A SIMPLE INCUBATION METHOD FOR THE EXTRACTION OF NEMATODES FROM SOIL

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ABSTRACT

Rodríguez-Kábana, R., and M.H. Pope. 1981. A simple method for the extraction of nematode from soil. Nematropica 11: 175-186.

A simple method for the extraction of nematods from soil was developed using commonly available materials. With the method 100 cm^3 soil were spread on 2-ply "Scottie" tissue paper on a sieve consisting of fiberglass screen glued between two PVC pipe sections. The sieve with the soil was placed in a plastic bowl and water was added to just cover the soil. The sieve with soil was incubated for 72 h when nematodes in the water were collected by passing the water through a 38 μm screen. Comparisons between this method and a flotation sieving technique indicated that numbers of nematodes extracted by the two techniques were significantly correlated. The bowl method resulted in extraction of higher numbers of *Pratylenchus* Filipjev larvae of *Heterodera glycines* Ichinohe and *Meloidogyne* Goeldi but less numbers of *Helicotylenchus* Steiner and *Hoplolaimus* Daday than the flotation technique.

Additional key words: methodology, diagnostic services, root-knot nematodes, soybean cyst nematodes, spiral nematodes, lance nematodes, lesion nematodes.

RESUMEN

Rodríguez-Kábana, R., y M.H. Pope. 1981. Un método sencillo para la extraccion de nematodos del suelo. Nematropica 11: 175-186.

El trabajo describe un método sencillo para la extracción de nematodos del suelo basado en el uso de materiales comunes. Con el método, se dispersaron 100 cm^3 de suelo sobre papel "Scottie" retenido por un tamiz hecho con una malla de fibra de vidrio pegada entre dos secciones de tuberia PVC. El tamiz con el suelo fué colocado en un recipiente plástico y se añadió agua en cantidad suficiente para cubrir el suelo. Después de 72 h de incubación los nematodos en el agua se recolectaron con un tamiz de 38 um. En unas comparaciones entre el nuevo método y uno de flotación-tamizado los números de nematodos extraidos por ambos métodos estuvieron altamente corre-

lacionados. Sin embargo, el nuevo método extrajo más *Pratylenchus* Filipjev y larvas de *Heterodera glycines* Ichinohe y de *Meloidogyne* Goeldi pero menos numeros de *Helicotylenchus* Steiner y *Hoplolaimus* Daday.
Palabras claves adicionales: metodología, servicios de diagnosis, nematodos noduladores, nematodos enquistadores, nematodos espiraliformes y lesionadores.

INTRODUCTION

The accurate determination of nematode numbers in soil is a prerequisite for development of control recommendations for farmers. In the past a number of methods have been developed for extraction of nematodes from soil (1,9). The methods rely either on direct extraction of nematodes from soil (2,3,4,5,7,8) or on incubation of the soil in water to permit movement of the nematodes out of the soil into the water (6,11). Although most of the methods are accurate they require specialized equipment such as semiautomatic elutriators (2) or centrifuges (5) which may not be available to nematologists working with limited resources. Other techniques although requiring simple equipment are not accurate and may give a limited view of the nematode population in soil samples (2). Another aspect of the determination of nematode numbers in soil is the amount of labor and manpower required. Direct extraction techniques such as sugar flotation or centrifugation methods (3,5,7) are efficient but have labor requirements that do not permit their routine use in laboratories processing large numbers of samples daily. This paper describes a method based on the use of inexpensive equipment that can be made from materials available to most nematologists. In addition, it presents results on conditions for the use of the method and data of comparisons from determinations on a variety of soils performed with the new method and with the molasses flotation-sieving technique (7).

MATERIALS AND METHODS

Soils for the study were collected from three locations in Alabama. One soil, a silty clay loam, was from the Tallassee substation at Tallassee, from a cotton field under a cotton-soybean-corn rotation; the soil was infested with *Hoplolaimus galeatus* (Cobb) Sher, *Paratrichodorus christiei* (Allen) Sidiqui, and *Meloidogyne incognita* (Kofoid and White) Chitwood. Another soil was a loamy sand from the Agronomy farm at Auburn University campus, with the same crop rotation as the Tallassee soil, which was with corn at the time of sampling and was infested with *Helicotylenchus dihystera* (Cobb) Sher.

The third soil was a silty clay loam from a field near Headland which had been under continous peanut culture for eight years; the soil was infested with *M. arenaria* (Neal) Chitwood.

In all three fields soils were collected at harvest time with a 2.54-cm-diameter probe from the root zone of the growing crop plants to a depth of 16-20 cm.

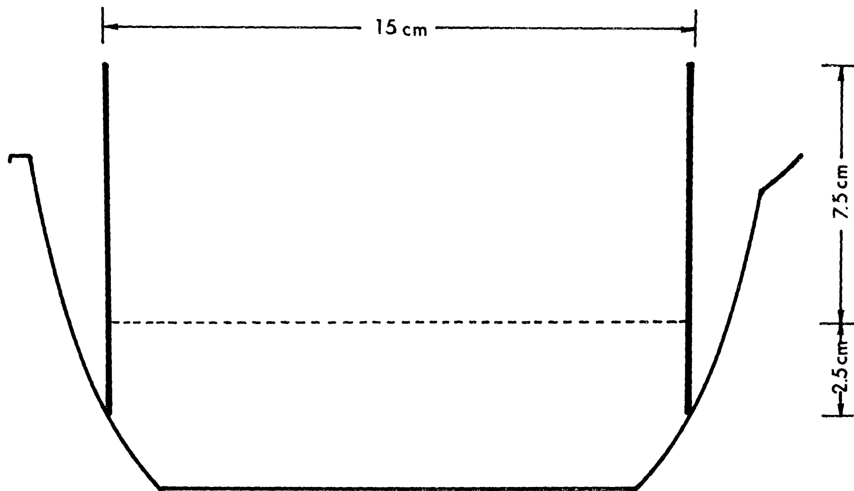


Fig. 1. Diagram of the "salad bowl" and PVC sieve used to extract nematodes from soil by incubation in water.

Description of the Method.

In the standard technique 100 cm^3 of soil were spread on a two-ply "Scottie" tissue paper on a sieve consisting of a 1-mm-mesh fiberglass screen (common window screen) glued between two 15 cm (6 inch) diameter PVC pipe sections; the upper section was 7.62 cm (3 inch) long and the lower section 2.54 cm (1 inch) long (Fig. 1). The sieve with the soil was placed in a 2.3 l capacity plastic mixing bowl and 1.3 l water was added slowly between the sieve and bowl walls so as to just cover the soil. The sieve with soil was incubated at room temperature (25-27C) for 72 hr when the sieve was removed and the bowl contents were passed through a 38 μm mesh 7.6 cm (3 inch) diameter stainless steel sieve. Nematodes retained in the sieve were transferred to a counting dish and their numbers determined by direct counting with a dissecting microscope.

Effect of incubation time and sample size.

The effect of the length of the incubation period and sample size on recovery of nematodes was examined by incubating 50, 100, 200, or 400 cm^3 of soil for 24, 48, 72, or 96 hr. Soil samples were processed as described for the standard assay except that 1.5 l of water was used to be able to cover with water the largest sample size. Each sample size-incubation time combination was represented by eight replicate determinations.

Comparisons.

Nematode recovery from soil with the standard bowl method was compared with that of the molasses flotation technique (7). A total of 95 soil samples collected as described above from farms throughout the state were assayed for nematodes following both methods. The samples represented soils with different textures and crop histories and were similar to those received for evaluation of nematode numbers by the extension advisory personnel at Auburn University.

Statistical analyses.

All data were analyzed according to standard procedures for analysis of variance; regression equations and simple correlation coefficients were calculated also following standard procedures (10). Unless otherwise stated all differences mentioned in the text were significant to the 5% or lower level of probability.

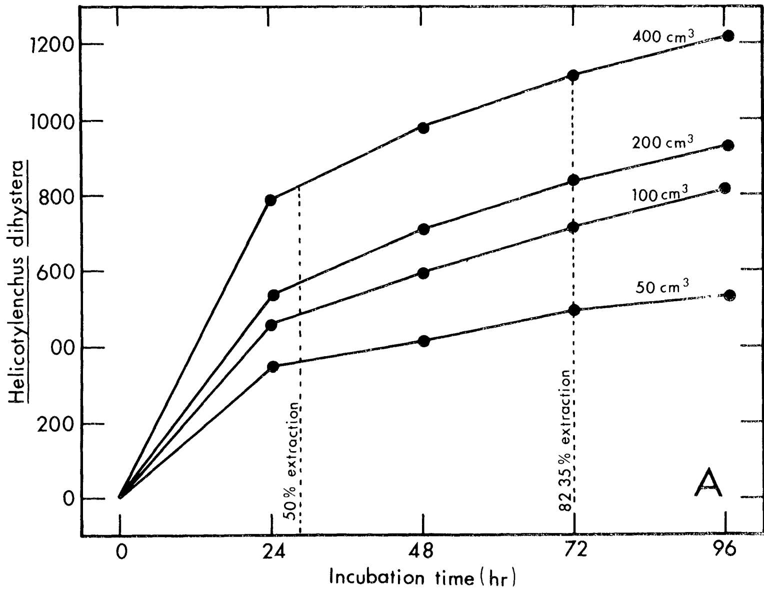
RESULTS

Effect of incubation time and sample size.

Data obtained for *H. dihystera* in the Agronomy farm soil are presented in Fig. 2A. Results showed that for all sample sizes numbers of *H. dihystera* increased with time and the greatest rate of extraction occurred during the first 24 h; thereafter, the rate of increase was almost constant. Differences between the average number of nematodes recovered for each sample size were significant for all incubation times; however, the number of nematodes recovered was not directly proportional to the volume of soil used (Fig. 2B) Proportionally more nematodes were recovered from the 50 cm^3 sample than from any of the other sample sizes. Similar results were obtained for free living and dorylaimoid nematodes which were counted on these samples (data not shown).

Results for *M. arenaria* in samples from peanut soil are presented in Fig. 2C. The data indicate that as for *H. dihystera* the maximum rate of recovery occurred during the first 24 h for all samples sizes. The relation between sample size and number of nematodes extracted was also not linear; the smallest sample resulted in proportionally more larvae extracted than larger samples.

The rate of extraction for *H. galeatus* varied little during the first 72 hr (Fig. 2D) and for all samples but the two smallest the rate of extraction declined significantly between the 72 and the 96 h incubation periods. A marked reduction in the number of *H. galeatus* recovered in relation to sample size was observed (Fig. 2E); significantly less numbers of *H. galeatus* were recovered after 24 h from the 200 and 400 cm^3 samples than were recovered from the two smallest sample sizes. Extraction patterns for *M. incognita* and *P. christiei* in this soil followed that described for *M. arenaria* in the peanut soil.



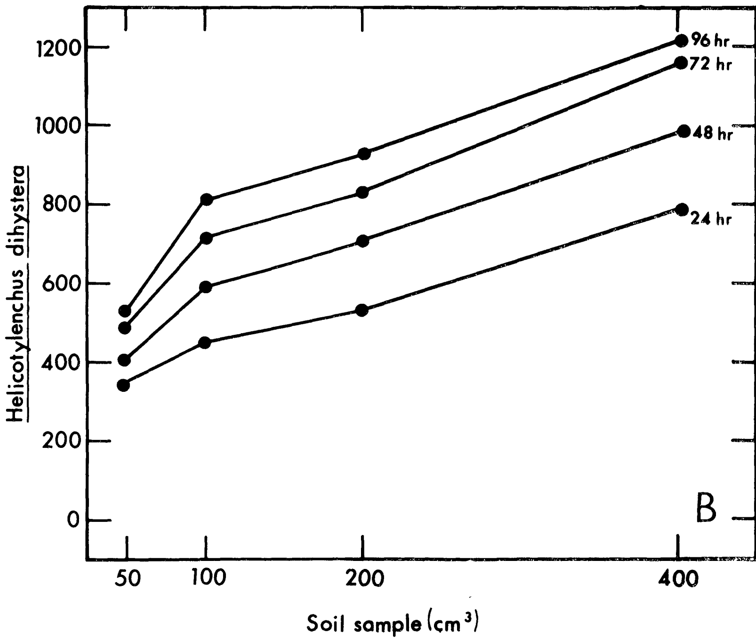
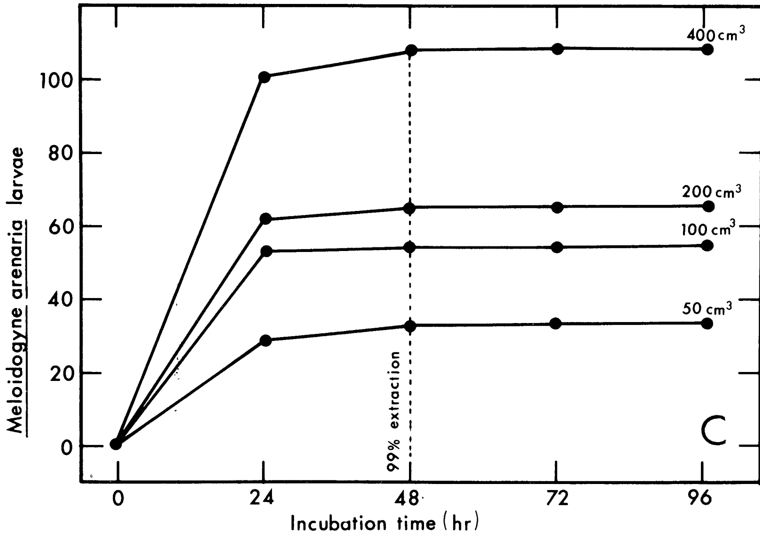
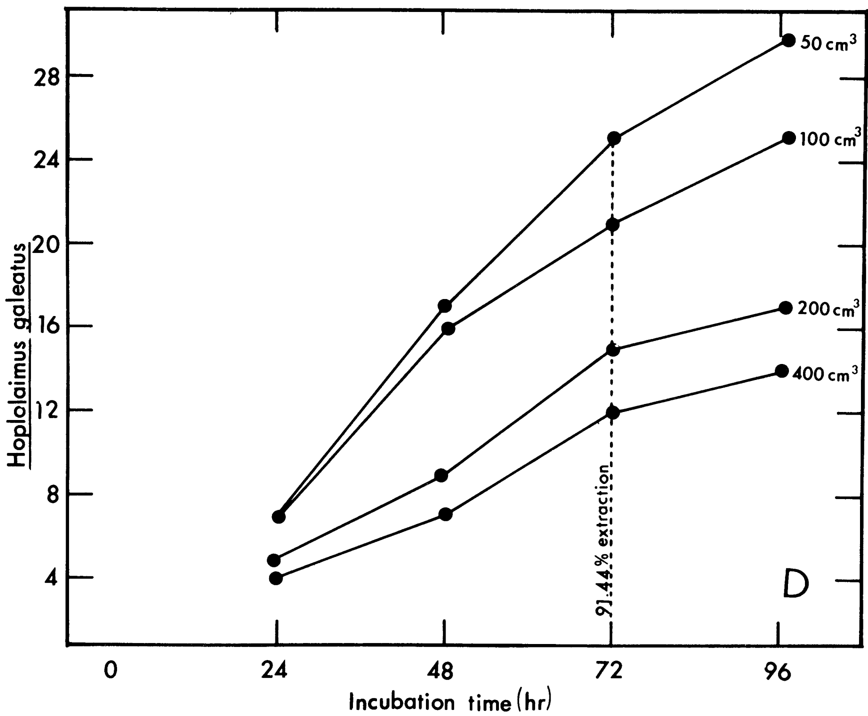


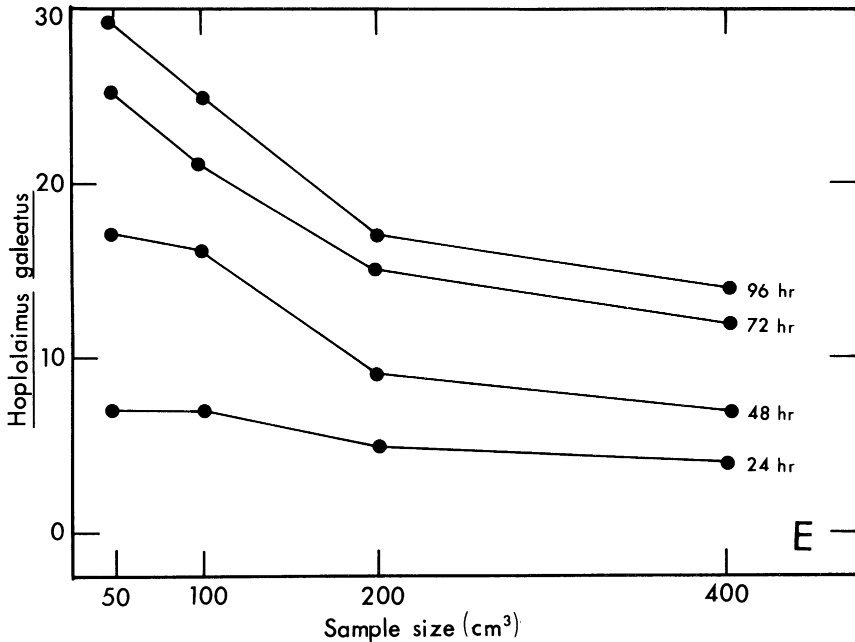
Fig. 2. Relation between incubation time, volume of soil, and the number of nematodes obtained from soil using the "salad bowl" method; A,B-*Heliocotylenchus dihystera;* C-*Melodogyne arenaria;* D,E-*Hoplolaimus galeatus.*



Meloidogyne arenaria larvae
100
80
60
40
20
0
400 cm³
200 cm³
100 cm³
50 cm³
99% extraction
C
0
24
48
72
96
Incubation time (hr)



Hoplolaimus galeatus
28
24
20
16
12
8
4
50 cm³
100 cm³
200 cm³
400 cm³
91.44% extraction
D
0
24
48
72
96
Incubation time (hr)



Comparisons.

Numbers of *Helicotylenchus* spp., *H. galeatus, Pratylenchus* spp., larvae of *Meloidogyne* spp., dorylaimoid and free living nematodes obtained with the bowl method were significantly correlated with corresponding numbers extracted with the molasses technique (Table 1); however, numbers of *Tylenchorhynchus* spp. extracted with both methods were not correlated. Slope values of the linear regression equations calculated with data for the bowl method as the independent variable indicated that the bowl method extracted more *Pratylenchus* spp. and larvae of *Meloidogyne* spp. than the flotation method but that it resulted in less numbers of *H. galeatus, Helicotylenchus* spp. and free living nematodes. In addition larvae of *Heterodera glycines* Ichinohe in soil samples were detected only with the bowl method (data not shown).

DISCUSSION

Results obtained with the bowl method indicated that the rate with which nematodes (n) left the soil, dn/dt, followed a first order process described by:

$$dn/dt = K(A - n). \qquad \text{I}$$

where K is a constant and A the maximum number of nematodes that could be obtained from each sample with unlimited time of incubation. The value of

Table 1. Comparison of the bowl method and the molasses flotation sieving technique for extraction of nematodes from soil samples collected from Alabama farms.

Genus	No. of Samples	Range		Average		Correlation Coefficients	Slope Values[x]	Y-intercepts[x]
		Bowl	Flotation	Bowl	Flotation			
Helicotylenchus	43	1-32	1-83	4.58 ± 5.86	15.16 ± 20.21	0.58**[y]	1.99	6.04
Hoplolaimus	28	2-71	5-119	15.86 ± 13.76	30.61 ± 25.25	0.54**	0.99	14.88
Meloidogyne	57	1-92	1-62	16.09 ± 23.44	9.12 ± 11.27	0.58**	0.28	4.65
Pratylenchus	49	1-25	1-6	6.06 ± 4.77	1.57 ± 1.18	0.41**	0.11	0.96
"Dorylaimoids"	93	1-23	1-35	4.25 ± 4.46	5.54 ± 5.32	0.33**	0.40	3.85
"Free Living"	95	14-442	i-486	139.87 ± 88.23	121.00 ± 110.44	0.68**	0.86	1.10

[x] Slope and Y-intercept values are from linear regression equations calculated considering numbers obtained with the flotation technique as the dependent variable (Y).

[y] ** Significant at $P = 0.01$.

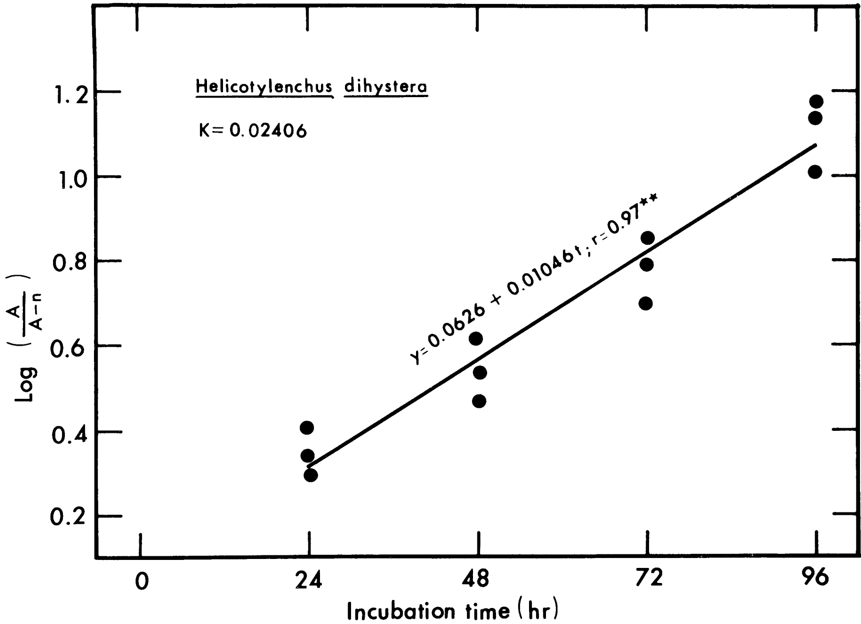


Fig. 3. Relation between incubation time and the ratio of nematodes *(H. dihystera)* recoverable with unlimited time of incubation (A) and the difference between A and the number of nematodes obtained at various periods of incubation (n).

the constant K for each nematode species can be calculated since equation I can be expressed as:

$$dn = Kdt(A - n) \qquad II$$

and

$$K \int dt = \int dn/(A - n) \qquad III$$

which results in:

$$Kt = 2.3 \log (A/A - n) \qquad IV$$

or

$$t = \frac{2.3}{K} \log A/(A - n) \qquad V$$

A plot with log A/(A -n) on the Y-axis and t (incubation time) on the abcissa results in a straight line with slope equal to K/2.3. The value of A for each sample size can be estimated graphically by determining the value of the horizontal asymptote for each of the curves representing nematode numbers and time of incubation in Fig. 2. Thus, for *H. dihystera* these values were: 550, 900, 1000, and 1300 for the 50, 100, 200 and 400 cm^3 samples, respectively. The resulting straight line for *H. dihystera* (Fig. 3) indicated that the K value for the species was 0.0241. Values for K may be expected to be directly related to the relative velocity with which a given nematode species moves out of the

Table 2. Relative motility constant (K value) and percent extraction of nematodes after 72 h incubation with the "bowl method".

Species	K Value	Percent Extraction
Helicotylenchus dihystera	0.0241	82.35
Hoplolaimus galeatus	0.0341	91.44
Meloidogyne arenaria	0.0602	99.87
Meloidogyne incognita	0.0882	99.99
Paratrichodorus christiei	0.0723	89.60
Pratylenchus brachyurus	0.0422	95.24
Tylenchorhynchus claytoni	0.0345	91.71
"Dorylaimoid" forms	0.0410	94.79
"Free Living" forms	0.0391	94.04

soil. Equation V also permits determination of the percent recovery of nematodes with time relative to the value of A. Thus, if $n = 0.5A$ equation V becomes $2.3/K \log 2$ and since $K = 0.0241$ for *H. dihystera*, then $t = 28.73$ h, the amount of time required to recover 50% of the extractable nematodes in the sample. Similar calculations indicated that 82.35% of the extractable *H. dihystera* would be recovered from the samples in the 72 h incubation period chosen for the standard assay.

Calculations for *M. arenaria* indicated that 99% of the larvae were extracted after 48 h incubation. Accordingly the K value for this species was 0.0602, a higher value than that obtained for *H. dihystera.* This is probably a reflection of the greater mobility of the larvae relative to that of *H. dihystera.*

Results of calculations for the other species in the study are presented in Table 2 together with the percent extraction expected for each species after 72 h incubation of the soil. K values ranged from 0.0241 for *H. dihystera* to 0.08824 for *M. incognita.* Since extraction for all species but one (*H. dihystera*) after 72 h incubation was of 90% or better we chose this incubation period for the standard assay.

Our results corroborate earlier findings with other methods (7) on the effect of sample size on number of nematodes extracted. Generally sample sizes of 50 or 100 cm^3 soil resulted in higher number of nematodes extracted per cm^3 soil. Apparently an increase in sample size beyond this range interferes with nematode movement and hence extraction with the bowl method. There is probably an optimal ratio between sample volume and sieve area beyond which efficiency of the method declines sharply. This problem needs further investigation to determine under what conditions larger sample sizes can be utilized without loss in extraction efficiency.

The superiority of the bowl method over the flotation-sieving method for species of *Meloidogyne, Heterodera* and *Pratylenchus* is probably due to the prolonged incubation time used with the first method. This may be expected to result in hatching of eggs and collection of *Pratylenchus* present in rootlets that would not be extracted by the flotation technique.

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