Journal of Nematology 31(1):45–53. 1999.

Changes in *Heterodera glycines* Egg Population Density in Continuous *Glycine max* over Four Years[1]

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Abstract: Soybean cyst nematode, *Heterodera glycines,* is found throughout soybean production areas of the United States, but the nematode's distribution is not uniform within states, counties, and individual fields. The goal of this research was to determine the spatial pattern of *H. glycines* population density in a field in southeastern Missouri and whether it changed over time in the absence of management practices. Geostatistical methods were used to describe and map the distribution of *H. glycines* over 4 years in a soybean (*Glycine max*) field in southeastern Missouri. Semivariograms and kriging, an interpolation method, were used to prepare isoarithmic contour maps and associated error maps. In the field studied, fall *H. glycines* population density (Pf) was poorly related to density the following spring (Pi). The distribution of peak *H. glycines* population density within the field changed from year to year, although high densities were often detected in the same general region of the field. The patchiness of *H. glycines* distribution within a field was verified. Yield was not related to *H. glycines* egg population density at planting, indicating that unmeasured variables were also reducing yield.

Key words: detection, distribution, *Heterodera glycines,* Missouri, nematode management, population density, precision farming, semivariance, soybean, spatial statistics, variable rate technology.

Soybean cyst nematode, *Heterodera glycines* Ichinohe, is a major yield-limiting pest of soybean (*Glycine max* (L.) Merr.) in the north-central United States and causes an estimated $250 million loss annually in that region (Doupnik, 1993; Wrather et al., 1995). Under stress conditions and in the southern United States, plants can be stunted, chlorotic, or die because of poor root growth. Under good growing conditions in the northern United States, there are no aboveground symptoms of the nematode's presence (Riggs and Niblack, 1993). Detection of the nematode involves examining roots for presence of cysts or white females on the roots followed by soil sampling. Once the nematode has been detected in a field, producers are advised to sample entire fields at harvest to monitor *H. glycines* egg population densities. Yield loss depends partially on Pi (egg population density in spring) (Francl and Dropkin, 1986). Yield loss from the nematode in successive years can be reduced by lowering the egg population density once the nematode has been detected in a field.

Because plant-feeding nematodes are distributed in patches in fields, estimating population density for management decisions is difficult and costly (Ferris et al., 1990; Francl, 1986; Goodell and Ferris, 1981; Noe and Campbell, 1985). Numerous studies have been conducted to determine the number of soil samples needed to accurately predict plant-parasitic nematode population density (Ferris et al., 1990; Francl, 1986; Goodell and Ferris, 1981; McSorley, 1982; McSorley and Dickson, 1991; McSorley and Parrado, 1982; Prot and Ferris, 1992; Schmitt et al., 1990; Schomaker and Been, 1992). However, the extensive sampling needed to provide precise estimates is not cost effective. New strategies are needed to optimize sampling of plant-parasitic nematodes for management decisions.

Received for publication 28 May 1998.
[1] Supported in part by agreement No. 060 from Missouri Soybean Merchandising Council. Contribution from the University of Missouri Agricultural Experiment Station. Journal Series No. 12,775. Mention of a trade name, warranty, proprietary product, or vendor does not constitute an endorsement of a product and does not imply its approval to the exclusion of other products or vendors that may also be suitable.
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The authors thank David Smith, Steve Taylor, Stephen Penn, Jose Cardozo, and Laura Hinshaw for their technical assistance, and Terry Niblack and James English for their critical review of this manuscript.
This paper was edited by A. F. Robinson.

Understanding why nematode distribution changes over time would allow focused sampling and may provide a better basis for recommending economical management strategies. Before a focused sampling pattern is devised, more information is needed on the spatial distribution of nematodes in fields over time. Geostatistics is a statistical tool that describes the spatial continuity essential to many natural phenomena (Journel and Huibregts, 1978) and is one approach that could be used to characterize or model the variability of nematode population density within fields. In geostatistical analysis, spatial relationships of sampled values for a regionalized variable are used for interpolation (i.e., prediction) of values at nearby unsampled locations. Isoarithmic maps, consisting of interpolated contours of nematode population density or other regionalized variables can be prepared with associated maps of error by means of kriging, an interpolation technique.

Research objectives were to: (i) determine if there was any spatial dependence during a single growing season of soybean yield or *H. glycines* Pi or Pf (egg population density in fall); (ii) measure the spatial variability of *H. glycines* Pi and Pf, and soybean yield in a field over time; (iii) measure the consistency of spatial dependence of *H. glycines* Pi and Pf as related to soybean yields over time.

Materials and Methods

The experimental field site was located at the Delta Center experimental farm of the University of Missouri near Portageville in southeastern Missouri. The site was level, and the soil type was a Tiptonville silt loam (fine, silty, mixed, thermic Typic Argiudoll) with pH 6.1 and 1.5% organic matter. The *H. glycines*-susceptible cultivar Essex was planted and grown in monoculture from 1990 through 1993. The field was divided into 100 plots (grid cells) that measured 12.2 m by 12.8 m.

Essex soybean was grown 1 year before nematode sampling was started in 1990. Soybean grain yield and plant height were measured from the center four rows of each plot from 1990 through 1993. Over the same period, soil samples for extraction of *H. glycines* eggs were collected at planting (20 May 1990, 10 June 1991, 20 June 1992, 18 June 1993) and at harvest (25 October 1990, 24 October 1991, 7 November 1992, 29 October 1993) in the center of each plot. Samples consisted of eight soil cores 2.5-cm diam. by 30 cm deep. Cysts were extracted from a 100-cm^3 subsample with a semiautomatic elutriator after the soil was passed through a 0.6-cm-pore screen (Byrd et al., 1976) following the techniques described in Niblack et al. (1993). Eggs washed from the sieve were combined with 1 ml acid fuchsin stain (Southey, 1970) and heated to boiling. Stained eggs and eggshells in the suspension were counted. For 1990 through 1992, one sample per plot was processed as described for both at planting and harvest sample dates; two subsamples were processed per plot at planting and harvest in 1993. Boxplots were prepared to describe variability in Pi and Pf, and soybean yield across the entire field over time (Helsel and Hirsch, 1992).

Egg counts were collected as part of another experiment that was overlaid on the plots in the field. The experiment was a strip-split-plot design with four replications. The preemergence herbicides alachlor, trifluralin, metribuzin, and imzaquin were applied at recommended rates to the main plots, and the soil insecticides carbofuran, tefluthrin, thiodicarb, and ethoprop were applied to the subplots. Weed control was visually rated 1 month after planting and before the postemergence herbicides bentazon plus acifluorfen were applied to the entire field to eliminate weed competition effects on yield. The major weeds present were common cocklebur (*Xanthium strumarium* L.), pigweed (*Amarathus* spp.), goosegrass (*Eleusine indicae* (L.) Gaertn.), ivyleaf morningglory (*Ipomoea hederacea* (L.) Jacg.), prostrate spurge (*Euphorbia humistrata* Engelm. ex Gray), crabgrass (*Digitaria* spp.) and jimsonweed (*Datura stramonium* L.). None of the weeds present are hosts for *H. glycines* (Riggs, 1992). Despite other reports in the literature (Altman,

TABLE 1. Semivariogram functions for various regionalized variables describing the distribution of *H. glycines* across a field.[a]

Variable	Time measured	Best semivariogram function	Nugget variance (C_0)[b]	Sill (Structural + nugget variance) ($C + C_0$)[c]	Model range parameter (A_0)[d]	Model range parameter (A_1)[e]	Model range parameter (A_2)[f]	r^2
Eggs	Spring 1990	Isotropic (linear)	73,357	124,548	142.4	—	—	0.73
Eggs	Spring 1991	Anisotropic (linear)	185,600	212,597	—	73.2	73.2	0.55
Eggs	Spring 1992	Anisotropic (exponential)	79,400	126,900	—	24.0	2,171.0	0.67
Eggs	Spring 1993	Anisotropic (spherical)	46,420	58,280	—	69.0	2,175.0	0.28
Eggs	Fall 1990	Anisotropic (exponential)	4,410,000	16,200,000	—	25.5	29.1	0.40
Eggs	Fall 1991	Isotropic (spherical)	1,400,000	2,008,000	129.1	—	—	0.68
Eggs	Fall 1992	Isotropic (spherical)	3,830,000	5,321,000	118.0	—	—	0.90
Eggs	Fall 1993	Anisotropic (spherical)	1,000	20,574,000	—	19.4	19.5	0.11
Yield	1990	Anisotropic (linear)	1,245,000	2,043,432	—	114.0	2,228.0	0.53
Yield	1991	Anisotropic (exponential)	145,600	192,300	—	22.0	2,141.0	0.78
Yield	1992	Isotropic (linear)	104,110	192,030	142.4	—	—	0.95
Yield	1993	Anisotropic (spherical)	411,000	362,260	—	12.3	74.27.0	0.49

[a] Based on a sample spacing of 12.2 by 12.8 m.
[b] C_0 = nugget variance ≥ 0 corresponds to unexplained measurement or sampling error independent of the sampling or lag distance.
[c] C = structural variance $\geq C_0$, which is a measure of spatial dependence on the sampling or lag distance.
[d] $C + C_0$ = sill, which is the lag distance at which the semivariance becomes independent of the sampling or lag distance.
[e] A_0 = range parameter for isotropic models.
[f] A_1, A_2 = range parameters for the major and minor axis of anisotropic models, respectively.

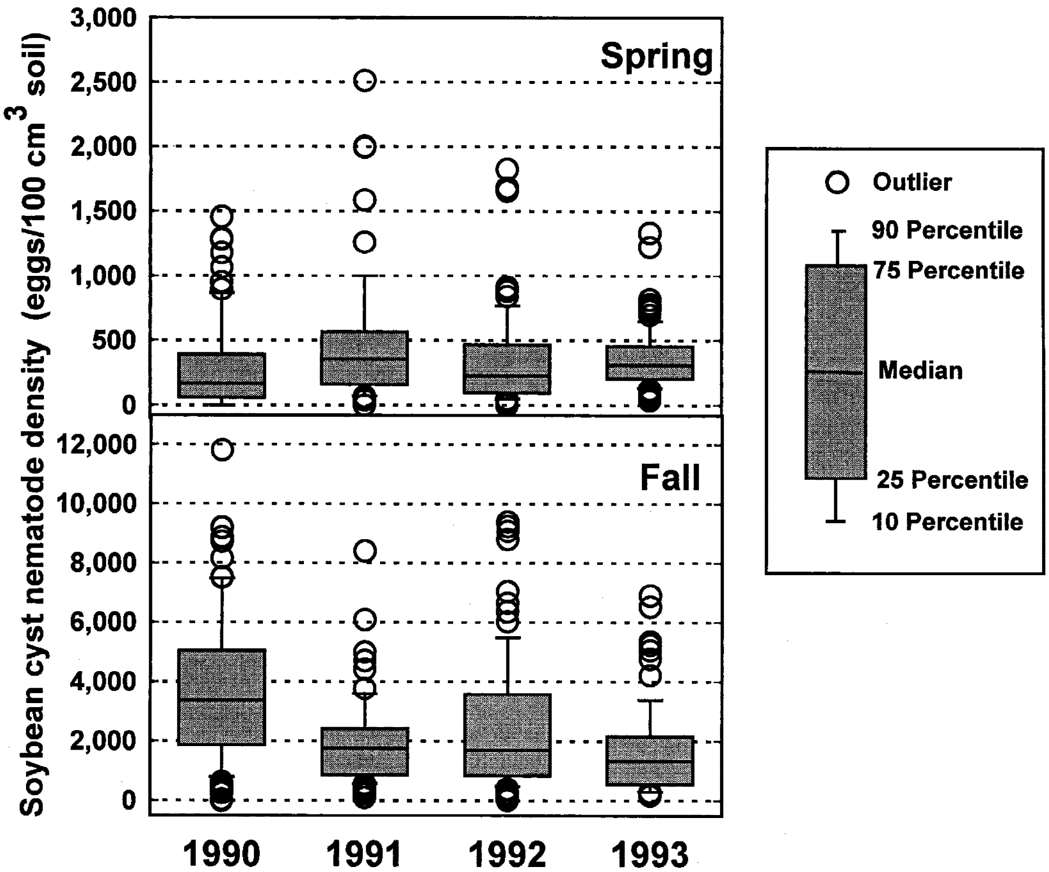

FIG. 1. Box plots of *Heterodera glycines* egg population densities at planting and harvest, 1990 through 1993.

1993), analysis of variance showed that there were no effects due to herbicide or soil insecticide treatments on egg population density in this study.

Geostatistical analysis: Semivariogram functions were graphed to relate semivariance (γ) of the regionalized variables, *H. glycines* egg population density and soybean yield, to the sample "lag" distance (h) (Phillips et al., 1992; Warrick et al., 1986). Semivariogram functions were estimated as described by Donald (1994) and Perrier and Wilding (1986).

Nugget variance (C_0) is the variance at zero distance (lag $h = 0$) in the semivariogram (Table 1). Nugget variance usually is considered to be unexplained random measurement or sampling error, such as an improperly chosen scale of sampling relative to the scale of natural variation of the measured regionalized variable (Trangmar et al., 1985). When the nugget variance approaches zero, variance can be explained almost totally by "structural" variance (C) due to the spatial dependence of the regionalized variable. The "sill" ($C + C_0$), which is the value of the asymptotic plateau of the semivariogram function, is used to estimate the "range" or distance (A_0) in multiples of lag h. The sill is the lag distance between measurements at which one value for a regionalized variable is independent of neighboring values.

Contour maps of egg population density and yield were prepared by means of block kriging (Burgess and Webster, 1980; Gambolati and Volpi, 1979; Phillips et al., 1992; Warrick et al., 1986). Block kriging employs weighted local averaging using the semivariogram range from the best-fit semivariogram function. It also gives unbiased predictions of interpolated values of regionalized values between sampled grid control points that minimize estimation variance (error)

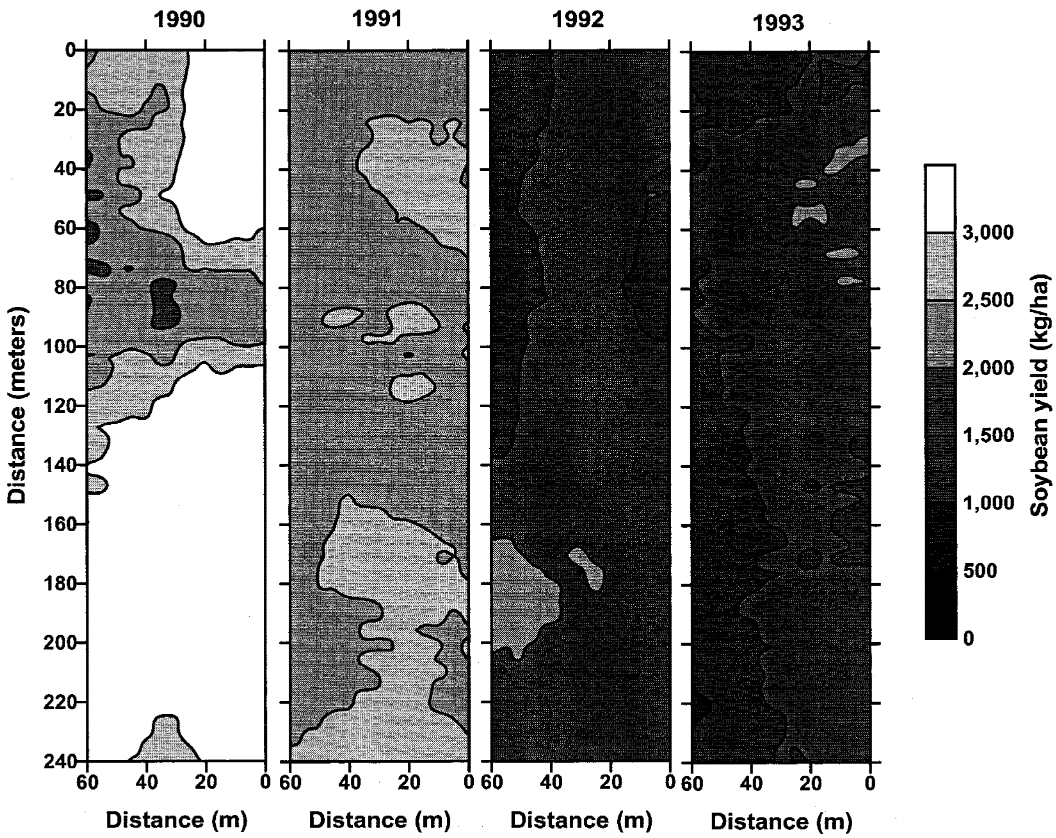

FIG. 2. Soybean grain yield in a field in Pemiscot County, Missouri, from 1990 through 1993.

(Trangmar et al., 1985; Webster, 1985). Kriging provides unbiased estimates of regionalized variables and their standard deviations in unsampled locations that depend only on semivariogram properties based on the data set. Geostatistical analysis and kriging were conducted on measured and calculated regionalized variables with GS^+ software (GS^+ ver. 2.3 software, Gamma Design Software, Plainwell, MI), and contour maps were generated with SURFER (GoldenSoftware, Golden, CO) contour mapping software based on GS^+ kriged values.

Results and Discussion

Changes in SCN egg density and soybean yield over time for the entire field: The extraction efficiency for SCN cysts was 40% using our technique as determined by adding known numbers of cysts to the elutriator and determining the recovery of those cysts. The percentage of soil samples below the detection limit (where no eggs were recovered) was greater for Pi than Pf ($n = 100$). At planting, the percentage of samples with Pi below the detection limit was 21%, 5%, 5%, and 0% in 1990, 1991, 1992, and 1993, respectively. In contrast, only one Pf sample (in 1990) was below the detection limit.

The Pi level was higher at the end of the experiment compared with the level at the beginning of the study (Fig. 1). The reverse was true for the Pf over the 4 years. Variability in Pf, reflected in either the range or standard error of the mean, increased linearly as mean Pf increased for the entire 4-year data set (data not shown). Averaged across the field, the Pi was always much lower than the Pf. Average Pi/Pf from the preceding fall was 11.2%, 17.2%, and 14.5% in the spring of 1991, 1992, and 1993, respectively.

Soybean yields decreased gradually over 4 years, as expected (Fig. 2). Overall field yield averaged 3,385, 2,415, 1,691, and 1,567

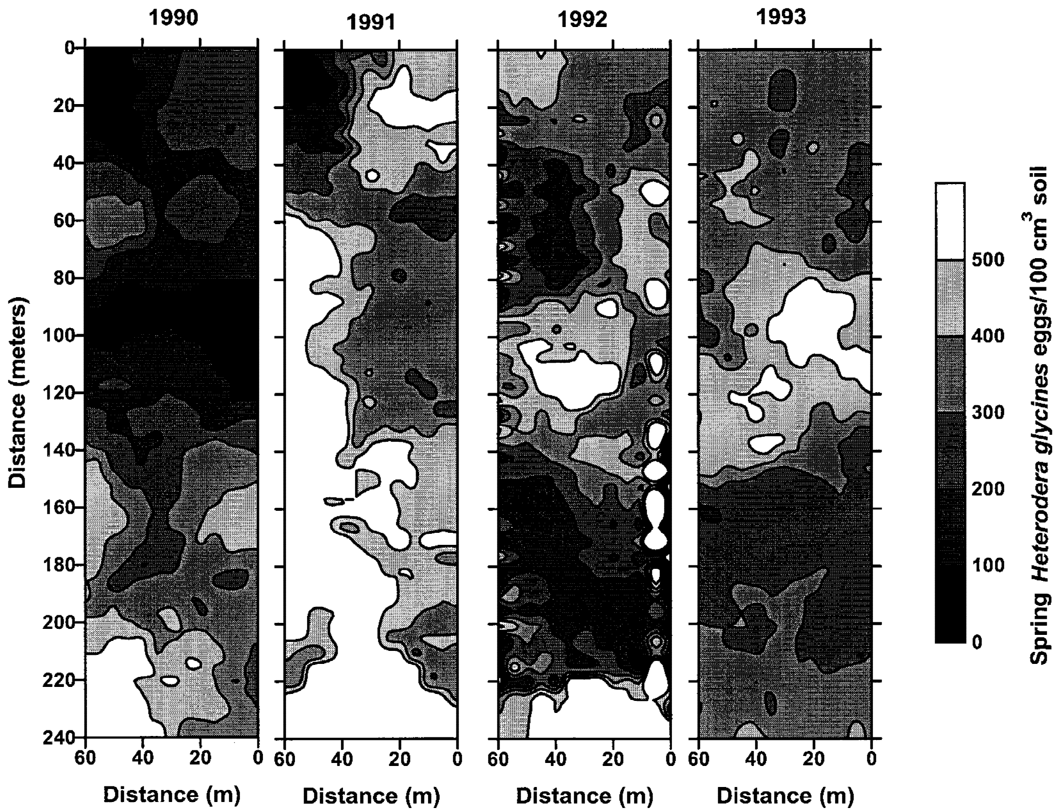

FIG. 3. *Heterodera glycines* egg population density levels (number of eggs per 100 cm^3 of soil) at planting (Pi) in a field in Pemiscot County, Missouri, from 1990 through 1993.

kg/ha in 1990, 1991, 1992, and 1993, respectively. Yield variability, measured as standard error of the mean, increased as mean yield increased (data not shown). At the field scale, decreases in mean yield over time were not directly related to corresponding increases in Pi or Pf.

Spatial dependence in H. glycines egg population density and soybean yields: Spatial dependence occurred for all measured variables at the grid distances used in this study. Spatial dependence in a semivariogram is indicated by an increase in the semivariogram from the nugget (C_0) to the sill ($C + C_0$). If there were no spatial dependence, the nugget equals the sill (Table 1). Spatial models using semivariograms showed that spatial dependence explained 0 to 100% of the variation in the data set, depending on the measured variable. Egg population density in the fall (Pf) varied widely, but the spatial dependence was least when sampled in the fall. Closer-spaced sampling would be needed to reduce the nugget variance further. Egg population density in the spring (Pi) was more uniform, and spatial dependence was observed more often.

Mapped spatial variability in egg population density and soybean yield over time: High Pi recurred in similar regions of the field in 1990, 1991, and 1992 but not in 1993 (Fig. 3). The areas of highest Pi expanded from 1990 to 1991. High Pi occurred in smaller, isolated areas in 1992 and 1993. The areas of the field with low Pi in 1990 and 1991 were not the same areas with low Pi in 1992 and 1993.

The spatial pattern of Pf was inconsistent from 1990 through 1993 (Fig. 4). The highest Pf occurred in 1990 (Figs. 1,4), and Pf decreased thereafter. In 1992 and 1993, the direction of spatial variation in yield was related to the direction of tillage but not to other factors such as soil type or weed distribution (data not presented).

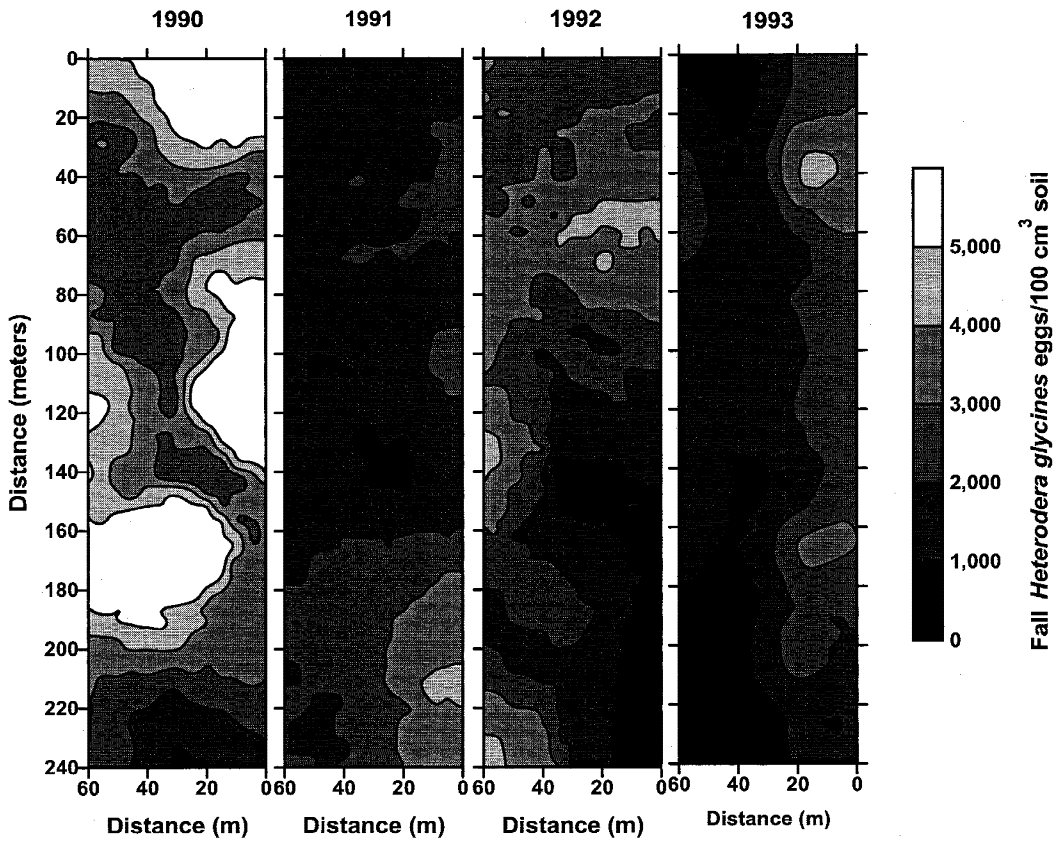

FIG. 4. *Heterodera glycines* egg population density levels (number of eggs per 100 cm^3 of soil) at harvest (Pf) in a field in Pemiscot County, Missouri, from 1990 through 1993.

Proponents of precision farming and variable-rate technology make several assumptions: (i) yield varies within fields over the landscape in a predictable way from year to year; (ii) yield-limiting factors can be identified and recur in the same regions of the fields repeated over time; (iii) yield will increase in response to correction in a predictable way, if yield-limiting factors are corrected; (iv) implementing variable-rate technology is cost-effective and even profitable (Lamb et al., 1996). Proponents also argue that applying variable rates of inputs using precision farming methods has economic advantage over conventional uniform application of inputs. Because fields are not uniform, blanket application of inputs may be wasteful of resources, and greater economic benefit may be gained if inputs are applied less uniformly, where they will increase yield the most. Data are available on nutrient variability across fields. However, before this study was started key data were lacking to determine whether there is adequate variation in *H. glycines* egg population density in a field to benefit from the cost of extensive soil sampling. Likewise, it was not known whether distributional patterns of *H. glycines* remain static over time. When variation in egg population density is sufficiently great, maps of *H. glycines* density across fields may be helpful in precision farming, management of the nematode, and understanding of *H. glycines* distribution changes over time throughout a field.

Although sites in fields with high nematode densities are expected to be surrounded by high nematode densities, and sites with low nematode densities are expected to be bordered by low nematode densities, variability can be large over small distances. The nematodes have a high reproductive potential, but they cannot move great distances by themselves. Distributional patterns of nematodes in fields have been linked to soil characteristics (Young, 1992), but *H. glycines* distribution in a field over time has not been studied. At one sampling date, Noe and Campbell (1985) found greater variability between rows than within rows when sampling soybean fields. However, Francl (1986) found that there were aggregates within rows that were equal to the variation between rows. In this study, there was variation in *H. glycines* egg population density not only above and below the detection threshold but also above and below the damage threshold of 500 eggs/250 cm^3 soil (T. L. Niblack, pers. comm.).

Data from this study emphasize the need to establish a field history of *H. glycines* infestation. Environment and other factors influence the reproductive rate of *H. glycines,* and generalizations or decisions concerning management made at one point in time may not accurately predict the situation in the future in a specific field. In our field study, there was not a good relationship between fall egg counts (Pf) and the following spring egg counts (Pi). In areas with high overwinter mortality, early-spring sampling for *H. glycines* management decisions would be more accurate than samples collected at harvest. Because of low Pi levels, fields should be more intensively sampled to improve the reliability of *H. glycines* population density estimate and thus reduce the risk of yield loss. Our results confirmed those of Lamb et al. (1996)—that areas with better grain yield were not consistent from year to year.

Most nematode management recommendations are not sufficiently refined to account for soil types and geographic changes within a region. For example, in this study in southeastern Missouri, there was a 10-fold decrease in egg counts from fall to spring. In contrast, Pi in northern areas of Missouri can be close to 100% of the Pf the previous fall (unpublished data).

Eventually, demographic modeling of nematode populations over time must address nematode spatial patterns. In addition, maps may help formulate testable hypotheses about the spread of plant-parasitic nematodes.

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