics users around the world (http://www.ai-geostats.org) offers a complete list of the software available with a brief description of main functions and links to their homepages, from where many of them can be downloaded.

There is not one program that has all of the tools required for a complete geostatistical analysis. The spatial analyst rather, needs a combination of packages to have a complete 'geostatistical tool box'. The selection of the right programs to choose should be made based on the tools required to achieve the research objectives, the platform available (DOS, Unix, Windows, Linux), the user's computer skills and preferences, and affordability.

Software for geostatistics, spatial analysis, and GIS are upgraded frequently with add-on codes, new versions, and new programs, constantly extending and improving the tools offered. It is recommended that the spatial analyst test several packages to find the one or the combination of packages that better fits the needs of the research project.

The software packages used for the geostatistical analysis presented in this dissertation were Surfer 7, Variowin 2.1, and SAS 8. GSLIB was initially used for semivariogram analysis, but it was soon replaced by the other packages, which are easier to work with. Surfer 7 was used to calculate empirical semivariograms, model semivariograms, kriging, and to draw contour maps. The overlaying tool only available in

Surfer, allowed showing more than one layer of information in one map. Variowin 2.1 was used mostly to calculate cross-correlograms. SAS 8 and GSLIB were used initially to calculate empirical semivariograms. ArcView (ESRI) was also tried for its mapping and GIS capabilities. Because of the lack of geostatistical tools and because it required high expertise level to take full advantage of its capabilities ArcView was not used further. For GIS projects however, it is probably the most complete packages available (http://www.esri.com). Following is a brief description of the main pros and cons I have come across in the software tested during this research (Tables B.1- 4). The lists shown in Tables B.1- 4 are meant to point out the obstacles encountered and highlight the positive aspects of each package used and not as an exhaustive software analysis. For detailed information and comparisons with more packages refer to the AI-GEOSTATS web site.

Table B.1. Summary of some of the benefits and drawbacks of the software package Surfer 7.†

PROS	CONS
Windows platform, user friendly	Cross-validation not available in version 7, new in version 8
Semivariogram modeling tools, with or without drift	Limited basic statistics
Probably the most complete set of semivariogram models offered	Univariate analysis only, no cross-correlograms or cross-variography.
Interactive isotropic and anisotropic semivariogram modeling	Does not provide empirical semivariogram values
Multiple interpolation and contouring tools available	Cost
Many functions for grid analysis	
Excellent plotting quality and capabilities	
Excellent mapping quality and capabilities	
Data sets can be used from Excel spreadsheets	
Data transformation tools	
Imported maps, images, figures can be digitized easily	
User's Manual is helpful and easy to follow	

† Version 8 available in 2002. Golden Software. Golden, Colorado. http://www.goldensoftware.com

Table B.2. Summary of some of the benefits and drawbacks of the software package Variowin 2.21.†

PROS	CONS
Windows platform	Data sets should be carefully prepared as simple text format (*.txt or *.dat)
Provides empirical semivariogram values	Restrictions on file name length
Calculates variograms, madograms, correlograms, standardized variograms, covariance	Poor graphing quality
Interactive modeling	No interpolation tools
Bivariate analysis capabilities (cross-correlogram and cross-variography)	The manual is out of print, may become available online soon
Semivariogram map	
Free	

† Yvan Pannatier. Shell International Exploration and Production. The Hague, Netherlands. http://www-sst.unil.ch/research/variowin/index.html

Table B.3. Summary of some of the benefits and drawbacks of the software package SAS/STAT.†

PROS	CONS
Excellent statistical analysis capabilities beyond spatial analysis	Limited semivariogram modeling
Variogram and ordinary kriging	Knowledge on SAS-code is a must
Improvements should be seen in the near future	Requires SAS. License should be renewed annually.

† The SAS Institute. Cary, North Carolina. http://www.SAS.com

Table B.4. Summary of some of the benefits and drawbacks of the software package GSLIB.†

PROS	CONS
Powerful capabilities	Executables and codes are needed to fully benefit from GSLIB capabilities
One of the few packages capable of 3D geostatistical analysis	Minor mistakes in data sets or variable inputs will cause the program to stop running
Jack-knifing and other cross-validation tools	Slow with large data sets
Platform DOS, UNIX, new version with Windows interface.	Data sets should be carefully prepared as simple text format (*.txt or *.dat)
DOS and UNIX versions are free	Programming skills required
Indicator semivariograms, and cross-variography	Poor graphing quality (Postscript output)
	The book is indispensable, even to accomplish the simplest tasks.

† Clayton V. Deutsch and Andre G. Journel (1992). Stanford University. http://www.gslib.com

APPENDIX C

Record of Deposition of Voucher Specimens*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: 2002-13

Title of thesis or dissertation (or other research projects):

Characterization of the spatial distribution of *Heterodera glycines* Ichinohe 1955 (Nematoda), soybean cyst nematode in two Michigan fields

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name(s) (typed)
Maria Felicitas Avendaño

Date 12/13/02

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America.
Bull. Entomol. Soc. Amer. 24: 141-42.

Deposit as follows:

Original: Include as Appendix 1 in ribbon copy of thesis or dissertation.

Copies: Include as Appendix 1 in copies of thesis or dissertation.
Museum(s) files.
Research project files.

This form is available from and the Voucher No. is assigned by the Curator, Michigan State University Entomology Museum.

Voucher Specimen Data

Page 1 of 1 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Heterodera glycines	Cysts extracted from cultures from cysts collected from two Shiawassee County, Michigan fields in 2000	-	-	-	-	6	-		Entomology MSU

(Use additional sheets if necessary)

Investigator's Name(s) (typed)
Maria Felicitas Avendaño

Date 12/13/2002

Voucher No. 2002-13
Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator 4/17/2003
Date

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