Journal of Nematology 40(1):30–34. 2008.

Comparative Efficiency of the Fenwick Can and Schuiling Centrifuge in Extracting Nematode Cysts from Different Soil Types

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Abstract: The Fenwick can and Schuiling centrifuge are widely used to extract nematode cysts from soil samples. The comparative efficiencies of these two methods during cyst extraction have not been determined for different soil types under different cyst densities. Such information is vital for statutory laboratories that must choose a method for routine, high-throughput soil monitoring. In this study, samples of different soil types seeded with varying densities of potato cyst nematode (*Globodera rostochiensis*) cysts were processed using both methods. In one experiment, with 200 ml samples, recovery was similar between methods. In a second experiment with 500 ml samples, cyst recovery was higher using the Schuiling centrifuge. For each method and soil type, cyst extraction efficiency was similar across all densities tested. Extraction was efficient from pure sand (Fenwick 72%, Schuiling 84%) and naturally sandy soils (Fenwick 62%, Schuiling 73%), but was significantly less efficient from clay-soil (Fenwick 42%, Schuiling 44%) and peat-soil with high organic matter content (Fenwick 35%, Schuiling 33%). Residual moisture (<10% w/w) in samples prior to analyses reduced extraction efficiency, particularly for sand and sandy soils. For each soil type and method, there were significant linear relationships between the number of cysts extracted and the numbers of cysts in the samples. We discuss the advantages and disadvantages of each extraction method for cyst extraction in statutory soil laboratories.

Key words: Fenwick can, potato cyst nematode, *Globodera rostochiensis*, Schuiling centrifuge, soil monitoring, method, statutory testing

The successful integrated management of cyst-forming nematodes depends on the accurate monitoring and estimation of cyst densities either before crops are planted or during crop growth (EPPO, 2004; EU, 2007). Furthermore, efficient quarantine protocols (including interception, sampling and laboratory analyses) are often required to prevent the spread of pathogenic nematodes between regions. The success of such protocols depends on the efficient detection of cysts in imported soils and other substrates (Kimpinski et al., 1993). A range of techniques has been developed to extract nematode cysts from soils and other materials (Southey, 1986; Turner, 1998; EPPO, 2004). Two devices (henceforth extraction methods), the Fenwick can and Schuiling centrifuge, that extract cysts from soil by flotation are widely used in statutory and quarantine laboratories in Europe, particularly for the detection and monitoring of potato cyst nematodes (PCN) (*Globodera pallida* (Stone) Behrens and *Globodera rostochiensis* (Wollenweber) Behrens) (Zaheer et al., 1996; Turner, 1998; Minnis et al., 2002; da Cunha et al., 2004). Both methods are based on the characteristic that cysts float when dried.

The Fenwick can is a metal can tapering at the top with a sloped base. The can has a sloping collar below the rim. Dry soil is placed in a receptor with a <2 mm screen over the can and washed through into the can. Heavy soil particles fall to the bottom of the can, whereas dried cysts and light soil debris float to the surface and are siphoned over the rim to a collecting sieve (Fenwick, 1940; Turner, 1998). The Fenwick can has been adapted by different manufacturers and laboratories and can vary in dimensions. The Schuiling centrifuge is a semi-automatic flotation method that was developed in the 1980s (Turner, 1998). The dried soil is placed in a transparent cylinder partly filled with water. The contents are swirled with a rotating two-pronged fork at high speed (450–500 rev/min), creating a vortex. Cysts and other floating particles are forced to the center through a wire-mesh cylinder (1.5 mm) and collected in a sieve for further processing. To our knowledge, no study has quantified the extraction efficiency of the Schuiling centrifuge under varying conditions of soil texture and cyst density. Furthermore, although the effects of soil-type and cyst density on Fenwick can efficiency have been evaluated (Caswell et al., 1985), no study has compared the two methods over a range of soil types and cyst densities. In particular, there is no experimental evidence to support the use of either the Fenwick can or Schuiling centrifuge for cyst extraction from clayey or high-organic soils.

The present study compares the efficiency of the Fenwick can and Schuiling centrifuge under varying soil types and cyst densities using artificially infested soil samples. The specific objectives of the study were: (i) to examine the effects of soil type on cyst recovery by both methods; (ii) to examine the effects of residual moisture on cyst extraction (though soils should be thoroughly dried, samples with up to 10% moisture can appear superficially dry); and (iii) to examine the effects of varying cyst density on extraction efficiency by both methods. The results from this study will aid in the choice of extraction techniques and design of extraction protocols for statutory nematology laboratories.

Materials and Methods

Experiment 1. Effects of residual soil moisture on cyst recovery from different soils: Samples (200 ml) of four soil types were seeded with PCN cysts at low (5 cysts/200 ml) and

Received for publication February 21, 2008.
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Funding for this study was provided by the Irish Department of Agriculture and Fisheries as part of the statutory PCN monitoring program (Teagasc-RMIS 5380); J Bellvert was funded through a Leonardo da Vinci education and training grant for transnational mobility.
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This paper was edited by Steve Koenning.

TABLE 1. Compositions of soils used in the experiments. Numbers are average percentages as estimated using Bouyoucos' (1962) hydrometer method.

Soil type	Sand %	Clay %	Silt %	Peat %
Children's play sand	100.00	0.00	0.00	0.00
Sand-soil (no clay)	80.80	0.00	19.20	Trace
Sand-soil (low clay)	64.74	7.15	28.11	Trace
Clay-soil	54.21	30.19	15.60	Trace
Peat-soil	48.48	0.00	11.52	40.00

high (50 cysts/200 ml) densities. Soils were selected based on our results of mechanical analyses using Bouyoucos' (1962) hydrometer method. The soils included play sand (sand) as a standard for which cyst recovery is generally high, naturally sandy soil without clay (sand-soil [no clay]), naturally sandy soil with low levels of clay (sand-soil [low clay]) and heavy clay soil (clay-soil) (Table 1). All soils were wet (approx. 30% w/w) before the cysts were added. The soil and cysts were thoroughly mixed within each sample. Samples were dried in a drying oven at 50°C for 48 hr. The moist samples were air dried until they reached a moisture level of approx. 10% w/w. At this moisture level, some samples appeared superficially dry. Samples for each extraction technique, moisture level, soil type and density combination were replicated five times. Cysts were extracted using procedures recommended for the Fenwick can by Turner (1998) and adhering to the operating instructions for the Schuiling centrifuge as supplied by Volkers and Zonen (The Netherlands). For both techniques, cyst-containing debris was collected in a 250-µm sieve, and cysts floated off using filter funnels (radius 15 cm). Filter papers were then laid on a rotating stage (turntable scanning unit) stereomicroscope (Zeiss-Germany) under ×20 magnification, and cysts counted. Cyst recovery was analyzed using a general linear model (GLM). The four-way model was as follows: *Cyst recovery = method + cyst density + soil type + moisture + error.* Pair-wise comparisons were conducted using Tukey's honestly significant difference (HSD) test. Cyst recovery was arcsine transformed, and residuals, plotted following each analysis, were normal and homogeneous. Statistical analyses for this and subsequent experiments were carried out using SPSS version 14.0 (SPSS Inc., Chicago, IL).

Experiment 2. Effects of soil type and cyst density on recovery efficiency: Cyst extraction was examined across a range of densities using four different types of soil: sand, sand-soil (no clay), clay-soil and peat-soil (Table 1). Peat-soil was prepared by mixing peat moss with sand-soil (no clay). Samples (500 ml) were seeded with cysts at the following densities: 1, 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 per 500 ml. The complete series of densities was replicated three times for each soil type, except sand, which was replicated twice. Samples were dried in an oven at 50°C for 48 hr. Cysts were extracted as indicated above. Because of high amounts of organic debris in the float from peat-soil samples, floats were re-suspended in acetone and drained through filter funnels. This significantly improved cyst detection ($F_{1,120}$ = 103.51, $P < 0.001$). Preliminary experiments indicated that re-suspension in acetone was not required for the other soil types. Cyst recovery was analyzed using GLM. The three-way model was as follows: *Cyst recovery = method + cyst density + soil type + error.* Low density samples (1, 2 and 5 cysts/500 ml) were excluded from the analysis because of instability caused by statistical artifacts; low density samples are disproportionately influenced by the magnitude of effect caused by the loss of each individual cyst. Pair-wise comparisons were conducted using Tukey's HSD test. Regression analysis was used to examine the relationship between the number of cysts recovered and the true density of cysts in the samples for each soil type. Regression slopes and intercepts were compared using two-way GLM (*slope* or *intercept = method + soil type + error*). Cyst recovery was arcsine transformed, and residuals, plotted following each analysis, were normal and homogeneous.

RESULTS

Experiment 1. Effects of residual soil moisture on cyst recovery from different soils: Cyst recovery was similar using both extraction methods ($F_{1,160}$ = 0.160, P = 0.689), was lower for low-density samples ($F_{1,160}$ = 24.782, $P < 0.001$) and clay-soil ($F_{3,160}$ = 11.905, $P < 0.001$), but similar among soils with high sand content (Tukey: $P > 0.05$) (Fig. 1). Low levels of moisture (approx. 10% w/w) reduced cyst recovery from all soil types by both extraction methods ($F_{1,160}$ = 63.258, $P < 0.001$) (Fig. 1). Cyst recovery from dried and moist clay-soil was generally similar; however, cyst extraction from moist sand was approximately four-fold lower than from dry sand. This resulted in a significant soil-type × moisture interaction ($F_{3,160}$ = 13.385, $P < 0.001$). All other interactions were not significant.

Experiment 2. Effects of soil type and cyst density on recovery effciency: Soil type had a significant effect on cyst recovery by both extraction methods (Fig. 2). Cyst densities had no effect on recovery above10 cysts/500 ml. Overall, cyst recovery was higher using the Schuiling centrifuge ($F_{1,220}$ = 8.756, $P < 0.001$), and soil type significantly affected recovery using both methods ($F_{3,220}$ = 65.540, $P < 0.001$) in a similar manner (interaction: $F_{2,220}$ = 2.271, P = 0.106). Cyst recovery differed between all soil types (Tukey: $P \leq 0.05$), with greatest recovery from sand and lowest recovery from peat-soil (Fig. 2). The relationship between the number of cysts recovered and the actual number of cysts in the sample differed between soil types (Fig. 2). Intercepts were similar (method: $F_{1,22}$ = 0.026, P = 0.874, soil type: $F_{3,22}$ = 0.906, P = 0.463). The extraction method had no effect on the slopes of the relationships ($F_{1,22}$ = 1.173, P = 0.297), but soil type significantly affected slopes ($F_{3,22}$ = 10.676, P = 0.001). Interactions were not significant.

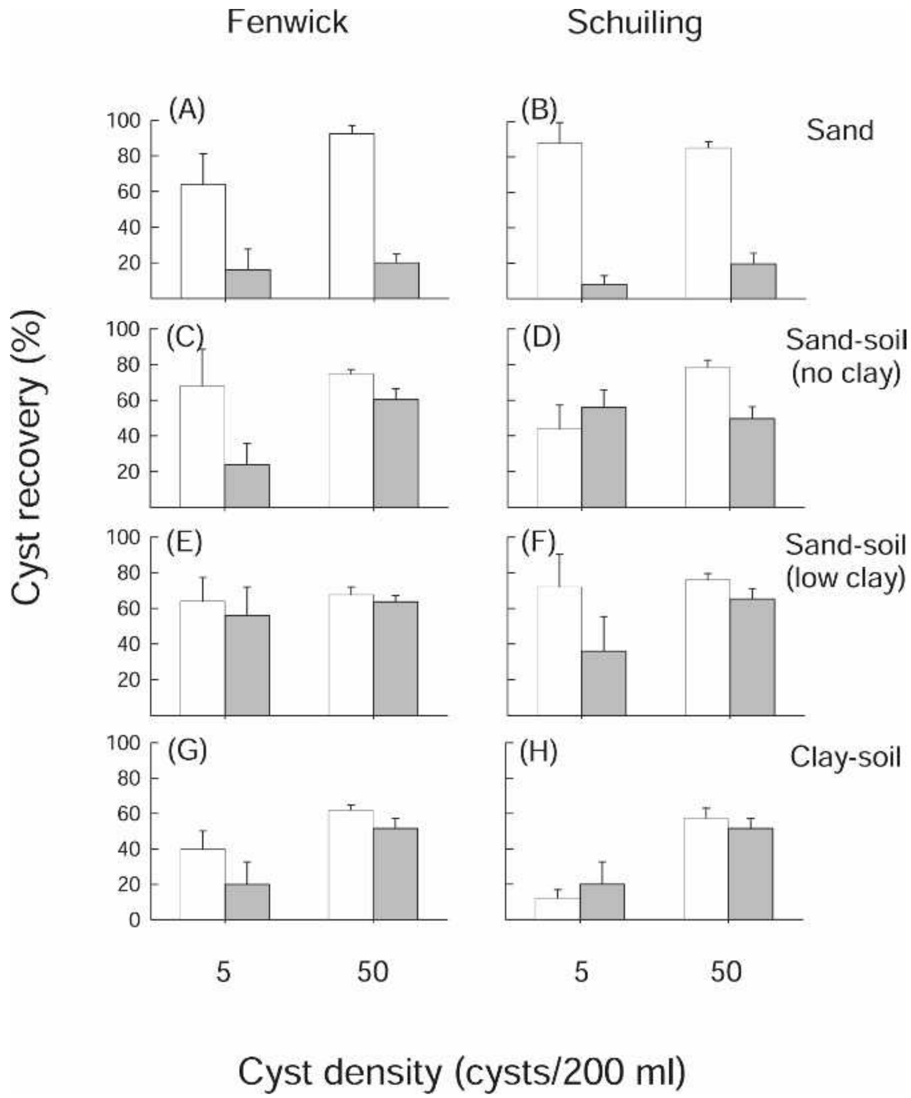

FIG. 1. Average recovery of *Globodera rostochiensis* cysts from 200 ml samples of different soil types seeded with cysts at two densities. Samples were oven dried (0% water) (open bars) and air-dried until approx. 10% water (solid bars). Soil types included sand (A and B), sand-soil with no clay (C and D), sand-soil with small amounts of clay (E and F) and clay-soil (G and H). Samples were processed using a Fenwick can (A, C, E and G) and Schuiling centrifuge (B, D, F and H). Standard errors are indicated.

DISCUSSION

Detailed, quantifiable data on the individual and comparative efficiencies of extraction techniques for cyst nematodes are difficult to find (Caswell et al., 1985; Clayden et al., 1985; Kimpinski et al., 1993) despite the requirements for such data when designing statutory laboratory protocols. The present study indicates that cyst extraction from 200–500 ml samples of different soil types is similar, over a range of cyst densities, when using either the Fenwick can or Schuiling centrifuge. However, in contrast to previous reports (e.g., Clayden et al., 1985, Kimpinski et al., 1993) extraction efficiency of the Schuiling centrifuge was often higher than of the Fenwick can. In the present study, differences between the two experiments in comparative extraction efficiency may be due to the different sample sizes used. Bellvert et al. (2008) have indicated different responses in extraction efficiency to varying sample sizes by the two methods: more cysts were lost from the Fenwick can as sample size increased (constant cyst numbers), whereas cyst recovery was constant for the Schuiling centrifuge for samples >100 ml. However, the effects of varying sample size were not directly tested in the present study. For both methods, cyst extraction was lower from soil samples containing high amounts of clay or peat.

We expected cyst extraction from children's play sand to be high because the relatively large sand particles sink rapidly, releasing the cysts. Therefore, play sand represented a good standard substrate with which to compare extraction efficiencies. Cyst extraction using this optimal substrate was >70% by both methods. Cyst recovery from natural sandy soils (sand-soil [no and low clay]) was often lower than from play sand. These results are comparable with previous studies for the Fenwick can (e.g., Caswell et al., 1985: 76–87%;

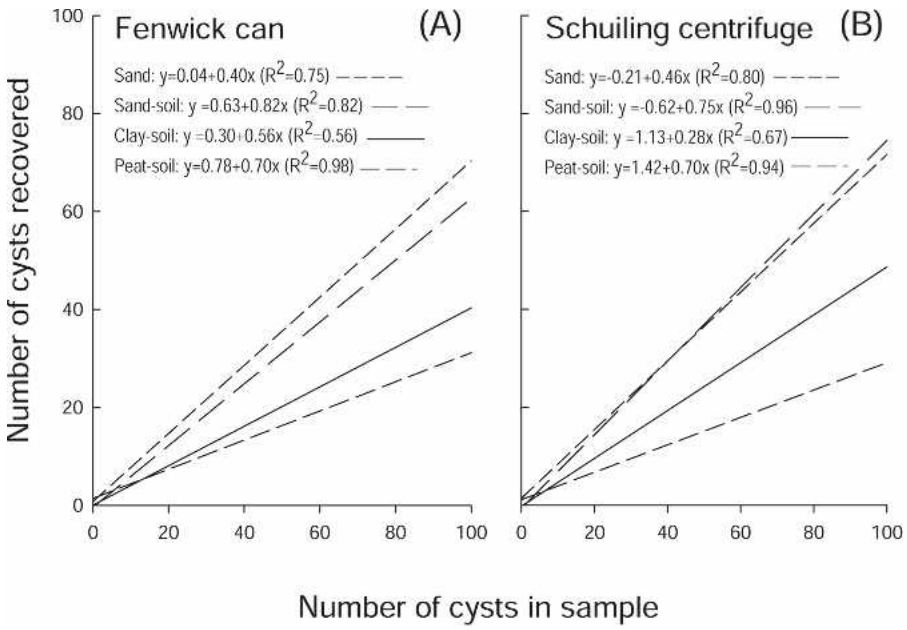

FIG. 2. Recovery of *Globodera rostochiensis* cysts from 500 ml samples of different soil types using (A) the Fenwick can and (B) the Schuiling centrifuge. Regression lines are averaged from three experimental replicates (two for sand). Average recoveries (>10 cysts) were as follows (mean % ± SEM): Sand: Fenwick, 72 ± 2; Schuiling, 84 ± 3; sand-soil: Fenwick, 62 ± 4; Schuiling, 73 ± 3; clay-soil: Fenwick, 42 ± 3; Schuiling, 44 ± 3; peat-soil: Fenwick, 35 ± 3; Schuiling, 33 ± 3.

Turner, 1998: 70–95%). Cyst extraction from clay-soil and peat-soil was generally less efficient (≥42 and ≥33%, respectively, in this study) than from sandy soils, but similar using both methods. This contrasts with claims by Clayden et al. (1985) that the Schuiling centrifuge is less effective in processing peaty soils and may be due to recent upgrading of the device. Using the Fenwick can, Caswell et al. (1985) found a low recovery of sugar beet nematode cysts from heavy clay-soil (24% clay) at low densities (63% recovered), but significantly better recovery at high densities (89% recovered). The clay-soil used in the present study had higher levels of clay than that used by Caswell et al. (1985), which may have reduced extraction efficiency.

In the present study, poor extraction efficiency using clay- and peat-soils led to a high proportion of false negatives at low cyst densities; both the Fenwick can and Schuiling centrifuge failed to detect cysts in 24% and 23% of low density (≤5cysts/500 ml) sand/sandy-soil samples, respectively, whereas 42% of low-density clay-soil samples were falsely declared negative using both methods. Furthermore, 44% and 66% of low-density peat-soil samples were declared negative after processing with the Fenwick can and Schuiling centrifuge, respectively. Improper drying of samples significantly increases the probabilities of false negatives. Based on these results, neither the Fenwick can nor Schuiling centrifuge are suitable for accurate detection of cysts from high clay and peat soils in statutory laboratories. Furthermore, adequate oven drying of all samples before sample processing is recommended. Improved cyst extraction from high clay samples may be achieved using other laboratory methods. Similar (e.g., semi-automatic elutriator—60%, Avendaño et al., 2004) and more efficient (e.g., Seinhorst elutriator—99%, Been et al., 2007) cyst recovery has been documented using elutriation methods, which process wet samples. However, direct comparisons between elutriators and the flotation methods examined in the present study are not available.

The choice of extraction methods employed in research and statutory laboratories depends on a number of criteria, including available budget, available personnel and equipment requirements for other laboratory functions (e.g., research). However, laboratories should also base their decisions on the nature of the soil that is to be tested. The Fenwick can and Schuiling centrifuge are easy to use and generally resource efficient. Both give good cyst recovery from sandy soils; however, overall the Schuiling centrifuge is more convenient to use and requires less water and processing time than the Fenwick can (Bellvert et al., 2008). Mechanical housing and electricals in the Schuiling centrifuge, which have been criticized in the past (Clayden et al., 1985), have now been substantially improved. Still, the relatively high cost of such semi-automated devices may limit their use in some laboratories. In choosing a suitable cyst extraction method, laboratories should be aware of the advantages of either the Fenwick can or Schuiling centrifuge, their generally similar efficiencies regardless of soil type or cyst density, and their overall inefficiency in extracting cysts from heavy clay soils and soils with a high content of organic matter.

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