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Extraction of Cyst Nematodes from Organic Soils[1]

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Abstract: The effects of extraction technique, sample size, soil moisture level, and overflow rate on recovery of *Globodera rostochiensis* and (or) *Heterodera schachtii* cysts from organic soils were investigated. A modified Fenwick can (MFC) and an underflow elutriator (UE) described in this paper were evaluated and compared for cyst recovery efficiency and amount of organic flotsam collected. The MFC and UE extracted similar numbers of cysts, but the UE collected 50% less flotsam than the MFC. Sample size was negatively correlated with cyst recovery and positively correlated with amount of flotsam. The amount of flotsam recovered with the MFC was correlated with overflow speed. Presoaking air dried samples for 30 minutes halved the amount of flotsam without affecting cyst recovery. Extracting cysts from wet soil without prior drying resulted in negligible recovery with both extraction techniques. There were no significant differences in cyst recovery of the two genera tested.

Key words: elutriation, extraction, *Globodera rostochiensis*, potato cyst nematode, *Heterodera schachtii*, sugarbeet cyst nematode.

Cysts of *Heterodera* and *Globodera* species are readily extracted by floating dried mineral soil in water (3,5,9,11) or by suspending undried soil in a column of water (6,11). With either technique, cysts, seeds, organic matter, and soil particles similar in size and density to cysts are extracted and retained on sieves. Further separation of cysts from debris is achieved by decanting in water (10), by flotation in ethanol and glycerine (1,2), by flotation in acetone (8), or by heavy sugar centrifugation (4). When cysts are extracted from organic soils (muck), the amount of organic matter retained on the sieves (flotsam) is often excessive. This large amount of flotsam generally restricts cyst recovery efficiency and greatly increases the time required to inspect each sample for cysts. This problem is especially evident in the USDA Animal and Plant Health Inspection Service (APHIS) Golden Nematode Survey Program, where more than 30,000 kg of organic soil is processed each year (D. Gaines, pers. comm.). The magnitude and cost of this problem was the stimulus for the work presented here. We tested the effects of sample size, nematode genus, cyst number per sample, presoaking, and overflow speed on the amount of flotsam collected and cyst recovery efficiency. The USDA cyst extractor (modified Fenwick can [10]) and a prototype underflow elutriator developed by the USDA APHIS were compared. A portion of this work was previously presented (7).

Materials and Methods

The modified Fenwick can (MFC) operates on the principle that dried cysts will float in a rising column of water. The overflow speed of this column of water can be varied tremendously, and up to 3,000 cm^3 of soil can be processed in a 4.5-minute cycle. The underflow elutriator (UE) developed by the USDA APHIS Whiteville Methods Development Center used for elutriating *Striga asiatica* seeds from soil (R. Eplee, pers. comm.) has not been previously described. The UE consists of a 90-cm cone-shaped elutriation column 5 cm d at the bottom and 30.5 cm d at the overflow point (Fig. 1). Water pressurized to 5.8 kg per cm^2 is introduced through a 5-mm orifice surrounded by four 1.6-mm orifices located in the cone gate. The high water pressure serves to break up soil particles in the samples. When the water level reaches 60 cm in the column, water is removed from the bottom of the column through a hydraulically operated, variable

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flow siphon tube located 10 cm above the water inlet. The underflow siphon tube removes particles concentrated at the bottom of the column, while cysts and lighter particles remain suspended higher in the column. Underflow is greatest during the first minute of elutriation and stops after 4 minutes, allowing maximum overflow at the top of the column. Cysts and particulate matter are kept free of the column wall by means of a vibrating shaker. Processing time for each sample cycle is 4.5 minutes; the sequence of events is controlled by cam-driven sequential timers. Cysts and flotsam were collected on a 850-μm-pore sieve nested under a 250-μm-pore sieve.

Unless otherwise specified, all experiments were performed using 1,000-cm³ soil samples seeded with 10 or 20 air-dried *Globodera rostochiensis* or *Heterodera schachtii* cysts of uniform size and content. All samples were processed in each machine for 4.5 minutes. In experiments not concerned with overflow speed, the MFC was operated at an overflow speed of 3.8 liters per minute. All flotsam retained on the 850-μm-pore sieve was dried, and cysts were further extracted in acetone in a milk filter sock (8). All remaining flotsam was carefully observed at 35× magnification to detect cysts that did not float free of debris in acetone.

The effect of sample size on cyst and flotsam recovery was determined for both the MFC and UE by processing 10 replicates each of dry muck samples of 500, 1,000, 1,500, 2,000, and 3,000 cm³. Twenty *G. rostochiensis* cysts 0.35–0.45 mm d were seeded in each sample.

The effect of presoaking air-dried muck on cyst recovery was determined by completely dispersing 1,000-cm³ samples seeded with 20 *G. rostochiensis* cysts for 5 or 30 minutes in water before MFC or UE extraction. Cyst extraction efficiency from wet muck (50% moisture by weight) was determined by using 1,000-cm³ samples seeded with 20 cysts previously soaked for 48 hours in water.

The effects of overflow speed on cyst

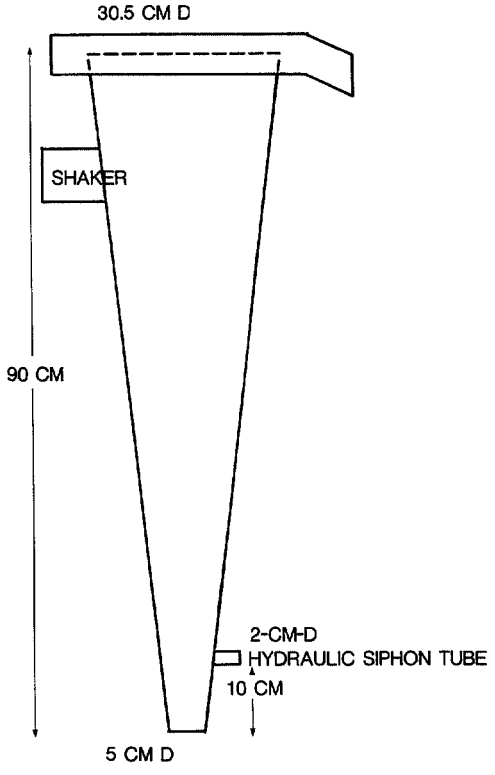

FIG. 1. Underflow elutriation column developed for extraction of cyst nematodes from organic soils.

recovery and amount of flotsam collected were determined for the MFC by varying the volume of water that overflowed the system in a given period of time.

RESULTS AND DISCUSSION

Because there were no significant differences between yields of nematode genera, data were combined for analysis. Recovery of *G. rostochiensis* and *H. schachtii* cysts from dry muck was negatively correlated with sample size. Numbers of cysts recovered with the MFC and UE did not differ significantly. The amount of flotsam collected was positively correlated with sample size (Table 1). The amount of flotsam collected with the UE was significantly less (50%) than that collected with the MFC, regardless of sample size.

Presoaking air-dried muck samples for 5 or 30 minutes in water did not affect cyst recovery with either the MFC or the UE,

TABLE 1. Effect of muck sample size on cyst nematode* recovery efficiency.

Sample size (cm^3)	USDA MFC		APHIS UE	
	Cyst recovery (of 20)	Flotsam† (cm^3)	Cyst recovery (of 20)	Flotsam† (cm^3)
500	18.5 ± 1.4	3.7 ± 0.4	18.6 ± 1.1	1.6 ± 0.7
1,000	18.2 ± 1.6	7.9 ± 1.8	18.1 ± 2.1	3.5 ± 0.4
1,500	16.3 ± 1.5	20.0 ± 3.9	16.9 ± 3.4	4.4 ± 0.5
2,000	15.8 ± 1.6	29.1 ± 11.9	13.7 ± 3.3	16.3 ± 8.1
3,000	17.0 ± 4.2	22.6 ± 1.8	15.2 ± 2.6	12.2 ± 5.8

Mean ± 1 SD.
* Recovery of *G. rostochiensis* and *H. schachtii* not different; combined.
† Flotsam retained on 250-μm-pore sieve only.

but it significantly reduced the amount of flotsam collected (Table 2). The amount of flotsam was greatest when dry samples were processed with the MFC. The 5-minute presoak reduced flotsam collected with the MFC, but not as much as did the 30-minute presoak. Flotsam from the MFC 5-minute presoak was similar to that recovered with UE 5-minute soak or UE dry muck treatments. Less flotsam was collected with samples that were presoaked 30 minutes before processing with the UE. Flotsam collected when samples were presoaked 48 hours (50% moisture by weight) before processing was less than that from dry samples, and cyst recovery was greatly diminished. Recovery of cysts from these samples was greater with the UE than with the MFC, but neither procedure was acceptable for APHIS use.

TABLE 2. Effect of presoaking dry muck on *Globodera rostochiensis* cyst recovery.

	Cyst recovery (of 20)	Flotsam (cm^3)
USDA MFC		
Air dry	18.2 ± 1.6 a	7.9 ± 1.8 a
5-minute soak	18.6 ± 0.6 a	5.2 ± 0.3 b
30-minute soak	19.0 ± 1.2 a	3.8 ± 0.7 c
48-hour soak	0.2 ± 0.4 c	1.5 ± 0.7 d
APHIS UE		
Air dry	18.1 ± 2.1 a	3.5 ± 0.4 c
5-minute soak	19.2 ± 0.8 a	3.3 ± 0.3 c
30-minute soak	19.8 ± 0.5 a	1.9 ± 0.2 d
48-hour soak	2.4 ± 2.8 b	1.8 ± 0.2 d

Mean ± 1 SD.
Means followed by the same letter not significantly different.

Increasing the overflow speed of the MFC above 7.6 liters per minute reduced cyst recovery and increased the amount of flotsam (Table 3). Operating the MFC at an overflow speed of 12.9 liters per minute resulted in the loss of samples due to plugging of sieves with flotsam.

Recovery of nematode cysts from organic soil and collection of flotsam during the extraction process were dependent on several factors including sample size, state of hydration of cysts and of soil, and extraction equipment. These factors can be adjusted to maintain or increase cyst recovery efficiency and decrease the amount of flotsam collected. Problems involved with an excess of organic flotsam in the extraction of cysts from organic soils may be overcome by use of an underflow elutriator, decreasing the sample size, presoaking samples before extraction, or a combination of the three methods. A further extraction in ethanol or acetone or centrifugation in heavy sugar solution would

TABLE 3. Effect of overflow speed on *Globodera rostochiensis* recovery efficiency from muck soil by the USDA cyst extractor (MFC).

Overflow speed (liters/min)	Cyst recovery (of 20)	Flotsam (cm^3)
2.7	19.0 ± 1.9	7.1 ± 1.1
3.8	18.2 ± 1.6	7.9 ± 1.8
7.6	19.5 ± 1.0	8.2 ± 1.1
9.1	13.0 ± 1.4	26.0 ± 9.9
11.4	15.0 ± 1.4	173.5 ± 89.8

Mean ± 1 SD.
Correlation between overflow speed and flotsam $r = 0.82$.

further decrease the amount of flotsam. The scale of the USDA regulatory program, concerns about cross contamination, and costs involved prohibit a second extraction of the same sample.

The amount of flotsam collected is roughly halved with the UE, which quickly removes much of the flotsam that would otherwise be recovered by the MFC. The MFC is currently in use by the USDA APHIS survey program to extract *G. rostochiensis* from both organic and mineral soils. Cyst recovery with the MFC operated at 3.8 liters per minute overflow rate is similar to the UE, and decreasing sample size and (or) presoaking dried samples would reduce the amount of flotsam recovered.

Sample size affects both the amount of flotsam and the recovery of cysts from organic soils. Cyst recovery from dry muck samples is greater than 90% for samples up to 1,000 cm^3 in size and decreases with increasing sample size. Just as important, the standard deviation increases with sample size, indicating a decrease in precision.

The recommendation to presoak muck samples to reduce flotsam must not be construed to mean that samples should not first be thoroughly dried. Extraction of wet cysts (cysts that do not float in water) from wet soil with the UE or MFC results in inefficient cyst recovery. In partially dried samples (50% moisture by weight), cysts do not dry sufficiently for acceptable recovery with either the MFC or UE.

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