Use of remote sensing, geographic information systems, and spatial statistics to assess spatio-temporal population dynamics of Heterodera glycines and soybean yield quantity and quality

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Use of remote sensing, geographic information systems, and spatial statistics to assess spatio-temporal population dynamics of *Heterodera glycines* and soybean yield quantity and quality

by

Antonio Jose de Araujo Moreira

A dissertation submitted to the graduate faculty in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Plant Pathology

Program of Study Committee:
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For the Major Program
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To Jozinha, Vicente, and Anna,

For your love.
CHAPTER 1

GENERAL INTRODUCTION

Dissertation Organization

This dissertation consists of five chapters. The first chapter is an introduction to the pathosystem involving soybean and soybean cyst nematode. Additionally, aspects of the methodology used in the research project are discussed and the rationale for the three following chapters is presented. The next three chapters are papers that will be submitted to the Crop Science. A general conclusion is presented in the last chapter.

Introduction

Soybean, *Glycine max* (L.) Merr., is a major source of vegetable oil produced worldwide (Wilcox, 2004), and soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is one of the principal causes of soybean yield losses in the world. Economic losses caused by SCN surpass US$ 1 billion in the United States annually (Wrather et al., 2001), and yield reductions can occur even in the absence of noticeable symptoms (Niblack et al., 1991; Wang et al., 2003; Young, 1996b). The economic importance of soybean crop, the large amount of agricultural land occupied with this crop throughout the world (Wilcox, 2004), the difficulties in precisely quantifying SCN population densities, and the difficulties of visually determining the impact of SCN on soybean yield make this pathosystem appropriate for being studied using alternative technological and statistical approaches. Remote sensing technologies may detect stress on soybean plants before the stress can be detected by the human eyes. The use of GIS tools allows creating maps of geo-referenced remote sensing data that could be related to SCN population densities and quantity and quality of soybean yield. Additionally, geo-referenced data are adequate to be analyzed using
spatial statistics methods. Finally, by integrating spatial statistics and regressions methods, spatio-temporal changes in SCN population densities may be described.

**Soybean cyst nematode population dynamics**

To understand the impact that SCN can have on soybean yield, it is necessary first to understand SCN population dynamics within a growing season and how SCN population dynamics may be related to soybean yield.

The factors affecting population densities of plant-parasitic nematodes could be classified into three categories: perfectly density dependent (intraspecific competition), imperfectly density dependent (interspecific competition, parasitism, and predation), and density independent (abiotic conditions) (Boag, 1989). These three types of factors can influence SCN population density within soybean seasons. However, it is hypothesized that only imperfectly density-dependent and density-independent factors affect SCN populations over winter. During this period, the lack of a susceptible host and the effect of extreme weather conditions, particularly low temperatures and/or low soil moisture, inhibit biological activities of this obligate parasite (Young, 1995).

Environmental factors may affect SCN development and reproduction. Soil temperature has been shown to affect all phases of the SCN life cycle. SCN juvenile survival in soil in the absence of the host is a result of the interaction among temperature, moisture, and soil type. SCN juvenile survive for longer in soil with moisture than in dry soil when the soil temperature is between 4 and 20 °C, but above this temperature, the survival will be greater in dry soils up to soil temperature of 36 °C (Slack et al., 1972). The rate of egg development and second-stage juvenile (J2) formation has a positive linear relationship with temperatures between 15 and 30 °C, but the nematode eggs do not survive soil temperatures above 36 °C (Alston and Schmitt, 1988). Infection of soybean roots by SCN juveniles does not occur at soil temperatures below 17 °C, and the ideal temperature range for SCN female
development is from 20 to 28 C (Melton et al., 1986), with 26 C being the optimum temperature for cyst production (Anand et al., 1995). Soil temperatures above 30 C are detrimental to J2 and adult nematodes (Alston and Schmitt, 1988).

Other environmental factors, such as soil pH, soil texture, and chemical elements in the soil also may affect SCN populations. Numbers of mature females were positively correlated with soil pH (Anand et al., 1995; Francl, 1993) and with soil magnesium (Francl, 1993). Moreover, population densities of SCN cysts and eggs in the fall were negatively correlated with copper concentration (Francl, 1993). Besides chemical characteristics of the soil, soil texture also may affect SCN biological activities because soil moisture content and atmosphere composition in the soybean rizosphere can be affected by soil texture.

Greenhouse experiments showed no difference in root penetration by SCN juveniles, but verified that SCN reproduction was greater in loam soils than in the clay soils (Young and Heatherly, 1990). Reproduction of SCN tended to be greater in coarse soil textures than in fine soil textures (Koenning and Barker, 1995), and high clay content in the lower horizons (15 – 45 cm) of soil profiles limited vertical growth of soybean roots and, thus, limited reproduction of SCN (Alston and Schmitt, 1987). Other factors that affect SCN reproduction are the genetic compatibility between SCN and its hosts (Chen et al., 2001b; Faghihi et al., 1986; Sipes, 1995; Wang et al., 2000) and the developmental stage of soybean plants, since SCN reproduction is greater in soybean plants during the reproductive than the vegetative stages (Hill and Schmitt, 1989).

Changes in environmental conditions caused by cultural practices also may influence SCN population dynamics. It was speculated that SCN reproduction may be stimulated by high oxygen levels since greenhouse experiments showed greater SCN reproduction occurred in disturbed than in undisturbed soils planted with soybean (Young, 1987). SCN reproduction in no-tillage production was reported to be equal (Chen et al., 2001a) or greater (Noel and Wax, 2003) than the reproduction in conventional soybean tillage production systems. Still,
the effects of tillage methods on SCN population densities can depend upon soil texture, and lower populations of the nematode were found in no-tilled than in tilled fields for soil textures with clay content equal or greater than silt clay loam (Workneh et al., 1999). However, different row spacing did not affect reproductive rates of SCN in a soybean/corn cropping system (Chen et al., 2001a).

Spread of SCN has followed the expansion of the soybean crop to new areas (Riggs, 1977), and the pathogen can be dispersed in association to soil particles for short and long distances (Slack et al., 1972). Wild animals also may disseminate SCN over long and short distances (Epps, 1971), however the importance of birds as agent of dissemination of SCN is not clear (Brodie, 1976). Besides physical dispersion of SCN, other factors, such as presence of host and environmental conditions, may affect the establishment of the pathogen in new areas. It has been suggested that the thresholds of establishment of SCN may vary with soil texture, and sand soils may have lower thresholds than fine-textured soils (Todd and Pearson, 1988).

**Effects of SCN populations on soybean yield**

It was determined that high J2 population density at planting was responsible for increasing root damage and reduction in root volume (Bonner and Schmitt, 1985). Thus, development of soybean root system has a better development in sites with low J2 population density at planting, and larger root systems support high SCN population densities at harvest (Bonner and Schmitt, 1985). Since the only source of J2 at planting are SCN eggs from previous seasons, it is preferable to quantify eggs than cysts or just J2 in the soil to predict damage (Bonner and Schmitt, 1985).

Initial SCN population densities have been shown to have a negative relationship with soybean yield (Francl and Dropkin, 1986; Koenning and Barker, 1995), and the yield benefit
of planting resistant instead of susceptible cultivars in SCN-infested fields has been shown to be linear and positively related to initial SCN population densities (Chen et al., 2001b).

Intensity of soybean yield losses is affected by the time when infection occurs and by soil texture characteristics. Yield losses in SCN-susceptible cultivars decreased when root infection was delayed from 2 to 6 weeks (Wrather and Anand, 1988). Also, differences in yield of resistant and susceptible soybean cultivars with comparable yield potentials decreased as the sand content of the soils decreased in infested fields (Koenning et al., 1988).

Knowledge about how SCN population dynamics impact soybean yield is very important for evaluating the efficacy of SCN management strategies and tactics. However, it is difficult to precisely determine SCN population densities and the effects these nematode population densities have on soybean yield because of the high sampling costs, inefficient soil extraction methods, and lack of knowledge concerning how both biotic and abiotic factors affect soybean yield in SCN-infested fields (Donald et al., 1999). Even in the presence of such limitations, a number of studies (Chen et al., 2001b; Ehwaeti et al., 2000; Francl and Dropkin, 1986; Francl and Wrather, 1987; Koenning and Barker, 1995; Noel and Edwards, 1996; Wrather and Anand, 1988; Young, 1996a) have been conducted to quantify the relationship between SCN population density and soybean yield, but these studies did not show how these relationships are spatially distributed within soybean fields or how SCN population dynamics affect soybean yield quantitatively and qualitatively. Likewise, although these previous studies evaluated both reproduction and survival of SCN, they did not examine or consider how these phenomena are spatially distributed in the fields. By sampling, estimating, and mapping SCN population densities in small quadrats for entire experimental areas, it should be possible to identify and characterize ecological factors affecting SCN population dynamics within and among seasons. Geographic information systems (GIS) offer the opportunity to generate maps of spatially referenced data. The tools present in GIS allow us to map and visualize how SCN population densities change in time
and space (Nutter et al., 2002). Additionally, utilizing GIS to display geo-referenced remote sensing data may provide means to visualize the impact of SCN population densities and other factors on quantity and quality of soybean yield.

Assessing the spatial distribution of SCN population densities

Spatial statistics have been shown to provide means to assess the spatial structure of nematode populations (Avendano et al., 2003; Avendano et al., 2004; Donald et al., 1999; Evans et al., 2002; Farias et al., 2002; Gavassoni et al., 2001; Morgan et al., 2002) and to assess soybean yield (Dobermann and Ping, 2004). However, the use of these statistical methods can be limited by the occurrence of errors in the determination of nematode population densities. Spatial statistical analyses have shown that soybean fields infested with *H. glycines* were initially aggregated and that no-tillage and ridge-tillage systems resulted in greater aggregation of *H. glycines* population densities over time compared to conventional and reduced tillage systems (Gavassoni et al., 2001). Although the spatial patterns of SCN distribution in soybean fields have been previously studied, very little is known about spatial patterns of reproduction and survival of the SCN within a field from season to season at a spatial scale that can lead to discovery of new ecological and biological information.

Errors and the difficulties related to prediction of population dynamics of the potato cyst nematodes (PCN), *Globodera* spp., limited the development of site-specific management practices to control these nematodes (Evans et al., 2002). The integration of spatial statistics and regressions to study spatio-temporal changes in SCN population densities may provide information that can be used in the development of new SCN control programs.
Using remote sensing technologies to assess SCN population densities and soybean yield

Optimizing the use of agronomic inputs in time and space, precision agriculture needs tools and techniques that facilitate site-specific management of crops (Pinter et al., 2003; Seelan et al., 2003). Remote sensing technologies that measure the percentage of sunlight reflected from soybean canopies may provide a means to obtain timely and accurate disease diagnoses and the means to differentiate and quantify plant stresses. Furthermore, reflectance measurements obtained from soybean canopies at different wavelength bands may indicate the presence of a plant stress, even when the stress cannot be visually detected. Thus, reflectance may be useful to detect stress caused by SCN in soybean plants, since, for this pathosystem, soybean yield reductions may occur even in the absence of visible symptoms (Niblack et al., 1991; Wang et al., 2003; Young, 1996b). Geographic information systems (GIS) allow displaying geo-referenced variables into maps. In this way, geo-referenced remote sensing data can be processed through GIS to provide spatial information about crop conditions that may be effective to site-specific management of crops. Additionally, mapping reflectance data obtained from soybean canopies may be used to provide early estimates of soybean yield.

To be incorporated in the decision-making process to optimize agricultural, economical, and ecological results, precision farming demands high quality remote sensing data to be obtained in a timely fashion and to be related to plant physiological status and crop yield (Pinter et al., 2003; Seelan et al., 2003). Ground-based remote sensing can provide the high-resolution data that are required to implement site-specific management programs required for precision farming. To verify if remote sensing can be automatically incorporated to the decision-making process, it is needed to determine if the remote sensing data remain stable across different assessment dates and are strongly related to plant growth and yield.
Jackson published a review on use of remote sensing to detect plant stress (Jackson, 1986), and several other researchers have addressed the use of these technologies to detect biotic (Carver and Griffiths, 1981; Gausman et al., 1975; Nutter, 1989; Nutter et al., 1990) and abiotic (Adams et al., 2000; Carver and Griffiths, 1981; Dale et al., 1982; Hansen and Schjoerring, 2003; Penuelas, 1998) plant stresses. Advantageously, these methods of assessing plant-health status can provide fast and reliable biological information with minimal disturbance of the crop (Nutter, 1989; Nutter et al., 1990). However, differences in atmospheric conditions (Liu and Huete, 1995; Xiao et al., 2003) and in soils characteristics (Gilabert et al., 2002; Haverkort et al., 1991; Huete, 1988; Huete et al., 1985; Liu and Huete, 1995; Rondeaux et al., 1996; Weidong et al., 2002) can affect the quality of the remote sensing data. Thus, different authors expressed their concern about the limitations of remote sensing techniques in agriculture (Tucker, 1979; Wiegand et al., 1972).

Knowledge of the potential sources of limitations of remote sensing should provide the framework to maximize its usefulness. Radiometers record reflectance data at specific wavelength bands and widths. These specifications can restrict the utility of the reflectance data since different wavelength bands and widths are affected differently by characteristics of the atmosphere, soil, and vegetation (Gitelson et al., 2002; Tucker, 1979). Height of the sensors, canopy type (Daughtry et al., 1982; Guan and Nutter, 2001), incident radiation, presence of water on the leaves (Guan and Nutter, 2001), and cultural practices (Kollenkark et al., 1982) influenced the quality of ground-based radiometer data.

Any plant stress causes color and/or morphological changes in plant parts (Jackson, 1986). Infection of soybean roots by SCN causes a reduction in the rate of above-ground plant growth that can result in a reduction in the amount of green leaf area (Koenning and Barker, 1995). The amount of green leaf tissue per unit of ground area is known as green leaf area index (GLAI) (Campbell and Madden, 1990). This index can be influenced by one or more plant stresses and often is highly related to yield (Guan and Nutter, 2000). To establish
the relationships between yield and GLAI and/or the change in GLAI with respect to time, the critical stages of crop development and frequency of data collection throughout the growing season need to be determined. Estimates of GLAI obtained during R4.5 and R5.5 soybean growth stages, that correspond to pod elongation and seed filling in soybean plants (Fehr et al., 1971), were found to provide an accurate estimate of yield losses in determinate and in indeterminate soybean cultivars (Fehr et al., 1981). Maximum soybean yields were achieved when soybean plants reached GLAI of 3.5 - 4.0 at pod formation and pod elongation (Board, 2004; Board et al., 1997). The relationships between soybean growth, light interception, and yield have been previously demonstrated (Adcock et al., 1990; Batista and Rudorff, 1990; Board, 2004), and spectral data have been used to predict the developmental stage of soybean (Badhwar and Henderson, 1985; Henderson and Badhwar, 1984). However, there is a lack of information about the variability and reliability associated with using ground-based remote sensing data to estimate aspects of quantitative and qualitative soybean yield throughout a season.

Usually, radiometers measure reflectance from a target object at several different wavelength bands. These measurements of reflectance can be correlated to some characteristics of the target object (Perry and Lautenschlager, 1984). However, the relationships among individual wavelength bands and the target may differ from each other (Holben et al., 1980). Thus, to assess various characteristics of plant canopies, information contained within single wavelength bands can be used alone or different wavelength bands can be empirically combined in different ways to form indices, called vegetation indices (VI’s). These indices may be correlated to canopy characteristics, such as leaf area index, fractional vegetation cover, biomass, and other vegetative conditions (Carlson and Ripley, 1997; Perry and Lautenschlager, 1984). Ratios of reflectance and differences in reflectance between two different wavelength bands form the two major categories of indices, although there are other ways of calculating VI’s.
Different VI’s may have specific relationships with individual canopy characteristics. Soybean leaf area index had a positive linear relationship with the radiance ratio (545 nm/655 nm) and with near infrared reflectance (750 nm) (Kanemasu, 1974). Infrared-to-red radiance ratios were found to have a positive linear relationship with soybean GLAI, soybean fresh biomass, and soybean yield (Batista and Rudorff, 1990; Holben et al., 1980; Kanemasu, 1974). Red radiance was found to be linearly and negatively related to soybean GLAI (Holben et al., 1980; Kanemasu, 1974). Unlike previous reports, quadratic equations best described the relationships between soybean (dry) biomass and soybean leaf area index with red and near infrared reflectance, with near infrared-red radiance ratio, and with a transformed greenness vegetation index (Kollenkark et al., 1982).

Positive, non-linear relationships between normalized difference vegetation index (NDVI), that is the ratio between the difference and the sum of near infrared and red radiances, and soybean GLAI were found to reach a saturation point at GLAI values between 2 and 3 (Kollenkark et al., 1982). This same VI obtained from soybean canopies in which soybean plants were at R4 and R5 growth stages showed to be positively correlated with soybean grain yield (Ma et al., 1996).

The difference vegetation index (DVI), that is the difference between infrared and red radiances, and the transformed vegetation index (TVI), that is a mathematical transformation of NDVI, were related to grass biomass (Tucker, 1979). The visible atmospheric resistant index (VARI) minimized atmospheric effects in the estimation of vegetation fraction of wheat and corn (Gitelson et al., 2002). The green normalized difference vegetation index (GNDVI), that is the ratio between the difference and the sum of near infrared and green radiances, accurately assessed chlorophyll content of plant canopies (Gitelson et al., 1996) and predicted corn yield when assessments were made during midgrain filling (Shanahan et al., 2001). The photochemical reflectance index (PRI), that is the ratio between the difference
and the sum of radiances at 531 nm and 570 nm, has been shown to be correlated with radiation use efficiency of several plant species (Gamon et al., 1997).

Distinct VI's are considered equivalent if they provide the same basis for decisions to be made (Perry and Lautenschlager, 1984). However, few evaluations were done to check equivalence among the VI used to assess soybean growth and yield.

It has been showed that reflectance from soybean canopies can be used to determine soybean growth stage, to estimate soybean growth indicated as GLAI, and to estimate soybean yield. However, there is a lack of information about how stable the relationships the between reflectance from soybean canopies and GLAI are during growing seasons. Thus, to study the relationships between reflectance from soybean canopies and soybean GLAI within and between growing seasons is the first objective of this research. The relationships between reflectance from soybean canopies and soybean grain yield have been described, however, to our knowledge, there is no report of the use of reflectance data to assess quantity and quality of soybean yield. Besides, little is known about how the relationships between reflectance from soybean canopies and soybean yield change during a soybean-growing season. In this perspective, our second objective is to study the usefulness of reflectance data from soybean canopies obtained during the soybean-growing season to assess quantity and quality of soybean yield. Additionally, it is our objective to verify the potential of using reflectance data to assess SCN population densities in soybean fields. Finally, there are few reports describing simultaneously spatial and temporal changes in SCN population densities within and between growing seasons. Then, our third objective is to study spatio-temporal changes in SCN population densities in fields under soybean monoculture.

References


CHAPTER 2
USE OF GROUND-BASED REMOTE SENSING TO ASSESS SOYBEAN GROWTH: EQUIVALENCE AND STABILITY OF THE INFORMATION
A paper to be submitted to Crop Science
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2 Abbreviations: GLAI, green leaf area index, VI, vegetation index, \( I - R_G \), green percentage reflectance; \( R_R \), red percentage reflectance; \( R_{NIR} \), near infrared percentage reflectance; \( R_{\text{infrared}} \), infrared percentage reflectance; \( R_w \), being \( W \) a wavelength band in nanometers (nm) from which percentage reflectance is measured; RR, radiance ratio; NDVI, normalized difference vegetation index; TVI, transformed vegetation index; PRI, photochemical reflectance index; GNDVI, green normalized difference vegetation index; VARI, visible atmospherically resistant index; DVI, difference vegetation index; RDVI, renormalized difference vegetation index; PDW, plant dry weight; \( R^2 \), linear coefficient of determination; \( \text{SEE}_y \), standard error of estimate for \( y \).
Abstract

A wide range of soybean [Glycine max (L.) Merr.] growth was generated in field experiments throughout 2002 and 2003. Soybean canopies were assessed periodically with a hand-held, multispectral radiometer measuring percentage reflectance at eight different narrow wavelength bands spaced 50 nm apart from 460 nm to 810 nm. After reflectance data were obtained, soybean plants were removed and green leaf area index (GLAI) determined. Within individual assessment dates, relationships among percentage reflectance of individual wavelength bands and vegetation indices with GLAI were studied using regressions. Percentage reflectance for 660 nm and for 810 nm had the best relationships with GLAI within assessment date and throughout each season. However, regression lines obtained for individual assessment dates were significantly different for the regressions obtained for entire seasons. The indices that best estimated variation in GLAI were radiance ratio (RR), difference vegetation index (DVI), and renormalized difference vegetation index (RDVI). Ground-based RDVI was not affected by environment conditions, and there was no significant difference between the regression lines of RDVI on GLAI obtained for individual assessment dates and those obtained for the entire 2002 and 2003 seasons. Results indicate that caution should be taken when percentage reflectance data obtained at different dates are combined to estimate GLAI. The ability of normalized difference vegetation index (NDVI), transformed vegetation index (TVI), and green normalized difference vegetation index (GNDVI) to estimate GLAI was equivalent throughout seasons. Plant dry weight (PDW) and GLAI were positive and linearly related. Similar relationships between percentage reflectance data and GLAI or PDW were observed.
Introduction

Remote sensing in crop production has been linked to the use of satellite imagery and aerial photography to infer soil properties (Muller and Decamps, 2000; Weidong et al., 2002), characteristics of plant canopies (Colombo et al., 2003; Colwell, 1974; Curran, 1983; Kanemasu, 1974; Mutanga and Skidmore, 2004; Mutanga et al., 2004; Sims and Gamon, 2002; Thenkabail et al., 1994; Tucker, 1979; Wiegand et al., 1972; Wiegand et al., 1991), effects of biotic and abiotic stresses on crops (Adams et al., 2000; Adcock et al., 1990; Carver and Griffiths, 1981; Ceccato et al., 2002; Dale et al., 1982; Gausman et al., 1975; Guan and Nutter, 2000; Hansen and Schjoerring, 2003; Jackson, 1986; Kobayashi et al., 2001; Ma et al., 1996; Nutter, 1989; Nutter and Littrel, 1996; Nutter et al., 1990; Nutter et al., 2002b; Penuelas, 1998; Roberts et al., 1987), and to predict yield (Aparicio et al., 2000; Guan and Nutter, 2000; Ma et al., 1996; Ma et al., 2001; Nutter and Littrel, 1996; Nutter et al., 2002a; Shanahan et al., 2001; Thenkabail et al., 1994). Even though the quality of satellite images of fields available has been improved and the availability of aerial images of fields has increased, problems still exist that restrict the use of these images in crop management. Image cost and resolution limit the minimum size of field areas assessed. Additionally, differences in atmospheric conditions (Liu and Huete, 1995; Xiao et al., 2003), in soil characteristics (Gilabert et al., 2002; Haverkort et al., 1991; Huete, 1988; Huete et al., 1985; Liu and Huete, 1995; Rondeaux et al., 1996; Weidong et al., 2002), and other environment conditions (Dale et al., 1982; Daugtry et al., 1982; Guan and Nutter, 2001; Kollenkark et al., 1982; Milton, 1982) affect the quality of the remote sensing data.

To be incorporated in the decision-making process to optimize agricultural, economical, and ecological results, precision farming demands high quality imagery to be obtained in a timely fashion and to be related to plant physiological status and crop yield (Pinter et al., 2003; Seelan et al., 2003). Ground-based remote sensing assessments can provide fast, non-destructive information about crop health that tend to be independent of individual evaluators (Adcock et al., 1990; Guan and Nutter, 2001; Nutter, 1989; Nutter et
al., 1990) and is not adversely affected by atmospheric conditions (Pontailler et al., 2003). Additionally, ground-based remote sensing can provide high-resolution data that are required to implement site-specific management programs required for precision farming. Thus, remote sensing can be automatically incorporated to the decision-making process if the extracted data remain stable across different assessment dates and are strongly related to plant growth and yield.

For an appropriate evaluation of the health of annual crops, it may be necessary to collect data throughout the growing season to capture information in critical developmental phases of the crop. However, frequency of crop assessments cannot be easily adjusted when assessments depend upon satellite images (Moran et al., 1997). Seed filling is a critical period for soybeans when yield is linearly and positively related to the amount of light interception by the canopies (Board, 2004; Board et al., 1997). Additionally, it was demonstrated that intensity of soybean defoliation between pod elongation and seed filling, the R4.5 and R5.5 growth stages of soybean (Fehr et al., 1971), respectively, is critical to determine yield losses (Fehr et al., 1981). The relationships between soybean growth, light interception, and yield have been demonstrated, but there is a lack of information about the stability of the use of remote sensing data to assess soybean growth among and during different growing seasons.

Remote sensing can obtain reflectance data at several wavelength bands that can be used individually or can be combined in different ways to test relationships with specific characteristics of plant canopies. By mathematically combining multispectral satellite data, different vegetation indices (VI) can be empirically obtained (Perry and Lautenschlager, 1984). Ratios and differences between two specific wavelength bands form the two major categories of vegetation indices; however, there are other ways of mathematically calculating many other VI. These indices often are correlated with physical properties of plant canopies, such as leaf area index, fractional vegetation cover, biomass, and vegetation conditions (Carlson and Ripley, 1997). To be adopted remote sensing of percentage reflectance from
soybean canopies needs to be stable for multiple situations. Our study compares the ability of
reflectance at different single wavelength bands and VI to estimate soybean GLAI among
and during different the growing seasons.

Different authors have expressed their concern about the limitations of the use of
remote sensing techniques in crop production (Baret and Guyot, 1991; Guan and Nutter,
2001; Huete et al., 1985; Jackson, 1986; Tucker, 1979). However, lack of published
unsuccessful results gives the impression that it is easy to work in this field (Wiegand et al.,
1972). Knowledge of the limitations of remote sensing should provide us the framework to
maximize its usefulness by applying it in the most appropriate manner.

Several factors can affect the quality of reflectance data. Ground-based reflectance
data can be affected by height of the sensors, canopy type (Daughtry et al., 1982), and
cultural practices (Kollenkark et al., 1982). Furthermore, the choice of different band
locations and band widths presented as an index creates restrictions to its use (Gitelson et al.,
2002; Tucker, 1979), since spectral measurements obtained at different wavelength bands are
affected differently by characteristics of the atmosphere and soil and type of the vegetation
(Jackson, 1986). Supporting this notion, mid-infrared data (from 1,550 to 2,350 nm) from
Landsat thematic mapper data to calculate VI showed stronger relationships with soybean
leaf area index, biomass, and yield than VI constructed using near-infrared data (from 760 to
900 nm) from the same satellite (Thenkabail et al., 1994).

Vegetation indices have been developed to minimize sources of errors in spectral
data, such as interference from soil background and atmospheric conditions that compromise
the reflectance measurements from crop canopies. Thus, different VI explore particular
characteristics of spectral data and present singular properties that make these indices more
or less affected by environmental factors and more or less related with some crop
characteristics.

There are several examples describing the relationships between VI and crop
characteristics. The radiance ratio ($R_{545}/R_{655}$) and the near-infrared reflectance ($R_{750}$) had a
strong positive linear relationships with soybean leaf area index (Kanemasu, 1974). An infrared-red radiance ratio ($R_{\text{infrared}}/ R_{\text{red}}$) and the infrared radiance were found to be linearly and positively related to soybean green leaf area index (GLAI) (Holben et al., 1980). Positive correlations were found between an infrared-red radiance ratio and soybean fresh biomass and soybean yield (Batista and Rudorff, 1990; Holben et al., 1980; Kanemasu, 1974). Red radiance was found to be linearly and negatively related with soybean GLAI (Holben et al., 1980). In contrast to previous reports with linear models, quadratic equations best described the relationships between soybean dry biomass and leaf area index with red reflectance, near-infrared reflectance, near-infrared-red radiance ratio ($R_{\text{NIR}}/R_{\text{R}}$), and also with a greenness transformation vegetation index (Kollenkark et al., 1982). It was reported that there was a positive, nonlinear relationship between normalized difference vegetation index, NDVI ($((R_{\text{NIR}} - R_{\text{Red}})/(R_{\text{NIR}} + R_{\text{Red}}))$, and soybean GLAI that reached a saturation point at GLAI values between 2 and 3 (Kollenkark et al., 1982). When assessing different soybean genotypes, soybean grain yield was found to be highly positively correlated to NDVI obtained at different assessment dates; however, correlations were the strongest between soybean growth stages R4 and R5 (Ma et al., 1996). The difference vegetation index, DVI ($R_{\text{infrared}} - R_{\text{Red}}$), and transformed vegetation index, TVI ($(\text{NDVI}+0.5)^{0.5}$), were related to grass GLAI (Tucker, 1979). The visible atmospheric resistant index, VARI ($(R_{560} - R_{660})/(R_{560} + R_{660} - R_{460})$), minimized atmospheric effects to estimate the vegetation fraction of both wheat and corn fields (Gitelson et al., 2002). A green normalized difference vegetation index, GNDVI ($(R_{\text{NIR}} - R_{\text{Green}})/(R_{\text{NIR}} + R_{\text{Green}})$), accurately assessed chlorophyll content at canopy level of several crops (Gitelson et al., 1996) and predicted corn yield when assessments were made during the midgrain filling period (Shanahan et al., 2001). Photochemical reflectance index, PRI ($(R_{531} - R_{570})/(R_{531} + R_{570})$, was correlated with radiation use efficiency of several plant species (Gamon et al., 1997).

Contradictory reports about the relationships of VI with soybean growth and with soybean yield may be due to differences in and of band locations and band widths used to
calculate the VI, lack of a sufficient numbers of observations to allow the description of the relationships to be accurately made, and the occurrence of temporal variation in the relationships of a specific vegetation index with soybean growth and yield. By generating a wide range of plant growth throughout multiple growing seasons and by intensifying sampling, it is possible to test the relationships between reflectance data and soybean growth over time.

The intensity of biotic and abiotic stresses on soybean crops may vary within seasons and/or within fields. As results of these stresses, a wide range of plant growth can be generated in soybean fields. The relationships between soybean growth, light interception, light reflectance, and soybean grain yield have been demonstrated, but there is a lack of information about the equivalence and stability of use of reflectance data and VI to assess soybean growth during the growing season. VI may be considered equivalents if they provide the same information about a particular crop condition in a given time (Perry and Lautenschlager, 1984). Stability is defined by the lack of variation in the relationships between percentage reflectance data and GLAI that is observed when the crop is assessed at different times within a season and between seasons. A number of indices have been used to assess plant growth in general and soybean growth in particular, but to our knowledge no evaluation has been done about VI equivalence and stability for estimating GLAI within and across soybean seasons. This information would be important to describe temporal variations in soybean growth in fields and to provide information about the impact of plant stresses and any crop management practice on crop growth. Additionally, the resolution of ground-based reflectance data may provide information about plant growth that is required for site-specific management of a soybean crop. Therefore, the main goal of this study was to evaluate the ability of assessing soybean canopy development as indicated by GLAI throughout growing seasons by constructing and comparing VI using ground-based reflectance data. Moreover, the relationships between different wavelength bands and between different VI were examined and their ability to estimate GLAI within and across seasons was evaluated.
Materials and Methods

This study was conducted at the Iowa State University Hinds Farm, Ames, Iowa, during the 2002 and 2003 seasons. The experimental field was located at 42.06321° north and 93.61766° west and contained a loam soil (20.2% clay, 45.0% sand, 34.8% silt) with 2.4% organic matter. Soybeans were planted every 5 to 10 days from late April to mid July in both seasons to generate a wide range of plant growth. Four rows of soybean cultivar AgriPro 1702 RR, 76 cm apart, were planted at a seeding rate of 30 seeds per meter at each planting date.

In 2002, four assessments of reflectance of sunlight from soybean canopies at intervals of 10 to 14 days from 23 July 2002 to 30 August 2002 were obtained. In 2003, seven assessments of canopy reflectance were obtained between 14 July 2003 and 27 August 2003 at intervals of 6 to 13 days. On each assessment date, canopy reflectance was measured from 12 arbitrarily selected circular plots (1.5-m diameter) that provided a wide range of plant growth as indicated by GLAI within each assessment date. A single, hand-held, multispectral radiometer (model MRS-87, CROPSCAN, Inc., Rochester, MN) was used to measure percentage of incident sunlight radiation that was reflected by soybean canopies. Percentage reflectance was measured at eight wavelength bands with midpoint values of 460, 510, 560, 610, 660, 710, 760, and 810 nm, and bandwidths of 27.0, 32.3, 25.0, 26.9, 25.5, 32.9, 28.0, and 31.7 nm (Cropscan, 1994), respectively, for each wavelength band. The radiometer was placed over the center of the plot between two rows with its sensors at 3-m height to measure reflectance from each circular plot. Five percentage reflectance measurements were obtained from each plot and averaged. Assessments were made under cloudless sky between 1100 and 1400 hours central standard time (CST) (Guan and Nutter, 2001).

Since the radiometric data, such as band location and width, are characteristics of the radiometer model, some of the indices presented here are approximations of previously published indices that have been used to detect differences in plant canopy development. The
percentage reflectance (R) at different wavelength bands (W subscripts) was used to obtain the vegetation indices evaluated in this study. The different indices evaluated were:

1. Green, \( R_G = \frac{(R_{510} + R_{560})}{2} \)
2. Red, \( R_R = \frac{(R_{610} + R_{660})}{2} \)
3. Near Infrared, \( R_{NIR} = \frac{(R_{760} + R_{810})}{2} \)
4. Radiance Ratio 1, \( RR_1 = \frac{R_{NIR}}{R_R} \) (Batista and Rudorff, 1990; Holben et al., 1980; Tucker, 1979)
5. Radiance Ratio 2, \( RR_2 = \frac{R_{810}}{R_{660}} \)
6. Radiance Ratio 3, \( RR_3 = \frac{R_{810}}{R_{610}} \)
7. Radiance Ratio 4, \( RR_4 = \frac{R_{760}}{R_{660}} \)
8. Radiance Ratio 5, \( RR_5 = \frac{R_{760}}{R_{610}} \)
9. Normalized Difference Vegetation Index 1, \( NDVI_1 = \frac{(R_{NIR} - R_R)}{(R_{NIR} + R_R)} \) (Holben et al., 1980)
10. Normalized Difference Vegetation Index 2, \( NDVI_2 = \frac{(R_{810} - R_{660})}{(R_{810} + R_{660})} \)
11. Normalized Difference Vegetation Index 3, \( NDVI_3 = \frac{(R_{810} - R_{610})}{(R_{810} + R_{610})} \)
12. Normalized Difference Vegetation Index 4, \( NDVI_4 = \frac{(R_{760} - R_{660})}{(R_{760} + R_{660})} \)
13. Normalized Difference Vegetation Index 5, \( NDVI_5 = \frac{(R_{760} - R_{610})}{(R_{760} + R_{610})} \)
14. Normalized Difference Vegetation Index 6, \( NDVI_6 = \frac{(R_{760} - R_{710})}{(R_{760} + R_{710})} \)
15. Transformed Vegetation Index 1, \( TVI_1 = (NDVI_1 + 0.5)^{0.5} \) (Tucker, 1979)
16. Transformed Vegetation Index 2, \( TVI_2 = (NDVI_2 + 0.5)^{0.5} \)
17. Transformed Vegetation Index 3, \( TVI_3 = (NDVI_3 + 0.5)^{0.5} \) (Huete et al., 1985)
18. Transformed Vegetation Index 4, \( TVI_4 = (NDVI_4 + 0.5)^{0.5} \)
19. Transformed Vegetation Index 5, \( TVI_5 = (NDVI_5 + 0.5)^{0.5} \)
20. Transformed Vegetation Index 6, \( TVI_6 = (NDVI_6 + 0.5)^{0.5} \)
21. Photochemical Reflectance Index, \( PRI = \frac{(R_{510} - R_{570})}{(R_{510} + R_{570})} \) (Gamon et al., 1997)
22. Green Normalized Difference Vegetation Index 1,
GNDVI\(_1\) = (R\(_{NIR}\) - R\(_G\))/(R\(_{NIR}\) + R\(_G\)) (Chang et al., 2003; Gitelson et al., 1996; Shanahan et al., 2001)

23. Green Normalized Difference Vegetation Index 2, GNDVI\(_2\) = (R\(_{810}\) - R\(_{560}\))/(R\(_{810}\) + R\(_{560}\))

24. Green Normalized Difference Vegetation Index 3, GNDVI\(_3\) = (R\(_{810}\) - R\(_{510}\))/(R\(_{810}\) + R\(_{510}\))

25. Green Normalized Difference Vegetation Index 4, GNDVI\(_4\) = (R\(_{760}\) - R\(_{560}\))/(R\(_{760}\) + R\(_{560}\))

26. Green Normalized Difference Vegetation Index 5, GNDVI\(_5\) = (R\(_{760}\) - R\(_{510}\))/(R\(_{760}\) + R\(_{510}\))

27. Visible Atmospherically Resistant Index, VARI = (R\(_{560}\) - R\(_{660}\))/(R\(_{560}\) + R\(_{660}\) - R\(_{460}\)) (Gitelson et al., 2002)

28. Difference Vegetation Index 1, DVI\(_1\) = (R\(_{NIR}\) - R\(_R\)) (Tucker, 1979)

29. Difference Vegetation Index 2, DVI\(_2\) = (R\(_{810}\) - R\(_{660}\))

30. Difference Vegetation Index 3, DVI\(_3\) = (R\(_{810}\) - R\(_{610}\))

31. Difference Vegetation Index 4, DVI\(_4\) = (R\(_{760}\) - R\(_{660}\))

32. Difference Vegetation Index 5, DVI\(_5\) = (R\(_{760}\) - R\(_{610}\))

33. Renormalized Difference Vegetation Index 1, RDVI\(_1\) = (NDVI\(_1\) * DVI\(_1\))\(^0.5\) (Broge and Leblanc, 2000; Roujean and Breon, 1995)

34. Renormalized Difference Vegetation Index 2, RDVI\(_2\) = (NDVI\(_1\) * DVI\(_2\))\(^0.5\)

35. Renormalized Difference Vegetation Index 3, RDVI\(_3\) = (NDVI\(_1\) * DVI\(_3\))\(^0.5\)

36. Renormalized Difference Vegetation Index 4, RDVI\(_4\) = (NDVI\(_1\) * DVI\(_4\))\(^0.5\)

37. Renormalized Difference Vegetation Index 5, RDVI\(_5\) = (NDVI\(_1\) * DVI\(_5\))\(^0.5\)

After measuring canopy reflectance, the aboveground portions of all plants within each plot were harvested, and ten plants were arbitrarily selected for measuring their green leaf area using an area meter (model LI 3100, LI-COR, Inc., Lincoln, NE). Between removal and measuring of green leaf area, the samples were kept refrigerated. All of the yellow and necrotic parts of leaves were removed prior measuring the green leaf area of the samples.

From each plot, the ten-plant sample and the remaining plants were kept separated and both were dried at 60°C for 48 hours using a forced air oven. After this period in the oven, the dry weight of the remaining plants within each plot and the dry weight of the 10
plants arbitrarily selected were obtained. The sum of the dry weight of the remaining plants within a plot and the dry weight of 10 plants arbitrarily selected from the same plot was calculated to obtain the total dry weight of the plants within a plot. The total dry weight of plants within a plot was called plant dry weight (PDW). The ratio between green leaf area and dry weight of the ten-plant sample was calculated and this ratio was multiplied by PDW to obtain the total green leaf area per plot. The total green leaf area for each plot was divided by the plot area (1.767 m²) to obtain the green leaf area index (GLAI).

Statistical analyses were done using S-Plus statistical software (Mathsoft, Inc., Cambridge, MA), and graphs were prepared using Sigma Plot (SPSS, Inc., Chicago, IL). To determine the relationships among wavelength bands, VI, GLAI, and PDW, scatter plot matrixes were prepared. Natural-logarithmic transformation of the data was done to eliminate problems with the residuals when linear regression was applied. Residual plots, P-values, coefficients of determination (R²), and standard error for estimate of y (SEEₚ) of regression models were considered to determine the wavelength bands and VI that had the best relationships with GLAI (Campbell and Madden, 1990).

The general linear test approach tested if the individual-assessment-date models within seasons could be reduced to full-season models describing the relationships between percentage reflectance data and GLAI within growing seasons (Neter and Wasserman, 1974). Similarly, this statistical test was used to compare regressions of reflectance data on GLAI obtained at different seasons. The relationships between PDW and GLAI with reflectance at different wavelength bands and vegetation indices were investigated, and the relationships between GLAI and PDW were described.

Results

The relationships among the percentage reflectance values for the individual wavelength bands are presented in Table 1. Positive linear relationships (P < 0.0001) were observed among percentage reflectance values for pairs of individual wavelength bands from
460 nm to 660 nm, and between 760 nm and 810 nm. There was no significant linear relationship between percentage reflectance values obtained for each infrared wavelength band (760 and 810 nm) and percentage reflectance values obtained at 710 nm (P > 0.5). Negative linear relationships (P ≤0.018) among percentage reflectance of visible wavelength bands (from 460 to 660 nm) and percentage reflectance of infrared wavelength bands were observed. Coefficient of determination (R²) values ranged between 0.97 and 0.99 for the regressions of percentage reflectance among 460, 510, 610, and 660 nm wavelength bands, and was 0.99 between percentage reflectance at 810 and at 760 nm within seasons. Linear relationships among percentage reflectance at 710 nm and any other wavelength band in the visible spectrum had R² varying from 0.44 to 0.80.

Percentage reflectance-GLAI scatter plots for specific wavelength bands for the 2002 and 2003 growing seasons are shown in Figs. 1 and 2. The best regression models describing the relationships between percentage reflectance for individual wavelength bands and GLAI were chosen based on the probability associated with the F-statistic, R², residual standard error, and the residual. For the 760 and 810 nm wavelength bands, linear models best described the relationships of GLAI and percentage reflectance within each assessment date and within seasons (Table 2). Linear relationship between percentage reflectance at 710 nm and GLAI was not significant in 2002 (P = 0.27), but it was significant in 2003 (P = 0.0049, R² = 9%). Natural-logarithmic transformations of percentage reflectance (460, 510, 560, 610, and 660 nm) and GLAI linearized the relationships between these variables and provided the best models for specific assessment dates and for the entire 2002 season (Table 3). For these same wavelength bands, a natural-logarithmic transformation of percentage reflectance regressed on untransformed GLAI provided the best models describing the relationships between percentage reflectance and GLAI in 2003 (Table 4). Among the visible wavelength bands (from 460 nm to 710 nm), percentage reflectance obtained at 660 nm best estimated GLAI in 2002 and 2003 (Tables 3 and 4).
The regressions of percentage reflectance for individual wavelength bands on GLAI had lower $R^2$ when analyzed for the entire seasons of 2002 and 2003 than when analyzed for individual assessment dates (Tables 2, 3 and 4).

The general linear test approach (Neter and Wasserman, 1974) was used to determine if the regressions of reflectance data on GLAI obtained at individual assessment dates could be reduced to single, overall, full-season regressions. The null hypothesis for this test is that there is no difference between reduced (annual regressions for individual wavelength bands) and individual-assessment-date models (models for each specific assessment date for a specific wavelength bands). The null hypothesis was rejected for all wavelength band-GLAI relationships in 2002 and in 2003 (Table 5).

It was not possible to use full-season models for describing the relationships between natural-log-transformed reflectance data at 660 nm and natural-log-transformed GLAI data within the 2002 ($F = 8.11, P = 0.001$) and 2003 ($F = 6.81, P = 0.002$) growing seasons. However, there was no significant difference ($F = 0.07, P = 0.93$) between the overall annual regressions of these two variables obtained 2002 and 2003 (Fig. 3A).

Regression of percentage reflectance at 810 nm on percentage reflectance at 760 nm showed a strong linear relationship ($Y = 0.73 + 1.08 X$, $R^2 = 0.99$; $Y = 0.90 + 1.10 X$, $R^2 = 0.99$) in 2002 and 2003 (Table 1). Positive linear relationships between the percentage reflectance for infrared wavelength bands and GLAI also were obtained within each assessment date and for the entire seasons (Table 2). Applying the general linear test approach, the regression lines obtained within each assessment date (individual-assessment-date model) with the regression lines obtained for the entire seasons (full-season model) were compared. For the regressions of percentage reflectance at 760 nm on GLAI, the reduced and individual-assessment-date models were dissimilar for the 2002 ($F = 6.79, P \leq 0.003$) and 2003 ($F = 19.75, P < 0.0001$) growing seasons. The same results were observed for the regressions of percentage reflectance at 810 nm on GLAI when individual-assessment-date model and full-season models were compared in 2002 ($F = 6.25, P \leq 0.004$) and 2003 ($F =$
Additionally, there was a significant difference \( F = 16.69, P < 0.0001 \) between regression lines of percentage reflectance at 760 nm on GLAI obtained for 2002 and 2003 (Fig. 3B), and between regression lines of percentage reflectance at 810 nm on GLAI obtained for 2002 and 2003 seasons \( (F = 17.09, P < 0.0001) \) (Fig. 3C).

Using percentage reflectance from slightly different wavelength bands to calculate VI, variations of the same type of vegetation indices were obtained. Each variation was identified by the subscripts following the index names. Correlation coefficients among the indices variations were greater than 0.99 for RR, DVI, RDVI, and GNDVI, and greater than 0.97 for NDVI and TVI. Due to the high similarity observed among the variations of single indices, just \( \text{RR}_1, \text{DVI}_1, \text{NDVI}_1, \text{RDVI}_1, \text{TVI}_1, \text{GNDVI}_1, \text{PRI}, \text{and VARI} \) were used to show the relationships among VI, GLAI, and PDW in scatter plot matrixes for 2002 and 2003 (Fig. 4). The relationships were similar across these two years, and all of the indices were positively related with each other, except PRI, which was negatively related with all other indices. Linear relationships were observed between NDVI, TVI, and GNDVI (Table 6), and between VARI, DVI, and RDVI (Table 7).

GLAI and PDW were positively related with all of the indices, except PRI. Similarly, positive linear relationships \( (P < 0.0001) \) were obtained between GLAI and \( \text{RR}_1, \text{DVI}_1, \text{and RDVI}_1 \) (Fig. 5). For the same years, natural-logarithmic transformation of GLAI had a positive linear relationship \( (P < 0.0001) \) with NDVI, TVI, and GNDVI (Fig. 6). The coefficients of determination \( (R^2) \) of the regressions of VI on GLAI or on natural-logarithmic-transformed GLAI throughout the 2002 and 2003 seasons showed that the relationships were stronger within assessment dates than within seasons (Fig. 5, 6, and 7).

Based on the general linear test approach, it was showed that the \( \text{RDVI}_1 \) full-season model analysis for the entire seasons of 2002 and 2003 did not differ statistically from the individual-assessment-date model analyses that considered each assessment date separately.
In 2003, the individual-assessment-date models involving RR, VARI, NDVI, TVI, and GNDVI did not differ statistically (P > 0.05) from the full-season models (Table 8). For all VI, the regression lines for 2002 and 2003 differed statistically (P ≤0.05).

Positive linear relationships between PDW and GLAI within each assessment date and within 2002 and 2003 seasons are presented (Fig. 8). The relationships between GLAI and VI were very similar to the relationships between PDW and VI (Fig. 4); however, more variability is present in the PDW-VI relationships.

Discussion

Precision agriculture requires real time information about crop conditions to allow implementation of management tactics to reduce pollution, improve yield, and to maximize the return on investment (Seelan et al., 2003). Ground-based reflectance sensors can provide the data about canopy conditions needed for site-specific management in a timely fashion. In order to test this hypothesis, it is necessary to evaluate the quality and quantity of sunlight reflected from crop canopies that can be measured by ground-based radiometers throughout seasons. The quality of the reflectance data can be assessed studying the relationships among percentage reflectance for individual wavelength bands or VI and the variables of interest, such as GLAI, PDW, plant stresses, and yield.

It was previously shown that measurements of soybean GLAI at critical developmental stages of the crop could be used to estimate yield (Board, 2004; Board et al., 1997; Fehr et al., 1981). However, periodical assessment of GLAI may be necessary to assess the effects of plant stresses on crop development throughout growing seasons (Pinter et al., 2003; Seelan et al., 2003). Our study showed that ground-based radiometer measurements of reflectance could be used to provide accurate estimations of GLAI during soybean growing seasons. We also illustrated how different assessments of reflectance from soybean are related to each other throughout crop seasons. These relationships are extremely important in
order to monitor the development of plant stresses after their detection and to verify efficacy of management practices implemented to mitigate those stresses.

Information about a crop is just one part of the composite data captured by radiometer readings (Jackson, 1986). Thus, it is necessary to understand how reflectance data relate with agronomic variables of interest throughout seasons and under different environmental conditions. We found that percentage reflectance for the wavelength bands tested in our work were not sensitive to low values of GLAI, and the reflectance for wavelength bands in the visible spectrum of light were not sensitive to GLAI above 2 or 3. Measuring percentage reflectance from soybean canopies, we found that wavelength bands from 460 to 660 nm were highly linearly related, and this relationship also was true for the near infrared wavelength bands (760 nm and 810 nm). This result suggests that percentage reflectance from those linearly related wavelength bands provide similar information about soybean canopies, a conclusion that was verified by the similarities in estimation of soybean GLAI by reflectance from those bands. Reflectance from individual wavelength bands can be considered functionally equivalent if they have an equal effect in the decision-making process (Perry and Lautenschlager, 1984). Consequently, percentage reflectance from wavelength bands from 460 to 660 nm and between 760 and 810 nm can be considered equivalent in their ability to estimate soybean GLAI. Then, the only justification to use any one of the visible or infrared wavelength bands is the ability of a particular wavelength band to improve the regression fit when regressed against the variable of interest (Perry and Lautenschlager, 1984). In this perspective, percentage reflectance from the wavelength bands centered at 660 nm and 810 nm provided the best relationships with GLAI for the wavelength bands in the visible and infrared spectra, respectively. Our research confirmed that percentage reflectance from red (660 nm) and infrared (810 nm) wavelength bands provided the best relationships with GLAI and these wavelength bands should be used to create VI, but it also showed the potential of using percentage of reflectance from blue (460
nm), green (510 nm), and orange (610 nm) wavelength bands to accomplish the same objectives.

The results of our work agree with those of Holben et al. (1980) who described the basic relationship between percentage reflectance of infrared wavelength bands and GLAI for soybean. However, we observed higher coefficients of determination than previously reported. Also, there were negative curvilinear relationships between all visible wavelength bands up to 660 nm and GLAI in our data, but Holden et al. (1980) found that the occurrence of outliers suggested a linear relationship instead of a curvilinear relationship between reflectance from red wavelength band and GLAI.

Slopes and intercepts for the regressions of individual wavelength bands on GLAI differed statistically among assessment dates. Additionally, our study showed that there are statistical differences among regression lines obtained for different assessment dates (individual-assessment-date models) and the regression lines obtained by combining all data for individual wavelength bands from one season (full-season models). Previous research (Batista and Rudorff, 1990; Holben et al., 1980; Kanemasu, 1974; Tucker, 1979) did not account for that possible sort of variability, and more additional work is needed to establish the set of conditions necessary to minimize the within-season variation in the dataset.

Considering that percentage reflectance can be used to estimate GLAI and that GLAI can be used to predict soybean yield, problems with the variability of reflectance-GLAI relationships during the season are eliminated if the yield prediction is made based on a single assessment of percentage reflectance during a critical period of crop development (Batista and Rudorff, 1990; Board, 2004; Board et al., 1997; Fehr et al., 1981; Holben et al., 1980; Kanemasu, 1974; Kollenkark et al., 1982). However, if percentage reflectance data obtained at different stages of crop development are integrated into crop yield models, the variability of the GLAI-percentage-reflectance relationship may negatively impact the model output. Moreover, this variability can reduce the usefulness of percentage reflectance data in
monitoring changes in crop development, assessing plant stresses, and evaluating efficacy of crop management practices.

Similarities of the slopes and intercepts for the individual-assessment-date models and for the full-season models indicate stability of the percentage reflectance measurements for multiple situations. Despite of the differences of regression lines of percentage reflectance on GLAI, it is important to note that regressions of percentage reflectance at 660 nm on GLAI obtained in 2002 and 2003 did not differ statistically. The wavelength band centered at 660 nm may provide data that will assess crop health status in specific fields over long periods of time.

Linear and curvilinear relationships between different pairs of VI were observed in our work. Positive linear relationships among NDVI, TVI, and GNDVI, and among VARI, DVI, and RDVI were observed. Similarities between percentage reflectance from soybean canopies for red (660 nm) and green (510 nm) wavelength bands explain the strong linear relationship between NDVI and GNDVI.

The VI that had the best linear relationships with GLAI were RR, DVI, and RDVI, and those with the best linear relationship with transformed GLAI were NDVI, TVI, and GNDVI during 2002 and 2003 seasons. The linear relationship between RR and soybean green biomass and soybean yield was previously reported (Batista and Rudorff, 1990). Linear coefficients of determination ($R^2$) for the relationships of NDVI, TVI, and GNDVI with GLAI were similar and varied uniformly throughout seasons. This common behavior supports the notion that NDVI, TVI, and GNDVI were equivalent to estimate soybean GLAI.

The stability of the relationships between percentage reflectance data and GLAI during the crop season justifies the use of full-season models combining all percentage reflectance data obtained within a season to estimate GLAI. Among all possibilities tested in our study, only RDVI allowed the use of full-season models to estimate GLAI in both 2002 and 2003 seasons.
Linear relationships between GLAI and PDW were observed within different assessment dates and for the entire seasons of 2002 and 2003. The relationships of VI and individual wavelength bands with GLAI were stronger than with PDW. Thus, there is the possibility of using percentage reflectance data to assess soybean biomass.

The work reported herein provides important information about the usefulness of percentage reflectance data to assess soybean growth during growing seasons. To expand the significance of our results, research verifying the effect of different environments, soybean varieties, and different soil types on the relationships between percentage reflectance data and GLAI should be implemented. The results of such work would provide the basis to justify the adoption of percentage reflectance in large scale to detect biotic and abiotic plant stresses that affect GLAI, to evaluate crop management practices, and to predict soybean yield.

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Table 3. Equation, coefficient of determination ($R^2$), and standard error of $y$ estimate (SEE$_y$) for the regressions of natural-logarithmic (Ln) transformed percentage reflectance for individual wavelength bands on natural-logarithmic transformed green leaf area index (GLAI) obtained within specific dates and for the entire year 2002.

Table 4. Equation, coefficient of determination ($R^2$), and standard error of $y$ estimate (SEE$_y$) for the regressions of natural-logarithmic (Ln) transformed percentage reflectance for individual wavelength bands on green leaf area index (GLAI) obtained within specific dates and for the entire year 2003.

Table 5. F statistics and P values for the general test approach that compared linear regressions of percentage reflectance for individual wavelength bands on GLAI for specific assessment dates (individual-assessment-date model) with the overall annual regressions of percentage reflectance on GLAI (full-season model) in 2002 and 2003.

Table 6. Linear relationships and coefficient of determination among vegetation indices (VI) obtained from reflectance from soybean canopies during 2002 and 2003 seasons. The different VI are: normalized difference vegetation index (NDVI), transformed vegetation index (TVI), and green normalized vegetation index (GNDVI).

Table 7. Linear relationships and coefficient of determination among vegetation indices (VI) obtained from reflectance from soybean canopies during 2002 and 2003 seasons. The
different VI are: renormalized difference vegetation index (RDVI), difference vegetation index (DVI), and visible atmospherically resistant index (VARI).

Table 8. F-statistics and P-values for the general test approach that compared linear regressions of vegetation indices (VI): radiance ratio 1 (RRi), difference vegetation index 1 (DVI1), renormalized difference vegetation index 1 (RDVI1), photochemical reflectance index (PRI), visible atmospherically resistant index (VARI), normalized difference vegetation index 1 (NDVI1), transformed vegetation index 1 (TVI1), and green normalized difference vegetation index 1 (GNDVI1) on GLAI or Ln GLAI for specific assessment dates (individual-assessment-date model) with the overall annual regressions of VI on GLAI or on Ln GLAI (full-season model) in 2002 and 2003. The null hypothesis for this test is that the lines are the same.

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Figure 1. Relationships among soybean green leaf area index (GLAI) and percentage reflectance from soybean canopies for individual wavelength bands centered at 460, 510, 560, 610, 660, 710, 760, and 810 nm obtained during 2002.

Figure 2. Relationships among soybean green leaf area index (GLAI) and percentage reflectance from soybean canopies for individual wavelength bands centered at 460, 510, 560, 610, 660, 710, 760, and 810 nm obtained during 2003.

Figure 3. Relationships among soybean green leaf area index (GLAI) and percentage reflectance from soybean canopies for 660 (A), 760 (B) and 810 nm (C) wavelength bands on soybean green leaf area index obtained throughout 2002 and 2003 seasons.

Figure 4. Scatter plots of the relationships among individual vegetation indices (RRi, DVI1, NDVI1, TVI1, RDVI1, GNDVI1, PRI, and VARI), soybean green leaf area index (GLAI), and plant dry weight (PDW) in 2002 (A) and 2003 (B).
Figure 5. Relationships among of soybean green leaf area index and vegetation indices: radiance ratio 1 (RR$_1$), difference vegetation index 1 (DVI$_1$), and renormalized difference vegetation index 1 (RDVI$_1$) throughout 2002 and 2003 growing seasons.

Figure 6. Relationships among natural-logarithmic transformed soybean green leaf area index (Ln (GLAI)) and vegetation indices: normalized difference vegetation index 1 (NDVI$_1$), transformed vegetation index 1 (TVI$_1$), and green normalized difference vegetation index 1 (GNDVI$_1$) throughout 2002 and 2003 seasons.

Figure 7. Coefficient of determination (R-square) for linear regressions relating different vegetation indices: radiance ratio (RR), difference vegetation index (DVI), renormalized difference vegetation index (RDVI), normalized difference vegetation index (NDVI), transformed vegetation index (TVI), and green normalized vegetation index (GNDVI), to soybean green leaf area index (GLAI) and to natural-logarithmic transformed GLAI during 2002 and 2003.

Figure 8. Relationships between soybean plant dry weight (PDW) (g) and soybean green leaf area index (GLAI) obtained within assessment dates and for the entire seasons 2002 and 2003.
Table 1. Relationships, coefficient of determination (R2), and standard error of estimate for \( y \) (SEE\(_y\)) among percentage reflectance from soybean canopies for individual wavelength bands obtained during the 2002 and 2003 seasons.
<table>
<thead>
<tr>
<th>Percentage Reflectance</th>
<th>Year</th>
<th>Regression</th>
<th>460 nm</th>
<th>510 nm</th>
<th>560 nm</th>
<th>610 nm</th>
<th>660 nm</th>
<th>760 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>510 nm</td>
<td>2002</td>
<td>( Y = 0.10 + 1.11 x )</td>
<td>0.98/0.015</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2003</td>
<td>( Y = 0.13 + 1.15 x )</td>
<td>0.99/0.008</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>560 nm</td>
<td>2002</td>
<td>( Y = 3.42 + 0.95 x )</td>
<td>0.87/0.053</td>
<td>3.27 + 0.83 x</td>
<td>0.91/0.050</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2003</td>
<td>( Y = 3.62 + 1.02 x )</td>
<td>0.90/0.030</td>
<td>-3.71 + 0.89 x</td>
<td>0.91/0.030</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>610 nm</td>
<td>2002</td>
<td>( Y = 1.25 + 1.42 x )</td>
<td>0.97/0.018</td>
<td>1.12 + 1.21 x</td>
<td>-2.91 + 1.36 x</td>
<td>0.99/0.010</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2003</td>
<td>( Y = 0.49 + 1.15 x )</td>
<td>0.99/0.008</td>
<td>0.67 + 1.31 x</td>
<td>-3.96 + 1.36 x</td>
<td>0.99/0.009</td>
<td></td>
<td></td>
</tr>
<tr>
<td>660 nm</td>
<td>2002</td>
<td>( Y = 1.15 + 1.75 x )</td>
<td>0.99/0.008</td>
<td>1.21 + 1.47 x</td>
<td>-5.68 + 1.59 x</td>
<td>-2.50 + 1.20 x</td>
<td>0.97/0.020</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2003</td>
<td>( Y = 0.60 + 1.81 x )</td>
<td>0.99/0.006</td>
<td>0.34 + 1.57 x</td>
<td>-5.31 + 1.56 x</td>
<td>-1.06 + 1.19 x</td>
<td>0.97/0.020</td>
<td></td>
</tr>
<tr>
<td>760 nm</td>
<td>2002</td>
<td>( Y = 42.54 - 2.85 x )</td>
<td>0.32/0.020</td>
<td>42.05 - 2.27 x</td>
<td>43.46 - 1.66 x</td>
<td>44.08 - 1.86 x</td>
<td>41.24 - 1.73 x</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2003</td>
<td>( Y = 39.82 - 2.45 x )</td>
<td>0.28/0.020</td>
<td>38.95 - 2.01 x</td>
<td>39.17 - 1.25 x</td>
<td>39.48 - 1.47 x</td>
<td>40.00 - 1.49 x</td>
<td></td>
</tr>
<tr>
<td>810 nm</td>
<td>2002</td>
<td>( Y = 45.29 - 3.09 x )</td>
<td>0.32/0.020</td>
<td>44.74 - 2.46 x</td>
<td>46.24 - 1.80 x</td>
<td>46.92 - 2.02 x</td>
<td>43.85 - 1.88 x</td>
<td>-0.73 + 1.08 x</td>
</tr>
<tr>
<td></td>
<td>2003</td>
<td>( Y = 42.42 - 2.73 x )</td>
<td>0.30/0.020</td>
<td>41.54 - 2.25 x</td>
<td>42.25 - 1.45 x</td>
<td>42.18 - 1.6 x</td>
<td>42.63 - 1.65 x</td>
<td>-0.90 + 1.10 x</td>
</tr>
</tbody>
</table>

\( R^2 \) and \( SSE \) values are also provided for each regression equation.
Table 2. Equation, coefficient of determination ($R^2$), and standard error of $y$ estimate ($\text{SEE}_y$) for the regressions of percentage reflectance for individual wavelength bands on green leaf area index (GLAI) obtained within specific dates and for years 2002 and 2003.
<table>
<thead>
<tr>
<th>Assessment Date</th>
<th>Regression</th>
<th>Percentage Reflectance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>760 nm</td>
</tr>
<tr>
<td>23 July</td>
<td>GLAI</td>
<td>5.36 + 0.23 x</td>
</tr>
<tr>
<td></td>
<td>R²</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>SEE&lt;sub&gt;y&lt;/sub&gt;</td>
<td>0.026</td>
</tr>
<tr>
<td>7 August</td>
<td>GLAI</td>
<td>1.76 + 0.12 x</td>
</tr>
<tr>
<td></td>
<td>R²</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>SEE&lt;sub&gt;y&lt;/sub&gt;</td>
<td>0.006</td>
</tr>
<tr>
<td>17 August</td>
<td>GLAI</td>
<td>1.13 + 0.11 x</td>
</tr>
<tr>
<td></td>
<td>R²</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>SEE&lt;sub&gt;y&lt;/sub&gt;</td>
<td>0.014</td>
</tr>
<tr>
<td>30 August</td>
<td>GLAI</td>
<td>4.05 + 0.18 x</td>
</tr>
<tr>
<td></td>
<td>R²</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>SEE&lt;sub&gt;y&lt;/sub&gt;</td>
<td>0.019</td>
</tr>
<tr>
<td>2002</td>
<td>GLAI</td>
<td>2.48 + 0.15 x</td>
</tr>
<tr>
<td></td>
<td>R²</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>SEE&lt;sub&gt;y&lt;/sub&gt;</td>
<td>0.01</td>
</tr>
<tr>
<td>14 July</td>
<td>GLAI</td>
<td>2.37 + 0.10 x</td>
</tr>
<tr>
<td></td>
<td>R²</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>SEE&lt;sub&gt;y&lt;/sub&gt;</td>
<td>0.012</td>
</tr>
<tr>
<td>21 July</td>
<td>GLAI</td>
<td>2.72 + 0.12 x</td>
</tr>
<tr>
<td></td>
<td>R²</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>SEE&lt;sub&gt;y&lt;/sub&gt;</td>
<td>0.008</td>
</tr>
<tr>
<td>29 July</td>
<td>GLAI</td>
<td>1.68 + 0.11 x</td>
</tr>
<tr>
<td></td>
<td>R²</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>SEE&lt;sub&gt;y&lt;/sub&gt;</td>
<td>0.004</td>
</tr>
<tr>
<td>4 August</td>
<td>GLAI</td>
<td>2.95 + 0.15 x</td>
</tr>
<tr>
<td></td>
<td>R²</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>SEE&lt;sub&gt;y&lt;/sub&gt;</td>
<td>0.013</td>
</tr>
<tr>
<td>14 August</td>
<td>GLAI</td>
<td>3.22 + 0.17 x</td>
</tr>
<tr>
<td></td>
<td>R²</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>SEE&lt;sub&gt;y&lt;/sub&gt;</td>
<td>0.013</td>
</tr>
<tr>
<td>27 August</td>
<td>GLAI</td>
<td>1.48 + 0.12 x</td>
</tr>
<tr>
<td></td>
<td>R²</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>SEE&lt;sub&gt;y&lt;/sub&gt;</td>
<td>0.014</td>
</tr>
<tr>
<td>8 September</td>
<td>GLAI</td>
<td>2.40 + 0.15 x</td>
</tr>
<tr>
<td></td>
<td>R²</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>SEE&lt;sub&gt;y&lt;/sub&gt;</td>
<td>0.009</td>
</tr>
<tr>
<td>2003</td>
<td>GLAI</td>
<td>1.71 + 0.11 x</td>
</tr>
<tr>
<td></td>
<td>R²</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>SEE&lt;sub&gt;y&lt;/sub&gt;</td>
<td>0.006</td>
</tr>
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</table>
Table 3. Equation, coefficient of determination ($R^2$), and standard error of $y$ estimate ($\text{SEE}_y$) for the regressions of natural-logarithmic (Ln) transformed percentage reflectance for individual wavelength bands on natural-logarithmic transformed green leaf area index (GLAI) obtained within specific dates and for the entire year 2002.

<table>
<thead>
<tr>
<th>Assessment Date</th>
<th>Regression</th>
<th>Ln (Percentage Reflectance)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>460 nm</td>
</tr>
<tr>
<td>23 July</td>
<td>Ln (GLAI)</td>
<td>3.75  - 2.39 x</td>
</tr>
<tr>
<td></td>
<td>$R^2$ (%)</td>
<td>94.0</td>
</tr>
<tr>
<td></td>
<td>$\text{SEE}_y$</td>
<td>0.19</td>
</tr>
<tr>
<td>7 August</td>
<td>Ln (GLAI)</td>
<td>3.30  - 2.76 x</td>
</tr>
<tr>
<td></td>
<td>$R^2$ (%)</td>
<td>86.7</td>
</tr>
<tr>
<td></td>
<td>$\text{SEE}_y$</td>
<td>0.34</td>
</tr>
<tr>
<td>17 August</td>
<td>Ln (GLAI)</td>
<td>4.33  - 3.87 x</td>
</tr>
<tr>
<td></td>
<td>$R^2$ (%)</td>
<td>91.0</td>
</tr>
<tr>
<td></td>
<td>$\text{SEE}_y$</td>
<td>0.38</td>
</tr>
<tr>
<td>30 August</td>
<td>Ln (GLAI)</td>
<td>2.99  - 2.01 x</td>
</tr>
<tr>
<td></td>
<td>$R^2$ (%)</td>
<td>90.2</td>
</tr>
<tr>
<td></td>
<td>$\text{SEE}_y$</td>
<td>0.21</td>
</tr>
<tr>
<td>2002</td>
<td>Ln (GLAI)</td>
<td>2.89  - 2.08 x</td>
</tr>
<tr>
<td></td>
<td>$R^2$ (%)</td>
<td>76.9</td>
</tr>
<tr>
<td></td>
<td>$\text{SEE}_y$</td>
<td>0.17</td>
</tr>
</tbody>
</table>
Table 4. Equation, coefficient of determination (R$^2$), and standard error of y estimate (SEE$_y$) for the regressions of natural-logarithmic (Ln) transformed percentage reflectance for individual wavelength bands on green leaf area index (GLAI) obtained within specific dates and for the entire year 2003.
<table>
<thead>
<tr>
<th>Assessment Date</th>
<th>Regression</th>
<th>Ln (Percentage Reflectance)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>460 nm</td>
</tr>
<tr>
<td>14 July GLAI</td>
<td>4.33 - 1.96 x</td>
<td>4.57 - 1.96 x</td>
</tr>
<tr>
<td></td>
<td>0.96</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>0.13</td>
<td>0.16</td>
</tr>
<tr>
<td>21 July GLAI</td>
<td>4.59 - 2.04 x</td>
<td>4.93 - 2.07 x</td>
</tr>
<tr>
<td></td>
<td>0.97</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>29 July GLAI</td>
<td>5.64 - 3.26 x</td>
<td>6.40 - 3.49 x</td>
</tr>
<tr>
<td></td>
<td>0.82</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>0.49</td>
<td>0.53</td>
</tr>
<tr>
<td>4 August GLAI</td>
<td>5.34 - 2.66 x</td>
<td>5.42 - 2.53 x</td>
</tr>
<tr>
<td></td>
<td>0.95</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>0.18</td>
</tr>
<tr>
<td>14 August GLAI</td>
<td>4.28 - 2.22 x</td>
<td>4.20 - 2.07 x</td>
</tr>
<tr>
<td></td>
<td>0.77</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>0.38</td>
<td>0.32</td>
</tr>
<tr>
<td>27 August GLAI</td>
<td>4.19 - 2.83 x</td>
<td>4.08 - 2.60 x</td>
</tr>
<tr>
<td></td>
<td>0.74</td>
<td>0.63</td>
</tr>
<tr>
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<td>0.53</td>
<td>0.63</td>
</tr>
<tr>
<td>8 September GLAI</td>
<td>3.30 - 1.67 x</td>
<td>3.28 - 1.53 x</td>
</tr>
<tr>
<td></td>
<td>0.96</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>2003 GLAI</td>
<td>4.15 - 2.03 x</td>
<td>4.17 - 1.90 x</td>
</tr>
<tr>
<td></td>
<td>0.72</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>0.14</td>
<td>0.14</td>
</tr>
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</table>
Table 5. F statistics and P values for the general test approach that compared linear regressions of percentage reflectance for individual wavelength bands on GLAI for specific assessment dates (individual-assessment-date models) with the overall annual regressions of percentage reflectance on GLAI (full-season model) in 2002 and 2003.

<table>
<thead>
<tr>
<th>Percentage Reflectance</th>
<th>Year</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>460 nm</td>
<td>2002</td>
<td>11.27</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>2003</td>
<td>7.91</td>
<td>0.0008</td>
</tr>
<tr>
<td>510 nm</td>
<td>2002</td>
<td>12.10</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>2003</td>
<td>7.13</td>
<td>0.001</td>
</tr>
<tr>
<td>610 nm</td>
<td>2002</td>
<td>11.67</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>2003</td>
<td>7.97</td>
<td>0.0008</td>
</tr>
<tr>
<td>660 nm</td>
<td>2002</td>
<td>8.11</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>2003</td>
<td>6.90</td>
<td>0.0018</td>
</tr>
<tr>
<td>760 nm</td>
<td>2002</td>
<td>6.79</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>2003</td>
<td>19.75</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>810 nm</td>
<td>2002</td>
<td>6.25</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>2003</td>
<td>18.47</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>
Table 6. Linear relationships and coefficient of determination among vegetation indices (VI) obtained from reflectance from soybean canopies during 2002 and 2003 seasons. The different VI are: normalized difference vegetation index (NDVI<sub>i</sub>), transformed vegetation index (TVI<sub>i</sub>), and green normalized vegetation index (GNDVI<sub>i</sub>).

<table>
<thead>
<tr>
<th>VI</th>
<th>Year</th>
<th>Regression</th>
<th>R&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>TVI&lt;sub&gt;i&lt;/sub&gt;</td>
<td>2002</td>
<td>0.75 + 0.48 NDVI&lt;sub&gt;i&lt;/sub&gt;</td>
<td>0.65 + 0.62 GNDVI&lt;sub&gt;i&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R&lt;sup&gt;2&lt;/sup&gt; = 0.99</td>
<td>R&lt;sup&gt;2&lt;/sup&gt; = 0.99</td>
</tr>
<tr>
<td></td>
<td>2003</td>
<td>0.75 + 0.49 NDVI&lt;sub&gt;i&lt;/sub&gt;</td>
<td>0.64 + 0.64 GNDVI&lt;sub&gt;i&lt;/sub&gt;,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R&lt;sup&gt;2&lt;/sup&gt; = 0.99</td>
<td>R&lt;sup&gt;2&lt;/sup&gt; = 0.99</td>
</tr>
<tr>
<td>NDVI&lt;sub&gt;i&lt;/sub&gt;</td>
<td>2002</td>
<td>-0.20 + 1.29 GNDVI&lt;sub&gt;i&lt;/sub&gt;</td>
<td>-0.23 + 1.32 GNDVI&lt;sub&gt;i&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R&lt;sup&gt;2&lt;/sup&gt; = 0.99</td>
<td>R&lt;sup&gt;2&lt;/sup&gt; = 0.99</td>
</tr>
<tr>
<td></td>
<td>2003</td>
<td>-0.23 + 1.32 GNDVI&lt;sub&gt;i&lt;/sub&gt;</td>
<td>R&lt;sup&gt;2&lt;/sup&gt; = 0.99</td>
</tr>
</tbody>
</table>
Table 7. Linear relationships and coefficient of determination among vegetation indices (VI) obtained from reflectance from soybean canopies during 2002 and 2003 seasons. The different VI are: renormalized difference vegetation index (RDVI$_i$), difference vegetation index (DVI$_i$), and visible atmospherically resistant index (VARI).

<table>
<thead>
<tr>
<th>VI</th>
<th>Year</th>
<th>Regression</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>DVI$_i$</td>
<td>2002</td>
<td>-5.80 + 7.81 RDVI</td>
<td>13.71 + 49.96 VARI</td>
<td>$R^2 = 0.97$</td>
</tr>
<tr>
<td></td>
<td>2003</td>
<td>-4.06 + 7.41 RDVI</td>
<td>15.52 + 54.37 VARI</td>
<td>$R^2 = 0.97$</td>
</tr>
<tr>
<td>RDVI$_i$</td>
<td>2002</td>
<td>2.48 + 6.48 VARI</td>
<td>2.62 + 7.45 VARI</td>
<td>$R^2 = 0.95$</td>
</tr>
<tr>
<td></td>
<td>2003</td>
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Table 8. F statistics and P values for the general test approach that compared linear regressions of vegetation indices (VI): radiance ratio (RRi), difference vegetation index (DVIi), renormalized difference vegetation index (RDVIi), photochemical reflectance index (PRI), visible atmospherically resistant index (VARI), normalized difference vegetation index (NDVIi), transformed vegetation index (TVIi), and green normalized difference vegetation index (GNDVIi) on GLAI or Ln GLAI for specific assessment dates with the overall annual regressions of VI's on GLAI or on Ln GLAI in 2002 and 2003. The null hypothesis for this test is that the lines are the same.

<table>
<thead>
<tr>
<th>VI</th>
<th>Year</th>
<th>F</th>
<th>P</th>
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</thead>
<tbody>
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<td>RRi</td>
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<tr>
<td></td>
<td>2003</td>
<td>1.17</td>
<td>0.31</td>
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<tr>
<td>DVIi</td>
<td>2002</td>
<td>3.38</td>
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<tr>
<td></td>
<td>2003</td>
<td>7.21</td>
<td>0.001</td>
</tr>
<tr>
<td>RDVIi</td>
<td>2002</td>
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<td>0.10</td>
</tr>
<tr>
<td></td>
<td>2003</td>
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</tr>
<tr>
<td>PRI</td>
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</tr>
<tr>
<td></td>
<td>2003</td>
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<tr>
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</tr>
<tr>
<td></td>
<td>2003</td>
<td>1.86</td>
<td>0.16</td>
</tr>
<tr>
<td>TVIi</td>
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<td></td>
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<td>GNDVIi</td>
<td>2002</td>
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<td>2003</td>
<td>7.04</td>
<td>0.002</td>
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Figure 1. Relationships among soybean green leaf area index (GLAI) and percentage reflectance from soybean canopies for individual wavelength bands centered at 460, 510, 560, 610, 660, 710, 760, and 810 nm in 2002.
Figure 2. Relationships among soybean green leaf area index (GLAI) and percentage reflectance from soybean canopies for individual wavelength bands centered at 460, 510, 560, 610, 660, 710, 760, and 810 nm in 2003.
Figure 3. Relationships among soybean green leaf area index (GLAI) and percentage reflectance from soybean canopies for 660 (A), 760 (B) and 810 nm (C) wavelength bands obtained throughout 2002 and 2003 seasons.
Figure 4. Scatter plots of the relationships among individual vegetation indices (RR_1, DVI_1, NDVI_1, TVI_1, RDVI_1, GNDVI_1, PRI, and VARI), soybean green leaf area index (GLAI), and plant dry weight (PDW) in 2002 (A) and 2003 (B).
Figure 5. Relationships among soybean green leaf area index and vegetation indices: radiance ratio 1 (RR$_{1}$), difference vegetation index 1 (DVI$_{1}$), and renormalized difference vegetation index 1 (RDVI$_{1}$) throughout 2002 and 2003 growing seasons.
Figure 6. Relationships among natural-logarithmic transformed soybean green leaf area index (Ln (GLAI)) and vegetation indices: normalized difference vegetation index 1 (NDVI$_1$), transformed vegetation index 1 (TVI$_1$), and green normalized difference vegetation index 1 (GNDVI$_1$) throughout 2002 and 2003 growing seasons.
Figure 7. Coefficient of determination ($R^2$) for linear regressions relating different vegetation indices: radiance ratio (RR), difference vegetation index (DVI), renormalized difference vegetation index (RDVI), normalized difference vegetation index (NDVI), transformed vegetation index (TVI), and green normalized vegetation index (GNDVI), to soybean green leaf area index (GLAI) and to natural-logarithmic transformed GLAI during 2002 and 2003.
Figure 8. Relationships between soybean plant dry weight (PDW) (g) and soybean green leaf area index (GLAI) obtained within assessment dates and for the entire seasons 2002 and 2003.
CHAPTER 3
USE OF REMOTE SENSING, GEOGRAPHIC INFORMATION SYSTEMS TO ASSESS SOYBEAN YIELD AND SOYBEAN CYST NEMATODE POPULATIONS IN SOYBEAN FIELDS

A paper to be submitted to Crop Science
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2 Abbreviations: GLAI, green leaf area index; VI, vegetation index; 1- RG, green percentage reflectance; RR, red percentage reflectance; RNIR, near infrared percentage reflectance; Rinfrared, infrared percentage reflectance; RW, being W a wavelength band in nanometer (nm) from which percentage reflectance is measured; RR, radiance ratio; NDVI, normalized difference vegetation index; TVI, transformed vegetation index; PRI, photochemical reflectance index; GNDVI, green normalized difference vegetation index; VARI, visible atmospherically resistant index; DVI, difference vegetation index; RDVI, renormalized difference vegetation index; PDW, plant dry weight; R², linear coefficient of determination; SEEy, standard error of estimate for y.
Abstract

Reflectance data obtained throughout the growing season may be related with soybean growth, health, and quantity and quality of yield. Two field experiments with 995 and 613 (2 x 3 m) quadrats were planted with SCN-susceptible cultivars from 2000 to 2002. Ground-based reflectance data from soybean canopies were obtained for each quadrat every 4 to 15 days throughout the growing seasons using a handheld, multispectral radiometer. Soybean cyst nematode population densities were assessed for each quadrat prior to planting and after harvest of each season. The quality and quantity of the soybean grain produced in each quadrat was determined. The relationships among reflectance data, SCN population densities, and quantity and quality of soybean grain were investigated using regressions. The best relationships ($R^2$ up to 0.8) between reflectance data and quantity of soybean yield occurred with reflectance data obtained late August to early September. During this time, the best relationships between reflectance data and seed size ($R^2$ up to 0.57) also occurred.

Relationships among seed size, seed protein content, and seed oil content with percentage reflectance data varied between experiments and among growing seasons. The best relationships between seed protein content and percentage reflectance data ($R^2$ up to 0.53) occurred with reflectance data from early August to mid September. There was not a specific period in which the best relationship between seed oil content and percentage reflectance data occurred, and the maximum $R^2$ value observed for these relationships was 0.49. The variation in SCN population densities was best described by the variation in percentage reflectance data obtained very early or very late in the season. It has yet to be determined how reflectance from soil early in the season and/or from the senescing soybean foliage and soil late in the season are related to SCN population densities.
Introduction

Soybean, *Glycine max* (L.) Merr., is a major source of vegetable oil produced worldwide (Wilcox, 2004), and soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is one of the principal causes of soybean yield losses in the world. Economic losses caused by SCN surpass US$ 1 billion in the United States annually (Wrather et al., 2001), and yield reductions can occur even in the absence of noticeable symptoms (Niblack et al., 1991; Wang et al., 2003; Young, 1996). The economic importance of soybean crop, the large amount of agricultural land occupied with this crop throughout the world (Wilcox, 2004), and the difficulties of visually determining the impact of SCN on soybean yield makes this pathosystem appropriate for study by means of remote sensing technologies.

Precision agriculture pre-supposes that any yield-limiting factor must be detected early and that viable control practice can be deployed before yield losses exceed an economic threshold (Seelan et al., 2003). Optimizing the use of agronomic inputs in time and space, precision agriculture needs tools and techniques that facilitate site-specific management of crops (Pinter et al., 2003; Seelan et al., 2003). Remote sensing technologies that measure the percentage of sunlight reflected from soybean canopies may provide a means to obtain timely and accurate disease diagnoses and the means to differentiate and quantify plant stresses. Furthermore, reflectance measurements obtained from soybean canopies at different wavelength bands may indicate the presence of a plant stress, even when the stress cannot be visually detected. Geographic information systems (GIS) allow displaying geo-referenced variables into maps. In this way, geo-referenced remote sensing data can be processed through GIS to provide spatial information about crop conditions that may be effective to site-specific management of crops. Additionally, mapping reflectance data obtained from soybean canopies may be used to provide early estimates of soybean yield.

To be incorporated in the decision-making process to optimize agricultural, economical, and ecological results, precision farming demands high-quality remote sensing
data to be obtained in a timely fashion and to be related to plant physiological status and crop yield (Pinter et al., 2003; Seelan et al., 2003). Ground-based remote sensing can provide the high-resolution data that are required to implement site-specific management programs required for precision farming. If remote sensing is to be automatically incorporated to the decision-making process the data collected need to remain stable across different assessment dates and be strongly related to plant growth and yield.

Jackson published a review on use of remote sensing to detect plant stress (Jackson, 1986), and several other researchers have addressed the use of these technologies to detect biotic (Carver and Griffiths, 1981; Gausman et al., 1975; Nutter, 1989; Nutter et al., 1990) and abiotic (Adams et al., 2000; Carver and Griffiths, 1981; Dale et al., 1982; Hansen and Schjoerring, 2003; Penuelas, 1998) plant stresses. Advantageously, these methods of assessing plant-health status can provide fast and reliable biological information with minimal disturbance of the crop (Nutter, 1989; Nutter et al., 1990). However, differences in atmospheric conditions (Liu and Huete, 1995; Xiao et al., 2003) and in soil characteristics (Gilabert et al., 2002; Haverkort et al., 1991; Huete, 1988; Huete et al., 1985; Liu and Huete, 1995; Rondeaux et al., 1996; Weidong et al., 2002) can affect the quality of the remote sensing data. Thus, different authors expressed concern about the limitations of remote sensing techniques in agriculture (Tucker, 1979; Wiegand et al., 1972).

Knowledge of the potential limitations of remote sensing should provide the framework to maximize its usefulness. Radiometers record reflectance data at specific wavelength bands and widths. These specifications can restrict the utility of the reflectance data since different wavelength bands and widths are affected differently by characteristics of the atmosphere, soil, and vegetation (Gitelson et al., 2002; Tucker, 1979). Height of the sensors, canopy type (Daughtry et al., 1982; Guan and Nutter, 2001), incident radiation, presence of water on the leaves (Guan and Nutter, 2001), and cultural practices (Kollenkark et al., 1982) influence the quality of ground-based radiometer data.
Any plant stress causes color and/or morphological changes in plant parts (Jackson, 1986). Infection of soybean roots by SCN causes a reduction in the rate of aboveground plant growth that can result in a reduction in the amount of green leaf area. The amount of green leaf tissue per unit of ground area is known as green leaf area index (GLAI) (Campbell and Madden, 1990). This index can be influenced by one or more plant stresses and often is highly related to yield (Guan and Nutter, 2000). To establish the relationships between yield and GLAI and/or the change in GLAI with respect to time, the critical stages of crop development and frequency of data collection throughout the growing season need to be determined. Estimates of GLAI obtained during pod elongation and seed filling in soybean plants provided an accurate estimate of yield losses in determinate and indeterminate soybean cultivars (Fehr et al., 1981). Maximum soybean yields were achieved when soybean plants reached GLAI of 3.5 - 4.0 during pod formation and pod elongation (Board, 2004; Board et al., 1997). The relationships between soybean growth, light interception, and yield have been previously demonstrated (Adcock et al., 1990; Batista and Rudorff, 1990; Board, 2004), and spectral data have been used to predict developmental stage of soybean (Badhwar, 1985; Henderson and Badhwar, 1984). However, there is a lack of information about the variability and reliability associated with using ground-based remote sensing data to estimate quantitative and qualitative aspects of soybean yield throughout a season.

Usually, radiometers measure reflectance from a target object at several different wavelength bands. These measurements of reflectance can be correlated to some characteristics of the target object (Perry and Lautenschlager, 1984). However, the relationships among individual wavelength bands and the target may differ from each other (Holben et al., 1980). Thus, to assess various characteristics of plant canopies, information contained within single wavelength bands can be used alone or data from different wavelength bands can be empirically combined in different ways to calculate vegetation indices (VI). These indices may be correlated to canopy characteristics, such as leaf area
index, fractional vegetation cover, biomass, and other vegetative conditions (Carlson and Ripley, 1997; Perry and Lautenschlager, 1984). Ratios of reflectance and differences in reflectance between two different wavelength bands form the two major categories of indices, although there are other ways of calculating VI’s.

Different VI’s may have specific relationships with individual canopy characteristics. Soybean leaf area index had a positive linear relationship with the radiance ratio (545 nm/655 nm) and with near infrared reflectance (750 nm) (Kanemasu, 1974). Infrared-to-red radiance ratios were found to have a positive linear relationship with soybean GLAI, soybean fresh biomass, and soybean yield (Batista and Rudorff, 1990; Holben et al., 1980; Kanemasu, 1974). Red radiance was found to be linearly and negatively related to soybean GLAI (Holben et al., 1980; Kanemasu, 1974). However, quadratic equations best described the relationships between soybean dry biomass and soybean leaf area index with red and near infrared reflectance, with near infrared-red radiance ratio, and with a transformed greenness vegetation index (Kollenkark et al., 1982).

Positive, non-linear relationships between the normalized difference vegetation index (NDVI), that is the ratio between the difference and the sum of near infrared and red radiances, and soybean GLAI were found to reach a saturation point at GLAI values between 2 and 3 (Kollenkark et al., 1982). This same VI obtained from soybean canopies during pod elongation and seed filling were positively correlated with soybean grain yield (Ma et al., 1996).

The difference vegetation index (DVI), that is the difference between infrared and red radiances, and the transformed vegetation index (TVI), that is a mathematical transformation of NDVI, were related to grass biomass (Tucker, 1979). The visible atmospheric resistant index (VARI) minimized atmospheric effects in the estimation of vegetation fraction of wheat and corn (Gitelson et al., 2002). The green normalized difference vegetation index (GNDVI), a ratio between the difference and the sum of near infrared and green radiances,
accurately assessed chlorophyll content of plant canopies (Gitelson et al., 1996) and predicted corn yield when assessments were made during midgrain filling (Shanahan et al., 2001). The photochemical reflectance index (PRI), which is the ratio between the difference and the sum of radiances at 531 nm and 570 nm, has been shown to be correlated with radiation use efficiency of several plant species (Gamon et al., 1997).

Distinct VI’s are considered equivalent if they provide the same basis for decisions to be made (Perry and Lautenschlager, 1984). However, few evaluations were done to check equivalence among VI’s used to assess soybean growth and yield.

The main goals of this study were to evaluate the ability of predicting soybean yield using reflectance from different wavelength bands and from different vegetation indices and to study the relationships between of reflectance data and SCN population densities in soybean fields under continuous soybean cultivation.

**Material and Methods**

The research was conducted from 2000 to 2002 in fields with a history of occurrence of SCN. In the first year, one field experiment located at the Iowa State University Woodruff Farm, Ames, IA, was planted with a SCN-susceptible soybean cultivar, AgriPro 1995. In the two subsequent years, a field experiment located at the Iowa State University Bruner Farm was planted in addition to the experiment at the Woodruff Farm. A SCN-susceptible cultivar, AgriPro 1702 RR, was planted in both fields in 2001 and 2002. In the three seasons, a row spacing of 75 cm was used and 30 seeds were planted per meter.

A grid of 995 2 X 3 m quadrats was established in the Woodruff Farm experiment, and a grid of 613 similarly sized quadrats was established in the Bruner Farm experiment. Latitude and longitude values were determined for each quadrat. The exact coordinates of the quadrats were maintained in successive seasons using a Trimble Differential GPS Unit (Trimble, Sunnyvale, CA). Each quadrat had four soybean rows 2 m in length. At harvest, the
two central rows of each quadrat were harvested. The soybean grain was dried at 27 C, then seed moisture and oil and protein content were determined using a Tecator Grain Analyzer model INFRATEC 1229 (TECATOR AB, Hoganas, Sweden) in 2000 and 2001. In 2002, seed moisture was determined by a Dole 400 grain moisture tester (Eaton Corp., Carol Stream, IL), and seed oil and protein analyses were done at the Iowa State University Grain Quality Laboratory using a similar grain analyzer (INFRATEC 1229). Quantity of yield per quadrat was determined and standardized to moisture content of 0.13 g H2O g−1 during the experiments. To assess seed size, weight of 100 seeds was obtained by arbitrarily selecting and weighing 100 seeds per quadrat.

Percentage reflectance from soybean canopies was measured throughout the growing seasons. Percentage reflectance is the percentage of incident sunlight that is reflected by a target object. Within each assessment date, two multispectral, hand-held radiometers (model MRS-87, CROPSCAN, Inc., Rochester, MN) were used to measure percentage reflectance from a sensor height of 3 m above the ground. At this height, percentage reflectance of sunlight from soybean canopies was obtained from a circular area (1.5-m diameter) located at the center of each quadrat. Percentage reflectance was measured at eight wavelength bands with midpoint values of 460, 510, 560, 610, 660, 710, 760, and 810 nm. The bandwidths for these wavelength bands were of 27.0, 32.3, 25.0, 26.9, 25.5, 32.9, 28.0, and 31.7 nm, respectively. Percentage reflectance measurements within each quadrat obtained from both radiometers were averaged. Assessments were made under cloudless sky conditions between 1100 and 1500 hours CST (Guan and Nutter, 2001). Planting date, assessment dates, and harvest date for the three seasons are presented in Table 1. At each assessment date, five quadrats were arbitrarily selected from which soybean growth stage was assessed.

During the growing season, each quadrat in the fields was visually inspected every seven to fifteen days, and plants showing disease symptoms were identified. Disease intensity per quadrat was recorded as incidence and/or severity. Incidence and severity were
defined as the proportion of the total number of plants that were expressing disease symptoms and the proportion of plant tissue presenting symptoms of the diseases, respectively. When it was necessary, plant tissue was sampled and observed under the microscope for identification of the pathogens. Quadrats showing iron deficiency chlorosis were rated at the Bruner Farm experiment during the 2002 growing season. The typical symptoms of iron deficiency chlorosis on soybean leaves are characterized by yellowing of interveinal areas of young leaves (McGlamery and Curran, 1989). Iron deficiency chlorosis symptoms were rated using a 0 to 2 scale where 0 is the absence of symptoms in plants within a quadrat, 1 is the presence of iron deficiency chlorosis symptoms in at least one plant within a quadrat, and 2 is the occurrence of foliar symptoms of iron deficiency chlorosis associated with stunting of plants within a quadrat.

Since a particular radiometer model measures reflectance at specific band locations and bandwidths, some of the VI's used in this study are close approximations of previously published indices. The reflectance (R) at different wavelength bands (R subscript) was used to obtain the VI's evaluated in this study. The VI's were:

1- Green, \( R_G = (R_{510} + R_{660})/2 \)
2- Red, \( R_R = (R_{610} + R_{660})/2 \)
3- Near Infrared, \( \text{NIR} = (R_{760} + R_{810})/2 \)
4- Radiance Ratio, \( \text{RR} = \text{NIR}/R_R \) (Tucker, 1979)
5- Normalized Difference Vegetation Index \( \text{NDVI} = (R_{\text{NIR}} - R_R)/(R_{\text{NIR}} + R_R) \) (Holben et al., 1980)
6- Transformed Vegetation Index, \( \text{TVI} = (\text{NDVI}_1 + 0.5)^{0.5} \) (Tucker, 1979)
7- Photochemical Reflectance Index, \( \text{PRI} = (R_{510} - R_{570})/(R_{510} + R_{570}) \) (Gamon et al., 1997)
8- Green Normalized Difference Vegetation Index, \( \text{GNDVI} = (R_{\text{NIR}} - R_G)/(R_{\text{NIR}} + R_G) \) (Gitelson et al., 1996)
9. Visible Atmospherically Resistant Index, VARI = \( \frac{(R_{560} - R_{660})}{(R_{560} + R_{660} - R_{440})} \)  
(Gitelson et al., 2002)

10. Difference Vegetation Index, DVI = \( (R_{NIR} - R_R) \)  
(Tucker, 1979)

11. Renormalized Difference Vegetation Index, RDVI = \( (NDVI_1 \cdot DVI_1)^{0.5} \)  
(Roujean and Breon, 1995)

Soybean cyst nematode population densities were determined immediately before planting and after harvest in each experiment each year. Within each quadrat, six soil cores (2 cm diameter and 15 – 20 cm deep) were collected in a zigzag pattern, 10 cm apart, from the two central rows, and the cores were bulked. SCN cysts were extracted from 100 cm\(^3\) subsamples using a semi-automatic elutriator (Byrd et al., 1976). Using a drill press with a shaft-mounted rubber stopper rotating at 2340 rpm, SCN eggs were extracted from the cysts by crushing the cysts on a 250-µm-pore diameter sieve (Faghihi and Ferris, 2000). The eggs were recovered on a 25-µm-pore diameter sieve that was mounted under a 75-µm-pore diameter sieve. The extracted SCN eggs were stained with acid fuchsin (Niblack et al., 1993), eggs were counted under a dissecting microscope at 50x magnification, and the resultant egg count was used to calculate the number of eggs present in a 100 cm\(^3\) sample of soil.

Maps of the SCN population densities, canopy reflectance, and soybean yield were created using ArcGIS 8.0 (ESRI, Redlands, CA). Regression analyses were performed to quantify describe the relationships among SCN population densities, soybean yield, and percentage reflectance data from each soybean quadrat canopy (individual wavelength bands and vegetation indices) using SAS (SAS Institute Inc. Cary, NC), S-Plus (Mathsoft, Inc., Cambridge, MA), and Sigma Plot (SPSS, Inc., Chicago, IL). For the regressions of percentage reflectance data on SCN population densities, logarithmic transformations \((\log_{10})\) of SCN population densities were done. The coefficients of determination for the relationships among percentage reflectance data, soybean yield quantity and quality, and
SCN population densities reported in our results were obtained from regressions that had P values equal to or lower than 0.05.

**Results**

There were few foliar diseases observed on soybean plants in both fields from 2000 to 2002. At the Woodruff Farm experiment, *Cercospora* sp. and *Septoria* sp. were observed infecting soybean plants at low disease incidence (< 4.0%) and severity (< 6.0%) levels. A general yellowing of the leaves was observed across the Woodruff Farm experimental field during drought periods; however, plants recovered after rain. At the Bruner Farm experiment, *Cercospora* sp. was observed infecting soybean plants at low incidence (~3%) and severity (< 2%) levels. Typical symptoms of iron deficiency chlorosis on leaves were observed at the northwestern and northern areas of the Bruner Farm experiment in both years. Coincidently, the SCN population densities were the greatest in these areas where symptoms of iron deficiency chlorosis were visible in soybean plants at the Bruner Farm experiment (data not shown).

Percentage reflectance from soybean canopies at 760 nm and at 810 nm had the best linear relationships with soybean yield at the Woodruff Farm experiment in 2000 and for both the Woodruff and Bruner Farm experiments in 2001 and 2002. For these same experiments, the relationships between percentage reflectance at 710 nm and yield had the lowest coefficients of determination ($R^2$). Among the VI’s, NIR, RR, DVI, NDVI, TVI, and GNDVI had better linear relationships with soybean yield for all seasons and locations than $R_G$, $R_R$, PRI, and VARI. For each experiment, coefficients of determination for the best relationships among quantity of yield, percentage reflectance for individual wavelength bands, and specific VI in each season are shown (Tables 2 and 3).

Coefficients of determination for the linear relationships between the vegetation indices NIR, RR, RDVI, and GNDVI and yield throughout the seasons are shown for both
experiments (Figs. 1 and 2). In general, $R^2$ values tended to increase from the beginning of
the season and reached a maximum value at late August or early September in both
experiments except at the Woodruff Farm experiment in 2002, where lodging of soybean
plants occurred after mid July.

Throughout growing seasons, from early season to late August / early September,
relationships between reflectance and yield deteriorated in some periods. However in two
instances, at the Bruner Farm experiment in 2001 and at the Woodruff Farm experiment in
2002, deterioration of the relationships between yield and reflectance was not similar among
different indices. In these two instances, the GNDVI-yield relationships were less affected
than other VI-yield relationships (Figs. 1 and 2).

Spatial similarities between yield and reflectance can be presented in maps if
geographic coordinates of quadrats from where reflectance and yield data are obtained are
known. Yield maps and maps of the vegetation indices with the best linear relationships with
yield at the best assessment date in each season for the Woodruff and Bruner Farm
experiments are shown in Figs. 3, 4, 5, 6, and 7.

The relationships among the seed protein content, seed oil content, seed size, and
percentage reflectance were assessed at both experimental fields throughout the seasons. The
best relationships between percentage reflectance data and seed protein content occurred for
both the Woodruff and Bruner Farm experiments during the 2001 growing season (Fig. 8).
The relationships between seed protein content and percentage reflectance data had the
highest $R^2$ with reflectance data obtained between the months of August and September (Fig.
8). Vegetation indices had better relationships with seed protein content than single-
wavelength-band reflectance data (Fig. 8). Variation of NDVI and TVI explained 21.2% of
the variation in seed protein content in the Woodruff Farm experiment with reflectance data
from 9 August 2000 (Fig. 8). The coefficient of determination between GNDVI and seed
protein content was 0.31 in the Woodruff Farm experiment on 7 August 2001. Relationships
among NDVI, TVI, and seed protein content had an $R^2$ of 0.53 in the Bruner Farm experiment with reflectance data from 11 September 2001 (Fig. 8).

In 2002, the relationships between reflectance and seed protein were variable among different assessment dates, and the variation in NDVI and TVI on 29 August explained 14.6% of the variation in soybean protein in the Woodruff Farm experiment (Fig. 8). Variation on reflectance at 710 nm on 28 June explained 18.9% of the variation in soybean protein (Fig. 8). Maps of percentage reflectance data and seed protein content were made for the date in which the relationship between percentage reflectance data and seed protein content had the highest $R^2$ (Figs. 9 and 10).

The relationships between seed oil content and percentage reflectance data among different assessment dates in the Woodruff Farm experiment from 2000 to 2002 were variable. For this experiment, the best relationships between seed oil content and percentage reflectance had $R^2$ lower than 0.1 in 2000 and 2002, and a $R^2$ of 0.27 was obtained for reflectance data from 15 June 2002 (data not shown). For the Bruner Farm experiment, the best relationships between seed oil content and reflectance had $R^2$ of 0.49 and 0.48 for percentage reflectance at 660 nm and $R_r$, respectively, from measurements of reflectance obtained on 4 September 2001 (Fig. 11). During the 2002 growing season, significant relationships between seed oil content and reflectance were observed earlier in the season than in 2001, and reflectance at 710 nm measured 28 June presented the highest $R^2$, 0.26 (Fig. 11). Maps of seed-oil content and percentage reflectance data were made for the Bruner Farm experiment for dates when the best relationships between reflectance and seed oil were determined (Fig. 12).

The highest $R^2$ between seed size and percentage reflectance data occurred for measurements of reflectance obtained late in soybean growing season, late August to early September, in both experiments and seasons (Fig. 13). In 2001 and 2002, $R^2$ values were higher for the Woodruff Farm experiment than for the Bruner Farm experiment (Fig 13). At
the Woodruff Farm experiment, the variation of seed size was best described by the variation in GNDVI obtained at 8 September 2000, 12 September 2001, and 29 July 2002 with $R^2$ of 0.58, 0.46, and 0.48, respectively (Fig. 13). At the Bruner Farm experiment, the relationships among DVI, TVI, and seed size had $R^2$ of 0.36 and 0.40 for measurements of reflectance obtained on 4 September 2001 and on 12 September 2002, respectively. For this same experiment, the relationships between seed size and percentage reflectance for the 710 nm wavelength band measured on 11 September 2001 had $R^2$ of 0.37. Maps of percentage reflectance data obtained for the assessment date in which the relationships between percentage reflectance data and seed size had the highest $R^2$ were made for the Woodruff and Bruner Farm experiments (Figs. 14 and 15).

For the two experiments, percentage reflectance data had better relationships with SCN densities obtained at planting than at harvest in four out of five experimental location-year combinations. Most of the best relationships between percentage reflectance data from soybean canopies and SCN population densities at planting were found very early or very late in the growing seasons. For the Woodruff Farm experiment, the best relationships were found early in the season for percentage reflectance at 810 nm ($R^2 = 0.27$ on 8 June 2001) and for the NIR index ($R^2 = 0.25$ on 30 May 2002). However, for this same experiment, the best linear relationships between SCN population densities at planting and percentage reflectance data were obtained for the percentage reflectance at 810 nm during mid season ($R^2 = 0.16$ on 13 July 2000) (Fig. 16 and 18). For the Bruner Farm experiment, the best relationships between percentage reflectance data and SCN population densities at planting occurred late in the season for RR ($R^2 = 0.13$ on 28 August 2001) and for NDVI and TVI ($R^2 = 0.27$ on 12 September 2002) (Figs 17 and 19).

At the Woodruff Farm experiment, reflectance data that best described the variation in SCN population densities at harvest were: 710 nm ($R^2 = 0.19$ on 13 July 2000), 510 nm ($R^2 = 0.11$ on 8 June 2001), and DVI ($R^2 = 0.23$ on 15 June 2002) (data not shown). At the
Bruner Farm experiment, the highest $R^2$ obtained for the relationships between percentage reflectance data and SCN population density at harvest was obtained for the RR index ($R^2 = 0.12$ on 11 September 2002) (data not shown).

**Discussion**

The relationships between soybean yield and percentage reflectance data from soybean canopies improved for measurements of reflectance obtained from May to late August / early September, and deteriorated when reflectance was measured after this period. The only exception to this yield-reflectance-relationship pattern was observed at the Woodruff Farm experiment in 2002. During this year, the relationships between yield and percentage reflectance data obtained from mid July to early August 2002 deteriorated. Relationships between measurements of reflectance data obtained late August / early September 2000 and yield improved. However, the reflectance-yield relationships were weaker in the Woodruff Farm experiment in 2002 than the ones observed in previous seasons for the experiment. In this experiment, it was observed that the soybean plants lodged beginning early July 2002. For other crops, lodging negatively affected the relationships between percentage reflectance of infrared wavelength bands and GLAI (Haverkort et al., 1991), and GLAI can be highly related to yield (Guan and Nutter, 2000). Since infrared reflectance is part of most VI evaluated in our study, the relationships among those indices, GLAI, and yield probably deteriorated for the Woodruff Farm experiment after mid July 2002. It is important to note that the GNDVI that is calculated from the green and near infrared wavelength bands was less affected in the ability to predict yield when lodging occurred than the other indices.

The relationships between grain yield and reflectance increased for measurements of reflectance obtained from early season to late August. However, in some assessments of reflectance during this period, the relationships deteriorated without any identifiable reason.
Future research should focus on identifying factors that negatively affect the usefulness of percentage reflectance data to predict soybean yield.

The best relationships between reflectance data and yield were obtained in 2000. However, the results obtained from 2001 and 2002 may not be directly comparable to results obtained in 2000 because the soybean cultivar grown in 2000 was different from the cultivar grown in 2001 and 2002. However, the trends observed here showed that for the two cultivars planted in the two different experimental fields, the same phenomenon involving the development of relationships between percentage reflectance data and yield was observed. Additional experiments under different conditions, such as different row spacing and soil backgrounds, are important and will need to be conducted to produce results broadly applicable. Nonetheless, one major outcome our study is that ground-based percentage reflectance data can be used to predict soybean yield of a soybean field one month before harvest. Such information will be valuable for farmers because they will be able to identify yield limiting factors in their fields prior harvest. These yield predictive capabilities also will be valuable for making marketing decisions concerning sale of soybean grain.

In addition to predicting quantity of yield, ground-based remote sensing measuring percentage reflectance is also adequate to assess soybean quality traits, such as protein and, oil content, and seed size. The best relationships between percentage reflectance data and seed protein were obtained for reflectance data obtained in the period between early August and early September, although the magnitude of the relationships varied in place and time. In contrast, it was not possible to identify a specific period for measuring reflectance from soybean canopies where the relationships between percentage reflectance data and seed oil content were the best; however it was possible to verify that the lowest seed oil content occurred in the same areas SCN population densities were the highest in the Bruner Farm experiment. At these same areas, iron deficiency chlorosis also was observed.
The relationships between percentage reflectance data and seed size were the best for measurements of reflectance obtained late in soybean seasons, late August /early September, in both experimental fields from 2000 to 2002. This period coincided with the best period for predicting yield using reflectance data. Then, percentage reflectance data obtained during the months of August and September showed to be very important to predict quantity and quality of soybean yield.

Initial SCN population densities and reflectance data were best related when reflectance data was obtained very early or very late in the season. Soil has a greater influence on the total reflectance obtained for each quadrat early in the soybean season than in the middle of the season, when foliage completely covers the surface area of the quadrats. Thus, the relationships between reflectance and SCN population densities at planting observed at the Woodruff Farm experiment may somewhat represent the relationships between SCN populations and soil characteristics. Early senescence of SCN-infected soybean plants can explain the relationships between reflectance measurements obtained late in the season and SCN population densities measured at planting. In this case, besides level of SCN infection, i.e. multiple infections that can occur in soybean roots, time of infection can be a factor affecting early senescence of soybean plants.

It was not possible to identify the type of reflectance data that had the best relationship with SCN population densities. The percentage reflectance for individual wavelength bands and VI that best described the variation in SCN population densities varied between experimental fields. While percentage reflectance in the near infrared spectrum (769 and 810 nm) had the best relationships with SCN population densities at planting for the Woodruff Farm experiment, RR and NDVI had the best relationships with SCN population densities at planting for the Bruner Farm experiment. Soybean plants infected by SCN may senesce early and this difference in plant development may explain the improvement in the SCN-reflectance relationships at the end of the season. Thus, the effects SCN can have on
soybean growth also could affect reflectance data, and these possible effects should be considered in addition to the effects that soil background may have on percentage reflectance data when the best relationships between percentage reflectance data and SCN population densities at planting were found at the end of the growing season.

We did not find any single wavelength band or VI to be the best for assessing SCN population densities at planting or soybean yield quantity and quality. The wavelength bands and VI's that provide the best relationships with SCN population densities at planting and yield quantity or quality varied between the two experimental fields and/or among growing seasons. However, some of the relationships among different types of reflectance data, SCN population densities at planting, and soybean yield had similar pattern of variation within seasons. These similarities may be explained by the fact that most of the indices were mathematical combinations of percent reflectance of wavelength bands in the visible spectrum (most of times red or green) and percentage reflectance of wavelength bands in the infrared spectra. The red and green wavelength bands were highly related to each other, and the infrared wavelength bands were highly related also. Thus, it can be expected the DVI, NDVI, TVI, RDVI, and GNDVI had similar reflectance properties. These similarities among wavelength bands and VI's show the potential of having estimation of soybean yield quantity and quality and SCN population densities using different types of percentage reflectance data. GIS tools can be used to map geo-referenced percentage reflectance, soybean yield quantity and quality, and SCN population densities at planting and harvest data. These maps may provide useful information for site-specific management of the crop, for early prediction of soybean yield and for describing the effects plant stresses may have on soybean yield.

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Figure 2. Variation in the linear coefficients of determination \( (R^2) \) of the relationships among near infrared reflectance (NIR), radiance ratio (RR), renormalized difference vegetation index (RDVI), green normalized vegetation index (GNDVI), and yield for the Bruner Farm experiment during 2001 and 2002.

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Figure 13. Coefficients of determination ($R^2$) of the relationships between seed size (100-seed weight) and percentage reflectance data from soybean canopies as percentage reflectance of narrow wavelength bands (A) and as vegetation indices (B) obtained at different assessment dates within growing seasons for the Woodruff and Bruner Farm experiments from 2000 to 2002.

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Figure 17. Coefficients of determination ($R^2$) of the relationships between SCN population densities at planting and percentage reflectance data from soybean canopies as
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Table 1. Dates of planting, reflectance assessments, and harvest at Woodruff and Bruner Farm experiments in 2000, 2001, and 2002.

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† On these dates, the reflectance measurements were obtained from just one radiometer.
Table 2. Coefficient of determination ($R^2$) of the linear relationships between percentage reflectance for individual wavelength bands and yield for the assessment date within a season in which the best relationships between remote sensing data and yield were found for the Woodruff and Bruner Farm experiments from 2000 to 2002.

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† $R_x$, percentage reflectance for the $x$ wavelength band.
‡ All the $R^2$ values were significant at 0.05 level of probability.
Table 3. Coefficient of determination ($R^2$) of the linear relationships between vegetation indices and yield for the assessment date within a season in which the best relationships between remote sensing data and yield were found for the Woodruff and Bruner Farm experiments from 2000 to 2002.

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$\dagger$ Vegetation indices: R$_G$, green reflectance; R$_R$, red reflectance; NIR, near infrared reflectance; RR, radiance ratio; DVI, difference vegetation index; NDVI, normalized difference vegetation index; TVI, transformed vegetation index; RDVI, renormalized difference vegetation index; PRI, photochemical reflectance index; GNDVI, green normalized difference vegetation index; VARI, visible atmospherically resistant index.

$\dagger$ all $R^2$ values were significant at 0.05 level of probability.
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CHAPTER 4

USE OF GEOGRAPHIC INFORMATION SYSTEMS AND SPATIAL STATISTICS TO ASSESS SOYBEAN CYST NEMATODE POPULATION DYNAMICS IN SOYBEAN FIELDS

A paper to be submitted to Crop Science

A. J. de A. Moreira, F. W. Nutter, Jr, and G. L. Tylka*. 1

1 Department of Plant Pathology, Iowa State University, Ames, IA 50010. First author is sponsored by CNPq – Brazil. *Corresponding author (gltylka@iastate.edu).

2 Abbreviations: GIS, geographic information systems; SCN, soybean cyst nematode; Pi, SCN population density at planting, Pf, SCN population density at harvest, t, event that occurs within a season, t+1, event that occurs next season: R², linear coefficient of determination; SEEy, standard error of estimate for y.
Abstract

Spatial statistics and geographic information systems (GIS) were useful in assessing spatial attributes of soybean cyst nematode (SCN) population densities in soybean fields. Additionally, spatial statistics, statistic regression, and correlation analyses coupled with GIS were important to quantify and to visualize the impact of SCN on quantity and quality of soybean yield. Experiments were conducted in two fields (with 995 and 613 quadrats) from 2000 to 2003. From each quadrat, SCN population density was assessed twice per year (planting and harvest) and soybean yield (total yield, 100-seed weight, and seed protein and oil contents) was obtained. Variogram models described the spatial processes involving geo-referenced SCN population densities. Limits of spatial structure (ranges) and variations in spatial dependency (partial sill to sill ratio) varied within and between seasons and were possibly affected by environmental factors. Also, changes in SCN population densities were possibly affected by environmental factors within and between soybean seasons, and the carrying capacity of soybean fields varied among growing seasons. Coefficients of determination ($R^2$) for the relationships of within-season changes in SCN population densities ($Pf/Pi$) and SCN population densities at planting ($Pi$) were greater in 2000 and 2001 than in 2002. Climatological data indicated that excessive moisture and high temperatures might have restricted SCN population density increases in both experimental fields in the 2002 growing season. Overwinter changes in SCN population densities were related to SCN population densities obtained at planting time in the next season. There was a significant negative linear relationship between log-transformed SCN population densities at planting and soybean yield. Yield reduction could be partially explained by the reduction in size of the soybean seeds (100-seed weight). Also, SCN infection caused an increase in seed protein content and a decrease in seed oil content. In one experiment, high SCN population densities were found in the same area in which soybean plants had symptoms of iron deficiency chlorosis. In that area, soybean yield was quantitatively and qualitatively affected; however
more research needs to be carried out to understand how SCN population densities and iron deficiency interact to cause yield losses.

**Introduction**

Soybean \([Glycine\ max\ (L.)\ Merr.]\) is the most important oil crop and a major source of protein in the world (USDA, 2003). It is largely cultivated in all continents where agriculture is practiced. The United States, Brazil, Argentina, China, and India are the primary soybean-producing countries (Wilcox, 2004). Several biotic and abiotic factors cause soybean yield losses, among them soybean cyst nematode \(Heterodera\ glycines\) Ichinohe), SCN. The known distribution of the pathogen has followed soybean expansion towards new agricultural lands (Noel, 1995), and the nematode has become one of the principal causes of soybean yield losses in the world (Wrather et al., 2001b). Economic reductions in production due to SCN surpass US $1 billion in the United States annually (Wrather et al., 2001a). Yield losses can be severe, even in the absence of noticeable symptoms (Niblack et al., 1991; Wang et al., 2003; Young, 1996).

To understand the impact that SCN can have on soybean yield, it is necessary first to understand SCN population dynamics within a growing season and how SCN population dynamics are related to the quantity and quality of soybean yield. Identification and understanding of ecological conditions that reduce reproduction and augment mortality rates of SCN can possibly lead to development of new management tactics. Considering one crop season as unit of time \(t\), within-season events occur in time \(t\) and next-season events happen in time \(t+1\). The ratio between SCN population densities obtained at harvest \((P_f)\) and at planting \((P_i)\) can be defined as within-season change in SCN population densities \((P_f/P_i)\). The ratio between the SCN population density obtained at next planting \((P_{i(t+1)})\) and SCN population density obtained harvest \((P_f)\) characterizes the overwinter changes in SCN.
population densities and can be defined as overwinter SCN survival ($\frac{Pi(t+1)}{Pi(t)}$) (Todd et al., 2003).

Boag (1989) hypothesized that factors affecting population densities of plant-parasitic nematodes could be classified into three categories: those that are perfectly density dependent (intraspecific competition), those that are imperfectly density dependent (interspecific competition, parasitism, and predation), and those that are density independent (abiotic conditions). These three types of factors can influence SCN population density within soybean seasons. However, it is hypothesized that only imperfectly density-dependent and density-independent factors affect SCN populations over winter. During this period, the lack of a susceptible host and the effect of extreme weather conditions, particularly low temperatures and/or low soil moisture, inhibit biological activities of this obligate parasite (Young, 1995). Both competition for limited resources and the presence of antagonist organisms in tomato fields were responsible for density-dependent reproductive and survival rates of *Meloidogyne incognita* (Kofoid and White) Chitwood (Ferris, 1985). *Meloidogyne incognita* suppressed population densities of SCN second-stage juveniles (J2) infecting soybean roots at low SCN egg population densities (Niblack et al., 1986). Changes in SCN population densities that occur within and between seasons were density dependent in SCN-infested fields when mixtures of susceptible and resistant soybean cultivars were planted (Wallace et al., 1995) and when different cropping sequences with SCN-nonhost crops, SCN-susceptible, and SCN-resistant soybean cultivars were deployed (Francl and Dropkin, 1986; Todd et al., 2003; Wang et al., 2000). The genetic compatibility between SCN and its hosts is one of the most important factors affecting reproduction of the nematode (Chen et al., 2001b; Faghihi et al., 1986; Sipes, 1995; Wang et al., 2000). Two SCN-resistant cultivars with different sources of SCN-resistance genes deployed in different rotations did not reduce SCN population densities at harvest by the end of the five-year experimental period (Francl and Wrather, 1987).
Factors controlling reproduction and survival rates in SCN populations vary in time and space. However, it is important to identify ecological factors that suppress nematode development and reproduction and increase its mortality rate. Environmental factors, such as soil temperature, soil pH, and soil fertility, may affect SCN development and reproduction. Infection of soybean roots by SCN juveniles does not occur at soil temperatures below 17 C (Alston and Schmitt, 1987). The ideal temperature range for SCN female development is from 20 to 28 C (Melton et al., 1986), with 26 C being the optimum temperature for cyst production (Anand et al., 1995). Numbers of mature females were positively correlated with soil pH (Anand et al., 1995; Francl, 1993) and with soil magnesium (Francl, 1993). Moreover, population densities of SCN cysts and eggs in the fall were negatively correlated with copper concentration (Francl, 1993). Soil texture characteristics may affect soil moisture content and atmosphere composition in the soybean rizosphere, thereby affecting SCN biological activities. Reproduction of SCN tended to be higher in coarse soil textures than in fine soil textures (Koenning and Barker, 1995), and high clay content in the lower horizons (15 – 45 cm) of soil profiles limited vertical growth of soybean roots and, thus, limited reproduction of SCN (Alston and Schmitt, 1987). It was shown that SCN cyst population densities could be predicted in some fields by the sand, silt, and clay content of the soil (Avendano et al., 2004).

It is important to verify if cultural practices affect soil ecological characteristics that negatively influence SCN population dynamics. Soybean row spacing of 25 and 75 cm did not affect reproductive rates and SCN population densities at harvest in a soybean/corn cropping system (Chen et al., 2001a). Soybean cyst nematode reproduction in no-tillage production was reported to be equal to (Chen et al., 2001a) or greater (Noel and Wax, 2003) than reproduction in conventional soybean tillage production systems. However, SCN population densities were lower in no-tilled compared to tilled fields for soil textures with clay content equal to or greater than silt clay loam (Workneh et al., 1999). This result showed
the importance of considering the effects of the interactions of tillage practices and soil textures on SCN population densities.

Initial SCN population densities have been shown to have a negative relationship with soybean yield (Francl and Dropkin, 1986; Koenning and Barker, 1995), and the yield benefit of planting resistant instead of susceptible cultivars in SCN-infested fields has been shown to be linear and positively related to initial SCN population densities (Chen et al., 2001b). However, yield losses in SCN-susceptible cultivars decreased when root infection was delayed from 2 to 6 weeks (Wrather and Anand, 1988). Also, differences in yield of resistant and susceptible soybean cultivars with comparable yield potentials decreased as the sand content of the soils decreased in infested fields (Koenning et al., 1988). For these soils, final SCN population densities were negatively correlated with sand content (Koenning et al., 1988).

Knowledge about how SCN population dynamics affect soybean yield is very important for evaluating the efficacy of SCN management strategies and tactics. A number of studies have been conducted to quantify the relationship between SCN population density and soybean yield, but these studies did not show how these relationships are spatially distributed within soybean fields or how SCN population dynamics affect soybean yield quantitatively and qualitatively. Likewise, although previous studies evaluated both reproduction and survival of SCN, they did not examine or consider how these phenomena are spatially distributed in the fields. By sampling, estimating, and mapping SCN population densities in small quadrats for entire experimental areas, it should be possible to identify and characterize ecological factors affecting SCN population dynamics within and among seasons.

Geographic information systems (GIS) offer the opportunity to generate maps of spatially referenced data. The tools present in GIS allow us to map and visualize how SCN population densities change in time and space (Nutter et al., 2002). Additionally, GIS provide
means to visualize the impact of SCN population densities and other factors on quantity and quality of soybean yield, thus GIS may provide geographic information to be utilized in site-specific management of soybean fields.

Spatial statistics can be used to determine how SCN populations are spatially structured. Mapping SCN population densities at planting and at harvest over several seasons and determining seasonal changes in spatially defined SCN populations may elucidate the role of specific environmental factors affecting these populations. The knowledge of how ecological factors impact SCN population densities may lead to development of new and more effective management practices to control this pathogen. Additionally, the identification of areas within fields where SCN population densities have been suppressed can direct the search for antagonist organisms for possible use as biological control agents and/or the manipulation of environmental factors that optimize the efficacy of SCN management programs.

Spatial statistics have been shown to provide means to assess the spatial structure of nematode populations (Avendano et al., 2003; Avendano et al., 2004; Donald et al., 1999; Evans et al., 2002; Farias et al., 2002; Gavassoni et al., 2001; Morgan et al., 2002). But errors in the determination of spring population densities and the difficulties related to prediction of population dynamics of the potato cyst nematodes (PCN), *Globodera* spp., limited the development of site-specific management practices to control these nematodes (Evans et al., 2002). Other limitations to adoption of site-specific management include high sampling costs, inefficient soil extraction methods, and lack of knowledge concerning how both biotic and abiotic factors affect soybean yield in SCN-infested fields (Donald et al., 1999). New sampling strategies have been discussed (Melakeberhan, 2002), and a nested-sampling design has been proposed to assess the spatial distribution of SCN at an affordable cost (Avendano et al., 2003).
Aboveground symptoms of SCN infection in soybeans include stunting and chlorosis of plants, often occurring in circular to oval patches that usually follow the direction of tillage (Donald et al., 1999). Spatial statistical analyses have shown that soybean fields infested with *H. glycines* were initially aggregated and that no-tillage and ridge-tillage systems resulted in greater aggregation of *H. glycines* population densities over time compared to conventional and reduced tillage systems (Gavassoni et al., 2001). Although the spatial patterns of SCN distribution in soybean fields have been previously studied, very little is known about spatial patterns of reproduction and survival of the SCN within a field from season to season at a spatial scale that can lead to discovery of new ecological and biological information. The use of GIS to map SCN population densities over time may supply useful information about the ecology of SCN populations within soybean fields.

The main goals of this study were to quantify and map SCN population densities in SCN-infested fields and to discern the relationships between nematode densities and soybean yield. Another goal was to study the spatio-temporal structure of SCN populations under monoculture of a susceptible soybean.

**Materials and Methods**

Field experiments were conducted during the 2000, 2001, and 2002 growing seasons in fields with a history of occurrence of SCN. In the first year, one experiment was conducted on a single field located at the Iowa State University Woodruff Farm, Ames, IA. An SCN-susceptible soybean cultivar, AgriPro 1995, was planted at a seeding rate of 30 seeds per meter. In the two subsequent years, an experiment was conducted at the Iowa State University Bruner Farm in addition to the experiment at the Woodruff Farm. An SCN-susceptible cultivar, AgriPro 1702 RR, was planted in both experiments in 2001 and 2002. In all three seasons, a row spacing of 75 cm was used. The soil was disked and cultivated once
before planting, and soybean rows were planted in an east-to-west orientation. Both farms had clay loam soils, and results of texture and fertility analyses are presented in Table 1.

A grid of 995 (2 X 3 m) quadrats was established at the Woodruff Farm experiment, and a grid of 613 similar-sized quadrats was established at the Bruner Farm experiment. Latitude and longitude values were determined for each quadrat. The exact coordinates of the quadrats were maintained in successive seasons using a Trimble Differential Geographic Positioning System (GPS) Unit (Trimble, Sunnyvale, CA). The quadrats in the grid formed a checkerboard design. Each quadrat had four soybean rows 2 m in length, and the two central rows were mechanically harvested.

After harvest, soybean seeds were dried at 27 C and weighed, then seed moisture, oil, and protein were determined using a Tecator Grain Analyzer, model Infratec 1229 (Tecator AB, Hoganas, Sweden) in 2000 and 2001. In 2002, yield per quadrat was determined and standardized to a moisture content of 13% using a Dole 400 (Eaton Corp., Carol stream, IL) grain moisture tester, and seed oil and seed protein analyses were conducted at the Iowa State University Grain Quality Laboratory with a Infratec 1229 grain analyzer. Weight of 100 seeds was obtained by arbitrarily selecting and weighing 100 seeds per quadrat from 2000 to 2002.

During the growing season, each quadrat in each field was visually inspected every seven to fifteen days, and plants showing disease symptoms were identified. Disease intensity per quadrat was recorded as incidence and/or severity. Incidence and severity were defined as the proportion of the total number of plants that were expressing disease symptoms and the proportion of plant tissue presenting symptoms of the diseases, respectively. When it was necessary, plant tissue was sampled and observed under the microscope to identification of the pathogens. Quadrats showing iron deficiency chlorosis were rated at the Bruner Farm experiment during the 2002 growing season. The typical symptoms of iron deficiency chlorosis on soybean leaves are characterized by yellowing of
interveinal areas of young leaves (McGlamery and Curran, 1989). Iron deficiency chlorosis symptoms were rated using a 0 to 2 scale, where 0 is the absence of symptoms in plants within a quadrat, 1 is the presence of iron deficiency chlorosis symptoms in at least one plant within a quadrat, and 2 is the occurrence of foliar symptoms of iron deficiency chlorosis associated with stunting of plants within a quadrat. Map of the distribution of plants with symptoms of iron deficiency chlorosis were generated using ArcGIS 8.0 software (ESRI, Redlands, CA).

Soybean cyst nematode population densities were determined immediately before planting and after harvest in each experiment each year. Within each quadrat, six soil cores (2 cm diameter and 15 – 20 cm deep) were collected in a zigzag pattern, 10 cm apart from the two central rows, and were bulked. SCN cysts were extracted from 100 cm$^3$ soil samples using a semi-automatic elutriator (Byrd et al., 1976). Using a drill press with a shaft-mounted rubber stopper rotating at 2340 rpm, SCN eggs were extracted from the cysts by crushing the cysts on a 250-μm-pore diameter sieve. The eggs were recovered on a 25-μm-pore diameter sieve that was mounted under a 75-μm-pore diameter sieve (Faghihi and Ferris, 2000). The extracted SCN eggs were stained with acid fuchsin (Niblack et al., 1993), eggs were counted under a dissecting microscope at 50x magnification, and the result of the egg count was used to calculate the number of eggs present in a 100 cm$^3$ sample with of soil.

Data collection of soybean cyst nematode population densities, changes in SCN population densities, soybean yield were obtained for each quadrat in the fields. Maps were created using the geographic coordinates of the quadrats to geo-reference the variables of interest in ArcGIS 8.0. Within a field, minimum and maximum values of SCN population densities obtained, as well as mean, median, variance, and skewness of SCN population density distributions were calculated in S-Plus (Mathsoft, Inc., Cambridge, MA) for each assessment date of SCN population densities.
Spatial structure in SCN population density data was determined for each assessment made of SCN population densities at the Woodruff and Bruner Farm experiments. Analyses of the datasets, histograms, scatter-plots where the quantiles of two distributions were plotted against each other (normal QQ plots), and trend analyses were conducted using a geostatistical extension of ArcGIS. Trends in the dataset can be removed and then the remaining residuals can be modeled (Webster and Oliver, 2001). After performing trend analyses of SCN population density data, trends were removed if they were detected and the residuals were modeled. In a spatial process, the limits of the spatial structure and the intensity of spatial dependence can be described using variograms. Variograms are functions that relate the variance of the difference between variables that are separated by a given distance with the distance that separate them (Oliver and Webster, 1990; Webster and Oliver, 2001).

Variograms are functions with which the dependent variable is the variance of the differences between variables separated by a given distance and the independent variable is the distance that separate these variables (Cressie, 1991). A variogram has some basic parameters that can be used to describe it: range, sill, and nugget (Cressie, 1991; Johnston et al., 2001; Webster and Oliver, 2001). Range is the maximum distance within which spatial dependence is observed, and places beyond the range are spatially independent (Johnston et al., 2001; Webster and Oliver, 2001). Sill (S) is the maximum variance that occurs when the range is reached (Webster and Oliver, 2001). Theoretically, the variance should be zero when the distance that separates two variables is zero. However, variograms usually have positive values of variance that are called nugget when the distance separating two variables is zero. Nugget is caused by measurement error, by spatial dependent variation that exists at distances shorter than the smallest sampling interval, and by spatially uncorrelated variation (Cressie, 1991; Oliver and Webster, 1990). The sill can be described as the sum of the nugget plus the partial sill (PS), with partial sill being the difference between sill variance and nugget variance (Johnston et al., 2001). Within a given range, the higher the partial-sill-to-sill
ratio (PS S$^{-1}$), the higher the spatial dependency in a spatial process (Webster and Oliver, 2001). There are several different variogram models (Cressie, 1991), however the exponential and spherical models are the most used in earth sciences (Oliver and Webster, 1990). For these two models, variance increases with distance until the range is reached.

Directional variograms can be used to check whether isotropy occurs in the spatial process. Isotropy is when changes in spatial dependence in a spatial process are only a function of the distance between locations (Johnston et al., 2001). Biologically, isotropy means that two biological variable of interest will be more similar as the distances that separate these variables decreases, and that the physical process affecting these variables changes uniformly in space (Cressie, 1991). Spatial process in which spatial dependence varies with both distance and direction is called anisotropic process (Webster and Oliver, 2001) and is caused some physical process that changes differentially in space (Cressie, 1991). In an anisotropic process, directional variograms can have different ranges and sills (Webster and Oliver, 2001). Occurrence of anisotropy in the SCN population density datasets was assessed prior to modeling.

Models fitting the SCN population density data were selected based on the mean prediction error, root mean square of the prediction errors, average standard error, and the prediction error maps generated after kriging. Kriging is a method of estimation by local weighted averaging (Oliver and Webster, 1990) or a statistical method of interpolation that uses geographically referenced data to predict values of the same data type in unsampled locations (Johnston et al., 2001).

The relationships among SCN population densities, changes in SCN population densities within and between soybean growing seasons, and quantity and quality of soybean yield were described using regressions and correlations in S-Plus (Mathsoft, Inc., Cambridge, MA) and Sigma Plot (SPSS, Inc., Chicago, IL). Logarithmic transformation ($\log_{10} P+1$) of the SCN population densities ($P$) was done prior to the regressions. Scatter plots were
generated to visualize the relationships between variables, and variables were transformed to avoid problems with residuals in linear regressions. To evaluate the linear models, F-statistics and the corresponding probability values (P-values), linear coefficient of determination ($R^2$), and the standard error for estimate of $y$ (SEE$_y$) were considered. Regression lines were compared using the linear general test approach (Neter and Wasserman, 1974).

**Results**

Soybean cyst nematode egg population densities had a right-skewed distribution in all assessments dates (Table 2). The mean and median of SCN egg population densities increased from planting ($\text{Pi}_i$) to harvest ($\text{Pf}_i$) in the 2000 growing season at the Woodruff Farm experiment and in both Woodruff and Bruner Farm experiments in the 2001 growing season. However, mean and median population densities decreased in both experiments within the 2002 growing season (Table 2). The mean and median of SCN population densities decreased over winter, from harvest ($\text{Pf}_i$) to planting of the next year ($\text{Pi}_{(t+1)}$), in both experiments from 2000 to 2003 (Table 2).

Spatial statistics were used to assess spatial structure of SCN egg population densities at planting and at harvest from 2000 to 2003 at the Woodruff and Bruner Farm experiments. Range, sill (partial sill and nugget), nugget, partial-sill-to-sill ratio, and angle of anisotropic ellipse values for the best models obtained at the Woodruff and Bruner Farm experiments at planting and at harvest from 2000 to 2003 are presented in Table 3. Spherical and exponential variogram models best fit the data at the both experiments from 2000 to 2003 (Table 3). Deterministic trends in SCN population density spatial processes were visualized in the preliminary analyses (trend analyses) of harvest 2000 and planting 2003 Woodruff Farm data and were removed using a second-degree polynomial, and then the residuals were modeled. Anisotropy was modeled when directional differences in the variograms were
observed at the Woodruff Farm experiment (planting 2000 and from harvest 2001 to harvest 2002) and at the Bruner Farm experiment (from planting 2001 to harvest 2002) (Table 3).

The maximum distance where spatial dependence is detected is called the range, which defines limits of the spatial structure of a spatial process. Contraction and expansion of the spatial structure of SCN egg population densities due to changes in range values within and between soybean growing seasons were observed at the Woodruff Farm experiment from 2000 to 2003. Spatial structure contracted within the 2000 growing season, but the ranges increased within the 2001 and 2002 growing seasons (Table 3). At the Bruner Farm experiment, the ranges were beyond or close to the limits of the experimental field (70 x 57 m) during both the 2001 and 2002 seasons. During overwintering, the ranges decreased in both experiments; however the ranges increased at the Woodruff Farm experiment from harvest of the 2000 growing season to planting of the 2001 growing season (Table 3).

The maximum semivariance that occurs when the range is reached defines the sill, and the minimum value of the semivariance that occurs when the distances between two locations is zero is called the nugget. Differences in directional variograms occurred at the Bruner Farm experiment in the 2001 and 2002 seasons, and anisotropy was modeled. The anisotropopic ellipses were in a northeast-southwest direction at planting time and in a northwest-southeast direction at harvest time with similar angles between different planting assessments, 31.6 and 40.2 degrees, and between different harvest assessments, 296.2 and 334.0 degrees, respectively, for the data from the 2001 and 2002 seasons (Table 3).

Kriging using the best variogram models (Table 3) was done to generate maps of SCN egg population densities (Figs. 1 and 2). Within a given range, the higher the partial-sill-to-sill ratio (PS S⁻¹), the greater the spatial dependency is in a spatial process (Webster and Oliver, 2001). The degree of spatial dependence (PS S⁻¹) decreased within the 2000 growing season, but increased continuously from harvest 2000 to harvest 2002 at the Woodruff Farm experiment. At this experiment, a decrease in the degree of spatial
dependence was observed from harvest 2002 to planting 2003 (Table 3). Spatial dependency decreased within season and increased over winter at the Bruner Farm experiment from 2001 to 2002.

Some spatial features of SCN egg population densities were present in the fields throughout each assessment date. The lowest SCN egg population densities were observed in the northeastern corner at the Woodruff Farm experiment from 2000 to 2003 (Fig. 1). The highest SCN egg population densities were observed along the northern and western borders of the Bruner Farm experiment in 2001 and 2002 (Fig. 2).

The greatest changes in SCN population densities at the Woodruff Farm experiment (2000 and 2001) and at the Bruner Farm experiment (2001) were observed in quadrats where SCN egg population densities were not detectable or at very low levels at the time of planting. SCN population densities were transformed ($\log_{10}(P+1)$) and regressed on logarithmic-transformed ($\log_{10}((P_{f+1}/P_{i}))$) within-season changes of SCN population densities ($P_{f}/P_{i}$) and logarithmic-transformed ($\log_{10}((P_{i(t+1)+1})/(P_{f+1})$)) between-season changes of SCN population densities ($P_{i(t+1)}/P_{f}$) (Table 4). Regressions of SCN population densities at planting on the within-season changes in SCN population densities had negative slopes in both experiments between the 2000 and 2002 growing seasons (Table 4). For the same period, there was a positive regression of SCN population densities at planting on the within-season changes in SCN population density (Table 4). The linear coefficients of determination ($R^2$) for the relationships between SCN population density at planting and within-season changes in SCN population density were greater when the mean SCN population density increased from planting to harvest (Woodruff Farm 2000 and 2001, Bruner Farm 2001) than when the mean SCN population density decreased in the same period (Woodruff and Bruner Farm 2002) (Table 4). The opposite occurred with the coefficients of determination for the relationships between SCN population densities at harvest and within-season changes in SCN population densities (Table 4). In 2000 and 2001,
slopes of the regressions of \( \log_{10} P_i \) on \( \log_{10} P_{ft}/P_i \) did not significantly differ \((P > 0.05)\) between the Woodruff and Bruner Farm experiments. Linear coefficients of determination for the regressions of SCN egg population densities on within-season changes in SCN population densities in 2002 were lower \((-0.55\) and \(-0.40\) for the Woodruff and Bruner Farm experiments respectively) than the ones obtained in 2000 \((-0.84\) for the Woodruff Farm experiment) and 2001 for both experiments \((-0.86\) and \(-0.92\) for the at Woodruff and Bruner Farm experiments, respectively). Soybean cyst nematode population densities at harvest best described the variation of within-season changes in SCN population densities in 2002 \((R^2 = 0.31\) and \(0.47\) for the Woodruff and Bruner, respectively) \((\text{Table 4})\). Variation in the between-season changes of SCN population densities was best estimated by the variation in the SCN population densities observed the next planting season; \(R^2\) ranged from 0.35 to 0.53 for the Woodruff Farm experiment \((2001/2002)\) and the Bruner Farm experiment \((2001/2002)\), respectively \((\text{Table 4})\).

The greatest changes in SCN population densities within a season and the lowest changes in SCN population densities between seasons were observed at the northeastern corner of the Woodruff Farm experiment from the 2000 growing season to the planting 2002. In this experiment, there was no evident pattern in the changes of SCN population densities from planting 2002 to planting 2003 \((\text{Fig. 3})\). At the Bruner Farm experiment, the greatest changes in SCN population densities were observed following the east to west direction at the southern part of the field in 2001. Within \((2002)\) and between seasons \((2001/2002)\), changes in SCN population densities followed the soybean-row direction \((\text{Fig. 4})\).

There were few foliar diseases observed on soybean plants in both fields from 2000 to 2002. At the Woodruff Farm experiment, \textit{Cercospora} sp. and \textit{Septoria} sp. were observed infecting soybean plants at low disease incidence \((< 4.0\%)\) and severity \((< 6.0\%)\) levels. A general yellowing of the leaves was observed across the Woodruff Farm experimental field during drought periods; however, plants recovered after rain. At the Bruner Farm experiment,
Cercospora sp. was observed infecting soybean plants at low incidence (~3%) and severity (< 2%) levels. Typical symptoms of iron deficiency chlorosis on leaves were observed at the northwestern and northern areas of the Bruner Farm experiment in both years. In 2002, iron deficiency chlorosis was mapped at the Bruner Farm experiment (Fig. 5).

Besides several other uncontrolled yield-limiting factors affecting soybean yield, SCN population densities were correlated with yield (Table 5). Soybean cyst nematode population density at planting (Pi) was negatively correlated with yield (ranging from 162 to 1745 g quadrat⁻¹) and seed size (100-seed weight ranging from 8.6 to 16.5 g 100 seed⁻¹). Except for the Woodruff Farm experiment in 2002, greater absolute values of correlation coefficients were observed in the relationships between Pi and yield than between SCN egg population density at harvest (Pf) and yield (Table 5). Absolute values of the correlation coefficients among seed protein and seed oil with SCN population densities at the Bruner Farm experiment ranged from 0.12 to 0.37 and from 0.15 to 0.36, respectively. At the Woodruff Farm experiment, absolute values of the correlation coefficients among seed protein and seed oil with SCN population densities ranged from 0.07 to 0.22 and from 0.10 to 0.16, respectively. However, there were assessment dates for which correlations between SCN population densities and seed protein or seed oil content were not significant at the Woodruff Farm experiment. Seed protein content was positively correlated with Pi (Table 5). Seed oil content was negatively correlated to seed protein content (data not shown), with correlation coefficient values varying from -0.31 to -0.90 at the Woodruff Farm (2002) and Bruner Farm (2001) experiments, respectively. Maps showing similarities in geographic patterns of the distribution of total soybean grain yield, seed size, seed protein content, seed oil content, and SCN population densities for the instances with the highest correlations between SCN egg population densities and soybean yield variables are presented for the Woodruff and Bruner Farm experiments (Figs. 6 and 7).
Discussion

In the experiments, the mean SCN population densities increased within season, from spring to fall, in 2000 and 2001, but decreased in 2002. During this latter season, simultaneous occurrence of high precipitation and high temperatures were probably detrimental to SCN reproduction.

Spatial statistical analyses showed that the spatial structure and spatial dependence of SCN population densities increased and decreased in both experiments within and between soybean seasons. Changes in the limits of the spatial structure of SCN population density were not clearly related with seasonal changes in the mean SCN population densities at the Woodruff Farm experiment. Ecological factors possibly affected differently the limits of the spatial processes involving SCN population densities and seasonal changes nematode population densities. At the Bruner Farm experiment, the ranges increased and decreased as the mean SCN population increased and decreased throughout assessment dates. However, overwintering resulted in a decrease in mean SCN population densities and a decrease in the ranges of variogram models in three out of four occasions. More research needs to be done to improve the understanding of the effect of factors, such as soil texture, soil fertility, soil temperature, and soil moisture, on spatial processes of SCN population densities.

The angles of the anisotropic ellipses observed at the Woodruff and Bruner farm experiments probably were related to tillage practices that occurred at oblique angles relative to the direction of planting. Tillage can be considered a physical process that affects the spatial structure and/or spatial dependency of SCN population densities in SCN-infested fields.

The nuggets observed in our experiments were large, and the high values of the nuggets can possibly be explained by efficiency of the method of extracting SCN from soil samples and sampling errors. Measurement errors in the extraction of SCN from soil can be related to efficiency of the method of extraction (Moreira et al., unpublished data), sampling
error (Francl, 1986), and to the level of nematode infestation at different parts of the field (Seinhorst, 1982). Other common factor that affects the size of the nugget is the spatial structure that can occur at distances that are shorter than the minimum sampling interval. The minimum distance between two samples in our experiments was 4 m. Additionally, we did not account for these possible spatial variations in SCN population densities that could exist at distances shorter than the dimensions of a quadrat (2 x 3 m), when we obtained the six soil cores spatially separate to form a composite soil sample for each individual quadrat. The use of these composite samples to represent SCN population densities within quadrats probably affected the size of the nuggets in our experiments. Future work should adopt different sampling designs that consider sources of spatial variation that can occur at distances shorter than the smallest sample interval.

Spatial statistics provided methods to describe the changes in the characteristics of spatial process of SCN population densities in soybean fields over time. It is interesting to note that a spatial process can change in different aspects, such as range, sill, nugget, and occurrence of anisotropy, within and between seasons. Thus, it will be important to establish in future studies what factors determine those changes and how they affect the spatial processes.

In our fields, soybean rows were planted in an east-to-west direction; however the orientation of the anisotropic ellipses occurred at angles that did not coincide to the east-west direction. This result demonstrates the necessity to fully understand factors such as topography and spatial variability in soil properties that could affect the establishment anisotropic processes of SCN population densities in soybean fields. Thus, research to understand the effect of agronomic practices on SCN population density spatial processes should take into consideration seasonal and annual changes that can affect spatial processes.

The variation in the 2000 and the 2001 within-season changes of SCN population density (Pf/Pi) was best explained in both experiments by the variation in SCN population
densities obtained at planting (Pi). In contrast, the variation in the 2002 within-season changes of SCN population density (Pf/Pi) was best explained in both experiments by the variation in SCN population at harvest (Pf). Linear coefficients of determination (R²) for the best relationships between SCN population densities and within-season changes of SCN population densities were greater in 2000 and 2001, than in 2002 (Table 4). The higher the R² quantifying the relationship between Pi and Pf/Pi, the greater the presence of density-dependent and partially density-dependent factors controlling population dynamics of nematodes in a field (Boag, 1989). Low R² values in these relationships indicate the presence of density-independent factors (environmental conditions) and/or imperfectly density-dependent (interspecific competition, parasitism, and predation) factors controlling within-season changes in SCN population densities. The R² values we obtained indicated that intraspecific competition and/or the presence of antagonistic organisms may have played major roles in affecting SCN population densities in the 2000 and 2001 soybean growing seasons. Density-independent and/or imperfectly density-dependent factors caused the deterioration of the relationships between SCN population densities and within-season changes of SCN population densities in 2002. Furthermore, within-season changes in SCN population densities had a small range (from 0 to 5) in both experiments and did not have a specific pattern that might suggest that the entire fields were under the influence of the same environmental factors. Climatological data obtained at the Ames Weather Station showed that the 2002 growing season had the greater precipitation (606 mm) and higher average temperature (20.8 C) compared to 2000 (356 mm and 20.6 C) and to 2001 (511 mm and 20.3 C) growing seasons (data not shown). Besides the volume of precipitation, also the timing of the rain may have had an effect on the changes in SCN population densities observed within the 2002-growing season. During the months of July and August, 30%, 27%, and 54% of the total seasonal precipitation occurred in the 2000, 2001, and 2002 growing seasons, respectively (data not shown). Additionally, the month of July 2002 had 20 days with
temperatures above 30 C while this same month in 2000 and 2001 had 5 and 12 days with temperatures above 30 C, respectively (data not shown) (IEM, 2004). Excess moisture and temperatures above the ideal range for SCN female development (Melton et al., 1986) may have affected the SCN populations throughout the entire experimental fields, causing the within-season changes in SCN population densities to be less dependent of the levels of the SCN population densities at planting and at harvest.

Overwintering reduced the mean SCN egg population densities in the experiments. The between-season changes in SCN population densities should be more affected by factors that are partially dependent or independent of SCN population densities since SCN is not biologically active during most over winter periods because of the low temperatures (Alston and Schmitt, 1987) and the absence of host during this time. These factors would explain the low $R^2$ for the regressions of between-season changes in SCN population densities and SCN population densities measured at planting and harvest.

The greatest within-season changes in SCN population densities observed in both experiments in 2000-2001 were in quadrats that had very low SCN egg population densities at planting. Overestimation of the changes in nematode population densities may be caused by false negative results of nematode extractions, by existence of minimum thresholds of detection for extraction methods, and by inaccuracies in determination of low nematode population densities (Chen et al., 2001a; Donald et al., 1999; Farias et al., 2002; Francl and Dropkin, 1986; Seinhorst, 1982; Tylka and Flynn, 2000). Sampling protocols can be designed and used to improve detection of SCN and the potato cyst nematode in fields (Been and Schomaker, 1996; Francl, 1986). However, the high cost of sampling may limit the circumstances in which new assessment strategies for SCN population density can be adopted.

Soybean-yield components, such as number of pods per area, number of seeds per pod, and the size of the seeds, can be affected by biotic and abiotic stresses, and multiple
stresses may affect soybean crop during growing seasons. Negative relationships between SCN population densities at planting and soybean yield were observed, and the yield reduction could be, at least partially, explained by the reduction that occurs in seed size. Besides quantitative losses, SCN infection may cause an increase on the seed protein content and a decrease in seed oil content. The results of the study show that the effects of SCN population densities on yield possibly vary as a function of environmental factors. More research is needed to verify how the quality and composition of the seed oil and seed protein is affected by SCN.

At the Bruner Farm experiment, SCN population densities at planting were negatively correlated with soybean yield, seed oil content, and 100-seed weight, but positively correlated with seed protein content. The map of iron deficiency chlorosis symptoms and the map of SCN population densities at planting showed that iron deficiency chlorosis and high SCN population densities occurred in the same areas.

Iron deficiency chlorosis and SCN population densities cause yield losses and affect soybean seed quality; however more research needs to be carried out to understand why SCN population densities are high in areas in which iron deficiency chlorosis symptoms are detected in soybean plants. Also, it is important to understand the combined impact of SCN infection and iron deficiency on soybean yield.

The occurrence of iron deficiency chlorosis and high SCN population densities in the same areas suggests that it may not be easy to distinguish between these two factors causing chlorotic symptoms in soybean fields. Incorrect or incomplete diagnoses can lead to implementation of inadequate or inappropriate control practices.

Mapping SCN population densities in soybean fields is important to identify ecological zones in the fields that are suppressive or conducive to SCN reproduction. Identification of such conditions possibly could lead to the development of new management strategies to control the nematode. Furthermore, the use of GIS software allowed the
visualization of the geographic similarities between the distribution of SCN population densities and soybean yield. In this way, this study showed the quantitative and qualitative impact this pathogen can have on soybean yield.

Allowing overlaying maps of different variables, such as soil fertility, soil texture, soil temperature, soil moisture, SCN population density, and of the changes in SCN population densities that may occur within and between seasons, GIS can be used to identify the effect of ecological variables on SCN populations and can provide information on the combined effect of those variables on soybean yield. The results presented here reinforce the necessity of more research to understand the impact SCN can have on soybean yield under different environmental conditions.

Spatial statistics and geographic information systems (GIS) were useful to study and visualize SCN populations in soybean fields, to verify the impact SCN has in the quantity and quality of soybean yields, and to detect simultaneous occurrence of SCN and iron deficiency in soybean fields. The GIS tools and statistical techniques utilized in the study showed that they can be used to expand scientific understanding of SCN population dynamics, the SCN-soybean pathosystem, and the factors that can impact them.

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susceptible cultivars in Minnesota. Plant Disease 85:760-766.


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Table 1. Texture and fertility characteristics of the clay loam soils at Woodruff and Bruner Farm experimental fields.

<table>
<thead>
<tr>
<th>Field</th>
<th>Clay</th>
<th>Silt</th>
<th>Sand</th>
<th>Organic matter</th>
<th>pH</th>
<th>Phosphorus</th>
<th>Potassium</th>
<th>Iron</th>
</tr>
</thead>
<tbody>
<tr>
<td>Woodruff</td>
<td>30.2</td>
<td>34.8</td>
<td>35.0</td>
<td>5.0</td>
<td>6.9</td>
<td>29</td>
<td>163</td>
<td>39.8</td>
</tr>
<tr>
<td>Bruner</td>
<td>30.2</td>
<td>34.4</td>
<td>35.4</td>
<td>4.4</td>
<td>7.1</td>
<td>66</td>
<td>167</td>
<td>46.5</td>
</tr>
</tbody>
</table>
Table 2. Statistical characteristics of SCN egg population densities from the 995 and 613 quadrats at the Woodruff and Bruner Farm experiments, respectively, at planting and at harvest from 2000 to 2003.

<table>
<thead>
<tr>
<th>Field</th>
<th>Population density†</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Planting</td>
<td>Harvest</td>
<td>Planting</td>
<td>Harvest</td>
</tr>
<tr>
<td>Woodruff</td>
<td>Min.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>900</td>
</tr>
<tr>
<td></td>
<td>Max.</td>
<td>12,800</td>
<td>55,200</td>
<td>123,000</td>
<td>76,200</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>1,500</td>
<td>10,100</td>
<td>6,800</td>
<td>14,500</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>2,274.6</td>
<td>11,912.0</td>
<td>8,746.3</td>
<td>16,678.0</td>
</tr>
<tr>
<td></td>
<td>Variance</td>
<td>5,370,343</td>
<td>74,898,640</td>
<td>67,394,248</td>
<td>118,026,496</td>
</tr>
<tr>
<td></td>
<td>Skewness</td>
<td>1.55</td>
<td>1.11</td>
<td>3.87</td>
<td>1.37</td>
</tr>
<tr>
<td>Bruner</td>
<td>Min.</td>
<td>N/D†</td>
<td>N/D</td>
<td>N/D</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Max.</td>
<td>N/D</td>
<td>N/D</td>
<td>10100</td>
<td>43,400</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>N/D</td>
<td>N/D</td>
<td>800</td>
<td>7,800</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>N/D</td>
<td>N/D</td>
<td>1,316.6</td>
<td>9,252.2</td>
</tr>
<tr>
<td></td>
<td>Variance</td>
<td>N/D</td>
<td>N/D</td>
<td>2,102,500</td>
<td>32,182,929</td>
</tr>
<tr>
<td></td>
<td>Skewness</td>
<td>N/D</td>
<td>N/D</td>
<td>2.18</td>
<td>1.63</td>
</tr>
</tbody>
</table>

† Min., minimum SCN egg population density quadrat; Max., maximum SCN egg population density quadrat; Median, median SCN egg population density quadrat; Mean, mean SCN egg population density quadrat; Variance, variance SCN egg population density quadrat; Skewness, degree of skewness presented by the distribution of SCN egg population density quadrat.

‡ N/D, not determined.
Table 3. Characteristics of the fitted variogram models for SCN population density data obtained before planting and after harvest at the Bruner and Woodruff Farm experiments from 2000 to 2003.

<table>
<thead>
<tr>
<th>Spatial Model</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Planting</td>
<td>Harvest</td>
<td>Planting</td>
<td>Harvest</td>
</tr>
<tr>
<td>Woodruff</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type</td>
<td>spherical†</td>
<td>spherical‡</td>
<td>spherical</td>
<td>exponential†</td>
</tr>
<tr>
<td>Range</td>
<td>35.4 - 19.1§</td>
<td>21.1</td>
<td>40.8</td>
<td>98.8 - 70.9</td>
</tr>
<tr>
<td>Sill Partial Sill</td>
<td>1,860,000</td>
<td>10,606,000</td>
<td>30,446,000</td>
<td>56,871,000</td>
</tr>
<tr>
<td>Nugget</td>
<td>3,244,800</td>
<td>53,047,000</td>
<td>44,116,000</td>
<td>70,130,000</td>
</tr>
<tr>
<td>Angle</td>
<td>285.9</td>
<td></td>
<td></td>
<td>346.3</td>
</tr>
<tr>
<td>PS S⁻¹#</td>
<td>0.36</td>
<td>0.17</td>
<td>0.41</td>
<td>0.59</td>
</tr>
</tbody>
</table>

| Bruner        | 2000       | 2001       | 2002       | 2003       |
|               | Planting   | Harvest    | Planting   | Harvest    |
| Type          | N/D††      | N/D        | spherical† | exponential† |
| Range         | N/D        | N/D        | 97.6 - 78.1 | 111.6 - 99.5 |
| Sill Partial Sill | N/D        | N/D        | 1,428,000  | 18,570,000 |
| Nugget        | N/D        | N/D        | 1,130,400  | 19,383,000 |
| Angle         | N/D        | N/D        | 31.4       | 39.6       |
| PS S⁻¹#       | N/D        | N/D        | 0.56       | 0.36       |

† Anisotropy was modeled
‡ Deterministic trends were removed from the spatial process by a second order polynomial, and the residual process was modeled
§ Range - minor axis of the anisotropic ellipse
¶ Angle of the major axis of the anisotropic ellipse – 0 degree points north
# Partial-sill-to-sill ratio
†† N/D, not determined
Table 4. Slopes, intercepts, and coefficients of determination ($R^2$) for linear regressions of transformed SCN egg population densities at planting ($\log_{10} (P_i+1)$) and at harvest ($\log_{10} (P_f+1)$) on transformed within-season changes in SCN egg population densities ($\log_{10} (P_f/P_i)$) and on between-season changes in SCN egg population densities ($\log_{10} (P_i(t+i)+1)/(P_f(t)+1)$) at the Woodruff and Bruner Farm experiments from 2000 to 2003.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>SCN egg population density</th>
<th>Within-season changes in SCN population densities</th>
<th>Between-season changes in SCN population densities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Year</td>
<td>Date</td>
<td>slope</td>
</tr>
<tr>
<td>Woodruff Farm</td>
<td>2000</td>
<td>Planting</td>
<td>-0.84</td>
</tr>
<tr>
<td></td>
<td>Harvest</td>
<td>0.60</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>2001</td>
<td>Planting</td>
<td>-0.86</td>
</tr>
<tr>
<td></td>
<td>Harvest</td>
<td>0.60</td>
<td>-2.07</td>
</tr>
<tr>
<td></td>
<td>2002</td>
<td>Planting</td>
<td>-0.55</td>
</tr>
<tr>
<td></td>
<td>Harvest</td>
<td>0.58</td>
<td>-2.36</td>
</tr>
<tr>
<td></td>
<td>2003</td>
<td>Planting</td>
<td>-0.58</td>
</tr>
<tr>
<td>Bruner Farm</td>
<td>2001</td>
<td>Planting</td>
<td>-0.92</td>
</tr>
<tr>
<td></td>
<td>Harvest</td>
<td>0.40</td>
<td>-0.48</td>
</tr>
<tr>
<td></td>
<td>2002</td>
<td>Planting</td>
<td>-0.40</td>
</tr>
<tr>
<td></td>
<td>Harvest</td>
<td>0.78</td>
<td>3.61</td>
</tr>
</tbody>
</table>

† Transformed SCN egg population density at planting, $\log_{10} (P_i+1)$; transformed SCN population density at harvest, $\log_{10} (P_f+1)$

‡ Transformed within-season changes in SCN egg population densities, $\log_{10} [(P_f+1)/(P_i+1)^{-1}]$

¶ Transformed between-season changes in SCN egg population densities, $\log_{10} [(P_i(t+i)+1)/(P_f(t)+1)^{-1}]$
Table 5. Linear correlation coefficients of SCN egg population densities at planting (log_{10} (Pi+1)) and harvest (log_{10} (Pf+1)) with soybean yield variables: seed yield (g quadrat^{-1}), 100-seed weight (g 100 seeds^{-1}), and seed protein (%), seed oil (%) obtained at Woodruff and Bruner Farms from 2000 to 2002.

<table>
<thead>
<tr>
<th></th>
<th>Woodruff Farm experiment</th>
<th>Bruner Farm experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2000</td>
<td>2001</td>
</tr>
<tr>
<td>Seed yield</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pf</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed yield</td>
<td>-0.24**</td>
<td>-0.07*</td>
</tr>
<tr>
<td>100-seed weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pf</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100-seed weight</td>
<td>-0.11**</td>
<td>NS</td>
</tr>
<tr>
<td>Seed protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pf</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed protein</td>
<td>0.07*</td>
<td>0.12**</td>
</tr>
<tr>
<td>Seed oil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pf</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed oil</td>
<td>0.10**</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, nonsignificant at the 0.05 probability level
* Significant at the 0.05 probability level
** Significant at the 0.001 probability level
Figure 1. Maps of SCN egg population densities obtained by kriging the best variogram models for each planting and harvest assessment date at the Woodruff Farm experiment from 2000 to 2003.
Figure 2. Maps of SCN egg population densities obtained by kriging the best variogram models for each planting and harvest assessment date at the Bruner Farm experiment in 2001 and 2002.
Figure 3. Maps of within-season changes in SCN egg population density (Pf/Pi) and between-season changes in SCN egg population density (Pi(Pt)/Pf) obtained at the Woodruff Farm experiment from 2000 to 2003.
Figure 4. Maps of within-season changes in SCN population densities ($P_{f}/P_{i}$) and between-season changes in SCN population densities ($P_{i(t+1)}/P_{f}$) obtained at the Bruner Farm experiment from 2001 to 2003.
Figure 5. Map of quadrats exhibiting visible symptoms of iron deficiency chlorosis (IDC) at the Bruner Farm experiment in 2002.
Figure 6. Maps of SCN population densities, soybean yield, and 100-seed weight for the Woodruff Farm experiment in 2000 and 2002.
Figure 7. Maps of SCN population densities, soybean yield, 100-seed weight, seed protein, and seed oil for the Bruner Farm experiment for 2001 and 2002.
CHAPTER 5

GENERAL SUMMARY

Three interdependent research projects comprise this dissertation. The first project was developed to understand how the relationships among percentage reflectance data for individual wavelength bands, vegetation indices (VI), and soybean growth indicated as green leaf area index (GLAI) vary within and between soybean seasons. The relationships among reflectance data and soybean GLAI were studied by generating a wide range of soybean development throughout the 2002 and 2003 seasons and by measuring percentage reflectance from those soybean canopies at different assessment dates within a season. The relationships between reflectance data and soybean GLAI were described using regressions, and the regressions obtained at different assessment dates were compared using a general linear test approach. Our result showed that within assessment dates, the percentage reflectance for the 660-nm and 810-nm-wavelength bands had the best relationship with GLAI. However, the regressions of percentage reflectance from these two wavelength bands on GLAI obtained at different assessment dates were significantly different from the regressions of these same variables obtained for the entire season. Among VI, radiance ratio (RR), difference vegetation index (DVI), and renormalized difference vegetation index (RDVI) had the best relationships with yield within assessment dates. But, only the regressions of RDVI on GLAI obtained within season were not significantly different from the regression of RDVI on GLAI for each season. Differences in the regressions obtained for different assessment dates indicate that the relationships between reflectance data and GLAI vary throughout the season. Probably, temporal changes in environmental factors that affect reflectance from soybean canopies and/or the soybean canopies themselves are affecting the slopes and intercepts of the regressions. More research needs to be conducted to determine and to
control those factors. Other results of our study indicate reflectance measurements from several wavelength bands and VI are highly related to each other. These relationships suggest that at least some of the wavelength bands and VI may be considered equivalent in the ability of assessing GLAI. For instance, equivalence was observed between normalized difference vegetation index (NDVI) and green normalized difference vegetation index (GNDVI).

Finally, our results showed that ground-based remote sensing measuring percentage reflectance from soybean canopies can be used to estimate soybean GLAI throughout seasons with a very high resolution.

Our second research project was to determine if ground-based assessment of percentage reflectance from soybean canopies could be used to assess quantity and quality of soybean yield and stresses caused in soybean plants by soybean cyst nematodes (SCN). This project was conducted in two different experiments with 995 and 613 2 X 3 m quadrats from 2000 to 2002. From each quadrat, percentage reflectance was measured every 7 to 14 days using a handheld, multispectral radiometer. Additionally, SCN egg population densities were determined for each quadrat at planting and at harvest. Soybean grain yield was obtained for each quadrat and also seed protein and seed oil contents and 100-seed weight. Our results show how the relationships among reflectance data, quantity and quality of soybean yield, and SCN population densities develop during the soybean season. Considering the soybean cultivar, the geographical location of our fields, and the planting dates, our results showed that reflectance data from soybean canopies obtained during August and September had the best relationships with quantity and quality of soybean yield. Soybean grain yield and reflectance data indicated as near infrared reflectance (NIR), radiance ratio (RR), renormalized difference vegetation index (RDVI), and green normalized difference vegetation index (GNDVI) had their best relationships when reflectance from soybean canopies was measured from late August to early September. However, these relationships between VI and grain yield were negatively affected by lodging of soybean plants. The
relationships among reflectance data, seed protein, seed oil, and 100-seed weight varied among years and fields. Soybean cyst nematode population densities at planting were best related to reflectance data at the very beginning or at the end of the growing seasons. It is logical to assume that soil has a greater influence on the total reflectance obtained for each quadrat very early and very late in the soybean season than in the middle of the season, when foliage completely covers the surface of the quadrats. Thus, the relationships between reflectance and SCN population densities at planting verified very early in the seasons may somewhat represent the relationships between SCN population densities and soil characteristics. When the best relationships between reflectance data and SCN population density was observed at the end of the growing season, effects of SCN on the soybean growth should be considered besides the characteristics of soil reflectance. Early senescence of soybean plants that were infected early in the growing season can possibly improve the relationships between SCN population densities at planting and reflectance data. There was not a single wavelength band or VI that showed to be superior to the others in the relationships with reflectance data for both experimental fields. Results from this work indicate that reflectance data is related to soybean growth and soybean yield. Additionally, it is important to note our results show that it is possible to estimate soybean yield using ground-based percentage reflectance measurements one month before harvest.

Finally, the third research project dealt with the study of SCN population density dynamics, the spatial analyses of the SCN population densities, and the relationships between SCN population densities and quantity and quality of soybean yield under monoculture of SCN-susceptible soybean cultivars. The SCN population density data and soybean yield data collected were the same as were described for the second project. It was observed that mean SCN population density per quadrat increased (2000 and 2001) and decreased (2002) within growing seasons, and these densities were probably affected by environmental factors, such as temperature and soil moisture. Limits of the spatial structure (range) and variations in
spatial dependence (partial sill to sill ratio) varied within and between seasons in both experimental fields. It was not clear how different environmental factors, such as temperature and soil moisture, affected the range and the spatial dependence of SCN population densities in the two experiments. Spatial statistics associated with geographic information systems has the advantage of allowing the visualization of the results in maps. Changes in SCN population densities in the experiments within and between seasons suggest that factors exist that affect the carrying capacity of soybean fields among growing seasons. Changes in SCN population densities within a season were best related to the SCN population densities found at planting. However, these relationships between SCN population densities at planting and the within-season changes in SCN population densities deteriorated when environmental factors possibly negatively affected reproduction and/or survival of SCN. Soybean yield was negatively related to SCN population density at planting, and reduction in grain yield could be partially explained by the reduction in seed size. Also, it was observed that high SCN population densities were associated with an increase in seed protein and a decrease in seed oil content. A final important aspect of this study was the observations of the symptoms of iron deficiency chlorosis in the same areas the highest SCN population densities were found. More study needs to be done to understand the combined effects of SCN infection and iron deficiency on soybean plants and on soybean yield. Moreover, it is important to determine how iron deficiency may affect SCN population dynamics.
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