Evaluation of Three Quantitative Assays for *Sclerotinia minor*

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ABSTRACT

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Three techniques to quantitatively assay field soil for *Sclerotinia minor* were compared for precision, bias, and time required to assay a sample. The techniques compared were wet sieving, wet sieving with Calgon, and hydropneumatic root elutriation. Precision was measured by the standard error of the mean for repeated assays of 40 100-g samples each of clay, loam, and sand soils artificially infested with 15 viable sclerotia. Bias was measured by the deviation between the number of sclerotia recovered with each of the three techniques and the true number of *S. minor* sclerotia introduced into the above autoclaved soil samples. Regardless of the soil type, the root elutriation technique was the most precise and unbiased method to assay soil for *S. minor*. The efficiency of the hydropneumatic root elutriation technique was higher for clay soil and was comparable with the other techniques for loam and sand. In general, 92–95% of the sclerotia added to soils were recovered with the root elutriation technique. Wet sieving was the least precise, most biased, least efficient, and gave the lowest recovery of sclerotia from a naturally infested clay soil. Efficiency of the wet sieving technique was primarily dependent on soil type. Adding Calgon to soil samples before wet sieving increased the precision, reduced bias, and improved the efficiency of wet sieving for all soil types except clay. Relative net precision, encompassing the bias, precision, and cost in time of assaying a sample, was also highest for the hydropneumatic root elutriation technique regardless of the soil type. Viability of recovered sclerotia was influenced by the assay technique employed and soil type used.

Additional keywords: lettuce drop, quantitative epidemiology.

Lettuce drop is caused by two species of *Sclerotinia, S. sclerotiorum* (Lib.) de Bary and *S. minor* Jagger. In the coastal valleys of California, the disease is predominantly caused by *S. minor* (9). Regardless of the species causing the disease, lettuce drop symptoms include a soft watery crown and root rot of both head and leaf lettuce. The disease can occur at any stage of the crop growth but predominantly occurs prior to harvest (9). Unlike *S. sclerotiorum, S. minor* does not produce apothecia and survives in the soil as sclerotia. Infection of lettuce plants is caused by eruptive germination of soilborne sclerotia (9,10). Disease incidence is correlated with the number of sclerotia in soil (5).

Ecological and epidemiological studies on soilborne pathogens require rapid and efficient methods of assaying soil for determining the density of pathogen propagules. Because the samples collected by researchers in the field usually consist of large volumes of soil, it is often impractical to assay whole samples. Instead, one or several fractions with a known mass or volume randomly taken from thoroughly mixed samples are assayed separately. The figures from these fractions are averaged to provide an estimate of the inoculum density of the pathogen propagules. However, soil sampling techniques to incorporate the concept of 'competence volume' of soilborne propagules (7) require assaying the soil samples in their entirety. The competence volume is defined as the volume of soil encompassing the maximum distance and depth from which a pathogen propagule can cause infection (7). The majority of lettuce drop infections are caused by *S. minor* sclerotia in the top 8 cm of soil located about 1 cm from the crowns. Regardless of the soil sampling concepts adapted, the significance of these techniques depends on three critical qualities of the soil assay: precision, bias, and efficiency. Precision is a measure of repeatability of a given technique and is usually measured by the magnitude of standard deviation or standard error of the mean. Bias is a measure of nearness of propagule estimates of a given technique to the actual number of propagules. It determines whether a given technique systematically under- or overestimates the propagule numbers. Efficiency is a measure of time required to process each sample. The utility of techniques that are efficient but imprecise, or vice versa, is limited.

A variety of techniques have been developed to detect and quantify *S. minor* in soil (2,3,6,11). Most of the quantitative methods reported rely on assaying a known amount of infested soil using wet sieving (3) or modifications of wet sieving (6), wet sieving followed by glycerol flotation (2), and adaptation of a semiautomatic elutriation method commonly used for nematode extraction (11). The number of sclerotia in the processed samples are then enumerated under a stereoscope (2,3,6,11). Development of several discrete soil assay techniques without comparison with previously developed techniques would make the choice of any one technique difficult. Comparison of these techniques for precision, bias, and efficiency enables the identification of techniques that provide satisfactory results. To our knowledge, there are no reports on comparing assay techniques for *S. minor* sclerotia in soil. Only the most commonly used techniques (3,6,11) were compared. This study was undertaken with two objectives. First, to determine the efficacy of an eight-unit hydropneumatic root elutriator (Gillison's Variety Fabrication, Inc., Benzonia, MI) (13) for assay of *S. minor*. This instrument is commonly used in ecological studies to extract roots from soil to determine root biomass and other related variables. Second, to compare the precision, bias, and efficiency of hydropneumatic root elutriation (13), wet sieving (3), and wet sieving with Calgon (6) on three types of soil artificially infested with *S. minor* sclerotia and on soils naturally infested with *S. minor* sclerotia. Preliminary results have been published previously (14).

MATERIALS AND METHODS

Hydropneumatic root elutriator. A detailed description of the equipment designed for quantitative separation of roots from soil

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has been provided by Smucker et al (13). Briefly, the equipment includes eight cylindrical columns that separate biological materials from soil by a combination of kinetic energy from pressurized spray jets and low energy air flotation. Each cylindrical column is composed of four parts: a) high kinetic energy washing chamber, b) elutriation chamber, c) transfer tube, and d) low kinetic energy primary sieve. Quantitative separation of biological materials is achieved by a closed system of mechanical separations using water and air to isolate and deposit the biological materials on the submerged primary sieve. The pressurized water creates a vortex that suspends buoyant particles for collection in the primary sieve. The contents on the primary sieve are emptied into suitable containers for quantifying the biological material in question. Each cylindrical column has its own timing device for adjusting the duration of elutriation depending on the soil texture, and concentration and density of the biological materials.

An experiment was conducted to test the efficacy of hydropneumatic root elutriation for assaying *S. minor* sclerotia and standardizing elutriation times for different types of soils. We assayed artificially infested clay, loam, and sandy soils using a 410-μm sieve for all three soil types. Constant air and water pressures of 48 and 450 kPa, respectively, were maintained during processing. Mean rate of water flow into each elutriation unit was approximately 5.6 L/min. Twenty-four 100-g samples of each soil type infested with 15 *S. minor* sclerotia were elutriated for 2, 4, 6, and 8 min. The contents on the primary sieve were emptied onto an 80-mesh sieve, washed in tap water, placed into petri dishes (90-mm) and the number of recovered sclerotia counted at 10× with the aid of a streoscope. Analysis of variance was conducted on the number of sclerotia recovered to determine differences in soil and elutriation times. Least significant difference values ($P = 0.05$) were calculated for mean comparisons.

Soil types for comparison of assay techniques. Three soil types common in the Salinas Valley were chosen for comparison from areas with no previous history of *S. minor* infestation. The soils included in the experiment were silty clay (50% clay, 44% silt, 6% sand, and 3.3% organic matter), loam (23% clay, 32% silt, 45% sand, and 2.2% organic matter), and sandy loam (10% clay, 20% silt, 70% sand, and 1.2% organic matter). Throughout the text, we have referred to these three soil types as clay, loam, and sand, respectively. From each soil type, 15 kg of soil was collected, thoroughly mixed, and allowed to air dry on greenhouse benches for 2 wk. Subsequently, moisture content of each soil was determined gravimetrically, and 120 100-g samples from each soil were prepared for the experiment.

Production of *S. minor* sclerotia. A monosclerotial isolate of *S. minor* (HL-16) was plated onto acidified (2.5 ml of 25% [v/v] lactic acid per liter of medium) potato-dextrose agar (APDA) medium and incubated at room temperature (23 ± 2 C). After 2 days of incubation, a 4-mm-diameter mycelial agar plug from the edge of this culture was centrally seeded on 25 APDA plates each, and incubated as described above for 2 wk. Uniform sclerotia produced on these cultures were picked, air-dried, and stored at room temperature until further use. Each of the 100-g soil samples was infested with 15 randomly chosen sclerotia for processing by the different techniques after 1 wk.

Soil assay techniques. We used the wet-sieving technique of Adams (3), modified wet-sieving technique of Dillard and Grogan (6), and the hydropneumatic root-elutriation technique (13) to compare for bias, precision, and efficiency. For both wet sieving and modified wet sieving we used a 425-μm sieve instead of the 385-μm sieve suggested by Adams (3). Repeated assays of 40 100-g samples of each soil type, infested with *S. minor* as described above, were made separately by each technique. For wet sieving, soil samples were assayed individually, the soil fraction remaining on the sieve poured into clean petri dishes, and the number of sclerotia recovered were enumerated at 10×. Samples intended for processing by modified wet sieving (6) were soaked in 100 ml of 1% Calgon (sodium polymetaphosphate) solution for 2 min before wet sieving. The total time to process a sample by this technique included soaking and processing.

Evaluation of the hydropneumatic root-elutriation technique indicated that most of the infested sclerotia could be recovered after 2, 2, and 4 min of elutriation for sand, loam and clay soils, respectively. Consequently, we processed all samples within each soil type according to these times. Contents remaining on the primary sieve (410 μm) were emptied onto an 80-mesh sieve, washed with tap water, and poured into clean petri dishes (90 mm). The number of sclerotia recovered was recorded. The times (in s) required for processing each sample and for enumerating the number of sclerotia were recorded separately.

Statistical analysis. All data analyses were performed with SAS (12) using procedures MEANS and ANOVA. Bias for individual samples was calculated with the following formula

$$\text{Bias (\%)} = [(15 - \text{Number of recovered sclerotia})/15] \times 100, \quad (1)$$

where 15 was the number of sclerotia added to each soil sample. Bias (%) was analyzed as a 3 × 3 factorial design with three types of soil and three assay techniques. Precision, a measure of repeatability of a given technique, was measured by calculating both the standard error of mean and standard deviation. To provide replications for analysis of variance as a 3 × 3 factorial design, we calculated the standard errors of the mean and standard deviations for groups of eight samples for all soil types and assay

TABLE 1. Recovery of *Sclerotinia minor* sclerotia after various elutriation times in three different types of soil by the hydropneumatic root-elutriation technique

Elutriation time (min)	Clay[a]	Loam[a]	Sand[a]
2	11.2	13.9	13.9
4	14.1	13.9	13.9
6	14.2	14.1	14.0
8	14.1	13.9	13.9
LSD ($P \leq 0.05$)	0.20	0.22	0.16

[a]Number of sclerotia recovered. Mean of 24 100 g samples of each soil type assayed for different elutriation times.

TABLE 2. Comparison of hydropneumatic root-elutriation (RE), wet-sieving (WS) and wet-sieving with Calgon (WSC) techniques for numbers of *Sclerotinia minor* sclerotia recovered, and the percent bias in recovery from artificially infested clay, loam, and sand soils

Technique	Clay		Loam		Sand		Overall Mean	
	Recovery[a]	Bias[b] (%)	Recovery	Bias (%)	Recovery	Bias (%)	Recovery	Bias
RE	14.2 ± 0.18	5.3 ± 1.21	13.9 ± 0.15	7.3 ± 0.97	13.8 ± 0.17	8.2 ± 1.10	13.9 z[c]	7.2 z[c]
WS	12.7 ± 0.35	15.3 ± 2.31	13.2 ± 0.21	12.2 ± 1.39	12.7 ± 0.28	15.3 ± 1.84	12.8 x	14.4 x
WSC	12.4 ± 0.32	17.7 ± 2.12	14.0 ± 0.15	6.5 ± 1.00	13.7 ± 0.24	8.7 ± 1.60	13.4 y	10.9 y
Overall mean	13.1 j[c]	12.9 j	13.7 k	8.8 k	13.4 jk	10.8 jk		

[a]Mean number of sclerotia recovered out of 15 added to aliquots of 40 100-g samples of each soil type and their corresponding standard errors of mean.

[b]Bias measured as the percentage of deviation between number of sclerotia recovered with each of three techniques and the 15 sclerotia introduced into each soil sample. Numbers followed by the mean bias are the corresponding standard errors of mean.

[c]Values within an overall mean column or row for the variables recovery or bias followed by the same letters are not significantly different at $P \leq 0.05$ according to the LSD test.

techniques because eight samples could be processed at one time with the hydropneumatic root elutriator. Subsequently, both standard deviation and standard error of the mean were subjected to ANOVA assuming a 3 × 3 factorial design with five replications. Since the sample sizes for all soils and assay techniques were equal, both the standard deviation and standard error of the mean provided identical results; therefore, only the results for standard error of the mean are reported. The total processing time needed by each assay technique for each soil type was considered as a measure of efficiency.

We also calculated the relative net precision (5) that takes into consideration the relative size of the recovered sclerotia (bias), variance associated with the recovered sclerotia (precision), and cost in time (efficiency) for the individual assay techniques and soil types, with the formula

$$\text{relative net precision} = X_r^2/(C_r S_r^2), \quad (2)$$

where X_r^2 is the mean of recovered sclerotia from an assay technique and soil type combination r, C_r is the cost in time, and S_r^2 is the variance associated with the recovered sclerotia. The smallest calculated relative net precision was considered as equivalent to 100 and all other values were expressed relative to the smallest value.

Viability of recovered sclerotia. The viability of the recovered sclerotia in each sample processed by the three techniques was tested by plating sclerotia surface-disinfested (in 1% sodium hypochlorite solution for 3 min) on 2% water agar. The sclerotia were air-dried for 3–5 h before plating as moisture status of the sclerotia determines the level of their germination (10). The plates were incubated at 23 ± 2 C for 3–5 days and the number of germinated sclerotia was recorded for each sample. Viability of sclerotia was expressed as the percent of recovered sclerotia. Analysis of variance was conducted on the data to detect differences in the viability as affected by the assay techniques and soil types and an LSD test was used to compare means.

Detection of inoculum in naturally infested field soil. Thirty-two soil samples were collected from commercial lettuce fields with a history of lettuce drop in coastal California. Of these, 16 samples came from fields with loam soil and the rest from fields with clay soil. Each of these soil samples was assayed by the three techniques, and the sclerotial density was determined. The time required to process each sample was recorded, and the viability of recovered sclerotia was tested as described above. Precision and efficiency were computed. Bias was not calculated for this data because we did not have the true inoculum density values for the naturally infested field soil samples.

TABLE 3. Summary analysis of variance for percent bias and standard error of the mean for three quantitative assays for *Sclerotinia minor* in three types of soil

Source of variation	Bias (%)			Standard error of the mean for recovered sclerotia		
	df[a]	SS[b]	$P > F$[c]	df	SS	$P > F$
Soil	2	1,041.7	0.0060	2	0.305	0.0082
Technique	2	3,083.2	0.0001	2	0.198	0.0379
Soil × Technique	4	2,182.7	0.0003	4	0.198	0.1515
Error	347	34,855.3		36	0.995	

[a] Degrees of freedom for the individual source of variation.
[b] Sum of squares.
[c] Probability associated with the individual F tests.

RESULTS

Hydropneumatic root elutriator. The hydropneumatic root-elutriation technique recovered 92–95% of the artificially infested *S. minor* sclerotia regardless of the soil type assayed. For sandy and loamy soils, recovery of the maximum number of sclerotia was achieved within 2 min of elutriation. Elutriation for longer periods did not significantly improve recovery of sclerotia. For clay soil, however, a minimum elutriation time of 4 min was required to achieve maximum sclerotial recovery (Table 1).

Comparison of the three assay techniques for bias, precision, and efficiency. In general, at least 83% of introduced sclerotia was recovered by all three assay techniques irrespective of the soil type assayed. Recovery of artificially infested sclerotia varied from 92 to 95% for hydropneumatic root elutriation, 85 to 88% for wet sieving, and 83 to 93% for wet sieving with Calgon, depending on the soil type assayed (Table 2). Analysis of variance detected a significant effect of the soil type and assay technique, and a significant interaction of the soil type × assay technique on the estimated bias (Table 3). Mean bias was lowest for loam, highest for clay, and intermediate for sand (Table 2). Bias for individual elutriation techniques was dependent on the soil type assayed. For hydropneumatic root elutriation, bias was least for clay and nearly identical for loam and sand. For wet sieving, bias was least for loam and nearly identical for clay and sand. For wet sieving with Calgon, bias was lowest for loam, highest for clay, and intermediate for sand (Table 2).

Precision, as measured by the standard error of the mean, was significantly affected by the soil type and the assay technique employed to process the soil. The precision estimates were consistent for the three soil types assayed by each technique as demonstrated by the lack of interaction between the soil type and the assay technique (Table 3). Regardless of the soil type, the hydropneumatic root-elutriation technique was more precise compared with either wet sieving or wet sieving with Calgon. Adding Calgon to all types of soil before wet sieving provided more precise estimates of the number of sclerotia compared with wet sieving without Calgon.

Efficiency, measured by the total time required to process each sample, was dependent on the soil type assayed. Within each assay technique, clay soil required the most time to process a sample, followed by loam and sand. Between the three assay techniques, differences in time for processing a soil sample were also clearly evident for only the clay soil. The hydropneumatic root-elutriation technique required the least time to process a

TABLE 4. Cost in time to determine the number of *Sclerotinia minor* sclerotia in aliquots of 100-g soil in three soil types using the hydropneumatic root-elutriation (RE), wet-sieving (WS), and wet-sieving with Calgon (WSC) techniques

Technique	Soil	Time for processing[a] (min:sec ± SE)	Time for recovery[b] (min:sec ± SE)	Total time (min:sec ± SE)
RE	Clay	6:24 ± 0:22	2:23 ± 0:36	8:47 ± 0:42
	Loam	3:45 ± 0:34	3:03 ± 0:36	6:48 ± 1:08
	Sand	3:58 ± 1:08	4:06 ± 2:31	8:04 ± 2:48
WS	Clay	12:43 ± 2:02	1:56 ± 0:36	14:39 ± 2:07
	Loam	2:59 ± 0:36	2:29 ± 1:50	5:28 ± 2:17
	Sand	3:18 ± 0:38	4:04 ± 2:28	7:22 ± 2:09
WSC	Clay	10:01 ± 2:19	2:09 ± 0:35	12:10 ± 1:04
	Loam	3:33 ± 1:13	3:34 ± 1:13	7:07 ± 1:11
	Sand	3:31 ± 2:01	4:52 ± 2:43	8:23 ± 3:20

[a] Includes sample preparation and elutriation or wet sieving times.
[b] Includes the time required for enumerating the sclerotia.

clay soil sample, and the wet-sieving technique the most. The time to process a sand or loam soil sample was comparable among the three techniques (Table 4).

In general, the relative net precision values were higher for hydropneumatic root elutriation regardless of the soil type, except for loam, and were highest for clay and sand. For wet sieving, regardless of the soil type, the relative net precision values were uniformly low. Wet sieving with Calgon had intermediate values for sand, higher values for loam and lower values for clay (Fig. 1).

Viability of recovered sclerotia. The viability sclerotia recovered after elutriation ranged from 77 to 90%. Analysis of variance showed that both the assay techniques and the soil types influenced the viability of recovered sclerotia. The viability was significantly ($P < 0.05$) lower for sclerotia recovered from the hydropneumatic root elutriator largely because of sclerotia recovered from loam and sand. The viability of sclerotia recovered from clay soil was significantly higher compared with the other two soil types (Table 5).

Quantification of *S. minor* sclerotia in naturally infested field soil. The mean numbers of sclerotia recovered in loam and clay soils were 3 ± 0.3 and 5 ± 0.3, respectively. The number of sclerotia recovered in the two soils was not significantly different among the assay techniques employed. Even though the magnitude of precision estimates was comparable among the three techniques, it was lowest for the hydropnuematic root-elutriation technique. The time required to process a loam soil sample was not significantly different for the three assay techniques; however, for clay soil samples, hydropneumatic root elutriation required the least time (Table 6).

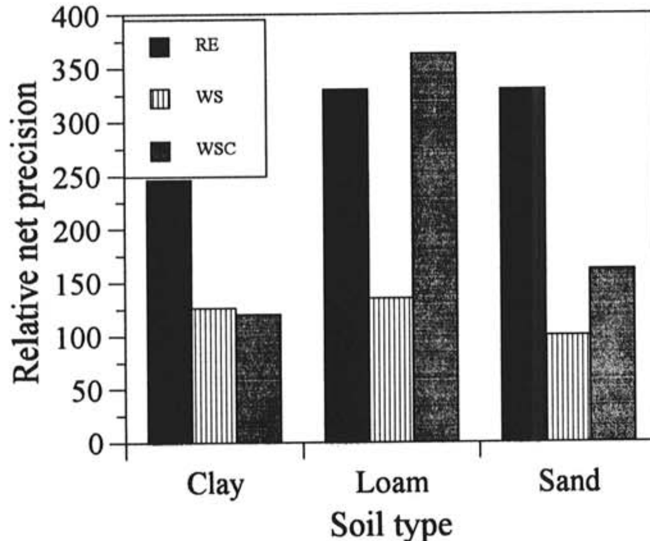

Fig. 1. The relative net precision values associated with the recovery of *Sclerotinia minor* sclerotia from artificially infested clay, loam, and sand soils assayed with the hydropneumatic root-elutriation (RE), wet-sieving (WS), and wet-sieving with Calgon (WSC) techniques. Values were calculated as the ratio of the square of the mean number of recovered sclerotia to the product of the variance among samples and the cost in total time, and expressed as relative to the smallest value obtained (= 100).

TABLE 5. Mean percentage of viable sclerotia recovered by the three assay techniques in three soil types

	Mean viability (%)[a]			
Technique	Clay	Loam	Sand	Overall mean[b]
Hydropneumatic root-elutriation	85.5	77.1	78.3	80.3 b
Wet-sieving	87.9	81.4	89.3	86.2 a
Wet-sieving with Calgon	86.1	83.2	80.2	83.2 a
Overall mean[b]	86.5 a	80.6 b	82.6 b	

[a] Mean of proportion of viable sclerotia obtained from 40 soil samples. Number of recovered sclerotia ranged from 7 to 15.

[b] Values within an overall mean column or row followed by the same letters are not significantly different at $P \leq 0.05$ according to the LSD test.

DISCUSSION

Among the three techniques tested in this study, both wet sieving and wet sieving with Calgon were less efficient than hydropneumatic root elutriation in recovering *S. minor* sclerotia in soil assays, largely due to the slower processing of clay soil. Hydropneumatic root elutriation also provided the most unbiased and precise method of assaying soil samples for *S. minor* sclerotia, followed by wet sieving with Calgon. In naturally infested soils, we could not detect differences in precision in the three assay techniques, but considerable differences in their efficiency were observed. Hydropneumatic root elutriation was also efficient for assaying naturally infested clay soils. The lack of differences in precision in naturally infested field soils was probably because of the low density of sclerotia in the soils assayed. Even though the density of sclerotia was low in naturally infested soils, each of the three assay techniques compared in this study was able to detect nearly an identical number of sclerotia in them, suggesting their high detection thresholds.

The hydropneumatic root-elutriation technique detected nearly 95% of the sclerotia introduced in clay soil. Approximately 15 and 17% of the sclerotia were lost during processing by wet sieving and wet sieving with Calgon, respectively. Sclerotia may inadvertently be crushed due to extensive handling while processing clay soil by wet sieving and wet sieving with Calgon. This perhaps explains the lower detection by these two techniques. No differences occurred between hydropneumatic root elutriation and wet sieving with Calgon methods in ability to detect sclerotia in loam and sand soils. Consequently, the bias in detection between hydropneumatic root elutriation and wet sieving with Calgon was nearly identical for the two soils. In contrast, for wet sieving, the bias was identical in clay and sand soils, and was only slightly lower in loam soil. For all three soil types, the bias for wet sieving was higher than the other two techniques indicating that, regardless of the soil type, wet sieving was unable to detect all of the *S. minor* sclerotia in the soil. Adams, (3) however, reported nearly 100% detection of artificially infested *S. minor* sclerotia with wet sieving.

The standard errors associated with the number of recovered sclerotia were consistently lowest for the hydropneumatic root-elutriation technique and highest for wet sieving regardless of the soil type. Therefore, estimates of inoculum density obtained with the hydropneumatic root-elutriation technique are likely to be more precise compared with the other two techniques regardless

TABLE 6. Comparison of hydropneumatic root elutriation (RE), wet sieving (WS), and wet sieving with Calgon (WSC) for *Sclerotinia minor* assay in naturally infested clay and loam soils

	Loam		Clay	
Technique	Number of sclerotia recovered[a] ± SE[b]	Processing time ± SE	Number of sclerotia recovered[a] ± SE	Processing time ± SE
RE	3.1 ± 0.21	3:41 ± 0:33	5.3 ± 0.22	7:04 ± 0:28
WS	3.3 ± 0.25	3:13 ± 1:13	4.9 ± 0.27	12:54 ± 2:02
WSC	2.8 ± 0.31	2:27 ± 2:47	4.8 ± 0.26	10:25 ± 2:34

[a] Mean of 16 samples of each soil type.

[b] Standard error of the mean.

of the soil type. The standard errors associated with inoculum density estimates from naturally infested soils also were lowest for the hydropneumatic root elutriation technique, but wet sieving provided statistically comparable results.

Efficiency, measured by the total time required for processing a soil sample among the three techniques, was comparable for all soil types except clay. Processing clay soil samples required significantly less time with hydropneumatic root elutriation than with the other two techniques. Adding Calgon to clay soil samples before processing by wet sieving reduced the processing time. Thus, the hydropneumatic root-elutriation technique is unlikely to be advantageous over the other two techniques for soil types other than for clay soil from the point of view of efficiency. However, processing soil samples using the hydropneumatic root elutriator reduces the potential differences in inoculum density estimates caused by different people processing soil. The time for enumerating the number of sclerotia in samples processed by wet sieving was lower for clay and loam soils compared with sandy soil because the processed samples had less residual sand mixed with sclerotia. The residual fractions of all processed samples were emptied into 90-mm petri dishes for enumerating the sclerotia. Use of larger petri dishes has the potential of improving the efficiency of all three techniques by reducing the time required to search for sclerotia.

Bias, precision, and efficiency are all taken into consideration in the calculation of the relative net precision (5), which collectively represents the relative usefulness of the different assay techniques for different soil types. The relative net precision values for hydropneumatic root elutriation were uniformly higher for all soil types, except for loam, indicating the usefulness of hydropneumatic root elutriation on different soil types. The uniform lower values for wet sieving and the improvement in values for wet sieving with Calgon suggest that modifications to wet sieving will make it a useful technique.

The viability of *S. minor* sclerotia is significantly influenced by the innate moisture status of the sclerotia (10) as well as the moisture status of the soil medium in which they reside (1). In this study, the viability of recovered sclerotia was tested after air-drying them for 3–5 h on laboratory benches, it is unlikely that the above two factors influenced the viability of sclerotia. While a significant reduction in viability was recorded for hydropneumatic root elutriation, it was not consistent for all soil types.

Porter and Steel (11) adapted an elutriation technique commonly used for nematode extraction to assay soil for *S. minor* sclerotia. Their procedure processes four soil samples at a time. Even though the extraction-procedure principles in their technique and in the hydropneumatic root-elutriation technique described here are similar, the latter processes eight samples at a time and thus appears to be a significant improvement over their technique. The technique of wet sieving followed by glycerol flotation developed for extraction of *S. minor* sclerotia in organic soils (2) was not compared in this study because it is not a commonly used technique elsewhere. Whether the hydropneumatic root-elutriation technique can extract sclerotia in organic soils as efficiently as wet sieving followed by glycerol flotation remains to be tested. Even though the hydropneumatic root-elutriation technique provides unbiased and precise estimates of *S. minor* sclerotia in soil, the cost of equipment might discourage its wider use. In such circumstances, wet sieving with Calgon should provide satisfactory results. Comparative studies of pathogen-assay techniques such as ours, and those of Butterfield and DeVay (4), and Nicot and Rouse (8), would significantly standardize the techniques used by different researchers and make inoculum-density comparisons between different studies consistent. Our study also provides similar comparative information for the three assay techniques used for the detection of *S. minor* sclerotia.

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