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Sampling for Regional Monitoring of Nematode Communities in Agricultural Soils[1]

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Abstract: Regional assessment of nematode communities to monitor the condition or ecological health of agricultural soils requires sampling programs with measures of known reliability and the ability to detect differences over time. Numbers of fields sampled in a region, samples taken per field, and subsamples assayed per sample must be balanced with cost to provide the best sampling scheme. We used components of variance from statewide surveys in North Carolina (1992) and Nebraska (1993) to estimate number of (i) fields to be sampled; (ii) 20-core, composite soil samples to be obtained for each field; and (iii) subsamples to be assayed for each composite sample to detect a specified amount of change in index values within a geographic region. Variances for these three components were used to estimate the degree of reliability for five ecologically based indices (four measures of maturity and one of diversity) of nematode communities. Total variance for maturity and diversity indices, based upon communities of free-living nematodes, was greater in North Carolina than in Nebraska; the opposite was true for indices based strictly upon maturity of communities of plant-parasitic nematodes or of all nematodes in soil. Variability within samples was greater in North Carolina than in Nebraska, especially for maturity indices based only upon free-living nematodes. We identified two possible sampling strategies for a regional survey: Option 1, with two independent samples per field and a single subsample assayed per sample, which would provide a reliability ratio value ≥ 0.6 for most indices; and Option 2, with three independent samples per field and two subsamples assayed per sample, which would provide a reliability ratio value ≥ 0.7 for several indices. When cost was considered, Option 1 was the better strategy. Number of fields to be sampled within a region or state varied with the index chosen; with specific indices, however, a 10% change in mean index value could be detected with a sample of 50 to 100 fields.

Key words: ecology, maturity index, monitoring, nematode community, power curve, regional, reliability ratio, survey, trophic diversity, variance component.

Nematode communities are ubiquitous in agricultural soils and encompass a range of trophic groups within the soil food web (27). Because of the variety of families and genera of free-living and plant-parasitic nematodes and their differential sensitivity to environmental disturbances (6,10,13,17, 20,21,32,37), nematodes may provide an indication of the overall diversity, maturity, or stability of soil ecosystems (28,29). When ecological attributes of soil nematode communities are quantified through measures such as a diversity index (34,36) or maturity index (5,38), an indication of relative soil biological or ecological health is obtained, which can be used as one measure to address issues of change in ecological condition of agricultural systems. We assume that a healthy soil is one with an intact food web and all positions in the food chain present and functioning properly.

Questions concerning the ecological health of soil in agricultural systems and the possibility for sustaining production of food and fiber in such systems over the long term require answers at the state, regional, or national levels. Most previous studies to determine optimum sampling strategy have had the goal of reaching a specified precision of nematode density estimates for management decisions within a field or group of similar fields and, thus, have focused appropriately on populations

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of plant-parasitic nematodes within a field or within small plots (1–4,14–16,18,23, 25,26,30,33). Sampling patterns used for plant-parasitic nematodes might be applicable to sampling free-living nematodes because examples of strongly or weakly aggregated genera can be found among nematodes with different feeding preferences (24). Only a few studies, however, have examined sampling strategies for nematodes on a large geographic scale (12) or in an extensive survey (31). Furthermore, few studies have been completed to determine an appropriate sample size for communities of both plant-parasitic and free-living nematodes on a scale much larger than a field (29,31).

Sampling to characterize densities of free-living and plant-parasitic nematodes in soil communities at a regional level comprises two aspects. The first results from the desire to compartmentalize sources of variance such that there is a greater proportion of variance among fields than within fields, i.e., a high signal-to-noise ratio. This is necessary to determine status or condition of the resource of interest on a regional basis at one point in time. The second aspect relates to the number of fields that must be sampled within a region to be able to detect a specified and meaningful level of change between two sampling times; this is important in the detection of trends in ecological condition over time. The objective of our research was to estimate the number of composite soil samples (transects) to obtain per field, and the number of subsamples to be assayed for each transect, in order to obtain a high degree of sample reliability. These results were then used to estimate the number of fields to be sampled in a geographic region to detect a prescribed amount of change in condition with specified levels of statistical power and confidence. North Carolina and Nebraska, two states with different types of agricultural systems, climate, and soils, were selected as initial study regions, and sampling of nematode communities was performed as a portion of a larger survey and sampling projects (8,19).

Materials and Methods

Survey design: The USDA-National Agricultural Statistics Service (NASS) has a well-established, nationwide sampling design used to conduct agricultural surveys each year for state, regional, and national estimates of agricultural production and associated management practices. NASS uses an area frame, probability sampling design (9) to conduct its surveys. June Agricultural Surveys are conducted on a statewide basis. Within a state, land is stratified by the proportion of land under cultivation; extensively cultivated areas are sampled at greater intensity than sparsely cultivated areas. A sampling frame is developed for each stratum in a two-stage process. First, the stratum is divided into primary sampling units; then a random subset of these is divided into small units called segments. Segments are chosen at random for data collection. NASS relies on a sample size of $n = 390$ segments, for example, to make statewide estimates of agricultural production for Nebraska. Complete land use data are collected for each sample segment. The location of each field in each sample segment is mapped on an aerial photograph and coded for identification. Each segment has an associated expansion factor, which is the inverse of the probability of that segment being included in the sample (9). Multiplying a hectare by its segment's expansion factor converts that hectare to the number of hectares it represents.

Because it has the reputation of providing reliable and acceptable estimates on statewide scales, we chose the USDA-NASS area frame as the experimental design of this study to determine appropriate sample sizes needed for measurements of soil fauna on a regional scale. We limited our selection of sites to fields that had annually harvested, herbaceous crops or that could be planted to these crops in North Carolina ($n = 164$) in 1992 and Nebraska ($n = 154$) in 1993 (8,19). Soil samples were collected from fields by enumerators (non-specialists) working in cooperation with the USDA-NASS.

Within each field selected, one 90-m linear transect was located randomly and diagonally to rows of current or previous crops and sampled to estimate variation among fields. In every sixth field, a second, independent transect also was sampled to estimate variability within fields. Variability within composite samples was quantified by splitting the composite soil samples of double volume taken from the second transect in every 12th field. For the first transect in each field, soils were sampled by taking one core (2-cm/diam. × 20-cm/deep) at each of 20 equally spaced sites along the 90-m transect (29). Soil cores were mixed thoroughly by hand to form a composite sample and to reduce variance associated with the aggregated spatial pattern of nematodes in soil (1) and to obtain a representative estimate of the nematode community within the field. For the second transect, two soil cores were taken at each sample site. After pooling and mixing the 40 cores, the composite soil sample was subdivided equally to provide two subsamples for laboratory assay and nematode identification. Each soil sample or subsample was placed in a plastic bag and sent via overnight courier to the identification laboratory (N & A Nematode Identification Service, Davis, CA).

Laboratory analyses: Nematodes were extracted from each soil sample or subsample by a modified Cobb's sifting and gravity method followed by sugar centrifugal-flotation (28,29). Numbers of nematodes in each taxonomic family and trophic group were counted in 500 cm^3 soil; counts were not corrected for extraction efficiency. Taxonomic families were assigned to a trophic group (plant-parasitic, bacterivorous, fungivorous, omnivorous, and predatory) according to Yeates et al. (39) (Table 1). Taxonomic families were also assigned a colonizer-persister (*c-p*) value according to Bongers (5) (Table 1).

Statistical analysis: Five indices were computed for the nematode community in each soil sample or subsample: (i) maturity index for all free-living nematodes (MI) (5); (ii) maturity index for free-living nematodes excluding opportunists (MINO) (Bongers, pers. comm.); (iii) maturity index for all plant-parasitic nematodes (PPI) (5); (iv) combined maturity index for free-living nematodes excluding opportunists, i.e., MINO, and plant-parasitic nematodes (ΣMI) (38); and (v) Shannon index of trophic diversity (SHAN) (34). The MI was calculated as the weighted mean of the values assigned to constituent nematode families (and the genera and species they contain) (5): MI or PPI = $(\Sigma v_i * f_i)/n$ where v_i = the colonizer-persister (*c-p*) value assigned to family i, f_i = the frequency of family i in a sample or subsample, and n = total number of individuals in a subsample. *C-p* values range from 1 to 5; plant-parasitic taxa are assigned *c-p* values only from 2 to 5, however, because there are no plant-parasitic colonizers designated as category 1 (5). Free-living nematodes with a *c-p* value of 1 are considered enrichment opportunists (7). Opportunist populations increase when nitrogen fertilizer and (or) organic matter are added to soil, changing the soil food web structure only temporarily. Therefore, the maturity index for free-living nematodes was calculated separately with (MI) or without (MINO) opportunists (*c-p* = 1)included. Diversity of nematodes by feeding preference was estimated with the Shannon diversity index, $NI = \exp[-\Sigma P_i(\ln P_i)]$, where P_i is the proportion of trophic group i in the total nematode community (22).

Variance components were estimated for three sources of variation: among fields, within fields, and within composite soil samples. Reliability ratios were estimated from the variance components to measure the relative ability of an index to differentiate among field sites. This approach is analogous to that used by Shokes et al. (35) in studying disease assessment procedures. Values were calculated such that

$$\text{Reliability ratio} = \{\sigma^2_{\text{among fields}}/[\sigma^2_{\text{among fields}} + (\sigma^2_{\text{within fields}}/t) + (\sigma^2_{\text{within samples}}/(t \cdot d))]\} \quad (1)$$

TABLE 1. Mean abundance of families of nematodes in soil samples collected in North Carolina (1992, n = 164) and Nebraska (1993, n = 154) that were used in calculation of the MINO[a], PPI[b], and SHAN indices. *C-p* values are from Bongers (5), and trophic groups are assigned as: 1 = bacterivorous, 2 = fungivorous, 3 = plant-parasitic, 4 = omnivorous, and 5 = predatory.

Family	*c-p* value	Trophic group	Number/500 cm^3 of fresh soil NC	NE
Alaimidae[a]	4	1	43.2	7.5
Anatonchidae[a]	4	5	3.0	0.0
Anguinidae[a]	2	2	23.3	47.1
Aphelenchidae[a]	2	2	99.4	210.1
Aphelenchoididae[a]	2	2	89.4	226.5
Bastianidae[a]	3	1	0.6	0.0
Belondiridae[a]	5	4	11.2	1.8
Belonolaimidae[b]	2	3	149.8	116.4
Bunonematidae	1	1	0.2	0.1
Carcharolaimidae[a]	4	5	1.0	1.4
Cephalobidae[a]	2	1	401.0	573.8
Chromadoridae[a]	3	5	2.6	0.3
Criconematidae[b]	3	3	103.9	6.9
Cyatholaimidae[a]	3	4	2.3	1.0
Cylindrolaimidae[a]	3	1	2.8	1.7
Diphtherophoridae[a]	3	2	15.4	4.9
Diplogasteridae	1	5	74.1	56.6
Diploscapteridae	1	1	15.2	4.4
Dorylaimellidae[a]	5	4	4.0	4.4
Dorylaimidae[a]	4	4	199.1	156.9
Heteroderidae[b]	3	3	132.4	3.0
Hoplolaimidae[b]	3	3	430.5	143.1
Iotonchulidae[a]	4	5	1.8	0.0
Isolaimidae[a]	4	1	0.1	0.0
Leptolaimidae[a]	3	1	1.1	0.0
Leptonchidae[a]	4	4	7.7	8.1
Longidoridae[b]	5	3	47.2	41.0
Microlaimidae[a]	3	1	1.6	0.1
Monhysteridae	1	1	24.3	5.3
Mononchidae[a]	4	5	78.2	12.2
Mylonchulidae[a]	4	5	17.9	10.3
Nygolaimidae[a]	5	5	4.0	1.4
Panagrolaimidae	1	1	5.4	3.8
Paraphelenchidae[a]	2	2	1.0	4.9
Plectidae[a]	2	1	68.2	11.6
Pratylenchidae[b]	3	3	95.1	99.6
Prismatolaimidae[a]	3	1	38.9	8.4
Rhabditidae	1	1	741.5	308.0
Rhabdolaimidae[a]	3	1	0.7	0.0
Seinuridae[a]	2	5	2.9	0.8
Teratocephalidae[a]	3	1	3.3	0.1
Trichodoridae[b]	4	3	32.1	1.8
Tripylidae[a]	3	4	13.3	0.4
Tylenchidae[b]	2	3	361.5	513.1
Tylencholaimellidae[a]	4	2	21.1	9.6
Tylencholaimidae[a]	4	2	11.1	8.4
Tylenchulidae[b]	2	3	18.8	74.4

where t = number of transects per field and d = number of subsamples per transect analyzed. The quantity, $(\sigma^2_{within\ fields}/t)$, is the mean variance per transect. Similarly, $(\sigma^2_{within\ samples}/(t \cdot d))$ is the mean variance per subsample. The reliability ratio is the correlation between independent measurements made on a sample unit. In our case, the measurements are from two independent transects and the sample unit is

a field. Because we are interested in making regional statements, differences among fields are of primary interest. Field estimates represent individual data points for a region that contains a population of fields. Thus, among field variability is the "signal," whereas variability within fields and within composite samples can be defined as "noise." High variability within fields and within samples relative to the variability among fields results in a smaller reliability ratio, i.e., the more noise, the less reliable an observation is for a field. When the reliability ratio is small, it is more difficult to detect differences among fields. We arbitrarily chose the criterion of having a value of 0.70 or larger for a reliability ratio to be considered satisfactory.

Power curves were also constructed with the variance components for North Carolina and Nebraska soil samples separately to estimate the number of fields that would need to be sampled if we wanted to detect a 10% change in mean value in a statewide region (i.e., 150,000–200,000 km^2) between two time periods with a power $(1-\beta)$ of 0.8 and an alpha (α, the probability of rejecting a true null hypothesis, Type I error) of 0.1. For this type of study, the null hypothesis (H_0) would be that no difference exists between the mean values for two time periods. The alternative hypothesis (H_a) is that a difference does exist. Thus, β is the probability of failing to reject the H_0 when it is false. Power $(1-\beta)$ is the probability of detecting the specific H_a (11, p. 62). We assumed that data points for the two time periods would be distributed normally. Power curves were calculated using the estimated variance components from the study. We allowed sample size to range from 25 to 200 fields per region and made the calculation with either two or three composite samples (transects) per field and one or two laboratory determinations per sample. All statistical analyses were performed with SAS Ver. 6.08 (SAS Institute, Cary, NC).

Cost factors were also recorded as an aid in selecting among various sampling options. Mean time to collect soil samples for each transect (=sample) and subsample was estimated. Cost to mail samples to the identification laboratory and costs associated with nematode extraction and identification were also recorded. Cost to locate and travel among fields was not used in our calculations. Field personnel were selected within multiple regions of each state, and travel time to a field did not exceed 1 hour. Thus, travel costs were small in relation to sampling and identification costs.

Results

Nematodes from a total of 47 and 41 families were found in North Carolina and Nebraska, respectively (Table 1). A total of 107 and 98 genera represented these families in North Carolina and Nebraska, respectively (data not shown). Abundances of families present ranged from 0.1 per 500 cm^3 of fresh soil for Isolaimidae to 741.5 for Rhabditidae in North Carolina and from 0.1 ml per 500 cm^3 of fresh soil for Bunonematidae, Microlaimidae, and Teratocephalidae to 573.8 for Cephalobidae in Nebraska (Table 1). In general, plant-parasitic (37–40%) and bacterivorous (34–40%) nematodes were the more abundant trophic groups in each state.

Overall, the total variance for nematode communities as characterized by the maturity indices, MI and MINO, and the trophic diversity index, SHAN, were greater in North Carolina than Nebraska; the opposite was true for maturity indices including populations of plant-parasitic nematodes, PPI and ΣMI. Total variability and components of total variability of ΣMI were less than either maturity of plant-parasitic (PPI) or free-living (MINO) nematodes calculated separately, except among fields in Nebraska (Table 2).

Total variance, especially in North Carolina, and within-sample variance in both states was less for MINO than MI, suggesting that inclusion of early-colonizing taxa (c-p = 1) increased variance or noise and may have reduced reliability of detection (see Eq. [1]). The proportion of total vari-

TABLE 2. Variance components for five nematode community indices. Actual variance values are presented for North Carolina (1992) and Nebraska (1993) soils.

Indices	Among fields		Within fields		Within samples	
	NC	NE	NC	NE	NC	NE
Maturity index (MI)	0.104	0.032	0.010	0.050	0.127	0.064
Maturity index (MINO)	0.028	0.059	0.032	0.052	0.093	0.027
Plant-parasitic index (PPI)	0.040	0.124	0.031	0.032	0.030	0.021
Combined maturity index (ΣMI)	0.011	0.071	0.031	0.027	0.025	0.011
Shannon trophic diversity (SHAN)	0.081	0.228	0.175	0.035	0.134	0.103

ance explained by variability within samples, however, was not consistent between states. In North Carolina, variance within samples was 53 and 61% of the total variance for MI and MINO, respectively. The reciprocal was true for Nebraska, with 44 and 20% of total variance within samples for MI and MINO, respectively (Table 2).

Values for the variance component, "within samples," was greater in North Carolina than Nebraska, especially for maturity indices with only free-living nematodes, i.e., MI and MINO (Table 2). To achieve a reliability ratio of 0.7, it would be necessary to analyze at least two subsamples for each of two or more transects sampled for MI in North Carolina and at least two subsamples for each of three or more transects for MINO in Nebraska (Table 3). The target reliability ratio of 0.7 could not be achieved even with three subsamples assayed for each of three transects for MINO, ΣMI, or SHAN in North Carolina or for MI in Nebraska. For PPI in Nebraska, variability among fields was about four times greater than either variability within fields and within samples. Thus, only one transect (=sample) with one subsample assayed per transect would be needed to achieve a reliability ratio of 0.7 or greater. Variance was distributed equally among the components of total variance for PPI values in North Carolina, with the result that three transects per field and two subsamples per transect would be necessary to achieve a reliability ratio of 0.7. Furthermore, if the goal is to achieve a reliability ratio of 0.8, additional transects and subsamples per field would be required to differentiate clearly among fields in either state. Given these results, we identified two possible sampling strategies for further evaluation for a regional survey: Option 1—two independent transects per field with single subsample per transect, which would provide a reliability of approximately 0.6 or greater for MI and PPI in North Carolina and for MINO, PPI, ΣMI, and SHAN in Nebraska; and Option 2—three independent transects with duplicate subsamples per transect, which would provide a reliability of 0.7 or greater for the same index-site combinations identified for Option 1.

Optimum sample size that would be needed to detect a 10% change (with power of 0.8) in index value between two sampling periods varied by index, state, and sampling option. For example, in North Carolina, an appropriate sample size for MINO would be 100 fields with Option 1 (Fig. 1A) and 50 fields with Option 2 (Fig. 1B). In Nebraska, 100 and 75 fields would be necessary for Options 1 and 2, respectively (Table 4). Including opportunistic nematodes ($c\text{-}p = 1$), as in MI, increases the required sample size substantially for North Carolina, but not for Nebraska (Table 4). The reverse is true for PPI, where sample sizes of ≥200 fields are necessary to sample a state such as Nebraska, but only 50 to 75 fields would be required in North Carolina (Table 4). Samples sizes required for ΣMI are 25 to 50 to sample North Carolina and similar to MINO for Nebraska, i.e., 100 and 75 fields for Options 1 and 2, respectively (Table 4). For SHAN, sample sizes >200 fields would

TABLE 3. Reliability ratios for several indices of nematode community structures for various sampling plans. Values greater than 0.7 are shown in bold italics.

Samples per field	Subsamples per composite	Reliability ratios[a] MI[b]	MINO[c]	PPI[d]	ΣMI[e]	SHAN[f]
		North Carolina, 1992				
1	1	0.432	0.183	0.397	0.170	0.209
1	2	0.587	0.263	0.467	0.208	0.252
1	3	0.667	0.307	0.496	0.225	0.270
2	1	0.603	0.310	0.568	0.290	0.345
2	2	***0.740***	0.416	0.636	0.345	0.402
2	3	***0.800***	0.470	0.663	0.368	0.426
3	1	0.695	0.402	0.664	0.380	0.442
3	2	***0.810***	0.516	***0.724***	0.441	0.502
3	3	***0.857***	0.571	***0.747***	0.466	0.526
		Nebraska, 1993				
1	1	0.219	0.427	***0.703***	0.655	0.623
1	2	0.281	0.473	***0.747***	0.689	***0.725***
1	3	0.310	0.491	***0.763***	***0.701***	***0.767***
2	1	0.360	0.598	***0.826***	***0.792***	***0.768***
2	2	0.438	0.642	***0.855***	***0.816***	***0.841***
2	3	0.473	0.658	***0.865***	***0.825***	***0.868***
3	1	0.457	0.691	***0.877***	***0.851***	***0.832***
3	2	0.539	***0.729***	***0.898***	***0.869***	***0.888***
3	3	0.574	***0.743***	***0.906***	***0.876***	***0.908***

[a] Calculated from the variance component estimates in Table 2.
[b] Maturity index for free-living nematodes (including taxa with $c\text{-}p = 1$ to 5).
[c] Maturity index for free-living nematodes (including taxa with $c\text{-}p = 2$ to 5).
[d] Maturity index for plant-parasitic nematodes (including taxa with $c\text{-}p = 2$ to 5).
[e] Maturity index for free-living and plant-parasitic nematodes combined (including taxa with $c\text{-}p = 2$ to 5).
[f] Shannon index of trophic diversity.

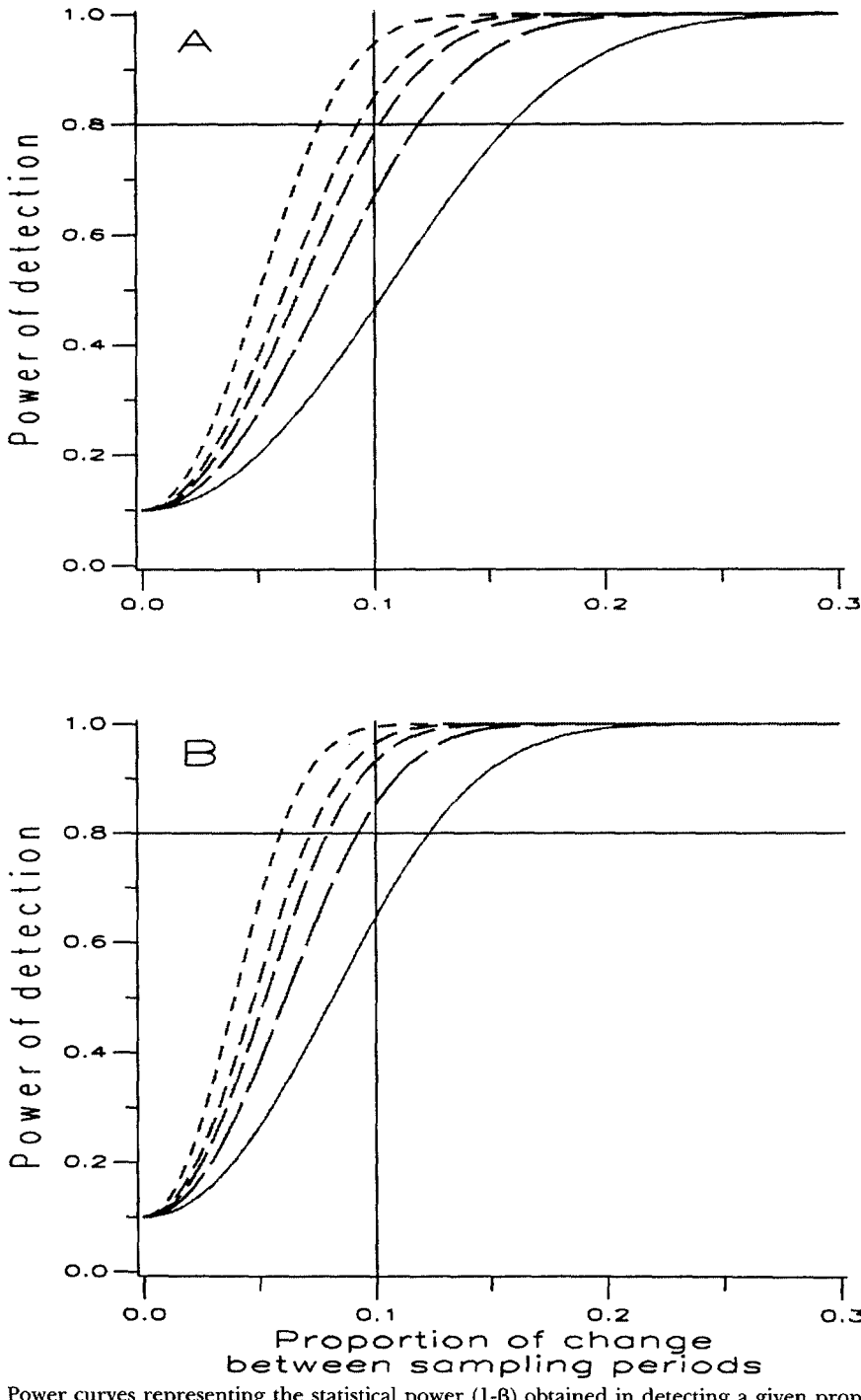

FIG. 1. Power curves representing the statistical power (1-β) obtained in detecting a given proportion of change, from one time period to another, in the mean value of MINO for North Carolina (1992) for a certain number of fields sampled. The power curves as shown in A) one composite sample for each of two transects (Option 1) and in B) two composite samples for each of three transects (Option 2) were analyzed per field. A single curve can be interpreted as representing the ability to detect x proportion of change with y power from one time period to another time period for the given number of fields sampled. Different line styles indicate the number of fields sampled in a state or region (solid = 25, long dash = 50, medium dash = 75, short dash = 100, mini dash = 200).

TABLE 4. Optimum sampling size for statewide surveys in North Carolina (1992) and Nebraska (1993). Two strategies for sampling within fields are compared: Option 1 with two transects per field and one subsample per transect, and Option 2 with three transects per field and two subsamples per transect. Estimates are based on variance components (Table 2) and reliability ratios (Table 3). Values represent proportion of change in the mean between sampling periods that can be detected with a range of sample sizes (number of fields per state) with power (1-β) = 0.8 and alpha (α) = 0.10.

				Number of fields per state				
Index	State/year	No. transects	No. samples	25	50	75	100	200
MI[a]	NC92	2	1	0.2196	0.1643	0.1411	0.1280	0.1051
		3	2	0.1895	0.1418	0.1218	0.1104	0.0908
	NE93	2	1	0.1605	0.1211	0.1047	0.0954	0.0796
		3	2	0.1310	0.0989	0.0855	0.0780	0.0650
MINO[b]	NC92	2	1	0.1594	0.1192	0.1025	0.0929	0.0764
		3	2	0.1234	0.0924	0.0793	0.0720	0.0591
	NE93	2	1	0.1678	0.1266	0.1095	0.0998	0.0833
		3	2	0.1520	0.1147	0.0992	0.0904	0.0754
PPI[c]	NC92	2	1	0.1409	0.1054	0.0905	0.0821	0.0675
		3	2	0.1248	0.0934	0.0802	0.0728	0.0597
	NE93	2	1	0.2077	0.1567	0.1355	0.1235	0.1030
		3	2	0.1991	0.1502	0.1299	0.1184	0.0988
ΣMI[d]	NC92	2	1	0.1044	0.0782	0.0672	0.0609	0.0500
		3	2	0.0847	0.0634	0.0544	0.0494	0.0406
	NE93	2	1	0.1601	0.1208	0.1045	0.0952	0.0794
		3	2	0.1528	0.1153	0.0997	0.0909	0.0758
SHAN[e]	NC92	2	1	0.2570	0.1923	0.1651	0.1497	0.1231
		3	2	0.2131	0.1594	0.1369	0.1241	0.1021
	NE93	2	1	0.2917	0.2200	0.1903	0.1735	0.1446
		3	2	0.2713	0.2046	0.1770	0.1613	0.1345

[a] Maturity index for free-living nematodes (including taxa with *c-p* = 1 to 5).
[b] Maturity index for free-living and plant-parasitic nematodes combined (including taxa with *c-p* = 2 to 5).
[c] Maturity index for plant-parasitic nematodes (including taxa with *c-p* = 2 to 5).
[d] Maturity index for free-living and plant-parasitic nematodes combined (including taxa with *c-p* = 2 to 5).
[e] Shannon index of trophic diversity.

be required for either Nebraska or North Carolina (Table 4). Numbers of fields that need to be sampled to detect the specified 10% change between two times increase as the variance among fields increases (Tables 1,3).

Costs also must be considered when determining an optimum sampling design. On average, it would require 3 hours (1.5 hours for each transect) to sample one field for Option 1 and 5.5 hours (1.5 hours for each transect + 20 minutes for each transect to obtain a second subsample) per field for Option 2. With a sample size of 75 fields for Option 1 and 50 fields for Option 2, it would take a total of 225.0 and 275.0 hours of actual time in the fields to complete field sampling for a regional survey for Options 1 and 2, respectively. Given the cost of $4 to mail each subsample to the laboratory and $70 per subsample to extract, identify, and count the nematodes in the sample, Option 1 would cost about $11,100 and Option 2 about $22,200, not including the cost of labor involved in collecting the soil in the field for a regional survey. Because it is more expensive to process samples (i.e., extract from soil, identify, and count) than to obtain them from fields, it is more cost-effective to choose Option 1 than 2. Based on costs associated with Option 1, reliability, and the power of detection, it would be more effective and efficient, especially in North Carolina, to use ΣMI rather than either MINO or MI because only 50 to 100 fields would need to be sampled for ΣMI, whereas ≥100 fields would be required for MI or MINO to achieve the same power of detection that ΣMI offers (Table 4). Required numbers of fields are greater for the indices PPI and ΣMI for Option 1 than Option 2 in both North Carolina and Nebraska; it would be less costly to increase the number of fields, however, and use Option 1 than to increase the laboratory determinations required per field for Option 2.

Discussion

Measures such as maturity or diversity indices, which describe community structure for nematodes, can provide an indication of the condition or ecological health of agricultural soils. Such measures provide important information about soil quality for a range of organisms in the soil food web and can serve as integral components of assessments for characteristics such as sustainability and biodiversity of agricultural systems. The focus of our assessments was on agricultural systems rather than individual fields. Because of the implied regionality of such systems-level assessments, we quantified measures of nematode community structure at a regional scale.

Assurance of quality estimates for ecological indices of nematode community structure at a regional level requires the development and evaluation of sampling schemes that are appropriate for the geographic scale of interest. Most studies on sampling for nematodes have, however, focused on detection and quantification at the field scale for assessing field-level risk or providing management options (e.g., 16, 18, 26, 33). Whereas these field-level studies provide an excellent basis upon which to design a sampling scheme that will achieve goals of detection and representativeness of samples *within fields,* little information from such studies can be extrapolated to prescribing sampling strategies to detect differences *among fields.*

Three sources of variation are important as inputs to regional sampling plans: among fields, within fields, and within composite samples. Each source can be quantified in the sampling scheme with the goal of ensuring that most of the variation encountered occurs among fields, because that is the level of information appropriate for inclusion in regional-scale statements. Large amounts of variation in any of the three sources will result in the need to allocate more sampling resources to that level in order to achieve the desired reliability and power to detect differences in condition through time.

We selected two states with contrasting agricultural landscapes in different land resource and ecological regions for this

study and, as expected, found differences in the nematode communities in these regions. Comparison of the occurrence and prevalence of specific nematode taxa and differences in maturity of the nematode communities in the two geographic areas are being addressed (D. A. Neher et al., unpubl.). Greater total variance for three indices, i.e., MI, MINO, and SHAN, was found in North Carolina than Nebraska, whereas larger total variance for PPI and ΣMI was found in Nebraska than North Carolina. This is due, in part, to the fact that hosts susceptible to plant-parasitic nematodes were grown on proportionally larger hectareages in North Carolina than in Nebraska, which results in greater overall uniformity across the state for plant-parasitic nematodes in North Carolina than in Nebraska. For both states, however, the largest variation for PPI was among fields rather than within fields or within samples. The relative magnitude of difference among the three sources of variation was much greater for Nebraska than for North Carolina. The differential allocation of variance within samples for MI and MINO between the states also reflects a difference in the relative success of free-living opportunists ($c\text{-}p = 1$) versus those families that signify greater community maturity ($c\text{-}p = 2$ to 5), i.e., the free-living opportunist nematodes have a greater degree of uniformity of occurrence within fields in Nebraska than in North Carolina. Relatively large total and within-sample variances for MINO in comparison to MI also suggest that, in both states, inclusion of the free-living opportunists ($c\text{-}p = 1$) in maturity indices decreases the reliability of detection by inflating noise (=within-field plus within-sample variability; see Eq. 1).

Reliability ratio values for ΣMI were less than MINO or PPI in North Carolina but between values of MINO and PPI in Nebraska. Reliability ratios that were relatively large for PPI indicated that it is more important to increase numbers of fields than transects within fields for a regional monitoring program in which plant-parasitic nematodes are of interest. For maturity of free-living nematodes, MI had greater reliability ratios than MINO and ΣMI in North Carolina, but ΣMI performed better than MINO and MI in Nebraska. Therefore, MI and ΣMI may be superior to MINO in terms of reliability for regional sampling.

The two sampling options that we examined in detail, i.e., Option 1 with two samples (transects) per field and one subsample per transect and Option 2 with three samples per field and two subsamples per transect, indicate that a general increase in reliability from 0.6 to 0.7 can be achieved by increasing numbers of samples per field and number of subsamples assayed per sample. The results were as expected. However, the relatively small gain in the signal-to-noise ratio for a relatively large increase in sampling effort ultimately reflects the large degree of variability associated with the ecological indices due to natural variation in nematode communities.

Based upon the results of our study, a proposed sampling scheme for regional studies would include 50 to 100 fields with three independent samples (transects) per field and two subsamples assayed per sample. If cost is a major limiting factor, a second choice would be a larger number of fields with only two samples and one subsample assayed per field. The information obtained using the second choice would have a lower degree of reliability; however, cost is often a driving factor in sampling programs. For states or regions such as North Carolina, in which plant-parasitic nematodes are major agricultural pests, an index such as MI may be a better choice than PPI if the focus of the study is to examine overall maturity or stability of nematode communities. For states or regions such as Nebraska, in which plant-parasitic nematodes occur but are less prevalent, ecological indices that include plant-parasitic nematodes such as PPI and ΣMI may be the better choices for use because they indicate variability among fields more reliably than indices that include only free-living nematodes such as MI or MINO.

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