Relationship Between Sclerotial Spatial Pattern and Density of *Sclerotinia minor* and the Incidence of Lettuce Drop

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ABSTRACT

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Soil samples taken from 15 naturally infested field plots near Salinas, CA, indicated inoculum densities at lettuce planting time ranging from 1.66 to 11.35 sclerotia of *Sclerotinia minor* per 100 cm^3 of soil. The spatial pattern of sclerotia within plots was best described by the negative binomial distribution. In all but four plots, variance-to-mean ratios were significantly greater than unity, indicating a clustering of inoculum. Infected plants were mapped weekly in five of the 15 plots, each with about 200 lettuce plants. At harvest, there was a random distribution of healthy and infected plants and no significant plant-to-plant spread had occurred. Disease progress curves constructed for all 15 plots showed no disease for 30 days after planting and a rapid increase during the last 10 days prior to harvest. Disease incidence at harvest was significantly correlated ($r = 0.90$) with the mean number of sclerotia per 100 cm^3 of soil at planting and with the percentage of soil samples with seven or more sclerotia at planting ($r = 0.94$).

In the coastal valleys of California, *Sclerotinia minor* Jagger is the principal cause of lettuce drop, a soft watery crown and root rot of head lettuce (*Lactuca sativa* L.). Until the early 1900s, lettuce drop was reported to be caused exclusively by *Sclerotinia sclerotiorum*, an inoperculate Discomycete that forms large sclerotia (2–20 mm in diameter). In 1900, Smith (22) recorded the occurrence of lettuce drop caused in greenhouses in Massachusetts by a fungus similar to *S. sclerotiorum* but with much smaller sclerotia (0.5–1 mm in diameter). Jagger (17) later reported lettuce drop in New York caused by a similar small sclerotial form of *Sclerotinia* and described a new species, *S. minor* Jagger. Although there has been some controversy as to whether *S. minor* and *S. sclerotiorum* are different species, most workers separate the two species on the basis of sclerotium, ascus, and ascospore sizes, as well as morphological, biochemical, and cytological characteristics (25).

The most important epidemiological differences between the two species involve the conditions required for infection. Sclerotia of *S. sclerotiorum* usually germinate by the production of apothecia that forcibly discharge windborne ascospores. These spores cannot infect directly but must first colonize a food base, which in the case of lettuce is senescent leaves (1). From this initial colonization, the fungus grows into the crown area and kills the plant. Apothecia of *S. minor* have not been found in Salinas Valley lettuce fields and most if not all infections are initiated by eruptively germinating sclerotia (1,4). *S. minor* infects lettuce plants at the base of the stem, on senescent leaves in direct contact with the soil, and on the roots (1). Therefore, the pathogen is strictly soilborne.

The objectives of this research were to determine the influence of inoculum density and spatial pattern on the incidence and progress of disease in naturally infested lettuce fields, and the pattern of diseased plants in the field when infections are initiated by soilborne sclerotia of *S. minor*.

MATERIALS AND METHODS

Soil sampling concept. Marcum (20) found that if sclerotia were located more than 1 cm horizontally from the plant or 5 cm below the soil surface their ability to cause lettuce drop was much reduced. We have determined experimentally that the maximum competence distance (10), ie, the maximum distance a sclerotium of *S. minor* can lie from a root surface and still have chance of causing infection in the presence of biological competition and without an extraneous food base is about 2 cm. Further, sclerotia below 8 cm in the soil rarely if ever germinate (20). From these data we estimated that the competence volume of soil per sclerotium is approximately 100 cm^3. Thus, we believe that a single sclerotium capable of eruptive germination and located within this competence volume around a lettuce root potentially can infect and kill the plant. All soil cores obtained in this study represent this competence volume for sclerotia around a lettuce root, and they were 8 cm in depth by 4 cm in diameter for a total volume of about 100 cm^3.

Fifty soil samples were randomly collected at planting time from between plants in five naturally infested field plots. Two of the plots were direct-seeded with lettuce, and three plots contained transplants. Each plot was 9.15 × 4.27 m (30 × 14 ft) and contained about 200 plants (cultivar Salinas), planted in the spring of 1983 on four double-row beds and thinned to 0.305 m (approximately 1 ft) spacing within and between rows. One hundred soil samples were obtained at planting time from the seed lines in 10 additional field plots (direct-seeded with lettuce) measuring 15.25 × 4.27 m (50 × 14 ft). All field plots were located on mineral soils (Clear Lake clay, Pacheco clay loam, and Salinas clay loam) near Salinas, CA.

Soil sample assays. All soil samples were processed identically and separately. Individual soil samples were stirred in 50 ml of 1% Calgon solution (sodium polymetaphosphate) in 500 ml of water for 2 min in a standard Waring blender assembly operated at low speed. The blended mixture was wet-sieved according to the method of Adams (2). The debris containing sclerotia was washed with distilled water into a 500-ml beaker and swirled to suspend buoyant particles which were poured onto Whatman #1 filter paper in a Büchner funnel. After moisture removal by suction filtration, the residue was air-dried and examined with a stereoscopic microscope (×30). Sclerotia were counted and incubated on washed, moist, quartz sand at room temperature (about 25 C) to determine percent eruptive germination.

The spatial pattern of sclerotia within plots was tested for goodness-of-fit to seven probability distribution models by using the FORTRAN program developed by Gates and Ethridge (6). The models tested were Poisson, Negative Binomial, Thomas Double

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Poisson, Neyman Type A, Poisson-Binomial, Poisson with zeros, and Logarithmic with zeros. The Poisson series describes a random spatial pattern of propagules for which the sample variance is approximately equal to the sample mean. The other models describe clustered spatial patterns for which the sample variance is significantly greater than the sample mean. These models for describing clustered spatial patterns differ in the biological and mathematical assumptions from which they were derived, their degree of skewness (referring to the bilateral asymmetry of the frequency distribution), and their ability to describe the number of peaks in a frequency distribution (5,6,23).

A ratio of sample variance to sample mean was calculated for sclerotia per sample. This test is based on the equality of variance and mean such that, if the ratio approximates unity, agreement with a Poisson series is expected. Significance of the departure from unity was determined by a chi-square statistic (5); a variance-to-mean ratio significantly greater than unity may indicate a clustering or aggregation of sclerotia.

Disease progress. At weekly intervals, the position of each symptomatic plant in five of the field plots was mapped. Two disease records were made at the time of harvest. The first was an assessment of lettuce drop obvious from above-ground symptoms. The second was obtained by digging roots of seemingly healthy plants and isolating *S. minor* from tan lesions if these were present.

Data were analyzed by the ordinary runs analysis (7,8,19) to determine if the pathogen had spread from plant-to-plant and if there were clusters of infected plants. A run in this analysis is a succession of one or more diseased plants that are followed and preceded by one or more healthy plants. The observed number of runs would be less than the expected number if the pathogen had spread from plant-to-plant resulting in clusters of infected plants.

The other 10 field plots measuring 15.25 × 4.27 m (50 × 14 ft) and planted with the cultivar Salinas were monitored for visible disease symptoms at weekly intervals. The number of diseased plants was recorded and a disease progress curve was constructed for each plot.

RESULTS

Inoculum density and distribution. Mean inoculum density of field plots ranged from 1.7 to 11.4 sclerotia per 100 cm^3 soil and most data sets were best described by the negative binomial distribution (Table 1). Variance-to-mean ratios and the dispersion index '*k*' were used in addition to the distributions to facilitate interpretation of the dispersion patterns (Table 1). Probability distribution models and the dispersion index '*k*' were estimated by the Gates and Ethridge program (6). All but four plots showed a variance-to-mean ratio significantly greater than unity, which confirmed clustered or aggregated spatial patterns of inoculum. Plots H, I, and K demonstrated variance-to-mean ratios not significantly different from unity, indicating a random spatial pattern of inoculum. The inoculum in these plots also was characterized by extremely high '*k*' values, which is another indication of an approach towards randomness. The variance-to-mean ratio of Plot E was not significantly different from unity, although the data set was best fit by the negative binomial distribution with a '*k*' value of 5.0. Plot J was not adequately described by any of the probability distributions tested, but the variance-to-mean ratio was significantly greater than unity, indicating a clustered inoculum spatial pattern.

TABLE 1. Indices of dispersion and best fit probability distribution for the sclerotial populations of *Sclerotinia minor* in 15 naturally infested field plots

Plot[a]	$s^2/\bar{x}$[b]	k[c]	Model with best fit[d]	Probability of exceeding χ^2 value
A	1.7	12.4	NB	0.19
B	1.7	17.3	NB	0.62
C	1.9	8.2	NB	0.44
D	1.9	2.2	LWZ	0.75
E	1.4†	5.0	NB	0.95
F	1.9	7.4	NA	0.47
G	1.5	15.6	NB	0.66
H	1.2†	25.7	PB	0.71
I	1.2†	27.9	PB	0.82
J	1.6	9.9	–	–
K	1.3†	21.6	NB	0.59
L	2.7	7.5	NB	0.78
M	1.9	8.8	NB	0.24
N	2.9	5.9	NB	0.50
O	2.4	7.1	NB	0.90

[a] Values for plots A to E are from 50 soil samples per plot. Values for plots F to O are from 100 soil samples per plot.

[b] Variance-to-mean ratio. † = Not significantly ⩾1, hypothesis of randomness not disproved.

[c] Dispersion parameter of the negative binomial distribution.

[d] Discrete frequency distribution models: NB = Negative Binomial, LWZ = Logarithmic with Zeros, NA = Neyman Type A, PB = Poisson Binomial, – = no significant fit to the discrete frequency distributions tested.

The numbers of isolated sclerotia that germinated on quartz sand ranged from 2 to 16%, and these were not correlated with disease incidence ($r = 0.08$). Lack of correlation may indicate a failure of the laboratory procedure to simulate field conditions conducive for breaking dormancy of the sclerotia. However, the mean number of sclerotia isolated per 100 cm^3 of soil was highly significantly correlated with disease incidence (Fig. 1), $r = 0.90$ significant at $P = 0.001$. In addition, linear regression analysis was performed to relate disease incidence and the observed number of soil samples falling into individual frequency classes from 1 to 20 sclerotia per 100 cm^3 soil. The number of soil samples with seven or more sclerotia gave the best correlation with disease incidence, $r = 0.94$ significant at $P = 0.001$ (Fig. 2). Similar correlations were found between the number of soil samples with seven or more sclerotia and disease incidence using expected frequency classes from clustered (negative binomial, $r = 0.92$) or random (Poisson, $r = 0.94$) distribution models.

Disease progress. An example of a mapped field plot demonstrating the position of each diseased plant is presented in Fig. 3. Ordinary runs analysis (8,19) failed to show a departure

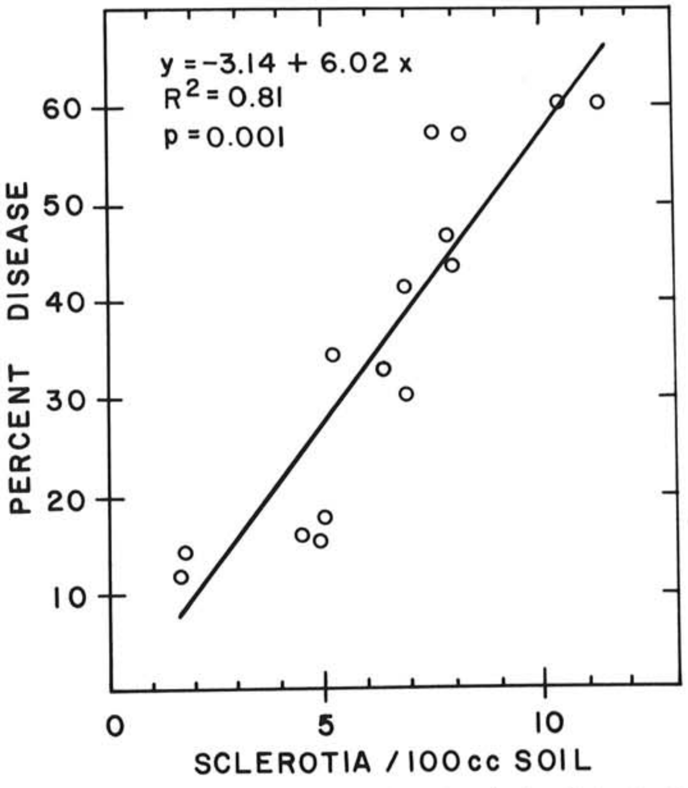

Fig. 1. Relationship between initial mean inoculum density of sclerotia of *Sclerotinia minor* in 15 field plots at planting and disease incidence of lettuce drop at harvest.

from randomness in the spatial pattern of healthy and infected plants (Table 2). In one row (Plot C, row 2) however, a significant aggregation of diseased plants was detected, but this was not sufficient to alter the random pattern of diseased plants in the plot.

Representative disease progress curves are presented in Fig. 4. Lettuce drop was not observed in any field until 30 days or more after planting, regardless of whether the lettuce was direct-seeded or transplanted. Incidence of lettuce drop was less in fields with low inoculum densities than in fields with high inoculum densities. The number of diseased plants increased rapidly during the last 10 days before harvest. In general, the shape of the disease progress curve in all plots was similar.

DISCUSSION

Inoculum patterns best described by the negative binomial distribution have been reported for the microsclerotia of *Cylindrocladium crotalariae* (11,24), propagules of *Rhizoctonia* spp. (21), and plant parasitic nematodes (9). Similarly, our analysis of frequency class data for sclerotia of *S. minor* indicated that the propagules were mostly clumped or clustered in the field and were best described by the negative binomial distribution. Clumping of inoculum in soil probably results when infested lettuce residue is dispersed by tillage and the mechanical action of disking does not disengage the sclerotia and thoroughly mix them in the soil.

According to Southwood (23), the negative binomial distribution can be derived from at least five different hypotheses. One hypothesis, which may be applicable to sessile soilborne pathogens such as *S. minor*, is that of randomly distributed clumps. Clumps of individuals may be distributed at random in the field according to a Poisson series, and the number of individuals in the clumps may be distributed logarithmically. Analysis of these types of data results in description of the data by the negative binomial distribution. Theoretically, if clusters of sclerotia of *S. minor* result in a high probability of successful germination and infection of lettuce, then the spatial pattern of propagule clusters should be the same as that of diseased plants in the field. We have shown that lettuce plants infected with *S. minor* are randomly distributed in the field indicating possible agreement with this theory.

Imolehin and Grogan (16) showed that a single sclerotium located adjacent to the root surface could cause an infection and death of an infected plant. Increased numbers of sclerotia per competence volume, however, increase the probability of successful germination and infection by *S. minor* but do not ensure it. Presumably, the more sclerotia per competence volume, the closer this probability would approach 1.0. However, the probability curve may plateau as sclerotial numbers increase because some sclerotia will participate in multiple infections. We estimated the number of sclerotia of *S. minor* needed per soil sample (of the competence volume) for a high probability of successful germination and infection. The highest correlation was found between the number of soil samples with seven or more sclerotia per 100 cc soil and percent disease incidence ($r = 0.94$). We concluded that seven sclerotia of *S. minor* per competence volume of soil have the highest probability of germinating and causing infection of lettuce with the greatest efficiency of inoculum.

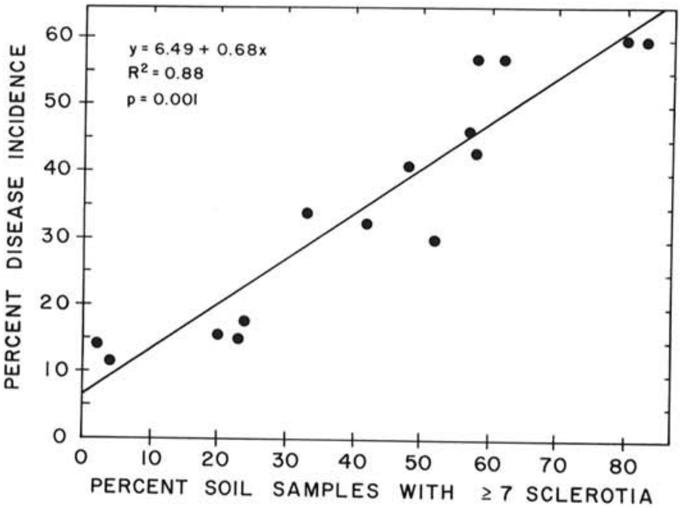

Fig. 2. Relationship between the percentage of soil samples with seven or more sclerotia of *Sclerotinia minor* at planting from 15 field plots and disease incidence of lettuce drop at harvest.

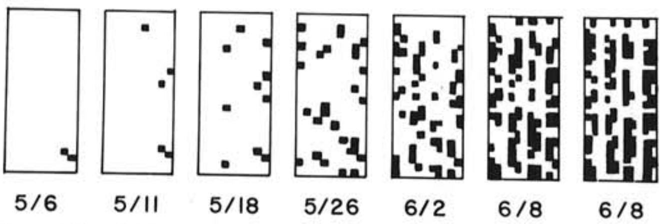

Fig. 3. Map of field plot (Field B) showing the position of each lettuce plant infected with *Sclerotinia minor* at intervals from 6 May to 8 June 1983. Maps are of four double-row beds planted to lettuce. Furrows are indicated by vertical white spaces between beds, and diseased plants by dark squares. Six maps on left indicate plants with above-ground symptoms. Map on right indicates plants with above-ground symptoms or root lesions characteristic of *S. minor*.

TABLE 2. Results of ordinary runs analysis to determine the pattern of lettuce plants infected by *Sclerotinia minor*

Plot	Observed runs	Expected runs	Standard deviation	Z^a	Pattern[b]
A	91	93	6.7	−0.2	random
B	97	91	6.6	1.0	random
C	75	83	6.0	−1.3	random
D	49	46	3.6	0.9	random
E	47	46	3.3	0.3	random

[a] Standardized variable; large negative values indicate clustering.

[b] Analysis combines adjacent rows. Analysis of individual rows demonstrated random patterns, except for Row 2 in Plot C where a significant aggregation of diseased plants was detected.

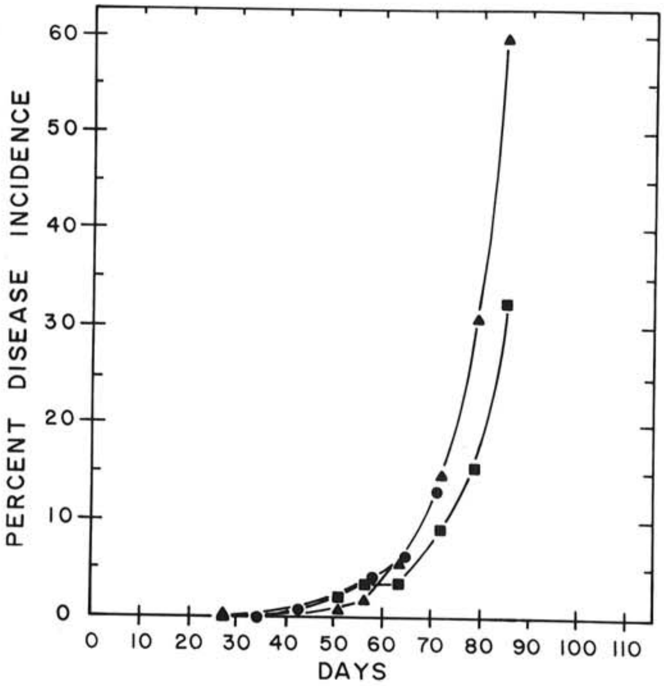

Fig. 4. Representative disease progress curves for lettuce drop at three initial inoculum levels of *Sclerotinia minor* at planting. Symbols: ▲, a field with a mean of 10.48 sclerotia per 100 cm³ of soil; ■, a field with a mean of 6.36 sclerotia per 100 cm³ of soil; ●, a field with a mean of 1.84 sclerotia per 100 cm³ of soil.

We also determined that either a random (Poisson) or clustered (negative binomial) probability distribution could be used to predict disease incidence. In the Poisson series, the frequency distribution is very asymmetrical at low means and approaches normality as the mean increases. The negative binomial distribution is asymmetrical for a large range of arithmetic means when '*k*' is small but approaches normality when '*k*' increases and the mean is large. Thus, either model can be used to predict lettuce drop because both distributions possess similar shapes (approaching normality) for the frequency distribution of numbers of sclerotia of *S. minor* per competence volume found in naturally infested fields. The comparable symmetry of the distributions at the higher means also explains why the arithmetic mean can be used to predict disease incidence. We did not attempt to predict disease with the other five frequency distributions tested because of their poor fit to the observed data sets.

The value of '*k*' of the negative binomial distribution gives a measure of dispersion, and the smaller its value the greater the extent of aggregation. Large '*k*' values (over about 8) indicate that the distribution approaches randomness (5). Theoretically, the degree of aggregation of a pathogen population should influence disease incidence. However, we found no correlation between disease incidence and the '*k*' value ($r = 0.14$) or the variance-to-mean ratio ($r = 0.62$). Thus, we found no effect on disease incidence associated with the dispersion statistics of the pathogen populations. The most useful mathematical parameter for characterizing disease incidence was the arithmetic mean inoculum density of the pathogen or the proportion of soil samples that contained seven or more sclerotia.

Adams and Tate (3) examined the relationship between inoculum density of *S. minor* and lettuce drop incidence in greenhouse studies using field soil infested with laboratory-grown inoculum. They reported that for 20% infection, 31 sclerotia per 100 g of soil were required. These high levels of inoculum required to produce a relatively low incidence of disease suggest that most of their sclerotia did not cause infection. Our data is perhaps better suited for predicting disease in the field because the experiments were conducted in naturally infested fields, where the resident sclerotia are stimulated to germinate by a combination of natural processes (3,4).

Although disease progress curves for lettuce drop were curvilinear upward, linear regressions of disease incidence and time using data transformations were not necessary because biological interpretations could be made from the nonlinearized data. Our disease progress curves confirm field observations that most drop caused in lettuce by *S. minor* becomes evident after head formation and near maturity (1,20). We did not observe any disease symptoms in the field until 30 days or more after planting. Marcum (20) found that infections of lower leaves and crown tissues resulted in plant death within 1 wk, below-ground infections of the tap root resulted in death within 7–14 days, and infections of secondary roots usually required 3 wk or longer before drop was evident. Slow development of subterranean infections may explain in part the delay in disease expression observed. Further, mature lower leaves in contact with the soil modify the soil microclimate by making it wetter and cooler and thus more conducive for sclerotial germination and infection of mature plants. Huisman (15) proposed that as roots grow they come in contact with increasing numbers of propagules, thus making root growth an important component in the progress of diseases caused by soilborne pathogens. However, since *S. minor* does not germinate below 8 cm and lettuce possesses a tap root system, we believe that lower leaf expansion is more important for exposing additional susceptible tissue to increasing numbers of sclerotia.

Jarvis and Hawthorne (18) analyzed disease progress curves of lettuce drop in New Zealand. They suggested that primary infections were attributable to airborne ascospores and reported significant plant-to-plant spread. They concluded later, however, that ascospores were not the main type of inoculum, and that mycelium from germinating sclerotia was the principal inoculum in New Zealand (12,13). We presented evidence to show that the pattern of infected plants in the field was random when infections were initiated by eruptively germinating sclerotia and no significant plant-to-plant spread had occurred. Huang and Hoes (14) found significant plant-to-plant spread of *S. sclerotiorum* in sunflower stands, and reported that plant spacing was an important factor in wilt development. This may be due to the greater volume of sunflower roots, closer plant spacing, and a more rapid and extensive growth of *Sclerotinia* in infected sunflower. We also observed plant-to-plant spread of *S. minor* in sunflower windbreaks in the Salinas Valley (*unpublished*).

The common cultural practice in California is to thin lettuce plants to approximately 1 ft (0.305 m) spacing within and between rows. At this spacing, lettuce heads and roots do not physically touch neighboring heads and roots, but at maturity, lower leaves of neighboring plants frequently overlap. Occasionally, mycelium was observed growing from lower leaf infections of mature lettuce plants that were touching neighboring healthy plants. However, plant-to-plant spread of *S. minor* apparently plays a minimal role in the epidemiology of lettuce drop.

LITERATURE CITED

1. Abawi, G. S., and Grogan, R. G. 1979. Epidemiology of diseases caused by *Sclerotinia* species. Phytopathology 69:899-904.
2. Adams, P. B. 1979. A rapid method for quantitative isolation of sclerotia of *Sclerotinia minor* and *Sclerotium cepivorum* from soil. Plant Dis. Rep. 63:349-351.
3. Adams, P. B., and Tate, C. J. 1975. Factors affecting lettuce drop caused by *Sclerotinia sclerotiorum*. Plant Dis. Rep. 59:140-143.
4. Adams, P. B., and Tate, C. J. 1976. Mycelial germination of *Sclerotinia sclerotiorum* on soil. Plant Dis. Rep. 60:515-518.
5. Elliott, J. M. 1977. Some methods for the statistical analysis of samples of benthic invertebrates. 2nd ed. Scientific Publ. No. 25. Freshwater Biol. Assoc. The Ferry House, Ambleside, Cumbria, England. 160 pp.
6. Gates, C. E., and Ethridge, F. G. 1972. A generalized set of discrete frequency distributions with FORTRAN program. Math. Geol. 4:1-24.
7. Gibbons, J. D. 1971. Nonparametric Statistical Inference. McGraw-Hill, New York. 206 pp.
8. Gibbons, J. D. 1976. Nonparametric Methods for Quantitative Analysis. Holt, Rinehart, and Winston, New York. 463 pp.
9. Goodell, P., and Ferris, H. 1980. Plant-parasitic nematode distributions in an alfalfa field. J. Nematol. 12:136-141.
10. Grogan, R. G., Sall, M. A., and Punja, Z. K. 1980. Concepts for modeling root infection by soilborne fungi. Phytopathology 70:361-363.
11. Hau, F. C., Campbell, C. L., and Beute, M. K. 1982. Inoculum distribution and sampling methods for *Cylindrocladium crotalariae* in a peanut field. Plant Dis. 66:568-571.
12. Hawthorne, B. T. 1974. *Sclerotinia minor* on lettuce: Effect of plant growth on susceptibility to infection. N. Z. J. Agric. Res. 17:387-392.
13. Hawthorne, B. T. 1975. Effect of mulching on the incidence of *Sclerotinia minor* on lettuce. N. Z. J. Exp. Agric. 3:273-274.
14. Huang, H. C., and Hoes, J. A. 1980. Importance of plant spacing and sclerotial position to development of Sclerotinia wilt of sunflower. Plant Dis. 64:81-84.
15. Huisman, O. C. 1982. Interrelations of root growth dynamics to epidemiology of root-invading fungi. Annu. Rev. Phytopathol. 20:303-327.
16. Imolehin, E. D., and Grogan, R. G. 1980. Factors affecting survival of sclerotia, and effects of inoculum density, relative position and distance of sclerotia from the host on infection of lettuce by *Sclerotinia minor*. Phytopathology 70:1162-1167.
17. Jagger, J. C. 1920. *Sclerotinia minor* n. sp., the cause of a decay of lettuce, celery, and other crops. J. Agric. Res. 20:331-334.
18. Jarvis, W. R., and Hawthorne, B. T. 1972. *Sclerotinia minor* on lettuce: Progress of an epidemic. Ann. Appl. Biol. 70:207-214.
19. Madden, L. V., Louie, R., Abt, J. J., and Knoke, J. K. 1982. Evaluation of tests for randomness of infected plants. Phytopathology 72:195-198.
20. Marcum, D. B. 1980. Studies on the biology and control of *Sclerotinia minor* on lettuce. Ph.D. thesis. University of California, Davis. 50 pp.
21. Martin, S. B., Campbell, C. L., and Lucas, L. T. 1983. Horizontal distribution and characterization of *Rhizoctonia* spp. in tall fescue turf. Phytopathology 73:1064-1068.
22. Smith, R. E. 1900. *Botrytis* and *Sclerotinia*: Their relation to certain

plant diseases and to each other. Bot. Gaz. 29:369-407.
23. Southwood, T. R. E. 1978. Ecological Methods. 2nd ed. Chapman and Hall, London. 524 pp.
24. Taylor, J. D., Griffin, G. J., and Garren, K. H. 1981. Inoculum pattern, inoculum density-disease incidence relationships, and population fluctuations of *Cylindrocladium crotalariae* microsclerotia in peanut field soil. Phytopathology 71:1297-1302.
25. Willets, H. J., and Wong, J. A. L. 1980. The biology of *Sclerotinia sclerotiorum*, *S. trifoliorum*, and *S. minor* with emphasis on specific nomenclature. Bot. Rev. 46:101-165.