EVALUATION OF METHODS FOR SEPARATING NEMATODES FROM SOIL

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Abstract

An investigation was carried out into the relative effectiveness of the Baermann funnel and the centrifugal sugar flotation technique for extracting nematodes from soil samples, and the results of retaining samples in the Baermann funnel for 48 or 72 hours were compared.

The effects of treating the soil in different ways and of storage of samples for different lengths of time before extraction were tested. Nematodes of the following genera were included in the investigation: *Meloidogyne* (larvae), *Pratylenchus*, *Criconemoides*, Hoplolaims, *Hoplolaimus*, *Rotylenchulus*, *Xiphinema*, *Dolichodorus*, *Trichodorus* and *Mononchus*.

Of the two extraction methods the centrifugal sugar flotation technique, especially after treatment of the soil with formalin, was generally better, although the Baermann funnel yielded greater numbers of *Rotylenchulus*. Numbers of nematodes extracted by either of the techniques were decreased after heating or drying of the soil, and heating caused the most noticeable reduction.

Introduction

Techniques used for separating various genera and species of nematodes from soil are known to vary in efficiency, both quantitatively and qualitatively.

Elmiligy and De Grisse,[3] and Dunn[2] have found centrifugal flotation techniques to be generally more satisfactory than either Baermann funnel or elutriation methods. Kimpinski and Welch[5] found that centrifugal sugar flotation was more efficient than the Baermann funnel.

Where nematode numbers are important, consistent and reliable extraction techniques are essential for drawing meaningful conclusions from research and for making sound recommendations in the field. It was therefore decided, in working with nematodes occurring in the sandy soils of the South African Sugar Industry, to carry out investigations on currently used nematode extraction methods. It was decided also to simulate some of the conditions which frequently arise when taking and handling soil samples.

This paper deals with the early results of what was intended ultimately to be a thorough investigation of sampling and extraction techniques. The discussions and conclusions are based on figures which have not been statistically analysed but which do indicate certain important effects and differences.

Materials and methods

A composite soil sample was taken from a field of sugarcane growing on a Clansthal sand (clay 7%, silt 2% and sand 91%) at field capacity (approximately 9% moisture). Roots and extraneous material were removed and the soil was thoroughly mixed. Subsamples of equal amounts of this soil were subjected to the following treatments, commencing within an hour after the sample was taken:

Treatment

No. 1. Soil stored in open tray (2,5 cm deep) at room temperature (23-26°C) and allowed to air dry (*d*).
2. Soil sealed in a plastic bag and stored at 5°C (*c*).
3. Soil sealed in a plastic bag and stored at 24°C (*w*).
4. Soil sealed in a plastic bag and placed outside, in direct sunshine, for 2 hours on two consecutive days before being stored at 5°C. The maximum temperature in the bag 2,5 cm below the soil surface on the first day was 40°C and on the second 41°C (*s*).
5. Soil sealed in a glass jar and stored at room temperature after addition of the following fixative at 20 ml per 100 ml soil:

   40% formalin 10% by volume
   Glycerine 1% by volume
   Distilled water 89% by volume

   This treatment (*f*) was similar to that of Emiligy and De Grisse[3] but differed in that the fixative added was at room temperature and not 80°C.

The soil in each treatment was used for the following extraction methods: (1) The Baermann funnel (BF) technique and (2) The centrifugal sugar flotation (SF) technique as described by Jenkins[4] but using a 400 mesh sieve. The soil with the fixative solution was used only for SF extracts.

Basic requirements for the Baermann technique are a funnel with a piece of rubber tubing attached to the stem and two spring clips. One, about 25 mm from the end of the tubing, is closed, and the other, about 75 mm from the end, is kept open. The funnel is placed in a support and a sieve 80 mm in diameter, lined with a single layer of paper tissue, is inserted. A subsample consisting of 100 ml of soil is put into the sieve and water is run into the funnel until the soil is just covered. The apparatus is left to stand, usually for 48 hours, and then the top spring clip is closed and the extract between the two clips is run off into a conical tube.

In the centrifugal sugar flotation technique, 100 ml of soil are washed through a 16 mesh sieve into a bucket and made up to approximately 6ℓ with water. After being stirred, the suspension is allowed to settle for 30 seconds before the supernatant is decanted through a 400 mesh sieve. The sievings are collected in a 50 ml centrifuge tube and spun at 1 750 rev/min for 4½ minutes. The supernatant is poured off and replaced by sucrose solution (456 g/ℓ). The tubes are balanced, shaken and spun for half a minute. The supernatant is poured through a 400 mesh sieve. The sievings are washed well and collected in a conical tube.

For this investigation all treatments were replicated four times and the entire nematode population extracted from each 100 ml sample was counted. Baermann funnel extraction was run for 48 hours for all treatments but an additional set of replications of treatment 2 was extracted for 72 hours. Using both BF and SF methods, extracts were made from the fresh soil immediately after mixing and before other treatments were carried out. After each treatment extracts were made by both methods at known intervals over a 51 week storage period, as indicated in Table 2 where the totals for 4 replicates of each treatment, at each extraction, are given for the following nematode genera:

TABLE 1
Total number of each nematode genus extracted for each treatment

Meloidogyne (larvae)			*Pratylenchus*			*Criconemoides*			Hoplolaims		
SF	*f*	1 000	SF	*f*	249	SF	*f*	615	SF	*f*	2 178
SF	*c*	476	SF	*c*	155	SF	*c*	266	SF	*c*	1 742
SF	*d*	161	BF	*w*	111	SF	*w*	163	SF	*s*	760
SF	*s*	146	SF	*w*	97	SF	*s*	151	SF	*d*	699
SF	*w*	82	SF	*s*	79	SF	*d*	25	SF	*w*	666
BF	*w*	42	SF	*d*	76	BF	*w*	1	BF	*w*	187
BF	*c*	27	BF	*c*	47	BF	*c*	1	BF	*c*	150
BF	*s*	2	BF	*d*	15	BF	*d*	0	BF	*d*	63
BF	*d*	3	BF	*s*	1	BF	*s*	0	BF	*s*	0
Hoplolaimus			*Rotylenchulus*			*Xiphinema*			*Dolichodorus*		
SF	*f*	135	BF	*c*	945	SF	*w*	41	SF	*f*	12
SF	*c*	95	BF	*w*	726	SF	*f*	31	SF	*d*	4
SF	*w*	44	SF	*f*	290	SF	*d*	18	SF	*c*	3
SF	*s*	22	SF	*c*	251	SF	*c*	9	SF	*s*	2
SF	*d*	18	SF	*w*	105	SF	*s*	7	SF	*w*	0
BF	*w*	1	SF	*d*	99	BF	*w*	7	BF	*w*	0
BF	*c*	0	SF	*s*	72	BF	*d*	2	BF	*c*	0
BF	*d*	0	BF	*d*	50	BF	*c*	1	BF	*d*	0
BF	*s*	0	BF	*s*	3	BF	*s*	0	BF	*s*	0
Trichodorus			*Mononchus*								
SF	*f*	125	BF	*w*	27						
SF	*c*	29	SF	*d*	14						
BF	*w*	24	SF	*c*	11						
SF	*w*	20	SF	*f*	8						
BF	*c*	9	SF	*w*	8						
SF	*d*	2	BF	*c*	5						
SF	*s*	1	BF	*d*	4						
BF	*d*	0	SF	*s*	1						
BF	*s*	0	BF	*s*	0						

SF = Centrifugal sugar flotation
BF = Baermann funnel
d = Treatment No. 1
c = Treatment No. 2
w = Treatment No. 3
s = Treatment No. 4
f = Treatment No. 5

RESULTS

Meloidogyne (larvae)
Pratylenchus
Dolichodorus
Criconemoides
Xiphinema
Hoplolaims (nematodes in the sub family Hoplolaiminae) excluding *Hoplolaimus*
Hoplolaimus
Trichodorus
Rotylenchulus
Mononchus

Results and conclusions

The extraction efficiency of each treatment is discussed in relation to total numbers extracted over a 51 week period (Table 1). These totals are listed vertically, in descending order, for each treatment. The effects of storage time are discussed here with reference to Table 2, which shows the numbers of each nematode genus extracted after each treatment, at all extraction dates and for both techniques.

Meloidogyne (larvae)

The SF/*f* treatment yielded the most larvae. SF was superior in each instance to BF. The BF/*d* and *s* treatments severely affected extraction by BF which was generally less efficient than SF at separating the larvae from soil.

TABLE 2
Counts of nematodes of 10 genera, showing the effects of 5 treatments and 19 storage times on the relative efficacy of 2 methods of extraction from soil.

	Storage time in weeks	*Meloidogyne*					*Pratylenchus*					*Criconemoides*					Hoplolaims				
		w	*c*	*d*	*f*	*s*	*w*	*c*	*d*	*f*	*s*	*w*	*c*	*d*	*f*	*s*	*w*	*c*	*d*	*f*	*s*
	Fresh soil	84	84	84	84	84	31	31	31	31	31	55	55	55	55	55	168	168	168	168	168
	1	11	50	21	94	28	14	18	13	23	16	25	18	0	80	29	119	157	62	221	130
	2	24	65	20	128	13	9	21	8	30	7	18	12	0	53	19	71	171	91	258	86
	3	7	51	11	92	10	14	14	8	36	8	21	39	0	64	13	72	220	43	223	62
	4	10	91	32	54	14	15	17	13	17	17	17	13	2	61	11	79	228	103	132	73
	5	3	30	6	84	4	2	15	3	17	2	19	25	3	77	7	45	121	48	122	26
	8	1	9	5	50	14	2	6	1	16	5	4	21	2	40	8	3	46	12	99	34
SF extraction	11	3	18	6	128	4	8	6	1	28	4	14	10	2	61	8	53	66	33	198	21
	14	2	32	14	60	8	8	6	4	17	2	18	29	2	39	8	28	117	35	166	37
	17	6	56	12	118	6	6	4	3	22	3	9	23	3	24	10	31	130	49	159	45
	20	7	33	11	37	11	7	10	3	9	3	6	9	0	17	8	53	104	20	106	41
	23	5	14	1	16	10	4	8	1	1	3	6	15	0	11	5	41	57	16	65	21
	26	2	2	6	15	1	8	3	7	4	1	6	11	1	17	3	54	76	39	63	30
	31	1	12	8	2	3	0	7	3	2	1	0	5	5	4	2	12	33	37	22	11
	35	0	1	4	10	2	0	2	2	1	1	0	12	0	5	2	0	21	30	35	19
	39	0	2	0	3	0	0	4	5	1	0	0	5	3	4	1	5	35	32	32	7
	43	0	9	3	90	9	0	9	1	19	0	0	3	0	23	8	0	37	13	109	18
	47	0	1	1	19	9	0	2	0	5	3	0	4	0	19	5	0	69	5	60	35
	51	0	0	0	0	0	0	3	0	1	3	0	12	2	16	4	0	54	31	108	64
	TOTAL	166	560	245	1 084	230	128	186	107	280	110	218	321	80	670	206	834	1 910	867	2 346	928
	Fresh soil	14	14	14	–	14	12	12	12	–	12	0	0	0	–	0	29	29	29	–	29
	1	5	18	0	–	0	12	14	4	–	1	1	1	0	–	0	19	21	14	–	0
	2	9	6	0	–	0	19	12	5	–	0	0	0	0	–	0	17	15	14	–	0
	3	4	0	0	–	0	12	2	2	–	0	0	0	0	–	0	9	11	11	–	0
	4	4	1	0	–	0	11	1	2	–	0	0	0	0	–	0	6	3	6	–	0
	5	7	0	0	–	0	7	7	0	–	0	0	0	0	–	0	13	20	6	–	0
	8	2	0	0	–	0	5	4	1	–	0	0	0	0	–	0	3	3	3	–	0
BF extraction	11	1	1	0	–	0	2	0	0	–	0	0	0	0	–	0	12	6	3	–	0
	14	0	1	0	–	0	2	2	1	–	0	0	0	0	–	0	11	7	3	–	0
	17	2	0	0	–	1	13	2	0	–	0	0	0	0	–	0	25	9	1	–	0
	20	2	0	0	–	0	17	0	0	–	0	0	0	0	–	0	22	9	2	–	0
	23	6	0	0	–	0	6	0	0	–	0	0	0	0	–	0	20	2	0	–	0
	26	0	0	0	–	0	5	3	0	–	0	0	0	0	–	0	29	11	0	–	0
	31	0	0	1	–	1	0	0	0	–	0	0	0	0	–	0	0	4	0	–	0
	35	0	0	0	–	0	0	0	0	–	0	0	0	0	–	0	0	7	0	–	0
	39	0	0	2	–	0	0	0	0	–	0	0	0	0	–	0	0	11	0	–	0
	43	0	0	0	–	0	0	0	0	–	0	0	0	0	–	0	0	3	0	–	0
	47	0	0	0	–	0	0	0	0	–	0	0	0	0	–	0	1	6	0	–	0
	51	0	0	0	–	0	0	0	0	–	0	0	0	0	–	0	0	2	0	–	0
	TOTAL	56	41	17	–	16	123	59	27	–	13	1	1	0	–	0	216	179	92	–	29

TABLE 2 (Continued)

Storage time in weeks	*Hoplolaimus*					*Rotylenchulus*					*Xiphinema*					*Dolichodorus*				
	w	*c*	*d*	*f*	*s*	*w*	*c*	*d*	*f*	*s*	*w*	*c*	*d*	*f*	*s*	*w*	*c*	*d*	*f*	*s*
Fresh soil	11	11	11	11	11	27	27	27	27	27	2	2	2	2	2	1	1	1	1	1
1	5	16	0	14	3	4	25	12	32	27	7	1	0	0	1	0	0	1	0	1
2	13	10	2	21	2	16	25	13	46	1	8	1	1	3	0	0	0	0	0	0
3	3	16	1	15	1	7	16	13	30	4	7	4	0	3	0	0	1	1	1	0
4	1	6	0	9	0	8	15	13	16	15	6	0	0	2	0	0	0	1	0	0
5	3	7	0	14	0	5	12	0	21	1	2	0	2	2	0	0	2	1	1	0
8	0	4	1	5	3	0	9	0	12	1	2	0	1	4	1	0	0	0	0	0
SF extraction 11	1	4	1	10	2	3	9	5	42	2	5	1	2	4	1	0	0	0	5	0
14	2	8	2	7	1	8	9	6	13	3	1	1	0	1	0	0	0	0	1	0
17	5	11	2	8	2	20	20	4	31	0	1	0	1	1	0	0	0	0	1	0
20	4	4	0	8	2	10	22	7	4	3	1	0	2	0	0	0	0	0	0	0
23	2	4	2	1	0	11	15	3	3	2	1	0	0	1	0	0	0	0	0	0
26	5	0	0	3	1	5	8	3	1	1	0	0	3	3	1	0	0	0	0	0
31	0	2	4	3	2	7	3	7	4	1	0	0	4	1	0	0	0	0	0	0
35	0	0	0	1	0	0	17	3	7	0	0	0	0	2	0	0	0	0	0	0
39	0	0	2	0	0	1	4	5	4	0	0	0	1	1	0	0	0	0	0	0
43	0	0	0	7	0	0	11	1	13	1	0	0	0	2	1	0	0	0	1	1
47	0	2	0	4	1	0	19	0	2	6	0	1	1	1	2	0	0	0	1	0
51	0	1	1	5	2	0	12	4	9	4	0	0	0	0	0	0	0	0	1	0
TOTAL	55	106	29	146	33	132	278	126	317	99	43	11	20	33	9	1	4	5	13	3
Fresh soil	0	0	0	–	0	188	188	188	–	188	0	0	0	–	0	0	0	0	–	0
1	0	0	0	–	0	34	103	15	–	0	0	0	0	–	0	0	0	0	–	0
2	0	0	0	–	0	66	88	12	–	0	0	1	0	–	0	0	0	0	–	0
3	1	0	0	–	0	40	36	5	–	0	1	0	0	–	0	0	0	0	–	0
4	0	0	0	–	0	30	28	4	–	0	2	0	0	–	0	0	0	0	–	0
5	0	0	0	–	0	37	86	8	–	0	1	0	2	–	0	0	0	0	–	0
8	0	0	0	–	0	20	16	1	–	3	1	0	0	–	0	0	0	0	–	0
BF extraction 11	0	0	0	–	0	28	16	1	–	0	0	0	0	–	0	0	0	0	–	0
14	0	0	0	–	0	23	19	0	–	0	0	0	0	–	0	0	0	0	–	0
17	0	0	0	–	0	96	47	1	–	0	1	0	0	–	0	0	0	0	–	0
20	0	0	0	–	0	144	37	0	–	0	1	0	0	–	0	0	0	0	–	0
23	0	0	0	–	0	81	23	0	–	0	0	0	0	–	0	0	0	0	–	0
26	0	0	0	–	0	126	58	0	–	0	0	0	0	–	0	0	0	0	–	0
31	0	0	0	–	0	1	100	0	–	0	0	0	0	–	0	0	0	0	–	0
35	0	0	0	–	0	0	97	1	–	0	0	0	0	–	0	0	0	0	–	0
39	0	0	0	–	0	0	57	2	–	0	0	0	0	–	0	0	0	0	–	0
43	0	0	0	–	0	0	104	0	–	0	0	0	0	–	0	0	0	0	–	0
47	0	0	0	–	0	0	18	0	–	0	0	0	0	–	0	0	0	0	–	0
51	0	0	0	–	0	0	12	0	–	0	0	0	0	–	0	0	0	0	–	0
TOTAL	1	0	0	–	0	914	1 133	238	–	191	7	1	2	–	0	0	0	0	–	0

Storage time in weeks	*Trichodorus*					*Mononchus*				
	w	*c*	*d*	*f*	*s*	*w*	*c*	*d*	*f*	*s*
Fresh soil	11	11	11	11	11	1	1	1	1	1
1	3	9	0	11	0	4	2	3	1	0
2	3	2	0	13	0	0	3	4	0	0
3	4	7	1	15	1	3	2	2	1	0
4	4	7	0	11	0	0	3	1	2	0
5	4	1	1	18	0	0	1	0	2	0
8	0	1	0	5	0	1	0	1	0	0
SF extraction 11	0	0	0	5	0	0	0	0	0	0
14	1	0	0	5	0	0	0	0	0	0
17	1	0	0	8	0	0	0	0	0	0
20	0	0	0	9	0	0	0	0	1	1
23	0	1	0	3	0	0	0	0	0	0
26	0	0	0	3	0	0	0	0	0	0
31	0	0	0	3	0	0	0	0	0	0
35	0	0	0	1	0	0	0	0	0	0
39	0	1	0	3	0	0	0	0	0	0
43	0	0	0	6	0	0	0	2	1	0
47	0	0	0	2	0	0	0	0	0	0
51	0	0	0	4	0	0	0	1	0	0
TOTAL	31	40	13	136	12	9	12	15	9	2

Storage time in weeks	*Trichodorus*					*Mononchus*				
	w	*c*	*d*		*s*	*w*	*c*	*d*		*s*
Fresh soil	4	4	4	–	4	2	2	2	–	2
1	3	3	0	–	0	4	4	3	–	0
2	8	3	0	–	0	8	1	0	–	0
3	3	2	0	–	0	7	0	1	–	0
4	2	1	0	–	0	1	0	0	–	0
5	0	0	0	–	0	3	0	0	–	0
8	2	0	0	–	0	1	0	0	–	0
BF extraction 11	1	0	0	–	0	1	0	0	–	0
14	0	0	0	–	0	1	0	0	–	0
17	3	0	0	–	0	0	0	0	–	0
20	0	0	0	–	0	1	0	0	–	0
23	1	0	0	–	0	0	0	0	–	0
26	0	0	0	–	0	0	0	0	–	0
31	1	0	0	–	0	0	0	0	–	0
35	0	0	0	–	0	0	0	0	–	0
39	0	0	0	–	0	0	0	0	–	0
43	0	0	0	–	0	0	0	0	–	0
47	0	0	0	–	0	0	0	0	–	0
51	0	0	0	–	0	0	0	0	–	0
TOTAL	28	13	4	–	4	29	7	6	–	2

Although the *d* and *s* treatments had severe adverse effects on numbers extracted by the SF technique, they were even more severe when using BF. This was presumably due to mortality as well as to reduction in motility. Among the BF treatments, BF/*w* yielded the highest numbers, probably because of increased motility with higher temperature. Unexpectedly, SF/*w* was more drastic than BF/*w* in reducing the numbers extracted.

Effect of storage

(*a*) SF

All treatments gave positive results at each extraction time, until the 47th week except *w* for which numbers fell below detectable levels after 31 weeks of storage. All treatments except *f* showed a fall in numbers after the first week of storage. The *f* and *c* treatments were clearly the most reliable and *f* was the most efficient.

(*b*) BF

The *d* and *s* treatments affected extraction, after only one week of storage, so severely as to cause numbers to fall below detectable levels. The *c* treatment did not result in a drop in numbers after the first week as *w* did but it gave less reliable results, particularly after the second week after which *w* yielded larvae until the 23rd week. Although numbers extracted were low, storage time decreased numbers more severely than it did in the SF extractions.

Pratylenchus

The SF technique, in particular again the SF/*f* treatment, was generally more efficient than BF. Of the BF treatments, BF/*w* was the best, probably because of increased motility with higher temperature. Differences between SF and BF, except for BF/*s* and *d*, were not large. This indicated that *Pratylenchus* can be successfully extracted by both techniques except that when the soil is allowed to dry or become heated, BF becomes less effective.

Effect of storage

(*a*) SF

A drop in numbers at the first week was not very marked in any of the treatments. Although there was a decrease after 5 and 17 weeks in *c* and *f*, respectively, positive results were obtained with both treatments over the whole period. The *w* treatment ceased to yield nematodes after 26 weeks while *c* and *f* were the most reliable. Although *d* and *s* decreased numbers, positive extracts were made during most of the storage period.

(*b*) BF

Although fewer individuals were recovered than with SF, the fall in numbers was not as marked after the first week in *c* and *w* where zero results were obtained only after the 26th week. Both *s* and *d* severely affected extractions, the former being the more drastic.

Criconemoides

The BF treatment is not discussed here as it is very inefficient at extracting this nematode.[1] Once again SF/*f* yielded the greatest numbers. Drying the soil lowered numbers more than did heating which had surprisingly little effect.

Effect of storage

The *f* treatment gave the most reliable and consistent results, *c* being the second most effective. After 26 weeks *w* gave zero results. The *d* treatment affected numbers more adversely than the *s*, which yielded fewer after 20 weeks. All treatments except *f* gave decreased numbers after one week of storage.

Hoplolaims

In all instances SF was superior to BF. In particular SF/*f* was again good. After storing the soil at 24°C and using SF, numbers were similarly affected by *d* and *s*. Although SF/*c* was not as efficient as SF/*f* it was second to it only in efficiency. When BF was used, *w* and *c* were the most successful. BF/*s* was much more severe than BF/*d* in decreasing numbers extracted.

Effect of storage

(*a*) SF

After the first week of storage the *d* and *s* treatments resulted in a marked drop in numbers. The *f* and *c* treatments yielded most nematodes with a decrease in numbers commencing after 20 weeks in storage. The *w* treatment was the only one to give zero results after 31 weeks. Indications were that these nematodes were somewhat tolerant of the heating and drying treatments because over the full storage period *d* and *s* yielded consistent results. The results with BF, however, were different.

(*b*) BF

The *s* treatment was so severe that no nematodes were extracted. In the other treatments a population decrease occurred from the first week, and with *d* in particular after the 20th week. The greatest numbers of nematodes were extracted from *c* and *w*, the latter of these yielding no nematodes after 26 weeks. Numbers recovered were well below those from SF.

Hoplolaimus

Only the SF technique is discussed here as BF was very inefficient at extracting this nematode, as was indicated by the results of the *w*, *c*, *d* and *s* treatments after the 3rd and 20th weeks. The *w* and *c* treatments yielded very little between the 23rd and 51st weeks. Yields from the *d* and *s* treatments indicated some tolerance to heating and drying. The SF/*f* treatment was, once again, the most efficient.

Rotylenchulus (immature)

Rotylenchulus differed from the nematodes already discussed since the treatments BF/*c* and *w* resulted in the most successful extractions. The *s* treatment, however, severely affected BF extraction. As far as the other treatments were concerned, SF was the most successful method. Numbers extracted by this method were almost equally affected by heating and drying.

Effect of storage

(*a*) SF

Numbers decreased after one week of storage in *w* and *d*, and a further drop occurred after 4 weeks in *d*. A large drop occurred after 2 weeks in *s*, and after 17 weeks in *f*.

(*b*) BF

After one week of storage numbers fell in *w*, *d* and *s*, and after 2 weeks in *c*. Both *d* and *s* had adverse effects on the numbers recovered, *s* being the most severe. When *c* and *w* were compared *w* was unreliable, yielding no nematodes after 32 weeks.

Xiphinema and *Dolichodorus*

No *Dolichodorus* and very few *Xiphinema* were extracted by BF and, although SF showed their presence, very few were extracted. Again the SF/*f* treatment was the most successful for *Dolichodorus*. SF/*w* yielded the most *Xiphinema* but only over the first 11 weeks of storage.

The BF technique did not indicate the presence of *Dolichodorus*, and extracted *Xiphinema* erratically. These results could have been due to a low population and to the relatively large body size of these nematodes which would make it difficult for them to move out of the soil and through the tissue paper. With *Xiphinema* the results of the SF technique suggest that *s* had decreased numbers more severely than *d*. With BF, heating, drying and storage depressed numbers of *Xiphinema*. Storage at 24°C increased numbers extracted by SF for at least the first 12 weeks.

Trichodorus

With SF the *f* treatment was the most successful, followed by *c* and *w* while BF *w* was as effective as the last two.

Effect of storage

(*a*) SF

Although a fall in population density was indicated after the fifth week with *f*, this treatment gave positive results for the full period. Poor extracts were made with *c* after four, and with *w* after five weeks. With both *d* and *s*, numbers were severely depressed one week after treatment.

(*b*) BF

Few individuals were extracted by this technique. Only *w* and *c* yielded nematodes for the first 4 weeks, after which *c* yielded nothing.

Mononchus

Numbers extracted indicated that BF/*w* was the most efficient treatment. It did, however, yield nothing after 20 weeks of storage. All other BF extractions yielded less than SF, with the exception of *s*, from which a total of four nematodes only were extracted.

Saprobiotic nematodes

No attempt was made to identify these nematodes but total counts were made. The results indicated that SF/*f* and BF/*w* were the most successful extraction methods. Using SF, the *d* and *s* treatments did not decrease numbers as severely as they did when using BF.

Modifications of BF technique

Using the *c* treatment, soil samples were run for 72 hours and compared with the standard 48 hour extraction. Results for nematodes which occurred in greater numbers are given in Table 3. The longer period did not significantly improve extraction.

No other attempts were made to improve on the BF technique, for example by using larger trays and shallower layers of soil. In BF, nematodes virtually extract themselves by their own movements so that only active nematodes can be obtained. By contrast, SF operates through differences in density and can even extract inert nematodes. For this reason it is considered probable that SF would normally give better results than BF even if the technique were improved.

TABLE 3

Results from Baermann funnel, comparing 48 with 72 hour extraction time

Storage time in weeks	*Meloidogyne*		*Pratylenchus*	
	48 hours	72 hours	48 hours	72 hours
1	18	18	14	14
2	6	5	12	18
3	0	2	2	11
4	1	2	1	10
5	0	2	7	3
8	0	0	4	0
11	1	0	0	3
14	1	0	2	2
17	0	0	2	7
20	0	1	0	1
23	0	0	0	0
26	0	0	3	2
31	0	0	0	0
35	0	0	0	1
39	0	0	0	0
43	0	0	0	1
47	0	0	0	0
51	0	0	0	0
TOTAL	27	30	47	73

Storage time in weeks	Hoplolaims		*Rotylenchulus*	
	48 hours	72 hours	48 hours	72 hours
1	21	26	103	157
2	15	33	88	133
3	11	21	36	67
4	3	18	28	66
5	20	17	86	38
8	3	5	16	27
11	6	4	16	17
14	7	4	19	33
17	9	8	47	99
20	9	6	37	97
23	2	4	23	39
26	11	18	58	66
31	4	4	100	72
35	7	6	97	106
39	11	18	57	126
43	3	4	104	68
47	6	5	18	6
51	2	1	12	40
TOTAL	150	202	945	1 257

Discussion

Although sugar flotation, particularly after certain of the treatments such as *f*, yielded higher numbers and more positive results, variability between samples made it difficult to determine which treatments gave the most consistent results. Since no method is known whereby all nematodes are extracted from the soil and since all determinations are merely estimates of the total population, consistency is often more important than efficiency. One source of error results from the fact that no single method can extract all stages, eggs in particular being difficult to extract.

The Baermann funnel yielded the highest numbers of *Rotylenchulus*. Although this nematode was formerly regarded as a doubtful parasite of sugarcane in South Africa, it was included in these investigations because it has now been successfully reared in pots in the laboratory. Swollen adult females identified as *R. parvus* (Williams) could be seen in these cultures.

During these investigations it became obvious that the different nematodes were very differently affected by the treatments. Both heating and drying of the soil depressed the numbers extracted and heating generally had the more noticeable effect. Storage, even for a week, depressed numbers which either dropped sharply or became progressively less. It is important to be aware of these effects which appear to be largely eliminated or overcome by using a fixative treatment such as the one described here. However, in certain instances, after

about 4 months of storage, even the formalin treatment resulted in a drop in numbers. This was probably because the quantity added to the sample was not sufficient to preserve the nematodes satisfactorily for prolonged periods. This was also demonstrated by an apparent increase in numbers, mainly at the 43rd week of storage (Table 2). The concentration of the fixative in these particular samples might have been high enough to preserve nematodes over that period. Unless it is intended to make extracts from soil samples immediately after taking them, it seems imperative to fix or stabilize the population in them, for example by using formalin. Storing at 5°C did not stabilize the population effectively.

This work was undertaken in an attempt at collecting nematodes for diagnosis and population studies. For this reason no differentiation was made between living or dead, mobile or immobile nematodes. Different species within the genera were not recorded separately nor was attention paid to different stages in the life cycle. Although the sugar flotation technique is quite adequate for obtaining living nematodes for starting cultures, some of the additional treatments, such as fixation with formalin would not be suitable for this purpose.

It is likely that the greater efficiency of sugar flotation is due to the fact that this method operates through differences in density and is therefore able to extract nematodes even when they are in an inert state. The Baermann funnel, by contrast, can extract only actively moving nematodes and is not very effective with large or sluggish genera such as *Dolichodorus*, *Criconemoides*, *Xiphinema*, *Hoplolaimus* and the Hoplolaims.

The formalin treatment improved extraction by SF, which might have resulted from changes in the density of the nematodes, making them more easily extracted by flotation.

Soil of only one type was used in these experiments although it should be appreciated that the physical properties of soil, as well as soil disturbance and seasonal fluctuations, may significantly influence the results of extractions. In the South African sugar industry it is in soils with low clay contents that nematodes are of the greatest concern.

This work demonstrates that extraction of nematodes from the soil can be influenced by variation in a number of factors. This should be taken into account and important factors should be kept as uniform as possible.

With present facilities and methods it is difficult to make accurate assessments of nematode numbers in sugarcane soils.

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