Effects of Deep Plowing on the Distribution and Density of *Sclerotinia minor* Sclerotia and Lettuce Drop Incidence

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ABSTRACT

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Effects of deep plowing on the density and distribution of *Sclerotinia minor* sclerotia and disease incidence were evaluated in a field with a history of severe lettuce drop. Incidence of lettuce drop was recorded on the crop prior to deep plowing. A 40 × 102 m area was divided into four replicate plots of 40 × 24 m with a 2-m space between replications. Each plot was further subdivided into 60 subplots of 4 × 4 m. Soil samples were collected and bulked from six random locations within each subplot to a depth of 15 cm before, immediately after, and one lettuce crop after deep plowing from each subplot. Aliquots of 100 g of soil from each subplot were assayed for *S. minor* sclerotia by wet sieving. In two successive lettuce crops following deep plowing, the total number of plants and the number showing lettuce drop symptoms were counted in each subplot prior to crop harvest. Significant reductions in the mean number of sclerotia and lettuce drop incidence occurred on the crop immediately after deep plowing; however, disease incidence was significantly greater in the second crop. Calculated values of Lloyd's index of patchiness showed that the distribution of sclerotia had changed from a highly aggregated pattern prior to deep plowing to less aggregated patterns approaching randomness subsequently. While the desired effect of reducing the number of sclerotia was accomplished with deep plowing, the altered distribution of sclerotia increased the likelihood of infection of a greater number of lettuce plants. Consequently, a higher lettuce drop incidence was recorded in the succeeding crops. The viability of sclerotia was also significantly higher following deep plowing. Deep plowing is therefore unlikely to be a successful disease management strategy for lettuce drop in the high inoculum density fields in the Salinas Valley.

Additional keywords: disease management, epidemiology, *Lactuca sativa*

Lettuce drop is an important disease throughout the lettuce (*Lactuca sativa* L.) growing areas of California. The disease causes a soft watery rot of both head and leaf lettuce. Lettuce drop is caused by two species of *Sclerotinia*, *S. sclerotiorum* (Lib.) de Bary and *S. minor* Jagger (1,7,15). The two species are differentiated on the basis of sizes of sclerotia, asci, and ascospores as well as on biochemical and cytological characteristics (15). Furthermore, they require different conditions for infection and differ in their mode of infection. In coastal valleys of California, lettuce drop is predominantly caused by *S. minor* (7,15).

S. minor survives in soil as sclerotia which seldom produce apothecia. All infections are therefore caused by direct germination of sclerotia, and thus *S. minor* is strictly a soilborne pathogen (1,6,7,8). Under optimal moisture conditions, the sclerotia germinate eruptively, producing masses of hyphae that come in contact with the roots, stems, and senescent leaves, infecting the host (15,16). Large numbers of sclerotia are produced in colonized tissues of lettuce. Disease incidence is correlated with the number of sclerotia in the soil (6), although a single viable sclerotium within the competence zone (8) can cause infection. The competence zone is defined as the maximum distance and depth from which a sclerotium can cause infection (8). The majority of lettuce drop infections are caused by *S. minor* sclerotia in the top 8 cm of soil located within about 2 cm of plant crowns (10).

Survival of *S. minor* sclerotia in soil is affected primarily by soil water and burial depth (2,3,8–10). Sclerotia either disintegrate or fail to germinate in saturated soils (0 MPa) and survive better in moist soils (–0.01 to –0.5 MPa) (2,11). Germination decreases progressively with time and depth of burial (10). Also, if sclerotia are moved from the top 10 cm of soil to depths greater than 10 cm, they will be unable to cause lettuce drop infections (10). With these principles in mind, deep plowing was developed as a disease management strategy, the object being to bury sclerotia at depths of 25 to 30 cm from the surface so as to reduce the number of sclerotia in the top 20 cm of soil (12). A specially designed moldboard plow, which inverts the soil profile to a depth of 30 cm, is used for the purpose and has been used as a disease management tool ever since 1985 (12). Growers deep plow highly infested fields about every 3 to 5 years but have noticed a general lack of benefit when it is practiced in the Salinas Valley in California. While the utility of this strategy is known intuitively, no data are available to confirm its efficacy nor on the optimal frequency of practicing deep plowing to prevent recolonization of the top 10 cm of soil by *S. minor* sclerotia (19). The efficacy of deep plowing is important, particularly in areas such as the Salinas Valley, where lettuce has been grown continually for over 75 years (4) and the likelihood of viable sclerotia in the different soil profiles is expected to be very high. This study was undertaken with the objectives of comparing the density, distribution, and viability of sclerotia before and after deep plowing and evaluating the effects of deep plowing on the incidence of drop in succeeding lettuce crops.

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MATERIALS AND METHODS

Field plot. The study was located in a 5.5-ha commercial field in Salinas with a history of lettuce cultivation since the 1930s. The soil texture was silty clay, and its chemical characteristics were determined. The field had a history of very high incidence of lettuce drop (percentage of diseased plants) caused by *S. minor*.

Soil sampling and processing. An area 40 × 102 m was demarcated in the field before deep plowing and flagged to facilitate precise location of the area for repeated sampling. This area was subdivided into four replicate plots of 40 × 24 m with a 2-m space between replications. Each plot was further subdivided into 60 contiguous subplots of 4 × 4 m. Soil samples from six random sites within each subplot were collected to a depth of 15 cm and bulked before deep plowing using a 2.5-cm-diameter soil probe. In November 1992, the field was disked, ripped, and disked again before deep plowing. A moldboard plow driven by a 265-hp Caterpillar Challenger tractor (Caterpillar Co., Decatur, IL) was used for deep plowing to a depth of 35 cm. Subsequently, the soil received various management inputs outlined in Table 1 before planting the first lettuce crop in March 1993.

Post-deep-plowed soil samples were collected in February 1993 to study the immediate effects of deep plowing on the distribution and density of *S. minor* sclerotia from each of the subplots as outlined above. Similarly, soil samples were again collected from each of the subplots in June 1993 after the first lettuce crop to determine the recolonization potential of the deep-plowed soil.

To record changes in the density, distribution, and viability of *S. minor* sclerotia, and incidence of lettuce drop in a field with tillage practices common to lettuce production but without deep plowing, a 40 × 24 m area was demarcated in a field with comparable incidence of lettuce drop. Soil characteristics of this area were: pH = 7.3, N = 0.154%, P = 60 ppm, CEC = 56.1 meq/100 g, OM = 3.1%, sand = 5%, silt = 43%, and clay = 52%. This area was further subdivided into 60 subplots of 4 × 4 m. Soil samples from these subplots were collected at the same time as those from the deep-plowed field and processed similarly.

All soil samples were air-dried for 4 weeks on greenhouse benches (23 ± 1°C) before processing. Aliquots of 100 g of soil from each subplot were soaked in 100 ml of 1% sodium polymetaphosphate solution for 5 min before assaying for *S. minor* sclerotia by the modified wet sieving technique of Dillard and Grogan (6) using a 425-μm sieve. The soil fraction remaining on the sieve was poured into clean petri dishes (90 mm diameter), and the number of sclerotia recovered were enumerated at 10× using a stereoscope.

Sclerotia recovered from each subplot were surface-disinfested in 1% sodium hypochlorite solution for 3 min, air-dried for 3 to 5 h, and plated on 2% water agar (16). The plates were incubated at 23 ± 2°C for 3 to 5 days, after which the number of germinated sclerotia was recorded. Viability of sclerotia was expressed as a percentage of the total recovered sclerotia.

Analysis of variance was conducted for the number of sclerotia recovered and their viability, to detect differences in the sampling times in plots with or without deep plowing. Least significant differences ($P \leq 0.05$) were used to compare treatment means.

Lettuce drop incidence and deep plowing. Prior to deep plowing, the incidence of lettuce drop was recorded at maturity in 20 random 4 × 4 m areas at the site. In two successive lettuce crops immediately after deep plowing, disease incidence was recorded at maturity in each subplot from which soil samples were collected, and expressed as a percentage. During both seasons after deep plowing, the lettuce cultivar Salinas was planted for commercial production in two rows 30 cm apart on beds (1 m between bed centers). Three weeks after emergence, the plants were thinned to a spacing of 30 cm between plants. The crops were irrigated by furrow irrigation. The mean lettuce drop incidence on crops before and after deep plowing was compared using the least significant difference test ($P \leq 0.05$). Similarly, lettuce drop incidence in each subplot was recorded in the plot without deep plowing.

Spatial analyses. Spatial distribution maps for both sclerotia and lettuce drop incidence were generated for each sampling period. To record the changes in the number of sclerotia per 100 g of soil in different subplots during the three sampling periods, the cumulative frequency of subplots in frequency classes 0, 1 to 5, 6 to 10, 11 to 20, 21 to 30, 31 to 50, and >50 (sclerotia/100 g of soil) was calculated. The aggregation index, Lloyd's index of mean patchiness (LIP) (17), was calculated for both sclerotia and lettuce drop incidence for each sampling period for the plots with or without deep plowing. The LIP was calculated for each replication within each sampling time using the equation: $[(y + s^2)/(y - 1)]/y$, where y and s^2 are the mean and variance for the number of sclerotia or percentage of lettuce drop incidence for individual replications within each sampling period. To determine the relationship between the LIP and mean number of *S. minor* sclerotia at different sampling periods, the LIP values for the number of sclerotia at different sampling periods were plotted against the corresponding mean number of sclerotia. The LIP is independent of the mean and the subplot size (13), and therefore can be used to compare changes in the distribution of sclerotia and lettuce drop incidence before and after deep plowing. Least significant difference tests were used to compare LIP for sclerotia and lettuce drop incidence among the different sampling periods in plots with or without deep plowing.

RESULTS

There was little variation in any of the measured soil characteristics across the four replications in the deep-plowed field. The mean pH, total nitrogen (%), total phosphorus (ppm), cation exchange capacity (meq/100 g), organic matter (%), sand (%), silt (%), and clay (%) for the site were 7.1, 0.146, 62, 52.2, 3.3, 6, 44, and 50, respectively. The number of tillage operations in deep-plowed soil were higher compared with the non-deep-plowed soil. The deep-plowed field, subsequent to the previous crop harvest, required 22 tractor passes in different tillage operations to prepare the soil for lettuce production. In contrast, subsequent to a crop harvest, the standard tillage operations to prepare soil for lettuce production required only 11 tractor passes (Table 1).

Table 1. Management practices to prepare soil for lettuce production in plots with deep plowing subsequent to lettuce harvest in October 1992 to reduce *Sclerotinia minor* sclerotia in soil compared with conventional tillage operations

	Number of passes	
Management practices	Deep plowing[x]	Conventional tillage[y]
Disking	2	4
Ripping to 50 cm	2	2
Disking	3	...[z]
Plowing to 35 cm	1	...
Disking (to break up clods)	4	...
Chiseling	1	2
Disking	3	...
Land planing	2	2
Disking	2	...
Chiseling	1	...
Listing beds	1	1

[x] A 133-hp D60 DSA tractor was used for all operations except plowing. A 265-hp Caterpillar Challenger tractor was used for plowing.

[y] A 150-hp 4450 John Deere tractor was used for all operations.

[z] Tillage operations not part of the standard land preparation activities for lettuce production.

Table 2. Effects of deep plowing on the number and viability of *Sclerotinia minor* sclerotia, lettuce drop incidence, and the Lloyd's index of mean patchiness for the distribution of sclerotia (LIP1) and lettuce drop incidence (LIP2) at different sampling times

Sampling period (date)	Sclerotia per 100 g soil	Viability (%)	LIP1	Drop incidence (%)	LIP2
Before deep plowing (Oct. 1992)	8.2 a[y]	71.9 b	5.0 a	21.6 b	...[z]
Immediately after (Feb. 1993)	5.1 b	80.6 a	2.8 b	...	...
After one lettuce crop (June 1993)	4.2 b	84.3 a	2.3 b	14.4 c	1.2 b
Second lettuce crop (Oct. 1993)	...	...	...	40.3 a	1.1 b

[y] Means within a column followed by the same letter are not significantly different according to LSD ($P = 0.05$).

[z] Data not collected or not appropriate.

Number of sclerotia, their distribution and viability in the deep-plowed field. The number of sclerotia in the sampled subplots before deep plowing varied between 0 and 201, with a mean of 8.2 per 100 g of soil (Table 2). Spatial distribution maps indicated a highly aggregated pattern for sclerotia before deep plowing (Fig. 1A). This was also indicated by the calculated LIP values (Table 2, Fig. 2). Results from the four replications were similar, and therefore data from only three replications are presented. Deep plowing reduced the number of sclerotia significantly ($P \leq 0.05$) in the sampled subplots, to a mean of 5.1 per 100 g of soil (Table 2) with a range of 0 to 60. However, it also altered the distribution of sclerotia. The sampled subplots after deep plowing showed greater uniformity in the distribution of sclerotia than prior to deep plowing (Fig. 1B). Even though the calculated LIP values indicated an aggregated pattern of sclerotia, the values were significantly lower ($P \leq 0.05$) after deep plowing than prior to deep plowing (Table 2, Fig. 2). The mean number of sclerotia per 100 g of soil in samples collected at the end of the first lettuce crop after deep plowing was 4.2 (range 0 to 31) and was not significantly different ($P \leq 0.05$) from the mean number of sclerotia immediately after deep plowing (Table 2). The spatial distribution maps showed less aggregated patterns of sclerotia than the two previous sampling periods (Fig. 1C). The LIP for the sampling period after deep plowing was significantly lower than that calculated for prior to deep plowing ($P \leq 0.05$), and was lower but not significantly different than the LIP immediately after deep plowing (Table 2). In contrast, the LIP values for the corresponding periods in the nonplowed soil (with routine tillage operations common to lettuce production) were 1.39, 1.36, and 1.53, respectively, and were not different significantly ($P \geq 0.05$).

Deep plowing altered the distribution of sclerotia from a highly aggregated pattern to less aggregated patterns approaching random distribution during the sampling period reported in this study (Fig. 2). This was also demonstrated by the cumulative distribution of subplots among frequency classes of sclerotial density (Table 3). Before deep plowing, there were 97 subplots with >5 sclerotia. Immediately after deep

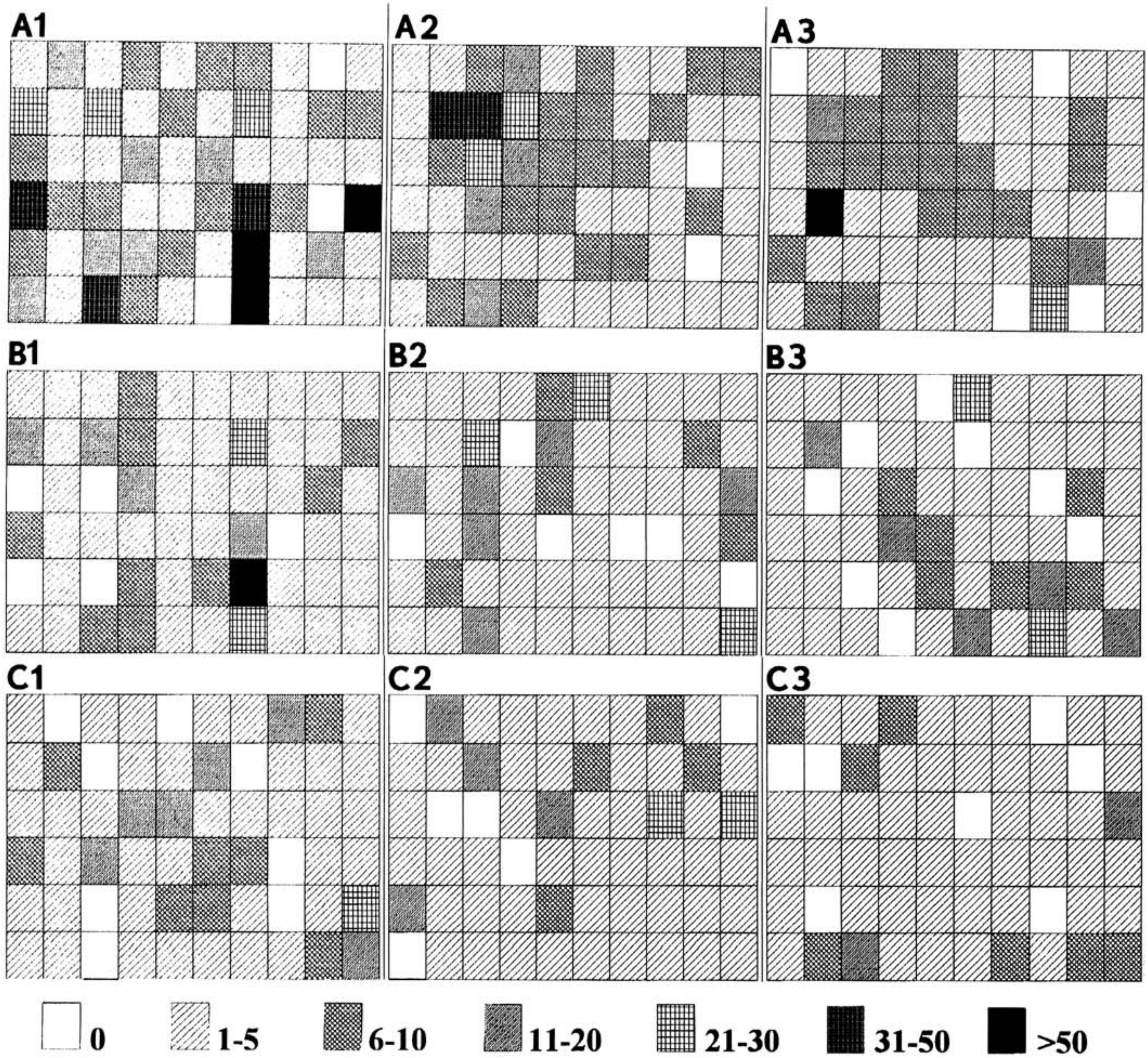

Fig. 1. Spatial distribution maps of *Sclerotinia minor* sclerotia in various discrete classes (numbers of sclerotia per 100 g of soil): **(A)** before deep plowing; **(B)** immediately after deep plowing; **(C)** at the end of the first lettuce crop after deep plowing. The numbers after **A**, **B**, and **C** represent data from the respective replications. Replicate 4 was similar to replicates 1, 2, and 3; therefore it was not illustrated.

plowing, however, their numbers decreased to 29, and after one lettuce crop, there were 49 subplots with >5 sclerotia per 100 g of soil (Table 3).

The mean viability (%) of recovered sclerotia from subplots after deep plowing was significantly greater ($P \le 0.05$) than prior to deep plowing. Although the viability (%) of recovered sclerotia one crop after deep plowing was higher than in samples collected immediately after deep plowing, the difference was not statistically significant (Table 2).

Incidence of lettuce drop after deep plowing. The lettuce crop prior to deep plowing had a mean lettuce drop incidence of 22%, with a range of 16 to 35%. Mean incidence of drop in the sampled area on the first lettuce crop after deep plowing was significantly lower ($P \le 0.05$) than on the crop prior to deep plowing (Table 2). However, the mean incidence on the second successive lettuce crop after deep plowing was nearly two times greater than on the crop prior to deep plowing, and nearly three times greater than on the first crop after deep plowing (Table 2). The spatial distribution maps of lettuce drop incidence showed a uniform distribution of lettuce drop incidence on both crops after deep plowing (Fig. 3A and B). Results from the fourth replication were similar and therefore are not presented. A uniform distribution was also indicated by the calculated LIP values (≅1) for both sampling periods, which were not significantly different ($P \ge 0.05$). Disease incidence did not differ significantly between successive lettuce crops for the nonplowed site (data not shown). The LIP values for lettuce drop incidence for the corresponding periods in the nonplowed soil were 0.89 and 0.92, respectively, and were not significantly different ($P \ge 0.05$). These values indicated that the distribution of lettuce drop was regular in the nonplowed soil.

DISCUSSION

This study provides the first data on the effects of deep plowing on the density and distribution of *S. minor* sclerotia and incidence of lettuce drop. Although a significant reduction in the mean number of sclerotia was recorded after deep plowing, sclerotial distribution was modified from a highly aggregated pattern into less aggregated patterns. All other edaphic and crop production practices being the same, this perhaps increased the likelihood of infection of a greater number of plants, and consequently, a significantly higher incidence of lettuce drop in the second successive crop after deep plowing was recorded. White mold severity on beans (5,18) was also not reduced by deep plowing of *S. sclerotiorum* sclerotia to a depth of 25 cm. In the white mold study (5), however, the authors did not consider the effect of deep plowing on the distribution of *S. sclerotiorum* sclerotia and disease severity. Deep plowing is therefore unlikely to be an efficacious option for lettuce drop management in the high inoculum density fields in the Salinas Valley of California.

S. minor sclerotia are formed on both aboveground (7) and belowground infected tissues (K. V. Subbarao, *unpublished*). Sclerotia formed aboveground are deposited on the soil surface along with the infected crop debris and are incorporated into the soil at various depths during land preparation for the next crop. The results from studies on the survival of sclerotia under a wide range of soil conditions have been variable, ranging from 1 month to 11 years (1,3,7). The primary factors that affect the survival of *S. minor* sclerotia are soil water (2,3,11), burial depth, and duration of burial (10). Survival, measured as the ability of sclerotia to germinate, decreased with increasing time and depth of burial in wet soils (10). Factors such as soil temperature, O_2, CO_2, and ethylene concentrations are expected to change with soil depth. However, Imolehin and Grogan (9) concluded that these factors are usually not limiting to the survival of *S. minor* sclerotia. They attributed the decrease in survival to colonization of sclerotia by various soil fungi. Furthermore, *S. minor* sclerotia are unable to infect lettuce plants from more than 2 cm away from the tap root or at greater than 8-cm depth (8).

The preceding information led to the development of deep plowing as a disease-management practice to remove the sclerotia located in the upper soil profile, where sclerotia are expected to be viable for longer periods and be close enough to the lettuce roots and crowns to cause infection. A specially designed moldboard plow was developed for this purpose (12), and the growers have been using this technique since 1985. However, subsequent to the development of this technique, a clearly designed study to test its efficacy was not undertaken. In the Salinas Valley, two crops of lettuce in a given field are commonly grown each year, and lettuce drop infections from these crops con-

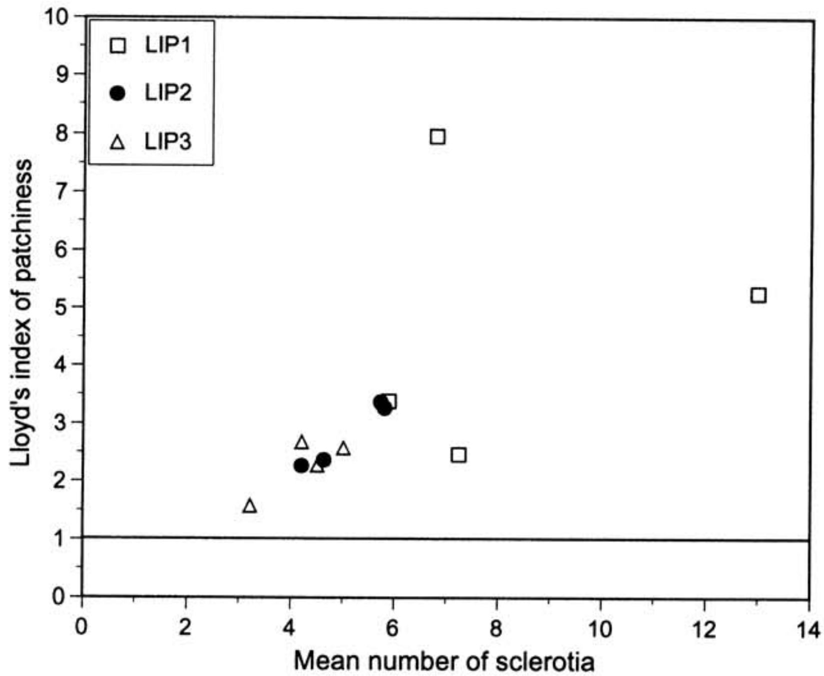

Fig. 2. Relationship between mean number of *Sclerotinia minor* sclerotia recovered from 100 g of soil and the Lloyd's index of patchiness (LIP) (13) at different sampling times: LIP1, before deep plowing; LIP2, immediately after deep plowing; and LIP3, at the end of the first lettuce crop after deep plowing. The horizontal line parallel to the x-axis represents the value of LIP at which the distribution of sclerotia will be random.

Table 3. Cumulative frequency of subplots with *Sclerotinia minor* sclerotia (per 100 g of soil) before and after deep-plowing in different discrete classes

	Frequency class						
Sampling period (date)	**0**	**1-5**	**6-10**	**11-20**	**21-30**	**31-50**	**>50**
Before deep-plowing (Oct. 1992)	18	125	64	13	6	4	10
Immediately after (Feb. 1993)	22	189	0	19	7	1	2
After one lettuce crop (Oct. 1993)	26	165	24	19	5	1	0

tinually add sclerotia to the soil. These sclerotia are then deposited at various depths during land preparation for the next crop. In these fields, therefore, viable sclerotia are probably always present in different soil profiles. Their distribution may be aggregated because many sclerotia form on infected plants and these are concentrated in the area containing the debris from infected plants. Large numbers of very large clods form as a result of soil inversion by deep plowing. Breaking these clods to prepare soil for lettuce production requires additional tillage operations not normally required during soil preparation in nonplowed soil. Thus, deep plowing and the subsequent land preparation activities may redistribute the sclerotia to areas that previously lacked them and also dilute the numbers of sclerotia from areas that had greater aggregation. Moreover, the viability of sclerotia recovered after deep plowing was significantly greater than before deep plowing.

S. minor sclerotia located at depths below 8 cm from the surface are unable to infect lettuce plants (8). In our study, even though many subplots had similar numbers of sclerotia, lettuce drop incidence was variable among subplots and increased between crops in the deep-plowed field. Lettuce drop incidence between the two crops in conventionally tilled fields was similar. Soil water plays a critical role in every phase of the lettuce drop disease cycle (2,11,20). The distribution of soil water is not likely to be uniform in large commercial fields, and this perhaps affected disease incidence in certain parts of the field.

To judge the benefits of deep plowing, one must examine both the mean number of sclerotia and their distribution before and after deep plowing. This is because sclerotial distribution is a key factor in determining the reliability of inoculum density estimates, in developing soil sampling strategies, and in assessing the impact of plant disease on crop yield (14). Examining the mean number of sclerotia alone may lead to erroneous conclusions and development of an energy-intensive disease-management practice (11). Our data suggest that the benefits of deep plowing, if any, are likely to disappear within one crop after deep plowing. Alternatively, deep plowing would have to be practiced every year, which in turn might return sclerotia buried the previous year to the surface.

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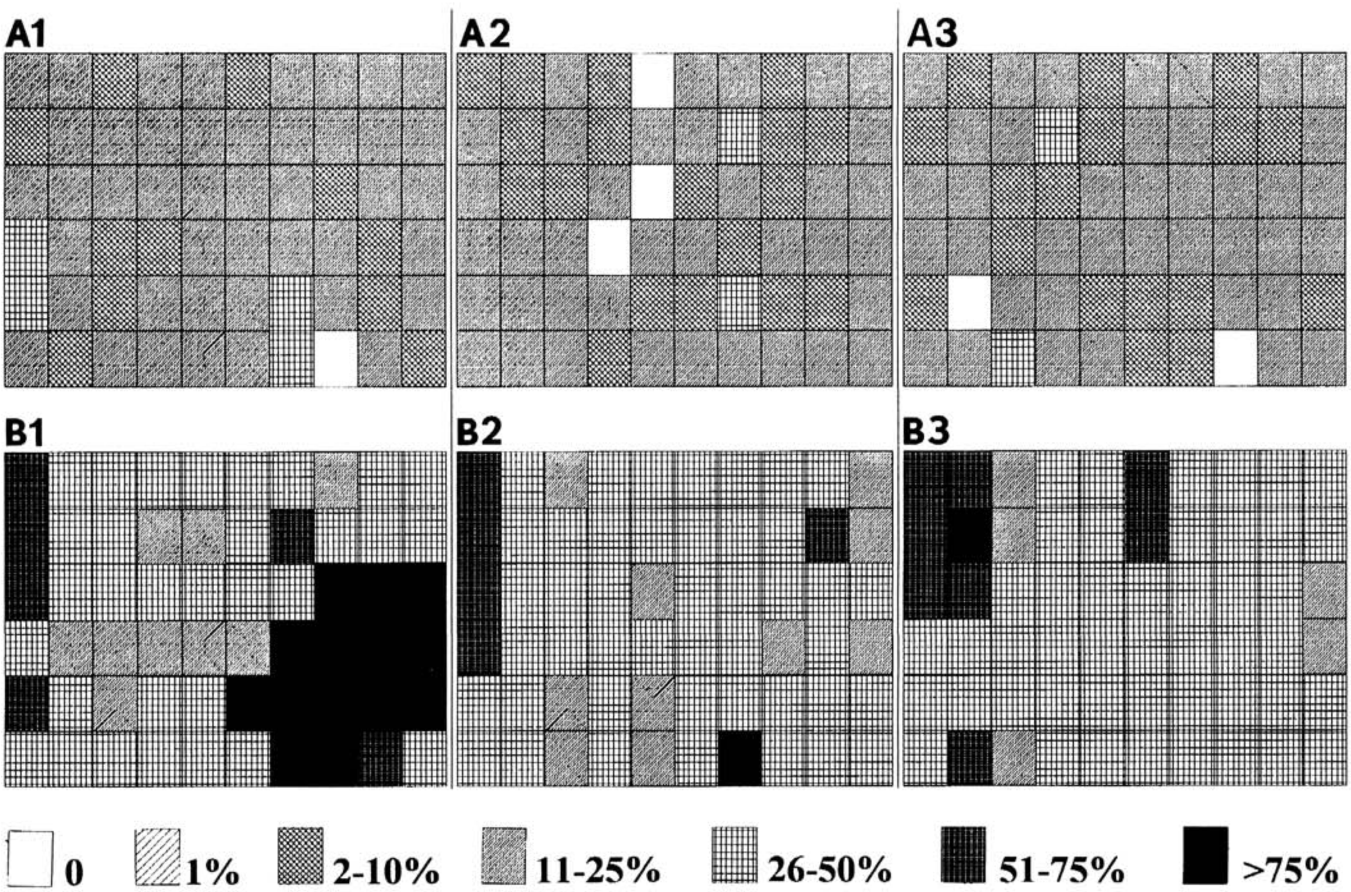

Fig. 3. Spatial distribution maps of lettuce drop incidence (%) in various discrete classes: **(A)** on the first lettuce crop immediately after deep plowing; **(B)** on the second successive lettuce crop after deep plowing. The numbers after **A** and **B** are data from the corresponding replications. Replicate 4 was similar to replicates 1,2, and 3; therefore it was not illustrated.

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