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Sampling Approaches for Extensive Surveys in Nematology

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Abstract: Extensive surveys of the frequency and abundance of plant-parasitic nematodes over large geographic areas provide useful data of unknown reliability. Time, cost, and logistical constraints may limit the sampling intensity that can be invested at any survey site. We developed a computer program to evaluate the probability of detection and the reliability of population estimates obtained by different strategies for collecting one sample of 10 cores from a field. We used data from two fields that had been sampled systematically and extensively as the basis for our analyses. Our analyses indicate that, at least for those two fields, it is possible to have a high probability of detecting the presence of nematode species and to reliably estimate abundance, with a single 10-core soil sample from a field. When species were rare or not uniformly distributed in a field, the probability of detection and reliability of the population estimate were correlated with the distance between core removal sites. Increasing the prescribed distance between cores resulted in the composite sample representing a wider range of microenvironments in the field.

Key words: abundance, detection, frequency, nematode, plant-parasitic nematode, population density, sampling, survey.

Extensive surveys are sometimes necessary in nematology to determine the occurrence, distribution, frequency, and relative abundance of species of nematodes over large geographic areas. Such surveys are indispensable in providing information on the probability and magnitude of crop losses due to nematodes. They may be an important basis for prioritizing research activities or for commercial development of nematode management tools (nematicides, biological control agents, resistant cultivars, etc.), and also for the design of regulatory programs to prevent nematode importation or spread.

Extensive survey data of nematode occurrence and abundance in a geographic area have been accumulated several ways. For example, a survey of Longidoridae, Trichodoridae, and Criconematidae of the British Isles (9) involved multiple-core samples from 100-m^2 areas in five vegetation types from a series of 100-km^2 regions. The survey was supplemented by advisory records, data from a separate survey, and a literature search. On a more crop-specific basis, McKenry and Kretsch (11) determined nematode occurrence and abundance in the almond orchards of California by sampling soil from three trees in each of 333 orchards. The orchards sampled were suggested by county personnel in 15 of 17 almond-producing counties. Siddiqui et al. (15) used accumulated records of regulatory agencies and of research and extension personnel to catalog nematode distribution and host associations in California. Although extremely useful for a variety of purposes, these data vary in their reliability according to the approach and purpose of the original observation. Usually there is no way of determining that reliability.

Considerable research and practical experience have resulted in sampling plans to provide information for research or diagnostic and advisory purposes (1,2,8,10). Optimal patterns for collecting cores into composite samples have been determined (1,2). Optimum numbers of samples per field and numbers of cores per composite sample have been calculated as a function of nematode species, crop value, and management cost (4,6,8,12–14). In general, these procedures require considerable sampling intensity to reach the precision required for management decisions; consequently, they are not well adapted to extensive surveys of large geographic areas.

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The goal of an extensive survey is to gather reliable and useful information over a large geographic area within available budgetary, time, and personnel constraints. The dilemma is the tradeoff between effort invested at individual sites and the number of sites that can be included. The minimum amount of effort to be expended at each site is that which will provide the quality of information desired from the study. The influence of sample optimization strategy on the detection of species occurring at low frequency in a field is of particular interest. Populations of various nematode species in fields differ in numbers and spatial pattern. With the constraint of using a single composite soil sample in fields with populations of several species of plant-parasitic nematodes, our objectives were (i) to determine the probability of detecting the presence of a species, (ii) to determine the accuracy of the estimate of the population density of each species, and (iii) to study the influence of the pattern of soil core collection on the detection of species and accuracy of the population estimate.

Materials and Methods

For these studies we used extensive datasets collected in previous studies on nematode distribution from 7-ha and 2.6-ha fields (6,7). The first dataset (7) was collected by superimposing a 44 × 44 grid pattern, with intersections 6 m apart, on a 7-ha alfalfa (*Medicago sativa* L.) field. An individual soil core (2.5 cm d, 45 cm depth) was collected at each of the 1936 grid intersections. The plant-parasitic nematode species present were *Meloidogyne arenaria* (Neal) Chitwood, *Pratylenchus neglectus* (Rensch) Filipjev & Schuurmans Stekhoven (= *P. minyus*), *Merlinius brevidens* (Allen) Siddiqui, *Helicotylenchus digonicus* Perry, and *Paratrichodorus minor* (Colbran) Siddiqi. The second dataset (6) was collected by superimposing a 25 × 25 grid pattern, with intersections 6.5 m apart, on a 2.6-ha cotton (*Gossypium hirsutum* L.) field, with individual cores (2.5 cm d, 30 cm depth) collected at each of the 625 grid intersections. Plant-parasitic species present included *Criconemella xenoplax* (Raski) Luc & Raski, *Helicotylenchus dihystera* (Cobb) Sher, *Meloidogyne incognita* (Kofoid & White) Chitwood, *Meloidogyne javanica* (Treub) Chitwood, *P. minor,* and *Xiphinema americanum* Cobb. In both studies, the number of individuals of each species extracted from each soil core was determined.

The datasets collected on each of the plant-parasitic nematode populations present in each field were the basis for simulation studies addressing the objectives. A computer program was written to simulate collection of soil cores for composite samples. The program allows user selection of the number of cores per sample, selection of cores at random, or selection with a specified minimum (*dmin*) and maximum (*dmax*) distance between them, and the choice of side-of-entry of a field. The point-of-entry along the side-of-entry was selected at random.

In initial simulations, we studied the probability of detection of various species in 100 replications of single samples consisting of different numbers of cores selected at random from the field. In subsequent analyses, we restricted sample size to a composite of 10 cores. Average volume of the 30-cm cores from the 2.6-ha field was 150 cm^3 and that of the 45-cm cores in the 7-ha field was 230 cm^3. A composite volume of around 2 liters of soil from 10 cores is a convenient and manageable amount considering the logistics of collection, mixing, and processing. We also considered 10-core composite samples to be at the upper limit imposed by the logistical constraints of extensive surveys.

The first core of each composite sample was selected at a random location within 20 m of the point of entry of the field. For random sampling schemes, subsequent cores were taken at random at any point in the field without allowing the same core to be used more than once in a single sample. For sampling patterns with spatial constraints, the second core was selected at random from an area between two circles,

one of radius *dmin* and one of radius *dmax,* centered on the location of the first core. Similarly, each of the following eight cores was selected at random from the area between two circles centered on the location of the previous core, but excluding any overlap of the sampling area with the sampling areas of any previous core (Fig. 1).

The *dmin* and *dmax* distances used for studies in the 2.6-ha field were 5 and 15 m, 7.5 and 17.5 m, 10 and 20 m, 15 and 25 m, 17.5 and 27.5 m, 20 and 30 m, 30 and 40 m, and 35 and 45 m. In the 7-ha field they were 10 and 20 m, 15 and 25 m, 20 and 30 m, 25 and 35 m, 30 and 40 m, 35 and 45 m, 40 and 50 m, 45 and 55 m, 50 and 60 m, 55 and 65 m, 60 and 70 m, and 65 and 75 m. To simplify presentation of the data, we use the average of *dmin* and *dmax* to indicate the average distance between soil cores. For each nematode species in each field and for each sampling distance, the simulation was repeated for all sides of the field. Each sampling strategy combination was repeated 100 times to provide data on probabilities of detection of each species, on the accuracy of each population density estimate, and on the influence of distance between cores on those parameters. Detailed analyses were concentrated on those species at low frequency in each field.

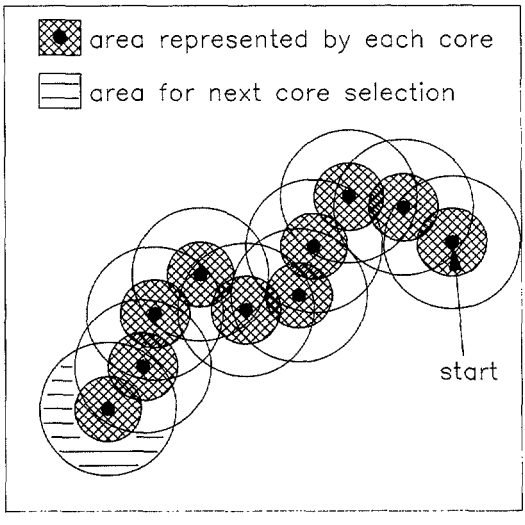

FIG. 1. Sampling pattern with spatial constraints; each core is selected at random from an area between two circles, one of radius *dmin* and one of radius *dmax,* centered on the location of the previous core. The area available for each core excludes any overlap of the sampling area with the sampling areas of any previous core.

RESULTS

When cores were selected at random from the field, the impact of number of cores on detection of a nematode species varied with the spatial pattern and abundance of the populations. Representative examples are *H. digonicus* and *M. brevidens* from the 7-ha field (Fig. 2). *Helicotylenchus digonicus* was spatially restricted to the west side of that field. *Merlinus brevidens* was also located primarily on the west side, but with lower frequency and in low numbers (7). The probability of detection of *H. digonicus* stabilized at about 95% for samples consisting of 14 and more cores. The probability of detection of *M. brevidens* reached about 80% at 17 cores per sample. At 10 cores per sample, the probabilities of detection of *H. digonicus* and *M. brevidens* were 87% and 62%, respectively (Fig. 2). All other species in both fields had probabilities of detection greater than 90% when samples consisted of 10 or more cores (Tables 1–3).

When species were abundant and dispersed across the 7-ha field (*M. arenaria, P. neglectus,* and *P. minor*), the populations

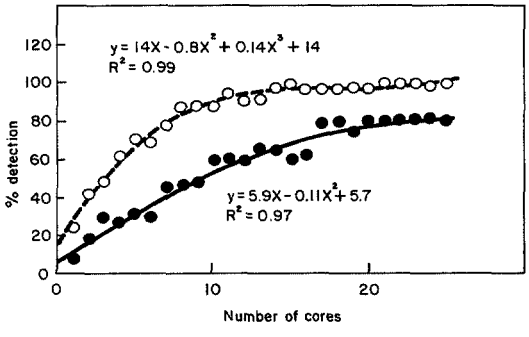

FIG. 2. Effect of number of cores constituting a composite sample on the probability of detection of *Helicotylenchus digonicus* (open circles, mean density = 1 per 100 g soil) and *Merlinius brevidens* (solid dots, mean density = 0.25 per 100 g soil). Cores were selected at random from 1,936 cores systematically removed from a 7-ha field. Each point is the number of samples (in 100 repetitions) in which the nematode was detected.

TABLE 1. Average number of individuals per 100 g soil (A), coefficient of variation (CV), and frequency of occurrence (F%) of three nematode species in a dataset of 1936 soil cores systematically collected from a 7-ha alfalfa field.

		Meloidogyne arenaria		*Pratylenchus neglectus*		*Paratrichodorus minor*	
1936 cores	A	2467		787		82	
	CV	109		135		176	
	F%	98		89		48	
10 cores	A	2532		850		78	
random†	CV	44		43		55	
	%D	100		100		100	
		East	West	East	West	East	West
10 cores	A	2245	2623	972	725	66	94
15 m‡	CV	48	32	67	38	58	55
	%D	100	100	100	100	100	100
10 cores	A	2319	2393	847	778	82	95
65 m‡	CV	32	32	48	38	59	53
	%D	100	100	100	100	100	100

† A, CV, and % detection (%D) of the species in 100 repetitions of 1 sample of 10 cores selected at random from the dataset.
‡ A, CV, and %D in samples selected with cores at prescribed distances apart (15 or 65 m) starting from opposite sides (east, west) of the field.

were detected in all composite samples of 10 cores collected by all strategies (random, defined distance, varying starting point) (Table 1). Irrespective of sampling pattern or starting point, the population level estimate, expressed as the mean of 100 samples, was close to the true mean of all cores in the field. The estimate differed by a maximum of about 20% for *P. minor*, which occurred only in about 48% of all cores from the field. Of greater interest is that the coefficient of variation for repeated estimates of the population mean was relatively consistent for a species across all sampling strategies, indicating that all approaches were similar in their reliability for these frequent species. The side of entry of the field influenced only the coefficient of variation, and therefore the reliability of the population estimate, for samples collected with a short distance between cores (Table 1).

TABLE 2. Average number of individuals per 100 g soil (A), coefficient of variation (CV), and frequency of occurrence (F%) of three nematode species in a dataset of 625 soil cores systematically collected from a 2.6-ha alfalfa field.

		Helicotylenchus dihystera		*Meloidogyne* spp.		*Paratrichodorus minor*	
625 cores	A	399		166		295	
	CV	66		83		29	
	F%	20		98		48	
10 cores	A	394		181		291	
random†	CV	67		82		25	
	%D	100		100		100	
		South	North	South	North	South	North
10 cores	A	156	582	81	289	199	346
10 m‡	CV	139	63	111	72	54	32
	%D	96	100	97	100	100	100
10 cores	A	412	373	187	156	299	291
40 m‡	CV	68	66	75	112	26	31
	%D	100	100	100	100	100	100

† A, CV, and % detection (%D) of the species in 100 repetitions of 1 sample of 10 cores selected at random from the dataset.
‡ A, CV, and %D in samples selected with cores at prescribed distances apart (10 or 40 m) starting from opposite sides (north, south) of the field.

TABLE 3. Average number of individuals per 100 g soil (A), coefficient of variation (CV), and frequency of occurrence (F%) of nematode species in datasets of 1936 and 625 soil cores systematically collected from a 7-ha field and a 2.6-ha field, respectively.

		7-ha field 1936 cores		2.6-ha field 625 cores	
		Helicotylenchus digonicus	*Merlinius brevidens*	*Criconemella xenoplax*	*Xiphinema americanum*
All cores	A	84	22	26	24
	CV	360	570	260	480
	F%	20	7	16	19
10 cores random†	A	81	22	28	30
	CV	143	177	79	185
	%D	87	62	92	93

† A, CV, and % detection (%D) of the species in 100 repetitions of 1 sample of 10 cores selected at random from the datasets.

In the 2.6-ha field, the most frequent and abundant species (*H. dihystera, Meloidogyne* spp., and *P. minor*) were always detected in composite samples of 10 randomly selected cores not constrained by distance apart (Table 2). Coefficients of variation associated with the population level estimates varied with the spatial patterns and aggregation characteristics of the individual species. When cores were separated by a predetermined distance, the probability of detection of these frequent species was again high, increasing with distance between cores. Again, the coefficient of variation of repeated estimates of the population level was not influenced by distance between cores (Table 2). In this field there were dramatic differences in the population estimate, depending on the side of entry for samples consisting of cores separated by predetermined distances. The differences diminished as distance between cores increased (Table 2).

When infrequent species in each field were measured by composite samples of 10 cores selected at random with no distance constraints between cores, detection approached 90% for those species with a frequency around 20% (Table 3, Fig. 2). When cores were selected at predetermined distances apart, the probability of detection depended on the side of entry of the field and on the distance between cores (Figs. 3,4). In the 7-ha field, there was a high probability of detecting *H. dihystera* in samples started from the west side of the field, where it was more common. When sampling was started from the east side of the field, the probability of detection of *H. dihystera* was positively correlated with the distance between cores (Fig. 3). The probability of detection of *M. brevidens* was greater when the sampling was initiated at the west side of the field, and again the probability increased with distance between cores when sampling was initiated on the east side of the field (Fig. 4). In both cases, when the distance between cores was sufficiently large so that the composite sample covered most of the field, the probability of detection was not affected by the side of entry of the field (Figs. 3,4). Comparable results, with a north-south influence, were obtained in the 2.6-ha field with the infrequent species *C. xenoplax* and *X. americanum*. These two species were concentrated in cores removed from the northern part of that field.

The population level estimates for *H. dihystera* and *M. brevidens* in the 7-ha field were negatively correlated with distance between core sampling sites when the sampling was started at the side of the field where the nematodes were frequent and (or) abundant. The correlations were positive when sampling was started from the side of the field where the nematode species were rare (Figs. 3,4). When the distance between cores was sufficiently large so that the composite sample covered most of the field, the number of individuals per sample approached the theoretical mean

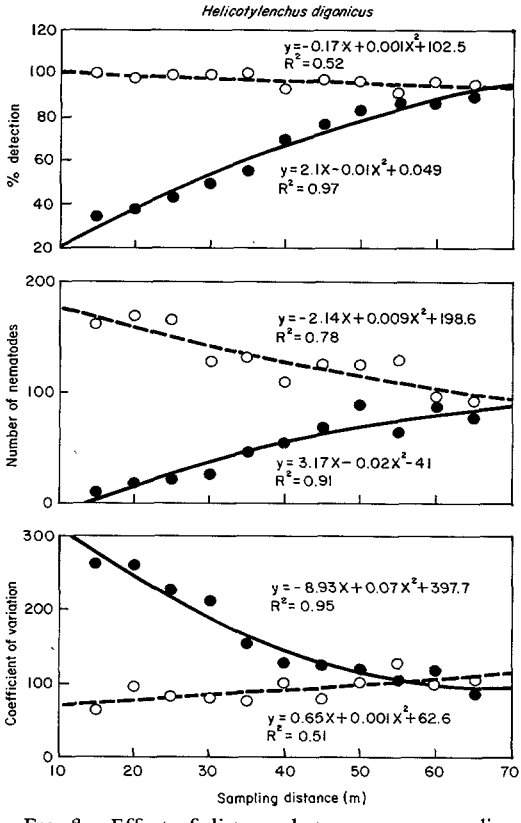

FIG. 3. Effect of distance between core sampling sites on the probability of detection, estimate of abundance (nematodes per 100 g soil), and the reliability of that estimate, of *Helicotylenchus digonicus* using a single composite sample of 10 cores from a 7-ha field. The sampling pattern was initiated with the first core on the west (open circles), or east (solid dots) side of the field. Each point is based on 100 repetitions.

obtained from all cores removed from the field (Table 3, Figs. 3,4). Similar results, not presented, were obtained for *C. xenoplax* and *X. americanum* in the 2.6-ha field.

The coefficients of variation for *H. dihystera* and *M. brevidens* among 100 samples from the 7-ha field were negatively correlated with distance between cores when sampling was initiated from the side of the field where the nematodes were rare (Figs. 3,4). Again, similar results were obtained for *C. xenoplax* and *X. americanum* in the 2.6-ha field.

DISCUSSION

Environmental, temporal, and behavioral factors result in aggregated spatial patterns for many biological populations. Plant-parasitic nematodes are no exception. The microscopic size of soil-dwelling nematodes and the density of the soil medium compound problems of detection and population assessment. The population density and spatial dispersion characteristics of a species provide insights into the probability of its detection. If the spatial distribution of a population can be characterized by a defined mathematical distribution, for example the Poisson series for a random distribution, the positive binomial for a regular distribution, or the negative binomial for an aggregated distribution, the probability that individuals will not be present in a sample unit can be cal-

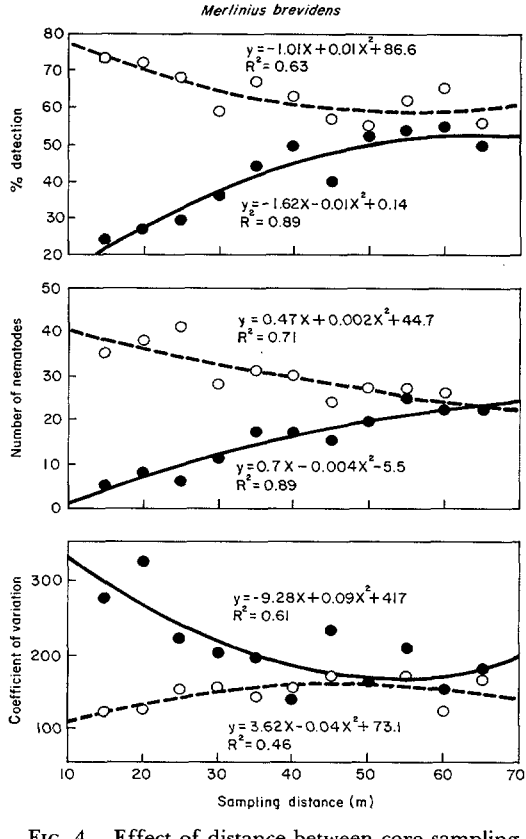

FIG. 4. Effect of distance between core sampling sites on the probability of detection, estimate of abundance (nematodes per 100 g soil), and the reliability of that estimate, of *Merlinius brevidens* using a single composite sample of 10 cores from a 7-ha field. The sampling pattern was initiated with the first core on the west (open circles), or east (solid dots) side of the field. Each point is based on 100 repetitions.

culated (3). These characteristics of nematode populations that may or may not be present in a field are unlikely to be known before survey sampling. However, in most instances, the spatial pattern of nematode populations is aggregated and can be characterized by the negative binomial model (2,7,12).

The first term of the negative binomial model is:

$$P_0 = (1 + \mu/k)^{-k}$$

where μ is the mean population density (e.g., per liter of soil) and k an index of dispersion (3). For multiple-core composite soil samples, k varies with the aggregation characteristics of the nematode species. It is usually less than 2 and frequently as low as 0.5 for species with highly aggregative biology (5). For a population with $k = 0.75$ and $\mu = 1$, 10, 100, or 1000 individuals per liter of soil, the respective probabilities of nondetection will be 0.53, 0.136, 0.025, or 0.005. Clearly, it is easier to detect an abundant species even when aggregated than to detect one that is at low population densities. Prior knowledge of the dispersion parameters of a population would allow assessment of the quality of the information derived from a sample. In the above example, if a species was not detected in a sample, there is a 47% chance that it occurs in that field at population densities up to 1 individual per liter of soil. Unfortunately, the population numbers and dispersion patterns of nematodes are not known a priori for the individual fields that may constitute an extensive survey.

In a practical sense, we consider that collecting a composite soil sample from a field by selecting cores purely at random to be logistically difficult. Walking across the field and selecting random sites for core collection is fraught with potential bias and is more likely to result in a systematic pattern of core collection distributed across the field than one that is purely random. One unbiased approach to collecting cores at random would be to select core-removal sites as random map coordinates before entering the field; however, that process appears cumbersome. Consequently, the designation of distances between cores (e.g., number of paces to be taken) is appealing and practical. In the system we have used in these studies, an individual would be instructed to select a core-removal site "at random," at least *dmin* paces from the previous site, but within *dmax* paces from that site.

Where nematode species were frequent and abundant, as in the case of *M. arenaria, P. neglectus,* and *P. minor* in the 7-ha field and *H. dihystera, Meloidogyne* spp., and *P. minor* in the 2.6-ha field, any of the sampling plans tested was sufficient for detection. However assessment of abundance in the field was more accurate and reliable when 10-core samples were selected purely at random from the field or with a large distance between cores. In both cases, there is a high probability that the composite sample will contain representative cores from any region of the field. When the distance between cores is smaller, the composite sample will represent a restricted region of the field, which may not represent the field as a whole.

For nematode species that are infrequently represented in the field, the probability of detection was relatively independent of distances between cores if the sampling was started in an area of the field where the nematodes were frequent (Figs. 3,4). That is because at least one core was removed from the high-frequency area. However, the existence of such an area in a field is usually not known a priori during an extensive survey. When the sampling was started in any other area of the field, the probability of detection of infrequent species increased with prescribed distance between cores until that distance was great enough that cores were removed from all areas of the field, including that of highest frequency. When the nematodes were infrequent even in their area of highest concentration, for example *M. brevidens* (Fig. 4), the probability of detection actually decreased with increasing distance between cores when sampling was started in that area. As before, because that area is prob-

ably unknown, the convergence of detection probability, and of accuracy and reliability of population estimates, in samples with cores widely spaced indicates the optimal strategy for survey purposes.

Based on these studies, we conclude that the objectives of an extensive survey, including detecting the presence of a species and providing an estimate of the population density of each species, can be achieved by taking one composite sample of 10 soil cores from each field. The cores should be spaced so that they are dispersed over the entire field and represent the range of microenvironments occurring in that field. We recognize that increasing the number of samples or size of samples, where possible, will further increase probabilities of detection, and the precision and reliability of the sample estimate.

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