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Multivariate Analysis of Selected Edaphic Factors and Their Relationship to *Heterodera glycines* Population Density

L. J. Francl

Abstract: The influence of selected soil physical and chemical factors on population density of *Heterodera glycines* was investigated in 1988 and 1989 in two different locations of a soybean (*Glycine max*) field. Soil variables of a Norfolk loamy sand were measured after planting soybeans susceptible to *H. glycines*. Cyst and egg populations were determined after harvest. Nematode population density was found to be clustered. Up to 91% of the eggs were parasitized by a sterile fungus. Principal component analysis with orthogonal VARIMAX rotation grouped 12 variables into five uncorrelated factors in 1988 and three in 1989. In 1988, the factor "pH and Mg" was positively correlated ($P < 0.001$) with cyst and egg population density. Also, the factor "fine texture and Cu" was negatively correlated ($P < 0.05$) with egg population density. In 1989, the factor "pH, Mg and Cu" was positively correlated ($P < 0.05$) with levels of cysts and percentage of parasitized eggs, but not with total egg number. Across 2 years, factors containing soil pH and Mg were positively associated with cyst nematode population density. Copper appeared to be negatively associated with populations of *H. glycines*.

Key words: Glycine max, Heterodera glycines, multivariate analysis, soil fertility, soybean cyst nematode, spatial distribution.

The soil environment is not neutral in its effect on host–nematode interactions and resultant nematode population densities. Nematode population dynamics may be affected directly by edaphic factors or indirectly influenced through the host's response to its environment. Soil parameters conducive to yield loss might be predictable, and many of these could be manipulated to favor plant health.

Norton (14,15) reviewed the many effects that abiotic edaphic factors have on plant-parasitic nematodes. *Merilinius brevidens* (Allen) Siddiqi and *Helicotylenchus digonicus* Perry had highly skewed, nonrandom dispersions in an alfalfa field, and their population density was positively related to fine soil texture (9). This apparently was the first report to suggest that the clustered spatial distribution commonly found for plant-parasitic nematodes in a field might be attributed in part to edaphic factors. Koenning et al. (12) found that an increasing sand content along a soil texture gradient was negatively correlated to soybean cyst nematode, *Heterodera glycines* (Ichinohe), population density and soybean (*Glycine max* L.) yield.

Several multivariate analysis methods have the property of forming new, uncorrelated variables from data, making these techniques suitable for unraveling complex interrelationships found among edaphic factors (10). Noe and Barker (13) used discriminant analysis and canonical correlation to suggest that clay content, Na, and Cu helped explain within-field variation in population density of *Meloidogyne incognita* (Kofoid & White) Chitwood, *Tylenchorhynchus claytoni* Steiner, and *Helicotylenchus dihystera* (Cobb) Sher. Quénéhervé (16) used principal component analysis to learn if textural classes, pH, and organic matter content of soils from banana plantations were related to nematode dispersion. Clay content explained most of the variation in population densities of *Helicotylenchus multicinctus* (Cobb) Golden (positive correlation), *Hoplolaimus pararobustus* (Schuurmans Stekhoven) Sher (negative), and *M. incognita* (negative); however, populations of *Radopholus similis* (Cobb) Thorne were largely unaffected by the edaphic variables measured.

The objectives of this study were to determine the suitability of principal components analysis for summarizing the infor-

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Department of Plant Pathology, North Dakota State University 58105; formerly, Nematology Laboratory, USDA ARS, Beltsville, MD 20705.

mation contained in 12 edaphic variables and to research the relationship between these selected edaphic variables and population density of *H. glycines*. A preliminary report of this work has been published (6).

Materials and Methods

Field plots were located in Wicomico County, Maryland, in a Norfolk loamy sand (mapped as a fine-loamy, siliceous, thermic, Typic Paleudult; 86% sand, 8% silt, 6% clay). The field had been monocultured to soybeans for an unknown number years and had become infested with *H. glycines*. Plots were three rows spaced 0.76 m apart and 4.9 m long. One hundred contiguous plots were laid out in a 10 × 10 array, with 10 additional plots outside the array in 1988. Field plots (10 × 10) in 1989 were similarly established about 100 m from the 1988 site. The areas were planted on 6 June 1988 with 'Asgrow 4271' (Maturity Group IV) and 13 June 1989 with 'Essex' (Maturity Group V). Both soybean cultivars were susceptible to *H. glycines*. Plots were harvested on 25 October 1988 and 2 November 1989.

Soil was sampled after planting on 20 June 1988 and 13 June 1989 and at harvest in 1988 and 1989. Twenty-four cores were taken with a 19-mm probe to a depth of 20 cm from each plot: eight from the middle row, four from each adjacent furrow, and four from each intermediate area. The bulk soil sample was mixed and a 100-g subsample was dried (105 C for 24 hours) to obtain soil dry weight. Subsamples of the June samples were sent to the University of Maryland soil testing laboratory for measurement of P_2O_5, K_2O, Ca, Mg, SO_4, Zn, Mn, Cu, pH, and percentage of organic matter. Another subsample of 85 g from each plot was shaken mechanically through nested sieves to determine particle size distribution by standard methods (8). A 500-g subsample of the harvest sample was saturated with water overnight at 2 C. Cysts were separated from this subsample by suspending in water and decanting three times onto nested sieves having 0.42- and 0.25-mm apertures. Cysts were further separated from soil material under a dissecting microscope. Cysts then were opened with forceps and the viability of eggs within was assessed by visual examination. Discolored eggs were assumed to be diseased, and their numbers were counted. Nondiscolored eggs were assumed to be healthy and were counted directly or were estimated by aliquot if numbers were high.

The nematode population at harvest was categorized into number of cysts, total number of eggs, number of healthy eggs, and percentage of diseased eggs. Spatial distribution of cysts was assessed for randomness by a χ^2 goodness-of-fit test to the Poisson distribution (7). Goodness-of-fit tests for distributions of cyst and other nematode population categories to other nonrandom distributions were not attempted because the high frequency of null counts would have forced an extreme regrouping of data for a valid χ^2 analysis (7).

Twelve edaphic variables (Table 1) were analyzed using PROC FACTOR from SAS (17). Edaphic variables were grouped by principal component analysis with VARIMAX orthogonal rotation. Scree plots, eigenvalues, correlation analysis, and Kaiser's measure of sampling adequacy were used in preliminary analyses as diagnostic tests and to select the appropriate number of principal components or factors. Eigenvalues measure the variance accounted for by a given component and thus are useful as a criterion for determining the number of significant factors (4). Components with eigenvalues greater than 1.0 are retained according to the Kaiser criterion, or a scree plot of the eigenvalues is drawn and the components with low eigenvalues (i.e., the rubble or scree) at the toe of the "slope" are judged as insignificant.

Edaphic variables were linked to factor scores by selecting the largest loading for a variable and assigning that edaphic variable to the corresponding factor. Other

TABLE 1. Statistical summary of *Heterodera glycines* populations measured at harvest and selected edaphic variables at planting.

	Mean†		Std. Dev.		Minimum		Maximum	
Variable‡	1988	1989	1988	1989	1988	1989	1988	1989
Cysts	839	468	300	168	312	218	1,952	976
Total eggs	66,617	39,968	22,403	13,754	22,287	15,234	116,925	66,859
Healthy eggs	39,406	26,593	20,217	10,895	6,388	6,770	106,516	51,874
Diseased eggs (%)	39	33	24	17	2	3	91	71
P_2O_5	270	268	62.5	104.0	125	126	434	668
K_2O	118	135	29.6	55.7	60	62	179	401
Ca	296	350	68.0	94.1	174	223	555	778
Mg	96	149	29.4	41.8	38	68.5	163	238
SO_4	3.43	9.88	1.50	11.54	0.89	0.09	8.1	53.4
Mn	11.15	5.32	12.81	2.45	0.00	0.00	42.7	10.7
Zn	1.25	1.68	0.38	1.00	0.80	0.71	3.92	6.41
Cu	0.54	0.68	0.20	0.20	0.00	0.36	0.89	1.16
pH	5.99	5.59	0.28	0.31	5.40	5.00	6.40	6.40
OM (%)	0.68	1.10	0.09	0.33	0.50	0.50	0.90	2.00
SM (%)	5.30	7.00	0.58	0.94	3.20	5.50	7.20	10.80
PS (%)	10.98	13.83	1.64	2.60	7.65	9.75	13.62	20.17

† There were 109 observations in 1988 and 72 in 1989.
‡ Nematodes per kg soil dry weight; soil nutrients in (kg/ha); OM = organic matter; SM = soil moisture content; PS = particle size <0.10 mm.

edaphic variables were judged to have some practical significance if their contribution to that factor was greater than an absolute value of 0.3 (4). Another criterion imposed for linkage was the ratio of the factor loading to that variable's own highest loading; this criterion was judged significant if it was greater than 0.75. Last, a meaningful name was attributed to each factor (4).

To relate factors to nematode population density, the edaphic variables from each field plot were multiplied by their factor-scoring coefficients and the result summed by plot (17). Factor scores were then correlated with cyst nematode populations at soybean harvest.

RESULTS

There were 109 observations analyzed in 1988. Heavy rains in 1989 shortly after planting reduced the stand in some plots, leaving 72 observations for analysis. Many of the edaphic variables did not seem to differ greatly in their means between years, but the data ranges were broad (Table 1). The nematode population density was about 40% lower in 1989 than 1988, but the degree of fungal parasitism was similar. More than one-third of the nematode eggs were parasitized by a dark, sterile fungus. The fungus could not be distinguished from a similarly sterile fungal pathogen of *H. glycines* from Arkansas (11, R. D. Riggs, pers. comm.).

The Poisson distribution did not fit the dispersion of cysts among plots in either year according to a χ^2 test ($P < 0.0001$). Because a uniform distribution can be ruled out as even less probable based on the high variance to mean ratio (Table 1), the dispersion of cysts was most likely nonrandom and clustered at the scale at which the population was sampled.

Results from correlation analysis and Kaiser's measure of sampling adequacy (not shown) supported elimination of particle size categories other than percentage of particle size < 0.10 mm (i.e., very fine sand, silt, and clay fractions). A scree plot was used to determine the number of relevant factors in the 1988 data because there was an eigenvalue very close to 1.0 (Fig. 1). The scree plot showed a clear separation in eigenvalues between factors 5 and 6. Therefore, the edaphic variables

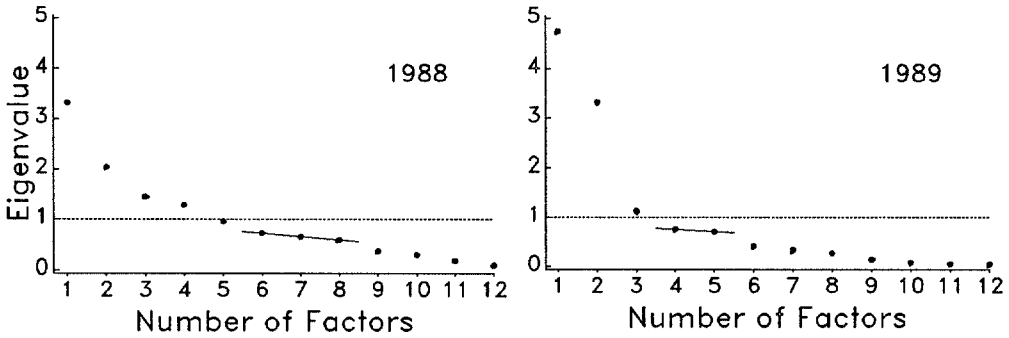

FIG. 1. Scree plots of eigenvalues from two principal components analyses of 12 selected edaphic variables. A horizontal dotted line is drawn at an eigenvalue of 1.0 in each plot to illustrate factor selection based on Kaiser's criterion. The solid line in each plot is drawn through the first nonsignificant points to illustrate graphic factor selection.

were grouped into five factors for 1988 data and three factors for 1989 data (Table 2). The first five factors from 1988 data and first three factors from 1989 data accounted for 76% and 77% of the variance in their respective data sets.

Because the original variables contribute in varying amounts to newly formed factors, these factors are commonly interpreted and renamed based on factor scores. The factor interpretations differed between the 1988 and 1989 data in several respects. For 1988, factor 1 may be interpreted as "soil fertility," factor 2 as "pH

TABLE 2. Principal component factor patterns of 12 edaphic variables after VARIMAX orthogonal rotation.†

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
			1988		
K_2O	0.88	−0.02	0.13	0.11	0.06
Ca	0.78	0.33	0.00	0.32	0.07
P_2O_5	0.68	0.21	0.37	−0.16	0.15
pH	−0.01	0.90	−0.23	0.00	0.16
Mg	0.43	0.86	0.03	0.06	0.00
PS‡ (%)	0.13	−0.03	0.87	−0.03	−0.09
Cu	0.17	−0.21	0.76	0.14	0.09
SM‡ (%)	−0.09	0.13	0.06	0.82	0.13
Zn	0.32	−0.15	−0.38	0.59	−0.11
OM‡ (%)	0.32	−0.02	0.31	0.58	0.20
SO_4	0.26	−0.06	−0.13	0.00	0.85
Mn	−0.07	0.27	0.18	0.23	0.76
			1989		
K_2O	0.94	0.19	−0.08		
P_2O_5	0.88	−0.09	−0.06		
Ca	0.87	0.24	0.24		
Mg	0.70	0.27	0.54		
SO_4	0.70	0.00	0.03		
Cu	−0.53	0.13	−0.44		
PS‡ (%)	0.04	0.91	−0.24		
OM‡ (%)	0.07	0.89	−0.32		
SM‡ (%)	0.05	0.83	−0.12		
Zn	0.00	0.80	0.14		
Mn	0.53	0.63	0.23		
pH	0.02	−0.27	0.90		

† Each factor coefficient is scaled from −1.00 to 1.00. The contribution of a variable to a factor is reflected in the closeness of that variable's coefficient to either of these two extremes and the coefficient's size relative to that for other factors (i.e., values in a row).

‡ PS = particle size <0.10 mm; OM = organic matter, SM = soil moisture content.

and Mg," factor 3 as "fine texture and Cu," factor 4 as "soil moisture capacity, organic matter and Zn," and factor 5 as "SO_4 and Mn" (Table 2). For 1989, factor 1 again represented "soil fertility" (macronutrients loaded similarly to 1988 with the exception of SO_4), factor 2 "soil moisture capacity, Zn and Mn," and factor 3 "pH, Mg and Cu." Mg and Cu contributed almost as much to factor 3 as to factor 1, with Cu having a negative effect in each instance, so Mg and Cu were of practical significance to the meaning of factor 3.

The spatial orientation in the first three factorial dimensions of the edaphic variables and their interpreted grouping into factors graphically illustrate their interrelationships (Fig. 2). These spatial relationships were plotted after VARIMAX orthogonal rotation, which maximizes the separation of variables. With the exception

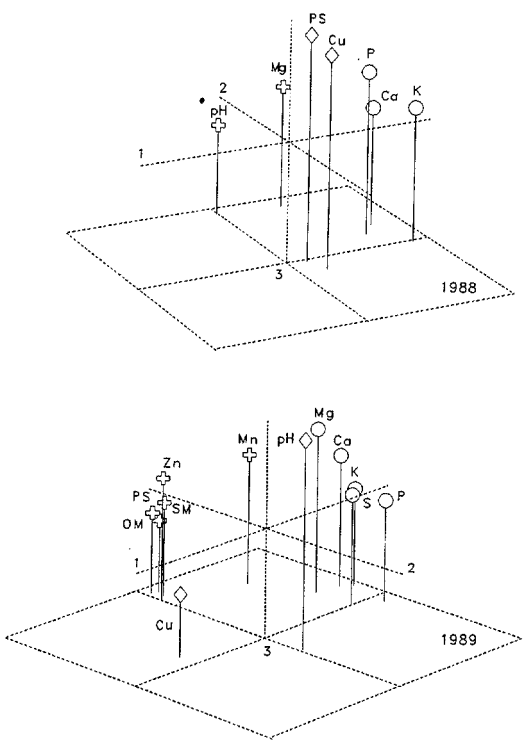

FIG. 2. Three-dimensional coordinates of edaphic variables mapped for the first three factors labeled 1 to 3 on the X, Y, and Z axes (not all variables for 1988 are represented). The grouping of variables according to their contribution to each factor is indicated by symbols atop the vertical lines: circle, factor one; cross, factor two; diamond, factor three.

of the negative contribution of Cu to factor 3 in 1989, the spatial arrangement clearly shows the factor grouping in positive associations.

Factor 1 was positively correlated ($P < 0.05$) to numbers of total eggs but not to numbers of healthy eggs or cysts in 1988 (Table 3). In contrast, the similarly formed 1989 scores for factor 1 were correlated negatively to percentage of diseased eggs ($P = 0.05$). Factor 4 from 1988 and the similarly formed factor 2 from 1989 also had different correlation patterns.

Factor 3 in 1988, "fine texture and Cu," was negatively correlated with numbers of total and healthy eggs. The analysis of 1989 data apportioned these two variables among all three factors. There was a negative correlation of factor 1 with percentage of diseased eggs. Cu contributed negatively to factor 1, indicating higher fertilities at lower Cu levels. But when Cu was a part of factor 3 (again contributing negatively), the correlation with percentage of diseased eggs was positive and a significant positive correlation with number of cysts was found. The pattern for fine soil texture was also inconsistent.

Number of cysts and eggs was positively correlated to factor 2 scores ("pH and Mg") in 1988, while the number of healthy eggs was not. Likewise, cyst number in 1989 was positively correlated with factor 3, "pH, Mg and Cu" (with Mg and pH positively and Cu negatively associated with cyst number). In addition, percentage of diseased eggs was positively correlated with these similarly formed factors at $P = 0.06$ in 1988 and $P = 0.02$ in 1989. Across the 2 years, factors containing pH and Mg exhibited the most consistent correlation pattern.

DISCUSSION

Edaphic factors are interrelated, and they interact with soil biota in many ways. Multivariate analysis therefore appears to be an appropriate tool for the giant task of unraveling possible cause-and-effect pathways and influential side effects because newly formed factors are uncorrelated. In

TABLE 3. Correlation coefficients (r) and associated levels of probability (P) from a correlation between population levels of *Heterodera glycines* and factor scores from principal components analyses of 12 edaphic variables.

Variable		Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
			1988			
Cysts/kg	r	0.07	0.36	−0.05	0.07	0.12
soil dry wt.	P	0.49	<0.001	0.60	0.47	0.20
Total eggs/kg	r	0.21	0.31	−0.21	0.15	0.02
soil dry wt.	P	0.03	0.001	0.03	0.12	0.86
Healthy eggs/	r	0.09	0.12	−0.24	0.12	−0.14
kg soil dry wt.	P	0.34	0.20	0.01	0.21	0.15
Diseased eggs	r	0.02	0.18	0.09	−0.04	0.19
(%)	P	0.84	0.06	0.37	0.65	0.05
			1989			
Cysts/kg	r	−0.02	−0.21	0.29		
soil dry wt.	P	0.89	0.07	0.01		
Total eggs/kg	r	−0.09	0.01	0.15		
soil dry wt.	P	0.44	0.93	0.20		
Healthy eggs/	r	0.06	0.24	−0.03		
kg soil dry wt.	P	0.59	0.04	0.82		
Diseased eggs	r	−0.23	−0.38	0.27		
(%)	P	0.05	0.001	0.02		

this particular case, principal components analysis resolved the intercorrelations among 12 edaphic variables in two different data sets. An integrated interpretation of the results across two data sets was difficult, however, because the selected edaphic variables were grouped somewhat differently between years.

Populations of *H. glycines* appeared to be spatially aggregated in both data sets. The cause of high between-plot variance could not have been due to sampling method because the systematic sampling methodology negated within-plot effects (5). The sterile fungus appeared to have a density-dependent depressant effect on egg populations (results not shown). Plant host-mediated and soil environmental effects are logical causes of the nematode spatial aggregation found in these data.

Having two independent data sets was an advantage in discerning important relationships among edaphic variables and nematode population densities. For example, correlations between *H. glycines* and soil fertility (factor 1) were inconsistent between environments, but pH and Mg were consistently correlated to nematode population variables. Specifically, factors that included pH and Mg consistently were correlated positively with cyst nematode population density at harvest (i.e., higher populations were found as pH increased from 5.0 to 6.4 and Mg increased from 38–68 to 163–238 kg/ha). A counterbalance of percentage of diseased eggs also seemed to be associated in both years with factors representing increasing pH and Mg. Calcium did not contribute much to the factor loading in either year; therefore, an investigation concerning the effects of calcic versus dolomitic limestone on population density of *H. glycines* in an acid soil would be interesting. Hydrogen ion concentration *per se* or its mediating effects on other chemical or biotic species could be affecting populations of *H. glycines*. Tefft et al. (18) found that encysted egg hatch of *H. glycines* had an optimal pH of 6.0, where 50% more hatch occurred than at pH 5.4. The range of pH values in this study encompassed these amounts. If the soybean plant and cyst nematode do indeed have different pH response curves, there may be a pH range where yield is not adversely affected but damage caused by the nematode is minimized.

Common salts in solution can have stimulatory or inhibitory effects on nematodes. Barker et al. (1) showed that NO_3^- can

reduce egg hatch and juvenile penetration and edaphic of *H. glycines*. Zinc and ferrous salts increase hatch of encysted eggs (3). Castro et al. (2) discovered that second-stage juveniles of *M. incognita* are repelled by K^+, NH_4^+, NO_3^-, Cs^+, and Cl^- ions. Principal components containing Cu, a plant micronutrient typically present in soils in trace amounts, were generally negatively associated with cyst nematode density, supporting previous work on different plant-parasitic species by Noe and Barker (13). The evidence in this study of the role of Cu was more tentative.

Principal component analysis appears useful for exploratory analysis of nematode population ecology as affected by soil physicochemical variables. Relatively low correlation coefficients and differences between years in factor groups found in this study suggest a limited predictive value for edaphic variable analysis by multivariate methods. The effect of the plant host component on parasite population density over the course of a growing season is also undoubtedly important.

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