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Quantitative Relationship of Soil Texture with the Observed Population Density Reduction of *Heterodera glycines* after Annual Corn Rotation in Nebraska

OSCAR PÉREZ-HERNÁNDEZ, LOREN J. GIESLER

Abstract: Soil texture has been commonly associated with the population density of *Heterodera glycines* (soybean cyst nematode: SCN), but such an association has been mainly described in terms of textural classes. In this study, multivariate analysis and a generalized linear modeling approach were used to elucidate the quantitative relationship of soil texture with the observed SCN population density reduction after annual corn rotation in Nebraska. Forty-five commercial production fields were sampled in 2009, 2010, and 2011 and SCN population density (eggs/100 cm³ of soil) for each field was determined before (Pi) and after (Pf) annual corn rotation from ten 3 × 3-m sampling grids. Principal components analysis revealed that, compared with silt and clay, sand had a stronger association with SCN Pi and Pf. Cluster analysis using the average linkage method and confirmed through 1,000 bootstrap simulations identified two groups: one corresponding to predominant silt-and-clay fields and other to sand-predominant fields. This grouping suggested that SCN relative percent population decline was higher in the sandy than in the silt-and-clay predominant group. However, when groups were compared for their SCN population density reduction using Pf as the response, Pi as a covariate, and incorporating the year and field variability, a negative binomial generalized linear model indicated that the SCN population density reduction was not statistically different between the sand-predominant field group and the silt-and-clay predominant group.

Key words: corn rotation, ecology, *Heterodera glycines*, multivariate analysis, negative binomial, soil texture, soybean cyst nematode.

Nebraska is an important soybean (*Glycine max* L.) producing state in the United States. (NASS, 2013). The soybean production value in the state in 2012 was estimated at close to \$2.9 billion (ASA, 2012), yet the economic losses attributable to *Heterodera glycines* Ichinohe (soybean cyst nematode: SCN) were estimated at \$40 million (Wilson and Giesler, 2013). Corn rotation (predominantly annual rotation) is a major practice to manage SCN field infestations in virtually all areas of the state where SCN occurs (Giesler and Wilson, 2011). The benefit of corn rotation is consistently a significant SCN population density reduction at the end of the growing season (Ross, 1962; Koenning et al., 1993; Porter et al., 2001; Niblack, 2005; Chen, 2007). However, although it is fairly well established that soil SCN population levels decline with corn rotation, soil factors associated with such a decline are poorly understood.

Soil texture has been previously associated with SCN population densities (Slack et al., 1972; Koenning et al., 1988; Heatherly and Young, 1991; Workneh et al., 1999), but such an association has been mainly described qualitatively—that is, only in terms of soil textural classes. Moreover, descriptions have focused on soybean and have been limited to within field, a few fields, or a single sample per field in many fields. The general observation is that higher SCN population densities occur in sandy soils, or conversely, that lower population densities occur in clay soils (Heatherly et al., 1982; Heatherly and Young, 1991; Workneh et al., 1999). For instance, in a greenhouse study, the number

of SCN cysts in the soil of potted soybean plants increased in silt loam soil and decreased in clay soil 60 d after inoculation (Heatherly and Young, 1991). Within two individual soybean fields, SCN cyst densities were higher in loamy sand than in sandy clay loam (Avenidaño et al., 2004). In a regional survey of soybean fields in the U.S. (one sample per field), lower SCN population densities in no-till fields were found in clay soils than in sandy loam, sandy clay loam, loam, clay loam, silt, and silt loam soils (Workneh et al., 1999).

The relationship of specific proportions of sand, silt, and clay with SCN population densities and population density changes in field cropping conditions is not clearly defined. Elucidation of such a relationship will unravel uncertainties in the suggested general association between soil texture and SCN. Importantly, description of the association of specific soil textural proportions with the observed SCN population density reduction after annual corn rotation will enhance our understanding of the role of soil texture in SCN field mortality. In addition, it will provide quantitative basis for SCN modeling studies that include soil texture as an input variable to describe effects on SCN population density changes. Ultimately, understanding this relationship will help to better assess the effect of management practices on SCN population densities and contribute to improving SCN management. The primary objective of this research was to elucidate the relationship of sand, silt, and clay proportions in soil with the observed SCN population density reduction after annual corn rotation in Nebraska.

MATERIALS AND METHODS

Field selection: Commercial production fields (37 to 55 ha in size) with a history of SCN and in annual soybean-corn rotation were identified in the major soybean-producing areas of Nebraska in 2009, 2010, and 2011.

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Each of the identified fields had tested positive for SCN in at least one composite sample diagnosed in a nematology laboratory of the University of Nebraska-Lincoln. Only fields diagnosed with ≥ 250 SCN eggs/100 cm³ of soil were preliminarily selected for the study. This field selection criterion was based on the SCN detection limit of our method (40 eggs/100 cm³ of soil) and on the consideration that 250 SCN eggs/100 cm³ of soil was a minimum, reasonable density on which to evaluate SCN population density change. Twenty-nine fields were selected in 2009, 30 in 2010, and 20 in 2011.

Field sampling: Before sampling, the boundaries of all selected fields were mapped and added to ArcPad field software (ArcPad 7.0, ESRI, Redlands, CA) installed on a GeoXT handheld GPS unit (GeoExplorer 2008 series Trimble, Westminster, CO). The purpose was to enable visualization of the area and shape of the fields to attain a well-structured sample distribution within fields during sampling. In each field, ten 3 × 3-m sampling grids were selected systematically in a zig-zag pattern and the center of each grid was georeferenced for site-specific resampling. From each grid, twenty 2.5-cm-diam., 15- to 20-cm deep soil cores, spaced at 60 cm, were collected and mixed in a bucket to obtain a single composite sample. Samples were collected in plastic bags, placed in a cooler, and stored at 4°C in a cold room until they were processed. Attempts were made to avoid sampling areas such as entry ways or low-lying areas that could represent unusual SCN population density observations. Also, for sampling consistency, the actual collection of the soil cores in each sampling grid was done in an east-west direction at all times. Each field in each set was sampled twice: in midspring before corn planting (before corn rotation), and in midspring of the following year, before soybean planting (after annual corn rotation).

Sample processing for SCN egg extraction and counts: In the laboratory, each composite soil sample was thoroughly mixed to obtain a homogeneous sample. Then a 100-cm³ subsample, measured by volumetric displacement, was processed to determine the number of SCN eggs/100 cm³ of soil. Processing was done using standard sieving techniques for cyst nematodes: decanting the sample content onto a 710- μ m-pore sieve stacked over a 250- μ m-pore sieve to collect the cysts and then decanting and grinding the collected cysts on a 125- μ m-pore sieve stacked over a 25- μ m-pore sieve to extract the eggs (Khan, 2008). The extracted eggs from each sample were collected in a 100-ml beaker, rinsing with water and maintaining the volume of the collecting solution at < 20 ml for all the samples. One ml of a solution containing 750 ml distilled water, 250 ml lactic acid, and 0.35 g acid fuchsin (Hooper, 1986) was added to each sample and the sample was microwaved for 70 to 90 sec (at 700 watts power) to stain the eggs. After the sample was raised to a standard volume of 20 ml, 1 ml from each sample was taken and placed in a counting dish, observed under a dissecting microscope at ×35 magnification, and the

number of eggs was recorded. Three counts were performed per sample to calculate an average representing one field sampling grid.

Soil texture and SCN field population density determination: To utilize fields represented by a reasonable number of SCN-positive samples, fields in which SCN was not detected in four or more sampling grids on both sampling occasions were excluded from the study. Moreover, to meet the field selection criterion sought at the inception of the study, fields whose average SCN population density was below 250 eggs/100 cm³ of soil at the first sampling also were excluded. Also, because only one soil sample was used to represent the texture of a field, samples of any field that exhibited a contrasting soil texture to the majority of samples from the same field were ruled out. This occurred only in a few fields, often with one or two samples, and rarely with three or four samples showing a contrasting texture. Whether or not a sample had a contrasting texture to the majority of the samples of the same field was determined visually and by feel analysis according to the protocol proposed by Thien (1979). Following the described screening process, 45 fields out of the 79 initially sampled in the three field sets were used in the final analysis. Soil texture (relative proportions of sand, silt, and clay) was determined for each of the 45 fields from a single composite sample analyzed by a commercial soil testing laboratory (Wards Laboratories, Inc., Kearney, NE) using the hydrometer method (Gee and Bauder, 1986). An average SCN population density was determined for each field using the egg counts of the sampling grids (up to ten per field).

Relationship of soil texture with SCN population density reduction after rotation: To elucidate the relationship of the proportions of sand, silt, and clay with SCN population density reduction after annual corn rotation, we used multivariate analysis and a generalized linear mixed modeling approach. Multivariate methods included principal components and cluster analysis using the proportions of sand, silt, clay, and the SCN population density before (Pi) and after annual corn rotation (Pf) (both transformed by log x) as measures of association. The use of a generalized linear model was anticipated for comparison of the SCN population density reduction between/among any field grouping detected by the cluster analysis. Because Pf and Pi are discrete random variables (counts), Pf was used as the response variable in a model that is specific for counts. Use of the relative population density decline (%) or the difference between Pf and Pi as the response requires the assumptions of the Gaussian distribution, which in this situation appears to be an inadequate distributional choice.

Principal component analysis (PCA): PCA was mainly used as a method to screen the data set and to verify clustering results. The analysis was applied to the correlation matrix of the variables using the PRINCOMP procedure

of SAS (SAS Institute, Cary, NC). Selection of the appropriate number of principal components was based on the percentage of variance explained (predetermined at $\geq 90\%$ and ascertained from the eigenvalues of each of the components) and on a scree plot (Johnson and Wichern, 2002). With the eigenvalues ordered from largest to smallest, the number of components in a scree plot is taken to be wherever an elbow or bend in the plot is identified, or equivalently, the point at which the remaining eigenvalues are relatively small and about the same size.

Cluster analysis: The analysis of clusters was used to unveil any type of true or natural grouping in the data set and further guide field comparisons for SCN population density reduction. Because the clustering algorithm computes the distances among variable values, the resulting clustering might directly foreshadow underlying relationships among variables and thus among fields or suggest relationships to be investigated. Essentially, cluster analysis might indicate whether or not fields with similar soil texture exhibited similar SCN population density change after annual corn rotation. The values of the five measures of association (sand, silt, clay, Pi, and Pf) were standardized to give each variable an equitable weight in the analysis. The standardization consisted in dividing the data values of each variable by the variable's range (Milligan and Cooper, 1988) with the formula

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

Z_{ij} = the transformed value of each j th datum of the X_i variable, $i=1,2,\dots,p$, and $j=1,2,\dots,n$

x_{ij} = j th datum of the X_i variable

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

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

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

As a final assessment of the cluster analysis results, the uncertainty in the clustering produced by the average linkage method was evaluated with a bootstrap resampling method (Efron et al., 1996; Shimodaira, 2004). The method consisted of 1,000 bootstrap replications implemented with the Pvcust package of R software (Suzuki and Shimodaira, 2006). The Pvcust provides two kinds of P values that indicate how strong the cluster is supported by the data. One value is the approximately unbiased (AU) P value, calculated by a multiscale bootstrap resampling, and the other value

is the bootstrap probability (BP) value, calculated by the ordinary bootstrap resampling. In the multiscale AU, which was used in this study, for a cluster with AU P value > 0.95 , the hypothesis that the cluster does not exist is rejected with significance level 0.05.

Generalized linear mixed model for group comparison: A Poisson model was used initially for comparison of any groups identified by the cluster analysis. This model is a standard starting equation suitable for modeling count data (Stroup, 2013). The response variable was the SCN population density after annual rotation (Pf), which is modeled through a logarithmic link function specific to generalized linear model for counts (Stroup, 2013). The SCN Pi (transformed to $\log x$) in each of the samples of each field was included in the model as a covariate to adjust the between/among group comparisons of Pf by Pi. To determine whether or not the Pf difference between the groups depended on Pi, a test for homogeneity of slopes was conducted by including the group by Pi interaction term in the model. Because groups included fields from three different rotation years, the year was incorporated as a fixed effect. Field sample variability and the field-by-year interaction were considered in the model as random effects. At this stage, maximum likelihood with Laplace's likelihood approximation was chosen as the estimation method of the parameters (GLIMMIX procedure of SAS). The statistical model representing all effects and sources of variation is written as follows:

$$\log(Pf_{ijk}) = \eta + F_i + G_j + (\beta_1 + \delta_i)X_{ij} + (FY)_{ik}$$

where

Pf_{ijk} is the mean SCN population density after annual corn rotation in the i th field ($i = 1,2,\dots,45$) of the j th group (1,2) and k th year (1,2,3)

$Pf_{ijk}|F_i, (FY)_{ik} \sim \text{Poisson}(\eta_{ijk})$

Linear predictor: $\eta_{ijk} = \eta + F_i + G_j + (\beta_1 + \delta_i)X_{ij} + (FY)_{ik}$

Link function: $\eta_{ijk} = \log(pf_{ijk})$

η is the intercept or grand mean

F_i is the effect of the i th field ($i = 1,2,3,\dots,45$)

G_j is the effect of the j th group ($j = 1,2$)

X_{ij} is the covariate (P_i transformed to the natural log) measured at the i th field of the j th group

δX_{ij} is the group by covariate interaction

Y_k is the effect of the k th year ($k = 1,2,3$)

β_1 is the regression coefficient for the covariate

FY_{ik} is the field*year interaction effect

$$F \sim iid N(0, \sigma_F^2);$$

$$FY \sim N(0, \sigma_{FY}^2)$$

A negative binomial model, which is also specific for count-type response variables, was considered as an alternative to the Poisson if the assumption of equal

mean and variance (equidispersion) was not met. In the negative binomial model, the variance is modeled as a function of the mean. In this alternative model, the assumed distribution is negative binomial: $Pf_{ijk}|F_b (FY)_{ik} \sim \text{Negative binomial}(\eta_{ijk}, \phi)$ and all sources of variation and effects are kept the same as in the Poisson model. Optimization of nonlinear parameters in this model was performed with a maximum number of iterations = 150 with the Dual-Quasi-Newton technique (Stroup, 2013) and the denominator df for the *F* test of fixed effects were approximated by Kenward-Roger's method (Kenward and Roger, 1997). Goodness-of-fit of the final model was determined with the residual log pseudolikelihood compared with the intercept-only model (Hilbe, 2011).

RESULTS

Relationship of soil texture with SCN population density reduction after rotation: Pairwise scatterplots of sand, silt,

clay, SCN Pi, and SCN Pf suggested positive correlations between silt and clay and between SCN Pi and Pf (Fig. 1). Negative correlations between sand and silt and between sand and clay also were apparent (Fig. 1). The first two principal components explained approximately 92% of the total variability in the data, as indicated by the eigenvalues of the correlation matrix (Table 1). The first component accounted for 60.5% of the variability and the second accounted for 31.5% (Table 1). A scree plot of the eigenvalues against each principal component also showed that the eigenvalues tended to level off at the third principal component, suggesting that the first two principal components were enough to describe the data (Fig. 2). The eigenvalues of the third, fourth, and fifth principal components were close to zero, suggesting such components need not be considered. The percent of explained variance and the scree plot implied that the five measured variables nearly fell within a two-dimensional subspace of the

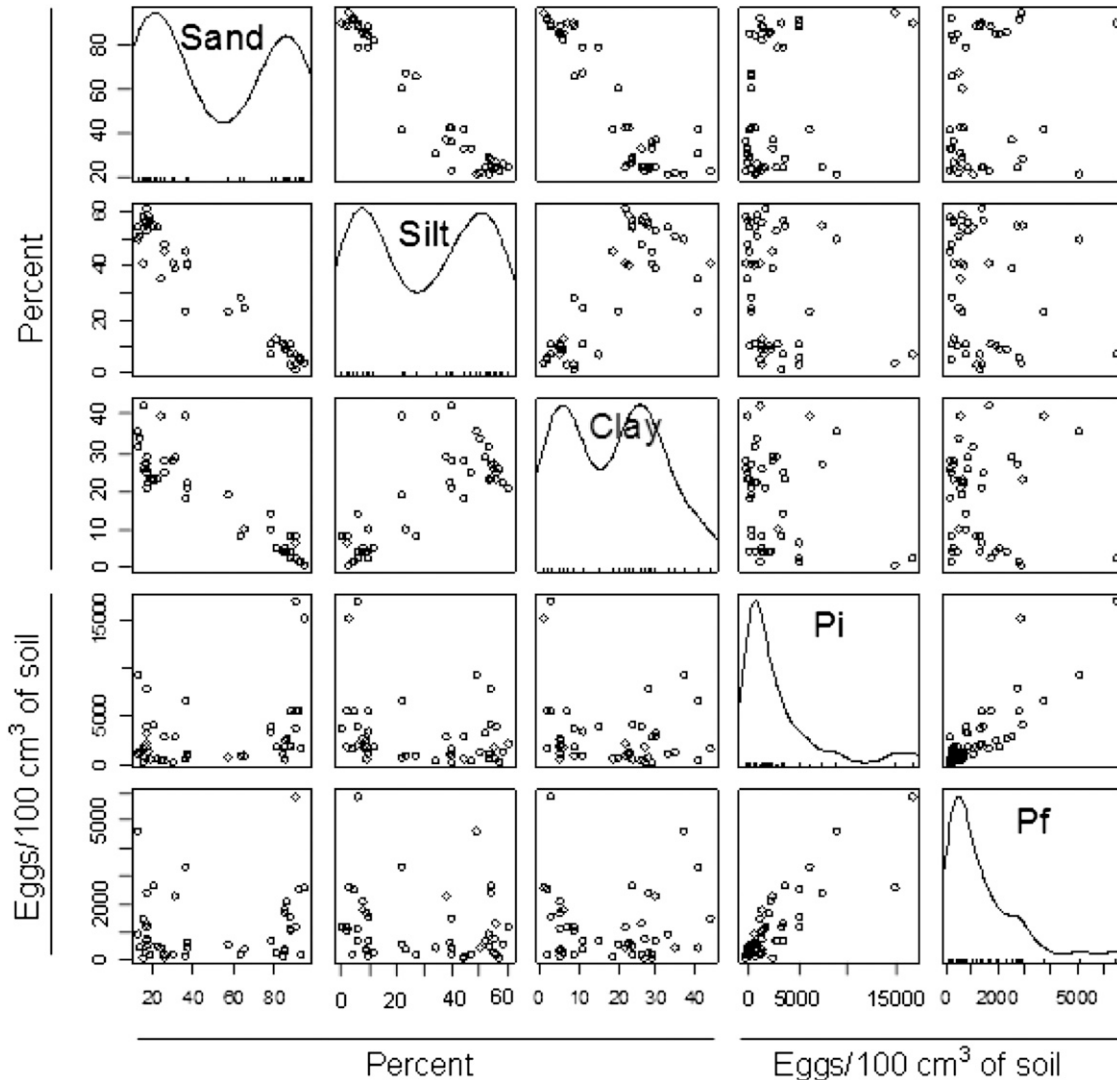


FIG. 1. Scatterplot matrix displaying the relationship of sand, silt, clay, SCN population density before (Pi; sampled in midspring before corn planting) and after annual corn rotation (Pf; sampled in midspring the following year before soybean planting). The labeled diagonal panel represents the distribution of each variable.

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

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

^a Each principal component represents a different linear combination of the variables sand, silt, clay, Pi, and Pf. SCN Pi = SCN population density (eggs/100 cm³ of soil) in midspring before corn planting (before corn rotation). SCN Pf = SCN population density (eggs/100 cm³ of soil) in midspring of the following year before soybean planting (after annual corn rotation).

sample space, or more simply, that some redundancy is implicit in the five variables. Overall, the first two principal components had a univariate normal distribution and meaningful interpretation, as indicated by their eigenvectors (Table 2), correlation coefficients of the variables with each component and significance level of those correlations (Table 3). The eigenvectors of the first principal component (PC1) represented a contrast of sand, Pi, and Pf vs. silt and clay: PC1 = 0.55 sand – 0.54 silt – 0.50 clay + 0.33 Pi + 0.23 Pf. Based on the values of the eigenvectors (Table 2) and their respective correlations (Table 3), this principal component was essentially a measure of soil texture itself. The second principal component (PC2) was also a contrast between sand and the rest of the variables: PC2 = – 0.22 sand + 0.16 silt + 0.29 clay + 0.60 Pi + 0.69 Pf. This component represented Pi and Pf.

Cluster analysis: The cubic clustering criterion (CCC) suggested the existence of two major clusters in the data, which was indicated by the occurrence of peaks

TABLE 2. Eigenvectors of the three principal components (PC1 to PC3) for five variables measured in 45 fields.

Variable	Eigenvectors		
	PC1 ^a	PC2 ^a	PC3 ^a
Sand	0.55	–0.22	0.13
Silt	–0.54	0.16	–0.52
Clay	–0.50	0.29	0.55
Pi ^b	0.33	0.60	–0.51
Pf ^c	0.23	0.69	0.38

^a Each principal component (PC1 to PC3) represents a linear combination of the variables sand, silt, clay, Pi, and Pf.

^b SCN population density (eggs/100 cm³ of soil) measured in midspring before corn planting (before corn rotation).

^c SCN population density (eggs/100 cm³ of soil) measured in midspring of the following year before soybean planting (after annual corn rotation).

at two and four clusters in the graphs of CCC vs. number of clusters (Fig. 3A). The plot of the pseudo T^2 statistics suggested that clustering occurred at seven, four, and two clusters. This is interpreted by looking at the plot from right to left until a value that is markedly larger than the previous value is found, then moving back to the right in the plot by one step in the cluster history (Fig. 3B). The hierarchical tree diagram revealed the existence of two major clusters or at least four subclusters (Fig. 4). This clustering result seemed plausible based on the plot of the scores of the first two principal components when two and four clusters were used for inspection of the clustering (Fig. 5). The clustering results of both the complete linkage and Ward's method also suggested two major groups or at least four subgroups in the data. The multiscale bootstrap analysis showed that the two clusters previously discerned were statistically supported by the data at $\alpha = 95\%$; AU values for both clusters were of 96 and 95

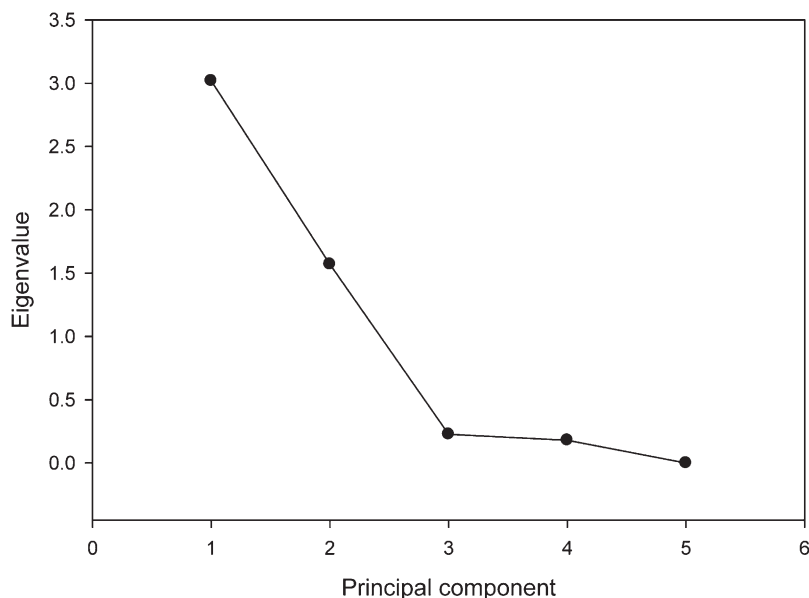


FIG. 2. Scree plot of the eigenvalues of the principal components from a data set consisting of 45 fields and five variables: sand, silt, clay, Pi, and Pf. Pi = SCN initial population density before rotation (sampled in midspring before corn planting), and Pf = SCN final population density after annual corn rotation (sampled in midspring the following year before soybean planting).

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

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

^a Each principal component (PC1 to PC3) represents a linear combination of the variables sand, silt, clay, Pi, and Pf.

^b SCN population density (eggs/100 cm³ of soil) measured in midspring before corn planting (before corn rotation).

^c SCN population density (eggs/100 cm³ of soil) measured in midspring of the following year before soybean planting (after annual corn rotation).

(Fig. 6) and standard errors of the P values were below 0.05 in most of the clusters.

SCN population density reduction in the identified clusters: Upon fitting the Poisson model, the equidispersion assumption was violated (overdispersion

parameter $\phi = 287.90$), thus justifying use of the negative binomial model. Overdispersion was no longer an issue with the negative binomial model ($\phi = 1.0$) and model goodness-of-fit was better than the intercept-only model (Table 4). The interaction between the covariate (Pi) and group was not statistically significant thus suggesting a common slope between the groups. The interaction term was dropped from the model. Significant linear association (slope = 0.68, $P < 0.0001$) was observed between Pi and Pf. The statistical model representing all the effects and sources of variation is written as:

$$\log(Pf_{ijk}) = \eta + F_i + G_j + \beta_1 X_{ij} + (FY)_{ik}$$

where

Pf_{ijk} is the mean SCN population density after annual corn rotation in the i th field ($i=1,2,\dots,45$) of the j th group (1,2) and k th year (1,2,3)

$$Pf_{ijk}|F_i, (FY)_{ik} \sim \text{Negative binomial}(\eta_{ijk})$$

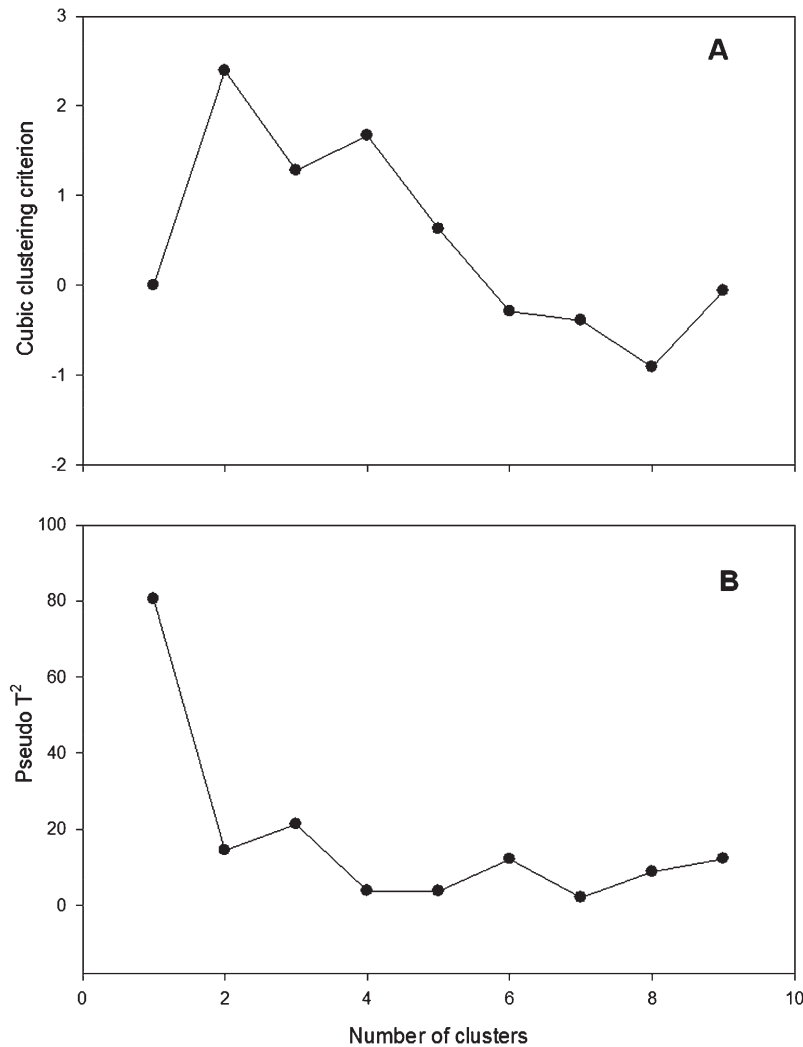


FIG. 3. Cubic clustering criterion (CCC; A) and Pseudo T^2 statistic (B) vs. number of clusters used to decide on the number of clusters in the data set. A peak in the CCC graph line and a depression in the Pseudo T^2 line are indicative of the number of clusters to choose.

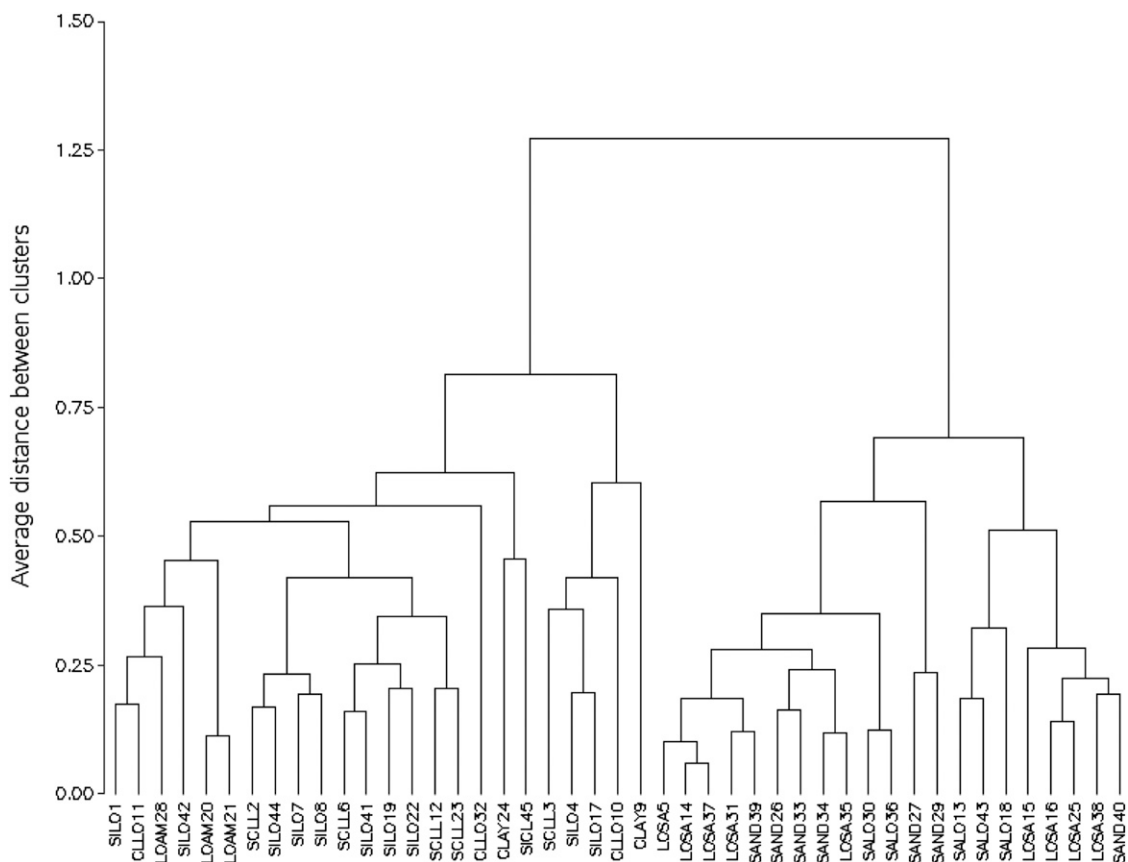


FIG. 4. Hierarchical tree diagram produced by the average distance clustering method. The leaves of the tree represent each of the nine soil textural classes determined in 45 fields sampled in the study along with the field identifier. SILO = silt loam, CLLO = clay loam, LOAM = loam, SICL = silty clay, CLAY = clay, SCLL = silty clay loam, LOSA = loamy sand, SAND = sand, and SALO = sandy loam.

Linear predictor: $\eta_{ijk} = \eta + F_i + G_j + \beta_1 X_{ij} + (FY)_{ik}$

Link function: $\eta_{ijk} = \log(pf_{ijk})$

η is the intercept or grand mean

F_i is the effect of the i th field ($i = 1, 2, 3, \dots, 45$)

G_j is the effect of the j th group ($j = 1, 2$)

X_{ij} is the covariate (P_i transformed to the natural log) measured at the i th field of the j th group

Y_k is the effect of the k th year ($k = 1, 2, 3$)

β_1 is the regression coefficient for the covariate

FY_{ik} is the field*year interaction effect

$$F \sim iid N(0, \sigma_F^2);$$

$$FY \sim N(0, \sigma_{FY}^2)$$

Considering the sources of variation included in the model, the estimated SCN Pf in the sand-predominant group was 543.7 eggs/100 cm³ of soil and in the silt-and-clay predominant group was 654.4 eggs/100 cm³ of soil. The analysis indicated that there were no significant differences in SCN Pf between the two groups ($F = 0.84$, $P = 0.36$) thereby also suggesting no significant differences in SCN population density reduction (Table 4). Year effect was not significant. Examination of the two major clusters revealed that fields with predominant silt and clay (textural classes silt loam, silty clay loam, silty clay, loam, clay loam, and clay) and lower SCN Pi were

placed in one group while fields with predominant sand (sand, loamy sand, and sandy loam) and higher SCN Pi were placed together in another group (Table 5). Because the clustering algorithm formed the clusters by computing the Euclidian distance among the textural proportions and SCN Pi and Pf, it follows from this clustering that SCN population density change after the annual corn rotation was similar in soils with a silt range of 23% to 61%, clay 6% to 44%, and sand 13% to 37%. Population density change also was similar in soils with a sand range of 57% to 95%, silt between 1% and 28%, and clay between 7% to 20%. The mean percent difference for each textural component between the two groups was 61% for sand, 38% for silt, and 22% for clay. Mean percent SCN population density decline after the annual corn rotation was 44% in the silt-predominant group and 56% in the sand-predominant group.

DISCUSSION

The main focus of this research was to describe the quantitative relationship of soil texture with the observed SCN population density reduction in fields annually rotated with corn in Nebraska. Supported by data representing multiple site-specific locations within

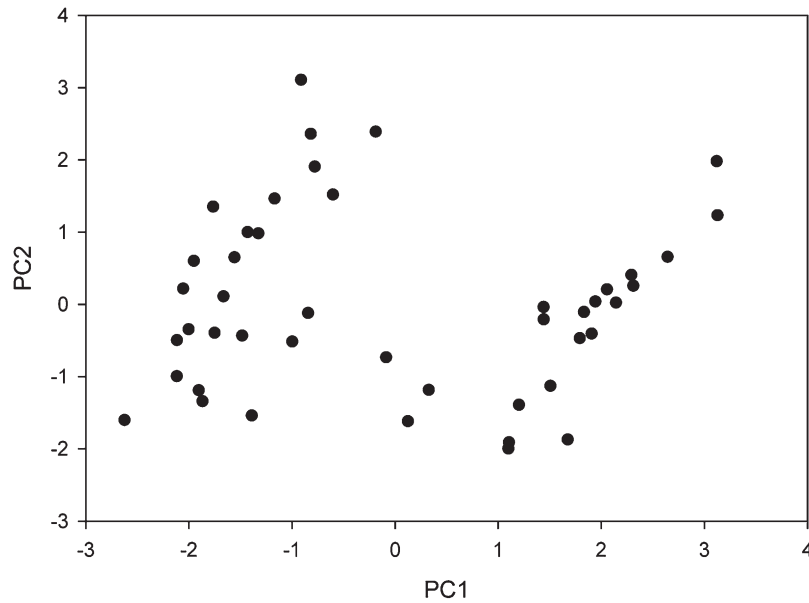


FIG. 5. Scatterplot of the first two principal components (PC1 and PC2) chosen to represent most of the variation in the data set in this study. PC1 represents the linear combination $0.55 \text{ sand} - 0.54 \text{ silt} - 0.50 \text{ clay} + 0.33\text{Pi} + 0.23\text{Pf}$ and PC2 the linear combination $-0.22 \text{ sand} + 0.16 \text{ silt} + 0.29 \text{ clay} + 0.60 \text{ Pi} + 0.69 \text{ Pf}$.

fields and a population of fields across the major corn and soybean growing areas in Nebraska, the present study is the first to examine SCN-texture relationship using the actual proportions of sand, silt, and clay. In the study, principal components and cluster analysis were instrumental to identify relationships between soil textural proportions and the observed SCN population densities before (Pi) and after annual corn rotation (Pf). In particular, cluster analysis was useful to guide a comparison of fields for SCN population density reduction, thus avoiding comparisons based on arbitrary criteria.

The two multivariate methodologies used in the present study are suitable to investigate relationships among variables (Johnson, 1998; Manly, 2005). Yet, although applicable in a wide range of studies dealing with multiple-variable data sets, use of these methodologies in SCN-related studies is scarce. In the present research, PCA produced two indices or components that were uncorrelated in order of their importance and that described most of the variation in the data. The first component not only revealed that the relative amount of sand increases whereas that of silt and clay decreases and vice versa, but also identified sand as the textural constituent that had the strongest association with SCN Pi and Pf in the corn rotation year. This relationship was not disclosed by the scatterplot matrix and may still not be apparent by simple correlation or other graphical or numerical univariate methods. The observed relationship between sand and SCN population densities suggested by PCA is in agreement with the results of studies in soybean in which higher SCN population densities were observed in coarse-textured soils than in fine-textured soils (Workneh et al., 1999).

Our data set encompassed a broad spectrum of different textural classes representing nine of the 12 classes of the USDA texture triangle and at least two main contrasting textural components. In reports of SCN-texture relationship in soybean, frequently two and rarely seven or more textural types have been assessed (Workneh et al., 1999; Avendaño et al., 2004). The second principal component revealed that SCN Pi and SCN Pf increased or decreased jointly during the annual corn rotation cycle. In previous studies on the effects of texture on SCN population densities, texture has been mainly considered as a categorical variable through traditional analysis of variance (Heatherly and Young, 1991; Workneh et al., 1999). Therefore, relationships between specific proportions of sand, silt, and clay with SCN population densities are inherently ignored and no direct quantitative associations can be inferred. The two principal components identified in the present analysis advise cautious consideration of the simultaneous correlations that exist between proportions of the three soil textural constituents and between sand and SCN population densities. Specifically, the first component suggests that if texture is to be used as an input numerical variable in a regression model, converting it into a texture index would be recommended to prevent collinearity problems and increase reliability in parameter estimation.

The analysis of clusters in the present study classified fields according to their similarity in soil texture and SCN population densities (Pi and Pf). The grouping identified by the main clustering algorithm concurred with that of the complete linkage and Ward's minimum variance clustering methods. The two major clusters, identically determined by the three clustering algorithms,

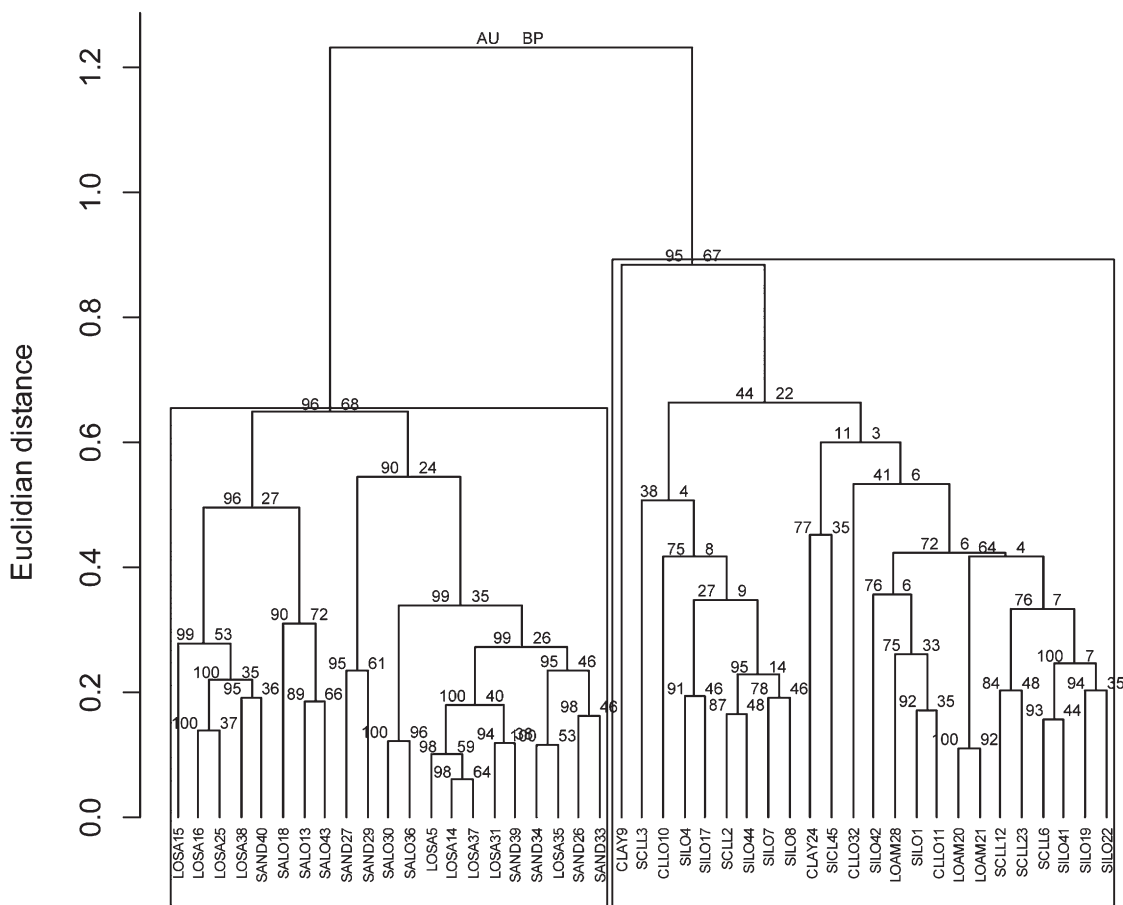


FIG. 6. Hierarchical clustering of 45 fields and five variables (sand, silt, clay, Pi, and Pf). Values at branches are the approximately unbiased (AU) *P* values (left); and bootstrap probability (BP) *P* values (right). Clusters with AU \geq 95 are indicated by the rectangles. The first rectangle from left to right is a cluster with AU = 0.96 and BP = 0.68.

were substantiated by the first two principal components and statistically supported by 1,000 simulations.

With the evidence gathered, it was concluded that two well-defined natural clusters existed in the data: one group with predominant silt and clay and other group with predominant sand. The SCN population density in both the silt- and sand-predominant clusters declined after the annual corn rotation. Observed declines of 44% to 56% fall within the 23% to 86% decline range observed in a clay loam soil in Minnesota after a single corn season (Pf measured at harvest) (Chen

et al., 2001). In the group comparison achieved by the negative binomial model, the lack of interaction between the covariate (Pi) and group suggested that the groups could be compared at a common Pi. A linear relationship between Pi and Pf, consistent with the results of the PCA, was identified with the analysis, but Pf did not differ significantly between the groups.

Altogether, the results of our study suggest that a trend of a slightly higher SCN population density reduction in sandy soils than in silt-and-clay soils may occur after annual corn rotation, but such a difference

TABLE 4. Tests of fixed effects for group comparison considering year and SCN population density before rotation (Pi) (left columns); and fit statistics (right columns) of the negative binomial (NB) generalized linear model used for the comparison. Last column corresponds to the intercept only model (IOM).

Effect	F value	P > F	Fit statistics		
				NB	IOM
Group ^a	0.84	0.36	-2Res Log Pseudo-Likelihood ^b	1045.16	5642.86
Year ^c	1.57	0.22	Generalized Chi-square/df ^d	1.0	1.26
Pi ^e	271.19	<0.0001			

^a Group corresponds to the two field groups identified by the cluster analysis.

^b Smaller value of the -2Res Log Pseudo-Likelihood indicates better fit of the NB model compared with the IOM.

^c Year effect.

^d Overdispersion parameter of the model.

^e Pi is the covariate SCN population density before rotation transformed to the natural log.

TABLE 5. Summary statistics of the five variables for each of the two major groups identified by the cluster analysis.

	Number of observations (field)	Variable	Mean	Standard deviation	Minimum	Maximum
Group 1 ^a	24	Sand	22	8.2	13	37
		Silt	49	9.3	23	61
		Clay	29	6.6	19	44
		Pi ^b	2,397	2,451	363	9,307
		Pf ^c	1,221	1,288	138	5,101
Group 2 ^c	21	PDC ^d	44	25	1	95
		Sand	83	10	57	95
		Silt	10	7	1	28
		Clay	7	4	1	20
		Pi ^b	3,861	4,339	651	16,954
		Pf ^c	1,420	1,428	208	6,461
		PDC ^d	56	27	-9 ^f	86

^a Fields with silt- and clay-predominant soil texture.

^b SCN population density (eggs/100 cm³ of soil) measured in midspring before corn planting (before corn rotation).

^c SCN population density (eggs/100 cm³ of soil) measured in midspring of the following year before soybean planting (after annual corn rotation).

^d Relative SCN population density change [(Pf-Pi/Pi) × 100] during the rotation year.

^e Fields with sand-predominant soil texture.

^f Value corresponds to a field in which Pf was higher than Pi.

appears to be not significant, at least under the agroclimatic conditions of Nebraska. These results, however, should be interpreted carefully because SCN population density change in our study was measured during one nonhost (corn) growing season followed by an overwintering period. The soil texture effect on *H. glycines* as assessed in this study can be conceptualized as to occur during two periods: the corn season and the off season or overwintering. In both periods, the texture-SCN association is affected by other physical and chemical soil properties, soil biological communities, agronomic practices, and weather. Tillage, pH, soil temperature, and soil moisture for example, are reported to affect SCN and to depend on soil texture (Alston and Schmitt, 1988; Koenning et al., 1988; Francl, 1993; Workneh et al., 1999; Pedersen et al., 2010). In particular, texture is directly related to water retention in the soil, and during the overwintering period it largely determines soil thermal conditions, hence the survival and reproduction of SCN. Therefore, other factors not considered in the study could have contributed directly or through interaction with texture to the observed SCN population density reduction.

Soil texture governs several hydrological, chemical, and biological soil processes (Hillel, 2004) and its effects on population densities of *H. glycines* (mainly in soybean) have been described in several studies (Koenning et al., 1988; Heatherly and Young, 1991; Workneh et al., 1999; Avendaño et al., 2004). A common observation is that higher population densities occur in coarse-textured soils than in fine-textured soils, which was also suggested in our study by the PCA. The data in the present research included nine different textural classes and a broad range of textural proportions so it is probable that cyst extraction varied among soil samples of different textures. Extraction efficiency of cysts of other cyst nematodes is reported to be affected by soil texture and found to be higher in sandy than in clay soils

(Bellvert et al., 2008). In any event, if the SCN-sand relationship observation is true, then increased SCN population densities would be consistently found in sand, but whether or not SCN mortality during annual corn rotation is exacerbated by sand remains unclear.

Soils with high clay content have increased particle-to-particle contact thus they exhibit prolonged saturation after irrigation or rain events, lower oxygen diffusion rates, and higher electric conductivity than do soils with coarse textures (Hillel, 2004). Sandy soils tend to have increased aeration, but when retaining moisture in winter they freeze faster than finer-textured soils. Based on this rationale, the hypothesis that sand may be more closely associated to SCN mortality than silt and clay during the overwintering period seems plausible. However, within the latitudinal range of the sampled areas in this study the soil climatic gradients, especially winter freezing temperatures, may not be drastic enough for the effect of texture on SCN to be fully manifested. To this regard, Jackson et al. (2005) found that *H. glycines* egg viability did not differ between overwintering months in Missouri. Assessments in broader latitudinal ranges, encompassing more northern areas than Nebraska would be useful to test this hypothesis.

Numerous studies have documented the benefit of using corn rotation in reducing SCN population densities in infested fields, most of them primarily assessing reduction at the end of the corn growing season (Ross, 1962; Koenning et al., 1993; Chen et al., 2001; Porter et al., 2001). In our study, SCN population density change was assessed for an annual period that encompassed a single corn season and an overwintering period. However, no similar assessments were made during years in which host soybeans were grown to allow for a comparison between such scenarios. Corn rotation almost always results in greater SCN population density suppression than resistant or susceptible soybean cropping sequences (Chen et al., 2001), but suppression owing to the overwintering period is still not fully understood.

This study confirms the claim that higher SCN population densities have a stronger relationship with sand than with silt and clay, yet our results are insufficient to make definitive conclusion whether or not SCN population reduction after annual corn rotation is higher in sand than in silt or clay soils. Assessment of additional factors such as amount of rainfall in the season, winter soil temperature, soil pH, and tillage will enhance understanding of SCN mortality during annual corn rotation and will be key pieces to better understand the effect of management practices on SCN population densities.

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