On the Methodology of Nematode Extraction from Field Samples: Baermann Funnel Modifications

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Abstract: Routine quantitative nematode extraction for pest management purposes remains a problem. There is need for more knowledge of the parameters limiting efficiency of the various available methods. Sedimentation rates for several species of nematodes have been confirmed as slow and highly variable and therefore not suitable for quantitative separation of nematodes. Funnel losses with clean and unpitted glassware, whether closed or open stemmed, with or without misting, are negligible so long as misting periods are neither inadequate nor excessive; i.e., approximately a 1.5-min water spray period in a 10-min cycle. Tissue paper used to retain soil, sievings, or other substrate in the funnel extraction can greatly inhibit the passage of nematodes depending upon the tissue properties and the nematode species. *Key words:* sedimentation, tissue paper, extraction efficiency. Journal of Nematology 15(3):438-444. 1983.

The extraction of nematodes from their environment continues to be a vexing problem. Initially the extraction objective was to obtain clean specimens for microscopic observation; however, it soon became desirable to extract nematodes in quantity and free of associated debri for use in experimental studies. The methods devised to accomplish this objective have been summarized in several reports (2,15,17,19). Over the years the number of modifications of basic methods have proliferated prodigiously. Usually the modifications were developed to satisfy the particular needs of individual investigators; motivation was usually due to particular characteristics of a nematode, soil or plant tissue substrate, or expediency requirements of experimentation (1,5,6,10,12,13,14,16,18,20,21,22,23). The methodology has served satisfactorily as long as the desired results were qualitative or relative. In recent years the demand for quantitative evaluations has intensified. Toward this goal there have been efforts comparing extraction methods for selected nematodes in specific substrates (1,3,4,7,9, 11). Others have described processes and apparatus in detail to achieve quantitative results in specific situations (8,18,19).

With increasing pressure to reduce pesticide use and pollution problems, there comes a need for quantitative nematology to determine field population levels of nematodes, population levels at the threshold of plant injury, standards of nematode extraction, including optimum efficiency, and corrective factors to reflect true field values. Quantitative nematology is therefore basic to improved nematode control tactics and integrated pest management practices. Consequently it was important to explore mechanical and biophysical aspects of selected extraction processes. A first step involved the examination of some of the basic parameters utilized in the various applications of modifications of the Baermann funnel.

MATERIALS AND METHODS

Sedimentation rates: Nematode sedimentation properties are intrinsic to many of the extraction procedures. It was of interest to approximate the sedimentation rates of various nematodes in water for possible comparative use of these values. Boiled water was used to prepare the sedimentation column which consisted of a 10.0-mm glass tube surrounded by an intermediate 25.0-mm tube comprising the water jacket in turn surrounded by a 45.0-mm tube forming a static air jacket. The entire assembly was 1 m in length and aligned vertically with a plumb. The sedimentation tube was marked 200 mm from the upper end to indicate the starting point of the measurement zone. The measurement zone consisted of 200 mm of column marked at 50.0-mm intervals. Individual nematodes were placed at the center of the upper end of the sedimentation tube and allowed to fall 200 mm to achieve dynamic equilibrium. The times required for a nematode to fall through each of two measurement zones was measured to the nearest .01 sec and averaged for that nematode. The sedi-

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mentation rates of at least 30 individuals were determined for each species.

To approximate what might be expected in a heavy concentration of nematodes, groups consisting of at least 10 individuals for large nematodes, and substantially more for smaller ones, were added to the sedimentation column. The sedimentation rates of the fastest and the slowest individual in each group were measured as before, with averages indicating the range for each population.

After preparation, the sedimentation column was allowed to come to rest undisturbed for a minimum of 24 hours. The jacketing reduced thermal convections and allowed the water column to approximate a static condition.

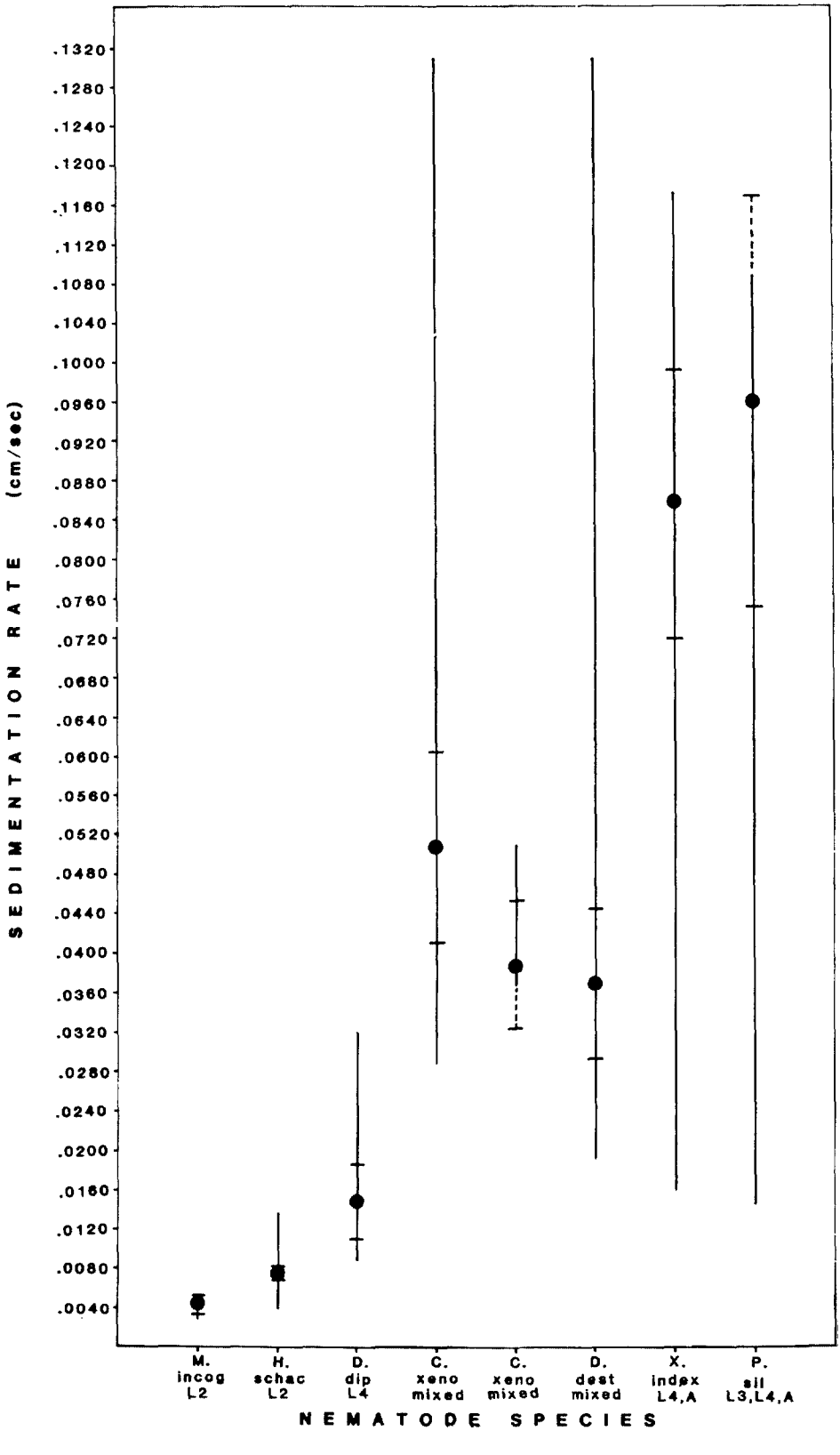
Funnel conformation in misting: The normal funnel conformations are stem closed and stem open. The closed stem configuration involves a short segment of rubber tubing at the end of the stem which is closed off by a pinch clamp or by the insertion of a small culture tube. The open stem configuration involves a short segment of rubber tubing to extend the stem so that the funnel washings are released near the bottom of a culture tube (usually 22 × 200 mm) enveloping the funnel stem. For comparative purposes, an additional modification was employed using an oil centrifugation tube (35 × 200 mm), thereby reducing the upward flow of water by a factor of 2.5. The usual misting apparatus involved an aqueous intermittant spray in a 10-min cycle. Several spray times were used to estimate yields and overflow losses. In the closed funnel stem, screens covered with micro-wipe tissue were placed in a holder maintaining the screen above the overflow water level. In the open stem funnel, a shaped screen, also covered with micro-wipe tissue, nested within the upper portion of the funnel with the flat bottom of the screen approximately 1 cm below the funnel edge. An aliquant of 500 nematodes was added by pipette to the moistened paper tissue, and the funnel racks containing replicates of 12 were placed in the mister. The overflow from the collection tubes or the funnels were collected in conical flasks. After 48 hours the nematodes in the collection tube, in the overflow, and in the funnel washing were counted.

Tissue paper permeability: Quality of tissues amenable to the Baermann (i.e., retaining debri while allowing nematodes to pass through) is known to vary in terms of porosity, texture, and wet strength. It was of interest to evaluate different sources of tissue as to the efficiency in allowing nematodes to pass through the closed stem conventional Baermann funnels with nested screens to support various tissues. The funnels were filled with tap water sufficient to cover the tissue with about 1 mm of water. Aliquants of nematodes were then added by pipet to the surface of water with a minimum of roiling. The funnel racks were covered with plastic sheets to retard evaporation. After 24 hours the funnel contents and funnel washings were collected and counted. The funnels were refilled with water as before and allowed to extract again. The process was repeated after 48 and 72 hours which terminated the trial. Except for parchment paper, tissues were used as two-ply, either as supplied by the manufacturer or folded if supplied as single-ply. The test consisted of 12 replicates for each tissue tested.

RESULTS

The sedimentation rates of different nematodes in a static water column are illustrated in Fig. 1. The median sedimentation rate of small nematodes appears to be less than that of larger ones. The range of sedimentation rates of a concentrated group of nematodes appears to be large; however, if the range is viewed as a percentage of the median, the comparative disparity is much reduced.

The observations obtained with various Baermann funnel configurations and different spray times are illustrated in Table 1. It is evident that the fraction of the inoculum found in the collection tubes is essentially the same as the total collected so that there is negligible loss due to overflow or adherence to the funnel. With spray periods of 10–15% of the cycle time (10 min), the recovery of inoculum in the collection tube was high; with 50% spray time, recovery was much reduced. It appears that the general recovery in relation to the inoculum was poor.


SEDIMENTATION RATE (cm/sec)
.1320
.1280
.1240
.1200
.1160
.1120
.1080
.1040
.1000
.0960
.0920
.0880
.0840
.0800
.0760
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.0480
.0440
.0400
.0360
.0320
.0280
.0240
.0200
.0160
.0120
.0080
.0040
M. incog L2
H. schac L2
D. dip L4
C. xeno mixed
C. xeno mixed
D. dest mixed
X. index L4,A
P. all L3,L4,A
NEMATODE SPECIES

Table 1. Nematodes recovered from different collector tube configurations with different spray periods. Total collected includes nematodes in the collection table, adhering to funnel, and in the overflow.

System	% Spray time*	Nematode	Collection tube	Total collected	Overflow (liters)
Sealed collection tube, elevated screen, overflow from funnel lip	10	*P. vulnus*†	158	187	0.1–1.9
Oil centrifuge tubes	10	*P. vulnus*	188	195	0.5–2.1
Reg. test tubes	10	*P. vulnus*	177	178	0.2–1.8
LSD ($P = .01$)				19	
Sealed collection tube	15	*M. incognita*‡	229	237	0.9–1.8
Oil centrifuge tube	15	*M. incognita*	191	198	0–1.2
Reg. test tubes	15	*M. incognita*	204	218	0.6–1.2
LSD ($P = .01$)				11	
Reg. test tubes	50	*M. javanica*§	130 ± 12		not collected

*10-min. cycle.
†*Pratylenchus vulnus* inoculum 300 ± 15.
‡*Meloidogyne incognita* inoculum 500 ± 30.
§*Meloidogyne javanica* inoculum 500 ± 28.

The property of different tissues to allow various nematodes to pass through them are illustrated in Table 2. Parchment paper is impermeable; however, all other tissues tested appeared to vary in their permeability, not only to one nematode but between nematodes. Under the conditions of these experiments, the bulk of the nematodes passing through the paper did so within 24 hours.

DISCUSSION

It appeared that as the heterogeneity of a nematode population increased, so did the variability of the sedimentation rate of individuals and the range for a concentrated population. The results of the two trials with *Criconemella xenoplax* illustrate the different sedimentation properties of two populations differing in age of culture and host plant. The cogeneric *Ditylenchus dipsaci* and *D. destructor* appeared to vary substantially in sedimentary properties. Since the sedimentation rate of an individual nematode measured over two segments of the track could vary, factors other than nematode density affected sedimentation; e.g., differential drag as the wriggling nematode presented different geometrical configurations to the waterfront and undetectable thermal gradients. These observations suggest that elutriation which utilizes high rates of turbulent flow may have some merit in separating light organic particles from dense mineral ones, but the method's usefulness in quantitative nematode separation is open to question.

Funnel and collector configurations appeared to have little effect on collection efficiency, provided the spray time fell within certain limits and the interior funnel walls were clean and pit free. Generally, misters utilize spray nozzles providing a cone shaped distribution of water droplets. The volume of water delivered per unit area through a plane normal to the axis of the cone decreases with radial distance. Funnels in racks placed in such a cone do not all receive the same volume of water. The variations in volume of overflow indicated in Table 1 for a 48-hour period indicate that spray in 10-min cycles of less than 10% are likely to be insufficient to wash nematodes into the collection tube in some funnel

Fig. 1. Sedimentation rates of nematodes in a static water column. Filled circles indicate the mean of individual release measurements and the cross bars SD about the mean. The long bars through the means indicate the range of sedimenting rates of nematodes released as groups. Nematodes tested include *Meloidogyne incognita* (M. incog.), *Heterodera schachtii* (H. schach.), *Ditylenchus dipsaci* (D. dip.), *Crinconemella xenoplax* (C. xeno.). *Ditylenchus destructor* (D. dest.), *Xiphinema index*, (X. index), *Panagrellus silusiae* (P. sil).

Table 2. Permeability of paper tissues to *Meloidogyne incognita** and *Ditylenchus dipsaci*† in a normal Baermann funnel extraction apparatus. (TT = toilet tissue.)

Tissue	Nematode recovery							
	1st 24 hours		2nd 24 hours		3rd 24 hours		Total	
	M. incognita	*D. dipsaci*	*M. incognita*	*D. dipsaci*	*M. incognita*	*D. dipsaci*	*M. incognita*	*D. dipsaci*
Scott (TT)	42 ± 24		27.6 ± 20	0.7 ± 1.7	35.5 ± 21		105	
Davis Lumber	137 ± 25	186 ± 28	11.8 ± 10	1.1 ± 1.14	2.83 ± 4.3	0.4 ± 0.9	152	188
Dennison	161 ± 42	174 ± 26	18 ± 18	0.6 ± 1.0	2.7 ± 2.7	0.3 ± 0.7	182	176
Rice paper	150 ± 34	143 ± 31	9.8 ± 7.3	2.4 ± 2.2	1.8 ± 2.3	0.7 ± 1.2	162	144
Northern (TT)	55.3 ± 36.1	174 ± 16	32.3 ± 29.4	1.7 ± 3.5	12.8 ± 8.8	0.8 ± 1.0	100	178
MD (TT)	235 ± 27.6	207 ± 31	6.8 ± 5.6	1.8 ± 3.2	2.6 ± 2.5	0.7 ± 1.1	245	209
Truly Fine (TT)	154 ± 22	207 ± 22	9.6 ± 4.9		2.4 ± 1.9	1.3 ± 1.8	166	210
Micro-wipes	120 ± 13.7	182 ± 39	27.8 ± 26.5	2.1 ± 1.9	2.9 ± 2.9	1.1 ± 1.4	150	186
					LSD (P = .01)		29	29
					(P = .05)		22	22

**Meloidogyne incognita* inoculum 272 ± 11.
†*Ditylenchus dipsaci* inoculum 219 ± 16.

preparations. In contrast, prolonged spray periods would cause some funnels to collect large volumes of water and to effect turbulence in the collector tube and loss of nematodes in the overflow, as indicated by the 50% spray time (Table 1). In all cases, the proportion of the number of nematodes recovered to that of the original inoculum was low; since all possible losses were accounted for, those nematodes not recovered must have been retained by the paper tissue. This notion is confirmed by the data of Table 2 which indicates that retention of *Meloidogyne incognita* larvae may vary between nearly 5 and 80% depending upon the particular tissue, and that of *D. dipsaci* from 5 to 35% in a 24-hour period. The bulk of the nematodes passing through the tissue did so within 24 hours, although in some cases appreciable numbers succeeded in the next 48 hours. The intent of Table 2 is to call attention to the nematode permeability properties of tissues used in Baermann extractions. The permeability of tissues to two nematodes used in these experiments could vary, so it remains to be established how other nematodes would respond. Furthermore, there is no assurance as to how other lots of the same brand of tissue would respond; therefore, each analyst needs to know the properties of the tissues available to him locally.

This report should focus additional attention on several sources of error inherent to specific kinds of extraction methodology and stimulate the development of quantitative processes. Recommendations for technique standardization must await more extensive studies on other impinging factors.

LITERATURE CITED

1. Ayala, A., J. Roman, and A. C. Tarjan. 1963. Comparison of four methods for isolating nematodes from soil samples. J. Agricult. Univ. of Puerto Rico. 47:219-225.

2. Ayoub, S. M. 1977. Plant nematology, an agricultural training aid. Sacramento: State of California, Dept. of Food and Agriculture.

3. Barker, K. A., C. J. Nusbaum, and L. A. Nelson. 1969. Seasonal population dynamics of selected plant-parasitic nematodes as measured by three extraction procedures. J. Nematol. 1:232-239.

4. Barker, K. A., C. J. Nusbaum, and L. A. Nelson. 1969. Effects of storage temperature and extraction procedure on recovery of plant-parasitic nematodes from field soils. J. Nematol. 1:240-247.

5. Bravo, M. A. C. 1977. Comparison of the Christie and Perry and the centrifugal flotation methods for extracting Xiphinema meditteraneum from soil. Agronomia Lusitana 38:203-212.

6. Caveness, F. E., and H. J. Jensen. 1955. Modification of the centrifugal-flotation technique for the isolation and concentration of nematodes and their eggs from soil and plant tissue. Proc. Helm. Soc. Wash. 22:87-89.

7. Coolen, W. A., and C. J. D'Herde. 1972. A method for the quantitative extraction of nematodes from plant tissue. State Nematology and Entomology Research Station. Burg. van Gansberghelaan 96, 9110 Merelbeke, Belgium.

8. Coolen, W. A., and C. J. D'Herde. 1977. Extraction de Longidorous et Xiphinema spp. du sol par centrifugation en utilisant du silice colloidal. Nematol. Medit. 5:195-206.

9. Decraemer, W., W. A. Coolen, and G. J. Hendrickx. 1979. Evaluation of extraction methods for a survey of Trichodoridae in fields of seed potatoes. Nematologica 25:494-495.

10. DeGrisse, A. T. 1969. Vergelijking van resultaten bekmen met de opspoelwattenfiltermethode (OWFM) en met de suikercentrifugedrijfmethode (SCDM) voor de extractie van plantenparasitaire nematoden wit de bodem. Overdruk Uit: Mededlingen Rijksfakulteit. Landbouwwetenschappen Gent 34:57-69.

11. Dunn, R. A. 1971. Comparison of two centrifugal flotation techniques with Seinhorst elutriation for efficiency of nematode extraction from soil. J. Nematol. 3:308-309. (Abstr.).

12. Flegg, J. J. M. 1967. Extraction of Xiphinema and Longidorus species from soil by a modification of Cobb's decanting and sieving technique. Ann. Appl. Biol. 60:429-437.

13. Gibbins, L. N., and G. S. Grandison. 1967. An improved centrifugal-flotation technique for the isolation of Ditylenchus dipsaci. Nematologica 12: 642.

14. Gooris, J., and C. J. D'Herde. 1972. A method for the quantitative extraction of eggs and second-stage juveniles of Meloidogyne spp. from soil. State Nematology and Entomology Research Station. Burg. van Gansberghelaan 96, 9220 Meulebeke, Belgium.

15. Harris, R. H. G., and J. M. C. Braithwaite. 1976. Evaluation of methods for separating nematodes from soil. Proc. S. African Sug. Tech. Ass'n. 50:23-28.

16. Mishra, S. P., K. Vijayalakshmi, and A. Seshadri. 1975. A rapid seiving-sedimentation method for extracting nematodes from soil. Ind. J. Nematol. 5:259-260.

17. Oostenbrink, M. 1960. Estimating nematode populations by some selected methods. *in* J. N. Sasser and W. R. Jenkins, eds. Nematology. Chapel Hill: University of North Carolina Press.

18. Sano, Zen-ichi. 1975. A modification of the sieve-funnel technique for the quantitative extraction of living nematodes from soil. Jap. J. Nematol. 5:41-47.

19. Southey, J. F. 1970. Laboratory methods for work with plant and soil nematodes. Ministry of Agriculture, Fisheries and Food. Ed. Technical

Bulletin No. 2. London: Her Majesty's Stationary Office.

20. Szczygiel, A. 1971. Zastosowanie metody wirwkowej do ektrakcji nicieni z gleby. Zeszyty Problemowe Postepow Nauk Rolniczych nr 121:169-179.

21. Trudgill, D. L., K. Evans, and G. Faulkner. 1973. A fluidizing column for extracting nematodes from soil. Nematologica 18:469-475.

22. Walker, J. T., and J. D. Wilson. 1960. The separation of nematodes from soil by a modified Baermann funnel technique. Plant. Dis. Rept. 44: 94-97.

23. Willard, J. R., and M. S. Petrovich. 1972. A direct centrifugal-flotation method for extraction of nematodes from clay soils. Plant. Dis. Rept. 56:808-810.