

NEMATODE LOSSES DURING SIEVING FROM TWO SOIL TYPES¹

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ABSTRACT

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Estimates of losses at each stage of a multistep sieving procedure were obtained for nematodes infesting Rockdale fine sandy loam and Perrine marl soils from southern Florida. The steps examined in the sieving procedure ranged from preparation of the soil subsample for extraction to concentration of a sieved subsample for centrifugation. Losses in the early stages of the sieving process (suspension of a soil subsample in water, screening through a coarse sieve) were much greater than losses in the latter stages (passing through a 38- μ m sieve, rinsing sieves, decanting). Estimates of maximum extraction efficiency through all of the sieving steps were 62.2% for *Criconebella onoensis*, 67.6% for *Rotylenchulus reniformis*, and 71.4% for *Helicotylenchus dihystera* on Rockdale soil; and 88.0% for *C. onoensis* and 75.8% for *R. reniformis* on Perrine marl soil. Experiments varying methodology at each step provided means for optimizing procedures at each step in the sieving process. *Additional key words:* *Criconebella onoensis*, *extraction efficiency*, *Helicotylenchus dihystera*, *methodology*, *Meloidogyne incognita*, *population assessment*, *quantitative nematology*, *Quinisulcius acutus*, *Rotylenchulus reniformis*, *Tylenchorhynchus martini*.

RESUMEN

McSorley, R., y J. L. Parrado. 1987. Pérdidas en la extracción de nematodos por tamizado en dos tipos de suelo. *Nematropica* 17:125-146.

Las estimaciones de pérdidas en cada etapa de un procedimiento múltiple de extracción por tamizado fue obtenido para los nematodos que infestan los suelos de textura franco arenosa fina de Rockdale y marl de Perrine al sur de la Florida. Los pasos en el procedimiento de tamizado examinado fluctuaron desde la preparación de la submuestra de suelo hasta la concentración de la submuestra tamizada lista para la centrifugación. Las pérdidas en las primeras etapas del proceso de tamizado (suspensión de la submuestra en agua, paso a través de un tamiz grueso) fueron mayores que las pérdidas en las etapas posteriores (paso a través del tamiz 38- μ m, enjuague de los tamices, decantado). Los estimados de la máxima eficiencia de extracción a través de todos los pasos del tamizado fueron 62.2% para *Criconebella onoensis*, 67.6% para *Rotylenchulus reniformis*, y 71.4% para *Helicotylenchus dihystera* en los suelos Rockdale; y 88.0% para *C. onoensis* y 75.8% para *R. reniformis* en los suelos marl de Perrine. Variando la metodología, los experimentos en cada paso proporcionaron la forma de optimizar el procedimiento en cada etapa del proceso de tamizado.

Palabras claves adicionales: *eficiencia de extracción*, *evaluación de la población*, *metodología*, *nematología cuantitativa*.

INTRODUCTION

Increased emphasis on numerical data in nematology has made it imperative that sources of error in commonly-used extraction procedures be identified and a firm quantitative basis for the methods be developed (15). Extraction efficiency, or that percentage of nematodes present in a sample which is recovered by the extraction method, has been proposed as a useful standard for quantifying and comparing methodologies (2,4). Published estimates of extraction efficiencies range from 0-100%, depending on the nematode, method, and soil type (9). Clearly, additional studies are necessary before the concept of extraction efficiency can be applied even to rather specific situations.

Many methods for extracting nematodes from soil involve multistep procedures (1,9,13), and so the determination of the exact points at which nematodes are lost can become quite complicated. Since the time of Cobb (3), many laboratories have used sieving as a convenient step in separating and concentrating large quantities of nematodes from soil suspensions. The reported efficiency of sieving is low however (14,16), and so it is most useful to determine the elements of the sieving process which result in substantial losses of nematodes.

The purpose of the current study is to examine a typical sieving process to determine estimates of losses at various stages of the process, and to determine the influence of varying methodology at specific points in the sieving process. The steps of the sieving procedure examined range from preparation of the soil subsample for extraction to concentration of sieved material for centrifugation, as part of a modified sieving-centrifugation process (8,10). The method was applied to soil samples of plant-parasitic nematodes from vegetable crops grown on two different soil types in southern Florida.

MATERIALS AND METHODS

Soil for these experiments was obtained from vegetable fields in the vicinity of Homestead, Florida. Two different types of soil were used in the various experiments (7): Rockdale fine sandy loam (Lithic Eutrochrept, 40.4% sand, 31.4% silt, 28.2% clay, pH = 7.8) and Perrine marl (Typic Fluvaquent, 3.6% sand, 64.2% silt, 32.2% clay, pH = 7.8). Nematodes were extracted from soil samples using a modification (10) of Jenkins' (8) sieving and centrifugation procedure. The individual steps in the sieving procedure are outlined (Fig. 1), and were identical for all experiments, except as modified below. For ease in comparing data from different experiments, nematodes obtained using this standard method (Fig. 1) are hereafter referred to as a "standard count". When several treatments and replications were needed for an experiment, the required number of separate 100-cm³ subsamples were removed from

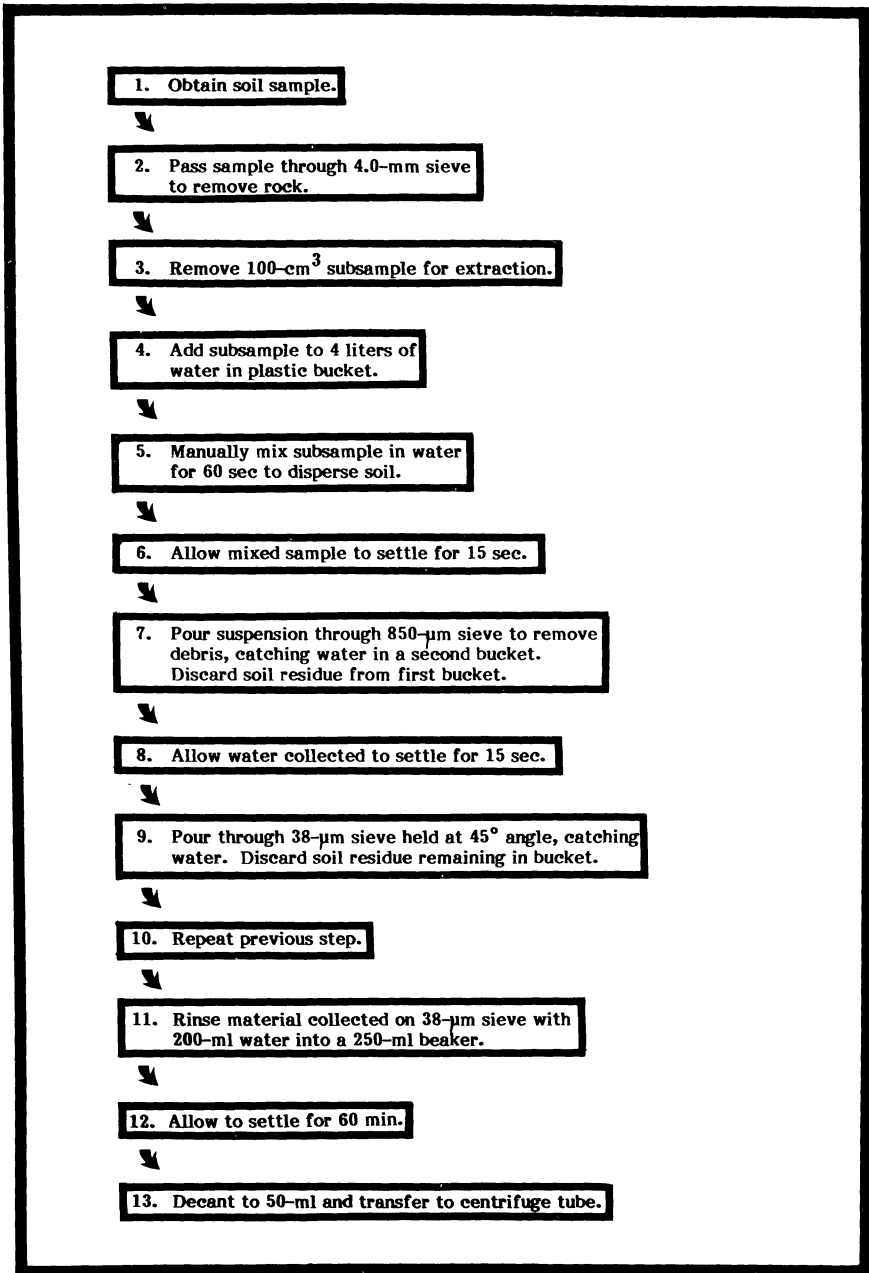


Fig. 1. Steps involved in a typical sieving process for extracting nematodes from soil subsamples.

the same large sample. All experiments were performed at least twice, once using the Rockdale soil and once with the Perrine marl soil. All data were analyzed by the analysis of variance procedure appropriate for the particular experiments, followed by curve fitting to linear and nonlinear models, if appropriate (5).

Operator variability. A large soil sample was passed through a 4.0-mm sieve to remove rock, the soil was mixed well, and 18 subsamples of 100-cm³ each were removed. Six 100-cm³ subsamples were given to each of three different operators to extract according to the sieving procedures outlined (Fig. 1, step #3 onward). Two operators (B and C) had considerable previous experience in processing soil samples, but the third operator (A) had no experience and was trained in the procedure the morning before the experiment. After the extractions were completed (Fig. 1, step #13) by the three operators, all samples were further processed by operator B, using a modification (10) of Jenkins' (8) centrifugation procedure.

Soil mixing prior to sieving. In preparing soil for the extraction process, the screening out of rock through a 4.0-mm sieve (Fig. 1, step #2) provides some mixing of the soil prior to removal of a 100-cm³ subsample for extraction. This experiment examined the effect of mixing the soil sample in a rotary mixer (9.5-L capacity, 31 rpm) prior to passing it through the 4.0-mm sieve. A bulked portion of more than 38 L of soil was divided into 7.5-L portions which were mixed in the rotary mixer for 0, 1, 2, 4, or 8 min. After each sample was passed through a 4.0-mm sieve, 5 replicate 100-cm³ subsamples were removed from each sample and processed as outlined previously (Fig. 1).

Volume of water for dilution of subsample. For this experiment, the extraction process was identical to Fig. 1, except that step #4 was varied, using 1, 2, 3, 4, or 5 L of water in the bucket to dilute the subsample. The 5 treatments were replicated 4 times, requiring 20 subsamples (100-cm³ each) for this experiment. In addition, for each subsample, soil residue remaining in the bucket after the first sieving (Fig. 1, step #7) was saved, and later resuspended in 5 L of water for extraction (Fig. 1, step #4). In this way, an evaluation could be made of nematodes remaining in the residual soil which is normally discarded.

Mixing time of subsample in water. After subsamples are added to 4 L of water, the usual procedure is to mix the suspension manually for 60 sec to break up the soil and disperse nematodes (Fig. 1, step #5). This experiment investigated the effect of five manual mixing times, 15, 30, 45, 60, and 90 sec, with each mixing time replicated three times. Fifteen 100-cm³ subsamples were thus used in this experiment. As in the previous experiments examining subsample dilution, residues were saved and reprocessed to obtain an indication of nematodes which did not go into suspension.

Settling time in water. Before a soil suspension is poured through the first sieve, it is permitted to stand 15 sec to allow large soil particles to settle (Fig. 1, step #6). In this experiment, settling times were varied (0, 15, 30, 60, and 90 sec) with other steps remaining the same. Fifteen 100-cm³ subsamples taken from the same soil sample provided 5 treatments x 3 replications. As in previous experiments, the residue remaining in the first bucket after pouring through the 850- μ m sieve was resuspended and reprocessed to determine the numbers of nematodes which had settled out.

Losses on the first sieve. The first sieve, a coarse 850- μ m-mesh sieve, is useful in removing root fragments, small rock particles, and other debris from the soil suspension (Fig. 1, step #7). To evaluate losses on this sieve, a soil subsample was extracted according to the usual procedure. However, debris remaining on the 850- μ m sieve was removed and resuspended for a second extraction. Debris caught on the 850- μ m sieve in that extraction was also resuspended and extracted, and so forth, until four successive 850- μ m sievings had been performed. The procedure was replicated three times, using three 100-cm³ subsamples.

In a second pair (Rockdale and marl soil) of experiments, extraction with a 850- μ m sieve at the first sieving was compared with a 600- μ m sieve. Twelve 100-cm³ subsamples provided 6 replications with each sieve size.

Settling time before second sieving. In this experiment, the settling time prior to the first pass through a 38- μ m sieve was varied (Fig. 1, step #8). Settling times used were 0, 15, 30, 60, and 90 sec, with each treatment replicated three times. Residues remaining in the bucket, after pouring the suspension through the sieve, were resuspended and extracted separately to give an indication of losses in the residues.

Losses through a 38- μ m sieve. Usually, the soil suspension is passed twice through a 38- μ m sieve (Fig. 1, steps #9-10). To evaluate losses through this sieve, a single pass through the sieve was made, catching the soil suspension in a second bucket. Nematodes were washed from the sieve and processed through the further steps of the extraction process. The soil suspension caught after passing through the sieve was sieved again and extracted a second time, and so on, until 5 successive sievings through a 38- μ m sieve had been made. The procedure was replicated three times.

Settling and decanting. The 38- μ m sieve is usually rinsed with 200 ml water and residues collected in a 250-ml beaker (Fig. 1, step #11), before decanting to a smaller volume. To investigate potential losses during decanting and to optimize settling time, four experiments were carried out, two utilizing Rockdale soil and two using Perrine marl soil. Subsamples from a large sample were extracted and allowed to settle for various times in the 250-ml beakers before decanting. In the first two experi-

ments (one Rockdale and one marl), 3 treatments (30, 60, and 120-min settling times) were replicated 6 times, while in the second two experiments, 4 treatments (15, 30, 60, and 120-min settling times) were replicated 4 times. After decanting, extractions proceeded using the standard centrifugation technique (8), and counts were made. However, water decanted from each subsample was also saved for direct examination for nematodes.

RESULTS AND DISCUSSION

Nematodes recovered from the Rockdale soil used in this experiment included *Rotylenchulus reniformis* Linford & Oliveira, *Meloidogyne incognita* (Kofoid & White) Chitwood, *Criconebella onoensis* (Luc) Luc & Raski, *Helicotylenchus dihystera* (Cobb) Sher, and *Quinisulcius acutus* (Allen) Siddiqi. Perrine marl soil contained *R. reniformis*, *M. incognita*, *Tylenchorhynchus martini* Fielding, *Psilenchus hilarulus* De Man, and *Aphelenchus* sp., although not all of these nematodes were recovered from samples in every experiment.

Operator variability. Although similar numbers of nematodes were extracted by all three operators in most cases (Table 1), operator B recovered significantly more *H. dihystera* from the Rockdale soil and significantly more *R. reniformis* from the marl soil. Measurement of the pellet heights (mm) in the centrifuge tubes following the first centrifugation (in water) suggested that operator B also recovered more soil particles in the sieving process from the Rockdale soil. The inexperienced operator A never recovered significantly fewer nematodes than did operator C, and often his coefficients of variation were lower than those of either experienced operator (Table 1). Coefficients of variation were similar to those achieved in a previous study using sieving and centrifugation (11). This study indicated the occurrence of some differences and variability in the sieving process due to different operators, and suggested a more in-depth investigation of the steps involved in the sieving process to determine which are the most critical and subject to error.

Soil mixing prior to sieving. Several nematode species showed significant linear or quadratic relationships between numbers recovered and mixing time (Table 2). For example, *C. onoensis* recovered from marl soil (y) was linearly related to mixing time in minutes (x), by $y = 2410 - 124x$, with $r^2 = 0.529$. All significant ($P = 0.05$) linear equations were of similar form, with a negative slope and maximum value when the mixing time equalled zero. The data (Table 2) reflect this general trend toward decreased numbers with increased mixing time, and so the mixing process was discontinued, since it would be an innovation which might reduce nematode recovery.

Table 1. Effect of the operator (A, B, or C) performing the sieving steps on nematode recovery from 100-cm³ soil subsamples.

Nematode	Numbers per 100-cm ³ soil by operator ^z			Coefficients of variation (%) by operator		
	A	B	C	A	B	C
Rockdale fine sandy loam						
<i>Criconemella onoensis</i>	296 a	283 a	342 a	41.9	30.4	30.6
<i>Rotylenchulus reniformis</i>	767 a	1004 a	792 a	11.2	30.0	18.4
<i>Helicotylenchus dihystera</i>	308 b	467 a	354 b	13.2	24.1	25.8
Pellet size in first centrifugation (height in mm)						
	15.0 b	22.0 a	13.7 b	10.3	17.5	25.2
Perrine marl						
<i>Criconemella onoensis</i>	323 a	376 a	328 a	10.8	4.9	18.0
<i>Rotylenchulus reniformis</i>	27 b	62 a	39 b	52.6	29.0	14.9
<i>Tylenchorhynchus martini</i>	8 a	12 a	11 a	31.0	70.4	34.7
Pellet size in first centrifugation (height in mm)						
	16.0 a	16.0 a	15.8 a	18.1	14.2	24.8

^zData are means of 6 replications. Means in rows followed by the same letter are not significantly different ($P=0.05$), according to the Waller-Duncan K-ratio test (5).

Although efficiency of recovery was reduced by the mixing process used here, mixing of soil is considered a process which usually improves reliability and reduces subsample error (6). Coefficients of variation from this experiment did not show a definite improvement with mixing time (data not shown). For example, *M. incognita* and *Q. acutus* from Rockdale soil and *C. onoensis* from marl soil all had coefficients of variation between 10-20%, even at the zero mixing time. On the other hand, coefficients of variation for pellet size in the first centrifugation decreased from 11.5% at zero mixing time to 5.2% after an 8-min mixing time with Rockdale soil, and from 12.3% at zero mixing time to 2.7% after 8-min with Perrine marl soil. Thus, the homogeneity of the soil seemed to improve with mixing, but the distribution of nematodes did not. The mixing process used here was unusual in that it involved large quantities of rock in the soil samples. Improved reliability with nematode extraction could possibly be obtained if the mixing was performed after the sieving step to remove rock (Fig. 1, step #2), rather than before it.

Table 2. Effect of mechanical mixing of the soil sample prior to extraction on nematode recovery from 100-cm³ soil samples.

Nematode	Nematodes per 100-cm ³ soil by mixing time (min) ^y							Significant effects ^z	
	0	1	2	4	8	8	Linear	Quadratic	
Rockdale fine sandy loam									
<i>Meloidogyne incognita</i>	76	72	68	58	54		n.s.	n.s.	
<i>Quisqualis acutus</i>	686	720	700	580	604		*	n.s.	
Pellet size in first centrifugation (height in mm)	23.0	24.6	26.6	23.0	24.8		n.s.	n.s.	
Perrine marl									
<i>Criconemella oncoensis</i>	2265	2520	2290	1600	1510		***	***	
<i>Rotylenchulus reniformis</i>	45	30	45	30	20		n.s.	n.s.	
<i>Tylenchorhynchus martini</i>	125	80	95	170	70		n.s.	n.s.	
<i>Aphelenchus</i> sp.	130	85	115	85	45		**	**	
<i>Psilenchus hilarulus</i>	55	75	40	20	20		*	*	
Pellet size in first centrifugation (height in mm)	22.6	25.2	25.0	25.4	26.0		n.s.	n.s.	

^yData are means of 5 replications.^zAsterisks (*, **, ***) indicate significant effects at P = 0.05, P = 0.01, and P = 0.001, respectively; n.s. = not significant.

Volume of water for dilution of subsample. Varying the amount of water used prior to the initial sieving had no significant ($P=0.05$) effect on numbers of nematodes obtained during a typical extraction or on sizes of pellets obtained in the centrifugation step (Table 3). For both nematode species extracted from the Rockdale soil, significant ($P=0.05$) quadratic relationships were observed between numbers of nematodes recovered from the residues (normally discarded after the first sieving) and the volume of water used for extraction. In both cases, peaks in nematode recovery from residues were obtained at extraction volumes of 3 to 4 L. The significant patterns observed with nematode losses in residues were not observed in the standard counts of nematodes recovered during a typical extraction. This lack of significant differences in nematode recovery with extraction volume is in agreement with other

Table 3. Effect of volume of water used for diluting the subsample on nematode recovery from 100-cm³ subsamples.

Nematode and Location	Nematodes per 100-cm ³ soil by volume of water (L) to dilute subsample ^x				
	1	2	3	4	5
Rockdale fine sandy loam					
<i>Meloidogyne incognita</i> in standard count ^y	112	100	138	108	150
in residue ^z	15	16	36	28	15
Residue as % of standard	13.4	16.2	26.2	25.5	10.0
<i>Quinaculcius acutus</i> in standard count	845	725	682	685	732
in residue	52	64	82	102	46
Residue as % of standard	6.2	8.8	12.1	15.0	6.3
Pellet size in first centrifugation (mm)	13.5	14.2	13.2	13.8	17.2
Perrine marl					
<i>Criconemella onoensis</i> in standard count	1980	2165	1840	1980	1835
in residue	54	32	58	48	45
Residue as % of standard	2.7	1.5	3.1	2.4	2.4
<i>Rotylenchulus reniformis</i> in standard count	90	85	65	70	55
in residue	4	6	2	4	8
Residue as % of standard	4.2	7.4	3.8	5.4	13.6
<i>Tylenchorhynchus martini</i> in standard count	130	140	115	115	120
in residue	10	12	9	8	6
Residue as % of standard	7.7	8.9	7.6	6.5	5.2
Pellet size in first centrifugation (mm)	20.2	19.2	20.2	18.2	23.0

^xData are means of 4 replications. No significant ($P=0.05$) differences with treatment, except that on Rockdale soil, numbers of *M. incognita* and *Q. acutus* lost in the residue showed significant quadratic relationships to the volume of water used.

^yThe standard count is the number of nematodes normally recovered in the extraction process.

^zSoil residue remaining in the bucket after pouring the suspension through the first sieve.

studies in which volume of the subsample was varied while volume of the extraction water was held constant (10,12). In one study, reduced recovery was observed as the ratio between extracting solution and soil volume became as low as 3.5:1 (12). At higher ratios, no differences were observed (10,12), as anticipated for the larger water-soil ratios used here (range 10:1 to 50:1).

Although effect of soil dilution on final nematode counts was negligible, examination of residues for evidence of incomplete suspension indicated some losses of nematodes in these residues, which would normally be discarded. In general, the magnitude of these losses (as a percent of the standard count) was somewhat greater on Rockdale soil (*M. incognita* range 10.0-26.2%; *Q. acutus* range 6.2-15.0%) than for those found in marl soil (*C. onoensis* range 1.5-3.1%; *R. reniformis* range 3.8-13.6%; *T. martini* range 5.2-8.9%).

Table 4. Effect of mixing time of subsample in water on nematode recovery from 100-cm³ subsamples.

Nematode and Location	Nematodes per 100 cm ³ soil by mixing time (sec) of subsample ^x				
	15	30	45	60	90
Rockdale fine sandy loam					
<i>Criconemella onoensis</i> in standard count ^y	417	483	492	450	358
in residue ^z	58	37	33	12	17 **
Residue as % of standard	14.0	7.6	6.8	2.6	4.7
<i>Rotylenchulus reniformis</i> in standard count	700	842	542	1292	975
in residue	137	145	152	98	73
Residue as % of standard	19.6	17.2	28.0	7.6	7.5
<i>Helicotylenchus dihystrera</i> in standard count	250	308	242	333	225
in residue	50	60	27	32	27
Residue as % of standard	20.0	19.5	11.0	9.5	11.9
Pellet size in first centrifugation (mm)	13.0	16.0	16.7	18.0	17.3
Perrine marl					
<i>Criconemella onoensis</i> in standard count	277	303	297	322	293
in residue	35	18	18	17	8 **
Residue as % of standard	12.6	6.0	6.2	5.2	2.8
<i>Rotylenchulus reniformis</i> in standard count	31	28	33	45	50
in residue	6.7	3.3	1.7	0	0 **
Residue as % of standard	21.0	11.8	5.0	0	0
Pellet size in first centrifugation (mm)	15.0	15.7	18.0	18.0	19.7

^xData are means of 3 replications. Asterisks (**) indicate significant (P=0.01) linear and quadratic relationships.

^yThe standard count is the number of nematodes normally recovered in the extraction process.

^zSoil residue remaining in the bucket after pouring the suspension through the first sieve.

Mixing time of subsample in water. Varying the amount of time the subsample was mixed in water did not affect the number of nematodes obtained during a typical extraction (Table 4). The number of nematodes not entering suspension (i.e., those left behind in the residue) showed significant ($P=0.05$) decreases with mixing time for three of five nematode-soil combinations studied (Table 4). In these cases, minimal losses in residues were obtained with a 60-90 sec mixing time, with very short (15 sec) mixing times providing the greatest losses of nematodes in the residue left behind following this soil suspension step. At the 60-sec mixing time, losses in the residue ranged from 0-5.2% on Perrine marl soil and from 2.6-9.5% on Rockdale soil, depending on the nematode species. Percent losses on Rockdale soil at a 60-sec mixing time in 4 L of water in this experiment were lower than those observed in the previous series of experiments on Rockdale soil (15.0-25.5%, Table 3), but nematode species were different. From the data of these experiments, there is evidence that percent loss in the first residue is species-dependent.

Settling time in water. In one instance, *C. onoensis* recovery from marl, nematodes extracted showed significant differences with settling time (Table 5). This relationship was quadratic ($R^2=0.459$, $P\leq 0.05$), and given by:

$$y = 304.2 + 4.018x - 0.0452x^2$$

where $y = C. \textit{onoensis}/100 \text{ cm}^3$ soil and $x =$ settling time in sec. The first derivative, dy/dx , is given by:

$$\frac{dy}{dx} = 4.018 - 0.0904x$$

and when set equal to zero, would yield a maximum nematode recovery at a settling time of 44.4 sec. Losses of *C. onoensis* in residues of Rockdale soil are given by ($R^2=0.460$, $P\leq 0.05$):

$$y = 43.47 - 1.00x + 0.0126x^2$$

Setting the first derivative equal to zero yields a minimum loss in the residue at a settling time of 39.7 sec. Both of these examples suggest that a settling time of 30-60 sec is optimal for maximum recovery of *C. onoensis* and least loss in residues. With other nematode species, settling time did not influence recovery, although results were highly variable for *H. dihystra* on Rockdale soil (Table 5). Pellet size in the first centrifugation decreased as longer settling times permitted more debris to settle out of the soil suspension. However, too much settling time (e.g., 90 sec)

Table 5. Effect of settling time of the soil suspension prior to the first sieving on nematode recovery from 100-cm³ subsamples.

Nematode and location	Nematodes per 100-cm ³ soil by settling time (sec) of suspension ^x						Significant effects	
	0	15	30	60	90	Linear	Quadratic	
Rockdale fine sandy loam								
<i>Criconemella onoensis</i> in standard count ^y	280	292	350	267	242	n.s.	n.s.	
in residue ^z	38	37	30	20	58	n.s.	*	
Residue as % standard	13.7	12.6	8.6	7.5	24.1			
<i>Rotylenchulus reiniformis</i> in standard count	1002	1275	1592	1275	1033	n.s.	n.s.	
in residue	285	248	263	260	272	n.s.	n.s.	
Residue as % of standard	28.4	19.4	16.5	20.4	26.3			
<i>Helicotylenchus dibystrera</i> in standard count	490	558	775	533	425	n.s.	n.s.	
in residue	90	103	110	105	295	n.s.	n.s.	
Residue as % of standard	18.4	18.5	14.2	19.7	69.4			
Pellet size in first centrifugation (mm)	28.3	24.3	19.3	16.0	16.3	***	***	
Perrine marl								
<i>Criconemella onoensis</i> in standard count	290	392	357	387	300	n.s.	*	
in residue	13	8	15	8	13	n.s.	n.s.	
Residue as % of standard	4.6	2.1	4.2	2.2	4.4			
<i>Rotylenchulus reiniformis</i> in standard count	48	37	47	40	18	n.s.	n.s.	
in residue	5.0	1.7	0	1.7	5	n.s.	n.s.	
Residue as % of standard	10.4	4.6	0	4.2	27.3			
Pellet size in first centrifugation (mm)	22.0	20.7	17.3	14.0	15.3	***	***	

^xData are means of 3 replications. Asterisks (*, ***) indicate significant linear or quadratic effects at P=0.05 and P=0.001, respectively; n.s. = not significant.

^yThe standard count is the number of nematodes normally recovered in the extraction process.

^zSoil residue remaining in the bucket after pouring the suspension through the first sieve.

Table 6. Losses on an 850- μ m sieve through successive sievings of 100-cm³ subsamples.

Nematode	Nematodes per 100-cm ³ soil standard count ¹	Nematodes per 100-cm ³ soil remaining on 850- μ m sieve ²				Total of 4 passes	Total loss as percent of standard count ³	Relationship between number of nematodes lost (y) and number of sievings (x) ^z
		1st pass	2nd pass	3rd pass	4th pass			
Rockdale fine sandy loam								
<i>Cricotemella onoensis</i>	242	75.0	33.3	10.3	7.0	126	52.0	not significant, $r^2 = 0.318$
<i>Rotylenchulus reniformis</i>	875	181.7	63.3	34.3	12.0	291	33.3	$y = 170.4 - 122.9 \ln x$, $r^2 = 0.884^{***}$ x-intercept = 4.00
<i>Helicobolenchus dibystrera</i>	1250	200.0	70.0	29.3	9.3	309	24.7	$y = 188.2 - 139.8 \ln x$, $r^2 = 0.911^{***}$ x-intercept = 3.84
Perrine marl								
<i>Cricotemella onoensis</i>	355	23.3	7.0	0.7	0	31.0	8.7	$y = 21.71 - 17.57 \ln x$, $r^2 = 0.862^{***}$ x-intercept = 3.44
<i>Rotylenchulus reniformis</i>	28.3	6.7	1.0	0	0	7.7	27.2	$y = 5.87 - 4.98 \ln x$, $r^2 = 0.489^*$ x-intercept = 3.25

¹Data are means of three replications. The standard count is the number of nematodes normally recovered in the extraction process.

^zAsterisks (*, ***) indicate significant relationships at P=0.05 and P=0.001, respectively.

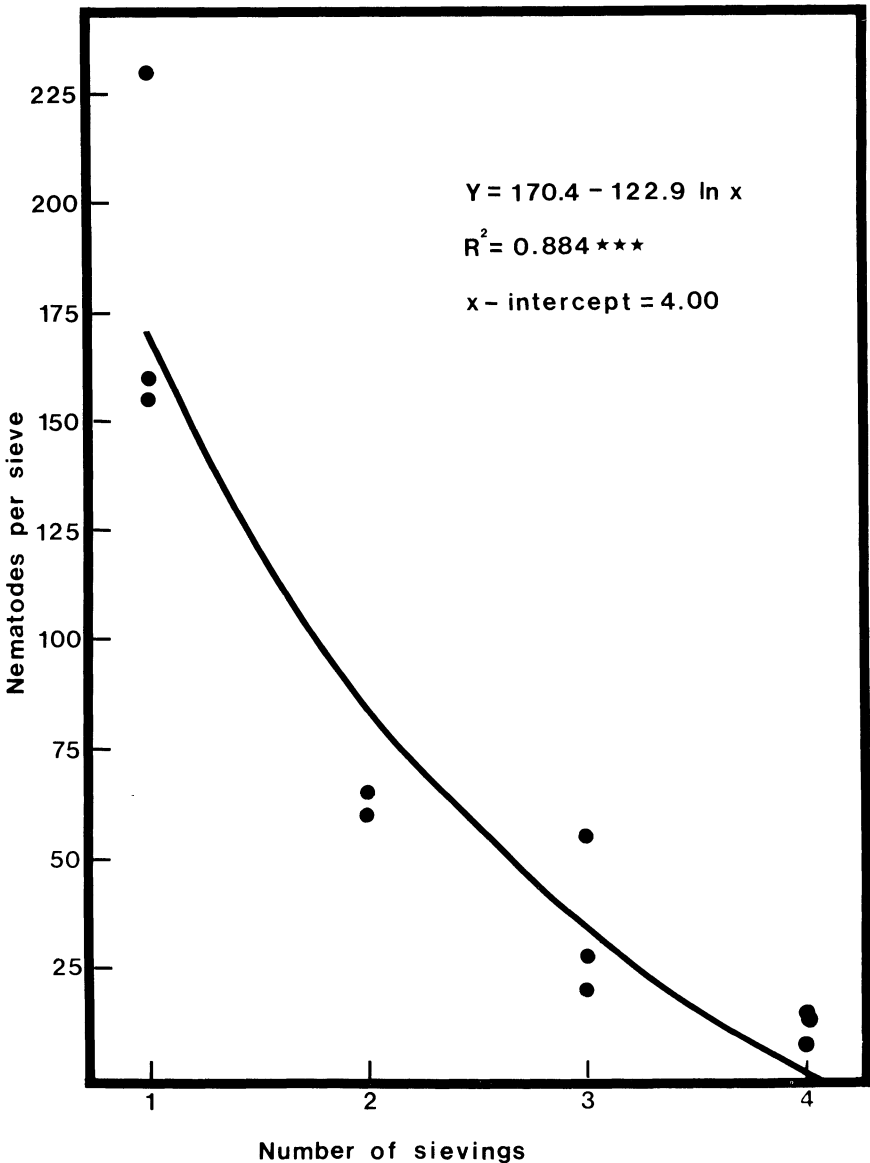


Fig. 2. Relationship between numbers of *Rotylenchulus reniformis* trapped on an 850- μm sieve and number of sievings.

would permit increasing numbers of nematodes to settle out, exerting a significant effect in the case of *C. omoensis*.

Nematode losses in the residues remaining after the first sieving were affected by settling time to some extent, as well as by volume of

water used and time of mixing. Here, losses (as a percent of nematodes in a standard count) ranged from 12.6-19.4% for a 15-sec settling time with Rockdale soil, depending on species, while a range of 2.1- 4.6% was observed for identical conditions with marl soil.

Losses on the first sieve. Substantial numbers of nematodes were trapped in the debris on the first sieve (Table 6). Numbers trapped in the debris declined with successive sievings, following a logarithmic function. For example, on Rockdale soil, numbers of *R. reniformis* obtained on the 850- μm sieve (y) was related ($R^2 = 0.884$, $P \leq 0.001$) to the number of sievings (x) of the debris by (Fig. 2):

$$y = 170.4 - 122.9 \ln x$$

Solution of this equation yields an x -intercept of 4.00, meaning that this equation predicts complete extraction from the residue (zero recovery) after 4 sievings. Similar intercepts were obtained for the other nematode-soil combinations (Table 6), and so it appears that the four sievings used were sufficient to remove almost all nematodes from these residues. If the sum of nematodes lost in these four sievings is considered the total loss on a 850- μm sieve, then it can be seen that losses on this sieve were substantial, ranging from 8.7-52.0% of the nematodes normally obtained in a counted sample. Losses on the 850- μm sieve generally appeared to be greater with Rockdale soil than with marl, probably because of the numerous small rock particles obtained from this soil type which were caught on the sieve, blocking or reducing the effective size of some of the pores.

Nematode recovery was not significantly ($P = 0.05$) affected by substituting a 600- μm sieve for an 850- μm sieve (Table 7), although significantly more debris (g dry wt) was retained on a 600- μm sieve than on an 850- μm sieve in some cases.

Settling time before second sieving. Varying the settling time before passing the soil suspension through a 38- μm sieve had no significant ($P = 0.05$) effects on nematodes recovered from the subsample or on nematodes lost in the residues (Table 8). Losses in these residues were much lower than those observed before the first sieving (Table 5). The higher figures observed in those previous experiments include not only nematodes settling out of the suspension (which should be comparable to results here), but more importantly, those nematodes which did not become suspended in the first place. Thus, settling time at this stage did not seem particularly critical in influencing nematode recovery. Pellet size in the centrifugation step showed linear decreases with settling time, since longer settling times allowed more debris to settle out.

Losses through a 38- μm sieve. Numbers of nematodes recovered from the soil suspension using a 38- μm sieve decreased rapidly following the

Table 7. Comparison of number of nematodes extracted using an 850- μm sieve or a 600- μm sieve for the first sieving of 100- cm^3 subsamples.

Parameter evaluated	Sieve size (μm) ^y		t-statistic ^z
	850	600	
Rockdale fine sandy loam, first test			
<i>Criconemella onoensis</i> /100 cm^3 soil	108	125	-0.58
<i>Rotylenchulus reniformis</i> /100 cm^3 soil	1546	1479	0.62
<i>Quinisolcius acutus</i> /100 cm^3 soil	54	71	-0.82
Pellet size in first centrifugation (mm)	18.2	17.3	1.43
Debris remaining on sieve (g dry wt)	0.38	0.47	-1.63
Rockdale fine sandy loam, second test			
<i>Criconemella onoensis</i> /100 cm^3 soil	33	71	-1.83
<i>Rotylenchulus reniformis</i> /100 cm^3 soil	1550	1738	-1.56
<i>Quinisolcius acutus</i> /100 cm^3 soil	33	62	-2.15
Pellet size in first centrifugation (mm)	20.3	21.8	-1.41
Debris remaining on sieve (g dry wt)	0.48	0.79	-4.27**
Perrine marl			
<i>Criconemella onoensis</i> /100 cm^3 soil	1325	1555	-1.55
<i>Tylenchorhynchus martini</i> /100 cm^3 soil	80	45	1.19
Pellet size in first centrifugation (mm)	22.6	25.0	-1.06
Debris remaining on sieve (g dry wt)	0.11	0.33	-2.48*

^yData are means of 6 replications.

^zAsterisks (*,**) indicate significant differences at $P=0.05$ and $P=0.01$, respectively.

first sieving (Table 9). Subsequent sievings recovered significantly fewer nematodes than the first sieving, with numbers recovered by successive sievings declining in a logarithmic manner (Table 9). When numbers recovered in all 5 sievings were totalled, 96-99% of all nematodes recovered were obtained in the first sieving. These results are in agreement with previous work (10) which found no differences in recovery whether one pass or two passes through a 38- μm sieve were used. Thus, only one sieving with a 38- μm sieve should be sufficient in most cases. When two sievings are used, as for the standard procedure outlined here (Fig. 1), only 0-1.5% of the number of nematodes counted could be expected to be lost by passing through these sieving steps (Table 9).

Settling and decanting. In no instances were nematode counts significantly affected by settling time and subsequent decanting (Table 10). Nematodes were rarely found in water decanted from the samples. Only with *R. reniformis* in one test on Rockdale soil were low numbers recovered from decanted water, averaging only 0.12 to 0.41% of the nema-

Table 8. Effect of settling time of the soil suspension prior to the second sieving on nematode recovery from 100-cm³ subsamples.

Nematode and location	Nematodes per 100-cm ³ soil by settling time (sec) of suspension ^x				
	0	15	30	60	90
Rockdale fine sand loam					
<i>Criconebella onensis</i> in standard count ^y	264	172	324	278	216
in residue ^z	6	2	4	6	8
Residue as % of standard	2.3	1.2	1.2	2.2	3.7
<i>Rotylenchulus reniformis</i> in standard count	1008	1116	1308	1036	1128
in residue	4	6	8	4	6
Residue as % of standard	0.4	0.5	0.6	0.4	0.5
<i>Helicotylenchus dihystera</i> in standard count	588	830	780	876	660
in residue	8	8	8	10	6
Residue as % of standard	1.4	1.0	1.0	1.1	0.9
Pellet size in first collection (mm)	19.0	18.3	16.3	12.3	9.7***
Perrine marl					
<i>Criconebella onensis</i> in standard count	312	296	284	254	283
in residue	4	5	6	9	8
Residue as % of standard	1.3	1.7	2.1	3.5	2.8
<i>Rotylenchulus reniformis</i> in standard count	24	44	42	38	30
in residue	2	0	0	0	1
Residue as % of standard	8.3	0	0	0	3.3
Pellet size in first centrifugation (mm)	17.3	19.0	15.0	13.0	12.0***

^xData are means of 3 replications. Asterisks (***) indicate significant (P=0.001) linear and quadratic relationships.

^yThe standard count is the number of nematodes normally recovered in the extraction process.

^zSoil residue remaining in the bucket after pouring the suspension through the second (38 µm) sieve.

todes counted in a standard sample, and showing no significant differences with settling time (data not shown). Rinsing sieves with 200 ml of water prior to settling and decanting (Fig 1, step #11) appeared to be adequate, since no additional nematodes could be rinsed from 38-µm sieves which had already been rinsed with 200 ml of water.

Although losses involved in rinsing sieves and decanting were minimal, substantial losses of nematodes did occur at some points of the extraction process. Losses occurring at specific steps are summarized (Table 11) for selected nematode species. Losses are expressed as a percent of the nematodes counted in a standard sample, since methods for determining losses varied, but the standard methodology (Fig. 1) did not. Furthermore, it should be noted that these tabled values are probably conservative, since obtaining counts of nematodes lost, using

Table 9. Numbers of nematodes retained on a 38- μ m sieve following successive sievings of a 100-cm³ subsample suspension.

Nematode	Nematodes per 100-cm ³ retained on a 38- μ m sieve ^y					Relationship between number of nematodes per sieve (y) and number of sievings (x) ^z
	1st pass	2nd pass	3rd pass	4th pass	5th pass	
Rockdale fine sandy loam						
<i>Criconemella oncoensis</i>	283	1.7	0	0	0	y = 218.1 - 168.2 ln x, r ² = 0.690***
<i>Rotylenchulus reniformis</i>	1192	15.0	2.0	0.3	0.3	y = 920.1 - 708.3 ln x, r ² = 0.704***
<i>Helicotylenchus dihystrera</i>	750	16.7	1.7	0.7	0.3	y = 581.7 - 446.8 ln x, r ² = 0.715***
Perrine marl						
<i>Criconemella oncoensis</i>	415	9.0	0.3	0	0	y = 321.8 - 247.4 ln x, r ² = 0.716***
<i>Rotylenchulus reniformis</i>	43	1.0	0	0.7	0	y = 33.6 - 25.7 ln x, r ² = 0.687***

^yData are means of three replications. In the standard procedure (Fig. 1), nematodes recovered in the first two sievings are normally saved and become the "standard count," while those recovered in sievings 3-5 would normally be lost by passing through the sieve.

^zAsterisks (***) indicate significant relationships at P = 0.001.

Table 10. Effect of settling time of sieved sample on nematode recovery from 100-cm³ subsamples.

Nematode	Nematodes per 100 cm ³ soil by settling time (min) of the sieved subsample ^x			
	15	30	60	120
Rockdale fine sandy loam, first test ^y				
<i>Criconemella onoensis</i>	—	106	103	122
<i>Rotylenchulus reniformis</i>	—	534	572	577
<i>Helicotylenchus dihystera</i>	—	169	157	152
<i>Quinisulcius acutus</i>	—	14	10	15
Rockdale fine sandy loam, second test ^z				
<i>Criconemella onoensis</i>	1338	1319	1394	1610
<i>Rotylenchulus reniformis</i>	38	56	31	35
<i>Quinisulcius acutus</i>	94	50	106	124
Perrine marl, first test ^y				
<i>Criconemella onoensis</i>	—	162	165	194
<i>Rotylenchulus reniformis</i>	—	22	22	22
Perrine marl, second test ^z				
<i>Tylenchorhynchus martini</i>	462	472	480	478
<i>Meloidogyne incognita</i>	72	52	48	88

^xNo significant ($P=0.05$) differences with settling time found in any test.

^yData are means of 6 replications.

^zData are means of 4 replications.

methods which do not extract 100% of all nematodes, will consistently underestimate loss. Losses occurring in several steps were quite low, losses due to inadequate suspension of the initial soil sample were moderate, and those on the coarse sieve (850- μ m) were quite large. This loss was especially severe with Rockdale soils, since small rock particles accumulated on this sieve to some extent, and evidently were quite effective in retaining nematodes. Total losses ranged from 13.7 to 60.8%, depending on nematode species and soil type (Table 11). Extraction efficiency (2,4,16) could be calculated from these loss figures as efficiency = $100/(y + 100)$, where y is the number of nematodes lost per 100 nematodes counted from a standard extraction. Calculated extraction efficiencies ranged from 62.2 to 88.0%, but these are maximum extraction efficiencies (loss estimates are conservative), and are for the sieving process only (Fig. 1), without considering centrifugation.

Results of this study suggest that efficiency of the sieving process outlined here (Fig. 1) could be improved in several ways. An optimized

Table 11. Estimated nematode losses during a sieving process.

Extraction loss (Source of data)	Losses as percent of nematodes counted in a standard count by soil type ^x					
	Rockdale fine sandy loam			Perrine marl		
	<i>Criconemella onoensis</i>	<i>Rotylenchulus reniformis</i>	<i>Helicotylenchus dihystera</i>	<i>Criconemella onoensis</i>	<i>Rotylenchulus reniformis</i>	
Incomplete suspension, settling out of first suspension (Table 3)	—	—	—	—	2.4	5.4
" " (Table 4)	2.6	7.6	9.5	5.2	0	0
" " (Table 5)	12.6	19.4	18.5	2.1	4.6	4.6
Mean of above three lines	7.6	13.5	14.0	3.2	3.3	3.3
Losses on 850- μ m sieve (Table 6)	52.0	33.3	24.7	8.7	27.2	27.2
Settling out of second suspension (Table 8)	1.2	0.5	1.0	1.7	0	0
Losses through 38- μ m sieve (Table 9)	0	0.2	0.4	0.1	1.5	1.5
Losses during decanting (text)	0	0.4	0	0	0	0
Total losses ^y	60.8	47.9	40.1	13.7	32.0	32.0
Maximum extraction efficiency ^z	62.2	67.6	71.4	88.0	75.8	75.8

^xA standard count is the number of nematodes normally recovered in the extraction process, according to steps outlined in Fig. 1. Dashes (—) indicate no data obtained.

^ySum of above 5 lines.

^zExtraction efficiency = 100/(total loss + 100); expressed as percent in table (x 100%).

sieving process would include: no mechanical mixing of the bulked soil sample (step #1); utilizing a large volume of water for extraction (step #4); mixing the sample into suspension for 60-90 sec (step #5); allowing the suspended sample to settle 30-60 sec before pouring (step #6); using a sieve much larger than 600-850 μ m to remove debris and particles from these soils (step #7); maintaining a 15-sec settling time before fine sieving (step #8); using only one pass through a 38- μ m sieve (steps #9-10); maintaining rinsing and decanting procedures (steps #11-13) for minimal losses.

It is evident that nematode extraction methodology must be greatly improved to facilitate reliable quantitative work (4,9,15). Individual stages in multistep extraction processes must be carefully evaluated to determine which are error-prone and in need of improvement. Such studies can be useful in estimating efficiency of extraction, although more extensive and detailed studies are probably needed before reliable extraction efficiency figures can be utilized in practice. Although nematode soil counts may contain a high degree of imprecision resulting from the sampling process, it may be unnecessarily risky to attempt to "adjust" soil counts to some hypothetical level of "actual" nematodes present using extraction efficiency figures which themselves may contain a high degree of imprecision. The present study has revealed some variation in estimates of extraction efficiency, depending on nematode species, soil type, operator, and even within steps of the extraction procedure itself.

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