Sampling and Extraction Procedures to Estimate Numbers, Spatial Pattern, and Temporal Distribution of Sclerotia of *Sclerotium rolfsii* in Soil

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ABSTRACT

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The mean inoculum density of *Sclerotium rolfsii* in six commercial fields sampled during 1983 and 1984 ranged from 0.3 to 53.7 sclerotia per 300 cm^3 of soil. The range of sclerotial density in the least and most heavily infested field was 0–5 and 2–225, respectively. The means and ranges varied with time of sampling and were influenced by previous cropping history and cultural practices. The frequency distribution of sclerotia among soil samples from the six fields at all sampling dates was best described by the negative binomial probability distribution. Values of the dispersion parameter, k, ranged from 0.15 to 4.4 and variance-to-mean ratios were significantly greater than unity for all samples, indicating a clustering of inoculum. The range of sclerotial density, frequency distribution of sclerotia, and extent of variability among samples obtained from one field were influenced by sample probe size. Samples taken with a 10.2-cm-diameter probe inserted 7.5 cm deep had a wide and variable range of inoculum density, and the distribution of inoculum was described by the negative binomial. Bulked samples obtained with a 1.8-cm-diameter soil auger inserted 12 cm deep had a smaller and less variable range of inoculum density, and the distribution was best described by the Poisson. Samples taken along simulated diagonal, parallel, or diamond-shaped paths in each of three nonuniformly infested fields provided similar mean inoculum density values, all of which were within 5% of the estimated population mean determined by the quadrat method. Total numbers of samples required were considerably reduced with these sampling patterns, however. Maximum recovery of laboratory- or soil-produced sclerotia from artificially infested soil samples was high (91–95%) with a wet-sieving assay method and intermediate (77–86%) with a flotation-sieving method. A methanol assay procedure provided a low recovery (28–33%) of viable soil-produced sclerotia and intermediate recovery (57–59%) of viable laboratory-produced sclerotia.

Additional key words: disease incidence, southern blight

Sclerotia of the soilborne pathogen *Sclerotium rolfsii* Sacc. are produced in large numbers on or adjacent to infected plant tissue and can survive in soil for 1–3 yr. They are capable of initiating infection with or without a food base of organic matter (9) and serve as the primary source of inoculum for initiation of disease in the field.

Southern blight or stem rot caused by *S. rolfsii* on various crops is generally difficult to control and may require repeated fungicide applications at high rates (10) or use of tolerant cultivars where available. Avoidance of fields with high inoculum densities, as determined by sampling before planting, may be an alternative approach to reduce losses to this pathogen. Leach and Davey (7) showed that through extensive multiyear sampling of sugar beet fields in California, threshold inoculum levels could be established on which predictions of disease incidence could be based. Such an approach requires knowledge of the spatial pattern and temporal distribution of inoculum in infested fields and requires accurate and efficient methods of sampling, extracting, and quantifying the numbers of sclerotia in soil.

In previous studies, sampling to estimate inoculum density of *S. rolfsii* within fields was conducted by taking 10–20 cores at random or along diagonal paths at specific locations within a plot or field by inserting a 2.2- or 2.5-cm-diameter soil auger 15–20 cm deep and bulking the samples (7,14–16). Subsequently, replicate subsamples were removed after air-drying (14–16) or the entire sample was assayed (7). Sclerotia were extracted from soil by wet-sieving (7), or by flotation-sieving (14), or by using a methanol assay procedure (15). Although each method has its advantages, a comparative study of the precision and efficiency of each procedure has not been conducted. Furthermore, the efficacy and efficiency of taking random samples with a small (2.5-cm-diameter) soil auger and bulking these samples, a procedure frequently used to estimate numbers and distribution of sclerotia, has not been evaluated.

The objectives of this study were to: 1) compare three extraction procedures for precision and efficiency in recovery of sclerotia of *S. rolfsii* from artificially infested soil, 2) determine the spatial pattern, frequency distribution of sclerotia among soil samples, and temporal distribution of inoculum in six naturally infested fields by sampling, 3) evaluate the efficacy of two sample probe sizes for recovery of sclerotia from a nonrandomly infested field, and 4) compare five sampling patterns for accuracy in estimating the mean number of sclerotia in three infested fields.

MATERIALS AND METHODS

Comparison of extraction procedures. The percentage recovery of sclerotia produced in laboratory cultures or in soil from artificially infested samples and the efficiency of each procedure were compared for three extraction methods. Sclerotia from laboratory cultures were produced on oat kernels as described by Punja and Grogan (8); sclerotia from soil were produced in the greenhouse following the procedure of Beute and Rodriguez-Kabana (1). Sclerotia were added to uninfested soil samples and thoroughly mixed; levels ranging from 1 to 60, 25, or 15 sclerotia (at increments of one sclerotium) per sample were tested depending on the assay method used. Viability of sclerotia was determined before mixing with soil by surface-disinfesting them with 0.5% NaClO and incubating on 1.5% water agar at 28 C for 72 hr. Uninfested soil was obtained from two fields in North Carolina (field A in Smithfield and field B in Maxton). Both soils were characterized as coarse sandy loams with organic matter contents and bulk densities of about 1 and 3% and 1.4 and 1.5 g/cm^3 for A and B, respectively. The soils were air-dried and sieved through a 4-mm opening before use. Procedures for the extraction methods

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were as follows:

Wet-sieving. Sclerotial densities ranging from 1 to 60/610 cm^3 of soil were tested. Each soil sample was washed for 1.5–2.5 min with continuous agitation, through nested sieves (4-, 2-, 0.85-, and 0.6-mm-diameter openings) with water flowing at a rate of about 30 L/min. Sand particles, debris, and sclerotia retained on the lower two sieves were washed into a 500-ml beaker, swirled to suspend buoyant particles, and decanted into a 15-cm-diameter, vacuum-suction Buchner funnel lined with Whatman No. 4 filter paper. Additional water was added to the beaker and the swirling and decanting steps were repeated twice to ensure that all buoyant particles were recovered. The debris on the filter paper was examined with a stereoscopic microscope at ×10. Sclerotia were distinguished from seeds and sand particles by their light brown to tan color and almost spherical shape and were picked out with forceps and counted.

Flotation-sieving. Sclerotial densities ranging from 1 to 15/50 cm^3 of soil were tested. Each soil sample was mixed in a 600-ml beaker with extracting solution of specific gravity of 1.07 (250 ml of molasses, 750 ml of water, and 0.0125 mg/ml of Calgon [sodium phosphate-carbonate]) to give a total volume of 350 ml. The mixture was stirred for 30 sec with a magnetic stirrer and allowed to settle for an additional 30 sec. The liquid was then poured through a sieve (0.6-mm opening) and sclerotia and debris were washed into a petri dish (100 × 15 mm). Sclerotia were picked out with forceps and counted. This method was adapted from the study by Rodriguez-Kabana et al (14).

Methanol assay. Sclerotial densities ranging from 1 to 25/220 g of soil were tested. Each sample was spread evenly in an aluminum baking pan (30 × 20 × 5.5 cm) and misted with a 1% (v/v) aqueous solution of methyl alcohol using a hand-operated CO_2-pressurized sprayer (pressure of 2.2 kg/cm^2) until the soil was at about field capacity (about 20–25 ml/100 g of soil). The pans were placed in polyethylene bags, sealed, and incubated on a greenhouse bench (temperature range 25–29 C). Colonies of *S. rolfsii* visible on the soil surface were counted after 3 days. This method was adapted from the study by Rodriguez-Kabana et al (15).

For each extraction procedure, four replicate samples were included for each inoculum density, source of sclerotia, and soil type; each assay was repeated twice. The coefficient of variability was calculated from these data. The time required to process one sample with each extraction method was calculated from the total time required for one person to assay 30 samples after air-drying and sieving the soils (not required for the wet-sieving procedure). Included are times required for preparing solutions, recording data, and cleaning up.

Sampling of fields. Five commercial fields in North Carolina and one field in Georgia were selected for sampling on the basis of previous reports of disease incidence caused by *S. rolfsii* on various crops. Portions of these fields ranging from 0.23 to 0.42 ha were divided into contiguous 7.6^2-m quadrats. The number of quadrats ranged from 40 to 72. A single soil sample was taken from the center of each quadrat with a golf green cup-cutter (10.2 cm in diameter) inserted 7.5 cm deep. Each sample (about 610 cm^3) was placed in a polyethylene bag and labeled with the quadrat coordinates. In three fields sampled sequentially over a 15-mo period, soil samples taken at each date were obtained from about the same location within the quadrat. In 1984, this sampling method was modified by taking a smaller volume of soil (about 300 cm^3) with a 7.2-cm-diameter probe inserted 7.5 cm deep. Samples were stored at 4 C for periods not exceeding 4 wk; they were processed separately and identically using the wet-sieving method described earlier. Observed numbers of sclerotia in 610-cm^3 samples taken in 1983 were expressed on a basis of 300 cm^3 of soil, the average competence volume of infection around carrot roots (13). Mean number of sclerotia and ratio of the variance to the mean (an index of dispersion [3]) were calculated for each field and sample date. The distribution of sclerotia among soil samples was tested for goodness of fit (using a chi-square test) to seven statistical probability distribution models (Poisson, Poisson with zeros, negative binomial, Thomas double Poisson, Neyman type A, Poisson-binomial, and logarithmic with zeros) using a FORTRAN program (4). For two fields (field 4, March 1983, and field 5, November 1983 [Table 1]) data in tail classes were combined to facilitate analysis.

Influence of sample probe size. A golf green cup-cutter (10.2 cm in diameter) and a soil-sampling auger (1.8 cm in diameter) were compared for efficacy and efficiency in recovery of sclerotia from a non-randomly infested field (field 4) in Maxton, NC. Sixty-four individual samples were taken in November 1983 from the centers of 7.2^2-m quadrats using the large probe as described earlier. The small auger was used to obtain 20 cores (each 30.5 cm^3) to a depth of 12 cm; samples were taken along the perimeter of a circle of radius 0.6 m (about 0.19 m between samples) whose center was the site of sampling with the large probe. The 20 cores from each quadrat were bulked (to give 610 cm^3), thoroughly mixed, and assayed as a single sample. Sclerotia were recovered from all samples with the wet-

Table 1. Spatial and temporal distribution of sclerotia of *Sclerotium rolfsii* among six fields sampled in 1983 and 1984[a]

Field	Location	Sampling time	No. of samples[b]	No. of sclerotia/300 cm^3 of soil			Probabilities for fit of data by two frequency distribution models[c]		
							Negative binomial		Poisson
				Range	Mean	Variance/mean	*k*-parameter	P^d	P^d
1	Clinton, NC	April 1983	72	0–5	0.5	1.68	0.80	0.85	0.0770
2	Clinton, NC	April 1984	72	0–11	1.3	1.93	1.34	0.99	0.0038
3	Tabor City, NC	April 1983	72	0–7	0.3	3.22	0.15	0.95	0.0015
4	Maxton, NC	March 1983	72	0–77	5.4	11.40	0.34	0.52[e]	0.0000
		June 1983	72	0–30	3.1	3.40	2.20	0.87	0.0000
		June 1984	72	0–17	3.0	2.89	2.27	0.93	0.0000
5	Maxton, NC	November 1983	64	2–225	53.7	6.70	2.01	0.24[e]	0.0000
		April 1984	64	0–112	17.5	3.29	2.27	0.99	0.0000
6	Cairo, GA	April 1983	40	1–26	5.9	3.17	4.40	0.89	0.0180
		June 1983	40	0–17	3.9	2.80	3.10	0.58	0.0000
		August 1983	40	0–6	2.3	1.80	2.12	0.69	0.0050
		April 1984	40	3–96	24.2	5.35	1.95	0.99	0.0000

[a] Fields ranged from 0.23 to 0.42 ha and were selected on the basis of reported incidence of disease the preceding year.
[b] Each sample was taken from the center of a 7.6^2-m quadrat. Samples taken in March and April were before planting, those in June and August were taken while the fields were cropped to carrots, and the sampling in November was done after harvest.
[c] None of the data were fit by the other five frequency distribution models tested.
[d] Probability of a greater chi-square value.
[e] Data in tail classes were combined.

sieving method. The mean number of sclerotia and standard deviation were calculated for each probe size, and the data in frequency classes were tested for goodness of fit to the seven distribution models described previously.

Comparison of sampling patterns. Five sampling patterns of varying shapes and areas of coverage (Fig. 1) were compared with the quadrat method used earlier to sample commercial fields. Sclerotial counts from the original quadrats were used to simulate the five sampling patterns in each of three infested fields. Distance intervals for samples were 7.6–10.7 m, depending on the sampling pattern. The mean number of sclerotia and standard deviation were calculated for each of the five sampling patterns in each field and compared with that of the population estimate obtained by the quadrat method.

RESULTS

Comparison of extraction procedures. Total percentage recovery of sclerotia from artificially infested soil samples was highest with the wet-sieving method (Table 2). One sclerotium in 610 cm^3 of soil was recovered in 92–95% of the experimental trials. Inoculum density did not influence percentage recovery. Source of sclerotia (laboratory or soil) did not affect recovery by the wet-sieving method but the time required to process one sample was greater in soil B, which contained a higher level of organic matter. Variability among replicates or trials was lowest with this procedure, although the time required per sample was the greatest (Table 2).

The flotation-sieving procedure required the least amount of time of the three extraction methods, and percentage recovery was not affected by source of sclerotia but was lower in soil B. With the methanol assay, recovery was not influenced by soil type but was lower when soil-produced sclerotia were employed. Maximum recovery of laboratory- and soil-produced sclerotia (expressed relative to total numbers of viable sclerotia present) was 59 and 33%, respectively, on the basis of average viability of 100 and 86%. Coefficient of variability in the

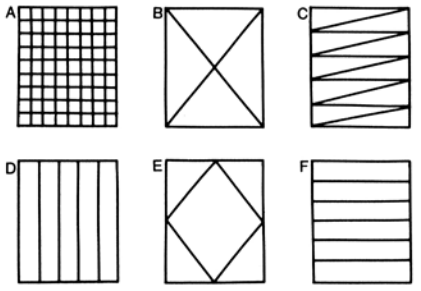

Fig. 1. Simulated sampling patterns used to obtain samples from three nonuniformly infested fields based on predetermined inoculum densities of *Sclerotium rolfsii* in 72 quadrats sampled according to **(A)** the quadrat method. The other sampling patterns were **(B)** diagonal path, **(C)** zigzag path, **(D)** parallel path (top to bottom), **(E)** diamond-shaped path, and **(F)** parallel path (left to right).

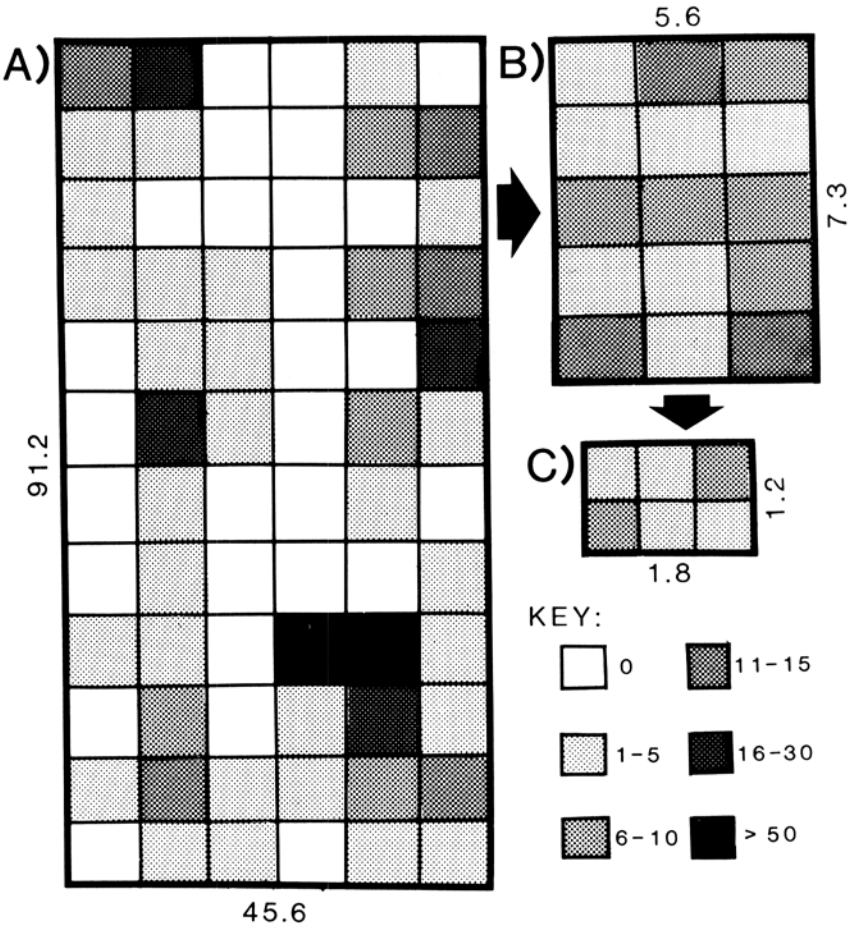

Fig. 2. Spatial pattern of sclerotia of *Sclerotium rolfsii* in 300-cm^3 soil samples taken from a 0.42-ha field in Maxton, NC (field 4, March 1983). The mean and range of sclerotial levels in this field were 5.4 and 0–77, respectively. **(A)** Each square represents a 7.6^2-m quadrat from which a single sample was taken. Other samples were taken from the centers of quadrats **(B)** 1.86 × 1.46 m or **(C)** 0.6^2 m. The intensity of the shading represents increasing numbers of sclerotia in each frequency class. Quadrats with no shading represent samples in which no sclerotia were recovered by the wet-sieving method.

Table 2. Comparison of three extraction procedures for recovery of sclerotia of *Sclerotium rolfsii* from artificially infested soil

Extraction procedure	Amount of soil assayed and range of sclerotial levels tested[a]	Soil type[b]	Average time required to process one sample (min)[c]	Percentage recovery of sclerotia from two sources[d]			
				Laboratory-produced		Soil-produced	
				Mean	C.V.	Mean	C.V.
Wet-sieving	610 cm^3, 1–60	A	11.0	92	15.5	91	16.4
		B	16.0	95	18.9	94	19.1
Flotation-sieving	50 cm^3, 1–15	A	2.5	81	18.4	86	17.3
		B	3.0	78	33.1	77	32.5
Methanol assay	220 g, 1–25	A	8.5	59	45.3	28	69.3
		B	8.5	57	44.1	33	97.1

[a] Sclerotial levels were tested at increments of one sclerotium.
[b] Soil A = Smithfield and B = Maxton. Both soils were coarse sandy loams.
[c] Calculated from the total time required for one person to assay 30 samples.
[d] Average of four replicates; each experiment was repeated twice.

methanol assay was the highest of the three methods (Table 2).

Sampling of fields. The mean number of sclerotia per 300 cm^3 of soil in samples from six commercial fields during 1983 and 1984 ranged from 0.3 to 53.7 (Table 1). The range of sclerotial density in the least and most heavily infested field was 0–5 and 2–225, respectively. Spatial pattern of inoculum in 72 quadrats (7.6^2 m) in field 4 (March) and in quadrats of decreasing size are illustrated in Figure 2.

The variance-to-mean ratio was significantly greater than unity (according to a chi-square test) for the six fields at all sampling dates, indicating a clustering or clumping of inoculum (3). The mean number of sclerotia and variance-to-mean ratios generally were reduced in sequential samples taken from fields that had been cultivated, bedded, and planted to carrots, i.e., in June (fields 4 and 6) or after several months of overwintering (field 5). The inoculum density in field 6 was higher in 1984 as a result of 35–43% disease incidence on carrots planted in that field in 1983. Similarly, the high inoculum density in field 5 in November reflected the high incidence of disease (about 45–53%) during August and September 1983. In both fields, the crop residue was disked to a depth of 15–20 cm in October.

The frequency distribution of sclerotia among soil samples from the six fields at all sampling dates was best described by the negative binomial distribution (probability of a greater chi-square = 0.24–0.99) and values of the dispersion parameter, k, indicated clumping and ranged from 0.15 to 4.4 (Table 1). The k values increased numerically in sequential samples from fields 4 and 5, indicating an increasing tendency toward randomness, but decreased over time for field 6, suggesting inoculum was more clustered. None of the data sets was well described by any other probability distribution model tested. The probabilities of exceeding the chi-square value for the Poisson distribution, which indicates a random dispersion of inoculum, ranged from 0 to 0.077 (Table 1).

Influence of sample probe size. The range of sclerotial density among samples taken from a nonrandomly infested field and the spatial pattern were influenced by sample probe size (Table 3). When samples were obtained with the large probe, the range was 2–225 sclerotia per 300 cm^3 of soil and the data in frequency classes were described by the negative binomial distribution (k = 2.01). When samples were taken with a 1.8-cm-diameter probe and bulked to give the same volume of soil, the range of sclerotial density in the same quadrats was lower (14–98) and the distribution of sclerotia among samples was described by the Poisson (P = 0.25). Although the mean inoculum density was similar using either probe, the standard deviation was higher with the large probe; however, about three times as much time was required to obtain samples with the small probe (Table 3). An insignificant correlation coefficient was obtained for linear regression analysis of sclerotial numbers in samples taken with the two probes (Z. K. Punja et al, *unpublished*).

Comparison of sampling patterns. The accuracy of five simulated sampling patterns in estimating the population mean varied with the field selected for sampling. In field 4 (k = 0.34), a diamond-shaped path (sample size 24) was the only pattern that yielded a sample mean within 5% of the estimated population mean if the standard deviations were not considered (Table 4). In field 5 (k = 2.01), samples taken along parallel paths across the field (sample size 32) provided the best population estimate with the lowest standard deviation. In field 6 (k = 4.4), three patterns (diagonal and horizontal or vertical parallel paths) with sample size of 20 were equally effective in predicting the estimated population mean and their standard deviations were comparable.

DISCUSSION

In this study, inoculum density was expressed per 300 cm^3 of soil, which is the average competence volume of infection (C-Vol) for *S. rolfsii* (5,8,13). The maximum distance and depth in soil from which a germinating sclerotium can infect a carrot root is 3 and 8 cm, respectively (13). Using these values, calculated C-Vols for 8-cm-long roots of radii 1.5 and 4 cm (3 and 5 mo after planting) are 190 and 403 cm^3, respectively (average C-Vol = 300 cm^3)(13). Thus, soil cores were taken by inserting a 7.2-cm-diameter probe 7.5 cm deep to obtain 300 cm^3. Expressing inoculum density per soil volume reflects the three-dimensional spatial pattern of propagules in soil and can be converted to propagules per gram of air-dry soil (ppg) using soil bulk density values. (Note: converted values may be 2–5% higher since bulk density is expressed on an oven-dry weight basis.)

Table 3. Efficacy of two sample probe sizes for estimation of numbers and distribution of sclerotia of *Sclerotium rolfsii* among soil samples taken from a nonrandomly infested field[a]

Diameter of probe (cm)[b]	Volume of soil sampled (cm^3)	Time required to obtain a sample (sec)	No. of sclerotia/300 cm^3 of soil			Probabilities for fit of data by two frequency distribution models		
						Negative binominal		Poisson
			Range	Mean	SD	k-parameter	P[c]	P[c]
1.8	610[d]	60	14–98	51.7	20.4	...	...	0.25
10.2	610	20	2–225	53.7	44.7	2.01	0.24	0.00

[a] Field 5 (November 1983).
[b] Probes were inserted 12 and 7.5 cm deep, respectively.
[c] Probability of a greater chi-square value.
[d] Represents bulked volume of soil from 20 cores.

Table 4. Comparison of five simulated sampling patterns for estimation of the mean number of sclerotia of *Sclerotium rolfsii* per 300 cm^3 of soil taken from three nonrandomly infested fields

Sampling pattern[a]	Field 4 (March)			Field 5 (November)			Field 6 (April)		
	No. of samples	Mean	SD	No. of samples	Mean	SD	No. of samples	Mean	SD
A	72	5.4	12.1	64	53.7	44.7	40	5.9	4.5
B	24	6.2	11.2	32	60.8	42.6	20	5.9[b]	4.3
C	24	3.0	4.2	32	48.8	45.4	20	4.5	2.7
D	24	8.3	12.1	32	52.1	55.7	20	5.9[b]	4.9
E	24	5.1[b]	11.7	32	60.6	43.0	20	5.3	3.3
F	36	7.2	16.0	32	56.3[b]	21.2	20	5.9[b]	4.3

[a] A = quadrat method, B = diagonal path, C = zigzag path, D = parallel path (top to bottom), E = diamond-shaped path, and F = parallel path left to right) (Fig. 1).
[b] Sample means were within 5% of the estimated population mean (determined by the quadrat method, pattern A) using these patterns if standard deviations (SD) were not considered.

The ranges and mean numbers of sclerotia of *S. rolfsii* among six fields were, with one exception (field 5), lower than those reported for populations in sugar beet fields in California (7). The largest range of inoculum density among samples from five commercial fields (determined by assay of 200-g samples by wet-sieving) reported by Leach and Davey (7) was 0–680/300 cm^3 of soil (using a bulk density of 1.33 g/cm^3); means ranged from 6 to 182/300 cm^3. The population in sugar beet field A (expressed per 0.67 ft^3) (7) following 70% disease incidence was 156/300 cm^3 (0.4 ppg); the lowest population reported was 5/300 cm^3 (7). Other investigators have reported lower inoculum levels in soil; the greatest range of inoculum density in samples from two peanut fields in Alabama (determined by assay of 50-cm^3 samples by flotation-sieving) was 0–23/300 cm^3, with a mean of 4.5 (14). The highest inoculum level in samples from three additional fields (determined by assay of 50-g samples by the methanol procedure) (15) was 11.5 sclerotia per 300 cm^3 (using a bulk density of 1.5 g/cm^3). These values may not, however, be directly comparable since time of sampling and extraction procedures differed.

Ranges and mean number of sclerotia among fields in this study varied with time of sampling and were influenced by previous cropping history and cultural practices. Leach and Davey (7) reported similar findings. Values of the dispersion index k in a given field also varied over time. Thus, time at which samples are taken should be considered in view of the purpose for sampling. Samples taken early in the season may overestimate inoculum densities present during growth of the crop, and this could influence the magnitude and nature of inoculum density-disease incidence relationships.

To correlate disease incidence with inoculum density, only sclerotia within the upper 7.5–8 cm of soil adjacent to a growing root should be considered since sclerotia of *S. rolfsii* do not germinate below this depth (11) and probably do not contribute to initiation of disease. An average of one and three viable sclerotia per 300 cm^3 of soil (0.002 and 0.006 ppg) may result in 20 and 30% disease incidence, respectively, on processing carrots (13). Comparable converted values from Leach and Davey's study (7) indicate that sugar beet fields from which samples with >1.6 viable sclerotia per 300 cm^3 were obtained (0.004 ppg) had about 10% disease incidence, whereas samples with 3.2 sclerotia per 300 cm^3 (0.008 ppg) resulted in >15% disease (7). In these crops (carrot and sugar beet), plant-to-plant spread of the pathogen may be extensive (7,13); therefore, low inoculum levels may result in high disease incidence. On peanut, however, in which plant-to-plant spread is not extensive, an average inoculum density (determined by the methanol assay) of 0.0002 ppg was associated with 6.1% disease incidence (16). Such a low inoculum density and high corresponding disease incidence may be due to poor detection of viable sclerotia with the methanol assay as shown in this study. Although stem rot of peanut was detected in 99% of the quadrats sampled (16), viable sclerotia were recovered from only 10% of the quadrats by the methanol assay. The method of obtaining soil samples (using a 2.5-cm-diameter auger and bulking the cores) (16) also may have influenced the proportion of sclerotia recovered. Low and variable recovery of soil-produced sclerotia compared with laboratory-produced sclerotia by the methanol assay observed in this study may be due to contaminating microorganisms present on the sclerotial surface that inhibited germination (12). The methanol assay may thus be much less effective in recovering naturally formed, surface-contaminated, viable field sclerotia.

The flotation-sieving method was the most rapid of the three assay procedures tested but it used the lowest amount of soil (50 cm^3) and may not be adaptable for use with larger (300-cm^3) soil samples. The increased volume of undiluted molasses required for these samples (about 450 ml/sample at a cost of $1.50/750 ml) would make the procedure uneconomical. The wet-sieving method is not limited by the volume of soil that can be processed and it is precise, even at extremes of inoculum density. The time required to count sclerotia may be greater in samples with high organic matter. However, using 300 cm^3 of soil, the time for each sample was about 5 min; a follow-up viability test that requires surface-disinfesting sclerotia with NaClO and incubating them on water agar can be used in conjunction with this method and requires an additional 2–3 min per sample (Z. K. Punja et al, *unpublished*).

Sampling with a large probe and keeping individual samples separate gave a wide range of sclerotial density, and variability among samples was high. The time and effort required for sample collection were reduced, however. With smaller samples taken with a soil auger and bulked, the range of sclerotial density was lower and the distribution among samples was described by the Poisson (16). In actuality, the range of sclerotial levels could be higher and the distribution may be clumped. Since these samples were obtained from greater depths in soil, however, the lower numbers of sclerotia could in part be due to fewer sclerotia being present deeper in soil (7). The time and effort required for sample collection were increased using the small probe but variability among samples was reduced. Selection of probe size for field sampling would therefore depend on whether efficiency or precision was desired and on the purpose for which sampling is conducted.

Sampling patterns influenced estimates of the population of *S. rolfsii* both within a given field and between fields. Although no single sampling pattern provided the best estimate of the population in three fields, samples taken along diagonal, parallel, or diamond-shaped paths gave estimates within 5% of the mean obtained using the quadrat method, depending on the degree of clustering of inoculum within the field. Although variability was quite high with each of these sampling patterns, the total number of samples taken was lower than that required for the quadrat method. The distances between samples could possibly be increased to cover larger areas and still retain the minimum number of samples without losing accuracy. In fields with a high degree of clustering, however, a more intense sampling pattern may be required.

The reported clustered spatial pattern of inoculum of *S. rolfsii* in the six fields sampled in this study is not unique among soilborne sclerotium-forming plant pathogens (2,6,17). Previous investigators noted that the spatial pattern of inoculum of *S. rolfsii* in fields was nonrandom (7,14); therefore, the resulting pattern of diseased plants also may be clustered. This was recently demonstrated for southern stem rot lesions on peanut (16). The spatial pattern of plants infected by *S. rolfsii* in three carrot fields sampled during this study also was clumped (Z. K. Punja et al, *unpublished*). To reduce variability among replicates when evaluating efficacy of fungicides, cultural practices, or tolerant cultivars for disease control in naturally infested fields, plot size and number of replicates should be sufficiently large to accomodate this clustered spatial pattern of inoculum. Fields with an average inoculum density of one or more viable sclerotia per 300 cm^3 of soil may sustain high losses to *S. rolfsii* if planted to a susceptible crop such as processing carrot.

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