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Spatial Analysis of *Heterodera glycines* Populations in Field Plots[1]

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Abstract: Spatial heterogeneity in nematode population densities presents an obstacle to the precise determination of infestation levels. Three field plots were intensively sampled for soybean cyst nematode (*Heterodera glycines* Ich.) cysts before and after spring cultivation to quantify the spatial attributes of the population. Population density strata were detected running parallel to plant rows. Highest population densities before cultivation were found in the plant row and the middle furrow. Population density in the plant row averaged 26% higher and 4% lower than the whole-plot mean before and after cultivation, respectively. Cysts containing fewer than 25 eggs were not stratified, indicating that most were produced before the previous season. Sample population counts were fit to the negative binomial distribution model before cultivation, but distributions differed among plots. The Neyman type A and negative binomial distributions both fit the data after cultivation disturbed the soil. Population clusters 1–3 m long were detected in plant beds before cultivation. Heterogeneity in population density increased with plant row length after cultivation. Optimum plot length for minimal spatial heterogeneity in four-row mechanically tended field plots was estimated at 6 m after trimming plot ends.

Key words: dispersion, *Heterodera glycines*, soybean cyst nematode, population ecology, stratification.

Estimates of nematode field populations require sampling procedures that are accurate and affordable. Nematode mobility, population density, and dispersion are spatial characteristics of a population that affect the error rate of a sampling procedure. Reports on nematode spatial analysis primarily have dealt with providing a scientific basis for nematode management in areas larger than 0.45 ha (2,7,10). Population density estimates often must be more accurate in field experimentation conducted in small plots than in nematode management (3,14). This paper reports a spatial analysis of soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, populations in field plots prior to an evaluation of sampling procedures.

Materials and Methods

Three contiguous field plots in Portageville, Missouri, were selected for study. The soil was a Tiptonville silt loam (Typic Arguidolls) with a sandy loam overwash. Each plot consisted of four 13-m-long rows on 96.5 cm centers. Soybeans, *Glycine max* (L.) Merr. cv. Forrest, were grown in 1982.

Plots were sampled on 21 April and 31 May 1983. The first date reflects a soil undisturbed over the winter. The first sample therefore represented the spatial dispersion of SCN cysts at the end of the previous season. The second sample was taken after the plots were disced and bedded.

A 2 × 12-m area covering the inner two rows and bordering furrows was sampled by dividing the area into a 9 × 25 grid. Each quadrat in the grid was 25 × 50 cm, with the long axis parallel to the plant row. A 19-mm-d × 20-cm-deep soil core (58 cm^3) was removed from each grid intersection. A second sample was taken from each plant row quadrat, 10 cm from the first core. Each soil core was identified as to location and processed separately. Vertical stratification was not examined, but the bulk of the SCN population presumably was included in the core since about 90% of the soybean root biomass may occur in the upper 15 cm of soils (11). Finally, multiple-core samples were taken from the plant row, the furrow, and the bed shoulder to measure soil moisture gravimetrically.

Soil wet weight was measured for each sample, and cysts were extracted from the soil by a semiautomatic elutriator with an extraction efficiency of about 75%. Cysts were classified according to egg content while counting to determine if there was a relationship between the condition of the cyst and location. Cyst categories were fewer than 25 eggs, 25 eggs to ¾ full, and ¾ full to full of eggs. The data base consisted

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of 825 and 550 observations taken before and after cultivation, respectively.

Analyses: The hypothesis first tested was whether SCN populations were in density strata parallel to the direction of the plant row. Horizontal strata were identified as the approximate midpoints (± 3.5 cm) of the plant rows, the furrows, and the shoulders of the beds. Exploratory linear models indicated that strata were discernible ($P < 0.01$) in each plot before and after cultivation. Average population densities closely corresponded between the two plant rows, between the two outer furrows, and among the bed shoulders. The final model viewed strata as distance from the middle furrow with direction disregarded.

Frequency distributions of cysts within plant row strata were analyzed to determine SCN population dispersions. The analysis was restricted to the plant rows as a conservative approach to avoid mixing possibly heterogeneous dispersions while maximizing the number of observations per plot (n = 100). Dispersion first was tested by subjecting a mean-to-variance index of dispersion (ID, equation 1) to a chi-square test (8). ID approximates a chi-square variate with n − 1 degrees of freedom and thus the mean-variance ratio is tested for probability of departure from equality. The variance should equal the mean if dispersion is random. A uniformly dispersed population would have a variance lower than the mean, whereas population aggregation would result in a variance higher than the mean.

$$ID = \frac{(n-1)s^2}{\bar{x}} \qquad (1)$$

The sample frequency distribution of SCN in plant rows was tested further for goodness of fit to the Neyman type A (NTAD) and the negative binomial (NBD) distributions (5). The NTAD and the NBD model aggregated populations and are generated mathematically by compounding two Poisson distributions and by compounding a Poisson and a logarithmic distribution, respectively (12). The NTAD describes a dispersion that is less aggregated than the NBD, but overlap in the goodness of fit is possible because of the flexibility of using two parameters to shape the distributions. Goodell (6) posited edaphic and rooting pattern factors that should cause the sample frequency distributions of most plant-parasitic nematode field populations to be fit by a model of aggregation.

The scale of heterogeneity was determined for SCN populations in plant beds by two-term local variance analysis (9). Quadrats 0.5 m square were formed by averaging two counts from the plant row and two counts from the bed shoulders. The local variance between SCN densities in quadrats was calculated as a moving average along the length of the plant bed (equation 2). Contiguous quadrats then were combined to form larger blocks (e.g., equation 3) and a FORTRAN program was written to compute the average local variance between block lengths of 0.5–6.0 m in 0.5-m intervals. An additional quadrat size of 2 × 0.5 m was formed by averaging data across the width of the sampled area, and local variances were computed as before.

$$\text{Variance}_{(0.5\text{ m})} = \frac{1}{n-1} \cdot \left[\frac{1}{2}(x_1 - x_2)^2 + \frac{1}{2}(x_2 - x_3)^2 + \ldots + \frac{1}{2}(x_{n-1} - x_n)^2\right] \qquad (2)$$

$$\text{Variance}_{(1.0\text{ m})} = \frac{1}{n-3} \cdot \left[\frac{1}{4}(x_1 + x_2 - x_3 - x_4)^2 + \frac{1}{4}(x_2 + x_3 - x_4 - x_5)^2 + \ldots + \frac{1}{4}(x_{n-3} + x_{n-2} - x_{n-1} - x_n)^2\right] \qquad (3)$$

SCN population density in plot 1 was contour mapped to visualize the two-dimensional dispersion pattern before and after cultivation. A SAS/GRAPH algorithm (13) was used with contour intervals set at the 10th, 25th, 75th, and 90th percentiles to point out high and low density areas.

TABLE 1. Summary statistics for *Heterodera glycines* population densities in field plots before and after primary cultivation in the spring.

	Mean cysts/ core	Variance	Median	CV
Before cultivation				
Plot 1	18.6	277	14	89
Plot 2	10.2	77	7	86
Plot 3	12.7	90	10	75
After cultivation				
Plot 1	11.3[1]	61	9	69
Plot 2	12.5	70	10	67

TABLE 2. Horizontal stratification of *Heterodera glycines* population densities in field plots. Distance is measured from the middle furrow with the plant row located at 50 cm.

Distance (cm)	Cysts/ soil core	¾ full to full (%)	25 eggs to ¾ full (%)	24 eggs or fewer (%)
Before cultivation				
0	16.5 a	49 bc	38 a	13 a
25	11.0 b	48 bc	38 a	14 a
50	18.2 a	55 a	30 b	15 a
75	12.0 b	51 ab	36 a	13 a
100	11.8 b	44 c	40 a	16 a
*P**	< 0.01	< 0.05	< 0.01	
After cultivation				
0	8.7 c	41 a	32 a	26 a
25	14.6 a	44 a	31 a	24 a
50	11.4 b	46 a	31 a	24 a
75	13.8 a	45 a	31 a	24 a
100	9.3 c	48 a	29 a	23 a
*P**	< 0.03			

* Probability that values in a column followed by different letters are equal according to an LSD mean separation test.

RESULTS AND DISCUSSION

Summary statistics of SCN populations in each field plot are presented in Table 1. The mean number of cysts per soil core averaged 35% and 25% more than the median before and after cultivation, respectively. Expressing population density as average cysts per soil core volume resulted in values that averaged 5% more than the alternative expression of cysts per 100 g soil dry weight. The coefficients of variation were similar, so there was no advantage to either expression.

Horizontal stratification: The highest cyst population densities before cultivation were located in the plant row and middle furrow, whereas low densities occurred in the bed shoulders and outer furrows (Fig. 1A). The plant row populations averaged 26% more than whole plot populations before cultivation. Polynomial regression models included 3rd and 4th power terms and lacked the ability to predict population density as a function of strata due to low coefficients of determination (r^2).

In small areas, the spatial dispersion of sedentary nematodes similar to SCN logically is related to root distribution (4,6). It is hypothesized that root density was high under the plant row and in furrows where the root systems from the two rows overlapped. However, low SCN populations in all outer furrows but one before cultivation remains unexplained by this hypothesis. Soil bulk density (core dry weight/58 cm^3) was examined because of a suspected relationship between soil compaction due to wheel traffic in the outer furrows and low cyst density. Bulk densities before cultivation in the outer furrows and adjoining shoulders were significantly higher ($P < 0.05$) than the strata enclosed by the plant rows. Bulk density before cultivation, however, had no correlation with cysts per soil core ($r = -0.05$, $P = 0.16$). Small errors in taking a soil core of the proper length would lower the precision of bulk density estimates and decrease correlation with cyst counts.

Greatest SCN population densities after cultivation were found in newly formed bed shoulders, and lowest cyst densities occurred in furrows (Fig. 1B). The plant row strata had a population density that averaged 4% fewer cysts, compared with the whole-plot density. These results can be attributed to tillage practices which displaced soil from furrow areas to build up plant bed height.

The cyst classification scheme was analyzed to determine the relationship between the putative age of the cysts and strata. Stratification was found before but not after cultivation for the categories ¾ full to full and 25 eggs to ¾ full (Table 2). The percentage of cysts containing fewer than 25 eggs was not significantly different among strata on both sampling dates, indicating that nearly all of these cysts were produced prior to the previous season. The

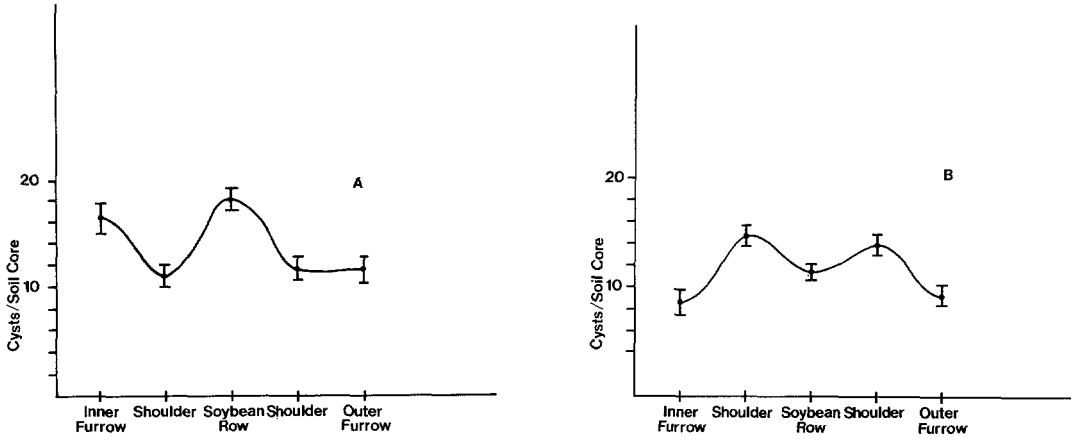

FIG. 1. Number of *Heterodera glycines* cysts ± one standard error in relation to plant row. A) Before cultivation. B) After cultivation.

percentage of full and partially full cysts declined from the first to the second date in roughly equal proportions, most likely releasing juveniles into the soil and replenishing the number of cysts with few eggs. The oldest cysts seemed to be adequately categorized as containing fewer than 25 eggs.

Dispersion analysis: The random and uniform dispersion hyoptheses, as indicated by chi-square tests on the indices of dispersion, were rejected ($P < 0.001$) for every frequency distribution both before and after cultivation. The sample variance was greater than the mean in every instance, implying aggregation in the population (8). The category of cysts with fewer than 25 eggs was also tested for randomness because previous cultivations presumably could redistribute older cysts. Once again, random dispersion was rejected ($P < 0.001$) in every case because of large sample variance.

Before cultivation, the NBD fit the frequency count data but the NTAD fit the data from only one plot (Table 3). The fit of NBD to sample frequency counts could be due to randomly located colonies within a stratum compounded with a logarithmic distribution of individuals within a colony, whereas the fit of the NTAD would imply randomly located colonies within a stratum compounded with a random placement of individuals within a colony (12). The latter explanation either does not hold for the SCN population before cultivation or the resolution of colonies and individuals was too coarse. When analyzed separately, the two component rows of plot 1 had greater probabilities of fit to the NBD ($P = 0.28$

TABLE 3. Chi-square goodness of fit for *Heterodera glycines* frequency distribuutions (n = 100/plot) to the Neyman type A and negative binomial distributions.

	Neyman type A parameters			Negative binomial parameters		
	v	a	$P > \chi^2$	$\bar{x}$	k	$P > \chi^2$
Before cultivation						
Plot 1	7.28	3.19	< 0.01	23.2	1.88	0.06
Plot 2	4.05	3.21	0.55	13.0	2.31	0.99
Plot 3	5.67	3.23	0.01	18.3	2.68	0.26
Combined	6.00	3.03	< 0.01	18.2	2.00	< 0.01
After cultivation						
Plot 1	2.38	4.34	0.75	10.3	3.68	0.96
Plot 2	3.44	3.62	0.51	12.4	2.58	0.95
Combined	3.00	3.79	0.16	11.4	2.93	0.85

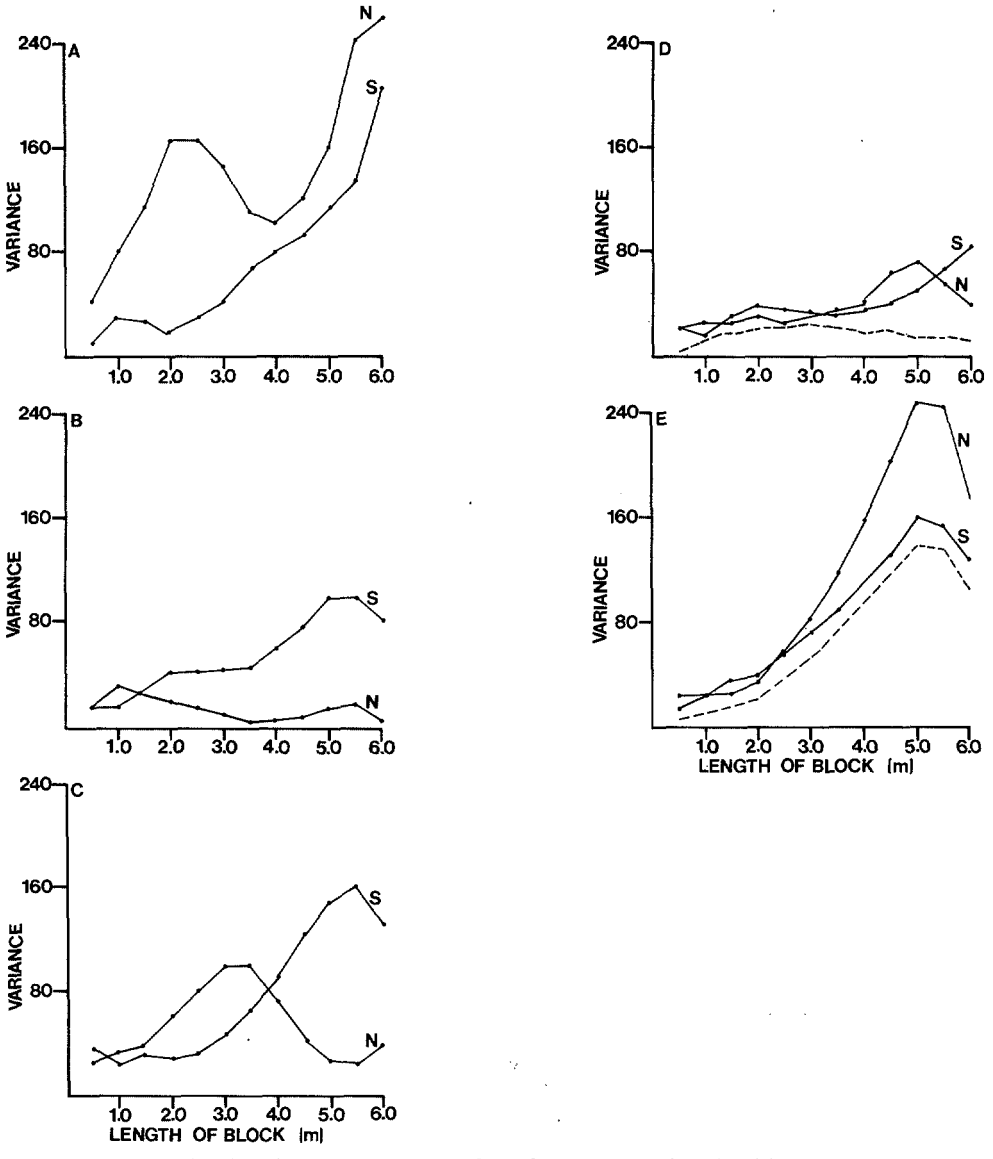

FIG. 2. Two-term local variance among *Heterodera glycines* population densities. A–C) Plots 1–3 before cultivation. D, E) Plots 1 and 2 after cultivation. North and south rows designated by N and S. Dashed lines in D and E are from a quadrat analysis that covered the width of the sampled area.

and 0.34) than did the two rows combined ($P = 0.06$). This result, as well as the low probability of fit to the NBD of the frequency distributions in plots 1–3 combined demonstrates that merging heterogeneous distributions can result in a poor fit.

After cultivation, the frequency distributions of the two plots apparently were homogeneous and their combination resulted in fits to the NBD and NTAD (Table 3). The fit of the NTAD and higher k values for the NBD after cultivation indicate that tillage dispersed population clumping (see also Fig. 3B). Soil mixing by tillage was insufficient to break up population clusters entirely.

Local variance analysis: Dispersion analysis indicates the presence or absence of population aggregation, whereas local variance analysis graphically displays the size of discontinuous areas (Fig. 2). The scale of heterogeneity was variable from row to row. Before cultivation, two rows contained a peak at the 2–3-m block length

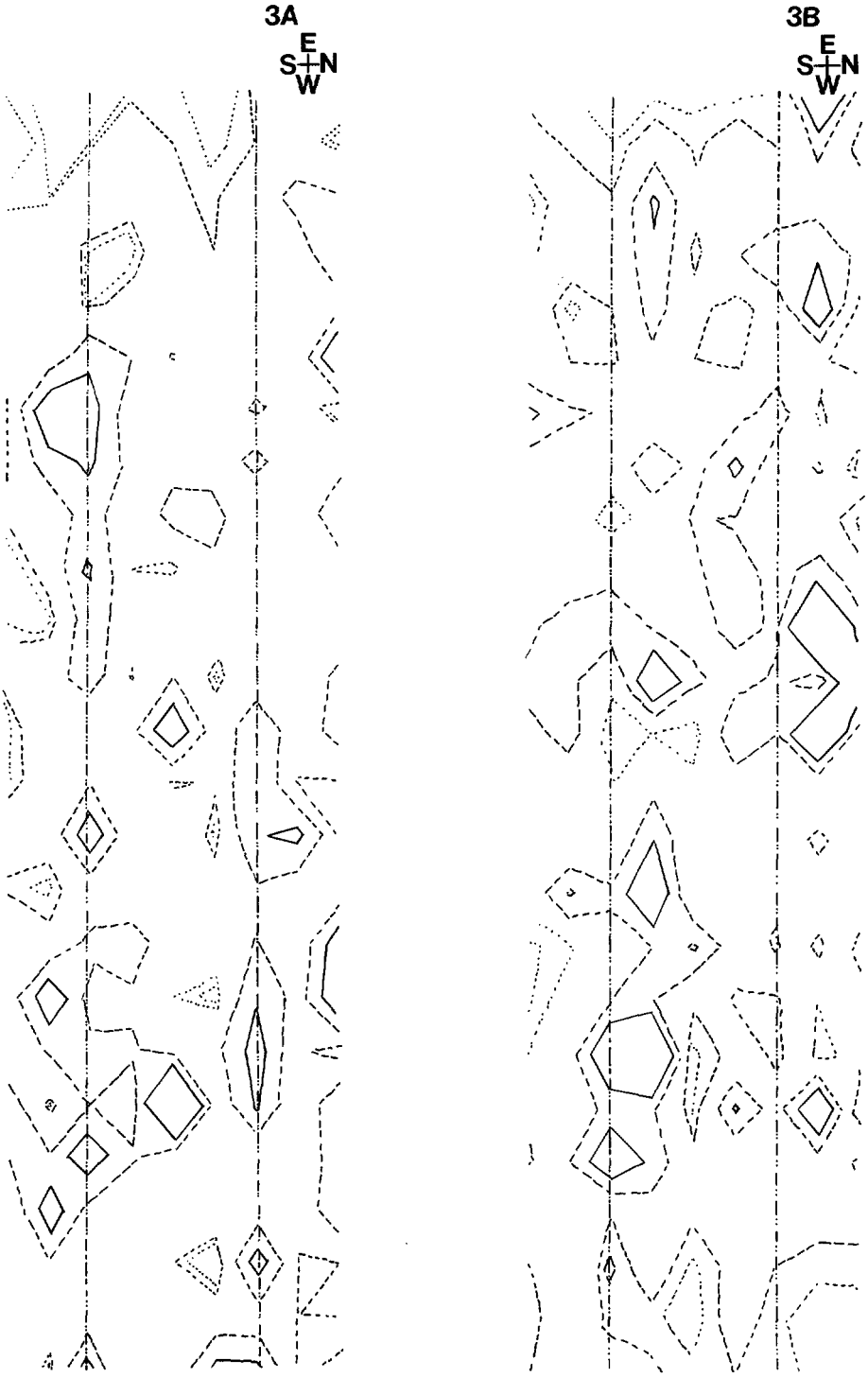

FIG. 3. Contour maps of *Heterodera glycines* population densities in plot 1 with vertical lines to mark the soybean rows. Contour lines drawn at 10th, 25th, 75th, and 90th percentiles. A) Before cultivation, percentiles set at 5, 7, 25, and 43 cysts per soil core. B) After cultivation, percentiles set at 3, 6, 15, and 22 cysts per soil core.

and two other rows had a small peak at 1 m. Results from using the mean of four sample counts were comparable to those using only in-row data. The in-row local variance of population densities 10 cm apart was greater than variation among 50 cm spacings in three of six rows before cultivation, suggesting that there may be a scale of heterogeneity at or below 10 cm. Therefore, a peak at 1–3 m may or may not represent a nematode colony. This problem should be studied further in a soil that is not disturbed annually.

After cultivation, local variance generally increased as the block size became larger, reaching maxima at 5–6 m. Variation in plot 1 was stable until reaching a block size of 4 m, while variation in plot 2 began to increase markedly at 2 m. Therefore, since two blocks were being contrasted the longest lengths of rows that had some degree of homogeneity were 8 and 4 m. A change in quadrat size to one that spanned the width of the sampled area stabilized the local variance in plot 1 but had no effect in plot 2. These results imply that greater local control over experimental plots could be achieved by plot lengths shorter than 12 m. A lower limit of about 6 m in plot length after late season end trimming (15) usually is imposed by machinery considerations. The optimum size for yield determination in manually tended soybean plots is 8 m^2 (1). Therefore, the plot length for four-row mechanically tended SCN-soybean field plots should be approximately 6 m, resulting in a 24-m^2 plot, as close to optimum as is practically feasible.

Contour maps illustrate heterogeneity qualitatively for plot 1 (Fig. 3). High population clusters can be seen in the plant rows, in the middle furrow, and in one of the outer furrows before cultivation. The low density at the east end of the plot in Figure 3A could indicate a border effect between the soybean plot and grass strip. The north soybean row shows high and low density areas, each 6 m in length. The south soybean row has several features which resulted in an average scale of heterogeneity at 2.0–2.5 m.

Spatial heterogeneity in SCN population densities presents an obstacle to the precise determination of nematode infestation levels. This problem exists even in the relatively small area of field plots and is an important source of uncertainty in field experimentation.

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