A Bioassay Sampling Plan for *Meloidogyne incognita*

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ABSTRACT

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Two hundred okra bioassay samples for *Meloidogyne incognita* from each of two large fields were used to simulate sampling plans consisting of various numbers of composited samples per field, with each composite sample representing soil from 20 different field locations. In fields of 2.3 and 6.1 ha, 3–4 composite samples were needed to achieve a standard error to mean ratio of 25% or less in the number of galls per plant; five composite samples were needed to obtain a standard error to mean ratio of 20%. In smaller fields of 1.2 ha, two and three composite samples were needed to achieve standard error to mean ratios of 25 and 20%, respectively. Instead of composite samples, use of 20 samples, each from a different location, resulted in a standard error to mean ratio of 49.6%.

Accurate assessment of field populations of plant-parasitic nematodes is essential before making control recommendations. In southern Florida, bioassay of soil samples for *Meloidogyne* spp. is more successful than traditional soil extraction for estimating relative population levels before planting in the autumn vegetable season (6). Use of bioassay plants can also facilitate *Meloidogyne* species identification (8).

Previous work with bioassay plants for nematode detection has been reviewed (1), but few guidelines are available for developing sampling plans involving bioassay plants. Godfrey (4) recommended planting indicator plants in at least 20 locations in a large field. For traditional soil samples, 20 cores of soil taken from a 1.6-ha field have given acceptable results (1). This may or may not be an appropriate number of soil samples for all areas so it is necessary to determine the number of such samples to be taken per field and provide some estimate of the errors involved in alternative sampling schemes.

Two sampling methods were compared for bioassay tests in southern Florida (6): 1) the composite sample from 20 different field locations, which would be most practical for bioassay plants that are grown in pots away from the field; and 2) individual samples from 20 different field locations, which should be most appropriate when bioassay plants are grown directly in the field. In each case, the objective is to determine the smallest numbers of samples to be taken while keeping sampling error within predetermined limits (standard error to mean ratios of 20–25%).

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MATERIALS AND METHODS

Sampling. Two fields near Homestead, FL, were sampled for this bioassay study during late summer in 1981. Both fields were uniform Rockdale fine sandy loam infested with *Meloidogyne incognita* (Kofoid & White) Chitwood. Both fields were planted to tomato during the spring of 1981 and maintained in a clean fallow condition during the summer of 1981. Site TC was 6.1 ha and was sampled on 25 August 1981. Site TK was 2.3 ha and sampled on 21 September 1981. Two hundred bioassay samples of 946 cm^3 of soil each were collected from each field in a stratified random sampling pattern; soil for each bioassay was collected with a hand trowel from one location in the field to a depth of 15 cm after removing the top 2.5 cm of soil.

Bioassay. To ensure that bioassay

plants were initially nematode-free, seed of okra, *Hibiscus esculentus* L. 'Clemson Spineless,' was grown in a sterile mix of 1:1 peat moss:vermiculite in Speedling trays (5 × 5 cm) for 3 wk. Each bioassay sample contained enough soil from one field location to fill one 946-cm^3 plastic pot. Immediately after the field soil was collected, a 3-wk-old bioassay plant was transplanted into each pot and maintained as described previously (6). Six weeks after transplanting, plants were removed and counts were made of galls in each root system plus any extra egg masses when more than one egg mass per gall occurred. Counts of galling on bioassay plants were then available from 200 locations in each field.

Data analysis. A FORTRAN program (3) was used to test goodness of fit to common statistical distributions of actual root gall counts and of counts transformed on a 0–5 scale (8). Because the data could not be fitted to Poisson or negative binomial distributions, the formulas for determining sample error based on those distributions (2,7) could not be used. Thus, it was necessary to use empirical methods for computing relative errors in samples. The methodology used here is similar to that developed by Goodell and Ferris (5). This method involves simulation of sampling plans from a large data base and is not restricted to predetermined sampling plans.

Two distinctly different sampling patterns were simulated from the data base of this experiment. The first pattern (single location per sample) was a single sample from a single location; the estimate of gall count per plant for the sample was the actual gall count for that location. This sampling pattern is comparable to the situation in which bioassay plants would be grown directly in the field, with one plant per location. The second pattern (multiple locations per sample) was a single sample with 20 subunits or cores, each from a different location in the field. In this case, the estimate of gall count per plant for the sample was computed as the mean of the gall counts over each of the 20 locations involved. This approach assumes that the cores obtained from each location are uniform in size and evenly mixed together. This second pattern is analogous to the case in which cores from several different field locations are composited and planted to a single bioassay plant.

Single location per sample. To simulate the situation in which all soil for a single sample was taken from one location, each large field was divided into 20 subunits of 10 bioassay counts each. One bioassay count was selected at random from each of these 20 subunits. Because each bioassay count in this scheme represented soil from only one location, each bioassay count was considered a single sample for this analysis. Estimates of the field mean, standard error, and standard error to mean ratio were computed over the 20 individual samples for a given field. The procedure was replicated five times by randomly choosing a different combination of 20 samples each time.

3	0	0	9	5	8	4	9	5	6	9	3	9	6	5	8	5	6	8	7
2	8	7	2	8	5	2	5	2	8	0	4	0	9	1	3	0	1	2	1
5	9	8	6	9	3	3	3	4	2	2	5	5	4	4	9	2	0	9	4
4	2	1	4	0	6	1	6	9	4	5	2	3	2	0	2	9	2	6	8
9	5	2	0	3	2	7	0	3	0	7	1	8	7	7	5	4	8	4	6
0	7	9	7	6	4	0	1	1	5	8	7	7	8	6	0	8	3	0	5
1	3	6	5	1	7	5	7	8	1	6	9	6	1	2	4	1	9	1	9
8	1	5	3	4	1	6	2	0	3	1	0	1	3	8	6	3	4	3	3
7	6	3	1	2	0	9	4	7	9	3	6	2	0	3	1	7	7	7	2
6	4	4	8	7	9	8	8	6	7	4	8	4	5	9	7	6	5	5	0

Fig. 1. Simulated sampling pattern for 10 composite samples from 20 locations each. Each number represents one bioassay count; field has been divided into 20 strips (subunits) of 10 counts each. 1 = 20 Core locations composited to make simulated sample No. 1; 2 = 20 core locations composited to make simulated sample No. 2, etc.; 0 = 20 core locations composited to make simulated sample No. 10.

This experiment was repeated, using fields divided into 40 subunits to give 40 samples from individual locations. This procedure was also replicated five times for comparison with the 20-sample case.

Multiple locations per sample. Each field was subdivided into 20 subunits of 10 bioassay counts each. To simulate a sampling plan of one composite sample of 20 cores, one bioassay count was selected at random from each of the 20 subunits and a mean count of galling was computed over the 20 cores comprising that sample (Fig. 1). The process was repeated for a second composite sample from the 20 locations (Fig. 1), up to a total of 10 composite samples. At each step, means, standard errors, and standard error to mean ratios were computed over all the composite samples collected to that point. The entire procedure was replicated five times. In this case, one sample consisted of cores from 20 different locations, represented by the mean count from the 20 locations, whereas in the single location per sample case, each sample was represented by the bioassay count from only one location.

Subdivided fields. Each field (sites TC and TK) was divided into two equal portions to be sampled as two separate fields. These new fields were subdivided into 20 subunits each and up to five composite samples of 20 cores each were selected at random from each according to the methods described for multiple locations per sample. Statistics were computed at each step as above, and average standard error to mean ratios were computed over five replicates.

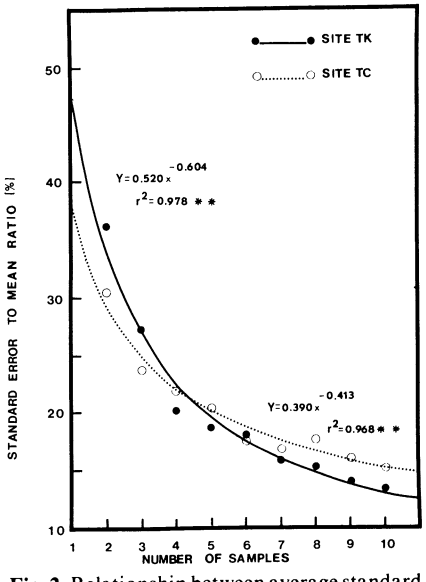

Fig. 2. Relationship between average standard error to mean ratios and number of bioassay samples (20 cores/sample) for two sites, with actual points and fitted cures of the form $y = ax^b$.

RESULTS

Single location per sample. When samples represented only one location in a field rather than a composite from 20 locations, more bioassay plants were needed to achieve a given level of error. With 20 such samples, an average standard error to mean ratio of 49.6% was obtained, whereas with 40 samples

Table 1. Number of composite samples of 20 cores each needed to achieve standard error (SE) to mean ($\bar{x}$) ratios of 20 and 25% for various sites

Field description	Field area (ha)	Minimum number of samples needed for SE/$\bar{x}$ of: 25%	20%
Site TC	6.14	3	5
Site TC, east half	3.07	4	6
Site TC, west half	3.07	3	4
Site TK	2.32	4	5
Site TK, north half	1.16	2	3
Site TK, south half	1.16	2	3

from single locations each, an average standard error to mean ratio of 34.7% was achieved.

Multiple locations per sample. For a given number of composite samples, an average value of the standard error to mean ratio was computed for the five replicates. The resulting values are illustrated for each field (Fig. 2), along with fitted curves of the form $y = ax^b$. To obtain a standard error to mean ratio of 25%, three samples of 20 cores each would be needed from site TC, whereas four such samples would be required from site TK. Five samples from 20 locations each would be required from either field to achieve a standard error to mean ratio of 20% or lower. Few differences are apparent between the two curves of Figure 2 despite the difference in size of the two fields. It is apparent from these results that estimates within a standard error to mean ratio of 25% could be obtained even for the 6-ha field.

Subdivided fields. Data comparing results obtained by dividing the larger fields in half are shown in Table 1. Subdivision of the large field TC into smaller units had little effect on the number of samples needed to achieve standard error to mean ratios of 20–25%. These "smaller" units, however, are still larger than site TK. Subdivisions of site TK into fields of 1.16 ha each indicated that fewer samples were needed from these smaller fields to maintain a given relative error.

DISCUSSION

It is evident that with single samples from one location each, many bioassay samples would be needed to obtain standard error to mean ratios of 20–25%. Such a situation exists when bioassay plants are grown directly in the field. Fewer bioassay plants would be needed if composite samples from 20 locations each were used to obtain a mean level of galling on a bioassay plant. With fields of 2.3–6 ha, four composite samples from 20 locations each were needed to obtain a standard error to mean ratio of 25%, whereas five samples were needed for 20%. In a smaller field of 1.16 ha, only two and three bioassay samples were needed to achieve standard error to mean ratios of 25 and 20%, respectively.

Division of large fields into units of about 1 ha is recommended, with two composite samples from 20 locations each per hectare. Although this may require collecting more samples than if fields were left undivided, it can provide information on localized large populations of *Meloidogyne* spp. and allows for possible spot treatment of such areas. A single mean representing an undivided 10-ha area may not be very meaningful because of the irregular distribution characteristic of root-knot nematodes.

Godfrey (4) attempted to estimate actual field populations from bioassay plants. This may not be possible with the present bioassay method (6) because the various ages of *Meloidogyne* eggs and larvae in the soils make it possible for asynchronous and even multiple generations to cause galling in bioassay plants. Nevertheless, the method can be useful for revealing relative infestation levels in various fields and can provide a standard of comparison when several fields are involved. Because plant yield can be inversely related to galling on host plants, it may be possible for integrated pest management programs to use the relative estimates obtained from bioassay plants to estimate the potential yield losses.

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