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AN EXAMINATION OF METHODS USED TO EXTRACT VIRUS-VECTOR NEMATODES (NEMATODA: LONGIDORIDAE AND TRICHODORIDAE) FROM SOIL SAMPLES

by
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Summary. The efficiency of the different methods used to collect and extract virus-vector nematodes (*Longidorus, Paratrichodorus, Trichodorus* and *Xiphinema*) were examined as part of a collaborative assessment by staff at the principal Nematology laboratories in Scotland, of methods for field sampling, handling and excracting these nematodes. Vehicular transportation of soil samples from field to laboratory did not reduce the numbers of nematodes recovered but several short drops did, by up to 39%, largely by soil compaction. The two-flask method was found to be much slower and was less effective than the decanting and sieving method for recovering longidorid nematodes. When using the decanting and sieving method a 200 g soil sample stirred into suspension in a 5 l pail, given a 15 s settling time and the supernatant poured through a sieve with a 150 μm aperture mesh held over an empty 5 l pail was found to be satisfactory. This collects the large longidorid nematodes. The residue on the sieve is washed onto a 95 μm aperture mesh plastic sieve which is then placed in a water-filled Baermann funnel. Smaller trichodorid nematodes and juvenile longidorids were collected by passing the supernatant collected in the second pail through individual 75 μm and 53 μm aperture mesh sieves. The residue on both sieves is washed onto a 2-ply tissue supported in a plastic sieve which is placed in a second water filled Baermann funnel. Most longidorids had fallen to the bottom of the funnel after 24 h, but if required for biological studies were collected after 4 to 6 h. Trichodorids took longer and were recovered after 48 h or, 24 h if required for biological studies. Water temperature in the funnels should be maintained at 15 to 20°C.

Staff in the Nematology laboratories at the Scottish Crop Research Institute (SCRI), Department of Agriculture and Fisheries for Scotland (DAFS) and at the Scottish Agricultural College's (SAC) collaborated in an examination of the methods and techniques they use for field sampling, handling and extracting virus-vector nematodes. This was done to standardize methods when working with these nematodes. Factors affecting the efficiency of the two methods mostly used for extracting *Longidorus, Paralongidorus, Paratrichodorus, Trichodorus* and *Xiphinema* nematodes were investigated at the SCRI. Also the relative susceptibility of virus-vector nematodes to handling of soil samples during transport from the field and in the laboratory was examined and the results from these studies are reported here.

Materials and Methods

Two methods for extracting virus vector nematodes from soil samples are used in Scotland, namely Flegg's (1967) modification of Cobb's (1918) decanting and sieving method and Seinhorst's (1955) two-flask method. Both methods are combined with the Baermann funnel method (1917) and Pitcher and Flegg's (1968) sieves for final separation of nematodes from soil debris. Full descriptions of these methods, including schematic illustrations of extraction methods are given by the respective authors. Furthermore, all these methods are described by Southey (1970), therefore, they are not repeated here. The various components and factors influencing the extraction and final separation methods examined in the present study are described below.

The nematode populations used in the study were *Longidorus elongatus* (de Man) Thorne *et* Swanger in sandy loam field soil under a crop of *Lolium perenne* L., Dundee, Scotland; trichodorids, 80% *Paratrichodorus pachydermus* (Seinhorst) Siddiqi and 20% *Trichodorus cylindricus* Hooper in loamy sand field soil under *L. perenne*, Carnoustie, Scotland and *Xiphinema index* Thorne *et* Allen originally from Italy, in sandy loam under *Ficus carica* L., maintained as a glasshouse culture at the SCRI.

Bulks of soil containing the nematodes were gently but thoroughly mixed and 200 g samples were used for the experiments. In the experiments the water temperature in the Baermann funnels was 20°C and the final separation time was 24 hours unless stated otherwise.

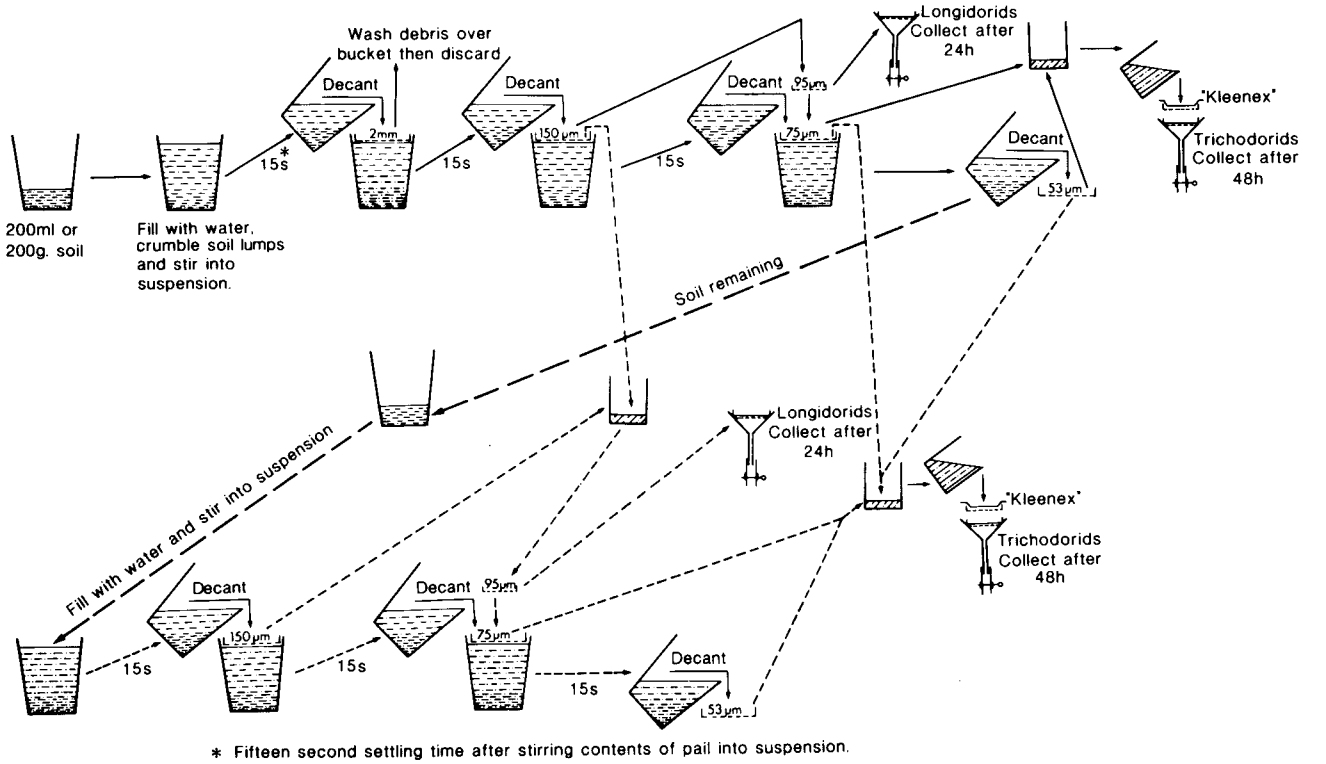

Fig. 1 - Schematic diagram of the decanting and sieving procedure used at the SCRI to recover *Longidorus, Paratrichodorus, Trichodorus* and *Xiphinema* species from soil samples. The procedure connected by solid lines results in an 85% recovery of trichodorids and a > 90% recovery of longidorids. This procedure when combined with the procedure connected by hatched lines results in a > 95% recovery of trichodorids and longidorids.

The extraction methods, combinations and numbers of sieves, sieve-mesh and final separation filter-mesh aperture sizes are given in Table I. When decanting and sieving a 15s sedimentation time was used throughout. With the two-flask method only stages 1 and 3 were done, each with a 10 min inversion time (Southey, 1970). Where «Kleenex» tissues («Professional wipes», Kimberley-Clark, London) were used as final separation filters they were supported on polyethylene final separation rings with bases of 1 mm aperture nylon-mesh.

Methods CSDLA and CSDTF (see Table I for explanation of codes) were used for longidorids and trichodorids respectively when examining the recovery of nematodes on successive sieves. Nematodes and debris retained on each sieve was placed on separate 95 µm nylon supports for longidorids or 2 ply «Kleenex» for trichodorids.

Methods CSDLA and CSDTF were used and after placing the final separation filters in the Baermann funnels, nematodes were recovered at the times specified. When recovering the nematodes at each specified time a quantity of water was added to each funnel equal to that removed with the nematodes.

The effect of water temperature in the Baermann funnels on recovery of nematodes was examined in controlled environment rooms. Final separation filters containing nematodes and soil debris were placed in the filled funnels when the water temperature had stabilised at the desired temperature.

Polythene-bags containing 500 g of soil containing nematodes or 500 cm^3 of water to which nematodes had been added were dropped from various heights on to a concrete floor. Nematode survival was assessed from a 200 g subsample taken from each of the 500 g soil samples. Nematodes were recovered by using methods CSDLA and CSDTF for longidorids and trichodorids respectively. After being dropped from the specified heights water samples containing nematodes were poured on to a final separation filter of 95 µm for longidorids and 2-ply «Kleenex» for trichodorids and then placed in Baermann funnels. Trichodorids in cylinders of soil in polythene bags contained within lengths of polyethylene-pipe, 300 mm × 55 mm dia. were dropped from specified heights. After being dropped the soil cylinders were divided into three equal sections and the trichodorids were recovered from each part by method CSDTF. Numbers of trichodorids recovered were adjusted to compensate for differences in the weights of the three soil sections. Polythene bags con-

TABLE I - *Methods and sieves and final separation filter aperture sizes used to recover longidorid and trichodorid nematodes from soil samples.*

	Sieves	Final separation	Code
Modified Cobb's sieving and decanting			
Longidorids	(3×150 μm)[b]	95 μm[a] nylon support	CSDLA
	250 μm, 150 μm	95 μm nylon support	CSDLB
	250 μm over water	95 μm nylon support	CSDLC
	150 μm over water	95 μm nylon support	CSDLD
	150 μm over water	2 ply Kleenex	CSDLE
Trichodorids			
	150 μm, 75 μm, 53 μm, 53 μm	2 ply Kleenex	CSDTF
	150 μm, 75 μm, 53 μm, 53 μm	1 ply Kleenex	CSDTG
	150 μm, 75 μm, 53 μm, 53 μm	95 μm nylon support	CSDTH
	150 μm over water	95 μm nylon support	CSDTI
	150 μm over water	2 ply Kleenex	CSDTJ
Seinhorst two flask			
Longidorids	(3×150 μm)	95 μm nylon support	SFLK
Trichodorids	(3× 75 μm)	2 ply Kleenex	SFTL
	(3× 75 μm)	95 μm nylon support	SFTM

[a] Aperture diameter of mesh; [b] brackets used to indicate banks of sieves.

TABLE II - *Recovery of longidorids and trichodorids by different extraction methods.*

Methods[a]	Trichodorids ♂ + SD[b]		♀ + SD		θ + SD		Total + SD		*L. elongatus* Total + SD		*X. index* Total + SD	
CSDLA	na		na		na		na		130	16	855	241
CSDLB	na		na		na		na		103	18	865	144
CSDLC	na		na		na		na		96	15	382	88
CSDLD	na		na		na		na		125	35	652	125
CSDLE	na		na		na		na		85	28	321	126
CSDTF	32	9	42	12	211	26	285	33	na		na	
CSDTG	42	23	76	22	217	24	335	26	na		na	
CSDTH	20	12	40	14	175	31	235	39	na		na	
CSDTI	20	10	27	11	20	6	66	16	na		na	
CSDTJ	22	10	24	16	30	4	77	23	na		na	
SFLK	na		na		na		na		38	16	257	111
SFTL	49	19	88	34	277	56	414	81	na		na	
SFTM	49	28	75	30	332	85	456	108	na		na	

[a] For explanation of codes see Table I; [b] mean (n=10)+one standard deviation.

taining 500 g of infected soil were transported 250 km by car, at an average speed of 70 km/h and were kept in the stationary car for 4 hours at the mid-point of the journey. The car travelled on several different road surfaces including country, motorway and city roads. The ambient temperature within the car ranged from 15-20°C.

Results

When extracting trichodorids the two-flask method was significantly ($P < 0.001$) more effective than the decanting and sieving method and yielded *c.* 25% more nematodes (Table II). Using a single or double-ply «Kleenex» for the final separation filter did not significantly affect the numbers of nematodes recovered but a significant ($P < 0.1$) reduction in recovery did occur between these and the 95 μm aperture nylon mesh. The two-flask method yielded substantially fewer *L. elongatus* and *X. index* than did decanting and sieving where a bank of three 150 μm, or a 250 μm and 150 μm, or a single partially submerged 150 μm aperture sieves were used with a 95 μm aperture nylon mesh final separation filter. Using a single partially submerged 150 μm aperture sieve was very inefficient for recovering trichodorids and «Kleenex» were inefficient when recovering *L. elongatus* and *X. index* (Table II).

With *L. elongatus* and *X. index* 97% of the total nematodes recovered were obtained from the first 150 μm aperture sieve whereas with trichodorids only 26% were thus recovered. A further 72% of trichodorids were recovered on the succeeding 75 μm and 53 μm aperture sieves (Table III).

Nematode recovery from the first decanting yielded 85 to 94% of the total trichodorids, *L. elongatus* and *X. index* recovered. Less than 10% of the longidorids but 15% of trichodorids were recovered from a second and third decanting with two-thirds of the trichodorids being recovered in the second decanting (Table III).

After 4 to 6 hrs in the funnels, at which time nematodes usually are recovered for biological studies, less than half the total number of trichodorids were recovered whereas 80 to 92% of *L. elongatus* and 56 to 64% of *X. index* were recovered. Three-quartes of the trichodorids were recovered after 24 hrs with a further 20% recovered after a further 24 h. After 24 h 98% and 86% of *L. elongatus* and *X. index* respectively were recovered with a further 10% of *X. index* being recovered after a further 24 h (Table IV).

The optimum temperature range for recovering nematodes was between 10 and 20°C for trichodorids and 20 to 30°C for *X. index*. The recovery of *L. elongatus* was not significantly affected within the temperature range 5-30°C. Few *X. index* were recovered at 5°C

TABLE III - *Percentage recovery of trichodorids and longidorids on successive sieves and after successive decantings.*

Sieves	Yields from successive sieves[a]											
	Trichodorids								*L. elongatus*		*X. index*	
	♂ + SD[b]		♀ + SD		θ + SD		Total + SD		Total + SD		Total + SD	
150 μm	na		na		na		na		97	35	97	28
150 μm	na		na		na		na		2	2	2	0.8
150 μm	51	26	46	20	18	5	26	6	1	0.8	0.9	0.3
75 μm	46	23	51	17	54	9	53	10	0		0.1	0.2
53 μm	3	7	3	3	25	5	19	4	na		na	
53 μm	0		0		4	3	3	2	na		na	

Extraction method	Nematode	Yields from successive decantings[a] Decanting 1 Total + SD	2 Total + SD	3 Total + SD
CSDTF	trichodorids	85 + 25	11 + 5.5	4 + 1.8
CSDCA	*L. elongatus*	94 + 9	5.5 + 2	0.5 —
CSDCA	*X. index*	92.5 + 12	7 + 1.4	0.5 —

[a] Mean (n=10) given as column percentage + one standard deviation given as percentage of column row; [b] as for [a] above but given as row percentage.

whereas substantial numbers of trichodorids were recovered at this temperature although the recovery was less than at the optimum temperature of 15°C (Table V).

Recovery of active trichodorids, *L. elongatus* and *X. index* was only minimally reduced after the nematodes in 500 cm³ of water had been dropped from 1, 3 or 5 m or 1 m five times. A substantial reduction in the recovery of nematodes was caused when the nematodes in 500 g of soil had been dropped from the same heights. A single drop from 1 m, of soil containing trichodorids reduced the subsequent recovery of nematodes by almost one fifth whereas with *L. elongatus* and *X. index* the reduction was only 3%. Dropping from 1 m five times reduced the recovery of trichodorids by 35% which was similar to the reduction caused by a single drop from 5 m. Repeated dropping of *L. elongatus* and *X. index* in soil however resulted in substantially larger reductions in yields when compared with a single 5 m drop. Although the proportion of adult trichodorids to juvenile stages was small in this experiment a larger proportion of the adults than juveniles were killed if dropped in soil and water.

TABLE IV - *Cumulative percentage recovery of longidorid and trichodorid nematodes during 72 or 166 hours.*

Time (hours)	Trichodorids				*L. elongatus* Total	*X. index* Total
	♂	♀	θ	Total		
0.25	na	na	na	na	na	4
0.50	na	na	na	na	15	12
1	10	7	10	10	28	41
2	18	16	22	21	50	46
4	35	33	37	37	80	56
6	45	44	48	47	92	64
24	71	70	76	75	98	86
48	92	92	94	94	99	96
72	100	100	100	100	100	98
96	na	na	na	na	na	99
120	na	na	na	na	na	100
144	na	na	na	na	na	100
166	na	na	na	na	na	100

Discussion

Our examination of methods and factors affecting the recovery of virus-vector nematodes from Scottish soils complements and extends the study by Flegg (1967) in which he examined the recovery of longidorid species, including *X. diversicaudatum*, which most frequently occur in soils from southern England. Flegg's (1967) modified decanting and sieving method for recovering longidorids is recommended by Southey (1970) and Taylor (1972) and is the most frequently used method for this purpose in British laboratories (Brown and Boag, 1986). In our study this method was more efficient for recovering longidorids than the two-flask method, which was developed for the recovery only of small nematodes such as trichodorids. With the two-flask method substantially more time is required to process a sample than with decanting and sieving, 25 min vs. 5 min per sample, thus its value for use in routine work

TABLE V - *Effect of temperature on the recovery of longidorid and trichodorid nematodes*

Temperature °C	Time hours	Trichodorids ♂ + SD[a]		♀ + SD		θ + SD		Total + SD		*L. elongatus* Total + SD		*X. index* Total + SD	
5	6	6	5	10	6	81	17	97	15	79	13	46	13
	24	12	8	21	12	161	25	194	31	111	18	60	17
10	6	4	3	8	5	51	20	62	23	66	14	221	59
	24	15	10	26	11	215	23	256	16	100	17	324	68
15	6	9	7	15	9	75	22	97	28	58	11	262	99
	24	22	11	37	11	254	45	312	48	89	21	350	87
20	6	16	8	15	12	58	27	89	34	80	8	214	67
	24	26	11	30	14	195	51	251	59	100	12	382	61
25	6	10	7	15	12	45	19	70	13	66	12	279	93
	24	31	13	46	16	148	39	225	44	87	16	434	76
30	6	3	5	12	5	28	14	43	16	45	23	232	79
	24	26	16	39	11	99	23	163	31	81	19	403	47

[a] Mean (n=10) + one standard deviation.

is limited. Where a limited number of soil samples have to be processed the two-flask method might be used especially if the detection of trichodorids has to be determined by the most effective routine method available i.e. statutory samples for certification purposes or pre-planting advice where a high value crop is very susceptible to damage by small numbers of trichodorids and/or the viruses they transmit.

We have adopted the use of a decanting and sieving procedure, illustrated in Figure 1, for the routine processing of soil samples to determine the presence or otherwise of virus vector nematode species present in Scotland (Heath *et al.*, 1977). This method, based on the results from our study and that of Flegg (1967), combines simplicity and speed of operation with relatively good efficiency of recovery of all virus-vector genera as compared with the two-flask methods. Virus-vector nematodes are found mostly in sand, loamy sand and sandy loam soils which are the soil types present in the main arable areas in Scotland (Alphey and Boag, 1976; Taylor and Brown, 1976). The method we have adopted for recovering virus-vector nematodes from soil samples is suited to these soil types but requires modification if used with humic or clay soils. Also, the method is suitable for use in those countries with a virus-vector nematode fauna similar to that in Scotland i.e. Fennoscandia, New Zealand, etc. The applicability of this method for use with routine advisory and statutory soil samples in Scotland is being evaluated by staff in the Department of Agriculture and Fisheries for Scotland and the Scottish Agricultural Colleges.

To obtain longidorid nematodes for biological studies we use the method CSDLD incorporating a 15 to 20°C funnel water temperature and 4 to 6 h final separation time. For trichodorids the CSDTF method can be used, the final 53 μm aperture sieve may be omitted, the funnel water temperatures should be 15 to 20° C and the final separation time 24 h. When working with non-indigenous virus-vector species from warmer climates e.g. *X. index*, it may be necessary to increase the funnel water temperature by 5 to 10°C. This increase in temperature reduces the oxygenation of the water in the funnels and can result in asphyxiation of the nematodes. This problem does not affect the total yield of nematodes from a soil sample and is important only when live specimens are required for biological studies.

TABLE VI - *Estimated percentage mortality of trichodorid and longidorid nematodes in 500 g soil samples and 500 cc water samples dropped from different heights*

Height	Trichodorids ♂	♀	θ	Total	*L. elongatus* Total	*X. index* Total
Soil samples						
1 m	51	24	11	18	3	3
3 m	53	29	21	25	4	17
5 m	44	21	37	35	1	27
5×1 m	63	44	29	35	13	39
Water samples						
1 m	25	12	0	<1	0	3
3 m	25	20	0	<1	4	3
5 m	0	4	0	<1	10	4
5×1 m	19	8	0	4	8	2

TABLE VII - *Estimated percentage mortality of trichodorids in the top, middle and bottom sections of a cylinder of soil dropped from 5 meters*

Soil section	Trichodorids ♂	♀	θ	Total
Top	0	48	17	22
Middle	6	48	13	19
Bottom	6	49	28	43

When reporting a study by Bor and Kuiper (1966) of the mortality of trichodorids in soil samples dropped from different heights, Winfield and Cooke (1975) omitted to state that the samples used for each height were dropped ten times. This omission permits the erroneous conclusion that 80% of trichodorids may be killed in a soil sample dropped 1 m. Our results are similar to those of Bor and Kuiper (1966) and show that less than 20% of trichodorids in a soil sample are killed when dropped 1 m. The mortality of trichodorids in dropped samples seems to be a function of the total height dropped e.g. 22% mortality from ten drops of 0.25 m (=2.5 m; Bor and Kuiper, 1966) as compared with 25% mortality from a single drop of 3 m; similarly, 35% mortality occurred with five drops from 1 m or with a single drop of 5 m. *Xiphinema index* were more susceptible to rough handling than *L. elongatus* with the latter species only affected by repeated dropping. The mortality of trichodorids, *L. elongatus* and *X. index* in water when dropped from up to 5 m was less than 10%. Soil compaction seems the likely cause of nematode mortality because as many trichodorids were incapacitated in the bottom section as in the combined middle and top sections of a column of soil dropped 5 m. Care should therefore be taken when handling soil-samples containing virus vector nematodes and repeated dropping even from a short distance should be avoided. We also found that vehicular transportation of soil samples from the field to the laboratory had no significant effect on the final yield of virus-vector nematodes from the samples.

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