Plant-parasitic Nematode Distributions in an Alfalfa Field

P. Goodell and H. Ferris[1]

Abstract: A 7-ha alfalfa field (*Medicago sativa* L. cv Mesa Sirsa) was sampled systematically on a 6 × 6-m grid by removing individual cores (2.54 cm diam) to a depth of 45 cm from each of the 1,936 grid intersections. The soil was mainly coarse-textured with a fine-textured streak running centrally, north to south. Nematodes were extracted by a semiautomatic elutriator and sugar flotation-sieving technique. Five plant-parasitic species were consistently present: *Meloidogyne arenaria, Pratylenchus minyus, Merlinius brevidens, Helicotylenchus digonicus,* and *Paratrichodorus minor.* All species had a highly skewed nonnormal frequency distribution that departed significantly from randomness. Goodness-of-fit tests on the distribution of five populations in the entire field showed that three (*Meloidogyne, Merlinius,* and *Helicotylenchus*) were described by a negative binomial. When the samples were categorized by soil texture (coarse vs. fine-textured), all populations in the fine-textured areas, and three populations (*Meloidogyne, Pratylenchus,* and *Merlinius*) in the coarse areas, fitted a negative binomial distribution. Nearly all populations fitted a negative binomial when the frequency distributions from randomly located one-meter-square areas were examined for each species. *Key Words:* population distribution, negative binomial model, sampling.

Nematode distribution in fields is patchy or clumped (1,2,6,8,15,21,23), but little work has been done to quantify or describe that clumping. Small-plot studies (16) have supported the suggestion that nematode distribution will fit a negative binomial model (1). Valid description of nematode distributions is important for meeting assumptions of certain parametric statistical tests, for aiding in the development of sampling techniques, for assessing temporal changes in density and distribution, and for comparing interspecific distribution patterns. This study was done to gain insight into the distribution of nematodes on a field level, and to test observed frequency distribution against a negative binomial model.

MATERIAL AND METHODS

In July 1977, a 7-ha field with a 2-year-old stand of alfalfa (*Medicago sativa* cv Mesa Sirsa) in the Palo Verde Valley, Riverside Co., California, was divided systematically by a 6 × 6-m network of gridlines. A single 2.54-cm-diam soil core was removed to a depth of 45 cm at each of the 1,936 grid intersections. Each core was placed in a plastic bag, labeled with its grid coordinates, and stored in an ice chest until removed from the field. Each core was weighed (mean = 170 g), and a visual rating was given to the soil texture on a scale from 1 (coarse-textured; 61% sand, 19.5% silt, 19.5% clay) to 5 (fine-textured; 23% sand, 43% silt, 34% clay). The field was mainly a coarse texture with a streak of fine-textured soil running north to south (Fig. 1). The nematodes in the cores were extracted by a modified semiautomatic elutriator and sugar flotation-sieving technique (5). The extract was heated to kill the nematodes, and sufficient formalin was added to make a 5% solution. The preserved nematodes were counted in a rectangular counting dish with guidelines etched on the bottom. Only half of the dish was counted, and the results were doubled to give an estimate of the numbers in that core. Nematode counts were stored in data files in a Prime 400 mini computer. Although several models describe clumped distributions (e.g., Thomas, Neyman's Type A, and Polya-Aeppli), these were not used to test the data because of the specific biological assumptions upon which they are based (7). The negative-binomial model was used because it is a general model and can describe the clumping in a variety of situations. FORTRAN programs were used to compare observed distribution with expected negative binomial distribution by goodness-of-fit.

In September 1977 six areas within the field were re-sampled using a 1-meter-square grid. Forty-one cores were removed from each grid according to a pre-measured tem-

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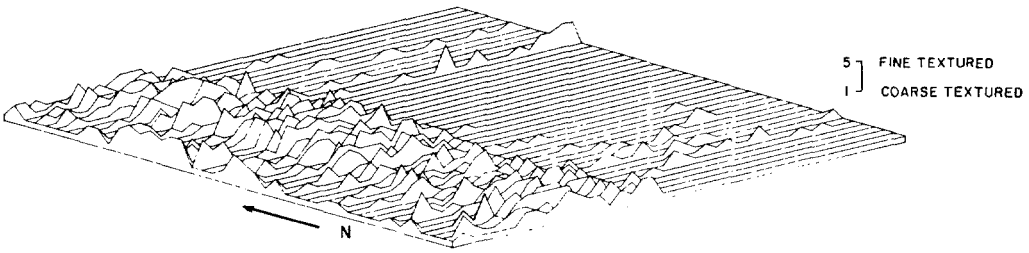

Fig. 1. Three-dimensional projection of soil textures at the study site. (*1* = coarse texture, 5 = fine texture).

plate. Each core was labeled individually and treated as above, except that the counts were made from unpreserved samples.

RESULTS AND DISCUSSION

The five genera of plant-parasitic nematodes consistently present were *Meloidogyne arenaria* (Neal) Chitwood; *Pratylenchus minyus* Sher and Allen; *Merlinius brevidens* (Allen) Siddiqi; *Helicotylenchus digonicus* Perry; and *Paratrichodorus minor* (Colbran) Raski. A *Criconemoides* species, observed infrequently, was excluded from the study because of inconsistent recovery with this extraction process (5). A topographical mapping computer program (Environmental Systems Research Institute, 380 New York Ave., Redlands, CA) was used to prepare three-dimensional projections of the nematode distribution (Fig. 2). This technique illustrated the patchy or clumped distribution through the field, as well as the variability among six single-meter sites.

Nematode densities in individual cores were standardized to nematodes/200 g soil (Table 1). In all populations, the variance exceeded the mean, indicating a clumped distributed (7,21). Departures from randomness were significant in all cases, according to a coefficient-of-dispersion test (7). Histograms of the frequency distribution (Fig. 3) were highly positively skewed, also indicating a clumped distribution (21).

In initial chi-square goodness-of-fit tests none of the populations fit a negative bi-

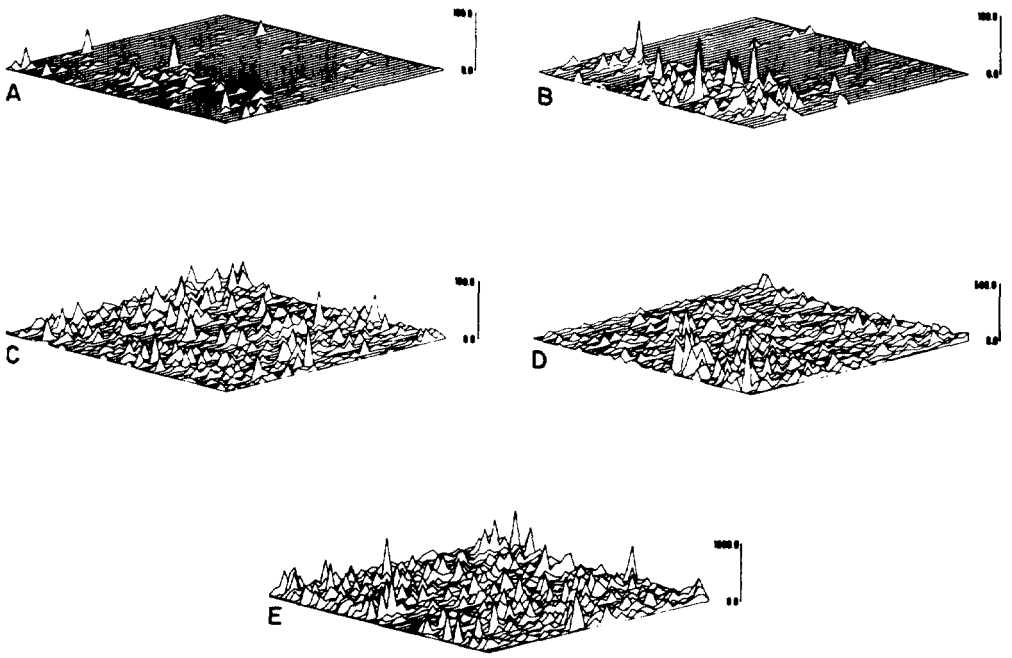

Fig. 2. Three-dimensional projection of population-density distribution in 200 g of soil: A) *Merlinius brevidens;* B) *Helicotylenchus digonicus;* C) *Paratrichodorus minor;* D) *Pratylenchus minyus;* E) *Meloidogyne arenaria.* Note that the scales differ between the projections.

Table 1. Statistics for population density estimates for five species in an alfalfa field in the Palo Verde Valley, California. All soil cores corrected to 200 grams of soil.

Species	Total in 1936 soil cores	$\bar{x}$ per core	SD between cores	Coefficient of dispersion
M. arenaria	162,739	84	91	555.8 ***
P. minyus	51,353	27	36	367.4 ***
M. brevidens	898	0.5	3	176.3 ***
H. digonicus	3,397	2	7	229.8 ***
P. minor	8,213	4	7	160.4***

***Significant departure from random at $P < 0.001$.

Table 2. Goodness of fit to negative binomial for entire field (actual acounts).

Species	χ^2	No. of observations	k
M. arenaria	228.8	196	1.0
P. minyus	117.8*	89	—
M. brevidens	9.6	15	0.04
H. digonicus	33.5	27	0.1
P. minor	45.0*	29	—

*Significantly different from negative binomial at $P < 0.05$.

nomial distribution. A large part of the deviation was related to the doubling of the counts, and therefore, the expected frequency at low counts was high. The goodness-of-fit was retested with actual counts from half a dish standardized to 200 g (Table 2). Three of the populations (*M. arenaria, M. brevidens, H. digonicus*) did not differ significantly from a negative binomial at $P < 0.05$. All further distribution testing was conducted on actual counts. One parameter of the negative binomial distribution, the k-value, can be considered an index of dispersion. The smaller the value of k, the more aggregated the population (21).

The fine-textured streak is shown in Fig. 1. Preliminary observation of the data indicated possible associations between populations and soil texture, especially in the fine-textured areas with *H. digonicus* and *M. brevidens*. Associations with soil texture have been reported for some genera present in this field (13,22). The correlation between population density and soil texture was tested by linear regression. *H. digonicus* and *M. brevidens* counts were correlated significantly with fine-textured areas, *M. arenaria* counts were correlated negatively with fine-textured areas, and *P. minyus* and *P. minor* counts were not correlated with soil texture.

The importance of soil texture in the distribution of some of the populations suggested that goodness-of-fit to the negative binomial be tested by separating cores from

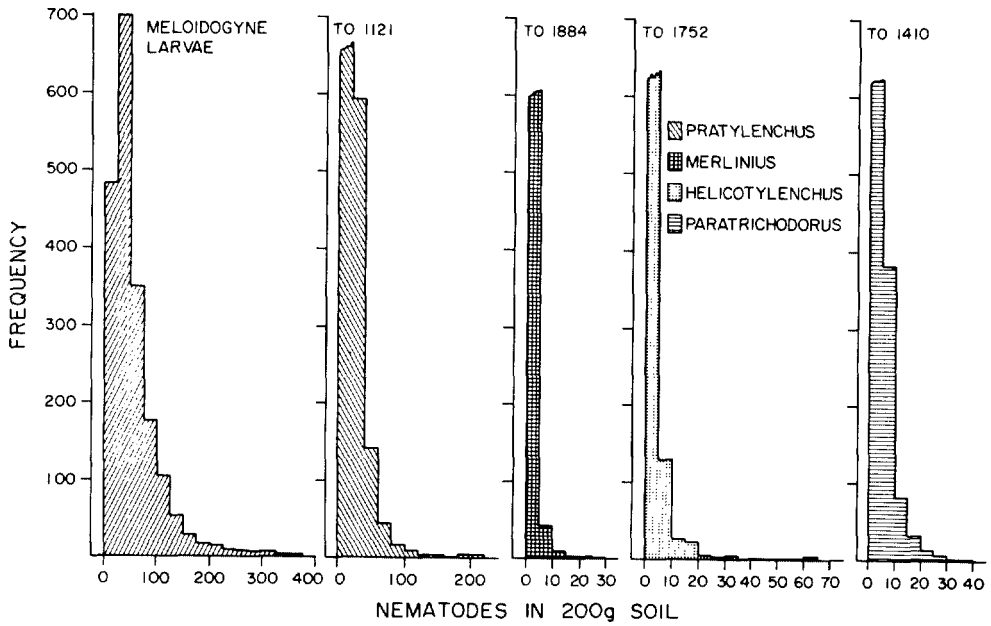

Fig. 3. Histograms figuring distributions of actual counts of the five nematode populations.

the coarse-textured area (texture rating of 1) and fine-textured area (rating 2–5). All populations in the fine-textured area fit a negative binomial, while three populations *M. arenaria, P. minyus,* and *M. brevidens* fit this distribution in the coarse-textured area. (Table 3). The k-values were similar to those for the entire field (Table 2).

The size of the area sampled may have affected the apparent distribution of these populations, as occurs with plant distribution (9,11). As quadrat size (here equated to sample unit) is increased, the apparent dispersion of a clumped population may be random, clumped, and finally regular, a result of increased area sampled with each quadrat (7). Although in nematology the sample unit (soil core) does not change, the size of the area sampled can vary greatly.

Table 3. Goodness of fit to negative binomial for field, separated by soil texture.

Species	χ^2	No. of observations	k
Coarse soil texture			
M. arenaria	213.6	186	1.04
P. minyus	100.5	83	0.75
M. brevidens	7.7	13	0.02
P. minor	40.9*	27	—
Fine soil texture			
M. arenaria	140.8	123	0.8
P. minyus	51.2	64	0.64
M. brevidens	14.9	20	0.1
H. digonicus	20.3	22	0.28
P. minor	19.2	20	0.31

*Significantly different from negative binomial at $P < 0.5$.

Table 4. Goodness of fit to negative binomial for six individual square meters.

Species	Location	Soil texture	χ^2	No. of observations	k
M. arenaria	SW1	Mixed	34.3	29	1.48
	SW2	Fine	23.8	33	2.26
	NW2	Coarse	26.9	28	1.59
	NE2	Coarse	22.2	21	2.3
	SE1	Coarse	26.9	29	2.57
	SE2	Coarse	26.4	31	2.11
P. minyus	SW1	Mixed	15.2*	8	—
	SW2	Fine	7.2	5	2.73
	NW2	Coarse	3.8	12	1.59
	NE2	Coarse	7.9	8	1.47
	SE1	Coarse	9.6	9	2.13
	SE2	Coarse	26.1	26	3.36
M. brevidens	SW1	Mixed	8.3	8	0.76
	SW2	Fine	2.8	4	0.80
	NW2	Coarse	N/A	0	—
	NE2	Coarse	1.2 N/A	3	—
	SE1	Coarse	N/A	0	—
	SE2	Coarse	N/A	0	—
H. digonicus	SW1	Mixed	6.7	8	0.86
	SW2	Fine	7.2*	5	—
	NW2	Coarse	0.0 N/A	2	—
	NE2	Coarse	0.0 N/A	2	—
	SE1	Coarse	N/A	0	—
	SE2	Coarse	N/A	0	—
P. minor	SW1	Mixed	8.2	11	0.56
	SW2	Fine	0.1	6	0.42
	NW2	Coarse	5.0	9	0.50
	NE2	Coarse	0.4 N/A	3	—
	SE1	Coarse	5.2	6	0.51
	SE2	Coarse	13.2	14	0.47

*Significantly different from negative binomial at $P < 0.05$.
N/A, not applicable.

Of the six areas sampled in September, one gave all fine-textured cores, one gave mixed coarse and fine-textured cores, and four gave coarse-textured cores. For *H. digonicus* and *M. brevidens,* whose numbers were low, several of the areas contained no individuals and several others contained so few individuals that distribution analysis would be meaningless.

Of the 30 potential frequency distributions among the five species in the six meter areas, 21 could be tested by goodness of fit (Table 4). All but two (*P. minyus* and *H. digonicus* in one location each) fit a negative binomial. The k-values for meter-square samples were larger in general than the other estimates but still indicative of highly aggregated populations. The almost consistent fit to the negative binomial can be attributed to the small sample area. Soil texture, salinity, and compaction are relatively consistent in such a small area, and the root distribution is more uniform. A sample from a meter-square may represent only one root system. In alfalfa, with its broad genetic base, this may be important because individual plants may vary in susceptibility and support different population densities.

The meter-square sample area may reflect the influence of host biology and parasite interactions on a microdistributional level. The 6m sample grid is more reflective of the edaphic and cultural influences on the macrodistribution of populations. The close association of some populations with certain soil textures supports current recommendations that edaphic factors should be considered in nematode sampling plans (1, 4,17).

Studies of this type are essential to nematode sampling plans. Mathematical models (e.g., normal, negative binomial) allow determination of the required number of cores and samples to achieve prescribed levels of accuracy in the sample estimate (21). If a sequential sample plan is being considered, the distribution must fit certain models (14, 21). If the results are to be analyzed with most parametric statistical procedures (analysis of variance, *t*-test, confidence limits) the normality of the data must be validated (18,23). Mathematical techniques are available for normalizing skewed data, with the most widely used transformation in biology being log (x + c) (16,20,23).

Population distributions varied with the size of area sampled. In the field studied, some populations would have been overlooked if samples were not taken over the entire area. Sampling recommendations by the Society of Nematologists (2) and information provided by several advising organizations (4,10,17) point out the importance of complete field coverage. It may be necessary to design sample plans for individual species where species are distributed differently and all species are economically important.

Alfalfa is a perennial crop with a deep spreading root system, planted uniformly across an entire field. The distribution of nematodes which are obligate plant parasites is influenced strongly by their food distribution. In other cropping situations (orchard, row-crop) the root distribution is substantially different, and nematode sampling patterns appropriate for them will require further study.

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