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Comparison of Four Methods for Isolating Nematodes from Soil Samples[1]

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INTRODUCTION

The extraction of nematodes from soil samples is possibly the most important task in nematology, not only for the economic nematologist but also for the soil scientist. For the latter, an accurate extraction gives important information on the relationships of organisms under natural conditions. For the economic nematologist use of a reliable method, in most cases, is the only means by which he can obtain knowledge of the plant nematodes in a specific soil and make predictions of crop damage. Subsequent recommendations on the proper method of control to be employed depend upon a conscientious study of the situation after isolating the parasites.

There are several methods for the isolation of nematodes from soil samples, but these are not always adapted to every given condition. Some investigators use the simplest and easiest method, while others have attempted to discriminate between procedures as necessitated by their special conditions. Accordingly, four methods currently in use were evaluated. Data will be presented on tests conducted in Puerto Rico and Florida, with different types of soil, and using the Oostenbrink Flotation Apparatus III (OFA), a modified Oostenbrink Elutriator (FCES), a modified sugar-flotation technique (SF), and a Sieving Petri-dish method (SPD).

PROCEDURES AND METHODS

Four main trials were conducted. In the first two trials, conducted independently in Puerto Rico by two of us, two types of soil were used: A

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Bayamón fine loamy sand containing 84.2 percent of sand, 12.9 of clay, and 2.9 of silt, and a Río Piedras clay containing 40.4 percent of sand, 47.8 of clay, and 11.8 of silt. Only two methods were tested: The Oostenbrink Flotation Apparatus III (OFA) and the modified sugar-flotation technique (SF), using 150-cc. soil samples, and replicated five times.

The third trial was conducted at the Florida Citrus Experiment Station, Lake Alfred, where three methods were used: The SF, the OFA, and the modified Oostenbrink Elutriator (FCES). Two types of soil were processed, one a Manatee fine sandy clay, made up to 17.5 percent of organic matter, 47 of sand, 10 of coarse silt, 2.2 of fine silt, and 40.8 of clay; and the other, a Lakeland fine sand was made up of 0.2 percent of organic matter, 97.9 of sand, 0.8 of silt, and 1.3 of clay. Nine replicate samples were processed for each method. Sand samples measured 250 cc. while clay samples of 200 cc. were used.

The fourth trial, conducted in Puerto Rico, consisted of five replicates of four kinds of soils in five different volumes, processed by three different methods. The SF, OFA, and the Sieving Petri-dish (SPD) methods were used in this test. A sandy soil from an abandoned pineapple field near Vega Alta, on the northern coast of Puerto Rico, was selected as the type sandy soil. The clay soil was obtained from a banana plantation close to Río Piedras.

After elimination of plant debris, the soils were thoroughly mixed in two proportions so as to obtain four experimental types. These were: 1, a "pineapple" soil containing 92.9 percent of sand, 2.1 of silt, and 5.0 of clay, designated as "sand"; 2, a "banana" soil containing 17.1 percent of sand, 32.0 of silt, and 50.9 of clay, designated as "silty clay"; 3, a mixture of 3 parts of "sand" and 1 part of "silty clay" containing 80.0 percent of sand, 12.5 of silt, and 7.5 of clay, designated as "loamy sand"; and 4, a mixture of 3 parts "silty clay" and 1 part "sand" containing 48.8 percent of sand, 20.0 of silt, and 31.2 of clay, designated as "clay loam". A total of 270 samples consisting of 75, 150, 300, 600, and 1,000 cc. of soil was processed. Only "sand" and "silty clay" were processed in 1,000-cc. volumes.

SUGAR FLOTATION TECHNIQUE (SF)

Caveness and Jensen (*1*)[3] originally used small volumes of soil mixed with water within tubes. After centrifugation for 5 minutes at 4,800 r.p.m. the supernatant liquid was decanted and a sugar-water syrup, 484.5 gm. of sugar per liter of water, was added to each tube which was then centrifuged an additional 5 minutes. Acknowledgment is made to Dr. A. A. DiEdwardo who proposed to one of the authors (ACT) the essential steps of the following modified technique. All samples except the sand were agitated with

[3] Italic numbers in parentheses refer to Literature Cited, p. 225.

water, using a Waring Blendor for 15 seconds. The supernatant mixture was passed through a 10-mesh sieve, additional water was added, and this mixture was passed three times through a 325-mesh sieve. The retained debris was placed in tubes and centrifuged for 4 minutes at 2,200 r.p.m., after which the supernatant liquid was discarded. A solution of 1 pound of sucrose per liter of water was added to each tube and the contents were thoroughly dispersed. Tubes were then centrifuged for 2 minutes and the supernatant fraction was passed twice through a 325-mesh sieve. Debris retained on the sieve was placed in water and nematode counts were made from suitable aliquots.

OOSTENBRINK FLOTATION APPARATUS III (OFA)

This Oostenbrink elutriator was used as specified (*4*) except for a few modifications. Soil samples were dispersed in water, using a Waring Blendor. For the three trials conducted in Puerto Rico, 800 cc. of water per minute were introduced into the apparatus for the first 10 minutes, after which the rate of flow was lowered to 400 cc. In Florida this underflow was 700 cc. throughout the washing period. After washing, the liquid contents in the apparatus were passed through a nested series of two 75- and 325-mesh-per-inch sieves. In Florida the collected residues were placed on special cotton-wool filters made in Holland and put in receptacles with a little water. In Puerto Rico the residues were placed in Petri dishes with facial-tissue filters supported by a 17-mesh screen. In all cases nematodes passing through the pores of the filters were collected and counted after 24 hours.

MODIFIED OOSTENBRINK METHOD (FCES)

This modified Oostenbrink elutriation method was used only for the trial conducted in Florida according to the procedure described by Tarjan, *et al.* (*5*), later altered by Malo (*2*). The collected debris was washed twice and passed through a 325-mesh sieve. Residues were placed on filters as described above.

SIEVING PETRI-DISH METHOD (SPD)

The sieving Petri-dish method was used only for the fourth trial conducted in Puerto Rico. Samples were dispersed in water in a Waring Blendor and then passed once through a 25-mesh sieve and twice through a 325-mesh sieve. The residues collected on the finer sieve were placed on facial tissue as previously described for the OFA method. In the Puerto Rican tests the volume of the nematode suspensions was increased to 50 cc. with water and the nematodes in four 1-cc. aliquots were counted. In the test conducted in Florida, the nematode suspensions from the clay samples were diluted to 50 cc., while those from the sand samples were diluted to 20 cc.; in both cases the nematodes in two 1-cc. aliquots were counted.

RESULTS AND OBSERVATIONS

In the first trial (table 1) the OFA technique was superior to the SF method when using a Bayamón fine loamy sand. However, the results were quite similar for both methods when a Río Piedras clay was used.

For the second trial (table 1), the OFA technique was slightly better than the SF method with the Bayamón fine loamy sand, but the opposite was true when using the Río Piedras clay.

TABLE 1.—*Average number of nematodes found per 150-cc. sample of soil in trials 1 and 2, using 2 methods and soils*

Trial No.	Data for type of soil and method indicated			
	Bayamón fine loamy sand		Río Piedras clay	
	Oostenbrink apparatus III (OFA)	Sugar flotation (SF)	Oostenbrink apparatus III (OFA)	Sugar flotation (SF)
1	1,467	256	1,023	1,057
2	691	462	675	903

TABLE 2.—*Average number of nematodes found per sample in trial 3, using 3 methods and 2 soils*

Type of soil	Oostenbrink apparatus III (OFA)	Sugar flotation (SF)	Modified elutriator (FCES)
Lakeland fine sand[1]	198	250	189
Mantee fine sandy clay[2]	1,169	558	1,208

[1] Sample size 250 cc.
[2] Sample size 200 cc.

In the third trial (table 2), which was conducted in Florida, the SF method was superior to the OFA and FCES methods when using a Lakeland fine sand. However, when a Manatee fine sandy clay was used, the SF technique was inferior to the other two methods.

The results of the fourth trial (table 3) were as follows: When a sand and a loamy sand were used, the SF technique was superior to the OFA and SPD methods, with the sole exception of the 1,000-cc. sample which gave a slightly higher count of nematodes when processed by the SPD method.

When a clay loam was used the SF technique was better than the OFA method, except for the 600-cc. samples. The SF technique was also supe-

rior to the SPD method when samples of 75 and 150 cc. were used, but was somewhat inferior with samples of 300 and 600 cc.

Higher nematode counts were obtained when silty clay samples were processed by the SPD method than when the SF method was employed. However, the method was inferior to the OFA method when samples of 600 and 1,000 cc. were used.

Ring nematodes (*Criconemoides* and *Hemicriconemoides*), in nearly all

TABLE 3.—*Average number of nematodes found per sample in trial 4, using 3 methods and 4 soils*

Type of nema[1]	Size of sample	Data for type of soil and method indicated[2]											
		Sand			Loamy sand			Clay loam			Silty clay		
		OFA[3]	SF	SPD	OFA	SF	SPD	OFA	SF	SPD	OFA	SF	SPD
	Cc.	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Total	75	58	138	28	338	1,540	320	493	933	643	693	1,013	1,053
	150	858	1,618	788	300	2,022	335	520	1,023	660	1,173	1,838	2,185
	300	1,358	2,035	1,600	370	2,168	370	995	1,462	1,568	2,670	2,140	3,602
	600	1,570	4,193	2,648	288	1,553	180	2,618	818	1,118	4,473	1,605	3,895
	1,000	2,670	3,156	3,602	—	—	—	—	—	—	4,025	1,188	3,898
Ring	75	13	38	3	10	370	7	3	58	3	8	30	3
	150	63	845	40	0	565	0	3	45	5	5	23	5
	300	33	795	45	0	333	5	0	50	5	0	58	0
	600	18	1,238	28	0	183	0	0	50	5	0	23	0
	1,000	23	693	5	—	—	—	—	—	—	0	60	0

[1] See text for composition of soil.

[2] Total = total nematode population; ring = ring nematodes, viz. *Hemicriconemoides* and *Criconemoides*.

[3] Processing method: OFA = Oostenbrink flotation apparatus III; SF = sugar flotation; SPD = sieving-Petri dish.

cases were much more numerous when the samples were processed by the SF method, as already reported by Martin (*3*), and Williamson and Harrison (*6*).

DISCUSSION AND CONCLUSIONS

In general, the number of nematodes recovered by each method varied according to the type and quantity of soil used. The SF technique was generally superior for sandy soils while the OFA and SPD methods seemed to be better adapted to clay soils. The FCES method was as efficient as the OFA method.

The SF technique has many advantages over other methods. It is simple

to use, quick, the extracted nematodes can be studied immediately, it is generally more efficient for sandy soils that the other methods used, and eggs are obtained.

The low number of nematodes recovered from clay soils using this method may be due to several factors. Possibly, when the soil was first centrifuged the colloidal particles in suspension may have retarded sedimentation of the nematodes during the initial 4-minute centrifugation. In Puerto Rico it has been observed repeatedly that, when clay soils are dispersed in water and allowed to settle, or are centrifuged, it takes much longer for small nematodes to sink than when sandy soils are used. This principle benefits the OFA and SPD methods because flotation of nematodes facilitates good recovery.

When sugar solution is added afterwards in the SF method, and the tube is centrifuged a second time, the settling colloidal particles may exert a mass action and pull down nematodes, despite the specific gravity of the sugar solution which would tend to impart buoyancy to the animals. Then, too, the colloids may have an electrical charge which might attract the nematodes and, in this manner, cause them to sink to the bottom. This phenomenon may also occur in sandy soils with a large clay content.

The OFA and FCES techniques required the most operation time. These methods are based on the flotation of nematodes by an underflow of water which, combined with the possible attraction of nematodes to floating colloidal particles, may have been responsible for the increased efficiency of these methods when clay soils were used. Ten to fifteen minutes are usually required for washing a sample, but this time is shortened when more than one apparatus is available. During the 24 hours allowed for passage of the nematodes in the debris through the filters, many nematodes can be lost because they are unable to work their way down through the debris and filter openings to the bottom of the dish. This is particularly true for nematodes that are normally inactive—such as ring nematodes. In all cases large and heavy nematodes may be lost in the washing process or otherwise in the deposition of residues on the filter paper. In conclusion, it may be said that these methods are efficient for clay soils and for samples of about 600 cc. of soil when an immediate examination of nematodes is not required. The FCES method, although used only for one set of trials, was comparable in results to the OFA technique.

The SPD method also proved to be applicable to clay soils. The equipment is inexpensive, easy to use, and the time required for washing one sample is brief. However, many nematodes may be lost during the washing process and by the deposition of residues on filter paper. Then, too, nematode suspensions thus obtained contained more dirt which hinders observations, than was the case with other methods.

These results indicated that a sample of 300 to 600 cc. is best suited for

nematode analysis, despite the method used, or the type of soil involved. Smaller samples usually are not representative while larger samples yield proportionately fewer nematodes and present more difficulties in the washing process.

SUMMARY

Four methods for extracting nematodes from soil samples were investigated, using soils ranging from predominately sand to mostly clay. These were: The Oostenbrink Flotation Apparatus III, a modified Oostenbrink elutriator, a sugar-flotation technique, and a sieving Petri-dish method. It was found that no one method can be regarded as completely satisfactory for all types of soils. The sugar-flotation technique was more effective with sandy soils, while the other three methods were more effective with clay soils. The sugar-flotation technique proved superior to the other methods for recovery of ring nematodes from all the types of soils used.

RESUMEN

Se investigaron cuatro métodos para extraer nemátodos de muestras de suelos, cuyos tipos variaron desde los arenosos hasta los más arcillosos. Los métodos usados fueron el "Oostenbrink Flotation Apparatus III", el "Oostenbrink Elutriator" modificado, la técnica de flotación de azúcar y el método convencional mediante cedazos. Se encontró que ningún método fue completamente eficaz para todos los tipos de suelos usados. La técnica de flotación de azúcar fue más eficiente en los suelos arenosos, mientras que los otros métodos dieron mejores resultados en los arcillosos. Una vez más, la técnica de flotación de azúcar demostró su superioridad sobre los otros métodos para extraer los nemátodos de anillos de todos los tipos de suelos usados durante las pruebas.

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