

Repeated Sampling to Determine the Precision of Estimating Nematode Population Densities¹

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Abstract: The first phase of this study involved repeated samplings of five fields using composite samples of 10, 20, 40, and 80 soil cores, to determine the precision of nematode assays. The second phase focused on randomly selecting two and four 2-ha subunits (data on *Meloidogyne* spp.) of 24 fields ranging from 6 to 40 ha and computing the precision of estimated means for these numbers of subunits versus the general field mean (based on all 2-ha subunits). Average numbers of nematodes from most samples containing *Meloidogyne* spp., *Heterodera glycines*, *Helicotylenchus dihystera*, *Scutellonema brachyurum*, and (or) *Hoplolaimus galeatus* were within 50% of the overall means. Coefficient of variation (CV) values were generally lower for 40 cores than for 10, 20, and 80 cores per sample. When data for all nematodes and fields were combined, this value was lowest for 40 and 80 cores. The CV values were higher for *Meloidogyne* spp. than for *H. glycines*. Means of two samplings increased the probability of obtaining numbers nearer the mean for that field than numbers from a single composite sample. For the second phase, population estimates of *Meloidogyne* spp. based on four 2-ha subunits generally were closer to field means than were those for two subunits. Sampling precision with these subunits diminished greatly in large fields with variable soils and (or) mixed cropping histories. Either two or four subunits gave population estimates within 3-20% of the field mean in most instances. The mean man hours required for sampling ca. 2-ha parcels of 4-20-ha fields was 0.54 hours.

Key words: assay, *Criconebella* spp., *Helicotylenchus dihystera*, *Heterodera glycines*, *Meloidogyne*, ring nematode, root-knot nematode, sampling, *Scutellonema brachyurum*, soybean cyst nematode, spiral nematode.

Efficient management of crops to limit economic losses due to plant-parasitic nematodes is possible only when the species and population densities of nematodes per unit of soil volume in a field are known. These parasites are typically clustered in most fields (1,5,7-13,15,16). Because of this aggregation, time-consuming and laborious sampling procedures are required to obtain reliable data (3). Sampling to estimate nematode populations usually is done by collecting a composite of 20-50 soil cores in a stratified or systematic pattern from 2-20 ha, depending on the crop and nematode species (3,14). Nematodes are usually

extracted from subsamples of 100, 250, or 500 cm³ soil. Data from intensive systematic samplings using single soil cores followed by computer simulated resamplings have been used to analyze spatial patterns of nematodes in order to develop practical sampling schemes (2,9,11). These studies have been concerned primarily with the precision of population estimates and not with accuracy of the estimate obtained. Ultimately, the objective of pest management sampling is to obtain a reliable estimate of mean nematode population densities in the field.

Estimates of time and costs required to achieve a desired level of precision with an assay based on single cores vary greatly. An estimated 7 hours of field sampling and laboratory analysis were needed to estimate a population density of *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans-Stekhoven within 20% of the mean with 95% confidence (15). Similarly, a procedure involving a 5-hour effort to collect six samples of 68 cores each per 7 ha was chosen as optimal for an alfalfa field (10). Even though optimal sampling schemes have

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been derived from computer-simulations based on single-core sampling, extrapolation of such data may not be comparable to actual multicore sample data.

The objective of this research was to determine the precision of nematode assays from repeated composite samples utilizing different numbers of cores with emphasis on *Heterodera glycines* Ichinohe and *Meloidogyne* spp. Determinations were made using actual multiple samples taken at the same date within each of five fields.

MATERIALS AND METHODS

Four fields in Cumberland County, and one field in Pasquotank County, in North Carolina, were sampled repeatedly soon after crops were harvested. The fields in Cumberland County were sampled in 1984 and the one in Pasquotank County in 1987. The cropping history of fields 1–4 (1982–84) and 5 (1986–87) were field 1, corn-soybean-corn; field 2, sweet potatoes-soybean-tobacco; field 3, soybean-soybean-corn; field 4, tobacco-tobacco-tobacco; field 5, soybean-corn. Nematode infestations per field were field 1, *H. glycines*; field 2, *Meloidogyne incognita* (Kofoid & White) Chitwood, *M. arenaria* (Neal) Chitwood, and *Helicotylenchus dihystrera* (Cobb) Sher; field 3, *H. glycines* and *Scutellonema brachyurum* (Steiner) Andrassy; field 4, *M. incognita* and *M. arenaria*; and field 5, *H. glycines*, *Tylenchorhynchus claytoni* Steiner, *H. dihystrera*, and *Hoplolaimus galeatus* (Cobb) Filipjev & Schuurmans-Stekhoven.

The fields were sampled identically. A 100 × 100-m grid was established in each field and then sampled in a systematic zig-zag pattern (4,13) that was similar for all repetitions of samplings. Cores were taken at approximately equal distances along the zig-zag path, although the distance between points varied according to the number of cores per sample. Composite samples consisting of 10, 20, 40, and 80 cores (2.5 cm d × 20 cm deep) were collected from each field. Eight sequential samplings were done for each core-number composite on the same date in each field. In Cumberland County, each composite sample was

mixed by passing it through a screen with 6.5- μ m openings, and a 500-cm³ subsample was packaged for transport to the laboratory. All soil was packaged in Pasquotank County, transported to the laboratory, mixed, and separated into 500-cm³ aliquants. All subsamples were assayed for nematodes by a combination of elutriation (6) and centrifugation (4). Eggs of *H. glycines* were freed from cysts with a glass tissue grinder (4).

Mean, percent deviation, and the coefficient of variation (CV) were calculated for each field from the eight replicated samples for each number of cores within each field. Then, data from the composite samples were paired in all possible combinations to determine sampling precision on the basis of change in the standard deviation compared with a single composite sample. The CV were analyzed across fields, nematode species, and numbers of cores using analysis of variance. Means were separated at $P = 0.05$ using Duncan's multiple-range test. Mean, range, and deviation were computed for the eight replications of each number of cores for each nematode species within fields. Deviations were computed as percentage of samples within a certain percentage of the mean of each core-number group.

A second study was conducted in 1988 at the R. J. Reynolds (Avoca) farm in Bertie County, North Carolina. The farm had approximately 500 ha cropped with both annual (peanut and wheat) and perennial crops (clery sage and basil). Fields were separated by natural barriers or crop history. Individual fields were divided into 1–3-ha (usually 2) parcels for sampling purposes. Wheat fields were sampled in June and July, whereas peanut fields were sampled in late September to November. Sage and basil fields were sampled June through November or within 3 weeks after harvest of these perennial crops. Samples were collected in a "W" pattern across parcels within fields with 20–25 soil cores (2.5 cm d × 20 cm deep) per composite sample. Soil samples were transported to the laboratory where a 500-cm³ subsample was processed

TABLE 1. Mean, range, coefficient of variation (CV), standard deviation of the mean (SD), and percentage of samples within 10–100% of the mean for *Meloidogyne* spp. following tobacco harvest in two fields near Falcon, North Carolina, 1984.

Cores/ sample	Mean	Range	CV	SD	Samples within given percent of mean (%)				
					10	25	50	75	100
Field 2									
10	1,033	370–2,180	65	668	13	38	50	88	88
20	735	200–1,300	47	343	13	50	75	80	100
40	870	320–1,300	43	371	13	13	75	100	100
80	893	530–1,240	30	271	13	50	100	100	100
Field 4									
10	817	240–1,280	40	324	25	75	75	100	100
20	704	370–1,310	50	354	25	38	75	88	100
40	748	250–1,060	40	300	25	25	88	100	100
80	796	110–1,330	49	394	0	25	75	88	100

by elutriation and centrifugation (1). Juveniles were extracted by Seinhorst mist early in the season, but clorox egg extraction (1) was used later in the season. Only data for *Meloidogyne* spp. (*M. hapla*, *M. arenaria*, and *M. incognita*) were included for this report. The mean and CV were calculated for each field. Means and CV were calculated from four and (or) two parcels selected at random from each field. The percent deviation from field mean was calculated for each subunit combination per field.

RESULTS AND DISCUSSION

Variation in numbers of nematodes from the mean of eight repeated samplings was usually less than 100% regardless of the number of cores per sample (Tables 1–5). Many samples for *Meloidogyne* spp., *H. glycines*, *H. dihystrera*, and *S. brachyurum* were within 50% of the overall sample means, and 66% of the samples for these nematodes were within 25% of the overall means (Table 1). Eggs and cysts of *H. glycines* were detected in every replicate, but this was not true for second-stage juveniles (J2).

The CV for various sample size means, combining nematode species and fields, decreased as the number of cores increased, dropping rapidly after 20 cores and leveling off at 40 cores per sample (Fig. 1). The CV for means of 10 core samples were significantly greater than those of 40 or 80 cores, with 20 cores resulting in interme-

mediate level CV. Nematode species occurring at mean densities less than 5/500 cm³ soil were excluded from these analyses because of unreliability in detection of nematodes at such low densities.

Overall CV for *Meloidogyne* J2 were 47% in field 2 and 49% in field 4 with ranges of ca. 30–70% (Fig. 2A). Means for *H. glycines* cysts across numbers of cores had a CV of 30% in field 1 and 40% in field 3 (Fig. 2B). The CV for mean number of eggs of *H. glycines* among numbers of cores was 50% in field 1 and 52% in field 3 (Fig. 2C).

Although the overall trend was clear in the combined analysis, CV of means differed among fields and nematode species. The two fields with *M. incognita* as the dominant species had higher CV than the two fields in Cumberland County with *H. glycines* as the dominant species (Fig. 2A–C). Coefficients of variation for *H. dihystrera* also were higher than those for females and cysts of *H. glycines* (Fig. 2B, D). Low numbers of *H. glycines* J2 resulted in variable and high CV (field 3), whereas samples with larger numbers (field 1) followed the pattern of other nematodes (Fig. 2). There were no interactions among fields, nematode species, or numbers of cores per sample in the analyses of CV. The general downward trend of CV with increasing core size was evident in 7 of 11 individual species (Fig. 2).

The range for CV was large among sub-

TABLE 2. Mean, range, coefficient of variation (CV), standard deviation of the mean (SD), and percentage of samples within 10–100% of the mean for cysts and eggs of *Heterodera glycines* following soybeans in fields 1 and 3 near Falcon, North Carolina, 1984, and field 5 near Elizabeth City, North Carolina, 1987.

Field	Cores/ sample	Mean	Range	CV	SD	Samples within given percent of mean (%)				
						10	25	50	75	100
Cysts										
1	10	20	14–38	39	7.9	25	75	88	88	100
	20	16	8–26	36	5.7	13	63	88	100	100
	40	25	10–32	30	7.5	13	50	88	100	100
	80	23	18–34	27	6.3	38	75	100	100	100
3	10	31	10–42	26	8.1	63	63	100	100	100
	20	24	14–38	30	7.1	38	63	88	100	100
	40	28	16–38	31	8.6	25	50	100	100	100
5	80	25	10–54	60	15.0	0	13	63	88	88
	10	22	2–64	91	20.0	0	13	50	75	88
	20	34	16–74	44	15.0	38	50	81	94	94
	40	36	4–72	42	15.0	31	47	78	91	100
80	41	14–112	46	19.0	27	44	70	94	95	
Eggs										
3	10	1,243	520–2,520	65	814.0	25	25	50	75	88
	20	1,208	640–2,200	47	570.0	0	50	75	88	100
	40	1,153	600–2,280	48	553.0	25	50	63	88	100
	80	998	440–1,780	42	421.0	25	50	75	88	100
5	10	325	40–760	88	285.0	25	38	38	63	75
	20	388	40–740	100	388.0	20	53	60	87	100
	40	701	220–1,980	47	330.0	32	45	84	97	97
	80	607	120–1,300	48	292.0	13	34	59	91	97

samples within a number of cores. The inherent clustering of nematodes may account for much of the sample-to-sample variation for each field. A sample of 40 cores had sufficient soil to process four sub-

samples. This 40-core quantity of soil apparently allowed for better mixing than 80 cores, as often reflected by lower CV for the former (Table 3, Fig. 1).

Means were similar at all levels of num-

TABLE 3. Means and coefficients of variation (CV) among subsamples within numbers of cores from two fields near Elizabeth City, North Carolina, 1987.

Cores (no.)	Sub-samples	<i>Tylenchorhynchus claytoni</i>		<i>Helicotylenchus dihystera</i>		<i>Hoplolaimus galeatus</i>		<i>Heterodera glycines</i>			
		Mean	CV	Mean	CV	Mean	CV	Cyst		Egg	
10	—	828	61	978	59	205	64	22	93	325	80
20	1	950	59	955	66	435	36	32	38	349	66
	2	731	46	716	59	215	58	37	46	423	47
40	1	806	26	1,214	69	249	36	26	47	588	57
	2	725	34	998	30	306	51	32	31	630	29
	3	836	43	961	33	288	25	45	39	649	21
80	4	794	32	959	36	324	46	41	26	933	57
	1	594	62	1,200	49	288	32	36	55	600	29
	2	568	66	955	51	190	28	32	22	610	28
	3	745	33	893	35	260	40	60	54	415	32
	4	820	35	1,093	42	300	48	31	40	773	51
	5	618	59	1,113	12	250	52	45	26	523	60
	6	785	39	1,005	42	265	47	40	43	693	42
	7	833	75	1,250	31	288	54	39	40	675	56
8	630	58	834	32	278	36	42	60	567	68	

TABLE 4. Mean, range, coefficient of variation (CV), standard deviation of the mean (SD) and percentage of the samples within 10–100% of the single mean for *Helicotylenchus dihystera* (Hd), *Scutellonema brachyurum* (Sb), and *Hoplolaimus galeatus* (Hg).

Field, species	Cores/sample	Mean	Range	CV	SD	Samples within given percent of mean (%)				
						10	25	50	75	100
2, Hd	10	251	50–600	70	176	0	13	38	75	88
	20	263	100–510	56	148	38	88	100	100	100
	40	256	120–560	56	143	25	50	88	100	100
	80	223	120–360	40	89	38	63	100	100	100
3, Sb	10	304	20–620	70	213	0	13	50	63	88
	20	318	210–410	22	69	0	25	63	88	88
	40	403	170–600	38	152	13	38	63	75	88
	80	379	250–560	26	100	38	38	88	100	100
5, Hd	10	978	400–2,000	56	547	0	38	63	88	88
	20	836	280–1,920	66	548	13	31	50	81	88
	40	1,033	490–3,060	48	462	22	47	94	94	97
	80	1,043	130–2,220	39	404	28	50	80	97	98
5, Hg	10	205	20–420	69	141	13	25	50	75	88
	20	325	40–900	71	231	6	31	50	75	88
	40	292	100–560	45	130	19	38	66	94	100
	80	265	40–640	45	119	25	52	81	88	98

bers of cores per sample for *H. dihystera* (Table 4). Ranges also were similar for 10, 20, and 40 cores per sample for this nematode (Table 4). The estimated mean number of *S. brachyurum* was 16–25% less for the 10 and 20 cores than for the 40 and 80 cores per sample. Ranges were variable among core numbers per sample for *S. brachyurum*.

The survey type of sampling used at the R. J. Reynolds farm revealed useful infor-

mation on related precision. The CV for individual fields of different sizes ranged from 33 to 242 for *Meloidogyne* spp. (mostly *M. incognita*) (Table 6). Means from two samples selected at random fell within 70% of the field mean provided there were at least six parcels from which to select. Similarly, all random selections of four samples were within 70% of the field mean with one exception (most of the assays gave numbers within 50% of the field mean).

TABLE 5. Analysis of variance of numbers of nematodes for subsamples per core size from a field near Elizabeth City, North Carolina.

Nematode species	Number		P value		CV
	Cores	Subsamples	Replication	Subsample	
<i>Tylenchorhynchus claytoni</i>	20	2	0.02*	0.12	29
	40	4	0.16	0.84	32
	80	8	0.31	0.75	56
<i>Helicotylenchus dihystera</i>	20	2	0.22	0.34	56
	40	4	0.02*	0.50	37
	80	8	0.26	0.38	38
<i>Hoplolaimus galeatus</i>	20	2	0.20	0.04*	53
	40	4	0.16	0.66	42
	80	8	0.05*	0.65	43
<i>Heterodera glycines</i> (cysts)	20	2	0.75	0.57	50
	40	4	0.26	0.04*	37
	80	8	0.46	0.09	46
<i>H. glycines</i> (eggs)	20	2	0.78	0.50	59
	40	4	0.09	0.10	40
	80	8	0.71	0.37	49

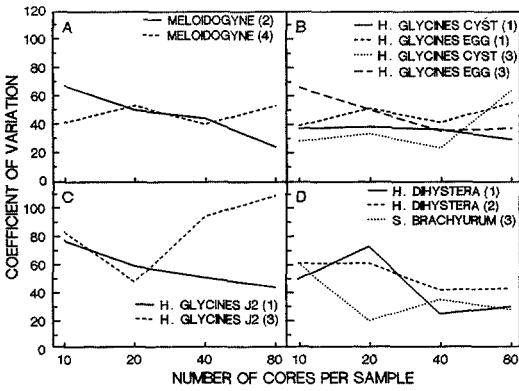


FIG. 1. Coefficients of variation versus number of cores per sample for four nematode species from Cumberland County, North Carolina. A) *Meloidogyne* spp. from fields 2 and 4. B) *Heterodera glycines* cysts and eggs from fields 1 and 3. C) *Heterodera glycines* second-stage juveniles (J2) from fields 1 and 3. D) *Helicotylenchus dihyстера* from fields 1 and 2 and *Scutellonema brachyurum* from field 3.

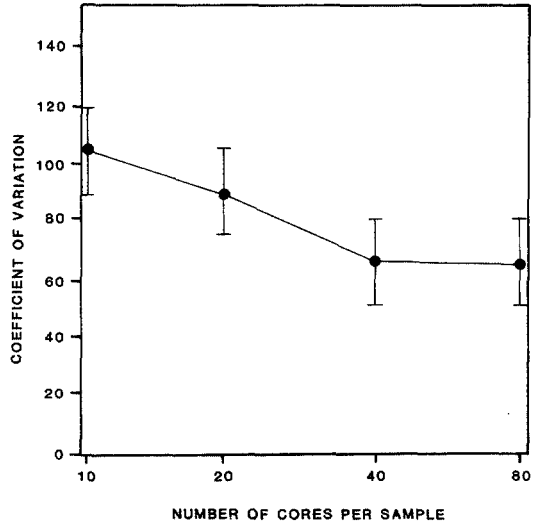


FIG. 2. Mean coefficients of variation versus number of cores per sample. Data combine nematode species and fields, excluding species occurring with mean densities less than 5/500 cm³ soil. Brackets indicate critical ranges on Duncan's multiple-range test, n = 95, P = 0.05.

The exception for the < 70% deviation (peanut field 21) is notable because it was discovered that this field had a varied cropping sequence which was not accounted for when sampling. The level of precision is surprising in view of the fact that 11 people were involved in sampling at different occasions.

A total of 169 man hours were involved in taking 313 samples for the R. J. Reynolds site, or approximately 0.54 man hours per sample, excluding travel time. Considerable savings in man hours could be achieved by limiting the number of samples (for randomly selected parcels) to 2-4 per large field. The gain in efficiency should compensate for the loss in accuracy, provided crop history and soil type are taken into consideration.

The probability of obtaining estimates approximating the true mean for a field can be increased by sampling twice instead of once. Using duplicate samples has been a standard practice in a few nematology laboratories (16). Egg numbers of *H. glycines* from the 40-core samples from field 1 illustrate this effect. Mean of the eight replicate samples was 2,280 eggs per 500 cm³ soil and range was 1,400-3,840. Individual samples were all within 75% of the mean and 50% were within 25% of the

mean. There are 28 possible ways of pairing the eight replicate samples. Averaging the paired samples reduces the range to 1,440-3,550, but more important, 25 of the 28 combinations have means within 25% of the overall mean and 16 combinations are within 16% of the overall mean. Similar results were obtained with other nematode species. Paired samplings would be better than processing two subsamples. In some instances, mixing soil before subdividing resulted in a reasonably uniform mixture (e.g., 680-880 eggs of *H. glycines* per 500 cm³ soil) for four subsamples from 40 cores. The average for eight replications was 701 eggs. The subsamples in another replicate ranged from 900 to 1,200. In neither case was subsampling a good representation of the mean.

Sampling fields for nematode advisory purposes usually is directed at unknown situations. Economic and time constraints allow the use of only one sampling strategy for each field. A systematic zig-zag pattern (4,13) was selected for our research. A composite of 40 cores per ha was found to be the optimal sample size. This number of cores per sample gave a CV of 62%, the

TABLE 6. *Meloidogyne* spp. means and coefficients of variation (CV) among subunit nematode assays of fields at the R. J. Reynolds farm near Merry Hill, North Carolina, 1988.

Field	Parcels (no.)	Field mean	Field CV	Mean of two samples	CV of two samples	Deviation from field mean (%)	Mean of four samples	CV of four samples	Deviation from field mean (%)
Peanut									
23	4	582	84	761	93	31			
57	21	7,350	198	306	47	96	26,053	103	254
Wheat									
76	6	101	66	31	5	69	116	58	15
80	13	528	75	850	45	57	806	53	53
109	14	53	97	176	141	232	26	134	51
Basil									
11	8	33,029	106	48,100	125	46	19,972	98	40
Sage									
35	3	22,447	38	25,264	39	13			
104	3	5,658	119	1,911	136	66			
105	3	30,728	29	25,744	11	16			
36	4	978	41	1,085	22	11			
106	4	4,386	57	4,632	59	5			
103	9	5,161	83	8,624	89	67	8,202	56	59
31	10	18,822	57	27,450	11	46	12,589	74	33
37	10	9,195	57	16,028	45	74	9,487	86	3
84	10	22,109	60	23,212	111	5	13,644	68	38
74	11	4,533	96	3,718	46	18	7,679	80	69
29	12	19,063	39	22,925	12	20	20,779	26	9
54	12	19,504	62	20,584	116	6	13,473	79	31
85	12	541	76	625	40	16	727	47	34
15	13	193	127	365	75	89	135	77	30
16	14	482	33	639	141	33	469	187	3
73	19	412	242	24	130	94	94	49	68
90	24	6,190	200	2,053	15	66	1,995	28	68

Subunit parcels of ca. 2-ha each; nematode data include eggs, where present, and second-stage juveniles.

same as for 80 cores, and would be acceptable for most advisory purposes. Where more critical evaluations are needed for predicting crop loss or potential damage, the field could be sampled twice and the numbers averaged to obtain an estimate with a higher probability of representing the true mean. Oostenbrink (14) indicated that it is advisable to take sample pairs for diagnostic work but that a bulk sample was adequate for quantitative estimations for research and advisory work.

Nematode population estimates obtained by repeatedly sampling a field did not vary as much as expected from previous computer simulated sampling using a data base of single cores. A maximum relative efficiency value for a 7-ha field was computed to be obtained with six samples of 68 cores, each requiring 5 or fewer hours

of sampling (10). Collection of 22 cores from 0.25–0.45 ha has been suggested as optimal (12). In reality, collecting a sample of 40 cores requires little more effort than collecting 20 cores, since the entire field needs to be covered in the systematic zig-zag pattern with several diagonals. For nematodes that occur in moderate to large numbers, a single 40-core sample appears adequate. However, with low, preplant infestations of highly pathogenic nematodes (e.g., *Belonolaimus longicaudatus* Rau, *M. arenaria*, *H. glycines*), which also tend to be highly clustered, the reliability of one sample may not be adequate, and two separately collected samples of 40 cores each would then be recommended.

Ideally, a sampling scheme should be tailored to each field and nematode species. However, such an approach would not be

acceptable to growers and would probably result in even fewer than the present level of samples being collected. Nevertheless, sampling can be tailored to nematode feeding habit, type of crop and soil, and season (4). Understanding of nematode spatial and temporal patterns also can be used to improve precision. As previously recommended (14), the use of separately collected duplicate samples is one means of greatly enhancing nematode sampling precision. For *H. glycines*, cyst and (or) eggs must be used for assessment (17).

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