A COMPARISON OF METHODS FOR EXTRACTING NEMATODES FROM SOIL AND ROOTS OF SUGARCANE

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Abstract

Five methods for extracting nematodes from soil were compared using three sandy soils and three clay soils. Although no one method was superior to the others for all the genera in any one particular soil, the decanting plus sieving plus Baermann tray method was generally the most efficient for sandy soils and the decanting plus sieving plus centrifugal sugar flotation method the most efficient for clay soils. The other methods tested were the Baermann tray, the two-flask sedimentation technique and direct centrifugal sugar flotation. Greater numbers of nematodes were extracted from chopped sugarcane roots incubated in a polythene bag in hydrogen peroxide solution than when incubated in water. When incubated on a Baermann tray with the peroxide solution macerated roots yielded more nematodes than did chopped roots. More nematodes were extracted from chopped roots incubated in a polythene bag than from macerated roots on a Baermann tray.

Introduction

A survey of the papers published in volumes one to ten of the Journal of Nematology shows that Jenkins'[9] decanting, sieving and centrifugal flotation (SCF) is the most popular method and, presumably therefore, one of the more efficient methods used by contributors to this journal for extracting nematodes from soil. This technique was used on 36% of 186 occasions when a method was referred to for quantitative work or simply to extract very large numbers of a particular genus. The second-most popular method (used on 19% of the occasions) was the decanting plus sieving plus Baermann funnel technique, described by Christie and Perry,[3] or slight modifications of this method.

A comparison between Jenkins' SCF and the original Baermann funnel method showed that the former was more efficient for extracting most of the nematode genera occurring in a sandy sugarcane field in South Africa.[6] Harrison and Green[7] found that, compared with a modified Christie and Perry technique (SBT) and two other standard methods of extraction as good as if not better results were generally obtained with their modification of the centrifugal flotation technique (DCF) of Caveness and Jensen.[2] The nematode fauna of the soils used by Harrison and Green differs markedly from that encountered in the sugarcane soils of South Africa and they did not include the SCF as one of their standard methods. In an attempt to find a suitable extraction method for work with the nematodes associated with sugarcane a comparison was made between their method, the SCF and SBT techniques and two other methods, using six soil types.

Various methods have been devised for extracting nematodes from plant roots.[8] Practically all of these rely on the fact that over a period of time non-sedentary nematodes will leave chopped or macerated roots incubated in a moist or saturated atmosphere. Extraction is improved if the roots are moistened with a weak hydrogen peroxide solution.[5, 12] As far as is known there are no published data to show which methods are suitable for sugarcane roots. A comparison was therefore made between a few simple techniques.

Materials and methods

Soils

The six soils (Table 1) were collected from sugarcane fields in Natal and processed within two days of collection. Six litres of each soil were first passed through a 4 mm aperture sieve to remove coarse debris and then coned and quartered six times. There were four replicates for each extraction method. All extractions (from both soil and roots) were carried out at room temperature, approximately 25° C, and 200 mm diameter sieves were used throughout.

TABLE 1

Textural composition (International classification) and organic matter content of soils used for the extraction comparison

Soil series[10]	% Clay	% Silt	% Sand			Organic matter %
			Fine	Medium	Coarse	
Whithorn sand	3	3	67	26	1	0,52
Fernwood sand	4	3	55	39	1	0,41
Cartref loamy sand	9	6	29	40	18	1,24
Inanda sandy clay	39	11	34	12	4	4,44
Rydalvale clay	43	14	29	8	6	4,44
Glendale clay	52	15	27	5	1	2,17

Extraction methods

(i) Baermann Tray (BT) (after Whitehead and Hemming[13]).

Fifty millilitres of soil were spread on double thickness two-ply Kleenex tissue supported on a 140 mm diameter wide-mesh plastic screen in a plastic tray. Water was added until the soil was just wet. The screen was removed after 24 hours and the nematode suspension condensed as described below.

(ii) Decanting plus sieving plus Baermann tray method (SBT) (after Christie and Perry[3]).

One hundred millilitres of soil were soaked in water for one hour and the clay fraction dispersed with a vibro mixer for five minutes. The slurry was then washed with 4 litres of water through a 1 mm aperture sieve into a jug and thoroughly mixed. The slurry was allowed to settle for 30 seconds, then decanted through a 53 µm aperture sieve over a 38 µm sieve. A further 4 litres of water were added to the remaining soil and the process repeated. The nematodes were separated from the residue on the sieves by the BT method.

In the original description of this method[3] the residue was poured from the sieves onto *submerged* woven muslin whereas in this work the residue was poured onto *wetted* tissue. To check whether there was any difference between using wetted and submerged tissue an extra SBT extraction was carried out with two of the soils where the residue was poured into water above submerged tissue (SBT (sub)).

(iii) Two flask technique[11] (2F).

One hundred millilitres of soil were soaked and dispersed as described above. The slurry was washed through a 2 mm aperture sieve into a 2 litre conical flask and the normal sedimentation procedure followed. The final suspension from all the soils was too dirty for the nematodes to be counted. The nematodes were therefore separated from the debris using the BT method except that an 80 mm diameter plastic screen was used.

(iv) Direct centrifugal sugar flotation (DCF). (Modification by Harrison and Green[7] of the method developed by Caveness and Jensen[2]). Fifty millilitres of soil were divided between four 50 ml centrifuge tubes and the modified procedure followed. The final suspension from all the soils was too dirty for the nematodes to be counted. It was therefore cleaned using a small BT as described for the 2F method.

(v) Decanting plus sieving plus centrifugal sugar flotation (SCF) (after Jenkins[9]).

One hundred millilitres of soil were soaked, dispersed, washed into a 5 litre jug, mixed, decanted and sieved as described for the first part of the SBT method. The residue from the sieves was then washed into two 50 ml centrifuge tubes and spun at 980 *g* for five minutes in a swing-out head centrifuge. The supernatant was carefully poured off and replaced with sugar solution (specific gravity 1,15). This solution and the sediment were thoroughly mixed with a vibro mixer and then centrifuged at 980 *g* for 30 seconds, after which the supernatant was poured into 2 litres of water. The nematode suspension was poured onto three 53 µm aperture sieves over two 38 µm sieves and further processed as described below.

The final sievings of the SCF method and the nematode suspension from the BTs of the other four methods were concentrated by sedimentation, first in a 100 ml measuring cylinder for at least four hours and then in a 25 ml test tube. All but 3 ml was siphoned off and one third of the nematodes counted in a Hawksley counting slide. All counts, adjusted to those present in 100 ml of soil, were transformed to log $(x + 1)$ and least significant differences (where $P < 0,05$) calculated after performing an analysis of variance.

Roots

The hydrogen peroxide solution used in the following three experiments was prepared by diluting 20 ml of 130 volume hydrogen peroxide to 600 ml with water.

Experiment 1. To compare hydrogen peroxide solution and water as incubation media for roots.

Sugarcane shoot roots were washed and excess moisture removed with absorbent paper. The roots were then chopped into approximately 10 mm lengths, mixed and a 5 g sub-sample incubated in a sealed polythene bag containing either 10 ml of hydrogen peroxide solution or 10 ml of water. Both treatments were replicated seven times. After six days the contents of each bag were washed onto a 140 mm diameter BT and the nematodes extracted from the root debris over a period of 24 hours.

Experiment 2. To determine whether the incubation period and separation of the nematodes from the root fragments can be combined into one operation. The two treatments were:

(1) A 5 g subsample of chopped roots, prepared as for experiment 1 but from a different field, was placed on an 80 mm diameter BT in a polythene bag. One hundred millilitres of hydrogen peroxide solution were added to the bag so that the roots were just wetted. After seven days the extracted nematodes were separated from the peroxide solution by sieving.

(2) A similar 5 g subsample of roots was incubated with 10 ml of hydrogen peroxide solution in a polythene bag for seven days. The contents of the bag were then washed onto a 1 mm aperture sieve over a 53 and 38 µm aperture sieve. The nematodes were extracted from the debris on the two lower sieves by the BT method. Both treatments were replicated eight times.

Experiment 3. To compare the extraction of nematodes from chopped and macerated roots and from chopped roots in a polythene bag and macerated roots on a BT. Chopped cane roots, prepared as for Experiment 1 but collected from a different field, were treated as follows (each treatment replicated six times).

(1) Incubated in a polythene bag with 10 ml of hydrogen peroxide solution for six days. The roots and solution were then washed onto a BT, fresh water added, and the nematodes extracted for 24 hours.

(2) As for treatment 1 but after incubating for six days the contents of the polythene bag were washed onto a 1 mm aperture sieve to remove the roots and the nematodes and root debris collected on a 53 µm over a 38 µm aperture sieve. The nematodes were separated from the debris using a BT.

(3) Incubated on a BT using hydrogen peroxide solution such that it just wetted the roots. After seven days the extracted nematodes were separated from the peroxide solution by sieving.

(4) The chopped roots were macerated in 250 ml of water for five seconds in a Moulinex kitchen blender. They were then washed onto a BT, incubated with the peroxide solution for seven days and the nematodes collected as for treatment 3.

(5) As for treatment 4 but the roots were macerated for 10 seconds.

(6) As for treatment 4 but the roots were macerated for 15 seconds.

The nematodes extracted from the roots in these three experiments were condensed and counted and the data analysed as described for the soils.

Results

Soils

In comparing the different extraction methods reference is made here only to the known root-feeding nematodes. *Tylenchus*, *Psilenchus* and other nematodes in the samples are included in Table 2 for the sake of completeness.

Only in a few instances did one method extract significantly more individuals of a particular genus from a particular soil than all the other methods, namely, SCF for *Macroposthonia* in the Whithorn sand and for *Pratylenchus*, *Scutellonema*, *Helicotylenchus* and *Macroposthonia* in the Rydalvale clay, and SBT ("dry" and submerged) for *Xiphinema* in the Fernwood sand (Table 2). Generally two or more methods were equally efficient (ie there was no statistically significant difference between the numbers extracted).

Methods that were efficient for *all* the sandy soils in which a particular genus occurred were as follows: BT for *Pratylenchus*, BT and SBT for *Rotylenchulus*, SBT for *Xiphinema* and *Paratrichodorus*, SBT and 2F for *Meloidogyne* and *Helicotylenchus*, BT, 2F and CSF for *Discocriconemella*, SCF for *Hopolaimus* and *Macroposthonia*, all methods for *Criconemella* and *Scutellonema* and all except BT for *Tylenchorhynchus*.

There was no significant difference between the extraction efficiency of SBT and SBT (sub) for any of the genera for the two sandy soils tested (Table 2).

Methods that were efficient for *all* the clay soils in which a particular genus occurred were as follows: BT and SBT for *Rotylenchulus*, BT and SCF for *Hemicycliophora*, SCF for *Pratylenchus*, *Scutellonema*, *Helicotylenchus*, *Macroposthonia* and *Discocriconemella*, all except DCF for *Paratrichodorus* and *Xiphinema* and all except BT and DCF for *Tylenchulus*.

TABLE 2

Antilog of the transformed means of the numbers of nematodes extracted per 100 ml soil

(BT — Baermann tray; SBT — sieving plus Baermann tray; 2F — 2 flask; DCF — direct centrifugal flotation; SCF — sieving plus centrifugal flotation; SBT (sub) — as for SBT but sieving poured onto submerged tissue.) Means for the same genus within the same soil followed by the same letter do not differ significantly ($P < 0,05$).

Whithorn sand

	BT	SBT	2F	DCF	SCF	SBT (sub)
Pratylenchus	153ab	182a	157ab	121b	51c	
Tylenchorhynchus	12a	13a	11a	4a	6a	
Hoplolaimus	9d	51b	32c	96a	85a	
Scutellonema	–	–	–	–	–	
Helicotylenchus	100b	244a	175a	106b	96b	
Meloidogyne	52ab	24ab	62a	46ab	14b	
Rotylenchulus	106a	46ab	10c	23bc	11c	
Tylenchulus	–	–	–	–	–	
Macroposthonia	2b	3b	0	3b	19a	
Discocriconemella	5a	2a	3a	2a	4a	
Criconemella	–	–	–	–	–	
Hemicycliophora	–	–	–	–	–	
Xiphinema	0	5ab	2b	2b	10a	
Paratrichodorus	8a	16a	6a	3a	12a	
Psilenchus	–	–	–	–	–	
Tylenchus	–	–	–	–	–	
Others	627a	393b	405b	433ab	133c	

Fernwood sand

	BT	SBT	2F	DCF	SCF	SBT (sub)
Pratylenchus	3ab	1b	0	5a	0	3ab
Tylenchorhynchus	7b	41a	25a	28a	16a	40a
Hoplolaimus	5a	7a	3a	0	3a	7a
Scutellonema	–	–	–	–	–	–
Helicotylenchus	–	–	–	–	–	–
Meloidogyne	50b	106a	89a	45b	112a	86a
Rotylenchulus	724a	508a	97c	195b	125b	503a
Tylenchulus	–	–	–	–	–	–
Macroposthonia	2a	3a	0	0	2a	2a
Discocriconemella	–	–	–	–	–	–
Criconemella	3a	6a	4a	2a	4a	8a
Hemicycliophora	–	–	–	–	–	–
Xiphinema	11b	41a	7b	2c	14b	37a
Paratrichodorus	0	1a	1a	0	0	1a
Psilenchus	–	–	–	–	–	–
Tylenchus	0	2a	2a	3a	0	4a
Others	508a	400a	197bc	252b	156c	408a

Cartref loamy sand

	BT	SBT	2F	DCF	SCF	SBT (sub)
Pratylenchus	147a	155a	142a	176a	82a	136a
Tylenchorhynchus	–	–	–	–	–	–
Hoplolaimus	–	–	–	–	–	–
Scutellonema	6a	8a	7a	10a	12a	11a
Helicotylenchus	520a	547a	590a	729a	327b	629a
Meloidogyne	–	–	–	–	–	–
Rotylenchulus	–	–	–	–	–	–
Tylenchulus	–	–	–	–	–	–
Macroposthonia	–	–	–	–	–	–
Discocriconemella	7a	1c	4ab	0	3ab	2bc
Criconemella	–	–	–	–	–	–
Hemicycliophora	–	–	–	–	–	–
Xiphinema	4b	17a	7ab	7ab	10ab	28a
Paratrichodorus	58ab	68a	40b	37b	58ab	44ab
Psilenchus	–	–	–	–	–	–
Tylenchus	–	–	–	–	–	–
Others	269a	180ab	117cd	160bc	90d	188ab

Inanda sandy clay

	BT	SBT	2F	DCF	SCF
Pratylenchus	–	–	–	–	–
Tylenchorhynchus	–	–	–	–	–
Hoplolaimus	–	–	–	–	–
Scutellonema	100bc	89c	83c	147ab	209a
Helicotylenchus	955ab	706b	1000a	1054a	1094a
Meloidogyne	–	–	–	–	–
Rotylenchulus	–	–	–	–	–
Tylenchulus	–	–	–	–	–
Macroposthonia	–	–	–	–	–
Discocriconemella	–	–	–	–	–
Criconemella	–	–	–	–	–
Hemicycliophora	–	–	–	–	–
Xiphinema	–	–	–	–	–
Paratrichodorus	25a	17a	13a	14a	23a
Psilenchus	–	–	–	–	–
Tylenchus	50a	3c	4c	31ab	9bc
Others	1622a	973b	1081b	1186ab	503c

Rydalvale clay

	BT	SBT	2F	DCF	SCF
Pratylenchus	127b	68c	140b	123b	251a
Tylenchorhynchus	–	–	–	–	–
Hoplolaimus	–	–	–	–	–
Scutellonema	115c	163b	165b	147bc	471a
Helicotylenchus	169b	116c	134bc	123bc	361a
Meloidogyne	–	–	–	–	–
Rotylenchulus	117a	96a	116a	94a	79a
Tylenchulus	–	–	–	–	–
Macroposthonia	0	2c	5b	8b	52a
Discocriconemella	3b	1b	1b	11ab	32a
Criconemella	–	–	–	–	–
Hemicycliophora	27a	11b	8b	9b	26a
Xiphinema	–	–	–	–	–
Paratrichodorus	6ab	7ab	10a	2b	16a
Psilenchus	208a	299a	284a	281a	851a
Tylenchus	5a	4a	4a	0	10a
Others	557a	151c	242b	265b	206bc

Glendale clay

	BT	SBT	2F	DCF	SCF
Pratylenchus	5b	20ab	10ab	15ab	33a
Tylenchorhynchus	–	–	–	–	–
Hoplolaimus	–	–	–	–	–
Scutellonema	24a	25a	9b	9b	19a
Helicotylenchus	25a	21a	9a	14a	31a
Meloidogyne	–	–	–	–	–
Rotylenchulus	159ab	188a	108c	66d	127bc
Tylenchulus	760b	984a	1101a	331c	1151a
Macroposthonia	2a	0	0	0	3a
Discocriconemella	178a	37c	53c	98b	222a
Criconemella	–	–	–	–	–
Hemicycliophora	–	–	–	–	–
Xiphinema	13a	7ab	8ab	2b	15a
Paratrichodorus	7a	7a	3a	0	9a
Psilenchus	–	–	–	–	–
Tylenchus	92a	105a	63a	19b	25b
Others	439a	247ab	164b	87c	356a

Combining all the genera, SBT extracted greater numbers of nematodes from the sandy soils than any other method, while SCF extracted the most from clay soils (Table 3). SBT (sub) was only used for two of the sandy soils and was not included in the calculations for Table 3. In fact the mean percentage number of nematodes extracted from the two soils by this method was virtually the same as by the "dry" SBT.

TABLE 3

Mean percentage number of nematodes extracted by five methods. (Assuming best method for each genus extracts 100% of the individuals)

	BT	SBT	2F	DCF	SCF
Sandy soils	58,9	76,5	48,3	47,3	58,6
Clay soils	64,2	48,8	46,7	36,7	94,9

No one method was more consistent than all the other methods for all the soils (Table 4). Considering the three sands together, BT and SBT (sub) (two soils only) were more consistent than the other methods while DCF and SCF were more consistent for the clay soils. SCF was relatively inconsistent for all the sandy soils.

TABLE 5

Mean number of nematodes extracted from 5 g samples of roots incubated in Experiment 1 with either water or hydrogen peroxide solution in a polythene bag

Genera	Water	Hydrogen peroxide
Pratylenchus . . .	138 b	375 a
Meloidogyne . . .	11 a	15 a
Rotylenchulus . . .	29 a	29 a
Helicotylenchus . . .	15 a	16 a

Means for the same genus followed by the same letter are not significantly different ($P < 0,05$)

Roots

Experiment 1. Incubating chopped roots in the hydrogen peroxide solution was superior to incubating in water for extracting *Pratylenchus* but there was no difference between these two media for the other genera present (Table 5).

Experiment 2. Significantly more *Pratylenchus* and *Meloidogyne* were extracted from roots incubated with hydrogen peroxide in a polythene bag than on a BT in a polythene bag.

TABLE 6

Mean number of nematodes extracted from 5 g samples of roots incubated in Experiment 2 with hydrogen peroxide solution in a polythene bag or on a Baermann tray within a polythene bag

Genera	Incubated in polythene bag	Incubated on Baermann tray in polythene bag
Pratylenchus . . .	573 a	193 b
Meloidogyne . . .	6301 a	1691 b

Means for the same genus followed by the same letter are not significantly different ($P < 0,05$)

Experiment 3. Greater numbers of *Pratylenchus* were extracted from macerated roots than from chopped roots on a BT (Table 7). There was no difference between the two methods for *Meloidogyne*, *Rotylenchulus* and *Helicotylenchus*. Significantly more *Pratylenchus*, *Meloidogyne* and to some extent *Rotylenchulus* were extracted from chopped roots incubated in a polythene bag than from chopped or macerated roots on a BT. Many more *Pratylenchus* were recovered using the polythene bag incubation method if the contents of the bag were poured directly onto a BT than if they were sieved prior to this final extraction process (cf treatments 1 and 2, Table 7).

Discussion

With a few exceptions SBT was the most suitable method for sandy soils, particularly if the final washing from the sieves

TABLE 4

Coefficients of variability of the log of the total number of plant feeding nematodes extracted by different methods

Method	Sandy soils			Clay soils			Mean of sands	Mean of clays
	Whithorn	Fernwood	Cartref	Inanda	Rydalvale	Glendale		
BT	2,28	0,83	2,12	6,11	1,55	1,55	1,74	3,07
SBT	0,78	1,25	4,46	3,10	2,50	0,65	2,16	2,08
2F	0,73	5,32	1,95	3,12	1,58	1,87	2,67	2,19
DCF	2,80	2,75	1,78	0,58	1,30	1,88	2,44	1,25
SCF	5,77	6,44	5,20	0,51	0,98	2,14	5,80	1,21
SBT (Sub) . . .	—	1,71	1,32	—	—	—	1,51	—

TABLE 7

Number of nematodes extracted from roots using six methods in Experiment 3

Genera	Mean number per 5 g roots					
	Polythene bag*		Baermann tray†			
	Chopped (1) ‡	Chopped and sieved (2)	Chopped (3)	Macerated 5 sec (4)	Macerated 10 sec (5)	Macerated 15 sec (6)
Pratylenchus	1581 a	624 b	34 d	161 c	135 c	217 c
Meloidogyne	287 a	233 a	4 b	7 b	11 b	7 b
Rotylenchulus	21 a	14 ab	1 c	3 c	2 c	3 bc
Helicotylenchus	17 a	11 a	7a	9 a	7 a	5 a

* Roots incubated with hydrogen peroxide in polythene bag for 6 days followed by 24 hours on BT.
† Roots incubated with hydrogen peroxide on BT for 7 days.
‡ Treatment number (see text).
Means for the same genus followed by the same letter are not significantly different ($P < 0,05$)

was poured into water over the paper tissue on the Baermann tray [= SBT (sub)]. In one of the sands this slight modification resulted in a more consistent extraction and, in both sands tested, no loss in efficiency. Possibly this was due to the more even distribution of the sediment from the sieves on the tissue.

SCF was the most efficient and consistent of the methods for clay soils but was unsuitable for sandy soils. In contrast Harris and Braithwaite[6] found that SCF was, with the exception of *Rotylenchulus*, more efficient than the Baermann funnel method (BF) for a Clansthal sand. The modified BF used in this study (= BT) was as good as SCF for the three sands. Probably the depth of soil on the funnel screen used by Harris and Braithwaite (100 ml of soil on an 80 mm diameter screen compared with 50 ml of soil on a 140 mm diameter tray screen used in this study) was too great to allow many of the nematodes to move from the soil during the extraction period, despite the fact that their BF extraction time was twice that of the BT method.

The DCF was generally less efficient than SBT and SCF for extracting the range of nematode genera occurring in the six sugarcane soils. It also resulted in more debris in the sample, to the extent that this had to be cleaned with a BT before the nematodes in 1 ml of a 3 ml extract could be counted. Similar conclusions were reached by Dickerson[4] although he found that in using a greater dilution for counting the additional cleaning process was unnecessary.

Chopped roots incubated in hydrogen peroxide solution in a polythene bag yielded more nematodes than did macerated roots incubated on a BT (Table 7). More nematodes were extracted from macerated roots than from chopped roots on a BT. The next logical test to further improve extraction efficiency would be to incubate macerated roots in hydrogen peroxide in a polythene bag. Such an experiment is planned for the future.

The difference between the results of treatments 1 and 2 of the third experiment on extraction from roots (Table 7) may be due to large numbers of *Pratylenchus* being lost during the sieving process. A 53 µm over a 38 µm aperture sieve was used to retain the nematodes in treatment 2. Bird[1] found that 23% of a population of *P. brachyurus* passed through a similar combination of sieves. This percentage would increase as the proportion of juveniles in the population increased, particularly second stage juveniles. However the possibility also exists that the large root fragments, discarded at the end of six days incubation in treatment 2, contained a significant number of nematodes, which in treatment 1 left the roots and passed through the tissue during the 24 hour final extraction period.

In view of their generally greater efficacy, SBT has been adopted for routine work with sandy soils and SCF for clay soils. For work with a particular nematode genus there are exceptions to these rules, eg BT and SBT for *Rotylenchulus* in all soils, and SCF for *Macroposthonia* in all soils. Until further comparisons have been made the polythene bag incubation method is being used to extract nematodes from sugarcane roots.

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