Nematologica 18 (1972): 469-475. E. J. Brill, Leiden

published in 1973

A FLUIDISING COLUMN FOR EXTRACTING NEMATODES FROM SOIL

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An apparatus which uses a controlled water current passing through a sintered plate to separate nematodes from soil particles is described. Nematodes of all types and sizes can be extracted, and the apparatus is particularly useful for extracting white *Heterodera* females. Construction is simple, so the apparatus is cheap, robust and valuable where only limited facilities are available. It may prove useful for extracting other small soil invertebrates, or their eggs.

Methods used to extract nematodes from soil are summarised in Oostenbrink (1960, 1970), Seinhorst (1956), Flegg & Hooper (1970) and Whitehead & Hemming (1965). They depend on the ability of nematodes to move through sieves or filters of appropriate mesh, on differences between the physical properties of nematodes and soil particles or on both. Sedimentation and elutriation and then sieving are the most widely used methods, but no single piece of apparatus extracts both cyst and vermiform nematodes efficiently.

The apparatus described below uses a controlled water current passing through a sintered plate and extraction depends only on differences in size and specific gravity between nematodes and soil particles. Because a wide range of steady rates of water flow can be established, the apparatus extracts nematodes ranging from the smallest larvae to the largest adults, including those of *Longidorus* and *Xiphinema* spp. and the swollen females and cysts of *Heterodera* spp.

Theory

Plastic sinters through which air is blown are used industrially to fluidise and separate powdered mixtures of minerals. The denser minerals move to the bottom of the fluidised bed and the less dense to the top. The fluidising column extractor described below uses the same principle, and separates nematodes from mineral particles and larger pieces of organic matter in an upcurrent of water. The terminal velocity of a spherical soil particle settling in a liquid is described by the equation:-

$$V = \frac{g\,(Sp - S1)\,D^2}{28\,n} \qquad \text{(Tanner \& Jackson, 1947)}$$

where V is the terminal velocity (cm/sec), g the acceleration due to gravity (981 cm/sec^2), Sp = the specific gravity of the particle, S1 the specific gravity of the liquid (0.998 for water at 23° C), D the diameter of the particle (cm) and n the viscosity of the liquid (0.00936 poises for water at 23° C).

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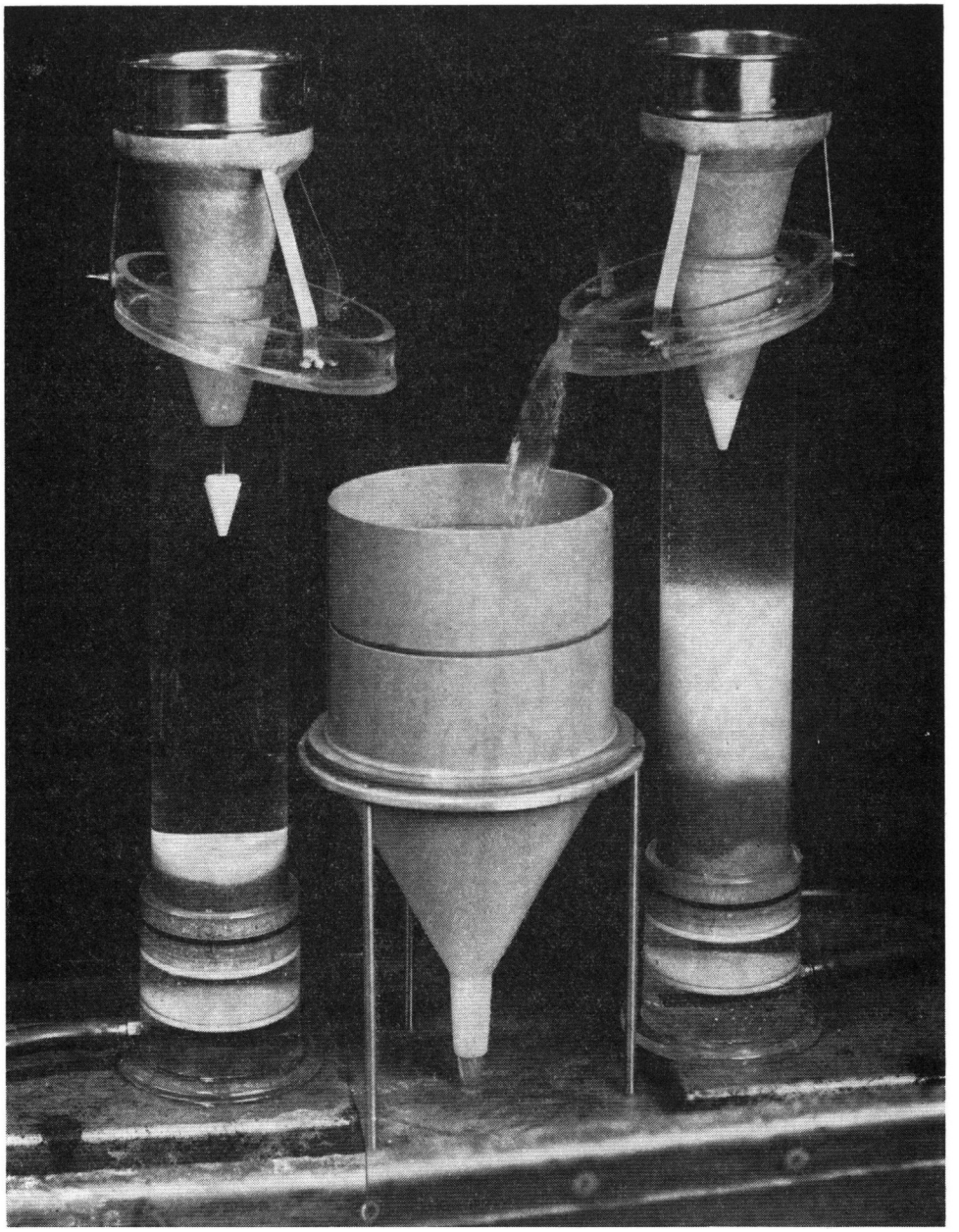

Fig. 1. The fluidising column containing sand in an unfluidised state (left) and a fluidised state (right). (Photograph by C. C. Doncaster).

For a particle to be carried upwards the upward rate of liquid flow must exceed the terminal settling velocity of the particle. Particles with settling rates greater than the water flow remain at the bottom of the column, occupying part of the cross-sectional area and so increasing the rate of water flow. Particles reach

equilibrium when their cross-sectional distribution is such that the increased rate of water flow equals their terminal settling velocity.

When a soil sample is added to the column it becomes fluidised with larger, denser particles at the bottom and smaller, less dense particles at the top of the fluidised layer and all particles separated from each other by water. The degree to which the soil sample expands and the type of particle carried to the top of the column are both determined by the rate of water input, which can be varied and accurately measured using a flow meter.

Slight turbulence occurs at the top of the column, so decreasing efficiency. This problem was overcome by putting an inverted cone in the top of the column, which increased the water flow uniformly over the top 12 cm. In a later model the top of the column was tapered to produce the same effect.

Descriptions of apparatus

Fig. 1 shows the first model containing sand of three particle sizes and colours, and in unfluidised and fluidised states. The column is a perspex tube 38 cm long of 63 mm inside diameter and 76 mm outside diameter. The base of the column is sealed by an 'O' ring into a short piece of perspex tube of 76 mm inside diameter,

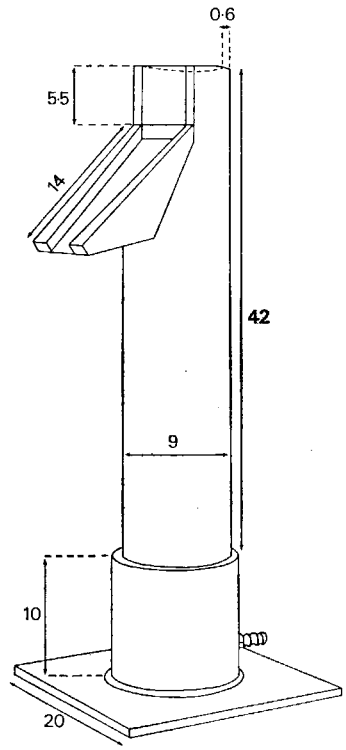

Fig. 2. Modified fluidising column, with dimensions in centimetres.

which contains two removable plastic sinters *) held in position by two short lengths of 76 mm outside diameter tube. Water is introduced from a constant head tank by a side arm into the chamber below the sinters. The rate of flow is monitored by a flow-meter and can be varied by an adjustable valve. The cone at the top of the column houses a simple valve and supports a 10 cm diameter sieve (12 mesh, aperture size 1.4 mm) through which the sample may be introduced; the cone is removable so that samples can be introduced through a larger funnel if necessary. The nematodes and other particles that rise to the top and overflow are directed onto sieves by a perspex collar. When small rates of water flow are used the column must be vertical, so levelling feet (not shown) are fitted.

After a series of trials a second model was made, incorporating all the essential features of the first but using standard parts and a simplified construction wherever possible. A perspex tube was sealed into a 7.6 cm "Durapipe" 'O' Ring Socket **), and this tube held a plastic sinter in place. The lower end of the socket was closed by welding a square of PVC to it, which also serves as a base. The top of the column was tapered by cutting it off at an angle, and gluing a block of perspex across the cut surface. A channel cut in this block directs the overflow onto a bank of sieves. A flat piece of perspex glued above the overflow protects the knife edge of the overflow, which is necessary for an even flow. All dimensions are in Fig. 2.

METHOD

The apparatus has a volume of approximately 2 litres and is designed to take 200 ml soil samples. All except samples of very sandy soil need pre-treatment to separate the particles before adding to the column. Samples from which vermiform nematodes were to be extracted were dispersed with a domestic sieve and large funnel (Seinhorst, 1956; Flegg & Hooper, 1970). Samples from which cysts or swollen females of *Heterodera* spp. were to be extracted were dispersed in water with a stirrer or a vibrator. The float from the column was collected on sieves of different mesh size according to the nematodes to be collected and the water flow rates used.

The flow rate to elutriate females of *H. rostochiensis* was determined by observing the relationship between mean diameter and the time taken to settle a distance of 34 cm in water. The specific gravity of females measured in sucrose solutions of different concentrations ranged from 1.07 to 1.08. Tanner & Jackson's (1947) formula predicted a terminal velocity of 2.1 cm/sec for the largest female examined, compared with observed terminal velocities of 1.3 cm/sec for the largest (0.7 mm diam.) and 0.4 cm/sec for the smallest (0.3 mm diam.). *Longidorus leptocephalus* females had terminal velocities not exceeding 0.11 cm/sec, and the settling rate of *H. rostochiensis* larvae was inferred by shaking a suspension of larvae in a large measuring cylinder and counting the number in a sample taken at a fixed point

*) 3 mm thick fluidising grade 'Vyon', supplied by Porvair Ltd., Estuary Road, King's Lynn, Norfolk.

**) Supplied by Wilford (Plastics) Ltd., 17a/21 Windsor Street, Luton, Bedfordshire.

(11 cm) below the surface at intervals. A mean terminal velocity of 0.01 cm/sec. was calculated from the results.

Terminal velocities of soil particles greater than 50 μm diam. were measured by Janke (1965) and can be calculated for smaller particles using Tanner & Jackson's formula.

The smallest sieves generally used to catch nematodes have apertures of 53 μm (300 meshes per inch, B.S. 410 test sieves). Soil particles small enough to pass through such a sieve (50 μm diam.) have a terminal velocity of 0.23 cm/sec, so flow rates are critical if a clean extract of vermiform nematodes is required. The flow rate of 1.3 cm/sec required to collect *Heterodera* females, using 60 mesh (250 μm aperture) sieves, will elutriate mineral particles of only 120 μm diam. and, as these easily pass through the collection sieves, flow rates are less critical here.

In practice it is unnecessary to run the apparatus continuously at these flow rates. As the soil becomes fluidised and the soil particles are sorted according to their density and diameter, the nematodes and smaller mineral particles move into suspension. All particles that are in suspension rather than fluidised may then be removed by increasing the flow rate until the fluidised layer expands to fill the column. This process may be repeated again to ensure that most of the nematodes are removed from the sample.

EXPERIMENTAL RESULTS

Most of the soils tested contained much sand, but whatever the soil type, aggregates must be broken up and particles too large to fluidise at the flow rate used removed.

Extraction of *H. rostochiensis* cysts was significantly better with the column extractor than the Fenwick can in all tests (Table I), particularly when the soil was undried. In another test the soil contained white females, new cysts full of eggs

TABLE I

Numbers of H. rostochiensis *cysts extracted from dried and undried soils. Means of eight replicates.*

	Fenwick Can	Column Extractor
Woburn soil (sandy)		
Wet	129 ± 7.7	174 ± 7.3
Dry	136 ± 6.3	171 ± 4.3
Rothamsted soil		
Wet	101 ± 3.5	140 ± 7.6
Dry	141 ± 3.2	170 ± 4.0
Soil from pot test (dried)	537 ± 13.2	604 ± 15.5

and old cysts containing few eggs (Table II). To remove white females, samples in the Fenwick can had to be washed vigorously, producing a dirty extract.

TABLE II

Numbers of white females, new and old cysts of H. rostochiensis, *extracted with Fenwick Can and Column Extractor. Cysts/200 ml of soil; means of nine replicates.*

	Fenwick Can	Column Extractor	Level of significance
White females	67	78*	*5%
New cysts	185	232**	**1%
Old cysts	299	283	
Total	551	593	

More of the denser white females and new cysts were collected with the column extractor, but the Fenwick can extracted the lighter, older cysts equally well.

Males and larvae as well as white females of *H. rostochiensis* were collected from two samples and, for comparison, the males and larvae were collected by the 2-flask method (Seinhorst, 1955) and Whitehead trays (Whitehead & Hemming, 1964); females were extracted with a Fenwick can. The column extracted more males and many more larvae from one of the samples than either of the methods (Table III) but there was no difference between the numbers of

TABLE III

Numbers of nematodes extracted by different methods. Numbers/200 ml of soil, means of four replicates from each of two soils.

Method	Sample+	*Heterodera* larvae	*Heterodera* males	*Heterodera* females	*Longidorus leptocephalus* females
Seinhorst 2-Flask (short method)	1	58	13		
	2	169	10		
Whitehead Tray	1	39	16		
	2	123	10		
Fenwick Can	1			63	
	2			37	
Column Extractor	1	38	17	60	96
	2	321**	15	40	102
Direct Sieving	1				92
	2				97

Significance *5%
**1%

+Samples 1 and 2 are from different bulks of soil

females collected from the column or the Fenwick can. In another test eight soil samples containing *H. rostochiensis* larvae were extracted, either with the column or the 2-Flask method, and each sample was then re-extracted by the other method. The Column extractor was always more efficient than the 2-Flask method (Table IV) but although much quicker it produced more sediment than the 2-Flask method.

Extraction of *Longidorus leptocephalus* by direct sieving was more tedious, but probably no less efficient than the column extractor (Table III).

TABLE IV

Numbers of H. rostochiensis *larvae extracted by the Seinhorst 2-Flask method and the column extractor. Larvae/200 ml soil, means of 8 replicates.*

	No. of larvae
Column extractor	1816
2-flask after column extractor	4
2-flask	1713
Column extractor after 2-flask	40

DISCUSSION

The results show that the fluidising column extracts soil-inhabiting plant-parasitic nematodes of all sizes as efficiently as, or more efficiently than, other methods in common use, and is simple, small and robust but it is no improvement for peaty soils. It is quicker than other methods and, as flow rates can be specified and accurately controlled and the whole process is visible, operator errors should be less than with other methods. The apparatus is easily cleaned by inverting and sluicing with water, and obviously is advantageous where only limited facilities are available. The principle of fluidising soil samples may also prove useful for extracting other small soil invertebrates, or their eggs.

ZUSAMMENFASSUNG

Eine Aufschwemmsäule für die Gewinnung von Nematoden aus dem Boden

Es wird ein Gerät beschrieben, in dem ein regelbarer, durch eine Sinterplatte fließender Wasserstrom zur Abtrennung von Nematoden aus Bodenproben benutzt wird. Damit können Nematoden aller Art und Größe extrahiert werden. Das Gerät ist besonders zur Gewinnung weißer *Heterodera*-Weibchen geeignet. Es ist einfach im Aufbau, preiswert, robust und besonders dort wertvoll, wo nur eine begrenzte Laborausstattung zur Verfügung steht. Das Gerät kann möglicherweise auch für die Gewinnung andere kleiner Bodenevertebraten oder ihrer Eier geeignet sein.

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Accepted for publication: September 13, 1972