Production of Sclerotia of *Sclerotinia minor* on Lettuce in the Field and Their Distribution in Soil After Disking

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ABSTRACT

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Fifteen romaine lettuce plants naturally infected with *Sclerotinia minor* on two farms in New Jersey produced an average of 3,450 sclerotia per plant with a range of 43 to 12,287 sclerotia per plant. One plant infected with *S. sclerotiorum* contained 63 sclerotia. When diseased lettuce plants were disked into the soil to a depth of 15 cm, about 70% of the sclerotia were in the top 8 cm of soil, and more than half of those were in the top 2 cm of soil.

Populations of fungi in soil are usually expressed as the number of propagules per unit of soil, normally spores per gram of soil or colony-forming units per gram of soil. This gross population figure has proven to be useful, but it also leads one to assume that the propagules of the fungi are uniformly distributed both vertically and horizontally throughout the soil mass. This is not true for many, and probably most, fungi. For example, it has been shown that *Rhizoctonia solani* is restricted to the top 10 cm of the soil profile (12) and is in a clustered distribution pattern situated horizontally in the field (7). *Sclerotium cepivorum*, however, has been shown to be uniformly distributed in the soil profile to a depth of 20–25 cm (approximate plow depth), below which the inoculum density markedly decreases (3). The same study described the great horizontal variability with a clustered distribution of *S. cepivorum* in the field. Areas of very high inoculum densities were adjacent to those of low inoculum densities. Similar horizontally clustered distribution patterns have been shown for *Cylindrocladium crotalariae* (10,11), *Sclerotium rolfsii* (6), and *Pythium aphanidermatum* (13). The distribution of most soilborne plant pathogens, especially those that produce survival propagules on their host plants, may be in both horizontally and vertically clustered patterns.

It was previously reported (1) that as many as 1,065 sclerotia of *Sclerotinia minor* Jagger were produced on a romaine lettuce plant. Dillard (8) has shown that the horizontal distribution of sclerotia in California lettuce fields follows the familiar negative binomial distribution. The purpose of this study was to estimate the number of sclerotia

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produced on infected lettuce plants and to determine their vertical and horizontal distribution in the soil profile after the plants were disked. Such information will give a better understanding of this plant pathogen and the diseases it causes and provide a basis for improving disease control.

MATERIALS FOR METHODS

Diseased romaine lettuce plants (*Lactuca sativa* L.) and surface soil beneath each plant were collected randomly from fields on two farms in Vineland, NJ, and assayed for number of sclerotia of *Sclerotinia minor*. Those from farm 1 were collected from a field about 1 wk before harvest, whereas those from farm 2 were collected from a field at harvest. Diseased plants and soil were put individually in plastic bags, which were placed in an ice chest. After arriving in Beltsville, the samples were put in a freezer (−15 C) until assayed. The number of sclerotia in soil (associated with each plant) was determined with the wet-sieving method (2). Sclerotia on the plant were counted by dissecting the plant under a microscope.

Distribution of the sclerotia (produced on diseased romaine lettuce) in soil after disking was determined on a third farm in Haleyville, NJ, on Hammonton loamy sand. Four dead plants infected by *S. minor* were selected. The surrounding healthy and diseased plants and surface soil (to a depth of 1 cm) for a distance of about 100 cm in all directions were removed to prevent possible interference with results. The soil under the four selected plants was not disturbed. Before disking, two soil samples were taken to a depth of 14 cm from opposite sides and immediately adjacent to each plant and combined. These samples were mixed and four 50-g samples assayed for number of sclerotia. These values were considered to be the background inoculum density in the vicinity of each plant. Each plant was photographed with a grid laid over the diseased plant. The grid was composed of 120 squares (10 × 12 squares), each square 5.5 cm on a side. The field was then disked twice, in opposite directions, with a finishing disk containing cylindrical blades 41 cm in diameter and 18 cm apart. The location of the plant, before disking, was determined from previously placed stakes at the edge of the disked area. The center of the grid was placed over the center of the former location of the plant. Soil samples were taken from alternate squares in the grid with a cylindrical soil sampler (5.2 cm i.d.) to a depth of 14 cm. Each 14-cm core sample was separated into samples representing depths of 0–2, 2–8, and 8–14 cm. The samples were placed individually in plastic bags. Thus, for each plant, core samples were taken from 60 of the 120 squares in the grid and each sample was subdivided into three vertical samples for a total of 180 samples from each plant. The soil samples were brought to Beltsville, MD, air-dried, weighed, and assayed for the number of sclerotia. Because the weight of the samples within a depth and between depths differed, the data were transformed to indicate the number of sclerotia per 100 g of air-dried soil.

Table 1. Number of sclerotia of *Sclerotinia minor* produced on diseased romaine lettuce plants on two farms in New Jersey

Sample	Farm 1[a] (1 wk before harvest)	Farm 2 (at harvest)
1	253	8,124
2	160	1,151
3	417	1,016
4	43	5,487
5	12,287	5,595
6	2,486	6,443
7	857	1,340
8	···	7,183
Average	2,358	4,542
Combined average	3,450	

[a] One plant from this farm contained 63 sclerotia of *Sclerotinia sclerotiorum*.

RESULTS

Eight diseased lettuce plants from each farm were assayed for the number of sclerotia produced on the plants. One plant from farm 1 was infected with *Sclerotinia sclerotiorum* (Lib.) de Bary and contained 63 sclerotia. The remaining 15 plants were infected by *S. minor*. Sclerotia were produced on and within the leaves, stem, and upper portion of the root. Many of the sclerotia were produced in association with the vascular bundles of the leaves. On plants that had been infected for several weeks and were nearly dead, *S. minor* had produced large numbers of sclerotia. Many of these sclerotia had fallen off the lettuce tissue and were found on the soil surface. Other plants showed typical wilting symptoms of lettuce drop but had not collapsed and produced relatively few sclerotia. These plants were collected about 1 wk before harvest (farm 1).

The number of sclerotia of *S. minor* produced on the seven plants from farm 1 varied from 43 to 12,287 per plant (average = 2,358), whereas the eight plants from farm 2 varied from 1,016 to 8,124 per plant (average = 4,542). The average production of sclerotia on the 15 plants from the two farms was 3,450 sclerotia per plant (Table 1).

Background inoculum densities for each of the four plants that were disked into the soil on farm 3 averaged 3.5–6.5 with a range of 2–16 sclerotia per 100 g of soil. Soil samples at the depth of 0–2 cm from sites of plants 1 and 3 generally did not have inoculum densities higher than the background inoculum density. Similarly, inoculum densities of selected samples from the depth of 2–8 cm of plant 1 were not greatly different from the background inoculum density. The remaining samples from these two plants were not assayed.

All 360 samples from plants 2 and 4 were assayed, and a summary of the results is presented in Table 2. About 27 kg of soil was assayed from each plant, resulting in the retrieval of 4,900 and 3,500 sclerotia from plants 2 and 4, respectively. Subtracting the background sclerotia from these numbers, it is estimated that 3,539 sclerotia were recovered from plant 2 and that 2,609 sclerotia were recovered from plant 4. Because only half of the soil beneath these plants was sampled (see Fig. 1 for

Table 2. Number and location of sclerotia of *Sclerotinia minor* in soil after disking diseased lettuce plants

	Soil depth (cm)							
	Plant 2[a]				Plant 4[a]			
Factor	0–2	2–8	8–14	Total	0–2	2–8	8–14	Total
Number of samples[b]	60	60	60	180	60	60	60	180
Soil sample wt (g)								
Total	3,974.0	11,083.6	11,872.6	26,930.2	3,958.5	11,307.3	12,053.0	27,318.8
Average per sample	66.2	184.7	197.9	···	66.0	188.5	200.9	···
Number of sclerotia								
Total	1,742	2,224	930	4,896	729	1,422	1,415	3,566
Average per sample	29.0	37.1	15.5	···	12.2	23.7	23.6	···
Average sclerotia								
Per 100 g of soil	43.8	20.1	7.8	18.2	18.4	12.6	11.7	13.1
Range	0–1,252	1–346	2–70	0–1,252	0–248	0–205	2–129	0–248

[a] Average background inoculum density and range: 5.0 (2–8 sclerotia per 100 g) for plant 2 and 3.5 (2–8 sclerotia per 100 g for plant 4.
[b] The area covered by these soil samples was 3,808 cm^2.

the pattern of collecting the soil samples), the actual number of sclerotia per plant was about 7,078 and 5,218, respectively.

From both plants, the average and range of inoculum densities were greatest at the soil depth of 0–2 cm and decreased at each successive depth. In soil samples in which high inoculum densities were detected, many of the sclerotia were attached to or embedded in lettuce tissue.

For plants 2 and 4, the maximum background inoculum density detected was eight sclerotia per 100 g of soil. On the basis of this finding, an arbitrary level of 10 or more sclerotia per 100 g of soil was considered to be substantially higher than background (Fig. 1). For plant 2, it appeared that most of the sclerotia produced on the plant remained in the sampled area after the plant was disked. The location of high inoculum densities from plant 4 indicated that some of the sclerotia produced on the lettuce plant were moved outside of the sampled area after disking. The distribution data also indicated that sclerotia at the depth of 0–2 cm were thrown farther from the center of the former location of the plant by the disk than sclerotia at lower depths.

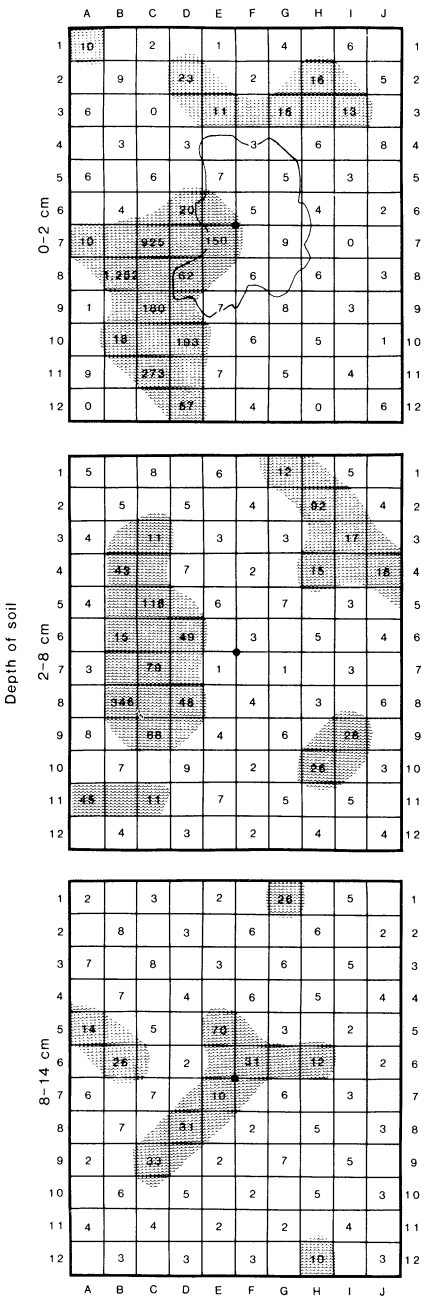

Fig. 1. Distribution of sclerotia of *Sclerotinia minor* produced on romaine lettuce after disking the diseased plant into the soil to a depth of 14 cm. The soil samples were collected at three depths of the soil profile. Each square on the grid represents a 5.5-cm square. The dot in the center of each grid represents the center and the curved line on the top grid (depth 0–2 cm) represents the outline of the diseased plant before disking. The numbers in the squares of the grids are the number of sclerotia per 100 g of air-dried soil for that sample. The shaded areas on each grid represent areas in which the inoculum density was 10 or more sclerotia per 100 g of soil. The background inoculum density of the soil before disking ranged from two to eight sclerotia per 100 g of soil.

DISCUSSION

The number of sclerotia of *S. minor* produced on lettuce plants ranged from 43 to 12,287. This great variation in number of sclerotia was probably due to variations in disease progress among plants. Obviously, plants infected 2–3 wk before harvest will have more sclerotia at harvest than plants infected 2–3 days before harvest. In normal lettuce production, each plant occupies about 1,860 cm^2 (2 ft^2). If an infected lettuce plant contained 3,450 sclerotia (as did the average plant in this study) and the sclerotia were mixed uniformly into the soil to a depth of 15 cm, the inoculum density of the soil would be increased by 7.9 sclerotia per 100 g of soil. In commercial agriculture, a disk does not mix sclerotia, especially those attached to plant tissue, uniformly into the soil. This study is the first to describe the large variation in inoculum densities among localized areas after disking. In Figure 1, 10% of the samples at 0–2 cm had 100 or more sclerotia per 100 g of soil, including two samples over 900/100 g. At 2–8 cm, 3% of the samples contained 100 or more sclerotia per 100 g of soil and 0% at 8–14 cm. The variation among localized inoculum densities was surprisingly large. On plant 2 at 0–2 cm, sample 8B (Fig. 1) contained 1,252 sclerotia, whereas sample 9A (within 7–8 cm) had only one sclerotium per 100 g. Similarly, at 0–2 cm, sample 11 C contained 273 sclerotia per 100 g of soil, whereas sample 11B contained nine sclerotia.

Most of the sclerotia disked into the soil were found in the top 8 cm of the soil profile, and more than half of these were in the top 2 cm. This result is similar to those obtained in a study in which a fluorescent dye was sprayed on the soil surface and incorporated into the soil (5). When two parallel passes were made with a finishing disk, nearly all of the dye was incorporated unevenly to a depth of 8 cm, with most of the dye in the upper 5 cm of the soil profile.

Few soil samples at the depth of 0–2 cm from plants 1 and 3 contained sclerotia higher than the background level in the field. This could be because 1) *S. minor* produced very few sclerotia on these plants, 2) the disk moved the sclerotia on the plant to regions outside of the sampled area, 3) the former location of the disked plant was not located as precisely as thought, or 4) a combination of these occurred. The latter three explanations seem plausible. In plant 4, some of the sclerotia from the plant were probably outside of the sampled area as indicated by the location of high inoculum densities.

These results have implications for cultural, chemical, and biological control of lettuce drop. It was previously shown in a greenhouse study that 61% of lettuce drop is initiated by sclerotia of *S. minor* on the soil surface and more than 90% by sclerotia in the top 2 cm of the soil profile (4). In a field study, it was subsequently shown that there was significantly more lettuce drop when sclerotia were placed at depths of 0, 1, or 2 cm than at depths of 4, 8 or 16 cm (9). The results of the present study show that disking diseased lettuce plants twice distributes most of the sclerotia in the top 8 cm, with most of them in the top 2 cm of the soil profile. Therefore, lettuce drop will not be controlled by disking but may be controlled by measures that will bury sclerotia deeper than 2 cm. More research is needed to determine if plowing will be adequate for this purpose.

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