PLANS FOR THE COLLECTION OF NEMATODE SOIL SAMPLES FROM FRUIT GROVES

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ABSTRACT

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Soil samples were used to estimate mean populations of plant-parasitic nematodes in groves of four tropical tree crops in South Florida: guava *(Psidium guajava)*, avocado *(Persea americana)*, mango *(Mangifera indica)*, and 'Tahiti' lime *(Citrus* x 'Tahiti'). For each nematode-crop combination, relationships were developed between the number of trees to be sampled in a grove and the number of sites to be sampled per tree to maintain a predetermined level of precision (standard error ≤ 25% of the mean). Cost functions were used to select the most economical sampling plan from several equally precise alternatives. On Rockdale soils in south Florida the most efficient sampling plan for most parasitic nematode species was to sample a number of trees in the grove at one site per tree, rather than fewer trees at several sites per tree. An exception occurred with *Tylenchulus semipenetrans* on lime, because of the high variablility in larval population levels at various sites around the same tree. For other nematode species, the number of trees to be sampled per grove varied with crop, grove size, and nematode species, and no one sampling plan was found to be suitable for all situations.

Additional key words: cost functions, spatial distribution, Criconemella sphaerocephala, Helicotylenchus dihystera, Hemicriconemoides mangiferae, Pratylenchus brachyurus, Quinisulcius acutus, Rotylenchulus reniformis, Tylenchulus semipentrans.

RESUMEN

McSorley, R., and J.L. Parrado. 1982. Plan para la recolección de muestras de suelo de nematodos en las arboledas de frutales. Nematrópica 12:257-267.

Muestras de suelo fueron usadas para estimar la población promedio de los nematodos parasíticos en arboledas de cuatro frutales tropicales en el Sur de la Florida: Guayaba *(Psidium guajava)*, Aguacate *(Persea americana)*, Mango *(Mangifera indica)*, y limón persa ('Tahiti' lime) (*Citrus x* 'Tahiti'). Para cada combinación nematodo-frutal se desarrollaron relaciones entre el número de plantas y el número de sitios por planta que deberian ser muestreados a fin de mantener un predeterminado nivel de precisión (error estandar ≤ 25% del promedio). Los costos de operación fueron usados para seleccionar el plan de muestreo más ecónomico entre varias alternativas igualmente precisas. En los suelos rocosos (Rockdale) del Sur de la Florida el más eficiente plan de muestreo para la mayoría de las especies de nematodos parasíticos fué muestrear un cierto número de plantas de la arboleda en un sólo sitio alrededor de la planta, más bien que, muestrear menos plantas con varios sitios por planta. Una excepción ocurrió con *Tylenchulus semipenetrans* en el limón persa, debido a la alta variabilidad de los niveles de la población de larvas en distintos sitios alrededor de la misma planta. Para otras especies de nematodos, el número de plantas que deben muestrearse por arboleda varia con el frutal, tamaño de la arboleda y la especie de nematodo y no fué encontrado un plan de muestreo apropiado para todas las situaciones.

Palabras claves adicionales: costos de operación, distribución espacial, Criconemella sphaerocephala, Helicotylenchus dihystera, Hemicriconemoides mangiferae, Pratylenchus brachyurus, Quinisulcius acutus, Rotylenchulus reinformis, Tylenchulus semipenetrans.

INTRODUCTION

Accurate assessment of nematode populations in groves is critical for nematological surveys and in making treatment decisions based on population levels (1). Few guidelines are available for estimating nematode populations in groves. The recommendation (6) to sample trees near the drip-line, or outer edge of the foliage, has proven useful in sampling tropical fruit trees (9). Usually, the nematode population of a field is estimated by collecting and pooling a number of cores or sample units from various locations in the field. In a grove, it is not clear how such sample units should be distributed to obtain a population estimate of the grove as a whole.

The current research uses the principles of cluster sampling (2) to demonstrate a methodology for developing sampling plans for nematode species in groves. This technique is useful in developing relationships between the number of trees to be sampled in a grove and the number of sites to be sampled around each tree to maintain a given level of precision. Methodology is also presented to assist in selecting the most cost-efficient soil sampling plan from several equally precise alternatives.

MATERIALS AND METHODS

Data bases for this study were obtained by sampling four different crops in five experiments during 1981-82: guava (*Psidium guajava* L.), avocado (*Persea americana* Mill.), mango (*Mangifera indica* L.), and 'Tahiti' lime (*Citrus* x 'Tahiti'). All trees were growing on Rockdale fine sandy loam soils (pH = 7.3-7.8) in Dade County, Florida.

Sampling dates and related information pertaining to the collection of soil samples are shown in Table 1. A number of trees were chosen at random from each grove for sampling, and individual sample units were collected from

Table 1. Sampling information for fruit crops sampled during 1981-82.

	Crop				
	Guava	Guava	Avocado	Lime	Mango
Date sampled	10-12-81	10-20-81	12-14-81	3-3-82	6-15-82
Plant age (yr.)	4	4	20	4	510
Planting distance in rows (m)	3.0	3.0	7.6	6.1	6.1
Planting distance between rows (m)	4.5-6.0	4.5-6.0	7.6	7.6	6.1
Size of grove (ha)	0.5	2.0	1.6	0.4	0.4
Number of trees in grove	312	1248	270	86	109
Number of trees actually sampled	5	20	20	5	6
Number of sites sampled per tree	8	4	4	6	8
Total number of sample units collected	40	80	80	30	48
Sampling distance from trunk (m)	1.5	1.5	3.0	1.5	1.5

several sites around each of the trees sampled, to give the total numbers of sample units shown in Table 1 for each crop. Individual sample units were taken from the drip-line of the tree involved, i.e., 1.5m from the trunk for all crops except avocado. Sample units were collected with a hand trowel to a depth of 15cm. In the laboratory, each soil sample unit was passed through a 4.0mm sieve to remove rock, and 100 cm^3 of soil was processed for plant-parasitic nematodes by a modified sieving-centrifugation procedure (5, 8).

For each experiment, counts of each nematode species in each sample unit were obtained and arranged by tree and by site around each tree. For each species, a mean count per $100cm^3$ of soil over all sample units in a given grove was obtained, and used as an estimate of the grove mean ($\bar{\bar{y}}$) for counts of that particular nematode. The relationship between the number of trees sampled in a grove (n) and number of sites sampled per tree (m) is given by (2):

$$V(\bar{\bar{y}}) = \left(\frac{N-n}{N}\right)\frac{S_b^2}{n} + \left(\frac{M-m}{M}\right)\frac{S_w^2}{mn} \qquad \text{(Eq. 1)}$$

Where:

$V(y)$ = variance of the grove mean, ($\bar{\bar{y}}$)

N = number of trees in the grove

M = number of possible sites that could be sampled per tree

S_b^2 = variance among mean nematode counts between trees

S_w^2 = variance among sites within the same tree

For each of the five experiments, the variance components, S_b^2 and S_w^2, were found for each species by using a nested analysis of variance program from the SAS package for data analysis (4).

RESULTS

Sampling for R. reniformis in a small guava grove — an example calculation. Eq. 1 was modified slightly for convenience in some of the calculations. The number of possible sites which could be sampled per tree (M) is considered to be large in relation to the actual number of sites sampled per tree (m). For example, if samples are taken 1.5 m from the trunk, then all possible sampling sites (M) are located on a circle with perimeter π x 3.0 = 9.42 m. Allowing 0.05 m of circumference per sampling site, there are 188 such sites around the tree. If five of these sites are sampled, then $\frac{M-m}{M}$ becomes 0.97 $\approx$ 1.0. Thus, Eq. 1 can be approximated by:

$$V(y) = \left(\frac{N-n}{N}\right)\frac{S_b^2}{n} + \frac{S_w^2}{mn}$$

(Eq. 2)

The choice of an index of precision depends on what the data are to be used for, but a standard error to mean ratio of 25% has proven useful in entomology for damage assessment and control studies (10). The standard error of the grove mean is given by $\sqrt{V(\bar{\bar{y}})}$. Thus, using this criterion the standard error to mean ratio is:

$$\frac{\sqrt{V(\bar{\bar{y}})}}{(\bar{y})} = 0.25$$

or

$$V(\bar{\bar{y}}) = .0625\ \bar{\bar{y}}^2$$

(Eq. 3)

By substitution, the following formula is useful for determing relationships between m and n when the standard error to mean ratio is 25%:

$$.0625\ \bar{\bar{y}}^2 = \left(\frac{N-n}{N}\right)\frac{S_b^2}{n} + \frac{S_w^2}{mn}$$

(Eq. 4)

These relationships were determined for each nematode species in each experiment. Counts of *Rotylenchulus reniformis* Linford & Oliveira in the smaller guava grove (Table 1) are used as an example. In the 40 samples collected from this grove, counts averaged 57.9 *R. reniformis* per 100cm³ of soil. Thus, the estimate of the grove mean is taken as $\bar{\bar{y}}$ = 57.9. The variance components were determined to be S_b^2 = 287.6 and S_w^2 = 920.3. There were 312 trees in the grove, so N = 312. When these values are substituted into Eq. 4, the relationship between the number of trees to be sampled (n) and the number of samples per tree (m) is given by:

Table 2. Relationship between number of sampling sites per tree and number of trees per grove needed to maintain a standard error to mean ratio of 25% in sampling a 0.5 ha guava grove for four nematode species.

Nematode species	m = number of sites per tree	n = number of trees per grove
Rotylenchulus reniformis	1	6
	2	4
	3	3
	4	3
	5	3
Helicotylenchus dihystera	1	44
	2	23
	3	16
	4	13
	5	11
Quinisulcius acutus	1	44
	2	41
	3	39
	4	39
	5	38
Criconemella sphaerocephala	1	67
	2	41
	3	32
	4	28
	5	25

$$n = 1.37 + \frac{4.37}{m} \qquad \text{(Eq. 5)}$$

Thus, if a small guava grove is to be sampled for *R. reniformis* and four sites per tree are chosen (m = 4), then n = 2.46 indicates that three trees should be sampled to maintain a standard error to mean ratio of 25% or less (n rounded up to nearest whole number).

For the conditions described here in sampling for *R. reniformis*, the calculated values of n from Eq. 4 are shown for various values of m, the number of sites per tree (Table 2). Analogous relationships useful in sampling for other nematode species in this grove are shown for *Helicotylenchus dihystera* (Cobb) Sher, *Quinisulcius acutus* (Allen) Siddiqi, and *Criconemella sphaerocephala* (Taylor) Luc & Raski (Table 2). Many more sample units were needed to measure populations of these nematodes than would be needed for

R. reniformis at the same level of precision in this grove.

Optimization of sampling plans using cost functions. From Table 2 it is apparent that a number of different combinations of trees and sites per tree to be sampled could be used with the same level of precision. If precision were the only concern in the sampling program, then any of the combinations for a given nematode species would be adequate. However, the optimum sampling plan is one which maintains the desired level of precision at the lowest cost (3).

A generalized cost function is represented by:

$$C = c_1 n + c_2 nm \qquad \text{(Eq.6)}$$

where C is the total cost, and $c_1 n$, the first component of cost, is proportional to the number of trees sampled, while $c_2 nm$, the second component of cost, is proportional to the total number of sample units. If t_1 is the time to locate and reach a given tree for sampling and t_2 is the time needed to dig a single soil sample of 100cm^3, at an hourly rate of k dollars, the cost function becomes:

$$C = kt_1 n + kt_2 nm = k(t_1 n + t_2 nm) \qquad \text{(Eq. 7)}$$

This function pertains only to the sampling process and does not include laboratory time or the time needed to travel to the grove. Under the conditions described for the groves in this study, timed tests over five replications indicated that it took an average of 53.4 sec to dig a soil sample, and 32.2 sec to move to and to prepare to sample a different tree in a 0.5 ha grove. The cost function then becomes:

$$C = k(32.2n + 53.4mn) = 53.4k(0.603n + nm) \qquad \text{(Eq. 8)}$$

for a 0.5 ha grove. For a 2.0 ha grove, the cost function is:

$$C = k(52.0n + 53.4mn) = 53.4k(0.974n + nm) \qquad \text{(Eq. 9)}$$

because of the longer time (average 52.0 sec) needed to locate trees in the larger grove.

From Eq. 8, the cost of sampling is proportional to (0.603n + nm), and so the most cost-efficient sampling plan is the one in which the quantity (0.603n + nm) is minimized with the constraint imposed by Eq. 5. A comparison of the relative cost of two sampling strategies can be obtained from the ratio:

$$\frac{C_1}{C_2} = \frac{53.4k(0.603n_1 + n_1 m_1)}{53.4k(0.603n_2 + n_2 m_2)} = \frac{0.603n_1 + n_1 m_1}{0.603n_2 + n_2 m_2} \qquad \text{(Eq. 10)}$$

where

Table 3. Relative costs of sampling plans for *Rotylenchulus reniformis* in a 0.5 ha guava grove that maintain a standard error to mean ratio of 25% or less.

m = number of sites per tree	n = number of trees per grove[x]	Actual time = 53.4 sec/sample (.603 n + mn)	Relative cost[y]	Hypothetical time = 10.0 sec/sample (3.22 n + mn)	Relative cost[y]
1	6	9.6	1.00	25.3	1.35
2	4	10.4	1.08	20.9	1.12
3	3	10.8	1.12	18.7	1.00
4	3	13.8	1.44	21.7	1.16
5	3	16.8	1.75	24.7	1.32

[x] Rounded up to nearest whole number, since trees must be sampled as units. A standard error to mean ratio of 25% or less is maintained by all sampling plans shown in this table; rounding down is not desirable since it will lead to a precision of >25%.

[y] For each column, relative cost = cost of the sampling plan ÷ cost of the sampling plan having the lowest cost.

C_1 = cost of first sampling strategy
C_2 = cost of second sampling strategy
n_1 = number of trees sampled per grove in first strategy
n_2 = number of trees sampled per grove in second strategy
m_1 = number of sites per tree in first strategy
m_2 = number of sites per tree in second strategy

The relative costs for various sampling plans for *R. reniformis* in a 0.5 ha guava grove were computed from the cost functions of Eqs. 8 & 10, and are shown in Table 3. All the combinations of numbers of trees and sites per tree shown in Table 3 will maintain a standard error to mean ratio of 25% or less, but sampling six different trees at one site per tree was the most cost-effective sampling plan.

Specific cost functions such as Eq. 8 may be useful only in certain situations. Eq. 8 is useful only in sampling Rockdale series soils. The time for digging a 100cm^3 soil sample is relatively great (53.4 sec), since a hand trowel must be used to penetrate the rocky soil. In soils where a cylindrical tool can be used to obtain a soil core, the time to take individual sample would be much less, possibly 10 sec or less to insert a tool and remove a soil core. An analogous cost function for a 10-sec subsampling time would be:

Table 4. Optimum values for number of sites per tree and trees per grove for various nematode and crop combinations sampled on Rockdale soils while maintaining a standard error to mean ratio of 25% or less.

	Crop and Grove size[x]														
	Lime, 0.4 ha			Mango, 0.4 ha			Guava, 0.5 ha			Guava, 2.0 ha			Avocado 1.6 ha		
	m_o	n_o	n_1	m_o	n_o	n_1	m_o	n_o	n_1	m_o	n_o	n_1	m_o	n_o	n_1
Criconemella sphaerocephala	—	—	—	2	13	22	1	67	67	—	—	—	—	—	—
Helicotylenchus dihystera	—	—	—	1	114	114	3	16	44	2	140	220	1	92	92
Hemicriconemoides mangiferae	—	—	—	1	17	17	—	—	—	—	—	—	—	—	—
Pratylenchus brachyurus	—	—	—	1	22	22	—	—	—	—	—	—	3	96	234
Quinisulcius acutus	—	—	—	—	—	—	1	44	44	1	25	25	—	—	—
Rotylenchulus reniformis	1	13	13	3	21	55	1	6	6	1	28	28	1	26	26
Tylenchulus semipenetrans	6	6	32	—	—	—	—	—	—	—	—	—	—	—	—

[x] m_o = optimum sites per tree; n_o = optimum number of trees per grove; n_1 = trees per grove if one site per tree is sampled ($m = 1$); dashes "—" indicate nematode/crop combinations not evaluated.

$$C = k(32.2m + 10.0nm) = 10.0k(3.22n + nm) \qquad \text{(Eq. 11)}$$

A comparison of relative costs at 10-sec and 53.4-sec digging times (Table 3) reveals that a different sampling plan would be more efficient for each soil type.

The optimum number of trees, n, and sites per tree, m, to be sampled can be found by an alternative method as well. If the components of the cost function (Eq. 6) and the maximum possible number of sites per tree, M, are known, then the optimum number of sites per tree, m_{opt}, can be computed by the formula (2):

$$m_{opt} = \frac{S_w}{\sqrt{S_b^2 - S_w^2/M}} \sqrt{\frac{c_1}{c_2}} \qquad \text{(Eq. 12)}$$

where the calculated value of m_{opt} is rounded to the nearest integer. The corresponding value of n can be found by substituting m_{opt} into Eq. 4 for m.

Optimum sampling plans for various fruit crops. Optimum numbers of sites per tree and trees per grove for the various nematode and crop combinations sampled in the various experiments were calculated (Table 4) from Eqs. 4, 8, and 9. The numbers of trees per grove needed when m = 1 are included for comparative purposes. All combinations in Table 4 maintain standard error to mean ratios of 25% or less.

DISCUSSION

It is apparent from Table 4 that no one sampling plan is adequate for every situation, but that individual plans must be developed for each nematode-crop combination. For example, if a general plan of one sampling site at each of 22 different trees were used for mango, the criterion of a 25% standard error to mean ratio would be met for *Hemicriconemoides mangiferae* Siddiqi, *Pratylenchus brachyurus* (Godfrey) Filijev & Schuurmans-Stekhoven, and *Criconemella sphaerocephala,* but more sampling error would have to be tolerated in the estimates of the other species. The magnitude of the standard error to mean ratios for each of the other species under this sampling plan could be found by substitution into Eq. 2 for n = 22 and m = 1, and finding the ratio between $\sqrt{V(\bar{\bar{y}})}$ and $\bar{\bar{y}}$. Using this sampling plan, standard error to mean ratios of 40% and 65% are calculated for *R. reniformis* and *H. dihystera,* respectively.

A few general trends are apparent from Table 4. In sampling the larger guava grove, more samples were needed for estimating *R. reniformis* and *H. dihystera* at the same level of precision obtained for the smaller grove. This was not the case for *Q. acutus*, since the data base for this nematode contained one tree with extremely high counts, resulting in a very high variance component between trees, a factor that was not duplicated in sampling the larger grove.

In the tree crops studied, *R. reniformis* was among the easiest nematodes to sample for, while *H. dihystera* was the most difficult. This may be due to the fact that *H. dihystera* was present in most groves in relatively low numbers and a relatively small error in terms of absolute numbers could represent a rather large standard error in relation to the small mean. However, it may be possible to tolerate relatively large standard error to mean ratios in sampling for this nematode since it has not been shown to be damaging to the crops studied. The most critical sampling plan needed for a given crop is that for the nematode with the greatest damage potential on the crop. Of the combinations shown here (Table 4), the most critical combinations would then likely be *Tylenchulus semipenetrans* Cobb on lime, *R. reniformis* on guava, *H. mangiferae* on mango, and *P. brachyurus* on avocado. The latter nematode is frequently found in roots (7, 11), and it is possible that a sampling plan involving root samples, rather than soil samples, may be more appropriate for it.

On Rockdale soils, the cost functions (Eqs. 8 & 9) dictated that, in general, sampling from relatively few sites around a large number of trees was more cost-efficient than sampling a number of sites around fewer trees. The latter strategy is probably more efficient for other soil types, however, because of the shorter time needed to extract soil cores. Even on Rockdale soils, however, the recommended sampling plan for larvae of *T. semipenetrans* on lime is quite different than the usual pattern for other nematodes, and multiple samples from fewer trees (6 x 6) were the most cost-efficient in estimating this nematode. The variability in counts of *T. semipenetrans* larvae at different sites around the same tree is extremely great and the within-tree variance comprised a much greater portion of the total variance than did the between-tree variance component for this nematode.

While the present study has presented a methodology for developing sampling plans for nematodes on tree crops, arbitrary decisions must be made at several points in the development of the sampling plan. In particular, the optimization of a sampling plan involves a balance between precision and cost. The individual investigator must determine the level of precision needed and the resources available, in order to choose the most cost-efficient sampling plan from an array of equally precise alternatives.

The sampling plans developed here are very specific to the tree crop-nematode combinations examined. They would be suitable plans to use in conducting further nematological work in these groves. However, since both variance components, S_b^2 and S_w^2, can vary with the grove mean, $\bar{\bar{y}}$, it would be necessary to perform identical studies in several groves of guavas, for example, to develop a general sampling plan for a wide range of guava groves. From the present study, it should be apparent that there may be no general sampling plan which is suitable for all grove crops, but that specific plans may have to be developed for each crop-nematode combination.

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