Inoculum Density of *Sclerotium cepivorum* and the Incidence of White Rot of Onion and Garlic

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ABSTRACT

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An improved soil assay for natural populations of *Sclerotium cepivorum* detected as few as 0.001 sclerotia per gram of soil. Survey data indicated that natural populations of sclerotia remained between 0.001 and 1.0 sclerotia per gram of soil for up to 8 yr, but decreased to between 0.001 and 0.01 sclerotia per gram of soil 10–15 yr after *Allium* spp. crop plants were grown. No sclerotia were recovered from soil in two fields in which allium crops were grown 17–20 yr prior to assay. Nearly all intact sclerotia recovered from infested soils germinated in soil when stimulated by garlic extract. Sclerotia which germinated were infective. A rapid, selective viability test was developed based on the characteristic growth of *S. cepivorum* on water agar. Disease incidence was dependent on preemergence inoculum density in uniform inoculum-density trials in different soils from several areas of California. Preemergence populations of $\leq$0.001, 0.001–0.01, 0.01–0.1 and $\geq$0.1 sclerotia per gram of soil resulted in $\leq$10%, 10–85%, 85–100% and 100% incidence of disease in onion and garlic plants by harvest, respectively. At inoculum densities greater than 1.0 sclerotia per gram of soil most plants were killed soon after emergence. At lower inoculum densities, distinct clusters of plants became diseased as the pathogen spread from plant to plant. Disease loci appeared progressively later and with reduced frequency with decreasing inoculum density. Data from a naturally infested field were similar to those obtained from controlled experiments. Sclerotia populations declined, presumably due to germination, during the season in soil planted with onion or garlic. In plots infested at preemergence with as few as 0.00004 and as many as 10.0 sclerotia per gram of soil, between 0.4 and 1.0 sclerotia per gram of soil were recovered 6 mo after harvest; up to 9.2 sclerotia per gram of soil were recovered 6 mo after harvest from plots infested between 0.001–0.01 sclerotia per gram of soil at preemergence.

Additional key words: *Allium cepa*, *A. sativum*, yield loss, crop rotation, inoculum potential, fungistasis.

White rot disease of onion (*Allium cepa* L.), garlic (*A. sativum* L.) and other *Allium* spp. results from attack by the soilborne fungus *Sclerotium cepivorum* Berk. Roots and leaf sheaths are penetrated directly and the invading hyphae advance inter- and intracellularly through underground host tissue (1). Primary inoculum of the pathogen consists of spherical, small (0.3–0.6 mm in diameter), black sclerotia produced on infected tissue of *Allium* spp.

White rot first occurs in small isolated areas with insignificant plant loss in fields previously free of the disease; however, plant loss usually is much greater when these fields are replanted. Presumably, abundant sclerotia produced on decaying plants in isolated locations become distributed across fields and through the soil profile in subsequent years. Soils remain infested for many years (5,18) even though *S. cepivorum* does not infect plants other than *Allium* spp. (6,12) or grow competitively as a saprophyte (12,22); persistence apparently is due to survival of individual sclerotia (6).

Sclerotia of *S. cepivorum* are normally held in fungistatic dormancy in nonsterile soil, but germinate in specific response to *Allium* spp. (7,8,10,16). However, sclerotia produced in vitro undergo a period of constitutive dormancy of $\geq$ 1 mo in field soil before stimulation of germination occurs (7). In experiments

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described here, sclerotia produced in culture were incorporated into field soil well in advance of planting to ensure that constitutive dormancy had terminated by the time plants began to grow.

The effect of inoculum density on disease incidence under field conditions has not been studied. Adams and Papavizas (2), in greenhouse experiments, suggested that the amount of white rot disease may be related to inoculum density, but the range used (5–200 sclerotia per gram of soil) was much higher than natural levels. Natural populations of 0 to 0.3 sclerotia per gram of soil were measured by McCain (17) and Papavizas (19), but the lower limit of both wet sieving assays used was 0.1 sclerotia per gram of soil. Ali et al (4) infested fungicide trial areas with approximately 0.1 sclerotia per gram of soil with inoculum taken directly from culture and reported 95–100% infection of onion bulbs by harvest in nontreated control plots.

Scott (23) and Crowe and Hall (12) reported that mycelium of *S. cepivorum* spread from plant to plant. The extent of plant-to-plant spread is an important consideration relating to effect of inoculum density on disease incidence because such spread could obscure the effect of increased inoculum density. In the experiments reported here, inoculum densities, distribution of sclerotia in fields, and viability of sclerotia from naturally infested soil were studied and the effect of inoculum densities on incidence of white rot was determined.

MATERIALS AND METHODS

Soil collection. Soil from naturally infested fields was sampled to determine inoculum density, distribution of sclerotia in the field and viability of sclerotia. Samples were collected from 15 fields with known histories of white rot disease. Ten to twenty 300-ml soil samples from the upper 10 cm of soil along each diagonal of a diamond pattern were combined into two or more composite samples for assay for sclerotia. To determine the within-sample variability, samples from selected areas within the sampling pattern were resampled and analyzed without compositing. In uniform inoculum-density plots, two composite samples of 20 soil cores were collected with a 2.5-cm-diameter soil sampling tube and assayed for sclerotia.

Soil assay. Each composite soil sample was screened through a 1-cm screen to remove larger particles. Clods were forced through the screen or coarsely ground and added to the screened fraction. Soil was mixed by tumbling for 2 min in plastic bags or in a large rotating drum. Samples were dried in open plastic bags at room temperature (21 C) until weight loss stabilized or were assayed without drying. When moist soil was assayed, inoculum density data were converted to an air-dry basis determined from the moisture loss in the remainder of the sample after air-drying. Inoculum density in soil sampled from areas planted with garlic or onion frequently declined during air drying, presumably as a result of sclerotium germination, induced by stimulant from *Allium* spp. Thus, samples from fields planted to *Allium* spp. always were assayed immediately. Inoculum density did not change during air drying of soil collected from areas free of *Allium* spp.

Sclerotia of *S. cepivorum* smaller than 0.250 mm have not been found in California soils. Sclerotia were separated from soil particles by wet sieving as described by McCain (17) with the following changes: 500-g portions of each soil sample were blended with 500 ml of water in a Waring Blendor at low speed for 30 sec and then passed through stacked 0.850- and 0.250-mm soil screens under a spray of water. If inoculum density was known to be greater than 1.0 sclerotia per gram of soil, only 100-g portions were assayed. Residue from the 0.250-mm screen was washed into a 1,000-ml beaker with 500 ml of water; residue floating or in suspension was decanted back onto the 0.250-mm screen and washed onto gridded counting dishes. Five hundred milliliters of sucrose solution (50% by weight, viscosity at 20–25 C = 1.23 g/cm^3) was poured onto the residue remaining in the beaker. This residue was stirred and the particles remaining in suspension after 20 sec were decanted onto the 0.250-mm screen, washed for 10 sec, and then washed onto gridded counting dishes. Residue remaining in the beaker was discarded. The total number of dull black, generally spherical, and firm sclerotia in the residue on all plates was counted at ×15 with a stereo microscope. The sclerotia in the two portions of each sample were counted separately and averaged. The efficiency of the soil assay was 96% for all inoculum densities as indicated by recovery of various known numbers of sclerotia that had been added to 500-g portions of noninfested soils. The coefficient of variability ($sd/\bar{X}$) between two 500-g portions averaged 0.61, 0.11, and 0.05 for samples with average inoculum densities near 0.001, 0.01, and 0.1 sclerotia per gram of soil. Considering time and resources, the lower limit of detectability was about 0.001 sclerotia per gram of soil per soil sample when two 500-g portions were assayed and the counts combined.

Identity and viability of sclerotia. Fifty sclerotia recovered from each soil sample were tested for viability; if fewer than 50 were recovered, all were tested. Sclerotia suspected of being in the process of germination were washed in sterile distilled water and placed directly on 1.5% water agar (WA). Nongerminating sclerotia were decontaminated by immersion for 2.5 min in 0.5% NaOCl, washed twice in sterile distilled water and placed with fine-pointed forceps 25 to a dish on WA. Each sclerotium was cracked (by pinching with the forceps) during placement to induce rapid and simultaneous germination. Petri dishes, stored in the laboratory in plastic boxes at 22–24 C for 2 wk, were examined at ×10 with a stereo microscope. The sparse hyphal growth that seldom extended beyond 1.5 cm from the sclerotia and the presence of microconidial sporodochia in and on the agar were presumptive evidence for *S. cepivorum*. Sclerotia of all pathogenic isolates collected from California and received from worldwide sources grew similarly on WA. Inoculum densities were expressed as the number of viable sclerotia per gram of air-dried soil.

Viability and infectivity of sclerotia recovered from naturally infested soil. The method of determining viability of sclerotia on WA was compared with other methods. Surface-sterilized sclerotia were placed without cracking on WA or potato-dextrose agar (PDA) in petri dishes. Sclerotia producing growth on PDA were transferred individually to fresh PDA to confirm the identity of *S. cepivorum* by formation of sclerotia. Viability also was determined by placing sclerotia without surface sterilization or cracking on nonsterile soil dampened with garlic extract or distilled water. Twenty-five grams (oven-dried equivalent) of uninfested Tulelake soil (unclassified soil, pH 6.8, 14% organic matter) dewetted from saturation to −300 mb (1,000 mb = 1 bar = 0.99 atmospheres) matric potential by the method of Duniway (14) were placed in petri plates. The garlic extract was prepared fresh as follows: garlic cloves (cultivar California Late) were blended at low speed in a Waring Blendor with distilled water (250 g/250 ml) for 30 sec. The extract was squeezed from the mixture through cheesecloth and filtered through Whatman No. 1 filter paper and diluted 100-fold. Sclerotia, placed on soil in petri plates covered with Saran plastic wrap to allow gas exchange, but to restrict water loss, were incubated at 18 C in covered plastic boxes with wet paper toweling in the bottom for 40 days. When mycelium grew from a sclerotium on soil, the sclerotium was placed next to a 3-wk-old onion seedling grown at 15 C in quartz sand to determine infectivity.

Production of inoculum. Isolate TL4-2 from an onion at Tulelake, CA, and isolate G-1 from garlic at Gilroy, CA, were used in all experiments. Inoculum was produced in 946.5-ml (1-qt) mason jars containing 150 g of wheat seed, 25 g of horticultural perlite (Sponge Rock, Paramount Perlite Co., Paramount, CA) and 125 ml of distilled water. Jars were sealed and autoclaved on two successive days for 40 min at 121 C and 1.05 kg/cm^2. Jars were shaken as they cooled to prevent clumping. Each jar of medium was inoculated with five 3-mm plugs of mycelium cut from the advancing edges of *S. cepivorum* colonies grown on PDA. During incubation for 6 wk at 22–24 C on a laboratory shelf, cultures were shaken two or three times each week to prevent clumping, to distribute the mycelium, and to promote growth. Sclerotia were separated from the growth medium by wet sieving through 0.850-, 0.425-, and 0.250-mm screens. Residues caught on the 0.250- and 0.425-mm screens were predominantly single sclerotia with a small amount of wheat debris and perlite. Sclerotia were air dried for 1 day and stored temporarily in open 946.5-ml

jars on the laboratory shelf at 22–24 C. Before use in each experiment, samples of sclerotia were tested to determine percent viability. Inoculum was not stored longer than 2 mo before use in field tests.

Elimination of constitutive dormancy of sclerotia. The burial time in field soil required to eliminate constitutive dormancy of sclerotia produced in culture was determined. Each isolate, in lots of 30,000 sclerotia each, was mixed with 150 g of noninfested Tulelake or Davis soil (Yolo fine sandy loam, pH 7.4, <1% organic content), placed in 8-cm × 13-cm nylon mesh (3.5 grids per millimeter) bags and buried 8 cm deep in field soils at Tulelake or Davis, CA. After various periods of time, the sclerotia were recovered and tested for viability, germinability, and infectivity as described previously.

Effect of inoculum density on disease incidence. The effect of various inoculum densities in the range recovered from infested soils in California was studied in trials at Tulelake and Davis, CA, in soils previously described and at Salinas, CA, in Salinas silty clay loam, pH 7.2, and low organic content. Five weeks before planting each trial, different numbers of sclerotia (equal proportions of bulk inoculum of TL4-2 and G-1) were added to plots as calculated on the basis of plot length, bed width, depth of incorporation, and bulk density of soil in the beds. At Tulelake, Salinas, and Davis, respectively, plots consisted of bed sections 1.80, 2.25, and 2.40 m long and treatments were replicated six, seven, and eight times. Sclerotia were sprinkled evenly over the bed sections and raked into the upper 10-cm of soil. Each trial was a randomized complete block design and included a noninfested control. Soil was sampled from each plot (to determine the inoculum density after incorporation of sclerotia) at planting time and (in the garlic trials only) when the plants began to emerge. Onion seed (cultivar Southport White Globe) was sown at 1.5-cm depth, 165 seed per meter of row, in two rows per bed on 1 June 1976 at Tulelake. In mid-November 1976, garlic cloves (cultivar California Late) were sown by hand at 4-cm depth, 32.8 cloves per meter of row, in two rows per bed at Davis and 52.5 cloves per meter of row in one row per bed at Salinas. Plants were recorded as diseased during the season if the leaves were wilted and the characteristic white mycelium and sclerotia of the pathogen were evident at the base of the plant. Plants were rated diseased at harvest if the stem plate or leaf sheaths were infected. Soil temperatures were recorded at 10-cm depth in planted rows of trial areas at Salinas and Davis; at Tulelake, soil temperatures were measured at 10-cm depth in an area adjacent to the trial.

RESULTS

Viability of sclerotia recovered from naturally infested soil. Sclerotia recovered with a plug of white mycelium erupted from the sclerotium surface, with or without hyphae emanating from the plug, were occasionally found. These formed microconidial sporodochia on WA and were presumed to be sclerotia of *S. cepivorum* which had germinated by the hyphal-plug type germination described by Coley-Smith (7). Other moldy sclerotia were rarely encountered and *S. cepivorum* never grew from these in culture. Apparently intact sclerotia which were firm but not brittle when gently squeezed with fine-pointed forceps were more than 99% viable and those which were brittle or nonresiliant were invariably nonviable. Other small sclerotium-forming fungi similar to *S. cepivorum* were not encountered. Various bacteria, *Fusarium* spp., *Penicillium* spp., *Aspergillus* spp., *Streptomyces* spp., and *Trichoderma viride* Pers. grew from fewer than 2% of surface-sterilized, intact sclerotia and prevented formation of microconidial sporodochia only when sclerotia were overgrown before growth of *S. cepivorum* occurred. Sclerotia recovered from all California soils were 85–100% viable.

Germinability and infectivity of sclerotia recovered from naturally infested soils. Twenty-five sclerotia, recovered from five different soils not previously air dried, were tested for viability by different methods. Each method was replicated four times. No sclerotia were recovered in a germinating state from any of the five soils. The germinability of: cracked, surface-sterilized sclerotia after 2 wk on WA; intact surface-sterilized sclerotia after 40 days on WA; intact surface-sterilized sclerotia after 40 days on PDA, and nonsurface-sterilized intact sclerotia after 40 days on soil dampened with garlic extract ranged from 85–100%. There were no significant differences among these treatments ($P = 0.05$) for each soil. In contrast, the germinability of sclerotia on soil dampened only with water was 10–18% after 40 days. On nonsterile soils only hyphal-plug germination occurred, but intact sclerotia on agar germinated in approximately equal portions by production of hyphal plugs and by growth of distinct hyphae from apparently intact rinds. Germination occurred predominatly (70% of total) within the first 20 days in all treatments but continued up to 40 days on both agar and soil. Infectivity (as measured by seedling collapse) averaged 88% of the sclerotia that germinated on soil.

Elimination of constitutive dormancy of sclerotia. Bulk inoculum of isolates TL4-2 and G-1 were both over 99% viable on WA, over 90% germinable on soil dampened with garlic extract and over 85% infective on onion seedlings after 5 and 16 wk of burial in field soil at both Tulelake and Davis, CA. When recovered before 5 wk, the percentages of stimulated germination with garlic extract were not as high as after 5 wk of burial; when placed without prior burial directly on soil dampened with garlic extract, less than 5% germination occurred after 40 days. Viability, germinability, and infectivity of the different isolates were similar.

Inoculum density of soil in naturally infested fields. Inoculum densities were determined for soil samples collected (eight per field)

TABLE 1. Effect of various preplanting inoculum densities of *Sclerotium cepivorum* on the cumulative percentage[a] of white rot diseased garlic plants and inoculum densities after harvest

Time after emergence (wk)	Incidence[b] of white rot disease in garlic grown in soil with preplant viable sclerotia populations[c] of:										LSD ($P = 0.05$)
	0	4×10^{-5}	1×10^{-4}	4×10^{-4}	1.7×10^{-3}	6.8×10^{-3}	2.0×10^{-2}	6.1×10^{-2}	1.8×10^{-1}	5.5×10^{-1}	
0	0	0	0	0	0	0.3	1.3	2.8	6.4	12.1	1.9[d]
7	0	0	0.5	0.1	3.1	16.2	35.9	75.4	90.3	93.9	7.0
11	0	0.7	1.6	2.8	13.5	51.9	73.9	91.1	94.1	95.9	6.9
13.5	0	2.1	2.8	5.4	26.2	68.8	87.3	95.7	96.9	96.4	5.8
16.5	0	2.8	4.0	6.3	36.4	76.0	91.7	97.2	97.5	97.0	6.4
19 (harvest)	0	3.2	3.8	7.5	53.1	87.5	94.9	98.9	99.3	99.7	7.3
	Viable sclerotia per gram of soil recovered 6 mo after harvest										
Mean	0	0.41	0.63	1.44	6.15	9.22	3.41	2.73	0.94	0.87	
Std. Dev.	···	1.24	1.36	0.94	1.02	0.50	0.58	0.30	0.18	0.52	

[a] Based on the total plant emergence in each plot.
[b] Averages of percent plants infected in seven replicates.
[c] Viable sclerotia per gram of soil (cracked sclerotia were incubated on water agar at 22–24 C for 2 wk).
[d] LSD ($P = 0.05$) calculated according to Duncan's new multiple range test.

from selected fields throughout California with known histories of white rot disease. No sclerotia were recovered from two fields, both in the Tulelake area, that had not been planted with *Allium* spp. crops for 17–20 yr prior to sampling. In three fields in which *Allium* spp. crops last had been grown 10–14 yr before sampling, the inoculum density in a total of 24 composite samples ranged from 0 to ~0.01 sclerotia per gram of soil; sclerotia were recovered from 18 of the 24 samples. In the other 10 fields, where *Allium* spp. crops were grown 1–8 yr prior to sampling, the inoculum density ranged from 0 to 1.3 sclerotia per gram of soil; of 80 composite samples, four samples contained more than 1.0 sclerotia per gram of soil, 27 samples contained between 0.1 and 1.0 sclerotia per gram of soil, 25 samples contained between 0.01 and 0.1 sclerotia per gram of soil, 14 samples contained between 0.001 and 0.01 sclerotia per gram of soil, and no sclerotia were recovered from 10 samples. For sections of fields in which the inoculum density of composite samples was measured near 0.001, 0.01, and 0.1 sclerotia per gram of soil, the coefficient of variability between duplicate composite samples averaged 0.45, 0.15, and 0.07, respectively. From six fields, 12 sample areas were selected in which duplicate composite samples averaged near 0.001, 0.01, or 0.1 sclerotia per gram of soil. Multiple composite samples were collected and additional samples also were assayed without compositing. Increasing the number of composite samples collected per sample area to three or four did not lower the coefficient of variability, except for samples averaging near 0.001 sclerotia per gram of soil for which four composite samples lowered the average coefficient of variability from 0.54 to 0.38.

Inoculum densities of 10–20 individual 300-ml soil samples ranged from 0–0.01, 0–1.0, and 0–2.0 sclerotia per gram of soil, respectively, in areas in which composite samples averaged about 0.001, 0.01, and 0.1 sclerotia per gram of soil; the average coefficient of variability was 2.2, 2.0, and 1.2, respectively.

Effect of inoculum density on disease incidence. The onion trial area at Tulelake was infested with a population of about 0.002 sclerotia per gram of soil prior to establishment of the inoculum-density test. Inoculum densities recovered after addition of sclerotia were 0.0017, 0.0067, 0.013,0.12,1.1, and 11.4 as compared with calculated levels of 0, 0.001, 0.01, 0.1, 1.0, and 10.0 sclerotia per gram of soil, respectively. The garlic tests at Salinas and Davis were conducted in noninfested soil as confirmed by soil assays of over 4,000 g of soil from the trial areas. The various calculated rates of inoculum densities at Salinas are shown in Table 1. At Davis, inoculum was incorporated at 0, 0.0001, 0.0005, 0.0018, 0.0072, 0.029, 0.12, 0.46, 1.9, and 7.4 sclerotia per gram of soil. No attempt was made to confirm inoculum density in plots in which less than 0.001 sclerotia per gram of soil were added because this was the lower limit of the soil assay. For higher rates of infestation there were no significant differences ($P = 0.05$) between artificially infested (calculated) and recovered inoculum densities in soil samples assayed just after incorporation in early October 1976, at planting time in mid-November 1976, and 11–12 wk after planting when garlic had begun to emerge in early February 1977.

Mean daily soil temperatures were between 10–15 C when garlic was planted at Davis and Salinas in November, but temperatures in December and January were 3–8 C. Garlic emerged in trial areas at Davis and Salinas when mean daily soil temperature at 10-cm depth had increased to 10 C. Temperatures generally increased through the season. Mean daily soil temperatures were 25 C in mid-June at Davis and 18 C in mid-July at Salinas when garlic was harvested. At Tulelake, the mean daily soil temperature at planting in June was 15 C, but increased to 23 C in August and declined to 10 C at harvest in October.

There were no significant differences ($P = 0.05$) between stands (plants established) in various treatments within any of the trials. Bulbs were harvested when mature 15.5, 17, and 19 wk after emergence at Tulelake, Davis, and Salinas, respectively. No postemergence plant loss resulted from other diseases or pests and white rot disease was expressed as a percentage of the stand in each plot.

In Tulelake, onions were fully emerged by 1 July, but white rot was not evident until 3 wk later. In Salinas and Davis, some garlic plants had symptoms at the time of emergence. At Salinas, the stand averaged 92 garlic plants per plot and the cumulative percentages of disease during the season for inoculum-density treatments are shown in Table 1. The pattern of disease development was very similar in all three trials. White rot symptoms first appeared on individual plants, but subsequently appeared on adjacent plants and formed a disease locus as the season progressed. As inoculum density increased, disease loci appeared sooner and in greater frequency and in many treatments disease loci eventually coalesced. At inoculum densities ≥ 1.0 sclerotia per gram of soil (only Davis and Tulelake trials had treatments with this many sclerotia) all plants developed symptoms rapidly after emergence and disease loci could not be distinguished. Percentage of diseased plants also increased with increasing inoculum density. Less than 10% disease occurred by time of harvest in plots infested with <0.001 sclerotia per gram of soil (only Davis and Salinas trials had treatments infested with so few sclerotia). Percentage disease increased considerably between 0.001 and 0.01 sclerotia per gram of soil and nearly all plants were diseased by harvest at inoculum densities ≥ 0.01 sclerotia per gram of soil. Inoculum densities between 0.001–0.004 sclerotia per gram of soil corresponded with 50% diseased plants by harvest in all three trials. No disease appeared in noninfested treatments at Davis and Salinas, although 52.7% of the onion stand at Tulelake was diseased by harvest in control plots for which a residual population of 0.0017 sclerotia per gram of soil was assayed.

Changes in inoculum density. After emergence, changes in inoculum density were determined in plots in inoculum-density trials infested with 0.1 or more sclerotia per gram of soil. Soil from the planted row itself was not sampled. In Tulelake, soil was only sampled 11 wk after emergence, when 0 and 0.003 sclerotia per gram of soil were recovered from treatments with preplant levels of 1.1 and 11.4 sclerotia per gram of soil. At Davis and Salinas only 20% of pre-emergence levels of sclerotia were recovered 5 wk after emergence and no sclerotia were recovered 14 wk after emergence in any treatments. Ten to 20% of the sclerotia recovered at all sampling times after emergence were in the process of germination.

Noninfected garlic bulbs were removed from plots at Salinas and Davis at harvest and each trial was irrigated three times during the summer before rototilling to a depth of 10 cm in September 1977 Two composite samples of ten 300-ml subsamples were collected from each plot with a small shovel. Post-season inoculum densities determined in November 1977 and February 1978 were similar. Data from samples collected in February are shown in Table 1 for the Salinas trial. In the Salinas trial, post-season inoculum densities from all inoculated plots increased from pre-emergence levels to average 0.41–9.22 sclerotia per gram of soil. In the Davis trial, post-season inoculum densities decreased to between 0.1–1.0 sclerotia per gram of soil from pre-emergence values of near 1.0 sclerotia per gram of soil or higher, but inoculum densities in all other treatments with lower initial inoculum densities increased to between 0.4–10.0 sclerotia per gram of soil as they did in Salinas. No sclerotia were recovered from any of the noninfested plots. In trials at Salinas and Davis, the highest post-season inoculum density levels resulted in plots with preemergence inoculum densities of 0.001–0.01 sclerotia per gram of soil. The number of sclerotia recovered from treatments with initially high inoculum densities (greater than 0.001 sclerotia per gram of soil) were uniformly high (coefficient of variability averaged 0.33) because many plants in these treatments were diseased. However, variation was high (coefficient of variability averaged 1.44) within treatments initially infested at low levels (less than 0.001 sclerotia per gram of soil) because sclerotia were concentrated in the areas where disease loci had developed.

Disease incidence in a naturally infested field. White rot disease was first detected and soil samples were collected in earch March 1977 in a field near King City, CA, half of which was planted commercially with garlic (cultivar California Late) in November 1976, and half of which was planted with tomatoes in spring 1977. The previous planting of an *Allium* sp. crop in this field was 8 yr earlier. Soil type was a gravelly loam. Between 0.001 and 0.01 sclerotia per gram of soil were recovered from soil samples collected from five 49-m (7 × 7-m) areas from both garlic and

tomato areas. Inoculum density at time of planting was assumed not to have changed since emergence in late February, although 3% germinating sclerotia were initially recovered from the garlic area. No germinating sclerotia were initially recovered from the tomato area. Soil also was collected in early March and disease was monitored in the garlic area in 27.5-m bed sections with inoculum densities of 0 (no recovered sclerotia), 0.0015, 0.0023, 0.0048, 0.0058, and 0.013 sclerotia per gram of soil. Bed lengths with initial inoculum densities greater than 0.005 sclerotia per gram of soil were not monitored through to harvest because white rot developed so severely that this part of the field was disked and replanted to a nonsusceptible crop. Bed lengths with inoculum densities of 0 (no recovered sclerotia), 0.0015, 0.0023, and 0.0048 sclerotia per gram of soil had 8.9, 40.4, 70.1, and 77.5% disease by the time of harvest (all plants were inspected for disease). Garlic was not harvested from most of the garlic planting due to the severity of white rot disease. The inoculum density in February 1978, in the half of this field in which white rot disease occurred, was generally 0.3 sclerotia per gram of soil. The half of this field planted with tomato remained generally infested with between 0.001 and 0.01 sclerotia per gram of soil. No germinating sclerotia were recovered during the 1977 season from the tomato field. As in the artificially infested field trials, 10–20% of the sclerotia were recovered in the process of germination when sampled at various times during the season in the area planted with garlic.

DISCUSSION

A simple and rapid test to determine the viability of sclerotia involved surface-sterilization and cracking of sclerotia to stimulate simultaneous growth on WA. The restricted growth on WA and formation of microconidial sporodochia in WA of all California isolates of *S. cepivorum* also was observed for a worldwide collection of isolates of *S. cepivorum* which were pathogenic on both onions and garlic. These features were not observed for any other soil fungi which produce small, black sclerotia and this development on WA is considered diagnostic for *S. cepivorum*.

Data herein confirm reports that most sclerotia of *S. cepivorum* recovered from soil are viable on agar (6–10,16), although a few reports indicate higher levels of germination following surface-sterilization (11,20). Coley-Smith (6,7) observed that large proportions of sclerotia of certain isolates of *S. cepivorum* germinated near roots of *Allium* spp. and most germinated sclerotia were infective. Similar observations were made here with sclerotia recovered from naturally infested soil throughout California. Germination on soil was always by eruption of a plug of mycelium, as described by Coley-Smith (7), but on agar hyphae sometimes grew from apparently intact sclerotia, as described by Adams and Papavizas (2). Sclerotia that tested viable on agar apparently were capable of infection in the field. Although the nature of the stimulated germination has been the subject of many studies (7,8,10,16), little attention had been placed on the generality of this phenomenon in the field and its relationship to disease incidence and sclerotial numbers. In field trials, preemergence populations of sclerotia declined to undetectable levels in the bed profile during the season and this decline was apparently due to stimulated germination. Sclerotia in the process of germination have not been recovered by the authors from naturally or artificially infested soil unless *Allium* spp. were actively growing or succulent *Allium* tissue was decaying in that soil. Sclerotia reported here and elsewhere (7,13) sometimes germinated at a low frequency when transferred in the laboratory from field soil to nonsterile soil which did not contain *Allium* factors. The nature of the reversal of fungistasis during this manipulation is not understood.

The incidence of both onion and garlic white rot disease (10–100%) reported here for inoculum densities ranging from less than 0.001 to 0.1 sclerotia per gram of soil may at first seem unusually high; however, presumably because most sclerotia germinate and each sclerotium can initiate an infection, less inoculum might be required to incite high disease incidence than would be necessary for pathogens with less efficient inoculum. The rhizosphere effect of *Allium* spp. (7) is perhaps the largest presently known (10 mm from the root surface) for a plant pathogen. Sclerotia from a large volume of soil appear to be stimulated to germinate as roots permeate that soil volume, thus helping ensure infection (12). Furthermore, disease in naturally infested fields and in inoculum-density trials occurs among distinct clusters of plants and these disease loci increase in size as the season progresses (12). In California, more than 20 plants per disease locus are common. The mycelium of *S. cepivorum* spreads from plant to plant (12,23) and the disease loci represent distinct infection centers. Because disease loci appear at various times and tend to merge as they expand, there is no simple mathematical relationship between inoculum density and either the numbers of disease loci or diseased plants.

It remains paradoxical that pre-emergence loss due to white rot is not observed in spring-planted onions in California since soil temperatures at planting are above the minimum for pathogen activity (13,24). Preemergence damping-off and early post-emergence seedling loss to white rot in fall-planted onions has been reported (3). No preemergence loss was observed in garlic in California, either, but soil temperatures decrease rapidly after planting in the fall and may remain too low for pathogen activity until spring when both garlic and *S. cepivorum* become active at about 10 C. Once white rot appears, temperatures remain in a range that allows pathogen activity until onions and garlic are harvested (13,24).

Since sclerotia distributed throughout the soil profile prior to planting of onions and garlic declined to undetectable levels during the season, the inoculum present following harvest was produced on plants which rotted during the season. Soil infested with between 0.001 and 1.0 sclerotia per gram of soil contained between 1.0 and 10.0 sclerotia per gram of soil after the crop was harvested. Many plants did not become infected until late in the season and thus provided a large nutrient base for production of sclerotia. Conversely, soil infested with 1.0 to 10.0 sclerotia per gram of soil resulted in 100% plant death when plants were small with a concomitant reduction of nutrients; thus, the post-season inoculum densities were below those originally present. Where the inoculum density was less than 0.001 sclerotia per gram of soil, a few plants became diseased late in the season; because these plants were large, a large nutrient base was available for production of many sclerotia. However, the size of nutrient base available for sclerotium production may not have been the only factor affecting postharvest inoculum densities. Quite possibly, many sclerotia produced early in the season were stimulated to germinate during the season and sclerotia present following harvest were mainly produced late in the season or after harvest on plants infected near the end of the season. In all areas, postharvest inoculum densities were high enough (greater than 0.4 sclerotia per gram of soil) to result in nearly total plant loss if *Allium* spp. crops were replanted. However, postharvest inoculum was not uniformly distributed in soil originally infested at low levels, therefore, extensive loss probably would not occur until sclerotia become more widely dispersed. Even though some dilution of inoculum density may occur during this dispersal, a very few cycles of white rot disease would result in the soil becoming highly and generally infested. With continued planting of *Allium* spp. crops in infested soil, inoculum density might stabilize or fluctuate somewhat, but it would probably remain at levels high enough to produce extensive disease, unless sclerotium populations declined rapidly during the years when *Allium* spp. crops were not grown.

In the absence of *Allium* spp. crops, sclerotia of *S. cepivorum* were recovered by Coley-Smith (6) for 4 yr with no decrease in numbers or percent viability. Rotations of 8 yr have failed to control white rot (18). Survey data presented here indicate that areas of fields may remain infested for at least 15 yr at inoculum levels high enough to cause 50% or more loss of both onion and garlic from white rot. Sclerotia may persist longer, but were not detected in the few sampled fields in which *Allium* spp. crops had not been grown during the 15-yr period prior to sampling. Additional surveys should be conducted to determine if a 15- to 20-yr rotation might adequately control white rot in most infested fields.

White rot has been recognized in California since 1939 (15) and

many fields are known to be infested. In addition, growers occasionally plant garlic in fields for which disease histories are unknown and suffer high losses from white rot. The soil assay described herein has been used to avoid these losses. The lower limit of the soil assay method is about 0.001 sclerotia per gram of soil. Although inoculum densities greater than 0.001 sclerotia per gram of soil corresponded to 50% or more disease, inoculum densities less than 0.001 sclerotia per gram of soil corresponded to 10% disease or less in the uniform inoculum-density trials, and in sections of the one naturally infested field in which the incidence of disease was monitored. These areas were located in diverse soil types and in various parts of California. Thus, if no sclerotia were detected in assays for sclerotia in commercial fields prior to planting, there probably would be little risk of heavy loss from white rot. White rot severity in 1976 and 1977 was considered typical of the disease as it occurs in most infested fields in most years in California. Other regions may experience white rot differently. In our experience variations from this pattern and severity may occur in two cases: In one case, onion pink root disease (caused by *Pyrenochaeta terrestis* [Hansen] Gorenz) occurs early and severely reduces the number of onion roots; few sclerotia of *S. cepivorum* germinate and white rot incidence is low. Inoculum density remains measurably unchanged during the season. In the other case, warm soil temperature and high soil moisture in winter and spring cause a reduction in numbers of sclerotia, presumably by decay (13), thus lowering the effective inoculum potential. Inoculum density declines and less disease develops than anticipated based on pre-season soil assays. Neither of these cases occurs frequently. Inoculum density varies widely both in magnitude and distribution across naturally infested fields, but when sclerotia are detectable economic loss is nearly certain based on our data and experience. We currently recommend not planting *Allium* spp. crops in fields with known histories of white rot or if any sclerotia of *S. cepivorum* are recovered, because no fungicides currently registered for use on onion or garlic provide adequate white rot control in California (F. Crowe, *unpublished*).

Although a few investigators conducting chemical control studies have considered the distribution of sclerotia (4,21), none has considered the magnitude of inoculum density as a variable. The close relationship between disease incidence and inoculum density less than 0.1 sclerotia per gram of soil, and the total plant loss resulting from inoculum densities above 0.1 sclerotia per gram of soil suggest that inoculum density should be considered in the experimental design and interpretation of results of fungicide trials.

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