

Sampling Citrus Fibrous Roots and *Tylenchulus semipenetrans*¹

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Abstract: Sampling precision was investigated for *Tylenchulus semipenetrans* juveniles and males in soil and females from roots and for citrus fibrous root mass density. For the case of two composite samples of 15 cores each, counts of juvenile and male nematodes were estimated to be within 40% of μ , at $P < 0.06$ (α) in orchards where $\bar{x} > 1,500$ nematodes/100 cm³ soil. A similar level of α was estimated for measurements of fibrous root mass density, but at a precision level of 25% of μ . Densities of female nematodes were estimated with less precision than juveniles and males. Precision estimates from a general sample plan derived from Taylor's Power Law were in good agreement with estimates from individual orchards. Two aspects involved in deriving sampling plans for management advisory purposes were investigated. A minimum of five to six preliminary samples were required to appreciably reduce bias toward underestimation of σ . The use of a Student's t value rather than a standard normal deviate in formulae to estimate sample size increased the estimates by an average of three units. Cases in which the use of z rather than Student's t is appropriate for these formulae are discussed.

Key words: citrus nematode, sampling, Taylor's Power Law, *Tylenchulus semipenetrans*.

The spatial patterns of citrus fibrous roots and of the citrus nematode, *Tylenchulus semipenetrans* Cobb, have been described for orchards with a variety of soil and cultural characteristics (1,2,4-6,15,18,20). Sampling plans to measure fibrous roots and *T. semipenetrans* have also been reported. McSorley and Parrado (18) estimated that 32 cores of soil taken at the dripline of randomly selected trees in a Florida lime orchard should provide an estimate of soil-stage population density within 50% ($P = 0.05$) of the mean. Five composite samples of 12 cores each were needed in a Texas grapefruit orchard to estimate population density within 20% of the mean (5). Abd-Elgawad (1) estimated that 20-60 samples are required to estimate population density within 40% of the mean in Egyptian orchards ranging in size from 2.1-8.4 ha.

Population density of *T. semipenetrans* is regulated in part by fibrous root availability. Thus, population density of the nematode (root-inhabiting stages or soil-inhabiting stages), expressed as a proportion of fibrous root mass density is a method of measuring the parasite burden on the tree (6). Although some information on citrus root sample optimization is available (11), no sampling protocol to measure fibrous root density concomitantly with nematode density has been reported.

In this paper, we analyze distributions of nematode counts and fibrous root mass density measurements from citrus orchards in Florida and Egypt to estimate sampling requirements. Orchards were selected to reflect a wide variety of cultural methods. We compare the output of several published methods for estimating sample size. Taylor's Power Law (23) is fitted to the data to evaluate a general sampling formula for this nematode. We consider sampling plans for management advisory purposes and discuss how the derivation of these plans may differ from the derivation of sampling plans for research plots.

MATERIALS AND METHODS

Survey sites: Six sites were used in these surveys. Five sites in Florida included a sin-

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gle bed (SBF) and a double bed (DBF) orchard, both of which were flood irrigated, a nonbedded overhead-irrigated orchard (NBO), a double bed drip-irrigated orchard (DBD), and a nonbedded microsprinkler irrigated orchard (NBM). A single site in Egypt was nonbedded and flood irrigated (NBF). Sour orange (*Citrus aurantium* L.) rootstocks were used in all orchards except NBM, which used rough lemon (*Citrus jambhiri* Lush). Orchard age ranged from 11 to >40 years. Soil characteristics, tree spacing, scion cultivar, and sampling dates are given in Table 1.

Sampling methods: Four survey methods were used to collect data used in this study: 1) 50 single soil cores per site were collected once; 2) 12 composite samples per site, each consisting of 15 soil cores, were collected once; 3) pairs of composite samples (15 cores each) were collected weekly from the same site for at least 9 months; and 4) single cores of soil were collected once from beneath five individual trees. For each survey method, selected trees were approximately evenly distributed throughout the sample area.

In the first survey, a single soil core (2.5 cm d × 30 cm deep) was obtained from the dripline (on the top and side of beds in double-bedded orchards) and another midway between the trunk and the dripline (on the top of the bed only, in bedded orchards) from 50 trees in 2-ha areas of the SBF, DBF, DBD, and NBO orchards. Six data sets of 50 soil cores each were ob-

tained from the driplines of trees and four data sets from under the tree canopies.

Samples were processed by sieving (1.25-mm openings) to remove fibrous roots from soil, then mixed by hand, subsampled (60 cm³), and extracted on Baermann funnels for 48 hours. Fibrous roots were rinsed, blotted, and weighed. Female *T. semipenetrans* were extracted from 2-g subsamples (3).

In the second survey, 12 composite samples were repeatedly obtained from the same 2-ha areas of the SBF, DBF, NBO, NBM, and NBF orchards. Each composite sample consisted of 15 soil cores (1 core per tree) taken midway between the dripline and trunk of the tree. Samples were processed as previously described, except that two subsamples of each sample were processed on Baermann funnels.

In the third survey, pairs of composite (15 core) samples were taken from the same 2-ha area of the NBF orchard weekly for 9 months beginning March 1992 and from the NBM orchard weekly for 27 months beginning May 1988 (10). Samples were collected and processed in the same manner as in the second survey, except that three rather than two subsamples of soil were processed on Baermann funnels in the NBF orchard.

In the fourth survey, 20 individual cores were collected systematically (8) from beneath each of four trees in the SBF orchard. A uniform grid pattern was used to collect 99 cores from beneath one tree in

TABLE 1. Edaphic and cultural characteristics of five orchards surveyed and first and second survey sample dates.

Cultural practice†	Soil texture (% sand:silt:clay)	Row × tree spacing (meters)	Tree age (years)	Single-core sample date	Multiple-core sample date	Rootstock	Scion
SBF‡	92:4:4	8.5 × 6.7	40+	31 July 1986	9 Feb. 1988	Sour orange	Valencia orange
DBF‡	90:5:5	9.1 × 6.0	14	14 Aug. 1986	25 Aug. 1988	Sour orange	Grapefruit
NBO‡	98:1.5:0.5	9.2 × 4.6	23	23 Aug. 1985	19 July 1988	Sour orange	Hamlin orange
DBD‡	98:1:1	7.6 × 5.2	11	7 Jan. 1987		Sour orange	Hamlin orange
NBM‡	98:0.5:1.5	9.2 × 4.6	40+		1 July 1988	Rough lemon	Grapefruit
NBF§	72:18:10	5.0 × 5.0	25		15 April 1993	Sour orange	Valencia orange

† SBF = Single bed orchard, flood irrigation; DBF = double bed orchard, flood irrigation; NBO = nonbedded orchard, overhead sprinkler irrigation; DBD = double bed orchard, drip irrigation; NBM = nonbedded microjet irrigation, NBF = nonbedded, flood irrigated.

‡ Orchards located in Florida.

§ Orchard located in Egypt.

the NBO orchard (6). Samples were processed as described for the first survey.

Data analyses: Formulae to estimate sample sizes necessary to achieve a specified accuracy at a specified probability level are frequently derived from the formula for a confidence interval about the mean (μ)

$$\mu \pm \frac{z_{\alpha/2}\sigma}{\sqrt{n}} \quad (1)$$

where σ = the population standard deviation, n = the number of samples, and $z_{\alpha/2}$ is the upper $\alpha/2$ point for the standard normal variate (12-14,17,19,22).

By estimating μ and σ using a sample mean (\bar{x}) and standard deviation (s), the right-hand term of the above expression (eq. 1) can be set equal to a given quantity as in

$$D\bar{x} = \frac{z_{\alpha/2}s}{\sqrt{n}} \quad (2)$$

where D = sample precision = the confidence interval half-length expressed as a proportion of the mean.

Various formulae to estimate the standard deviation (see below) can be substituted for s in equation 2. By defining the appropriate terms, equation 2 can be rearranged to solve for n , D , or z . In this study, we chose to define sample size (n) and precision (D) in order to estimate z . In effect, we are estimating α , or the probability that the confidence interval does not contain μ . Estimates of α were from a table (2-tail) of probability levels associated with z (21).

A Fortran program (16) was used to fit a negative binomial distribution model to the nematode counts from the individual cores from each orchard. The variance (s^2) of counts from single cores was derived using the formula (12)

$$s^2 \approx \bar{x} + \frac{\bar{x}^2}{k} \quad (3)$$

where k is the estimated aggregation parameter of the negative binomial model.

The formula

$$z_{\alpha/2} = \frac{D\sqrt{n}}{\sqrt{1/\bar{x} + 1/k}} \quad (4)$$

where n is the number of cores in a sample, was used to estimate α for each orchard for the arbitrary case where $n = 30$ cores and $D = 0.4$ (12).

The distributions of fibrous roots were often not significantly different than normal, and the formula (12)

$$z_{\alpha/2} = \frac{\sqrt{nD\bar{x}}}{s} \quad (5)$$

was used to estimate α for the arbitrary case where $n = 30$ cores and $D = 0.25$. From the Central Limit Theorem, nematode counts from composite samples approach normality with increasing numbers of cores per sample. Therefore, equation 5 was also used to estimate α based on composite samples for the case of two 15-core samples where $D = 0.40$.

To evaluate general (multiple site or time) sampling plans, Taylor's Power Law (23)

$$s^2 = a\bar{x}^b, \quad (6)$$

was also fitted to the nematode data. The natural logarithm of the variance was regressed against the natural logarithm of the mean to obtain estimates of a and b . For composite nematode samples, s^2 was from the standard formula assuming a normal distribution. For nematode counts from single-core samples, s^2 was from equation 3. Because nematodes were not detected under-canopy on 20 trees in the DBD orchard, the data set was considered a special case (9) and was omitted from the analysis. Data from Florida and Egypt were analyzed separately, because the numbers of subsamples from which nematodes were extracted differed. The formula (12)

$$z_{\alpha/2} = \sqrt{\frac{nD^2}{a\bar{x}^{b-2}}} \quad (7)$$

for the arbitrary cases where $D = 0.4$ and $n = 30$ for single-core samples and $n = 2$

for composite samples was used to estimate α .

From χ^2 distributions for various degrees of freedom, it can be seen that small sample size tends to result in bias toward underestimation of σ (21). To investigate the relationship between numbers of preliminary samples and bias of the standard deviation estimate (eqs. 4, 5, 7), random samples were drawn from a hypothetical, normally distributed population of known μ (1,000) and σ (500) (Minitab software). Standard deviations were calculated from 1,000 sets of samples of size 2, 3, 5, or 10. Median s as a proportion of σ was determined for each sample size.

Various studies of sample size requirements have used either Student's t_{n-1} (12,13) or the $z_{\alpha/2}$ statistic (1,19) in equation 2. To investigate the relationship between sample size predicted using Student's t values and those predicted using standard normal deviates, equation 5 was rearranged as:

$$\frac{z_{\alpha/2}}{\sqrt{n}} = \frac{D\bar{x}}{s} \text{ or} \quad (8)$$

$$\frac{t_{n-1}}{\sqrt{n}} = \frac{D\bar{x}}{s} \quad (9)$$

for the cases of large and small sample size, respectively. The left-hand term of each equation was solved for various n ($P = 0.05$) and compared to determine which values of n in each equation would make the two equations most nearly equivalent.

A Fortran program was written to simulate both random and systematic soil sampling from the 99 nematode counts from beneath a single tree in the NBO orchard. For a sample of a given size, the appropriate number of cores was randomly or systematically selected (with replacement). For systematic samples, an equal number of cores were selected randomly from each of four annuli whose midpoints were four equal distances between the trunk and the dripline. Mean feeder root weight and nematode population level were determined per soil volume in the composite

sample. The number of soil stages of the nematode per gram of fibrous root was also calculated from total nematode and root quantities in a simulated composite sample. These values were compared with the mean root, nematode, and nematode per gram root values for all sample cores. Specific sample strategies were simulated 1,000 times each. The proportion of the 1,000 sample estimates that were greater or less than a given proportion of the global mean was calculated.

RESULTS

Sampling orchards: The distributions of counts of all nematode populations, whether juveniles and males extracted from soil or females extracted from roots, were not significantly different from the negative binomial. Values of k ranged between 0.11 and 1.29 (Table 2). No relationship was apparent between values of k and the corresponding population means.

Population densities of *T. semipenetrans* and fibrous root mass density were generally higher beneath the tree canopy than at the dripline (Table 2). Therefore, α for the case of 30-core composite samples was usually estimated from equations 4 and 5 to be smaller for the under-canopy samples. For nematodes, however, differences in estimates of α between under-canopy and dripline samples were not great. In some cases, counts beneath the canopy were more aggregated than those from the dripline, resulting in lower α estimates for dripline samples despite lower mean density of nematodes.

Precision of population size estimates of soil-stage *T. semipenetrans* was higher than estimates of females (Table 2). Estimates of α , however, decreased on average by 24% when female nematodes were expressed per gram of fibrous roots (data not shown), rather than per volume soil. Expressing soil-stage juveniles per gram of fibrous roots had no effect on estimates of α .

There was close agreement between estimates of α from twelve 15-core samples

TABLE 2. Average *Tylenchulus semipenetrans* population density and citrus fibrous root mass density from 50 single cores of soil in four Florida citrus orchards.†

Orchard	Nematodes or roots per 100 cm ³ soil					
	Under-canopy			Dripline		
	Mean	(k)	α‡	Mean	(k)	α‡
		Juveniles and males				
DBF§ (top of bed)	2,586	(0.82)	0.05	1,912	(0.89)	0.04
DBF (bed wall)				14	(0.54)	0.11
DBD (top of bed)	1,114	(0.11)	0.47	8	(0.11)	0.47
DBD (bed wall)				2,146	(0.47)	0.13
SBF	738	(1.29)	0.01	636	(0.77)	0.05
NBO	850	(0.45)	0.14	486	(0.44)	0.15
		Females				
DBF (top of bed)	244	(0.42)	0.16	135	(0.56)	0.10
DBD (top of bed)	44	(0.29)	0.24	1	(0.10)	0.51
DBD (bed wall)				62	(0.54)	0.11
SBF	44	(0.74)	0.06	30	(0.34)	0.20
		Fibrous roots (mg)				
DBF (top of bed)	1.53	(0.99)	0.04	1.00	(0.62)	0.03
DBF (bed wall)				0.39	(0.14)	0.01
DBD (top of bed)	3.69	(1.91)	0.01	1.01	(1.03)	0.18
DBD (bed wall)				2.04	(1.52)	0.07
SBF	1.66	(1.00)	0.02	0.76	(0.78)	0.18
NBO	0.73	(0.51)	0.05	0.46	(0.39)	0.11

† Probability of a Type I error (α) is estimated for the arbitrary case of composite samples consisting of 30 cores (2.5-cm-d × 30 cm deep) of soil and roots. Sampling precision (D) is defined as confidence interval half-length equal to 40% of the mean nematode population density and 25% of the mean fibrous root mass density.

‡ For nematodes, $z_{\alpha/2} = D\sqrt{n}/\sqrt{1/\bar{x} + 1/k}$; for fibrous roots, $z_{\alpha/2} = (\sqrt{nD\bar{x}})/s$.

§ DBF = Double bed orchard; flood irrigation; DBD = double bed orchard, drip irrigation; SBF = single bed orchard, flood irrigation; NBO = nonbedded orchard, overhead sprinkler irrigation.

and those from Taylor's Power Law fit to data from those samples or to single-core data (Table 3). Power Law estimates of α

based on pairs of 15-core composite samples were lower, however, than those from the other methods. Parameter estimates

TABLE 3. Precision estimates for confidence interval half-lengths equal to 40% of the mean densities of juvenile and male *Tylenchulus semipenetrans* per 100 cm³ soil in four Florida citrus orchards† and in one citrus orchard located in Egypt.

Orchard	Mean density	Probability of greater z values by method			
		Normal‡	TPL1 ^c	TPL2 ^d	TPL3 ^e
NBO	687	0.14	0.12	0.08	0.01
NBM	1,595	0.03	0.05	0.06	0.01
DBF	1,584	0.06	0.05	0.06	0.01
SBF	2,762	0.04	0.03	0.04	0.01
NBF	508	0.04			0.01

† Estimates are from four different methods and are for the case where two samples of 15 composite cores are taken.

‡ Where $z_{\alpha/2} = (\sqrt{nD\bar{x}})/s$, for $n = 2$ where \bar{x} and s are estimated from twelve 15-core samples.

^c Where $z_{\alpha/2} = \sqrt{nD^2/(a\bar{x}^{(b-2)})}$, for $n = 2$ where a and b are from Taylor's Power Law ($s^2 = a\bar{x}^b$) fitted to estimates of \bar{x} and s from twelve 15-core samples.

^d Where $z_{\alpha/2} = \sqrt{nD^2/(a\bar{x}^{(b-2)})}$, for $n = 30$ where a and b are from Taylor's Power Law ($s^2 = a\bar{x}^b$) fitted to estimates of \bar{x} and s from 50 single-core samples.

^e Where $z_{\alpha/2} = \sqrt{nD^2/(a\bar{x}^{(b-2)})}$, for $n = 2$ where a and b are from Taylor's Power Law ($s^2 = a\bar{x}^b$) fitted to estimates of \bar{x} and s from 103 pairs of 15-core samples collected during 27 months (NBM), or from 36 pairs of 15-core samples collected during 9 months (NBF).

† Nonbedded, overhead irrigation (NBO); nonbedded, microjet irrigation (NBM); double-bedded, flood irrigation (DBF); single-bedded, flood irrigation (SBF); nonbedded, flood irrigated, Egypt (NBF).

for the Power Law from the four sets of twelve 15-core composite samples were $a = 3.97$ and $b = 1.48$ ($R^2 = 0.96, n = 4, P = 0.02$). For the nine sets of 50, single-core samples, parameter estimates were $a = 7.17$ and $b = 1.77$ ($R^2 = 0.96, n = 9, P = 0.01$). Power Law parameter estimates for the pairs of composite samples from Florida were $a = 0.07$ and $b = 1.83$ ($R^2 = 0.28, n = 103, P = 0.01$) and from Egypt were $a = 0.12$ and $b = 1.61$ ($R^2 = 0.23, n = 36, P = 0.01$). For the arbitrary case of two 15-core samples and $D = 0.40$, estimated α approached a level of $P = 0.05$ in four of the orchards sampled using composite samples (Table 3). Among the Florida orchards, only the NBO site ($P = 0.12$) had an estimated α appreciably greater than $P = 0.05$, due to the low population density.

Values of s from random sampling of populations of known mean and σ were not normally distributed. Median s were less than σ for all sample sizes (Fig. 1). As sample size increased from 2 to 10, the ratio of the median s to σ approached unity. The median s for 5 and 10 samples underestimated σ by 5.5% and 2.5%, respectively. Bias increased rapidly as sample size decreased from five samples.

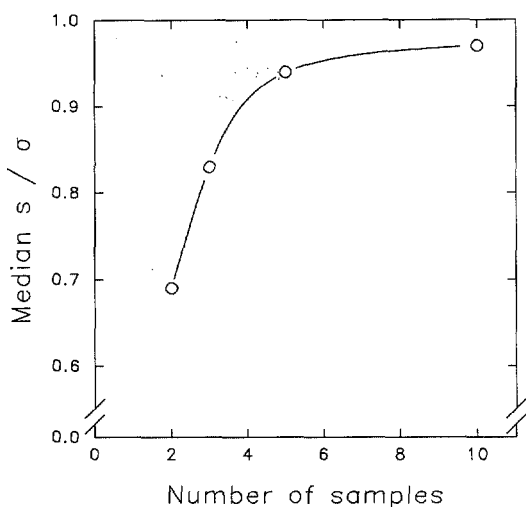


FIG. 1. Relationship between the median s and σ from 1,000 random samples for each of various sample sizes drawn from a population of known μ (1,000) and σ (500).

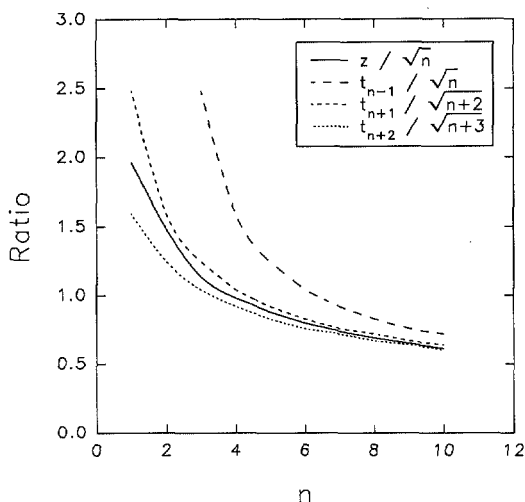


FIG. 2. Relationship of z/\sqrt{n} , t_{n-1}/\sqrt{n} , $t_{n+1}/\sqrt{n+2}$, and $t_{n+2}/\sqrt{n+3}$ for various values of n .

The ratios derived from equation 9 using $n, n + 1$, and $n + 2$ were greater than those from equation 8 using n , for all values of n (Fig. 2). The ratios from equation 9 using $n + 3$ were less than those from equation 8 using n , for all values of n .

Sampling individual trees: Taylor's Power Law parameter estimates from single trees in the SBF orchard were $a = 5.02$ and $b = 1.75$ ($R^2 = 0.97, n = 4, P = 0.01$). Compared to random sampling, required sample sizes were reduced by approximately half in the NBO orchard if samples beneath trees were collected systematically by specifying numbers of cores collected at specific distances from the trunk (Fig. 3). Root sampling required 29 or 15 cores per sample for 40% precision ($P = 0.05$), depending on whether samples were collected randomly or systematically. The corresponding numbers of cores per sample for nematodes were 36 and 18. A similar sample size of 21 cores for the NBO tree (1,274 nematodes/100 cm³ soil) is predicted from Taylor's Power Law derived from the four SBF trees sampled systematically.

DISCUSSION

According to Taylor's Power Law using data from single soil cores or composite

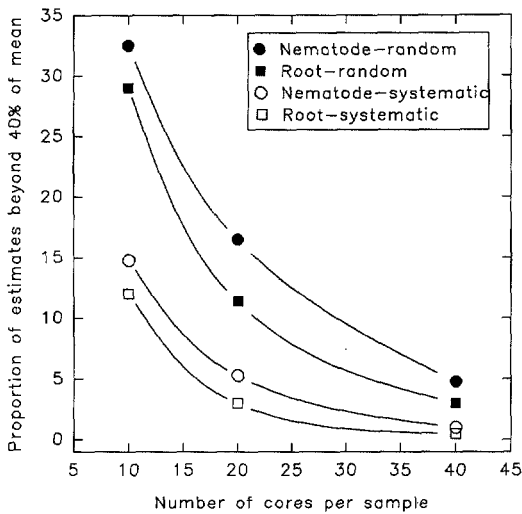


FIG. 3. Relationship between sample size (n) and estimated α (proportion of 1,000 estimates beyond 40% of the mean) from a FORTRAN program for samples obtained randomly or systematically from beneath a single citrus tree for nematode populations (juveniles and males) and fibrous root mass density.

samples of multiple cores, two samples of 15 cores each will measure *T. semipenetrans* population density in soil within 40% of the mean approximately 95% of the time when population density in soil exceeds 1,600–2,000 nematodes/100 cm³ soil. Most published action thresholds for management of this nematode on citrus tend to be at or above 2,000 juveniles/100 cm³ soil (7). At lower density (687 nematodes/100 cm³ soil), α was estimated to be as high as $P = 0.14$ in Florida, but remained low ($P = 0.04$) in an Egyptian orchard with 508 nematodes per 100 cm³ soil. The lower sample variability in the Egyptian orchard may have been due partly to the increased number of subsamples processed.

Results of this study were in reasonable agreement with other published estimates of sampling requirements for *T. semipenetrans*. Davis (5) randomly sampled from a database of counts from 625 cores of soil and estimated that five 12-core samples would provide estimates within 20% of μ (3,760 nematodes/100 cm³ soil) 95% of the time. Using those criteria and Taylor's Power Law parameters for the 15-core

samples in equation 7 results in a similar α estimate of $P = 0.06$ ($z = 1.91$). Similarly, two samples of 15 cores were estimated to result in α of approximately $P = 0.05$ in orchards with nematode density $>1,500/100$ cm³ soil, which is in substantial agreement with estimates by McSorley and Parrado (18), and Abd-Elgawad (1).

Two samples of 15-cores taken under-canopy were sufficient to measure fibrous root mass density within 25% of the mean at least 95% of the time in all orchards studied. Estimated α was considerably higher at the tree dripline. Because fibrous root mass density and fibrous root parasites in the surface horizon of the soil decrease with distance from the trunk (6), under-canopy sampling should increase root and nematode sample precision compared to sampling at the dripline.

Narrow diameter cores of roots and soil are convenient for advisory sampling and minimize disturbance to the rhizosphere in research plots. However, counts of female *T. semipenetrans* (which are generally less than 10% as numerous as juveniles and males) were much more variable than counts of juveniles and males from soil. Expressing females per gram of fibrous root helped to increase sampling precision. However, use of larger diameter augers or shovels to sample larger quantities of roots (which could be mixed and subsampled for processing) should probably be considered to significantly improve the precision of female counts.

The present study indicates a marked advantage of systematic over random (6) sampling to measure nematode parasitism of individual trees. Because fibrous roots and nematode counts decrease with distance from the trunk, simulated sampling on a systematic basis reduced a significant source of variability and reduced the estimated root and nematode sampling size by approximately half. Systematic sampling of the same surface area beneath each tree (e.g., a defined radius about the tree trunk) also permits comparison of one aspect of individual tree quality within an or-

chard based on root quantities recovered in the sample.

The fit of Taylor's Power Law to single core and composite sample data suggests that general sampling plans may be useful to measure population density of this nematode (1,9). Nevertheless, the number of cases used in this study was low, and additional work is likely to reveal orchards that are not well-described by a single, general variance-mean model (9). Indeed, in the under-canopy samples of the DBD orchard, the large number of 0 counts despite a relatively high mean density resulted in a variance to mean ratio considerably above that predicted from all other single core samples. This orchard was flood irrigated during the first 3 years of tree growth. Flood irrigation may have spread nematodes more uniformly along the root systems growing on the bed wall, while not affecting a patchy distribution on top of the bed. Orchards in which *T. semi-penetrans* appears to infect only patches of trees were previously identified (9). For a given mean density, orchards with patches of infected trees should have a higher variance than orchards in which most or all trees are infected. However, if a general sample plan can be determined for orchards in which most or all trees are infected, it will likely provide acceptable reliability for advisory purposes, even for orchards with patchy nematode distribution. The cost of current sampling methods precludes identifying small patches of infected trees, and mean nematode density in most such orchards is unlikely to exceed an action threshold.

Although there was close agreement in predictions of sampling precision derived from single core samples and large numbers of composite samples, the use of pairs of composite samples seriously underestimated sample variability. Clearly, to estimate parameters of Taylor's Power Law it is important to optimize between the number of observations (pairs of μ and σ estimates) and the number of samples taken per observation. To appreciably reduce

the degree of bias toward underestimating σ , a minimum of five to six samples are required. Sampling studies based on two to three samples per observation (10,14,17) tend to underestimate the variance to mean relationship, regardless of the number of observations employed. This fact constrains the use of experimental data to determine sample size for management advisory purposes, if the number of samples taken from each experimental unit is small.

Two important aspects in determining sample size are the expected precision and the probability that the predetermined confidence interval does not contain μ . In this work we evaluated sample size primarily in terms of the expected α for a given level of precision (17). The advantage of this approach is that sample size changes at a greater rate than α . For example, from equation 5, at a mean nematode density and standard deviation of 2,000 and 580, respectively, sample size must equal 3 to achieve $D \leq 40\%$ at $P = 0.05$. A sample size of 2 (which requires a third less time and labor to acquire) results in an α estimate of $P = 0.06$, for the case $D = 40\%$. Thus, for a given precision level, it may be more informative to estimate α for samples of a size that can be economically collected and processed than to estimate sample sizes necessary to achieve a predetermined α .

Nematode samples are characteristically composites, composed of large numbers of individual soil cores, because of the high cost and amount of time necessary to extract and count nematodes. For this reason, sample size to estimate nematode population density tends to be very small, often on the order of one to two composite samples of various size per 2–3 ha (13,14). Small sample size affects methods commonly used to estimate sampling precision by requiring the use of a Student's *t* value rather than the standard normal deviate (*z*) in such equations as 3, 5, and 7 (12,22). Use of *t* rather than *z* in those equations increases the estimated α and increases by

approximately 3 units the number of samples necessary to achieve a given level of α and precision. For example, equation 7 can be rearranged as

$$n = \frac{t_{n-1}^2 a \bar{x}^{b-2}}{D^2} \quad (10)$$

to estimate sampling size (13,14). In order to balance equation 10, n will be 2–3 units greater than if z^2 is substituted for t_{n-1}^2 (Fig. 2). When sample size is large, an increase of 2–3 units may be trivial. When sample size is typically small, however, such an increase may double or even triple the cost of sampling. For this reason, the purpose for collecting nematode samples should be considered when estimating appropriate sampling sizes.

For research purposes requiring mean separation, the use of Student's t_{n-1} is required in formulae to estimate sampling size because this term will be required in the anticipated calculations. If the samples are for management advisory purposes, however confidence intervals are not generally derived for mean estimates. Rather we are interested in the proportion of all mean estimates that are likely to be contained within a specified interval about μ . For this case, it may be more realistic to invest adequate resources in estimating σ and μ from s and \bar{x} in order to use $z_{\alpha/2}$ in these formulae. The rationale for such an approach is that n in formulae such as equation 10 will be the same for given estimates of μ and σ whether those estimates are based on very few or very large numbers of preliminary samples. As the number of preliminary samples increase, s and \bar{x} approach σ and μ , thereby approaching the case for which $z_{\alpha/2}$ rather than t_{n-1} is the more appropriate term in equation 10, regardless of whether the derived sampling size is large or small.

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