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GUIDELINES FOR SAMPLING TREE CROPS ON ROCKDALE SOILS FOR PLANT-PARASITIC NEMATODES[1]

R. McSorley and J. L. Parrado
IFAS, University of Florida,
Tropical Research and Education Center,
18905 SW 280 St., Homestead, FL 33031

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Abstract. **The spatial distributions of plant-parasitic nematodes in groves on Rockdale soils were used to develop sampling plans for 4 crops: 'Tahiti' lime** (Citrus latifolia **Tan.), mango** (Mangifera indica **L.), avocado** (Persea americana **Mill.), and guava** (Psidium guajava **L.). After removing rocks and leaves, soil samples are removed with a hand trowel to a depth of 15 cm from the "dripline", approximated by the other edge of the foliage, avoiding any weed growth beneath the tree. Many sampling plans are proposed, consisting of varying numbers of sampling locations per tree and trees sampled per grove, but plans consisting of one location per tree are usually most efficient on Rockdale soils. An exception occurred with the citrus nematode** (Tylenchulus semipenetrans **Cobb) on lime, for which more locations around fewer trees were recommended. For other situations, the number of trees to be sampled per grove varied with the host (avocado, guava, mango, or lime), the nematode species, and the predetermined level of precision. In sampling several species simultaneously on the same host, a level of precision and plan must be decided upon for one species (usually the most pathogenic). Other species are then simultaneously sampled by the same plan, but at different levels of precision, with less precise results tolerated for some of the less-important species.**

Many different species of plant-parasitic nematodes are associated with tropical and subtropical fruit trees (7, 9), some of which can cause serious tree damage or yield loss. The presence of plant-parasitic nematodes can easily be determined from a soil sample, but confusion exists concerning how such a soil sample should be taken, since no guidelines have been proposed. Specific questions must be answered if sampling plans for groves are to be developed. These include the number of trees to be sampled per grove, the number of locations to sample around each tree, the depth of sampling, and the distance from the trunk. Samples from citrus are usually collected from the "dripline", which is approximated by the outer edge of the foliage (5, 6, 12, 14). By taking soil samples just inside the outer edge of the canopy, it is presumed that the area with the most active feeder roots and highest nematode populations is being sampled. On Rockdale soils in southern Florida (10), sampling in the dripline was most effective in detecting the highest nematode populations on avocado (*Persea americana* Mill.), guava (*Psidium guajava* L.), mango (*Mangifera indica* L.), and 'Tahiti' lime (*Citrus latifolia* Tan.). A hand trowel was used to obtain soil samples to a depth of 15 cm from groves growing on Rockdale soils (10, 11). The underlying limestone rock may restrict sample depth to less than 15 cm in some cases when sampling in the dripline. In sampling beneath fruit trees, weeds and other vegetation should be avoided, since these may harbor nematode communities distinct from those directly associated with the tree crop (8).

If nematode sampling around a fruit tree is restricted to the circular area defined by the dripline, there are still a very large number of possible locations from which a trowel of soil can be removed from around a single tree. The purpose of this study was to develop guidelines for the number of sampling locations around an individual tree, and the number of trees to be sampled per grove.

Materials and Methods

Data for developing sampling plans were collected during 1981-82 from 5 fruit groves growing on Rockdale series soils (2) in Dade County, Florida:

Small guava grove. This grove was 0.5 ha and consisted of 4-yr-old trees planted 3 m apart in rows 4.5-6.0 m apart. The grove contained 312 trees.

Large guava grove. This grove was 2.0 ha and contained 1248 trees. Tree age and planting distances were the same as for the smaller grove.

Avocado. This 1.6 ha grove contained 20-yr-old trees planted at a distance of 7.6 m in all directions, for a total of 270 trees in the grove.

'Tahiti' lime. This grove was 0.4 ha and contained 86 4-yr-old trees planted 6.1 m in rows by 7.6 m between rows.

Mango. This grove was 0.4 ha and consisted of trees approximately 10 yr in age planted at 6.1 m in all directions, for a total of 109 trees in the grove.

Nematode sampling. Normally, nematode samples are collected by removing individual cores or trowels of soil from a variety of locations, and pooling these to provide a single soil sample for analysis. However, in this instance, soil from each different location was considered a separate sample for analysis. The numbers of samples collected ranged from 30-80, as described elsewhere (11), but in each grove several different locations were sampled around a number of individual trees. All samples were collected to a depth of 15 cm from the dripline of the trees involved. In the laboratory, each soil sample was passed through a 4.0-mm sieve to remove rock, and plant-parasitic nematodes were extracted from a 100 cm^3 portion of soil by a modified sieving-centrifugation procedure (4).

Mathematical analysis. As a result of the nematode sampling and extraction, counts of several species of nematodes were available at each of several locations around each of the trees sampled in a given grove. In addition, an average count for each nematode species was obtained over all samples collected in a given grove and used as an estimate of the grove mean density ($\bar{\bar{y}}$) for that species. Mean density of the grove is most often used for nematological survey samples, and the precision of its estimate depends on the variation in the nematode distribution around individual trees and from tree-to-tree. The relationship between the number of trees sampled in a grove (n) and the number of sites sampled per tree (m) can be obtained from (1, 11):

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

where

$V(\bar{\bar{y}})$ = variance of the grove mean, $\bar{\bar{y}}$
N = number of trees in the grove
S_b^2 = variance among mean nematode counts between trees
S_w^2 = variance among counts within the same tree

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The variance components, S_b^2 and S_w^2, were found for each nematode species in each grove by a nested analysis of variance procedure (3). Sampling error involved in estimating the grove mean, $\bar{y}$, can be estimated by various indices of precision, including the ratio between the standard error of the mean and the mean. This ratio is easily computed from the square root of the variance of the grove mean ($\sqrt{V(\bar{y})}$, equal to the standard error of the mean) and the grove mean (y) itself (11). If decisions are made about "acceptable" levels of precision (i.e., standard error to mean ratios), the above equation can be used to determine the relationship between the number of trees sampled (n) and the number of locations sampled per tree (m). These relationships were computed for a range standard error to mean ratios for each of the nematode-host combinations examined.

Results and Discussion

The large guava grove contained *Helicotylenchus dihystera* (Cobb) Sher (spiral nematode), *Quinisulcius acutus* (Allen) Siddiqi (stunt nematode), and *Rotylenchulus reniformis* Linford and Oliveira (reniform nematode) at mean densities of 2.9, 9.8, and 29 per 100 cm³ of soil, respectively. Overall mean densities of these 3 nematodes in the smaller guava grove were 3.1, 29, and 58/100 cm³ of soil. This grove also contained *Criconemella sphaerocephala* (Taylor) Luc and Raski (ring nematode) at a mean density of 5.8/100 cm³. The avocado grove contained *H. dihystera, Pratylenchus brachyurus* (Godfrey) Filipjev & Schuurmans Stekhoven (lesion nematode), and *R. reniformis* at mean densities of 2.8, 1.1, and 243/100 cm³, respectively. Five nematode species were recovered from the mango grove: *C. sphaerocephala, H. dihystera, Hemicriconemoides mangiferae* Siddiqi (sheath nematode), *P. brachyurus,* and *R. reniformis,* at mean densities of 84, 5.2, 18, 10, and 279/100 cm³ of soil, respectively. *Tylenchulus semipenetrans* Cobb (citrus nematode) and *R. reniformis* were recovered from the lime grove at mean densities of 2982 and 369/100 cm³ of soil.

Guidelines for sampling the various nematode species in the study sites were developed for the lime (Table 1), avocado (Table 2), and mango (Table 3) groves, as well as for the small (Table 4) and large (Table 5) guava groves. For each host-nematode combination, the tables indicate the number of trees to be sampled in a grove, given a predetermined level of precision and number of locations per tree. For example, to sample *R. reniformis* on limes with a 20% "error" (standard error to mean ratio), one could collect soil from 2 locations around each of 15 different trees or from 4 locations around each of 12 different trees. Both plans are equally precise. As more locations per tree are sampled, samples from fewer trees are needed to achieve the same level of precision. This effect is more evident with *T. semipenetrans* than with *R. reniformis* on lime (Table 1). With *R. reniformis,* increasing the number of locations sampled per tree from 3 to 6 does not affect the precision very much, but would double the amount of time required in sampling in most cases (Table 1).

Table 1. Numbers of trees to be sampled in a 0.4-ha lime grove to obtain a predetermined level of sampling error (standard error to mean ratio), using various numbers of locations per tree.

Standard error to mean ratio	Locations per tree					
	1	2	3	4	5	6
	Rotylenchulus reniformis, mean density (369/100 cm³ soil)					
0.10	56	43	38	36	35	34
0.20	19	15	13	12	12	12
0.25	13	10	9	8	8	8
0.30	9	7	6	6	6	6
0.40	6	4	4	4	4	4
0.50	4	3	3	3	2	2
	Tylenchulus semipenetrans, mean density (2982/100 cm³ soil)					
0.10[z]	—	—	67	51	42	36
0.20	50	26	18	14	11	10
0.25	32	17	12	9	7	6
0.30	23	12	8	6	5	5
0.40	13	7	5	4	3	3
0.50	8	5	3	3	2	2

[z]Dashes (—) indicate levels of precision which are unobtainable in the study site when using only few locations per tree.

Table 2. Numbers of trees to be sampled in a 1.6-ha avocado grove to obtain a predetermined level of sampling error (standard error to mean ratio), using various numbers of locations per tree.

Standard error to mean ratio	Locations per tree			
	1	2	3	4
	Helicotylenchus dihystera, mean density (2.8/100 cm³ soil)			
0.10[z]	—	227	202	189
0.20	130	98	87	82
0.25	92	69	61	57
0.30	67	50	45	42
0.40	40	30	27	25
0.50	26	20	18	17
	Pratylenchus brachyurus, mean density (1.1/100 cm³ soil)			
0.10	—	—	—	—
0.20	—	194	143	117
0.25	235	131	97	79
0.30	168	94	69	57
0.40	98	55	40	33
0.50	64	36	26	22
	Rotylenchulus reniformis, mean density (243/100 cm³ soil)			
0.10	118	102	96	94
0.20	39	34	32	31
0.25	26	22	21	21
0.30	19	16	15	15
0.40	11	9	9	9
0.50	7	6	6	6

[z]Dashes (—) indicate levels of precision which are unobtainable in the study site when using only few locations per tree.

To achieve very high levels of precision in sampling (e.g., standard error to mean ratio = 0.10), many trees must be sampled per grove. In most cases, there is a lower limit to the level of precision that can be achieved in a given grove using a specific plan. For example, in sampling *T. semipenetrans* in the lime grove (Table 1), a level of precision of 10% or less could not be achieved by sampling only one or 2 locations per tree, since these plans would require sampling more than the 86 trees available in the grove. Conversely, if only relatively few trees are sampled (e.g., less than 5), then relatively high sampling errors should be anticipated. Usually, a standard error to mean ratio of 0.25 is considered adequate for surveys of population density, with high precision (e.g., 0.10) needed only in certain critical research studies (13).

The difficulty in sampling for a rare nematode species is apparent from Table 2. Considerably fewer trees are required in estimating the common *R. reniformis* at a given level of precision than for *H. dihystera* or *P. brachyurus,* which were present at very low levels. Similar difficulties were encountered with *H. dihystera* in the mango (Table 3) and large guava (Table 5) groves, and to some extent with *C. sphaerocephala* in the smaller guava grove (Table 4). However, when densities are low (e.g., less than

Table 3. Numbers of trees to be sampled in a 0.4-ha mango grove to obtain a predetermined level of sampling error (standard error to mean ratio), using various numbers of locations per tree.

Standard error to mean ratio	Locations per tree 1	2	3	4
	Criconemella sphaerocephala, mean density (84/100 cm³ soil)			
0.10[z]	—	69	53	45
0.20	34	20	16	13
0.25	22	13	10	9
0.30	16	10	7	6
0.40	9	6	4	4
0.50	6	4	3	3
	Helicotylenchus dihystera, mean density (5.2/100 cm³ soil)			
0.10	—	—	—	—
0.20	—	98	80	70
0.25	—	73	59	53
0.30	87	56	45	40
0.40	55	35	28	25
0.50	37	24	19	17
	Hemicriconemoides mangiferae, mean density (18/100 cm³ soil)			
0.10	88	56	46	40
0.20	27	17	14	12
0.25	18	11	9	8
0.30	13	8	7	6
0.40	7	5	4	4
0.50	5	3	3	2
	Pratylenchus brachyurus, mean density (10/100 cm³ soil)			
0.10	—	67	53	46
0.20	33	20	16	14
0.25	22	14	11	9
0.30	16	10	8	7
0.40	9	6	5	4
0.50	6	4	3	3
	Rotylenchus reniformis, mean density (279/100 cm³ soil)			
0.10	—	—	—	87
0.20	84	45	32	25
0.25	55	29	21	16
0.30	39	21	15	12
0.40	22	12	9	7
0.50	14	8	6	5

[z]Dashes (—) indicate levels of precision which are unobtainable in the study site when using only few locations per tree.

10/100 cm³), relatively great sampling error can usually be tolerated. Even if such estimates are 50-100% from the true mean value, the absolute density involved is still very low, in most cases probably well below economic injury levels.

Considerable error could also be tolerated in sampling for nematodes such as *H. dihystera* or *Q. acutus*, which are relatively harmless to the crops examined here. In sampling several different species together on the same host, the sampling plan for the most damaging species can be used. When several pathogenic nematodes occur together, then the sampling plan for the nematode which is the most difficult to sample should be used. Other nematodes would then be sampled with even greater levels of precision. For example, on mango (Table 3), sampling 73 trees at 2 locations each is adequate for estimating *H. dihystera* with a standard error to mean ratio of 25%. This plan is also adequate for estimating the 4 other species at this level of precision or better (<10% for *C. sphaerocephala*, *H. mangiferae*, *P. brachyurus*). However, a considerable amount of work could be saved by recognizing that *H. dihystera* is relatively harmless and of little practical concern on mango, and instead sampling 29 trees at 2 locations each, the critical plan for *R. reniformis*, which is also adequate for estimat-

Table 4. Numbers of trees to be sampled in a 0.5-ha guava grove to obtain a predetermined level of sampling error (standard error to mean ratio), using various numbers of locations per tree.

Standard error to mean ratio	Locations per tree 1	2	3	4
	Criconemella sphaerocephala, mean density (5.8/100 cm³ soil)			
0.10[z]	—	204	161	139
0.20	102	62	49	42
0.25	67	41	32	28
0.30	47	29	23	20
0.40	27	17	13	12
0.50	18	11	9	8
	Helicotylenchus dihystera, mean density (3.1/100 cm³ soil)			
0.10	262	138	96	76
0.20	68	36	25	20
0.25	44	23	16	13
0.30	31	18	12	9
0.40	18	9	7	5
0.50	11	6	4	4
	Quinisulcius acutus, mean density (29/100 cm³ soil)			
0.10	170	156	151	149
0.20	65	59	58	57
0.25	44	41	39	39
0.30	32	29	29	28
0.40	19	17	17	17
0.50	13	11	11	11
	Rotylenchulus reniformis, mean density (58/100 cm³ soil)			
0.10	36	22	18	16
0.20	9	6	5	4
0.25	6	4	3	3
0.30	4	3	2	2
0.40	3	2	2	1
0.50	2	1	1	1

[z]Dashes (—) indicate levels of precision which are unobtainable in the study site when using only few locations per tree.

Table 5. Numbers of trees to be sampled in a 2.0-ha guava grove to obtain a predetermined level of sampling error (standard error to mean ratio), using various numbers of locations per tree.

Standard error to mean ratio	Locations per tree 1	2	3	4
	Helicotylenchus dihystera, mean density (2.9/100 cm³ soil)			
0.10	1102	700	566	499
0.20	335	213	172	152
0.25	220	140	113	100
0.30	155	99	80	71
0.40	89	56	46	40
0.50	57	37	30	26
	Quinisulcius acutus, mean density (9.8/100 cm³ soil)			
0.10	147	103	88	81
0.20	38	27	23	21
0.25	25	18	15	14
0.30	17	12	11	10
0.40	10	7	6	6
0.50	7	5	4	4
	Rotylenchulus reniformis, mean density (29/100 cm³ soil)			
0.10	160	121	108	101
0.20	42	32	29	27
0.25	28	21	19	17
0.30	19	15	13	12
0.40	11	8	8	7
0.50	7	6	5	5

ing the other 3 species within a standard error to mean ratio of 0.25 or less. This plan (29 trees x 2 locations) results in a standard error to mean ratio of 0.40-0.50 for *H. dihystera,* but this relatively large error should not be of much practical concern, since this species causes little damage.

A comparison of Tables 4 and 5 reveals that fewer samples were needed in estimating *H. dihystera* and *R. reniformis* in the smaller guava grove than in the large grove. The reverse of this trend was true for *Q. acutus,* although this irregularity probably resulted from the extremely high counts around one tree in the larger grove. More data are needed to establish relationships between grove size and sampling intensity.

Labor involved in sampling groves consists primarily of walking from tree to tree and removing individual cores or trowels of soil. On Rockdale soils, the labor involved in removing a trowel of soil is usually greater than walking from tree-to-tree, suggesting that the total number of locations sampled per tree should be minimized for maximum efficiency. For example, in sampling *R. reniformis* on avocado (Table 2), sampling 26 trees at one location per tree or 21 trees at 3 locations per tree results in equal precision, but the first plan requires digging 26 trowels of soil compared to 63 for the second plan. An economic analysis (11) has confirmed that, on Rockdale soils, sampling plans using only one location per tree are usually most efficient. This may not be true on other soil types where cores can be easily removed with a sampling tube. One notable exception to the one-site-per-tree plan recommended for Rockdale soils occurred with *T. semipenetrans* on lime (Table 1), for which sampling 6 trees at 6 locations per tree is slightly more efficient than sampling 32 trees at one location each (11). Another practical advantage of using one location per tree is that irregular weed growth beneath trees often causes difficulty in finding clean sampling sites if plans involving many locations per tree are used.

For the guava groves (Tables 4-5) and avocado grove (Table 3) examined here, the sampling plans for *R. reniformis* can be used, since the other species found were either present in low density or non-pathogenic. For these examples, sampling 28 trees at one location each can give a 0.25 standard error to mean ratio for *R. reniformis.* For limes, sampling plans for *T. semipenetrans* should be used, since that is the more important pest. The simultaneous sampling of 4 species in the mango grove (Table 3) has already been discussed. However, it is interesting to note that if a standard error to mean ratio of 40% were acceptable for *R. reniformis,* then 4 of the species could be sampled successfully by collecting soil from 22 trees at one location each.

In summary, tree crops growing on Rockdale soils can be sampled with a hand trowel to as great a depth as possible, usually about 15 cm. After removing leaves and rock from the soil surface, a trowel of soil should be taken from one location in the dripline of the tree, avoiding any weed cover which may be present. Based on the groves studied, which ranged from 0.4-2.0 ha, the most reasonable plans usually involved collecting soil from 20-35 trees per grove. At present, this may serve as a useful sampling guideline, until further research can provide more detailed information on number of trees required for specific crops or grove sizes.

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