Influence of Green Manure Crops and Lettuce on Sclerotial Populations of *Sclerotinia minor*

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ABSTRACT

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Continuous lettuce cropping resulted in the greatest mean inoculum density and proportion of soil samples with seven or more sclerotia of *Sclerotinia minor* during the 1-yr test period. In most plots, the frequency distribution of sclerotia among soil samples was best described by the negative binomial distribution. Of 50 data sets, only five had variance-to-mean ratios not greater than unity. Disease incidence in lettuce was greatest in plots previously planted to lettuce, vetch, or vetch/rye mix in the furrow-irrigated field and in plots previously planted to lettuce or vetch in the sprinkler-irrigated field.

Sclerotinia minor Jagger is the principal cause of lettuce drop, a soft watery crown and root rot of head lettuce (*Lactuca sativa* L.) that occurs frequently in the Salinas Valley of California. Most infections are initiated by eruptively germinating sclerotia that infect the base of the stem, senescent leaves in direct contact with the soil, or roots within about 7 cm of the soil surface (1). Soilborne sclerotia are the principal inocula and function as survival structures (1). Natural inoculum densities of sclerotia of *S. minor* found in previous surveys of lettuce fields ranged from 0 to 11 sclerotia per 100 g of mineral soil from the Salinas Valley of California (2,4,9), from 0 to 23 sclerotia per 100 g of New Jersey mineral soil (2), and 16 to 82 sclerotia per 100 g of New York muck soil (2).

Mathematical descriptions have been used to characterize the distribution of soilborne propagules of *Rhizoctonia* spp. (10), microsclerotia of *Cylindrocladium crotalariae* (8,11,12), and sclerotia of *S. minor* in naturally infested soils (4,5). The clumped microsclerotial pattern of *C. crotalariae* (12) was interpreted to contribute to low slope values obtained in $\log_{10}$-$\log_{10}$ plots of inoculum density vs. estimated infections. In a previous study, we found that the mean number of sclerotia per 100 cm^3 of soil and the number of soil samples with seven or more sclerotia were significantly correlated with disease incidence ($r = 0.90$ and $r = 0.94$, respectively).

Continuous cropping of lettuce in the Salinas Valley has resulted in increased losses from *S. minor* and decreased soil fertility, soil organic matter, and soil structure. Cultural practices to alleviate these problems have included green manuring with cover crops that are grown during the fall and early winter and disked during midwinter. Because of the wide host range of *S. minor* (3,13), it was important to determine the relative susceptibility of potential green manure crops to *S. minor*. The objectives of this study were to determine the relative effects of green manure crops and continuous lettuce cropping on sclerotial distribution and density in naturally infested soil and the resulting effect on disease incidence in the following lettuce crop.

MATERIALS AND METHODS

Cover crop and lettuce production. Purple vetch (*Vicia atropurpurea* Desf.), rye (*Secale cereale* L.), a 50/50 vetch-rye mix, a sorghum-sudangrass hybrid (Funk's G-88F), and lettuce (*Lactuca sativa* L. 'Salinas') were planted in two fields naturally infested with *S. minor*. Both fields were located near Salinas, CA; one field was sprinkler-irrigated (Pacheco clay loam) and the other was furrow-irrigated (Salinas clay loam). The sprinkler-irrigated field was planted on 22 June 1982, and the furrow-irrigated field, on 7 July 1982. Treatments were replicated four times in both fields in a completely random design. Each replicate plot was 50 ft (15.25 m) long and eight beds wide (8.13 m) with a surrounding 10-ft (3.05-m) fallow border strip.

Lettuce was planted by the grower with conventional equipment on raised 40-in. (101.6-cm) double-row beds and thinned to about 1-ft (0.305-m) spacing within and between rows. Cover crops were planted with an Earthway Precision Garden Seeder model 1001 B (Earthway Products Incorporated, Bristol, IN) on raised 40-in. (101.6-cm) triple-row beds with 2-in. (5.08-cm) spacing between plants. At intervals throughout the trial period, the cover crops were examined for disease symptoms and isolations were made from lesions that appeared to be due to *S. minor*.

The lettuce replicates were harvested by the grower with conventional equipment on 14 September 1982 in the furrow-irrigated field and on 30 August 1982 in the sprinkler-irrigated field. All treatments were disked on 22 September 1982 in the furrow-irrigated field and on 19 September 1982 in the sprinkler-irrigated field. Disking consisted of four passes parallel to the beds and two diagonal passes through the field with a double disk that had 24-in. (60.96-cm) seriate disk blades, 25–30 degrees off the horizontal. In the spring of 1983, lettuce was planted by the grower with conventional equipment in all replicate plots. The furrow-irrigated field was planted to lettuce on 15 March 1983 and harvested on 8 June 1983, and the sprinkler-irrigated field was planted on 6 May 1983 and harvested on 13 July 1983.

Henceforth, the cropping sequence treatments are abbreviated as follows: lettuce-lettuce (LL), vetch-lettuce (VL), vetch/rye mix-lettuce (VRL), rye-lettuce (RL), and sudangrass-lettuce (SL).

Soil sampling procedure. All soil samples were cores 8 cm deep and 4 cm in diameter with a total volume of about 100 cm^3 (4). Each soil core was bagged and processed individually. Twenty-five soil samples were taken randomly from the seed lines of the middle four rows of each replicate plot at five sampling intervals: planting in 1982, harvest in 1982 (before disking), 3–4 mo after disking, planting of lettuce in 1983, and harvest in 1983 (before disking). Soil samples obtained at harvest were taken between plants. The number of sclerotia per soil sample was determined by wet sieving (2,4).

Statistical analyses of soil samples. The spatial pattern of sclerotia within plots was tested for goodness of fit to seven statistical probability distribution models using a FORTRAN program developed by Gates and Ethridge (7). The models tested were Poisson, negative binomial, Thomas double Poisson, Neyman type A, Poisson binomial, Poisson with zeros, and logarithmic with zeros. A ratio of sample variance to sample mean was calculated for sclerotia per sample. The

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significance of the departure from unity was determined by a chi-square statistic (6).

Waller and Duncan's exact Bayesian k-ratio LSD ($P = 0.05$) was used to determine significant differences in lettuce drop incidence.

RESULTS

Disease incidence in green manure crops and lettuce, 1982. Neither rye nor the sorghum-sudangrass hybrid showed symptoms of disease caused by *S. minor* throughout the growing season. Purple vetch developed a dense canopy that covered the beds and furrows, and *S. minor* colonized both senescent debris and living tissue near the soil surface. Symptoms in vetch were not evident until 40 days after planting in the furrow-irrigated field and 50 days after planting in the sprinkler-irrigated field. Disease in vetch apparently was more prevalent in the furrow-irrigated field, but disease incidence in vetch was not recorded quantitatively in either field. Disease incidence in lettuce at harvest was 45.6% in the furrow-irrigated field, which had a mean inoculum density of 5.8 sclerotia per 100 cm^3 of soil at planting, and 34.1% in the sprinkler-irrigated field, which had a mean inoculum density of 4.8 sclerotia per 100 cm^3 of soil at planting.

Furrow-irrigated field. From planting (summer 1982) to postdisking (winter 1983), the mean number of sclerotia per 100 cm^3 of soil and the proportion of soil samples with seven or more sclerotia increased in all cropping treatments (Fig. 1). Conversely, sclerotial numbers decreased after the postdisking (winter 1983) period. The mean inoculum density and the proportion of soil samples with seven or more sclerotia in the LL plots was greater than in all other plots at planting (spring 1983) and harvest (summer 1983).

In most data sets, the frequency distribution of sclerotia among soil samples was best described by the negative binomial distribution, and variance-to-mean ratios for all data sets were greater than unity (Table 1). We interpreted this to mean that a clustering of inoculum occurred in this field in all cropping sequences. Disease incidence in lettuce at harvest (1983) was greatest in plots previously planted to lettuce, vetch, or vetch/rye mix (Table 1).

Sprinkler-irrigated field. From planting (summer 1982) to harvest (fall 1982), the mean inoculum density and the proportion of soil samples with seven or more sclerotia increased in all treatments and continued to increase through the postdisking (winter 1982) period in only the LL sequence (Fig. 2). Sclerotial numbers decreased from postdisking (winter 1982) to harvest (summer 1983) in all plots. At planting (spring 1983) and harvest (summer 1983), sclerotial numbers were greatest in the LL cropping sequence.

In this field, the frequency distribution of sclerotia among soil samples was most commonly described by the negative binomial distribution or other clustered distribution models (Table 2). Five data sets (of 25 total) had variance-to-mean ratios not greater than unity, indicating a random spatial pattern of inoculum. Variance-to-mean ratios less than unity occurred at planting (spring 1983) in the VL, VRL, and SL cropping sequences and at harvest (summer 1983) in the VRL and SL cropping sequences. Disease incidence in lettuce at harvest (summer 1983) was greater among plots previously planted to lettuce or vetch (Table 2).

DISCUSSION

An interesting result in this study was the increase in mean inoculum density from planting to harvest (1982) in all cropping sequences. Because soil samples were taken between plants, it is unlikely that the samples contained colonized lettuce tissue from the current crop. Inasmuch as these increases occurred in

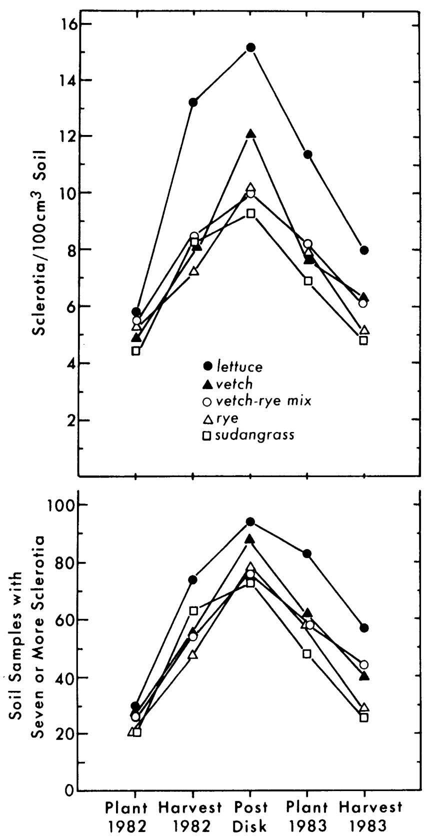

Fig. 1. Mean inoculum density and total number of soil samples with seven or more sclerotia of *Sclerotinia minor* in a furrow-irrigated field.

Table 1. Disease incidence, indices of dispersion, and best fit probability distribution for the sclerotial populations of *Sclerotinia minor* based on 100 soil samples per treatment at each sampled date in a furrow-irrigated field

Treatment	Sampling date	Lettuce drop incidence (%)[a]	$s^2/\bar{x}$[b]	k[c]	Best fit and probability of exceeding X^2[d]	
Lettuce	Planting, Jul. 1982		5.0	3.0	NB	0.50
	Harvest, Sept. 1982	45.6 y	9.3	···	–	–
	Postdisk, Jan. 1983		2.5	9.9	NB	0.77
Lettuce	Planting, Mar. 1983		2.7	7.5	NB	0.78
	Harvest Jun. 1983	60.3 x	2.1	8.1	NB	0.78
Vetch	Planting, Jul. 1982		2.1	5.1	NB	0.42
	Harvest, Sept. 1982		2.8	5.0	NB	0.60
	Postdisk, Jan. 1983		1.7	18.1	NB	0.75
Lettuce	Planting, Mar. 1983		1.9	8.8	NB	0.24
	Harvest, Jun. 1983	57.4 x	2.2	6.0	NB	0.21
Vetch/rye mix	Planting, Jul. 1982		5.6	2.9	NB	0.41
	Harvest, Sept. 1982		5.1	3.1	NB	0.55
	Postdisk, Jan. 1983		1.9	12.0	NB	0.30
Lettuce	Planting, Mar. 1983		2.9	5.9	NB	0.50
	Harvest, Jun. 1983	56.9 x	1.5	13.9	NB	0.12
Rye	Planting, Jul. 1982		3.7	···	–	–
	Harvest, Sept. 1982		4.0	4.1	NB	0.26
	Postdisk, Jan. 1983		1.7	15.0	NB	0.67
Lettuce	Planting, Mar. 1983		2.4	7.1	NB	0.90
	Harvest, Jun. 1983	46.4 y	1.5	11.2	NB	0.65
Sudangrass	Planting, Jul. 1982		1.8	···	TDP	0.95
	Harvest, Sept. 1982		2.2	6.9	NB	0.56
	Postdisk, Jan. 1983		1.7	13.7	NB	0.48
Lettuce	Planting, Mar. 1983		1.5	15.6	NB	0.65
	Harvest, Jun. 1983	41.2 y	1.6	8.0	NB	0.63

[a] Means in a column followed by the same letter do not differ significantly (Waller-Duncan's exact Bayesian k-ratio LSD, $P = 0.05$)

[b] Variance-to-mean ratio.

[c] Dispersion parameter of the negative binomial distribution.

[d] NB = negative binomial, TDP = Thomas double Poisson, and – = no significant fit to the discrete frequency distributions tested.

soil cropped to both susceptible (lettuce, vetch, and vetch/rye mix) and nonsusceptible crops (rye and sudangrass), we believe that the increases probably were due to formation of sclerotia on infected residue of the previous lettuce crop, which was disked about 1 mo before planting in both fields. This hypothesis is in agreement with Imolehin and Grogan (9), who found that sclerotia of *S. minor* formed on incipiently infected lettuce tissue buried 0–20 cm deep in the field.

From planting in the summer of 1982 to harvest in the summer of 1983, there were no large net increases in mean inoculum density within a cropping sequence in either the sprinkler- or furrow-irrigated field, although means were slightly higher in the LL treatment than in the others. Apparently, susceptible crops such as lettuce and vetch temporarily result in large increases in inoculum density, but rapid attrition of sclerotia ultimately results in nearly equal populations. These data support the conclusion of Imolehin and Grogan (9) that most sclerotia formed on infected lettuce tissue are ephemeral and the observation that consistent and relatively low inoculum densities are maintained in most Salinas Valley lettuce fields despite the growing of two crops of lettuce per year in most fields.

The sclerotial populations of *S. minor* in the sprinkler-irrigated field generally did not fluctuate as much in response to the cropping sequences as they did in the furrow-irrigated field. Mean inoculum density and total number of soil samples with seven or more sclerotia were always greater in the furrow-irrigated field, and consequently, overall disease incidence also was considerably greater. Better water management from use of sprinkler irrigation and physical, chemical, and biological differences in soil type perhaps restricted increase of the sclerotial populations.

There was indirect evidence of natural biological control of sclerotia of *S. minor*, especially in the sprinkler-irrigated field. After the winter fallow period, the sclerotial population decreased, variance-to-mean ratios were not significantly greater than one, and k values increased in most treatments. Thus, as the population density decreased, the extent of clumping decreased, which indicates that most of the formerly clumped sclerotia had rotted leaving smaller clumps, single sclerotia distributed more or less at random, or zero sclerotia in the competence volumes sampled.

Imolehin and Grogan (9) found that incidence of lettuce drop was significantly correlated with both the inoculum density of competent sclerotia ($r = 0.89$) and total sclerotia ($r = 0.78$). In another study (4), we also found that the mean number of sclerotia per 100 cm^3 of soil and the number of soil samples with seven or more sclerotia were correlated with disease incidence ($r = 0.90$ and $r = 0.94$, respectively). In this study, fields previously cropped to lettuce were most effective in increasing the inoculum levels for the fall planting of lettuce. In general, lettuce drop was maintained by the LL cropping sequence and other susceptible crops such as vetch may also contribute to increased disease incidence. Single-season crop rotations were ineffective for control of lettuce drop.

The portion of sclerotia surviving from one growing season to the next and the wide host range of *S. minor* (3,13) maintain stable sclerotial populations. We have observed *S. minor* infecting the following weeds in abandoned Salinas Valley lettuce fields (H. R. Dillard and R. G. Grogan, *unpublished*): pigweed (*Amaranthus retroflexus* L.), pineapple weed (*Matricaria matricariodes* (Less.) Porter), chickweed (*Stellaria media* (L.) Vill.), purslane (*Portulaca oleracea* L.), and shepherd's purse (*Capsella bursa-pastoris* (L.) Medic.). Thus, colonization of susceptible weeds by *S. minor* could sustain sclerotial populations in the absence of susceptible agronomic crops.

Research on the effects of multiple-season cropping sequences on sclerotial populations of *S. minor* is needed to facilitate biological control efforts and to predict the incidence of lettuce drop.

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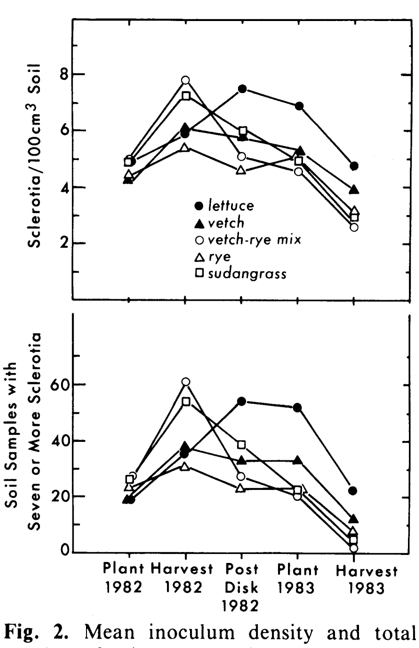

Fig. 2. Mean inoculum density and total number of soil samples with seven or more sclerotia of *Sclerotinia minor* in a sprinkler-irrigated field.

Table 2. Disease incidence, indices of dispersion, and best fit probability distribution for the sclerotial populations of *Sclerotinia minor* based on 100 soil samples per treatment at each sampled date in a sprinkler-irrigated field

Treatment	Sampling date	Lettuce drop incidence (%)[a]	$s^2/\bar{x}$[b]	k[c]	Best fit and probability of exceeding X^2[d]	
Lettuce	Planting, Jun. 1982		2.7	4.0	NB	0.24
	Harvest, Aug. 1982	34.1 x	2.2	5.3	NB	0.25
	Postdisk, Dec. 1982		2.7	···	NA	0.55
Lettuce	Planting, May 1983		1.9	···	NA	0.47
	Harvest, Jul. 1983	30.2 x	1.6	···	PB	0.73
Vetch	Planting, Jun. 1982		2.3	3.5	NB	0.78
	Harvest, Aug. 1982		2.1	5.7	NB	0.67
	Postdisk, Dec. 1982		5.1	3.2	NB	0.20
Lettuce	Planting, May 1983		1.2	···	PB	0.71
	Harvest, Jul. 1983	34.4 x	1.4	11.8	NB	0.94
Vetch/rye mix	Planting, Jun. 1982		3.2	3.1	NB	0.24
	Harvest, Aug. 1982		1.9	8.4	NB	0.84
	Postdisk, Dec. 1982		1.7	7.9	NB	0.52
Lettuce	Planting, May 1983		1.2	···	PB	0.82
	Harvest, Jul. 1983	15.8 y	1.0	···	P	0.78
Rye	Planting, Jun. 1982		2.4	3.3	NB	0.62
	Harvest, Aug. 1982		2.7	3.9	NB	0.16
	Postdisk, Dec. 1982		1.5	···	PB	0.66
Lettuce	Planting, May 1983		1.6	···	–	–
	Harvest, Jul. 1983	17.6 y	1.7	4.8	NB	0.98
Sudangrass	Planting, Jun. 1982		2.1	···	TDP	0.88
	Harvest, Aug. 1982		3.3	3.4	NB	0.40
	Postdisk, Dec. 1982		1.8	7.6	NB	0.58
Lettuce	Planting, May 1983		1.3	21.6	NB	0.59
	Harvest, Jul. 1983	15.2 y	1.1	···	P	0.64

[a] Means in a column followed by the same letter do not differ significantly (Waller-Duncan's exact Bayesian k-ratio LSD, $P = 0.05$)

[b] Variance-to-mean ratio.

[c] Dispersion parameter of the negative binomial distribution.

[d] NB = negative binomial, NA = Neyman type A, PB = Poisson binomial, P = Poisson, TDP = Thomas double Poisson, and – = no significant fit to the discrete frequency distributions tested.

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