

ERROR ESTIMATES FOR NEMATODE SOIL SAMPLES COMPOSED OF CORES OF UNEQUAL SIZES¹

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ABSTRACT

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Soil samples for nematode analysis are usually composed of a number of cores collected from random field locations. When collected with a soil sampling tube, they will be of nearly equal size, but if collected with a hand trowel, the size of each individual portion (also referred to as a "core" here) comprising the composite sample will vary. With certain soil types, however, a soil sampling tube cannot be used, and a hand trowel may be the only alternative. Distribution of core sizes collected with a trowel was found to be normal in three fields in south Florida having Rockdale fine sandy loam soil. Simulation was used to compare indices of precision obtained from samples composed of cores of unequal size with those obtained from samples composed of equal core sizes. Nematode density in the composite soil sample was the quantity evaluated, and the index of precision used was the standard error to mean ratio. For samples composed of 20 cores, differences in error estimates were small whether the samples had been composed of equal-size or unequal-size cores. Differences were greatest for simulations using the core size distribution with the greatest variance. With samples composed of less than 20 cores, differences in error estimates were also slight when comparing the equal- and unequal- core size cases. In most cases, a level of precision equal to or better than that obtained by collecting a given number of equal-size cores could be achieved by collecting only one more core per sample when cores of unequal size were used.

Additional key words: simulation, sampling precision, nematological techniques, *Meloidogyne incognita*.

RESUMEN

McSorley, R., y J.L. Parrado. 1983. Estimado del error en las muestras de suelo para nematodos compuestas por porciones de tamaños diferentes. *Nematropica* 13:27-36.

Las muestras de suelo para análisis de nematodos están usualmente compuestas por porciones recolectadas de distintos lugares del campo. Cuando las muestras se toman con un tubo especial para muestrear las porciones de suelo recolectadas son casi homogéneas, pero si se toman con una paleta de mano o transplantador el tamaño de cada paletada individual componente de la muestra varía. Debido a que en ciertos tipos de suelo el tubo muestreador no puede ser usado, la paleta de mano es la única alternativa para tomar la muestra, y por lo tanto estas estarán compuestas de por-

ciones heterogeneas. El tamaño de las porciones heterogeneas recolectados con la paleta de mano siguió la distribución normal de la población, en tres campos del Sur de la Florida de suelos de limo arenoso fino del tipo Rockdale. Se usó simulación para comparar los índices de precisión obtenidos de muestras compuestas de porciones heterogeneas con aquellos obtenidos de muestras compuestas de porciones homogeneas. La densidad de la población de nematodos en la muestra compuesta fue la cantidad evaluada y el índice de precisión usado fue la proporción error e standar al promedio. Para las muestras compuestas de 20 porciones las diferencias en el error estimado fueron pequeñas sin importar que estas fueran de porciones de igual o diferente tamaño. Las diferencias fueron mayores para simulaciones usando la distribución de las porciones desiguales con la varianza más grande. Con las muestras compuestas de menos de 20 paletadas, las diferencias en los estimados del error fueron también pequeñas cuando se compararon las porciones de igual y diferente tamaño. En la mayoría de los casos el nivel de precisión igual o mejor obtenido recolectando un determinado número de porciones iguales, puede ser alcanzado aumentando solo una paletada más por muestra en el caso de porciones heterogeneas. *Palabras claves adicionales: simulación, precisión del muestreo, técnicas nematológicas, Meloidogyne incognita.*

INTRODUCTION

Collection of soil samples has long been an important first step in the diagnosis of nematode problems in agriculture. Recent research has focused on the development of improved sampling plans and has attempted to assess the accuracy of sampling plans (3, 4, 5) All proposed plans involve the collection of a number of soil cores from a field, which are then pooled into a bag (or bags) to form one or more composite samples. Each composite sample is later assayed for nematodes in the laboratory. Various tools may be used to collect the individual soil cores making up the composite sample (1), but usually the cores are collected with a sampling tube (Fig. 1), which insures that the cores extracted will be of nearly uniform diameter and length. The hand trowel (Fig. 1) will obtain portions of unequal size, which, for convenience, will also be referred to here as "cores".

Recently, a simulator (4) has been developed to compare estimates of sampling error for sampling plans involving various combinations of numbers of composite samples and cores per sample. Input parameters needed for this computer program are the numbers of samples, cores per sample, and replicate simulation runs over which results are to be averaged for each sampling plan. In addition, an estimate of the field mean (\bar{x}) and an estimate of k for the negative binomial distribution of the nematode to be sampled are needed. The latter parameters may be related to field size in some cases, and estimates have been obtained for certain situations in south Florida (5). Output of the computer program consists of error estimates, such as standard error to mean

ratios, averaged over all the replicate simulation runs for each sampling plan examined. This simulator assumes that all cores making up a composite sample are of equal size.

The requirement of equal core size is easily satisfied if a soil sampling tube is used, but is not appropriate if a trowel (Fig. 1) is used to collect the individual cores making up the sample. However, rocky soils, such as the Rockdale series soils (2) of south Florida, cannot be penetrated with a sampling tube, and a trowel is needed to remove cores from these types of soil. In this situation, it is important to know the extent of the error introduced if cores of unequal size are mixed in a bag to form a composite sample, compared to samples formed from the mixing of cores of equal size. The present work involves modification of the previous simulator (4) to compare results obtained with cores of equal size to those obtained for cores of unequal size, as would be the case if a trowel were used to collect samples.

MATERIALS AND METHODS

The mean nematode density in a soil sample composed of multiple cores of equal size, where each core contributes a proportion $1/n$ to the mean, is given by:

$$\bar{c} = \frac{\sum_{i=1}^n c_i}{n} \quad (\text{Eq. 1})$$

where

- \bar{c} = mean nematode density
- c_i = nematode density in the i^{th} core
- n = number of cores per sample

With unequal core sizes, the contribution of each core must be weighted, depending on the relative size of the given core. If s_i is the size of a given core, then

$$\sum_{i=1}^n s_i = \text{the total amount of soil in the sample, and}$$

$$\frac{s_i}{\sum_{i=1}^n s_i} = \text{the relative size of the core compared to all cores comprising the sample (i.e., the proportion that the } i^{\text{th}} \text{ core contributes to the mean).}$$

With unequal core sizes, the weighted contribution of each core to the nematode density estimate in that sample becomes:

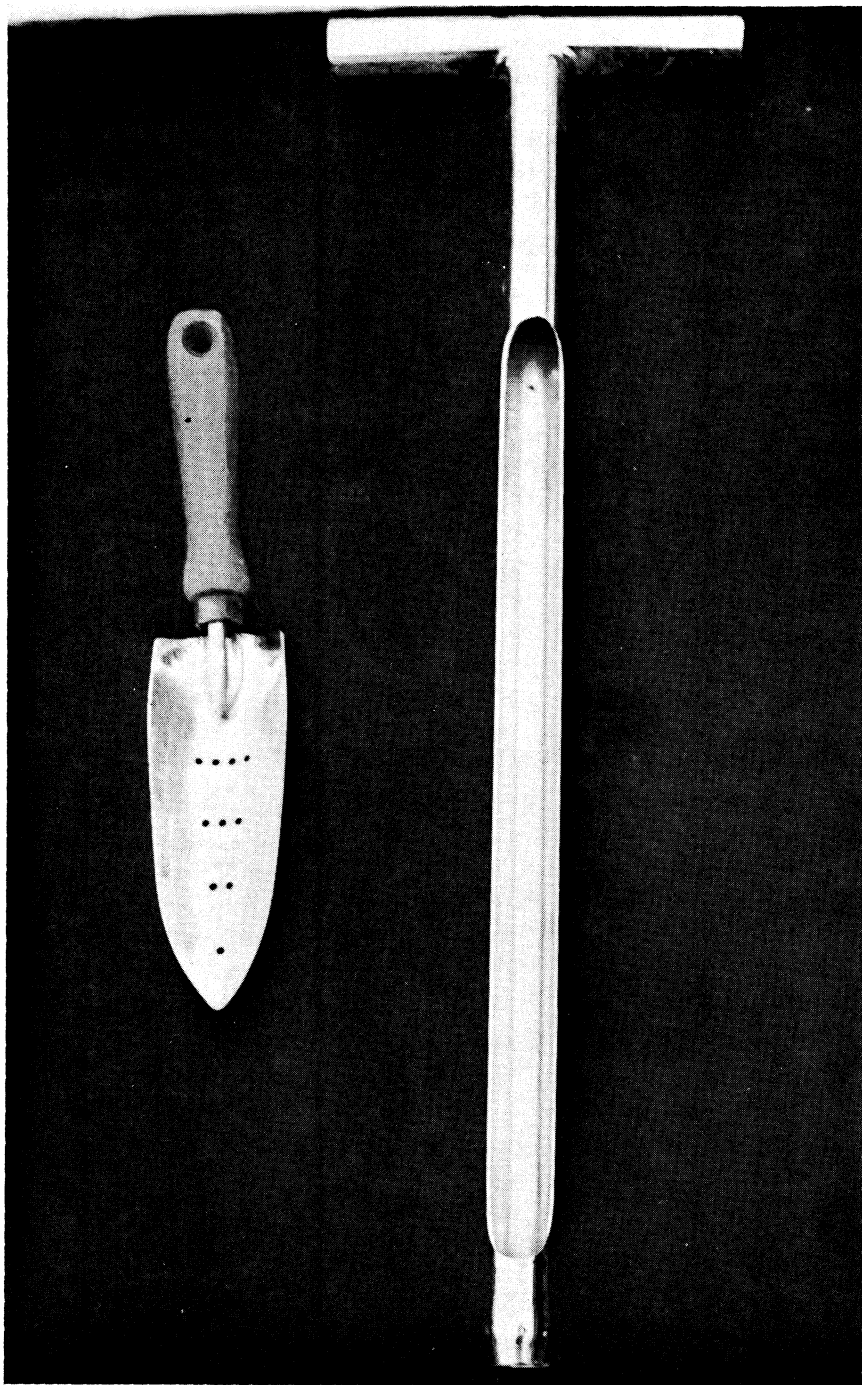


Fig. 1. Equipment for collecting nematode soil samples: sampling tube, which provides cylindrical cores of equal size; and trowel, which provides portions, or "cores", of unequal size.

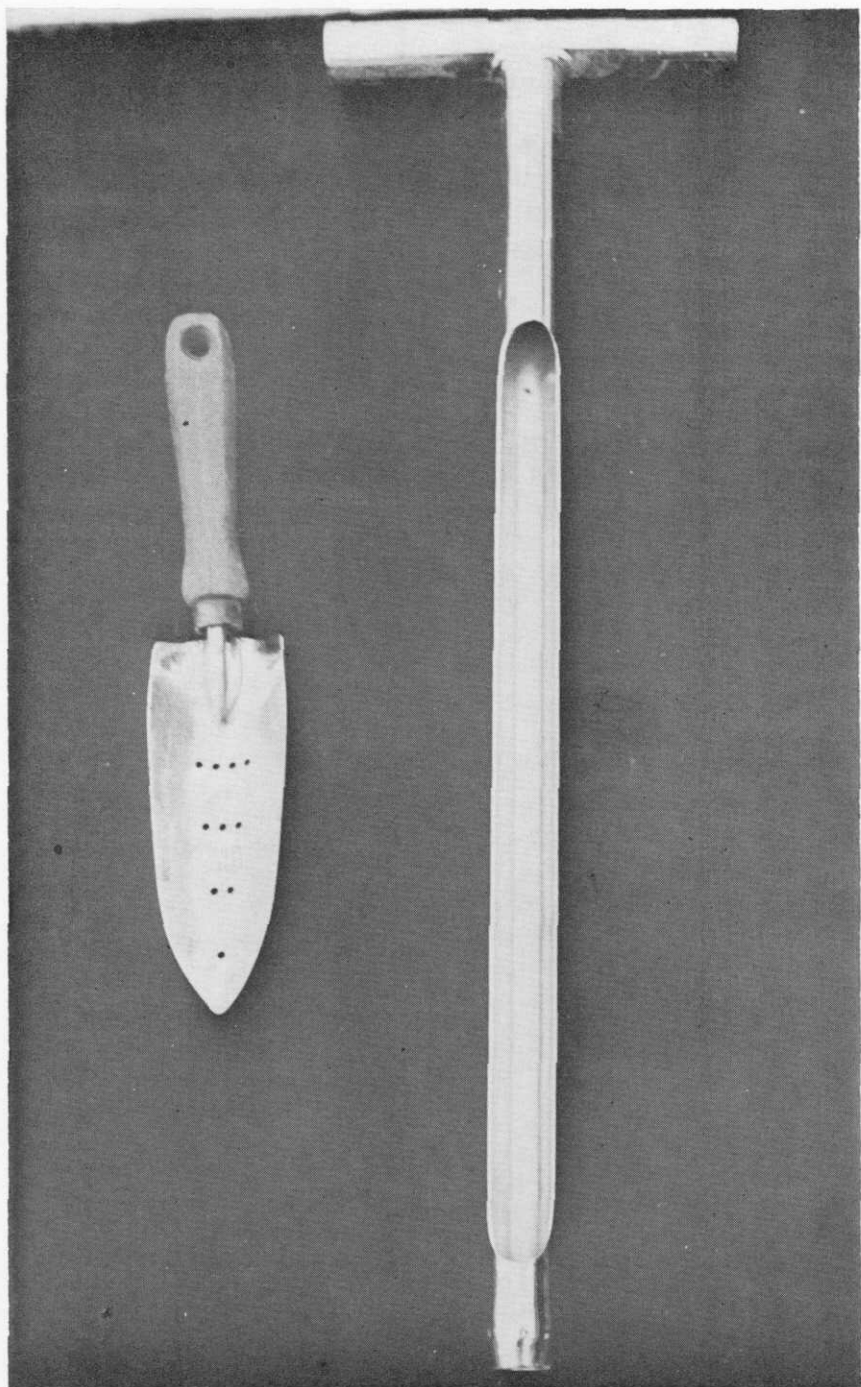


Fig. 1. Equipment for collecting nematode soil samples: sampling tube, which provides cylindrical cores of equal size; and trowel, which provides portions, or "cores", of unequal size.

$$c_i \left(\frac{s_i}{\sum_{i=1}^n s_i} \right)$$

Thus, the overall contribution to the mean becomes:

$$\begin{aligned} \bar{c} &= \frac{c_1 s_1}{\sum_{i=1}^n s_i} + \frac{c_2 s_2}{\sum_{i=1}^n s_i} + \dots + \frac{c_i s_i}{\sum_{i=1}^n s_i} \\ &= \frac{1}{\sum_{i=1}^n s_i} \left(\sum_{i=1}^n c_i s_i \right) \end{aligned} \quad (\text{Eq. 2})$$

This equation is useful in dealing with the case of unequal core sizes. Equation 1 is a special case of equation 2 where:

$$\sum_{i=1}^n s_i = nS$$

since for equal core sizes, $s_1 = s_2 = s_i = S$. Components of Eq. 2 were programmed into a pre-existing computer program (4) to make it possible to compare the equal and unequal core cases for a given sampling plan. However, a method for simulating the unequal core sizes, s_i , was still needed for the program.

Unequal Core Size Distribution. To determine the variability involved in collecting individual cores of soil with a hand trowel, 90-100 individual soil cores were collected by this method for each of three fields, each having a Rockdale fine sandy loam soil type (Table 1). Soil comprising each individual core was passed through a 4.0 mm mesh sieve to remove rock, the volume of the remaining soil was measured, and the mean and standard deviation of core sizes was determined for each field. Within each field, the core size distribution was roughly normal. In each case, goodness of fit to a normal distribution was tested by means of a χ^2 test, and the core size distributions did not differ significantly from the normal distribution (Table 1).

Thus, in a sampling program, unequal core sizes could be simulated from a normal distribution with a given mean, μ , and standard deviation σ . A technique used in simulation to generate a normal deviate has been discussed elsewhere (6). In this method, 12 pseudorandom numbers are summed to give a distribution which is approximately normal with a mean of 6 and a variance of one. A standard normal deviate can then be found by subtracting 6 from the sum, and then can be transformed

Table 1. Goodness of fit to normal distributions for unequal core sizes. Each soil sample represents one "core" of soil removed with a trowel.

Field number	Number of soil samples	Sample volume (cm ³)		Degrees of freedom	χ^2 P=0.05	Observed χ^2
		mean	S.D.			
1	90	98.6	20.8	19	30.14	17.92
2	100	121.8	26.9	14	23.68	9.87
3	100	158.7	61.4	14	23.68	11.71

to a normal distribution with mean, μ , and standard deviation, σ . The FORTRAN programming needed to incorporate this technique into the sampling simulation program previously reported (4) is shown (Table 2).

Simulations Comparing Equal and Unequal Core Sizes: 20 Cores per Sample. The revised computer program was used to compare sampling strategies involving equal and unequal core sizes. To compare samples of 20 cores each, a total of 27 different simulations was run. These involved all combinations of three different k values (0.294, 0.544, 1.35), three different mean core sizes for the unequal cores (Table 1), and three different numbers of 20-core samples per strategy (2, 5, or 10 samples). A mean nematode density of 2.0/10cm³ of soil was used in all simulations, and each strategy was replicated 10 times. The k values chosen for these simulations were obtained previously (5) from samples of *Meloidogyne incognita* (Kofoid & White) Chitwood collected in south Florida.

Simulations Comparing Equal and Unequal Core Sizes: Various Numbers of Cores per Sample. A second series of simulations were performed to examine strategies having various numbers of cores per sample. Input data for this series of simulations consisted of a mean nematode density of 2.0/10cm³ of soil, k = 0.294, and a mean core size of 158.7cm³ with a standard deviation of 61.4. Strategies consisting of 5 samples of 2, 5, 10, or 20 cores each were simulated and averaged over 20 replications.

RESULTS AND DISCUSSION

Simulations Comparing Equal and Unequal Core Sizes: 20 Cores per Sample. Standard error to mean ratios of estimated nematode densities were computed for sampling strategies simulated for 20-core samples in various situations (Table 3). Separate estimates of standard error to

Table 2. FORTRAN program for generating a normal deviate of mean core size RM and standard deviation RSD.

```

      RSUM=0.0
      READ(5,525)RM,RSD
525  FORMAT(2F7.3)
      DO 80 I=1,2
      80  RSUM=RSUM+RNDMF(1.0)
          V+RSUM-6.0
          RNORM=V*RSD+RM
          RSUM+0.0
          WRITE(7,618) RNORM
618  FORMAT(F8.3)
      STOP
      END

```

mean ratios were computed for estimates obtained from cores of equal size and for those obtained from cores of varying sizes. Results of all simulations were well below the 0.25 standard error to mean ratio desirable for pest survey work (7). As anticipated, the magnitude of the error estimates declined as k values increased (5) or as the number of samples increased from 2 to 10 per field (4).

In general, only slight differences in standard error to mean ratios were evident when comparing the equal-core-size estimate to the unequal-core-size estimate for a given situation. Differences were especially small when the largest k value ($k = 1.35$) was used. Greatest differences occurred when the unequal core sizes were simulated from a normal distribution with a mean of 158.7 and standard deviation of 61.4, since this distribution showed the greatest variance of those tested. The greatest difference with this core-size distribution was observed for the simulation involving two samples of 20 cores each and a k value of 0.294. However, even in this case, the difference in standard error to mean ratios (E) was still relatively small, with $E = 0.140$ in the equal core-size case and $E = 0.171$ in the unequal-core-size case.

Simulations Comparing Equal and Unequal Cores Sizes: Various Numbers of Cores per Sample. Data from a relatively sensitive situation ($k = 0.294$, mean core size = 158.7, standard deviation = 61.4, and 5 samples per field) were used to examine errors resulting from the use of different numbers of cores per sample. The observed relationships between the calculated standard error to mean ratio and the number of cores per sample were curvilinear and fit models of the form $y = ax^b$ (Fig. 2). Curves for the equal and unequal core size cases are similar.

Table 3. A comparison of sampling errors in 20-core samples having equal or unequal core sizes, using various mean values for unequal core size and various k values from a negative binomial distribution having a field mean of 2.0 nematodes/10cm³ of soil.

Negative binomial	Unequal core size:		Simulated standard error to mean ratios for 20-core samples ^a											
	Mean	S.D.	2 samples		5 samples		10 samples		5 samples		10 samples			
			Equal core size	Unequal core size	Equal core size	Unequal core size	Equal core size	Unequal core size	Equal core size	Unequal core size				
0.294	98.6	20.8	.140	.157	.131	.136	.122	.122	.131	.136	.122	.122		
	121.8	26.9	.140	.158	.131	.137	.122	.122	.131	.137	.122	.122		
	158.7	61.4	.140	.171	.131	.146	.122	.125	.131	.146	.122	.125		
0.544	98.6	20.8	.118	.129	.101	.107	.096	.096	.101	.107	.096	.096		
	121.8	26.9	.118	.130	.101	.107	.096	.096	.101	.107	.096	.096		
	158.7	61.4	.118	.138	.101	.116	.096	.100	.101	.116	.096	.100		
1.35	98.6	20.8	.089	.090	.078	.082	.071	.072	.078	.082	.071	.072		
	121.8	26.9	.089	.090	.078	.082	.071	.072	.078	.082	.071	.072		
	158.7	61.4	.089	.090	.078	.087	.071	.075	.078	.087	.071	.075		

^aEach value is a mean of 10 replications.

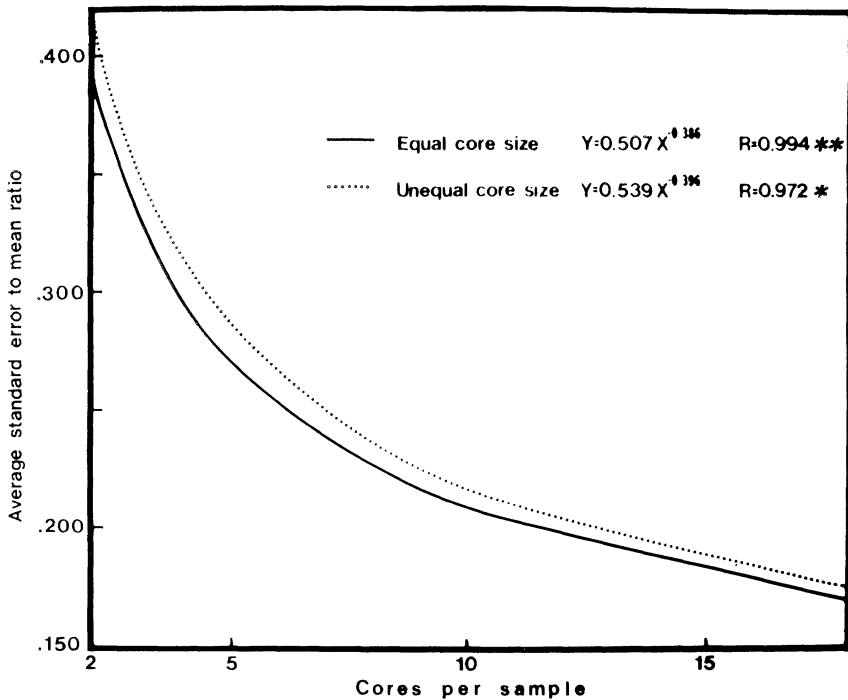


Fig. 2. Standard error to mean ratios of simulated nematode densities for varying numbers of cores per sample for the equal-core-size case and the unequal-core-size case, with a mean core size of 158.7 and standard deviation of 61.4. The nematode distribution was negative binomial with mean = 2.0 and $k = 0.294$. Curves were fit to data averaged over 20 replications of 5 samples each.

Results obtained from collecting cores of equal size gave only slightly less precision, especially when more than 10 cores per sample are involved. In most cases, a level of precision identical to or better than that observed for the equal-core-size case could be achieved with unequal cores simply by including an extra core in each sample.

In summary, errors in estimates of nematode density resulting from using soil cores of unequal size to form composite soil samples are relatively small and compare favorably with those obtained by using cores of equal size. This was especially true with 20-core samples, and errors should be minimized by making a reasonable effort to insure that the cores collected with a trowel are of approximately the same size. If this is done, then even with fewer than 20 cores per sample, only one extra core with a trowel should be needed to give results comparable to those achieved with a soil sampling tube.

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