Quantitative Assay by Elutriation of Peanut Field Soil for Sclerotia of *Sclerotinia minor*

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ABSTRACT

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Sclerotia of *Sclerotinia minor*, causal agent of Sclerotinia blight of peanuts, can be elutriated from soil with a semiautomatic elutriator. Sclerotia were collected on 425-μm-mesh sieves during elutriation. The recovery of sclerotia from artificially infested soil following 3.0, 4.5, 6.75, 10.0, and 15.0-min of elutriation (~64 ml of water per second and ~231 cm^3 of air per second per unit) was 65, 83, 92, 94, and 97%, respectively. Sclerotia can be found throughout the plow zone (20 cm depth) in fields with histories of Sclerotinia blight. The number of sclerotia at harvest in the top 2.5 cm of soil ranged from 10 to 35 per 100 g of soil in areas with symptoms of Sclerotinia blight. Sclerotia in the top 2.5 cm of soil readily survived winter temperatures of Virginia. One sclerotium per 100 g of soil was sufficient to cause severe disease. Continuous planting of nonhost crops, such as corn, greatly reduced soil populations of sclerotia. However, viable sclerotia in sufficient numbers to cause Sclerotinia blight remained in field soils not planted to peanuts for up to 41 mo. Based on these observations, rotations of up to 4 yr would not provide effective control of Sclerotinia blight in peanuts.

Additional key words: *Arachis hypogaea*, peanut, Sclerotinia blight.

Sclerotinia blight, caused by *Sclerotinia minor* Jagger (17), was first observed on peanuts (*Arachis hypogaea* L.) in Virginia in 1971 (21). This disease has steadily increased in prominence throughout the peanut growing region of Virginia (22). Disease estimates based on aerial infrared photography showed that over 50% of the peanut fields in 1979 exhibited symptoms of Sclerotinia blight. Yield reductions of ~2,250 kg/ha have been reported (20) where disease was severe. Losses in Virginia in 1979 due to *S. minor* exceeded $8 million (27).

Sclerotinia blight of peanuts is characterized by the presence of numerous sclerotia on and in all infected plant tissues (21). Peanut residue usually remains on the soil surface throughout the winter months. In early spring (March), the soil is disked, chiseled, and planted with corn. After harvest, the corn stalks are cut or shredded and the residue remains on the soil surface over winter. For peanut production, the soil is plowed to a depth of up to 20 cm and most surface residue from the preceding crop moves to a position either at or near the bottom of the plow zone.

Survival studies have shown that sclerotia of *Sclerotinia* spp. persist in soil for periods ranging from 1 to 8 yr (4,9,12,14,28). Where white mold of beans was severe (1), sclerotia of *S. sclerotiorum* were rarely found on the soil surface after plowing. Sclerotia buried at depths exceeding 7 cm were more likely to survive over winter than sclerotia at or near the soil surface (12,13,28). Inoculum densities of sclerotia of *S. minor* in fields having histories of disease ranged from 0 to 82 sclerotia per 100 g of soil (3,5,16,20, and D. M. Porter, *unpublished*). Sclerotia of *S. minor* require a period of dormancy (1,6). Rotations may reduce the severity of disease caused by *Sclerotinia* spp. (4,26,30), although white mold of dry beans (*S. sclerotiorum*) was not affected by rotations (11). The inoculum density of infested yields following harvest was correlated with disease severity (5,16,20); however, it was not correlated with subsequent disease epidemics (25).

Studies on the general ecology of soilborne fungi require rapid, efficient methods of soil assay. Procedures for recovering sclerotia of sclerotial-producing fungi from the soil usually includes direct plating of soil on specific media (7,8), wet-sieving followed by plating on specific media (12,18,19,23), flotation-sieving (24) or wet-sieving alone (3,5,20). Recently, a semiautomatic elutriator was developed for extraction of nematodes from soil (10). Recovery of microsclerotia of *Cylindrocladium crotalariae* by elutriation of soil samples was also recently demonstrated (23).

This investigation is concerned with determining the efficacy of elutriation of sclerotia of *S. minor* from artificially and naturally infested soil samples, the survival of sclerotia in field soil following specific tillage practices, and the relation of sclerotial inoculum densities to disease incidence.

MATERIALS AND METHODS

A complete description of the four-unit semiautomatic elutriator used in this study was provided by Byrd et al (10). A constant water pressure of 138 kPa (20 psi) was maintained at the inlet to the four-unit elutriator with a water pressure regulator. The mean rates of water flow per elutriation unit were ~63.5, 62.4, 64.4, and 66.3 ml/sec, respectively. The mean rate of water flow to each sieve spraying unit was adjusted to ~9.8 ml/sec and the mean rate of air flow to each elutriating unit averaged ~231.2 cm^3/sec.

Assay of artificially infested soil. Soil was obtained from a field with no history of Sclerotinia blight. The soil was an Aquic Hapludult loamy fine sand with an organic matter content of 1.5%. The soil, air-dried to ~1% moisture content, was screened over 3-mm wire mesh to remove debris and clods, and sterilized at 82 C for 4 hr. Sclerotia of *S. minor*, present in field soil having a history of Sclerotinia blight, were removed from the soil by wet-sieving using a 425-μm sieve. Sclerotia retained on the sieve were removed by hand with the aid of a steroscopic microscope (×10) and placed in plastic tubes (17 × 100 mm) containing 3 g of sterilized soil.

Determination of optimum soil sample size. Tubes of soil containing the designated number of sclerotia, either 5, 10, 20, or 40, were added to bags containing 47, 97, 197, and 397 g, respectively, of sterilized soil. Each of the 40 soil samples of 50, 100,

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200, and 400 g was thoroughly mixed. This produced artificially infested soil samples reflecting a constant density of one sclerotium per 10 g of soil.

Time of elutriation. The elutriator has an adjustable elutriation time range of 0–15.0 min. However, overflow of debris and sclerotia does not occur until 1.5 min after the elutriation process begins. Elutriation times of 3.0, 4.5, 6.75, 10.0, and 15.0 min were selected for efficacy evaluation of the artificially infested soil samples.

Soil elutriation procedures. After mixing the soil containing the designated number of sclerotia with the specific soil sample sizes, samples were elutriated at designated times. Each soil sample was poured into a different elutriating unit and four samples were elutriated simultaneously. In successive elutriations, soil samples were rotated through each of the four elutriating units to minimize any differences in air and water flow between elutriating units. Eight samples of each prepared size were elutriated for each selected elutriation time. Following elutriation, the debris, sand particles, and sclerotia collected on a 425-μm sieve were washed into a 100-ml beaker with a low-pressure mist nozzle. Sclerotia were enumerated by dumping contents of the beaker into a square sieve (5 × 10 cm) with a plastic mesh (425-μm) bottom divided into equal sections. Sclerotia in each section were counted with the aid of a steroscopic microscope (×10). Debris retained by the 425-μm sieve was dried for 2 wk at room temperature and weighed.

Soil sampling and sclerotial assay of naturally infested peanut fields. Soil samples were collected each year from designated sites in several peanut fields in Virginia during 1977 to 1981 where Sclerotinia blight had been observed. At each site, stratified soil samples measuring 32 × 32 × 2.5 cm were taken at 2.5-cm increments to a depth of 20 cm, air-dried to ~2% moisture content, screened over 3-mm wire mesh to remove clods and debris, placed in paper bags, and stored in the dark at 24 C. Prior to elutriation, the soil in each bag was mixed thoroughly and four 100-g samples were drawn and elutriated simultaneously for 6.75 min. Other elutriator settings and enumeration techniques were as previously described.

Sclerotial viability. Viability of sclerotia of *S. minor* was determined by placing surface-sterilized sclerotia (3 min in 10% sodium hypochlorite) in sterile plastic tubes containing potato-dextrose agar. Following 10 days incubation at 21 C, the sclerotia were observed for evidence of germination. Viability was considered successful when sclerotia typical of those produced by *S. minor* were produced on the agar surface.

TABLE 1. Relationship of elutriation time to the recovery of sclerotia and debris from soil artificially infested with sclerotia (one sclerotium per 10 g of soil) of *Sclerotinia minor*

Elutriation time (min)	Average sclerotia recovered (%)[y]	Debris (g)[y]
3.0	65.2 a[z]	0.6 a[z]
4.5	83.3 b	1.1 a
6.75	91.9 c	1.1 a
10.0	94.0 cd	1.7 b
15.0	97.4 d	1.8 b

[y] Average of 32 elutriation determinations.
[z] In a column, percentages followed by a common letter do not differ significantly ($P = 0.01$) according to Duncan's multiple range test.

TABLE 2. Relationship of soil sample size to the recovery of sclerotia and debris during elutriation from soil artificially infested with sclerotia (one sclerotium per 10 g soil) of *Sclerotinia minor*

Soil sample size (g)	Average sclerotia recovered (%)[y]	Debris (g)[y]
50.0	87.0 a[z]	0.4 a[z]
100.0	85.5 a	0.7 ab
200.0	86.6 a	1.2 b
400.0	86.3 a	2.7 c

[y] Average of 40 elutriation determinations.
[z] In a column, percentages followed by a common letter do not differ significantly ($P = 0.01$) according to Duncan's multiple range test.

RESULTS

Efficiency of recovery of sclerotia from artificially infested soil. Elutriation time significantly ($P = 0.01$) affected the recovery of sclerotia from seeded soil (Table 1). Recovery efficiency was 49% greater at an elutriation time of 15 min than at 3 min. Following 15-min elutriation, recovery of sclerotia ranged from 96.3 to 100% (mean 97.4%). The difference in recovery between elutriation times of 6.75 and 10 min was not significant.

Recovery of sclerotia of *S. minor* by elutriation was not significantly affected by soil sample size (Table 2).

The length of elutriation time influenced the amount of debris retained on a 425-μm sieve (Table 1). The amount of debris was significantly ($P = 0.01$) greater following elutriation for 10.0 or 15.0 min than for 3.0, 4.5, and 6.75 min. Soil sample size also directly influenced the amount of debris recovered during elutriation (Table 2).

The average recovery for all sample sizes is shown in Fig. 1 in relation to elutriation time. A logarithmic regression ($N = 20$) of percent sclerotia lost (Y_L) on elutriation time provided the trend and functional relationship shown. At an elutriation time of 10.0 min $\hat{Y}_L = 4.9\%$ or $\log \hat{Y}_L = 0.69$ and the confidence interval (CI) was 2.1 to 11.2% or $\log (\hat{Y}_L \pm CI) = 0.69 \pm 0.36$. These data suggest that for repeated 10-min elutriations of the sample sizes studied, about two-thirds of the unrecovered sclerotia percentages would lie between 2.1 and 11.2%. The CI width of ~8.4 percentage points for the 15-min elutriation time was nearly uniform, whereas at 6.75 min the CI was about 14.0 percentage points. The sclerotia recovery function may also be used to estimate an apparent sclerotial density in the soil. For example, the average number of sclerotia recovered by elutriating for 3 min was 6.5 and the estimated recovery percentage for 3 min was 68.0. Using 0.68 as a recovery factor suggests an apparent true density of 6.5/0.68 = 9.6 when the samples were seeded to a density of 10 sclerotia per 100 g of soil.

In a separate analysis, the coefficient of variation ($CV = s/[x]_{av}$) for each sample size and elutriation time was computed and related to size (w) and time (t). The relationship was based on the

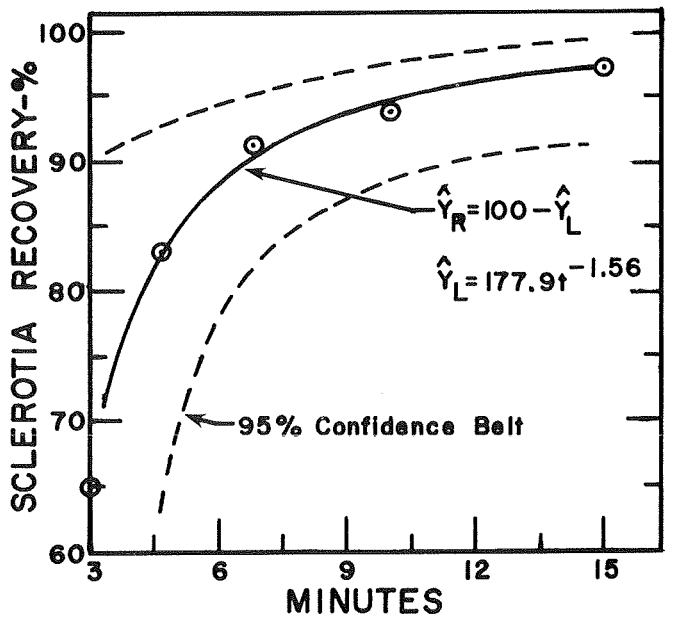

Fig. 1. Regression and 95% confidence belts based on a logarithmic regression of average sclerotia loss ($\hat{Y}_L$) on the length of elutriation time, pooled sizes, $N = 20$.

assumption that the CVs were related to the product of two functions $f(t)$ and $g(w)$. The functions as determined were $f(t) = 0.5 + 37.4\,t^{-2.55}$ and $g(w) = 71.1\,w^{-0.863}$ so that an estimate of CV in terms of elutriation time and sample size was $CV = 0.0742 \cdot f(t) \cdot f(w)$. This relation explained 83% of the variation in the observed CVs and may be used to estimate elutriation precision in terms of time and size (29). The relationship was based on eight determinations and may be used to determine number, sample size, and elutriation time combinations for target precisions when elutriating soil samples for sclerotia of *S. minor*. These results do not include field sampling errors nor potential differences resulting from nonuniform density samples.

Field soil sampling and sclerotial assay. In peanut fields having previous histories of Sclerotinia blight caused by *S. minor* and in fields where peanuts were currently exhibiting moderate to severe disease at harvest (October), sclerotia were found throughout the 20-cm plow zone but were concentrated in the uppermost 2.5-cm soil layer (Tables 3 and 4). At field sites A and B, the soil in the top 2.5-cm layer initially contained 21 and 24 sclerotia per 100 g soil, respectively. Sclerotia at the lower soil depths (7.5–20 cm) may be from previous peanut or soybean crops infected by *S. minor*. In December (3 mo following harvest), the sclerotial counts in the top 2.5 cm of soil remained the same (Table 3). Average viability of sclerotia in the top 5 cm of soil was 26%. However, viability of sclerotia recovered from the deeper depths (7.5–20 cm) averaged 75%. In preparation for corn production, the soil was plowed in April (1980) to a depth of 20 cm. Plowing the soil distributed the sclerotia throughout the plow zone but the sclerotia density was slightly greater near the bottom of the plow furrow (Table 3). Overall sclerotial viability was 48%. Twelve months later (April 1981) sclerotial counts throughout the plow zone decreased by 48%, but sclerotial viability increased substantially to 91%.

Planting of crops not susceptible to *S. minor* reduced the number of sclerotia recovered from the soil by elutriation (Table 4). In 1977 at field site B, Sclerotinia blight at harvest was severe and 24 sclerotia per 100 g of soil were recovered from the top 2.5-cm layer. Following chisel plowing in April 1978, sclerotia remained concentrated in the top 5 cm of the soil (19 sclerotia per 100 g of soil). In April 1979, the soil was plowed (20 cm depth) and again planted to corn. Sclerotial density in the top 2.5-cm soil layer was reduced to four sclerotia per 100 g of soil. In April 1980, the soil was again plowed (20 cm depth) in preparation for watermelon planting. Sclerotia were uniformly distributed throughout the plow zone. In April 1981, or 41 mo following the planting of three consecutive nonhost crops, the sclerotial density had steadily declined. Sclerotial counts per 100 g of soil were equal to or less than one at all soil depths. After deep plowing and planting, Sclerotinia blight was observed in the peanuts in August 1981. At harvest, the disease was severe and yields were greatly reduced. In April 1982, the soil was chisel plowed in preparation for corn planting. Sclerotial counts in the top 2.5-cm plow zone averaged 18 per 100 g of soil.

TABLE 3. Enumeration and viability of sclerotia of *Sclerotinia minor* in the plow zone (20 cm) of a naturally infested soil before and after plowing (field site A)

	October 1979[a]	Sclerotia per 100 g of soil (6.75 min of elutriation)					
		December 1979[b]		April 1980[c]		April 1981[d]	
Soil depth (cm)	Sclerotial density[e]	Sclerotial density[e]	Viability (%)[f]	Sclerotial density[e]	Viability (%)[f]	Sclerotial density[e]	Viability (%)[f]
2.5	21	22	23	4	54	2	91
5.0	4	6	39	4	46	2	83
7.5	3	4	69	5	39	3	100
10.0	3	3	96	5	44	4	93
12.5	3	4	71	6	54	2	92
15.0	4	3	82	6	53	3	80
17.5	4	3	54	7	47	3	100
20.0	5	4	80	5	50	3	85

[a] Sclerotinia blight was severe at harvest and soil samples were taken immediately following harvest.
[b] Soil undisturbed since harvest.
[c] Soil plowed (turned to a depth of 20 cm) and corn planted.
[d] Soil plowed (turned to a depth of 20 cm) and peanuts planted.
[e] Average of eight replications.
[f] Average of 40 tubes.

TABLE 4. Sclerotial density distribution of *Sclerotinia minor* in the plow zone (0–20 cm) of a naturally infested soil with a 3-yr rotation of nonhost crops (field site B)

	Sclerotia per 100 g of soil (6.75 min of elutriation)[a]					
Soil depth (cm)	October[b] 1977	April[c] 1978	April[d] 1979	April[e] 1980	April[f] 1981	April[g] 1982
2.5	24	19[g]	4	3	1.0	11
5.0	···	19	···	3	0.4	···
7.5	···	6	···	3	0.6	···
10.0	···	3	···	4	0.6	···
12.5	···	2	···	4	0.6	···
15.0	···	2	···	4	0.3	···
17.5	···	2	···	4	0.5	···
20.0	···	2	···	2	0.2	···

[a] Average of eight replications.
[b] Sclerotinia blight was severe at harvest and soil samples were taken immediately following harvest.
[c] Soil chiseled (not plowed) and corn planted.
[d] Soil plowed (turned to a depth of 20 cm) and corn planted.
[e] Soil plowed (turned to a depth of 20 cm) and watermelons planted.
[f] Soil plowed (turned to a depth of 20 cm) and peanuts planted.
[g] Soil chiseled (not plowed) and corn planted.

TABLE 5. Density distribution of sclerotia of *Sclerotinia minor* in plow zone (0–20 cm) of a naturally infested soil in relation to tillage practices (field sites C, D, and E)

Soil depth (cm)	Sclerotia/100 g soil (6.75 min. elutriation)[a] Field site C—1977 Corn[b] debris	After[c] plowing	At[d] harvest	Field site D—1978 Corn[b] debris	After[c] plowing	At[d] harvest	Field site E—1979 Corn[b] debris	After[c] plowing	At[d] harvest
2.5	16[d]	2	26	12	1	30	12	2	21
5.0	9	2	12	15	1	9	4	2	4
7.5	2	3	3	8	1	2	1	3	3
10.0	2	3	2	7	2	1	1	2	3
12.5	1	4	2	4	2	2	0	5	3
15.0	2	7	3	3	3	1	0	6	4
17.5	0	8	3	3	3	2	1	5	4
20.0	1	6	4	2	2	1	1	4	5

[a] Average of eight replications.
[b] Debris from previous corn crop. Cropping sequence was: corn planted following severe Sclerotina blight in peanuts; land was chisel plowed before corn was planted. Sclerotia counts were made in March following corn harvest, prior to plowing, and immediately following harvest.
[c] Soil plowed to a depth of 20 cm prior to planting peanuts.
[d] Sclerotinia blight was severe at harvest (October).

The relationship of tillage practices to the distribution of sclerotia of *S. minor* is given for three field sites, each having a history of Sclerotinia blight (Table 5). The normal crop rotation in Virginia is peanuts and corn. In preparation for planting corn, the soil was chiseled in April and not plowed; and the debris from the preceding peanut crop is left on the soil surface. Sclerotia of *S. minor* were found throughout the 20-cm plow zone in March, but were concentrated in the top 5 cm of soil. However, plowing the soil in preparation for peanuts distributed the sclerotia throughout the plow zone. The greatest numbers were observed in the deepest part of the plow zone. The top 2.5 cm of soil contained two, one, and two sclerotia per 100 g of soil at planting time in field sites C, D, and E, respectively. However, Sclerotinia blight was severe at all sites by the end of the growing season.

DISCUSSION

The elutriator provides an efficient method of recovering sclerotia of *S. minor* from the soil. Sclerotial enumeration can be done quickly and accurately provided the amount of debris recovered during elutriation is held to a minimum. The amount of debris can be minimized by elutriating for shorter periods and using a 50–100 g soil sample size. The recovery efficiency of sclerotia of *S. minor* from sclerotia-seeded soil exceeded 90% following elutriation of 100 g of soil samples for 6.75 min.

In this study, sclerotia of *S. minor* survived throughout the 20-cm plow zone. Adams (2) reported excellent survival at depths up to 30 cm, but not at 60 cm. Others (12,28) have noted that sclerotia on or near the soil surface are less apt to survive the winter than sclerotia buried at depths exceeding 7 cm. Davis (13) noted that sclerotia of *S. sclerotiorum* did not remain viable for more than 1 yr when they were located near the soil surface. In this study, sclerotia in the top 2.5-cm soil layer easily survived winter conditions. This is in contrast to the findings of others (1,16) who noted that sclerotia of *S. minor* in lettuce fields in California and *S. sclerotiorum* in bean fields in New York are shortlived. From the findings presented, *S. minor* survives in Virginia at all soil depths up to 20 cm. This difference in survival may result from warmer soil temperatures, difference in soil types, soil moistures, lack of biocontrol agents, etc., in Virginia. The survival of sclerotia throughout the plow zone and particularly on or near the soil surface ensures the presence of inoculum whenever susceptible host plants are present.

Sclerotia of *S. minor* persisted in a Virginia soil for almost 4 yr in the absence of a susceptible host. Other researchers (4,9,12,14,28) have presented similar findings. Thus, short-term rotations would not be effective in reducing Sclerotinia blight of peanuts in Virginia. Similar results were reported for dry beans (11). However, disease severity with other crops was reduced significantly by crop rotation (4,26,30). Crop rotation is generally ineffective when low inoculum densities can initiate disease. In this study, only one sclerotium of *S. minor* per 100 g of soil in the top 2.5-cm layer was required to initiate and cause severe Sclerotinia blight symptoms in peanuts at harvest. Fields with minimal sclerotial densities sometimes exhibit severe disease. Thus, the occurrence of epidemics may not be determined solely by inoculum densities. Schwartz and Steadman (25) presented similar findings. These findings are indicative of the importance of environmental factors such as those described by Dow (15) on disease initiation and subsequent epidemics.

Tillage practices influence the distribution of sclerotia of *S. minor* in the soil. When the soil is plowed to a depth of 20 cm, sclerotia are distributed throughout the plow zone. However, plowing increased the sclerotial density in the lower layers of the plow zone (10–20 cm), provided the sclerotial density was greatest in the upper soil layers before plowing. The practice of chiseling the soil before planting a nonhost rotational crop, such as corn, leaves the sclerotia from the preceding diseased peanut crop in the upper layers of soil throughout the corn crop year. Thus, peanuts are usually planted in soil having the least number of sclerotia; but sufficient sclerotia persist to initiate infection that may cause Sclerotinia blight.

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