

Extraction Efficiency of *Belonolaimus longicaudatus* from Sandy Soil¹

R. MCSORLEY AND J. J. FREDERICK²

Abstract: Numbers of *Belonolaimus longicaudatus* extracted from sandy soils (91-92% sand) by sieving and centrifugation were only 40-55% of those extracted by sieving and incubation on a Baermann tray. Residues normally discarded at each step of the sieving plus Baermann tray extraction procedure were examined for nematodes to obtain estimates of extraction efficiencies. For third-stage and fourth-stage juveniles, males, and females, estimates of extraction efficiency ranged from 60 to 65% in one experiment and 73 to 82% in another. Estimated extraction efficiencies for second-stage juveniles were lower (33% in one experiment, 67% in another) due to losses during sieving. When sterilized soil was seeded with known numbers of *B. longicaudatus*, 60% of second-stage juveniles and 68-76% of other stages were recovered. Most stages of *B. longicaudatus* could be extracted from these soils by sieving plus Baermann incubation with an efficiency of 60-70%.

Key words: *Belonolaimus longicaudatus*, extraction efficiency, nematode, population estimation, *Pratylenchus brachyurus*, quantitative nematology, sting nematode.

Extraction efficiency is a useful concept in quantitative nematology, but reported extraction efficiencies range widely (6). This is expected, because of differences in soil type, nematode population structure, slight modifications to standard extraction techniques, operator variability, and the experimental procedure by which extraction efficiency is estimated. Extraction efficiency can be determined by artificial seeding and recovery of nematodes from soil samples, by direct examination of small soil samples, or by examining residues discarded during extraction for nematodes that would be lost. Because error is involved in the estimation of extraction efficiencies, it may be undesirable to routinely apply extraction efficiencies to all quantitative nematode data (8). When comparing counts obtained by the same methodology, similar relative errors may occur in all samples. However, in studies requiring accurate population estimates, particularly at low densities, an estimate of extraction efficiency may aid in interpretation of data.

The sting nematode, *Belonolaimus longi-*

caudatus, can be damaging at low population levels (7), requiring accurate estimates of low densities. No estimates of extraction efficiency are available for this nematode, and few recommendations on extraction methodology exist. In North Carolina, recoveries of *B. longicaudatus* by centrifugation and by Baermann funnels were similar for various sampling times and locations (1). Baermann methodology continues to be modified and improved (12), however, and there has been some concern that large nematodes may not be extracted as well by centrifugation (6). For example, more *Xiphinema index* were recovered from three types of soil by sieving and Baermann funnel than by sieving and centrifugation (16). Potential loss of large nematodes is a major concern with *B. longicaudatus* because adults are longer than *Xiphinema* spp. The range of lengths observed among *B. longicaudatus* life stages may require the development of stage-specific extraction efficiencies (14).

The objectives of this study were to compare sieving and centrifugation to sieving and Baermann incubation for extraction of *B. longicaudatus* and to estimate the efficiency for extracting *B. longicaudatus* using sieving and Baermann incubation. Extraction efficiencies were estimated by examining nematode losses in extraction residues and by adding known numbers of nematodes to samples.

Received for publication 10 December 1990.

¹ Florida Agricultural Experiment Station Journal Series No. R-01209.

² Professor and Biological Scientist, Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611.

MATERIALS AND METHODS

Comparison of Baermann tray vs. centrifugation: This experiment was conducted twice in August 1989. Soils used were an Arredondo fine sand (92% sand, 3% silt, 5% clay; pH 6.1; < 1% organic matter) collected from a sugarcane (*Saccharum* sp.) field and a similar soil (91% sand, 3% silt, 6% clay; pH 6.0; 1.2% organic matter) obtained from an alyceclover (*Alysicarpus* spp.) field infested with *Pratylenchus brachyurus* as well as *B. longicaudatus*. The fields were 1 km apart on the University of Florida campus in Gainesville.

A 3-liter sample of each sandy field soil naturally infested with *B. longicaudatus* was collected and mixed. Sixteen 100-cm³ subsamples were removed for extraction, eight by Baermann tray and eight by centrifugation. The procedure used for extracting *B. longicaudatus* in Baermann trays is outlined in Figure 1. The incubation chamber was a covered plastic sandwich box, 13.5 cm square × 4 cm high. The residue rinsed from the 38- μ m sieve was placed on two layers of tissue paper (Kimwipes, Kimberly-Clark Corp., Roswell, GA) supported on a 1-mm-mesh window screen glued between two 10.5-cm-i.d. polyvinylchloride pipe sections (13); the upper section was 15 mm high and the lower section was 8 mm high. The total volume of water in the tray was ca. 100 ml. Samples for extraction by centrifugation were sieved similarly (Fig. 1), but residues from the 38- μ m sieve were placed in a sucrose solution (454 g/liter of water) in 50-ml centrifuge tubes for further extraction (5,9).

Extraction efficiency based on losses during extraction: Eight 100-cm³ subsamples of the sugarcane field soil were extracted by the standard Baermann tray procedure outlined in Figure 1. At the steps designated, residues which would normally be discarded were saved and checked for nematodes. These counts represent nematodes that would be lost during the extraction procedure and are reported as numbers lost per 100 counted from a standard extraction. If losses per 100 nematodes counted

are summed over all steps in the procedure, then extraction efficiency (9) is given by the following: Extraction efficiency = $100 / (100 + \text{sum of all losses})$.

This experiment was repeated with the alyceclover soil, although some check points in Figure 1 were omitted because few adult *B. longicaudatus* were found at these points in the previous experiment.

Extraction efficiency based on numbers added to soil: *Belonolaimus longicaudatus* were reared on bermudagrass (*Cynodon dactylon*) in a shadehouse and extracted on Baermann trays set up 3 days before inoculation. After 2 days of extraction, nematodes obtained from all trays were mixed and divided into 24 equal portions. Nematodes in each portion were counted and maintained at 24 C in a watchglass overnight.

Soil (ca. 3 liters) for the experiment was obtained from the sugarcane field and placed in an oven at 100 C for 24 hours. The four treatments, each replicated six times, involved addition of known numbers of nematodes at different points of the extraction procedure (Fig. 1). In the first treatment, "addition to soil," nematodes were added to the 100-cm³ soil subsample immediately before extraction (prior to step #1). For "addition to soil suspension," a 100-cm³ subsample was extracted, and nematodes were added directly to the soil suspension (prior to step #3). For "addition to Baermann with residue," a clean residue was added to the Baermann tray (step #4) and nematodes were added to the extraction at this point. Finally, Baermann trays without any soil residues were prepared and nematodes were added in water above the tissue for "addition to Baermann without residue." For all treatments, nematodes extracted after 48 hours were counted, percentages recovered were determined, and treatment differences ($P \leq 0.05$) were tested by analysis of variance, followed by mean separation using Duncan's multiple-range test (3).

A second experiment was conducted to determine the effect of residue in the Baermann trays on extraction efficiency. *Belonolaimus longicaudatus* was obtained from aly-

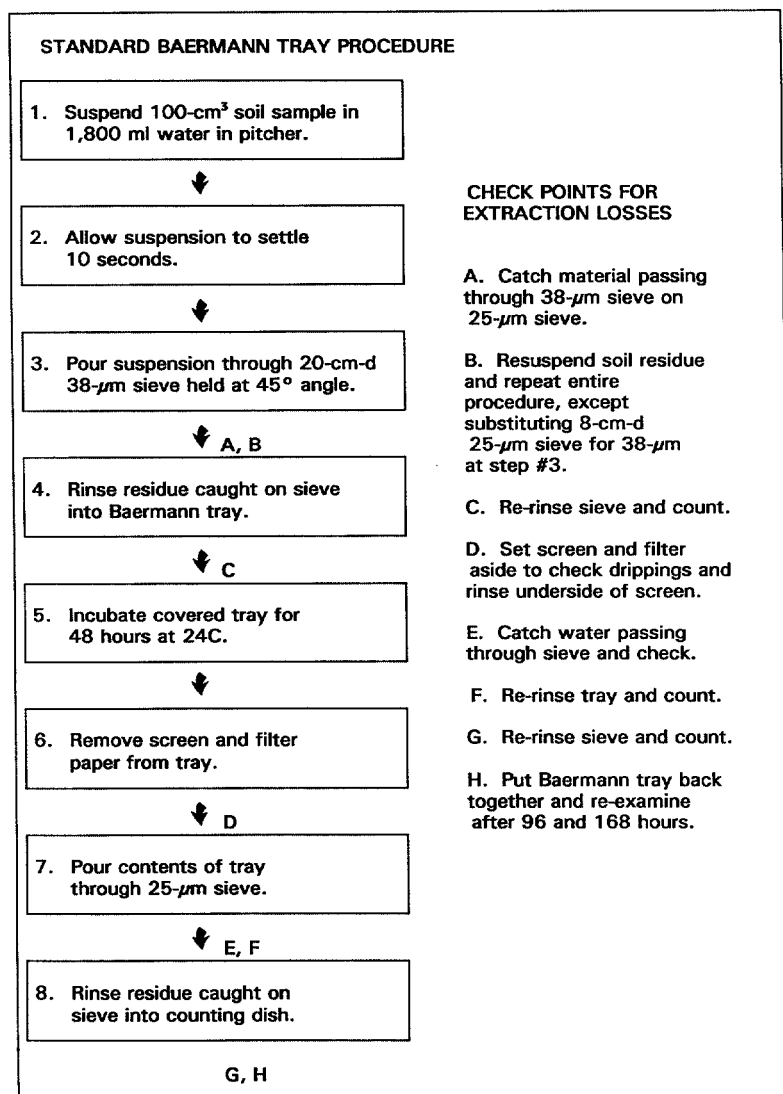


FIG. 1. Steps involved in extraction of nematodes using sieving followed by incubation on a Baermann tray, and points at which extraction residues were checked for losses of nematodes.

clover field soil by the procedure described for the bermudagrass soil. Treatments consisted of three different kinds of residues added to Baermann trays along with nematodes (step #4). Residues were obtained by extracting 100-cm³ sub-samples of either sand or potting soil, both of which were nematode free. The three treatments were potting soil residue (3.58 \pm 0.19 g dry weight of residue per tray, mostly organic matter), sand residue (0.1 \pm 0 g dry weight of residue per tray), and

no residue. Other methods and data analysis were identical to those of the previous experiment. All extractions in every described experiment were conducted by the same operator, in an effort to avoid the variability which can result from changing operators (8).

RESULTS

Comparison of Baermann tray vs. centrifugation: In both experiments and for all life stages, more *B. longicaudatus* were extract-

TABLE 1. Numbers of nematodes extracted from sugarcane or alyceclover field soil (100 cm²) using sieving plus centrifugation or sieving plus Baermann tray.

Extraction method	<i>Belonolaimus longicaudatus</i>				All stages	<i>Pratylenchus brachyurus</i> (all stages)
	J2†	J3-4†	Males	Females		
Sugarcane soil						
Centrifugation (CF)	30.2 ± 2.2	23.9 ± 1.8	5.6 ± 1.0	19.6 ± 1.7	79.4 ± 3.3	—‡
Baermann tray (BT)	74.8 ± 8.4	44.1 ± 4.9	12.8 ± 1.7	41.2 ± 4.9	172.9 ± 19.0	—
CF as % of BT	40.5%	54.1%	44.1%	47.6%	45.9%	—
Alyceclover soil						
Centrifugation (CF)	19.6 ± 3.1	17.8 ± 1.8	1.7 ± 0.5	4.2 ± 1.0	43.4 ± 4.6	40.9 ± 3.1
Baermann tray (BT)	43.6 ± 3.4	40.6 ± 2.5	4.1 ± 0.8	10.1 ± 0.8	98.5 ± 3.8	59.4 ± 6.2
CF as % of BT	45.0%	43.7%	42.5%	42.0%	44.0%	68.8%

All data are means ± standard error of eight replications. In all cases, numbers obtained using the Baermann tray were greater ($P \leq 0.05$) than those obtained using centrifugation, according to an F -test.

† J2 = second-stage juveniles; J3-4 = third-stage and fourth-stage juveniles.

‡ No *P. brachyurus* recovered.

ed by sieving plus Baermann tray extraction ($P \leq 0.05$) than by sieving plus centrifugation (Table 1). Numbers extracted by centrifugation were only 40–54% of those extracted on Baermann trays, a difference that was fairly consistent in both experiments and for all stages. In contrast, numbers of *P. brachyurus* extracted by centrifugation were almost 69% of those extracted on Baermann trays.

Extraction efficiency based on losses during extraction: Detailed examination of residues discarded during Baermann tray extraction revealed losses at many points during the 48-hour extraction period (Table 2). The greatest loss was in the soil residue remaining behind after sieving the soil suspension (step #3). When this residue was resuspended and the extraction process repeated, an additional 22–23 *B. longicaudatus*

TABLE 2. Estimated losses of *Belonolaimus longicaudatus* per 100 counted in a standard count during sieving plus Baermann tray extraction of soil from a sugarcane field.

Extraction loss†	J2	J3-4	Male	Female
Passed through 38- μ m sieve (A)	20.4 ± 2.4	4.0 ± 0.9	0.4 ± 0.2	0.4 ± 0.2
Passed through 25- μ m sieve (A)	3.1 ± 0.7	0	0	0
Recovered after resuspension of soil (B)	22.9 ± 5.5	23.1 ± 4.9	21.9 ± 2.7	22.9 ± 3.2
Passed through sieve during resuspension of soil (B, A)	1.2 ± 0.7	0	0.1 ± 0.1	0
Recovered after 2nd resuspension of soil (B)	3.7 ± 1.7	2.0 ± 0.8	4.7 ± 0.8	6.3 ± 1.3
Rinsed from 38- μ m sieve (C)	0.2 ± 0.2	0.9 ± 0.6	0.1 ± 0.1	0
Dripped from screen (D)	0.3 ± 0.3	0	0.1 ± 0.1	0
Passed through 25- μ m sieve (E)	11.5 ± 2.7	0.5 ± 0.5	0.1 ± 0.1	0.4 ± 0.2
Rinsed from tray (F)	0.3 ± 0.3	0	0.1 ± 0.1	0.7 ± 0.2
Rinsed from 25- μ m sieve (G)	0.8 ± 0.6	0.9 ± 0.6	0.2 ± 0.1	0.3 ± 0.2
Recovery at 96 hours (H)‡	92.9 ± 15.9	23.8 ± 5.3	18.9 ± 4.2	20.5 ± 3.9
Recovery at 168 hours (H)‡	46.0 ± 6.4	11.1 ± 3.3	6.6 ± 1.2	9.2 ± 2.9
Total losses	203.3	66.4	53.2	60.7
Extraction efficiency§	33.0%	60.1%	65.2%	62.2%

A standard count is the number of nematodes recovered after 48 hours following the steps in Figure 1. J2 = second-stage juveniles; J3-4 = third-stage and fourth-stage juveniles.

† Losses refer to check points A–H in Figure 1.

‡ These are considered losses because the standard count is made at 48 hours, at which time these residues would normally be discarded.

§ Extraction efficiency = 100/(100 + total losses).

TABLE 3. Estimated nematode losses per 100 counted in a standard count during sieving plus Baermann tray extraction of soil from an alycyclover field.

Extraction loss†	<i>Belonolaimus longicaudatus</i>				<i>Pratylenchus brachyurus</i> (all stages)
	J2	J3-4	Male	Female	
Passed through 38- μ m sieve (A)	10.1 \pm 1.6	1.2 \pm 0.6	0	0	8.4 \pm 2.3
Recovered after resuspension of soil (B)	13.1 \pm 2.4	15.9 \pm 3.0	13.6 \pm 4.4	8.1 \pm 2.3	29.2 \pm 6.5
Recovered after 2nd resuspension of soil (B)	1.8 \pm 0.7	0.9 \pm 0.4	3.1 \pm 3.1	0	19.2 \pm 7.9
Recovered after 3rd resuspension of soil (B)	0	0.6 \pm 0.4	0	0	6.6 \pm 3.5
Passed through 25- μ m sieve (E)	3.4 \pm 1.0	0	0	0	23.7 \pm 2.5
Rinsed from tray (F)	0.6 \pm 0.4	0.6 \pm 0.4	0	1.3 \pm 0.9	0.3 \pm 0.3
Rinsed from 25- μ m sieve (G)	1.4 \pm 0.8	0.3 \pm 0.3	0	0.8 \pm 0.8	2.6 \pm 0.6
Recovery at 96 hours (H)‡	11.4 \pm 1.9	11.0 \pm 1.5	5.0 \pm 5.0	7.6 \pm 2.1	70.4 \pm 6.5
Recovery at 168 hours (H)‡	7.1 \pm 2.1	6.3 \pm 1.4	6.9 \pm 3.6	3.7 \pm 1.2	77.2 \pm 8.0
Total losses	48.8	36.9	28.6	21.6	237.4
Extraction efficiency§	67.2%	73.1%	77.8%	82.3%	29.6%

A standard count is the number of nematodes recovered after 48 hours following the steps in Figure 1. J2 = second-stage juveniles; J3-4 = third-stage and fourth-stage juveniles.

† Losses refer to check points A-H in Figure 1.

‡ These are considered losses because the standard count is made at 48 hours, at which time these residues would normally be discarded.

§ Extraction efficiency = 100/(100 + total losses).

datus could be obtained for each 100 counted. Other losses, such as the number of nematodes passing through the sieves, were greater for second-stage juveniles (J2) than for third-stage and fourth-stage juveniles (J3-4) or adults. When Baermann trays were put back together, many additional nematodes (particularly J2) were extracted from them at 96 and 168 hours. This represents an additional and fairly large loss, since the standard 48-hour incubation time was insufficient to extract these nematodes from residues. Resulting extraction efficiency for J2 was only 33%, compared to 60-65% for other stages.

In the experiment using a different soil (Table 3), losses were not as great, with 67% of J2 and 73-82% of other *B. longicaudatus* stages extracted. In contrast, the calculated extraction efficiency for *P. brachyurus* from this soil was only 30%, since many individuals remained in the residue on the tray up to 96 or 168 hours.

Extraction efficiency based on numbers added to soil: When nematodes were added directly to sterilized sugarcane field soil (92% sand), 60-76% were recovered in a standard 48-hour Baermann extraction (Table 4). Recovery was similar whether *B. longicaudatus* were added to the soil, to the soil

suspension in water, or to residues in the Baermann trays, but was increased ($P \leq 0.05$) for J2 or all stages totalled if nematodes were added to Baermann trays without a residue. The importance of the tray residue on extraction efficiency is illustrated by the second experiment (Table 4) in which residues from potting soil resulted in lower extraction efficiencies of *B. longicaudatus* (33-46%) than did residues from sand (83-92%) or no residues (87-95%). Residue on the tray had less effect on *P. brachyurus* recovery (Table 4).

DISCUSSION

The relatively high recovery of *B. longicaudatus* from sand residues on Baermann trays is encouraging and probably applicable in most field situations, because the nematode is usually found in soils with high sand content (11). If soil organic matter is less than 1-2%, however, extraction efficiency should be checked, because organic matter on the trays can affect recovery.

Although for many nematode species extraction by centrifugation is considered to be more efficient than by modified Baermann methods (6), we found that *B. longicaudatus* was extracted much more effi-

TABLE 4. Nematode recovery as a percentage of known numbers added to samples in two experiments.

Treatment	<i>Belonolaimus longicaudatus</i>			<i>Pratylenchus brachyurus</i> (all stages)
	J2†	J3-4‡	Males	
	Nematodes added at various points of the extraction procedure			
Added to soil	60.0 ± 3.3 b	75.9 ± 5.8 a	68.0 ± 3.5 a	66.2 ± 1.5 b
Added to soil suspension	61.5 ± 0.9 b	83.9 ± 4.0 a	71.7 ± 7.7 a	71.4 ± 3.7 b
Added to Baermann with residue	67.4 ± 5.6 b	71.7 ± 6.8 a	74.5 ± 6.4 a	71.3 ± 2.2 b
Added to Baermann without residue	85.3 ± 6.6 a	88.0 ± 6.1 a	79.5 ± 3.5 a	84.8 ± 3.3 a
	Nematodes added to residues in Baermann trays			
Potting soil residue	34.1 ± 4.0 b	37.5 ± 3.1 b	45.8 ± 5.6 b	36.9 ± 2.8 b
Sand residue	91.7 ± 3.7 a	82.8 ± 7.1 a	90.2 ± 1.9 a	87.9 ± 1.9 a
No residue	87.4 ± 5.1 a	95.1 ± 2.1 a	91.4 ± 3.4 a	91.2 ± 1.8 a
				72.4 ± 4.3 b
				89.9 ± 3.9 a
				77.0 ± 5.3 ab

All data are means ± standard error of six replications. For each experiment, means in columns followed by the same letter do not differ ($P \leq 0.05$), according to Duncan's multiple-range test.

† J2 = second-stage juveniles; J3-4 = third-stage and fourth-stage juveniles.

‡ No *P. brachyurus* used.

ciently on Baermann trays. This contrasts with results from North Carolina (1) which indicate similar extraction of *B. longicaudatus* by centrifugation and a Baermann funnel technique. There were differences in soil types used in studies at the two locations (sandy loam or loamy sand in North Carolina vs. sand in Florida), but more important, the Baermann technique differed in the two studies. Our technique includes sieving before incubation instead of direct incubation of soil.

Extraction efficiencies estimated from cumulative losses were lower for J2 than for other life stages of *B. longicaudatus*. In the alyceclover soil, this difference probably was due to losses through sieves; in the sugarcane soil, a large proportion of J2 did not emerge from residues until after 48 hours. This increase in emergence could be due to egg hatch or in differences in J2 vigor between the two field populations. There was some evidence that J2 did not move through residues on the tray as easily as the other stages did. Egg hatch in Baermann trays can occur over time (2), and it is also possible that this emergence of J2 after 48 hours was due to egg hatch. Recovery of *P. brachyurus* increased markedly after 48 hours, possibly also due to egg hatch, but emergence of *Pratylenchus* spp. from root fragments also occurs commonly in Baermann funnels (1).

For life stages of *B. longicaudatus* other than J2, some differences were observed between estimated extraction efficiencies from the sugarcane field soil (60-65%) and the alyceclover field soil (73-82%). Slightly higher estimates were expected with the alyceclover soil, since fewer check points were evaluated for losses. Despite the similarity of the soils used in both experiments, there may be unmeasured differences in the association of nematodes with the soil particles, or in the health and activity of the two populations of *B. longicaudatus*. We hypothesize differences in population structure between the two sites because of the increase in J2 recovery, possibly from egg hatch in the sugarcane field soil.

In addition to examination of losses in extraction residues, artificial seeding of soil samples or direct examination of soil (6) can also be used to estimate extraction efficiency. We found direct examination of soil too tedious and samples too dirty to estimate nematode numbers reliably, and therefore we did not complete any experiments with this technique. The amount of soil typically examined in direct examination studies (10) is much less than the 100-cm³ used in our standard extraction procedure, which poses an additional difficulty in comparing methods. In our experiments, estimation of extraction efficiency by artificial seeding and recovery yielded estimates of 68–76% for J3–4, males, and females of *B. longicaudatus*. Comparable estimates of 60–65% were obtained for the same soil by tabulating extraction losses. Both methods of estimating efficiencies are subject to some errors. Despite thorough checking of many extraction steps, some losses probably went unnoticed, and so estimates of 60–65% extraction efficiency obtained by this method may be slightly high. When samples are artificially seeded, nematodes may not be completely incorporated into soil aggregates (4), and therefore extraction efficiency may be overestimated. Similar recovery of *B. longicaudatus*, whether they were added to soil, to suspensions, or to residues on trays, suggests incomplete incorporation into the soil by the seeding procedure used.

Although different methodology would be expected to result in somewhat different estimates of extraction efficiency, results obtained in the various experiments here were fairly similar. For the procedure outlined here, extraction efficiencies for most stages of *B. longicaudatus* were probably 60–70%. Centrifugation was only about 45% as efficient as Baermann tray extraction, giving probable extraction efficiencies for centrifugation of 27–32%. Because of increased losses through sieves, extraction efficiency for J2 was slightly less than for the other stages.

Some estimate of the egg fraction of a *B. longicaudatus* population could be ob-

tained by observing recovery of J2 beyond the normal 48-hour incubation time, but additional studies of the extraction efficiency for eggs are needed. In most cases, extraction efficiency can be increased by incubation beyond 48 hours. This is particularly true for *Pratylenchus* spp. (15), which can emerge from eggs and root fragments in soil over a week or more. The length of time for Baermann incubation depends on the goals of the particular study, and a 48-hour incubation time is, in a sense, a compromise between two extremes. If every infective unit (adults, juveniles, eggs in soil; all stages in roots) is to be assayed, then a long incubation time is preferable. If an instantaneous examination of population structure is desired, then a very rapid method such as centrifugation is preferred. Long incubation will result in an incorrect impression of population structure, since more eggs will hatch and be counted as juveniles.

LITERATURE CITED

1. Barker, K. R., C. J. Nusbaum, and L. A. Nelson. 1969. Effects of storage temperature and extraction procedure on recovery of plant-parasitic nematodes from field soil. *Journal of Nematology* 1:240–247.
2. Freckman, D. W., R. Mankau, and H. Ferris. 1975. Nematode community structure in desert soils: Nematode recovery. *Journal of Nematology* 7:343–346.
3. Freed, R., S. P. Eisensmith, S. Goetz, D. Reicosky, V. W. Smail, and P. Wolberg. 1987. User's guide to MSTAT. Michigan State University, East Lansing, MI.
4. Green, C.D., and D. M. Parrott. 1967. A reappraisal of extraction of *Heterodera* cysts from large volumes of soil. *Nematologica* 12:601–609.
5. Jenkins, W. R. 1964. A rapid centrifugal-floatation technique for separating nematodes from soil. *Plant Disease Reporter* 48:692.
6. McSorley, R. 1987. Extraction of nematodes and sampling methods. Pp. 13–47 in R. H. Brown and B. R. Kerry, eds. *Principles and practice of nematode control in crops*. Sydney: Academic Press Australia.
7. McSorley, R., and D. W. Dickson. 1989. Effects and dynamics of a nematode community on maize. *Journal of Nematology* 21:462–471.
8. McSorley, R., and J. L. Parrado. 1987. Nematode losses during sieving from two soil types. *Nematropica* 17:125–146.
9. McSorley, R., and J. L. Parrado. 1987. Nematode losses during centrifugal extraction from two soil types. *Nematropica* 17:147–161.
10. Minagawa, N. 1979. Efficiencies of two meth-

ods for extracting nematodes from soil. *Applied Entomology and Zoology* 14:469-477.

11. Perry, V. G., and H. L. Rhoades. 1982. The genus *Belonolaimus*. Pp. 144-149 in R. D. Riggs, ed. *Nematology in the southern region of the United States*. Southern Cooperative Series Bulletin 276, Arkansas Agricultural Experiment Station, University of Arkansas, Fayetteville.

12. Robinson, A. F., and C. M. Heald. 1989. Accelerated movement of nematodes from soil in Baermann funnels with temperature gradients. *Journal of Nematology* 21:370-378.

13. Rodríguez-Kábana, R., and M. H. Pope. 1981. A simple incubation method for the extraction of nematodes from soil. *Nematrópica* 11:175-186.

14. Schneider, S. M., and H. Ferris. 1987. Stage-specific population development and fecundity of *Paratrichodorus minor*. *Journal of Nematology* 19:395-403.

15. Townshend, J. L. 1963. A modification and evaluation of the apparatus for the Oostenbrink direct cottonwool filter extraction method. *Nematológica* 9:106-110.

16. Viglierchio, D. R., and R. V. Schmitt. 1983. On the methodology of nematode extraction from field samples: Comparison of methods for soil extraction. *Journal of Nematology* 15:450-454.