Estimating Relative Error in Nematode Numbers from Single Soil Samples Composed of Multiple Cores[1]

R. McSorley and J. L. Parrado[2]

Abstract: Spatial distributions of several species of plant-parasitic nematodes were determined in each of three fallow vegetable fields and in smaller subunits of those fields. Goodness of fit to each of several theoretical distributions was tested by means of a X^2 test. Distributions for most species showed good agreement with a negative binomial model. An exception occurred with *Criconemella* sp., which showed a better fit to the Neyman Type A distribution. For nematodes distributed according to the negative binomial model, the number of cores per composite sample needed to achieve specified relative errors was calculated. For a given nematode species, such as *Quinisulcius actus* (Allen) Siddiqi or *Meloidogyne incognita* (Kofoid & White) Chitwood, the k values for the negative binomial distribution increased as field size decreased, with the result that fewer cores were needed to achieve the same level of precision in a smaller field. Best results were achieved when the single sample was used to estimate populations in fields of 0.25–0.45 ha in size. When using only a single composite sample to estimate mixed populations of the nematodes studied here in a field of that size, approximately 22 cores per composite sample would be needed to estimate all population means within a standard error to mean ratio of 25%. Considerably more cores were needed to maintain a given level of precision in fields of 1.0 ha or greater, and it may be necessary to subdivide larger units (ca. 1.5 ha and up) for accurate sampling. *Key words:* spatial distribution, negative binomial distribution, Neyman Type A distribution, *Criconemella* sp., *Helicotylenchus dihystera, Meloidogyne incognita, Quinisulcius acutus, Rotylenchulus reniformis.*

Journal of Nematology 14(4):522-529. 1982.

The need for accurate sampling plans to estimate soil populations of plant-parasitic nematodes has become apparent with the greater emphasis by diagnostic services on nematode numbers and economic thresholds. Few plans are available for sampling agronomic and vegetable crops for nematodes other than *Heterodera* spp. Twenty cores of soil for a 1.6-ha field have given adequate results in North Carolina (1), but Proctor and Marks (11) found that precise data on *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans-Stekhoven in small plots could not be obtained without considerable effort and would be impractical in most cases. Goodell and Ferris (5) found that different combinations of sample and core numbers were needed to estimate populations of different plant-parasitic nematodes in a 7-ha alfalfa field. In most cases, five hours of collecting and laboratory work were needed to estimate populations within acceptable limits of error.

Because of the wide variety of crops, nematodes, and nematode distributions that may occur in any one geographical area, it is unlikely that any one sampling plan will suffice in all situations. It is desirable to demonstrate a methodology by which a sampling plan can be developed for a particular situation. The present study examines the feasibility of estimating mean nematode populations from a single composite sample consisting of multiple cores from fallow fields of various sizes. The single sample per field case is considered first because 1) the mathematics of the single sample case are more straightforward than the multiple samples per field case, 2) diagnostic laboratories may be required at times to make diagnoses from a single sample, and 3) it is desirable to demonstrate the smallest field unit that can be accurately

Received for Publication 6 April 1982.

[1]Florida Agricultural Experiment Stations Journal Series No 3760.

[2]University of Florida, IFAS, Agricultural Research and Education Center, 18905 S. W. 280 Street, Homestead, FL 33031.

sampled. The multiple samples per field case is the subject of a future study (9). Before sampling plans could be developed, data on the spatial distributions of the nematodes had to be obtained for these situations.

MATERIALS AND METHODS

A stratified random sampling plan (12) was used to collect cores of soil from three fallow vegetable fields in south Florida. According to this sampling plan, each field was divided into a number of equal-sized blocks, with one core collected at random from each block. All fields had been maintained free of weeds by disking and were uniform in soil type, a Rockdale fine sandy loam. Because of the nature of the soil, a cylindrical tool could not be used to obtain a traditional core of soil. Instead, a "core," as used here, was obtained with a hand trowel to a depth of 15 cm, after removing the top 2.5 cm of soil. The resulting material was passed through a 4.0-mm sieve to remove rock, and a 50-cm^3 portion was used as the core from which the nematodes were extracted. This extra step was necessary to obtain cores of uniform size for comparison, and the subsequent analyses assume that cores of uniform size are mixed to form a composite sample. The problems associated with mixing trowels of soil of unequal size are the subject of a future study.

Nematodes were extracted from each 50-cm^3 core by a combination of sieving and centrifugation (7,10). Extracted nematodes were killed by heating at 55–60 C, and 1/5 of each core was counted and recorded as nematodes per 10 cm^3 of soil.

Three different fallow fields were sampled in this study. Field 1 had an area of 0.25 ha and had been planted to yellow squash in the spring of 1980; 90 individual cores of soil were collected from it on 2 February 1981 for this study. Fields 2 and 3 were both planted to tomatoes during the spring of 1981 and had remained fallow during the following summer. Field 2 had an area of 6.14 ha and was sampled on 25 August 1981. Field 3, an area of 2.32 ha, was sampled on 29 September 1981. One hundred individual cores were collected from each of these fields.

A FORTRAN program (3) was used to calculate X^2 values for testing the goodness of fit to the Poisson, negative binomial, and Neyman Type A distributions for each common nematode species in each of the three fields. Field 2 was subdivided into two fields of 3.07 ha each (2Ba, 2Bb), into three fields of 2.05 ha each (2Ca, 2Cb, 2Cc), into four fields of 1.54 ha each (2Da, 2Db, 2Dc, 2Dd), and into six fields of 1.02 ha each (2Fa, 2Fb, 2Fc, 2Fd, 2Fe, 2Ff). Goodness of fit to the statistical distributions then was tested in each of these smaller fields, except in cases where not enough nematodes were present to produce a meaningful analysis. Field 3 was subdivided into two units, 3A and 3B, having areas of 0.93 and 1.39 ha, respectively; these in turn were subdivided to give five units of 0.45 ha each (3Aa, 3Ab, 3Ba, 3Bb, 3Bc). Goodness of fit was determined for each of these smaller units as well. Field 1 was not subdivided into smaller units.

RESULTS

Spatial distribution: Nematodes found in the study sites included *Meloidogyne incognita* (Kofoid & White) Chitwood, *Quinisulcius acutus* (Allen) Siddiqi, *Helicotylenchus dihystera* (Cobb) Sher, *Rotylenchulus reniformis* Lindford & Oliveira, *Criconemella* sp. (8), and *Pratylenchus brachyurus* (Godfrey) Filipjev & Schuurmans-Stekhoven. In some cases nematodes were not abundant enough in certain fields or subdivided fields to permit meaningful goodness of fit tests to the three frequency distributions. Data on *P. brachyurus* and *Criconemella* sp. are particularly sparse because these nematodes were relatively uncommon.

Nematode/field combinations for which goodness of fit tests to all three distributions were performed are shown in Table 1. In all of these cases, the variance (s^2) to mean ($\bar{x}$) ratios greatly exceeded 1.0, suggesting that the underlying distributions were clumped. In one case (for *M. incognita* in field subunit 3Bb), goodness of fit tests to the negative binomial and Neyman Type A distributions could not be performed, since $s^2 \approx \bar{x}$, indicating a noncontagious distribution (3). This distribution was not signifi-

Table 1. Distributions of nematode species by field units and subunits, showing k values for distributions which did not differ significantly ($P \leq 0.05$) from the negative binomial.*

Field unit	Size of unit (ha)	k values for nematode species					
		MI	QA	HD	RR	PB	C
2	6.14	0.27	0.04	0.08	...	...	...
2Ba	3.07	0.27	...	0.07	...	...	...
2Bb	3.07	0.28	...	...	...	...	...
3	2.32	0.84	0.37	0.11	0.15	0.05	0.03‡§
2Ca	2.05	0.26	...	0.07	...	...	...
2Cb	2.05	†	...	...	...	...	...
2Cc	2.05	0.25	...	...	...	...	...
2Da	1.54	0.14	...	0.09	...	...	...
2Db	1.54	0.56	...	...	...	...	...
2Dc	1.54	0.43	...	...	...	...	...
2Dd	1.54	0.17‡§	...	...	...	...	...
3B	1.39	0.66	0.25	0.11	0.15	...	0.06‡§
2Fb	1.02	1.08	...	...	...	...	...
2Fc	1.02	0.22	...	...	...	...	...
2Fd	1.02	0.48	...	...	...	...	...
2Fe	1.02	0.18	...	...	...	...	...
3A	0.93	1.20	0.74	0.12	0.29‡§	0.11	...
3Aa	0.45	1.76	0.58	0.18	†§	...	...
3Ab	0.45	0.84	0.95	...	0.39	...	...
3Ba	0.45	2.67	1.83	0.30	0.30§	...	0.21§
3Bc	0.45	0.41	...	...	...	...	...
1	0.25	...	1.56	1.27	1.30	...	...

*MI = *M. incognita*, QA = *Q. acutus*, HD = *H. dihystera*, RR = *R. reniformis*, PB = P. *brachyurus*, C = *Criconemella* sp. Dots (...) indicate too few individuals or classes to make analysis. Field units 2Fa, 2Ff, 3Bb not shown (see text).

†Significantly different from negative binomial at $P \leq 0.05$ according to X^2 test.

‡Marginal fit to negative binomial with $0.05 < P \leq 0.10$.

§Better fit to Neyman Type A distribution than negative binomial.

cantly ($P \leq 0.05$) different from the Poisson, according to the X^2 test.

Distributions for all other cases (Table 1) were significantly ($P \leq 0.05$) different from the Poisson distribution. On the other hand, most distributions did not differ significantly ($P \leq 0.05$) from the negative binomial, a fact which has also been observed by other investigators (4,11). The k values for the negative binomial distribution fitting each situation also are shown in Table 1. These k values were determined by an iterative procedure (3).

In general, the nematode distributions fit the negative binomial distribution more closely than the Neyman Type A distribution. However, in a few cases, the reverse was true. The only data sets for *Criconemella* sp. were obtained from one field and two of its subunits. The distributions of this nematode were not significantly ($P \leq 0.05$) different from the negative binomial according to the X^2 test, but the P values of 0.062, 0.089, and 0.141 for fields 3, 3B, and 3Ba, respectively, indicated that agreement with a negative binomial model was marginal. Respective P *values* for the X^2 test for the Neyman Type A distribution were 0.662, 0.677, and 0.733. Agreement with the negative binomial distribution was better in the smaller subunits of field 3. Distributions of *R. reniformis* also fit the Neyman Type A distribution in several cases (Table 1), but in the smallest field sampled, its distribution fit a negative binomial model.

Number of cores per composite sample: Various formulae are available for determining the number of sampling units in a random sample (2,12), but discrepancies exist in the formulae reported (6). For data fitting the negative binomial distribution, the most appropriate formula has been given by Southwood (12):

$$n = \frac{1}{E^2}\left(\frac{1}{\bar{x}} + \frac{1}{k}\right), \text{ where:}$$

n = number of sampling units per sample = cores per single sample
$\bar{x}$ = field mean = mean of all samples from a given field
k = k value from the negative binomial distribution
E = standard error to mean ratio

Provided that $\bar{x}$ and k for the underlying negative binomial distribution are known, the above formula can be used to find the required number of cores to be taken per sample if a predetermined standard error to mean ratio, E, is allowed.

The number of cores corresponding to various levels of E are calculated for the three common nematodes in Field 1, the smallest unit studied, with an area of 0.25 ha (Fig. 1). For this field, mean counts per core were 13.74 for *R. reniformis,* 2.50 for *H. dihystera,* and 1.53 for *Q. acutus,* and k values were 1.30, 1.27, and 1.56, respectively. The formula indicates that the number of cores is inversely proportional to the mean and k values. Hence, the curve for *R. reniformis* is the lowest, since that nematode had a much higher mean than the other two. In this example, to maintain a standard error to mean ratio of 20%, 21 cores were needed for *R. reniformis,* 30 cores of *H. dihystera,* and 33 cores for *Q. acutus,* while to maintain E = 25%, 14, 19, and 21 cores, respectively, are needed.

The k values for the fitted negative binomial distribution showed gradual increases as field size decreased for most of the nematodes studied (Table 1). Goodell and Ferris (4) demonstrated similar increases in k values when distributions in small (1 m²) plots were compared to distributions from a large field. While low k values are indicative of clumped populations (12), it is not anticipated that clumping of the populations should decrease as field size decreases, but rather that the influence of clumping on sampling declines as sampling area decreases. Thus, fewer cores are needed to estimate populations in

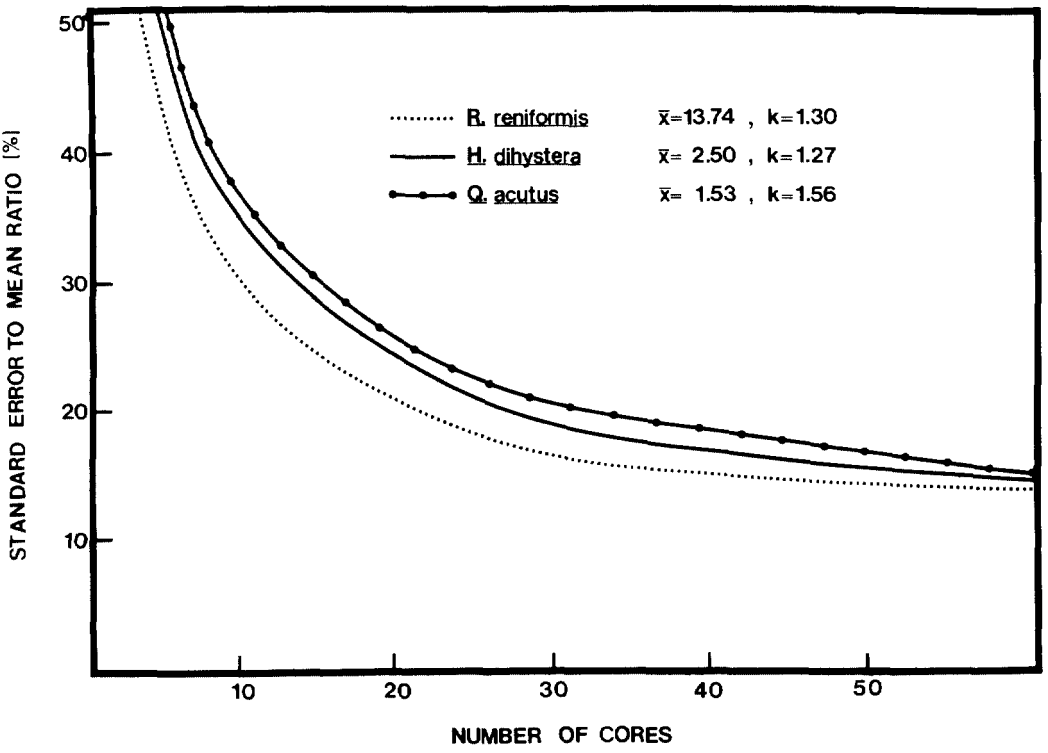


Fig. 1. Relationship between number of cores per sample and relative error in terms of standard error to mean ratio for three nematode species in a 0.25-ha plot. $\bar{x}$ = mean count per core; k = value from negative binomial distribution.

smaller fields. This trend can be illustrated with the data for *Q. acutus* (Table 1). The k value for the large (6.14 ha) field is extremely low, while that from the 0.25-ha field is relatively large. Using methods outlined elsewhere (2), estimates for a common k value (k_c) were obtained for the three samples from fields 0.93–2.32 ha in size and for the samples from subunits 0.45 ha in size. Estimates of k_c were 0.359 and 1.16, respectively. The corresponding relationships between the standard error to mean ratio, E, and the number of cores per sample, n, are shown for these k values and the appropriate means (Fig. 2). The sampling patterns were similar for *Q. acutus* in both small field situations (0.25 ha and 0.45 ha), but as field size increased to 0.93–2.32 ha, considerably more samples are needed to maintain the same relative error. The curve for the 6.14-ha field ($\bar{x}$ = 0.10, k = 0.042) could not be shown on the scale of Fig. 2, since a large number of cores are needed to obtain reasonable error estimates (e.g., 94 cores for E = 60%).

Larvae of *M. incognita* were present in most fields and subunits in sufficient numbers to produce a number of data sets for this economically important nematode (Table 1). Common k values were computed for field units of similar size. Respective k_c values were 1.35 for 0.45-ha units, 0.544 for 0.93–1.02-ha units, 0.294 for 1.39–1.54-ha units, and 0.283 for units of 2.05 ha and greater. For a given mean and k value, the number of cores required for a given standard error to mean ratio, E, can be calculated. These relationships are illustrated for several means at k values of 1.35 (Fig. 3A), 0.544 (Fig. 3B), and 0.294 (Fig. 3C). For a given k value, the number of cores required for a given level of error decreases as the field mean increases. As the k values decreases, more cores are required to maintain the same level of error. For comparative purposes, the curve for $\bar{x}$ = 1.0 of the k = 1.35 example (Fig. 3A) lies very close to the $\bar{x}$ = 100 case for the k = 0.544 example (Fig. 3B), while the curve for $\bar{x}$ = 100 case for the k = 0.294 example

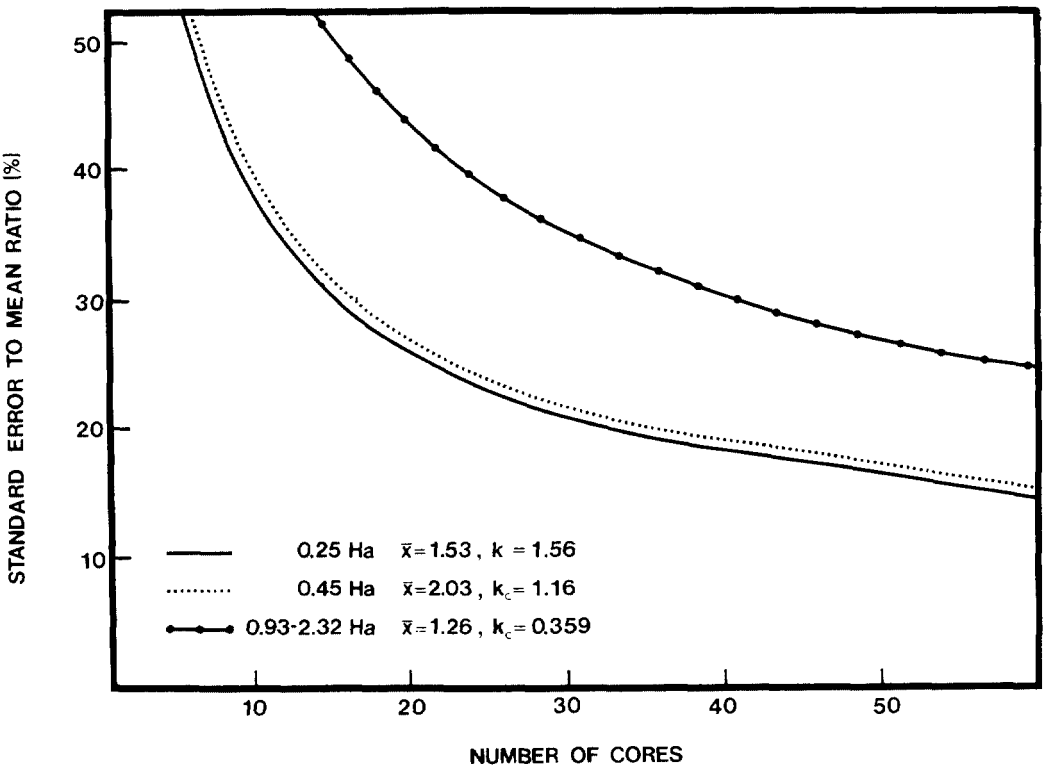


Fig. 2. Relationship between number of cores per sample and relative error in terms of standard error to mean ratio for *Q. acutus* in plots of various sizes. $\bar{x}$ = mean count per core; k, k_c = k values from negative binomial distribution.

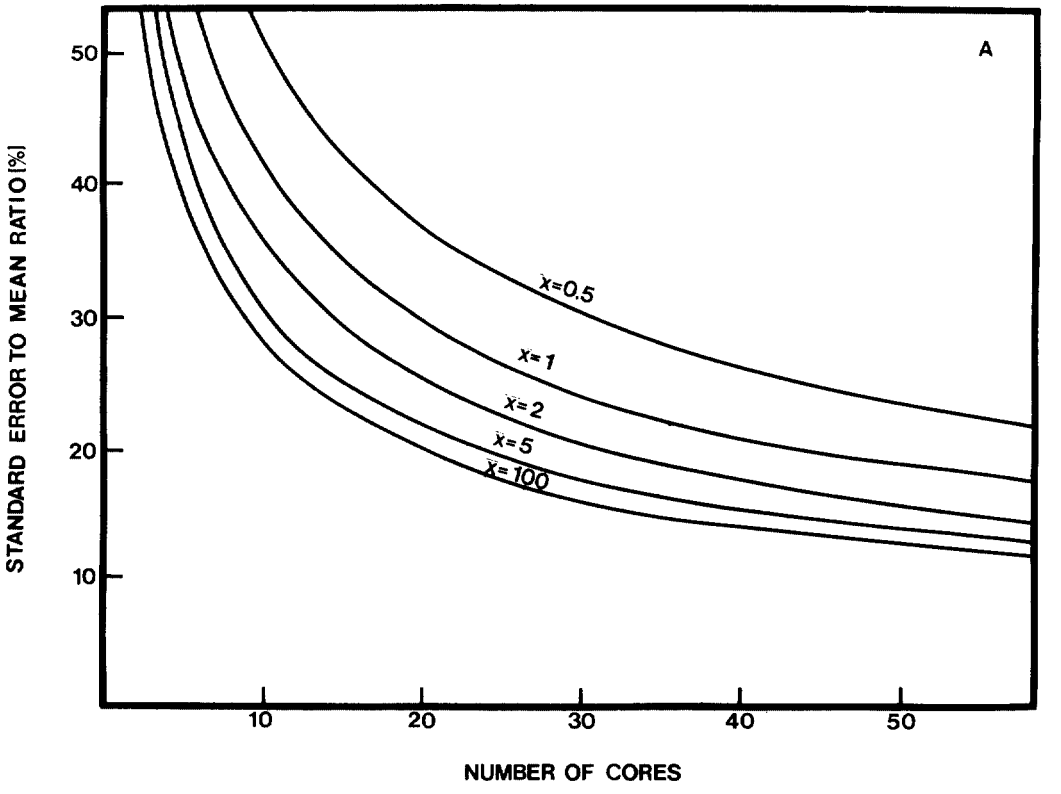
(Fig. 3C) lies between the curves for $\bar{x}$ = 0.5 and $\bar{x}$ = 1.0 for k = 0.544 (Fig. 3B). The curves from Figs. 3A and 3C do not overlap, as the $\bar{x}$ = 100 curve for Fig. 3C lies above the $\bar{x}$ = 0.5 curve for Fig. 3A. A comparison of the three graphs illustrates the combined interaction of mean and k values on the curves for the various sampling schemes.

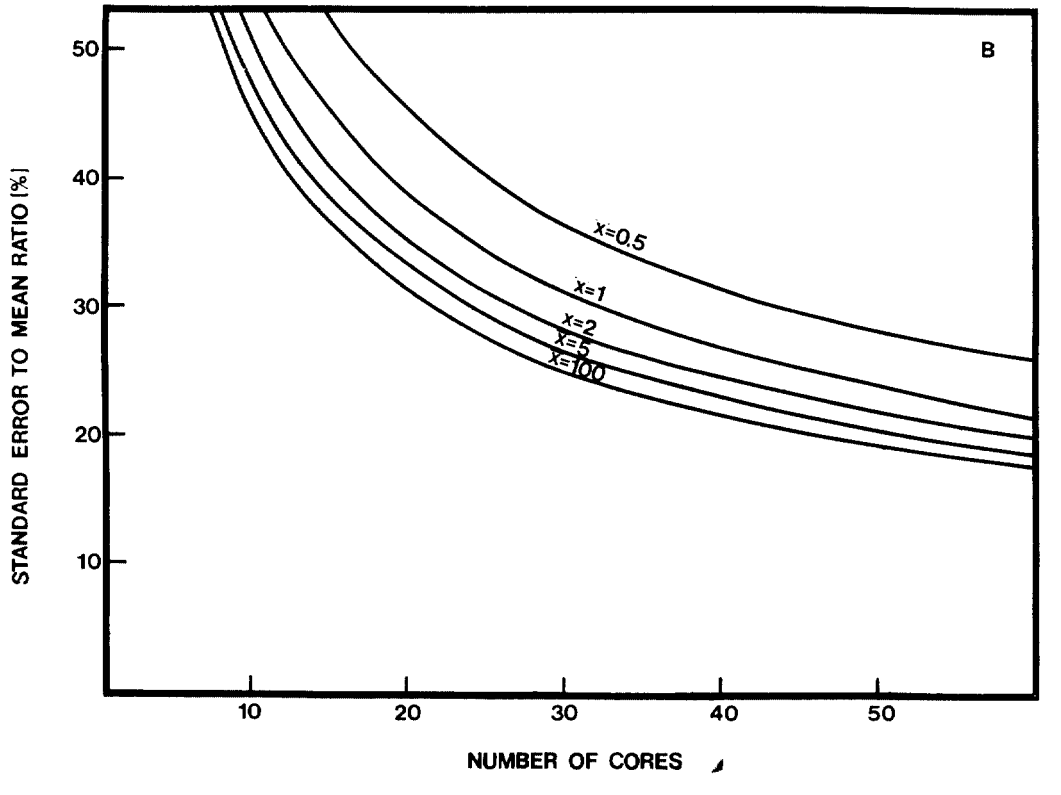
DISCUSSION

It is unlikely that one sampling plan will suffice in all situations, since the underlying spatial distributions vary with different nematode species and with the size of the field to be sampled. Some general guidelines may be suggested for deciding on the number of cores to comprise the single composite sample, if it is necessary to use a single sample to estimate nematode populations. Goodell and Ferris (5) have pointed out that the size of the relative sampling error which can be tolerated depends on the destructive potential of the nematode involved. Thus, the criteria for sampling for *M. incognita* (Fig. 3) are obviously more critical than those for *Q. acutus* (Fig. 2). Additionally, a larger relative error can usually be tolerated for a field having a low mean than for one having a high mean. For example, a mean count here of 1.0 corresponds to 10 nematodes/100 cm^3 of soil; a 50% error would allow for populations up to 15/100 cm^3. By contrast, a mean of 10.0 here corresponds to 100 nematodes/100 cm^3 of soil and a 50% error would be considerable in terms of actual population density. In general, the curves for $\bar{x}$ = 2 and $\bar{x}$ = 100 are fairly close for a given k value (Fig. 3), and so a conservative advisory service may be satisfied with a sampling plan based on the $\bar{x}$ = 2 curve. This can give a useful frame of reference in choosing an appropriate plan when the nematode density or history of the field to be sampled is unknown. If the nematode in question is damaging at low population levels, a curve for a lower mean would have to be used. In sampling fields with a history of nematode problems, a higher mean could be assumed, and therefore fewer cores would be needed. When sampling a field for several nematode species, the curve for the most difficult nematode to sample should be used. Examination of the $\bar{x}$ = 2 line for *M. incognita* (Fig. 3A) and for the other three common nematodes in this study (Figs. 1, 2) suggest that in sampling a small (0.25–0.45) field for all four species, 22 cores are needed for the single sample to maintain a standard error to mean ratio of 25%. If fewer cores are to be collected, greater errors must be tolerated.

The previous discussion assumes some estimates of k for the underlying negative binomial distribution of the nematode to be sampled. If k is very low, then the field may have to be subdivided into smaller units for individual sampling. In this study, fields of 0.25 and 0.45 ha required less sampling effort than fields of one ha. Populations in fields of more than one ha in size are difficult to estimate, because of the large number of cores that must be taken. Obviously, an unlimited number of cores cannot be collected, and a method has been developed (5) for setting an upper limit on the number that can be collected with the resources available. A cost function developed for California conditions (5) indicates that the cost per core would be greater for the sites sampled in south Florida, because of the extra time involved in insuring that uniform cores are collected from the local Rockdale soils.

Finally, there are cases where a single composite sample of multiple cores may be impractical even in a small field unit, particularly if a very low relative error is desired. In these cases, more efficient sampling schemes must be developed by comparing combinations of several composite samples, each containing multiple cores (5). It is evident from this and previous studies (5, 11) that the accurate estimation of field populations of plant-parasitic nematodes requires considerable effort and care in the collection of samples. If a single composite sample is collected, attempts to reduce the amount of work by taking fewer cores per sample can lead to increased errors in the estimates of the population. While high value crops, such as vegetables in south Florida, may support an intensive nematode sampling program, large acreages of low cash value crops may not be able to support the intensive sampling needed to achieve a


A
STANDARD ERROR TO MEAN RATIO [%]
50
40
30
20
10
x̄=0.5
x̄=1
x̄=2
x̄=5
x̄=100
10
20
30
40
50
NUMBER OF CORES


B
STANDARD ERROR TO MEAN RATIO [%]
50
40
30
20
10
x̄=0.5
x̄=1
x̄=2
x̄=5
x̄=100
10
20
30
40
50
NUMBER OF CORES

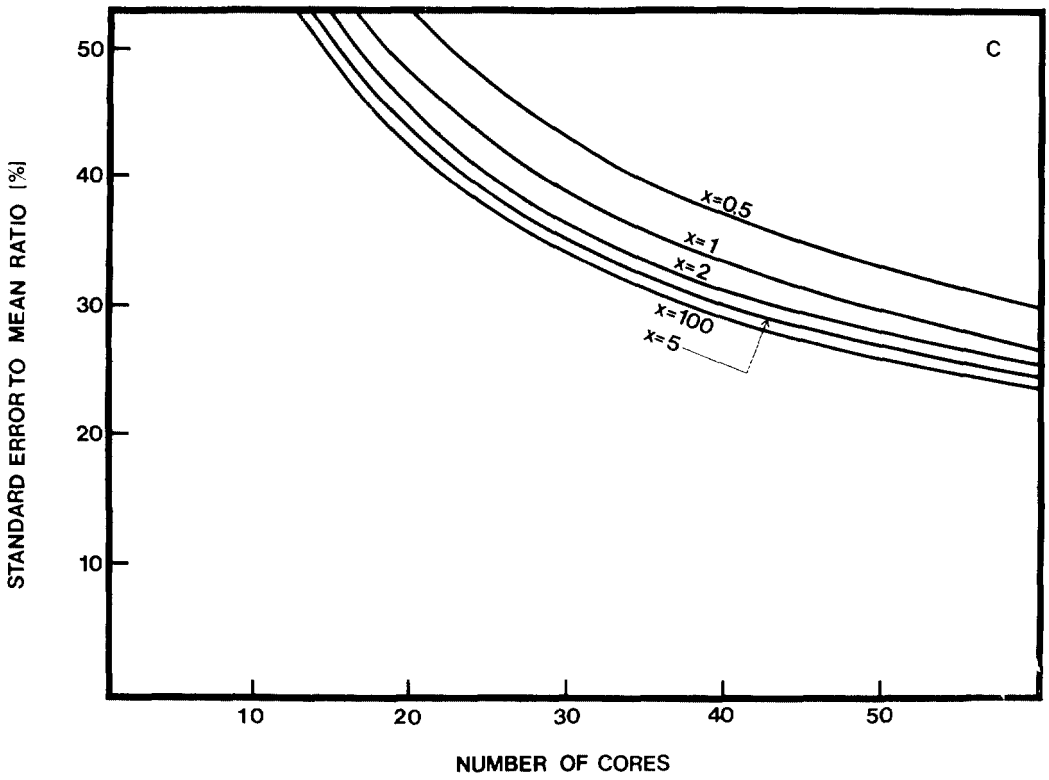


Fig. 3. Relationship between number of cores per sample and relative error in terms of standard error to mean ratio for various mean values ($\bar{x}$) of *M. incognita*. A) 0.45-ha plots, k_e = 1.35. B) 0.93–1.02-ha plots, k_e = 0.544. C) 1.39–1.54-ha plots, k_e = 0.294.

standard error to mean ratio of 25%, and in these situations it would be necessary to tolerate less precision if nematological data are needed.

It is apparent that sampling plans must be custom made for many different situations, since the relationship between number of cores and relative error changes in response to many factors, including nematode species and density, field size, crop, and soil type.

LITERATURE CITED

1. Barker, K. R., and C. J. Nusbaum. 1971. Diagnostic and advisory programs. Pp. 281-301 *in* B. M. Zuckerman, W. F. Mai, and R. A. Rohde, eds. Plant parasitic nematodes. Vol. 1. Morphology, anatomy, taxonomy, and ecology. New York: Academic Press.

2. Elliott, J. M. 1970. Some methods for the statistical analysis of samples of benthic invertebrates. Freshwater Biological Association Scientific Publication No. 25. Windermere, Eng.

3. Gates, C. E., and F. G. Ethridge. 1970. A generalized set of discrete frequency distributions with FORTRAN program. Int. Assoc. Math. Geol. 4:1-24.

4. Goodell, P., and H. Ferris. 1980. Plant-parasitic nematode distributions in an alfalfa field. J. Nematol. 12:136-141.

5. Goodell, P. B., and H. Ferris. 1981. Sample optimization for five plant-parasitic nematodes in an alfalfa field. J. Nematol. 13:304-313.

6. Karandinos, M. G. 1976. Optimum sample size and comments on some published formulae. Bull. Entomol. Soc. Amer. 22:417-421.

7. Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Dis. Rept. 48:692.

8. Luc, M., and D. J. Raski. 1981. Status of the genera Macroposthonia, Criconemoides, Criconemella, and Xenocriconemella (Criconematidae: Nematoda). Revue Nematol. 4:3-21.

9. McSorley, R. 1982. Simulated sampling strategies for nematodes distributed according to a negative binomial model. J. Nematol. 14: 517-522.

10. McSorley, R., and J. L. Parrado. 1981. Effect of sieve size on nematode extraction efficiency. Nematropica 11:165-174.

11. Proctor, J. R., and C. F. Marks. 1975. The determination of normalizing transformations for nematode count data from soil samples and of efficient sampling schemes. Nematologica 30:395-406.

12. Southwood, T. R. E. 1978. Ecological methods with particular reference to the study of insect populations. New York: Halsted Press.