EFFECT OF SIEVE SIZE ON NEMATODE EXTRACTION EFFICIENCY

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ABSTRACT

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A combination sieving and centrifugation procedure was used to compare the efficiency of recovering nematodes from soil suspensions using sieves having openings of various sizes: 53 μm, 45 μm, 38 μm, or 25 μm. With the 53 μm, 45 μm, and 38 μm sieve sizes, there were no differences in numbers extracted for larger nematodes such as *Helicotylenchus dihystera* or *Criconemella* spp., but the 38 μm sieve was the most efficient in recovering larvae of *Rotylenchulus reniformis*. Because the openings of the 25 μm sieve became clogged with soil particles, nematode recovery was poor from that sieve size for the Rockdale soil type tested here. The effect of decanting nematode suspensions in sucrose solutions onto sieves having 45 μm or 25 μm openings was also examined. In this case, recovery of *H. dihystera* was unaffected by sieve size, but the 25 μm sieve was more suitable for recovering *R. reniformis* larvae. In two tests, rinsing the nematodes on the sieve to remove the sugar solution resulted in a significant (P = 0.05) loss of *R. reniformis*. Results of these experiments indicate that the choice of sieve size in nematode extraction is very critical in obtaining quantitative data on small plant-parasitic nematodes.

Additional key words: reniform nematode, spiral nematode, soil type, Carica papaya, Ipomoea batatas, Manihot esculenta, Phaseolus aureus.

RESUMEN

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La eficiencia en la extracción de nematodos en suspensiones de suelos, fué estudiada usando combinaciones de tamices y procedimientos de centrifugación. Se usaron tamices con aberturas de varias medidas: 53 μm, 45 μm, 38 μm, y 25 μm. Con los tamices de 53 μm, 45 μm y 38 μm no se encontraron

diferencias en el número extraido para los nematodos más largos como el *Helicotylenchus dihystera* o el *Criconemella* spp. Sin embargo el tamiz de 38 μm fué el más eficiente en la recuperación de las larvas de *Rotylenchulus reniformis*. Debido a que la abertura del tamiz de 25 μm se obstruyó con las partículas de suelo, la recuperación de los nematodos con dicho tamiz y el tipo de suelo Rockdale usado fué pobre. El efecto de la decantación de las suspenciones de nematodos en soluciones de azúcar a través de tamices con aberturas de 45 μm y 25 μm fué también examinada. En este caso la recuperación de *H. dihystera* no fué afectada por el tamaño del tamiz pero el tamiz de 25 μm fué más aproriado para recobrar las larvas de *R. reniformis*. En los dos ensayos, el enjuagar los nematodos para remover la solución de azúcar resultó en una pérdida significativa (P = 0.05) de *R. reniformis*. Los resultados de estos experimentos indican que en la extracción de los nematodos la selección del tamaño del tamiz es muy crítica en la obtención de datos cuantitativos de los nematodos pequeños parasíticos de las plantas.

Palabras claves adicionales: Nematodo reniforme, nematodos espirales, tipo de suelo, Carica papaya, Ipomaea batatas, Manihot esculenta, Phaseolus aureus.

INTRODUCTION

The extraction of nematodes from soil is one of the most critical operations carried out by a nematology laboratory. A variety of extraction methods are available (1, 2, 3, 4, 5, 6, 7, 10, 11); however, at some stage of the procedure, most methods involve the use of wire mesh sieves. Debris is usually eliminated from soil suspensions in water by using coarse sieves, with most species of plant-parasitic nematodes actually being retained on a sieve with very fine pores. If several sieves are involved, the sieve with the smallest openings is used last, thus becoming the most critical sieve in nematode recovery. However, the size of the openings in the last sieve used varies widely from laboratory to laboratory. Pore sizes used in the final sieve have ranged from 53 μm (6, 7) to 45 μm (1, 7, 9) to 38 μm (11) to 25 μm (2). Such a range in sieve sizes makes it difficult to compare quantitative data obtained by various laboratories.

The present study compares nematode extraction from soil samples using sieves of various pore sizes to determine if differences in extraction efficiency are related to sieve size, and if so, to determine the most efficient sieve size to be used.

MATERIALS AND METHODS

In this study, the basic method used for extracting nematods from soil samples was the combination sieving-centrifugation procedure of Jenkins (7), modified as described elsewhere (9). This procedure involves sieving at two

stages: 1) initially, to remove nematodes from the soil suspension, and 2) after the centrifugation process, to remove nematodes from sugar solutions. Four experiments were performed to evaluate sieve size efficiency during these two stages. In Experiments 1 and 2, the initial sieve size was varied while the sieve size after centrifugation was held constant; in Experiments 3 and 4, the sieve size used after centrifugation was varied while the initial sieve size was held constant.

In each experiment, a large composite soil sample was collected from the rhizosphere of a crop growing on a Rockdale fine sandy loam soil in south-eastern Florida. Nematodes extracted during each experiment were killed by heating at 55-60°C and counted. Counts were analyzed by an analysis of variance followed by Duncan's New Multiple Range Test.

Effect of Final Sieve Size on Soil Suspensions

A. Experiment 1. The soil sample for this experiment was collected from white-fleshed sweet potato (*Ipomoea batatas* L.) on April 22, 1980. In the laboratory during the soil extraction step, the soil sample was passed through a 4.0mm sieve to remove rock, and 800cm^3 of soil was then suspended in five liters of water. Next, the soil suspension was passed through a 20-mesh sieve (850 μm openings) to remove debris, and the filtrate was divided into eight well-mixed and equal portions. Each of these portions was diluted with water to a total volume of five liters. Each of the eight diluted portions was then passed through one of four different sieve sizes: 270-mesh (53 μm openings), 325-mesh (45 μm openings), 400-mesh (38 μm openings), or 500-mesh (25 μm openings), and passed either once or twice through a given sieve. Processing was done in a random order for the eight combinations of sieve size and number of passes. After the last pass through a given sieve, nematodes were washed from the sieve and further separated by centrifugation.

In the centrifugation step, residues from each of the sieves were centrifuged in water for 5 minutes (R.C.F. = 1610 G), after which the supernatant solution was discarded. The pellet was resuspended in a sucrose solution (454 g/l of water) and centrifuged for one additional minute. The supernatant solution was decanted onto a 500-mesh sieve (25 μm openings). The entire procedure was replicated five times.

B. Experiment 2. The soil sample for this experiment was collected from cassava (*Manihot esculenta* Crantz) on March 12, 1981. Extraction procedures were identical to those in Experiment 1, except that the initial volume of soil suspended in five liters of water was 600 cm^3 rather than 800 cm^3, and the 500-mesh sieve was not used in the soil extraction step. The exclusion of the 500-mesh sieve eliminated two treatments, thus making a total of six combinations.

Effect of Sieving After Centrifugation

A. Experiment 3. Soil for this experiment was collected from papaya

(*Carica papaya* L.) on March 25, 1981. The soil sample was passed through a 4.0 mm sieve to remove rock, and 1000 cm^3 was suspended in five liters of water. The soil suspension was then passed through a 20-mesh sieve to remove debris, and the filtrate was divided into five equal portions which served as the five replications. The soil suspension from each replication was diluted with water to five liters, and passed twice through a 325-mesh sieve (45 μm openings) to collect the nematodes. Next, the nematodes were washed from the sieve, and the resulting suspension was divided into four equal volumes and poured into four 50 cm^3 centrifuge tubes. These were centrifuged for 5 minutes (R.C.F. = 1610G), after which time the supernatant solutions were discarded. The pellets were resuspended in sucrose solutions (454 g/1 of water) and centrifuged for one minute. Each of the four portions was subjected to one of the following procedures, in random order: 1) decantation onto a 325 mesh sieve (45 μm openings); 2) decantation onto a 500-mesh sieve (25 μm openings); 3) decantation onto a 325-mesh sieve followed by a 10-second rinse with a stream of water from a 500 cm^3 wash bottle; or 4) decantation onto a 500-mesh sieve followed by a similar 10-second rinse. Immediately after the decantations, nematodes were washed from each of the sieves and the centrifugation of the other replications was completed.

B. Experiment 4. Procedures used in this experiment were identical to those of Experiment 3, except that the soil sample was collected from mung bean (*Phaseolus aureus* L.) on July 6, 1981.

Additional Aspects of the Extraction Procedure

During the course of this study, two additional questions arose which were examined by two brief experiments. In the first case, the effect of passing a soil suspension once or twice through the final sieve was investigated further. A soil sample collected from *C. papaya* was processed according to the procedures used in Experiment 1, except that 300 cm^3 of soil was used initially. The following three treatments were used: 1) soil suspension passed once through a 38 μm sieve; 2) soil suspension passed twice through a 38 μm sieve; or 3) soil suspension passed twice through a 38 um sieve, but nematodes washed from the sieve after each pass. In the first two treatments, nematodes were washed from the sieve only after the last pass through the sieve (as in Experiments 1 and 2). This experiment was replicated five times.

A second brief experiment investigated the size of the soil sample used to make up the soil suspension to be sieved. Five soil samples of 100 cm^3 each were taken from a single large (1500-2000 cm^3) soil sample which had been sifted through a 4.0 mm sieve to remove rock, and each of these five samples were processed by sieving and centrifugation. In addition, a 500 cm^3 soil sample was also taken from the original large sample and processed by the same sieving and centrifugation procedure. After extraction and centrifugation, this sample (from the 500 cm^3 soil) was divided into five equal portions for comparison with the first five 100 cm^3 samples which had each originated from an individual 100 cm^3 soil sample. This procedure was repeated for ten nematode-crop combinations.

RESULTS

Effect of Final Sieve Size on Soil Suspensions.

Results of Experiments 1 and 2 are given in Table 1. The most common nematodes present in the sites sampled were *Rotylenchulus reniformis* Linford and Oliveira, *Quinisulcius acutus* (Allen) Siddiqi, *Criconemella* spp. (8), and *Helicotylenchus dihystera* (Cobb) Sher. Depending on the nematode species involved, the results were quite different.

With the larger nematodes, such as *Criconemella* spp., or *H. dihystera* with a mean length of 670 μm and width calculated at 23 μm (13), there were no significant (P = 0.05) differences in numbers extracted with sieves of different sizes. In comparison, *R. reniformis* larvae are among the smallest plant-parasitic nematodes likely to be present in soil samples, with average lengths of 350-410 μm and widths calculated at about 17 μm (12). Because of its small size, this nematode is capable of passing through pores of any of the sieves and provides a most critical test of sieve-size efficiency. Sieves having 38 μm openings appeared to be consistently the most effective in extracting *R. reniformis.* With larger pore sizes, some loss of very small nematodes may occur, but for larger nematodes, sieve size within the range used makes little difference.

Sieves having 25 μm openings proved unmanageable in sieving the soil suspensions in Experiment 1, and because of this, the use of a 25 μm sieve in the soil suspension step was discontinued for Experiment 2. While soil suspensions could easily be passed through sieves having pore sizes of 38 μm or greater, the pores of the 25 μm sieve became clogged with soil particles. As a result, approximately 20 min. were needed to pass the soil suspension through this sieve, which is normally a 1-2 min. process with the soil type used in these experiments. Over such a long period of time (20 min.), it may be possible for larger nematodes and soil particles to settle to the bottom of the container, or even for very small nematods to crawl through the pores of the sieve while the material is draining slowly. In either case, it would lead to an underestimation of nematode populations.

A soil suspension could be passed twice through a given sieve, rather than once, to catch nematodes that may have passed lengthwise through the pores the first time. Although there occasionally were trends toward increased nematode recovery by passing the soil suspension through the sieve twice, there were no significant (P = 0.05) differences between passing the soil suspension through once or twice for any sieve-nematode combination (Table 1). It is possible, however, that a second effect may have been involved here in that passing the soil suspension through the same sieve twice could wash through some nematodes that were retained after the first pass. These two effects could have balanced each other to produce the nonsignificant results obtained for this portion of Experiments 1 and 2.

Thus, the additional experiment described previously was performed to attempt to separate these two effects. In addition to the treatments of passing

Table 1. Numbers of nematodes extracted from soil samples by passing soil suspensions through sieves of various sizes.

Sieve size (μm)	Number of times passed through sieve	Nematodes per 100 cm^3 soil^x				
		Experiment 1			Experiment 2^y	
		Rotylenchulus reniformis	Quinisulcius acutus	Criconemella spp.	Rotylenchulus reniformis	Helicotylenchus dihystera
25	1	221 ab	20 ab	42 a	-	-
25	2	183 a	13 a	46 a	-	-
53	1	274 bc	36 b	62 a	54 a	743 a
53	2	307 cd	25 ab	76 a	60 ab	748 a
45	1	271 bc	37 b	73 a	68 abc	778 a
45	2	288 bc	28 ab	72 a	78 bc	731 a
38	1	338 cd	33 b	77 a	83 c	781 a
38	2	374 d	26 ab	76 a	79 bc	751 a

^{x}Mean of 5 replications. Means in columns followed by the same letter were not significantly different (P = 0.05), according to Duncan's New Multiple Range Test.

^{y}The 25 μm sieve was not used in Experiment 2.

Table 2. Numbers of nematodes obtained after centrifugation when sugar solution is decanted onto sieves of two different sizes with or without rinsing.

Sieve size (μm)	Treatment	Nematodes per 50 cm^3 soil[x]		
		Experiment 3	Experiment 4	
		Rotylenchulus reniformis	Rotylenchulus reniformis	Helicotylenchus dihystera
45	Rinse	2123 a	84 a	32 a
25	Rinse	2250 a	146 b	39 a
45	No rinse	2388 ab	142 b	44 a
25	No rinse	2793 b	218 c	46 a

[x]Mean of 5 replications. Means in columns followed by the same letter were not significantly different (P = 0.05), according to Duncan's New Multiple Range Test.

the soil suspension once or twice through the sieve, the third treatment consisted of passing the soil suspension twice through the sieve, but washing the nematodes from the sieve after each pass, as described previously. This third treatment avoids the possible loss of washing nematodes through the sieve during the second pass. Mean numbers of *R. reniformis* per 100 cm^3 of soil were: 963 when passed through the sieve once, 966 when passed through twice washing only the last sieve, and 946 when passed through twice washing each sieve. Differences among the three methods of sieving were not statistically significant. No loss in nematodes was found by passing the soil suspension twice through the same sieve, particularly if the suspension is poured through a different area of the sieve each time. Adding the extra step of washing the nematodes from the sieve between passes increased the coefficient of variation for the five replications from 5.8% to 9.8%.

Effect of Sieving After Centrifugation

Table 2 illustrates that the decanting of nematodes suspended in sugar solutions after centrifugation can also be a source of error in the extraction process. With larger nematodes, such as *H. dihystera*, pore sizes of the sieves onto which the solution is decanted made little difference. With the smaller *R. reniformis*, however, a sieve with 25 μm openings was superior and resulted in significantly (P = 0.05) greater nematode recovery in Experiment 4. Rinsing the sugar solution from the sieve containing the nematodes for even as little as 10 sec. led to a significant loss of *R. reniformis* regardless of sieve size. While it may be desirable to rinse specimens that will be used in taxonomic work, it is not to be recommended if quantitative data are desired since small nematodes can be lost.

DISCUSSION

Results of these experiments, along with previous information, makes it possible to develop an optimum procedure for quantitative extraction of nematodes from soil by the combination of sieving and centrifugation. However, one factor that was not considered here is the size of the initial soil sample to be extracted. The relationship between the size of the soil sample extracted and the volume of the extracting solution used to make a soil suspension has been previously investigated (11), with the result that nematode recovery from 100 cm^3 soil samples was reduced with a ratio between an extracting solution and soil volume of 3.5 to 1 compared to a ratio of 5 to 1 or greater. Since consistent recovery was obtained with the larger extracting solution to soil ratios, it is anticipated that the 50 to 1 volume ratio (5 liters water to 100 cm^3 soil) used here should be adequate. In the study (11) where varying initial volumes of soil were used, there was proportionally less nematode recovery at 50 cm^3 increments above a 100 cm^3 soil sample, suggesting that no advantage could be obtained by using larger initial soil samples.

In order to further investigate this factor, the additional experiment, dealing with size of the initial soil sample, was carried out as described in Materials and Methods. One may anticipate that the five samples taken from the 500 cm^3 soil sample and divided later when the nematodes were in water would be very well mixed, while the five 100 cm^3 individual samples would not be as well mixed. Although this experiment was repeated for a number of nematode-crop combinations, no consistent differences were found in the coefficients of variation (CV) obtained by either of the two methods. The CV was greater about as often when the larger (500 cm^3) sample was used as when the smaller (100 cm^3) samples were used. Mean numbers of nematodes recovered by these two methods were similar, except in one case, when significantly (P = 0.05) more *H. dihystera* were recovered from the five 100 cm^3 samples taken from malanga (*Xanthosoma* spp.), compared to the corresponding 500 cm^3 sample taken from this crop, with means of 186/100 cm^3 of soil and 94/100 cm^3 of soil, respectively. This observation of a greater number of nematodes recovered from the smaller initial sample is in agreement with the results of Rodríguez-Kábana and King (11). Thus there is apparently little or no advantage to using the larger initial soil sample, and 100 cm^3 should be sufficient.

Previous work (9) has demonstrated that not only is a combination of sieving and centrifugation more efficient than motility-dependent methods, but also that this method is less variable. Assuming that a combination of sieving and centrifugation is the chosen extraction method, an optimum protocol can be proposed to give the most efficient results for quantititive work. An initial soil sample of 100 cm^3 can be suspended in five liters of water and passed through a coarse sieve (such as 20-mesh) to eliminate debris. The soil suspension is then passed once or twice through a 400-mesh (38 μm openings) sieve, provided that the soil type used does not clog the sieve.

Residues washed from this sieve can be separated further by centrifuging for five minutes in water. The supernatant solution is then discarded and the pellet resuspended in a sucrose solution and centrifuged for one additional minute. Maximum recovery will be obtained if the sugar solution containing the nematodes is decanted onto a 500-mesh (25 μm openings) sieve without rinsing the sugar solution from the nematodes on the sieve. In this manner, small nematodes will not be forced through the sieve and lost. Nematodes then may be washed from the sieve with water (at least 40-50 ml) to lessen the effects of the sugar solution and to prepare them for further study. If nematode counts have previously been obtained using other sieve sizes or extraction techniques, they still can be compared quantitatively with counts obtained by the methods outlined here if the appropriate regression equations are developed. Methodology for obtaining regression equations relating two different extraction techniques has been developed elsewhere (9,11).

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