Extraction of Cysts and Eggs of *Heterodera schachtii* from Soil with an Assessment of Extraction Efficiency[1]

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Abstract: Techniques are described for the extraction and enumeration of cysts and contained eggs from soil samples. The average recovery of cysts from seeded soil samples of differing soil texture was 82.7 ± 2.1%. Recovery from sandy clay soil samples seeded at 1 cyst/100 g soil was 63.4 ± 5.5%; at 4.2 cysts/100 g soil recovery was 89.6 ± 1.8%. Recovery of cysts from naturally infested clay soil was 88.3 ± 2.05%. Egg extraction efficiency for seeded samples was calculated as 78%, and for naturally infested soil was estimated as 83%.

Key words: soil sample processing, cyst recovery.

The sugarbeet cyst nematode, *Heterodera schachtii* (Schm.) is a serious pest of sugarbeets. Preplant nematode egg densities (Pi) can be related to potential crop loss. Numerous methods have been suggested for recovering cysts and eggs from soil (1,6,10,12). Accurate information on Pi is required for making pest management decisions or evaluating cyst nematode population densities for research purposes.

The methods described here represent modifications of techniques described previously (1,3,6,7,11). The efficiency of extraction and processing techniques used in our laboratory (9) for several years is reported.

MATERIALS AND METHODS

Extraction of cysts from soil: Composite field soil samples of 1–1.5 kg are obtained according to protocols outlined elsewhere (2). The soil is placed in paper bags and allowed to air dry for 2 days at 25 C. Cysts in this soil lose water, making recovery in subsequent flotation steps more efficient.

The dry soil usually contains aggregates of various size and consistency. If the soil is not readily crumbled by hand, it is placed in a metal pan and comminuted using a rubber mallet or hammer. After thorough mixing, a 600-g subsample of soil is removed from which nematodes are recovered.

Cysts and organic material are separated

from the soil using a modified Fenwick flotation can (6) (Fig. 1). Water flows into the bottom of the can through 1.3-cm-i.d. pipe connected to a garden hose by a standard female connector. The rate of water flow is controlled by a ball valve which yields maximum water flow with a 90-degree turn of the valve. Water enters the can through six, 2-mm-d holes located in a 2.6-cm-d center nut. Each hole is drilled into the center nut at an angle of 32 degrees from vertical.

Soil is placed in the bottom of the can and water added through the holes at maximum rate to bring the water to within 10 cm of the spout. The water is turned off, and the contents of the can are vigorously stirred with a wooden rod. The water is turned on again at a flow rate of 6.4 liters/minute to bring cysts and organic matter, excluding silt or sand particles, over the spout of the can. The water flow from the can is directed onto a 850-μm-pore sieve nested over a 150-μm-pore sieve for 2 minutes (Fig. 1). Then ¾ of the water in the can is poured through the sieves, and the remaining contents of the can are stirred and run through another 2-minute cycle. After this final cycle the sieves are rinsed thoroughly to wash cysts caught on the 850-μm-pore sieve onto the 150-μm-pore sieve. The contents of the 150-μm-pore sieve are then washed off onto tissue paper supported by a 3.2-mm-mesh wire screen. The tissue paper and its contents are air-dried.

Separation of cysts from organic debris: Our method is modified from Andersson (1). Whatman No. 4 filter paper is placed into a 12.5-cm-d funnel (Fig. 2). The funnel stem valve is closed, the 3-way valve is adjusted to direct air to the flask, and the refill valve is opened. The T-joint is held
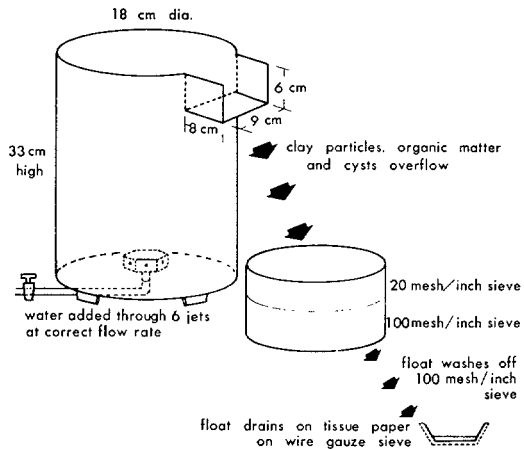
FIG. 1. Modified Fenwick can used to separate nematode cysts and organic debris from soil samples.
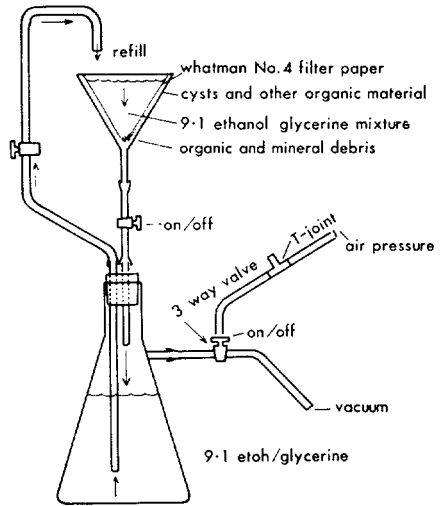

FIG. 2. Ethanol–glycerine flotation apparatus used for separation of nematode cysts from sample organic material.

closed, and the funnel is filled with the ethanol–glycerine mixture (9:1, v:v) (11) to within 2 cm of the top of the filter paper.

The dried organic debris containing cysts is loosened from the tissue paper by rubbing the paper between the fingers. The debris is scraped with a spatula off the tissue into the liquid in the funnel. Floating cysts will collect at the filter paper–liquid interface. The debris which sinks to the bottom of the funnel is stirred gently to release any trapped cysts. After approximately 15 seconds, all cysts should be aggregated at the edge of the liquid. At this point the refill stem valve is closed and the 3-way valve adjusted to apply vacuum to the flask. The funnel stem valve is opened to allow the vacuum to aid in removing the liquid from the funnel. Agitation at the liquid surface during opening and closing of the valves should be avoided, as cysts may be dislodged and carried away in the receding liquid front. After the liquid has been removed, the cysts will be concentrated in the uppermost band of residue on the filter paper (1). The filter paper is removed from the funnel, and, if desired, cysts counted. Subsequently, the cysts and debris in the uppermost ring are rinsed from the filter paper into a beaker.

Releasing eggs from cysts: Our technique is modified from Bijloo (3). Eggs are released from cysts using a tissue homogenizer with a clearance of 0.46–0.54 mm between the teflon pestle and the wall of the homogenizer tube. Cysts are concentrated by pouring the contents of the beaker through a 20-μm-pore sieve. Cysts are then rinsed off the screen with 5% NaOCl into a 40-ml pyrex homogenizer tube until the lower portion of the tube is approximately half full. Eggs are released from the cysts by 30 seconds of homogenization at 2,500 rpm.

The contents of the homogenizer tube are then rinsed onto a 20-μm-pore sieve on which the eggs are retained. The eggs are rinsed with tap water while on the 20-μm-pore sieve to remove residual NaOCl and are then rinsed into a 150-ml fluted beaker. Water is added to reach a final volume of 50 ml in the beaker. A 1-ml or 2-ml subsample is pipeted off while the sample is agitated on a magnetic stirrer, and the eggs and juveniles are counted.

Our objective was to determine the efficiency of cyst and egg extraction by the methods outlined above.

Experiment 1: Water containing 6, 15, or 25 cysts was added to 600-g portions of air dried soil of each of three soil types, and the soil was allowed to air dry. The soil types were sandy clay loam (60% sand, 16% silt, 24% clay), loamy sand (78% sand, 18% silt, 4% clay), and sand (94% sand, 4% silt, 2% clay). Each soil was steam-sterilized before use. Cysts were obtained from green-

house cultures of *H. schachtii* maintained on sugarbeet. Only brown cysts were selected, and cysts of various sizes were chosen at random. The average egg content of cysts was 76.1 ± 31.2 yielding equivalent soil egg population densities of 0.8, 1.9, and 3.2 eggs/g of soil, respectively, for sandy clay loam, loamy sand, and sand. Samples were processed using the modified Fenwick flotation can and ethanol–glycerine flotation, and the recovery of cysts determined.

Experiment 2: Naturally infested clay soil (6.2% sand, 28.7% silt, 65.1% clay) samples were processed using the Fenwick can and ethanol–glycerine flotation, and the number of cysts determined. The soil remaining in the bottom of the Fenwick can was poured onto nested 850-μm-pore over 150-μm-pore sieves, and the material collected on the 150-μm-pore sieve was processed by ethanol–glycerine flotation to determine the number of cysts remaining. Eight replicate soil samples were processed, and the percent recovery of cysts from the Fenwick can obtained.

Experiment 3: Three size classes of cysts (cyst length < 0.4 mm = small, 0.4–0.6 mm = medium, and >0.6 mm = large) and three different numbers of cysts (5, 15, 25) were homogenized to determine the effect of cyst size and number of cysts present on the efficacy of homogenization to release eggs. Treatments were replicated five times.

Experiment 4: Water containing a known number of eggs per 250 ml was poured through a 20-μm-pore sieve and the number of recovered eggs determined. This procedure was replicated 10 times.

RESULTS

Results are reported as means of replicate samples ± the standard error.

Experiment 1: At the low inoculum density (5 cysts/600 g soil), recovery of cysts from clay soil was lower than from sand or loam soils (Fig. 3). Recovery of cysts from other cyst densities among the soil types was similar. The average recovery of cysts for all soil types and cyst population densities was 82.7 ± 2.1%. Using this average, a regression equation of cysts recovered (x) vs. predicted recovery (y) is y = 0.88x + 1.42 (r = 0.97).
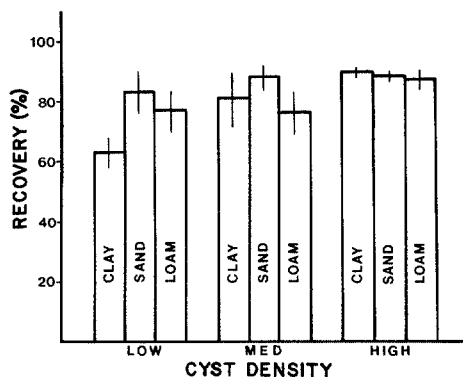

FIG. 3. The effect of cyst density and soil type on the recovery of cysts using the modified Fenwick flotation can and ethanol–glycerine flotation protocol (low = 6 cysts/600 g, med = 15 cysts/600 g, high = 25 cysts/600 g soil).

Defining predicted recovery as the mean percent recovery per population density per soil type multiplied by the number of cysts initially placed in the sample yields a regression equation for cysts recovered (x) vs. predicted recovery (y) of y = 0.94x + 0.72 (r = 0.97).

Experiment 2: The average recovery of cysts from naturally infested clay soil was 88.3 ± 2.1%.

Experiment 3: Homogenization crushed 100% of the large and medium cysts and 96 ± 1.5% of the small cysts. The average egg content of small cysts was 4.8 ± 0.8. The proportion of cysts crushed was not affected by the number of cysts present.

Experiment 4: The average egg recovery was 94.4 ± 1.8%.

DISCUSSION

Fenwick (6) reported a cyst extraction efficiency of 98% for the flotation can when sandy soil containing 2.2 cysts per gram of soil was processed. Seinhorst (10) reported a cyst extraction efficiency for soils naturally infested with *H. rostochiensis* (*sensu lato*) and *H. trifolii* with a range of 62–99.5% with maximum recovery occurring at a flow rate of 3.5 liters/minute. Our average cyst recovery is in close agreement with that of Fenwick and Seinhorst. The flotation technique works well across the soil types tested. Estimates of cyst extraction efficiency vary depending on the age structure and con-

dition of cyst populations used in the study. Older cysts tend to contain more air and float better than younger cysts.

The homogenization process works well for crushing cysts and releasing eggs. Cysts which are not crushed contain few eggs and contribute little to the total soil egg population density. Visual inspection showed no eggs remaining within the majority of crushed cysts. The sum of losses across all points in the extraction procedure can be obtained, and the egg extraction efficiency (EEF) can be determined. Using the cyst extraction efficiency obtained from seeded samples (82.7%), and the average of 76 eggs per cyst, the EEF for the entire procedure is 78%. If the higher cyst extraction efficiency obtained from naturally infested soil (88.3%) is used, the EEF for the entire process is 83%.

These recoveries are acceptable as a basis for management decisions in areas where the damage threshold is as low as 1 egg/g soil (4,5) or as high as 10 eggs/g soil (8). The error that occurs when estimating the actual population density is not critical at relatively high population densities. The estimated actual population density derived by use of the extraction efficiency correction factor will be conservative because the egg content per cyst is variable, and loss of cysts appears to be biased in favor of small cysts.

Knowledge of extraction efficiencies allows comparison of experimental results obtained from different sources using different extraction techniques which have different efficiencies. The egg extraction efficiency of 83% presented here can be used as a correction factor to estimate cyst nematode egg population densities in soil samples. It is suggested however, that each lab determine its own extraction efficiency for this procedure.

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