

# Effect of Inoculum Density of *Sclerotium cepivorum* and Some Soil Environmental Factors on Disease Severity

P. B. Adams and G. C. Papavizas

Plant Pathologist and Microbiologist, respectively, Plant Science Research Division, ARS, USDA, Plant Industry Station, Beltsville, Maryland 20705.

Appreciation is expressed to H. Russell and C. J. Tate for technical assistance, and to G. D. McCollum for the supply of seed of many of the *Allium* species used.

Accepted for publication 26 May 1971.

## ABSTRACT

Optimum temperature for white rot development on onion was 15 C. Optimum temperature for germination of sclerotia of *Sclerotium cepivorum* in autoclaved soil was 20 C. The optimum pH values for germination of sclerotia on agar and in autoclaved soil were 5.3 and 4.8, respectively. However, more than 50% of the sclerotia germinated in autoclaved soil at all pH levels tested between 4.5 and

7.8. At pH 5.0 or below, less than 30% of the plants were infected, whereas at 6.0 and above, 90% of the plants were infected. About 25 sclerotia/g soil were required to obtain infection of about 60% of the seedlings. Of numerous species tested, only *Allium caeruleum* appeared to possess significant resistance to white rot. *Phytopathology* 61:1253-1256.

*Additional key words:* white rot resistance, soil temperature, variation in pathogenicity, soil pH.

White rot caused by *Sclerotium cepivorum* Berk. has been reported from various parts of the world (4, 13). It has been reported in the United States from Virginia, Kentucky, New Jersey, Oregon, California, Louisiana, Washington (9, 12), and Illinois (M. B. Linn, *personal communication*). Losses may be severe, as evidenced by the abandonment of certain fields for onion culture in Washington (9), and the 100% losses reported in certain shallot fields in Louisiana (11).

The first control measures of white rot consisted of cultural practices, including crop rotation, exclusion of contaminated material, application of lime, and planting in noninfested field soils. As infestation became more widespread, these control measures became less effective and attention was directed to chemical control.

Walker (14) determined the optimum soil temperature for disease development. No one, however, has determined the effect of soil temperature on germination of sclerotia. There is also some disagreement in the literature concerning the effect of soil pH on disease severity and on the pathogen. Nothing exists in the literature on the relationship of inoculum density and disease severity.

The purpose of this investigation was to learn more of the effect of inoculum density as well as of some soil environmental factors on disease severity. A preliminary report of this study has been published (1).

**MATERIALS AND METHODS.**—Isolates of *S. cepivorum* were obtained from several sources (Table 1). Isolate J-11 was used in all experiments except in those designed to determine pathogenicity of isolates.

Sclerotia of various isolates were obtained by comminuting 4-week-old cultures, grown on Czapek-Dox agar (pH 5.2), in a homogenizer for 0.5 min. The resulting suspension was then poured onto an 80-mesh sieve (pore size, 0.18 mm) and washed with a stream of water. The sclerotia remained on the sieve while hyphal fragments and agar were washed through the sieve. The sclerotia were suspended in water 3 times, and the supernatant fluid was decanted. The sclerotia

were collected on filter paper, dried, and stored in desiccators at 20 C until used.

For testing germination of sclerotia on agar, sclerotia of J-11 were surface-disinfected with 0.5% NaClO solution for 2 min, rinsed with sterile distilled water several times, and plated out on the surface of potato carrot agar (PCA; 15 g potatoes, 15 g carrots, 20 g agar, 1 liter water) (J. R. Coley-Smith, *personal communication*) in petri dishes. One hundred sclerotia were plated on four dishes/replication. The dishes were incubated at 20 C, and the sclerotia were observed for germination after 6-7 days with a dissecting microscope.

Germination tests of sclerotia at various soil temperatures and pH levels were performed in plastic petri dishes (60 × 15 mm). A 5-mm layer of autoclaved soil (30 min at 121 C) was placed in each dish and packed down to form a smooth surface. A piece of aluminum foil with a 20-mm diam hole in the center was pressed onto the soil surface. Sclerotia were scattered over the soil exposed by the hole in the foil. Another 5-mm layer of autoclaved soil was then placed over the foil and pressed down. The dishes were then incubated at the desired temperature.

We removed the top layer of soil and the aluminum foil from the petri dish to observe the sclerotia. Three counts of 50 sclerotia in each of two dishes were made with the aid of a dissecting microscope for each treatment, and the average of the six counts was computed to obtain the percentage of germinated sclerotia.

The soil used was Hatboro loamy sand of pH 5.5, water-holding capacity (WHC) 30%, and contained 4.5% organic matter. It was treated with aerated steam at 71 C for 30 min (2). After cooling, onion seedlings were planted and grown for ca. 4 weeks. *Sclerotium cepivorum* was grown on sand-cornmeal (1,000 g quartz sand, 20 g cornmeal, 200 ml water) for 4 weeks, and added to soil at a rate of ca. 10% (w/w). The soil was then planted with two successive crops of onions, then air-dried and stored at ca. 15 C until used.

Hydrated lime or aluminum sulfate [ $Al_2(SO_4)_3 \cdot$

TABLE 1. Pathogenicity of various isolates of *Sclerotium cepivorum* on onion seedlings

Isolate <sup>a</sup>	Country of origin	% Infected seedlings
D-1-e	United States	24 a <sup>b</sup>
J-18	The Netherlands	48 b
F-5-a	United States	72 c
J-9	Norway	84 cd
J-14	France	86 cd
C-4-b	United States	86 cd
E-3-b	United States	90 d
J-3	United Kingdom	92 d
J-49	New Zealand	92 d
X	United States	98 d
E-1-b	United States	100 d
J-11	United States	100 d

<sup>a</sup> Isolates D-1-e, F-5-a, C-4-b, E-3-b, X, and E-1-b were obtained from S. B. Locke, Washington State University, Pullman; isolates J-18, J-9, J-14, J-3, and J-49, from J. R. Coley-Smith, The University, Hull, Yorks, Great Britain; and isolate J-11, from the American Type Culture Collection.

<sup>b</sup> Means with the same letter are not significantly different at the 1% level.

18H<sub>2</sub>O] were used to raise or lower the soil pH, respectively. They were added to moist soil which was kept at 25 C for at least 3-4 weeks. A soil slurry (1:1, v/v) was used to determine soil pH electrometrically. The soil pH was determined at the beginning and end of each experiment.

For studies on susceptibility to *S. cepivorum*, we used *Allium cepa* L. 'Yellow Globe Danvers' in all experiments except in those which included various species and cultivars of *Allium*. In all experiments, seedlings were transplanted into infested soil, grown in a growth chamber at 18 C with a 12-hr day length (1,200 ft-c, Sylvania VHO cool-white) for 4-5 weeks in plastic flats (9 × 12 × 5 cm) containing quartz sand, and irrigated periodically with Hoagland's solution (8).

All plant experiments were performed in No. 4.5 standard pots containing 900 g soil. Ten onion seedlings were transplanted into each pot. In the soil temperature experiments, the pots were placed in stainless steel beakers (12 × 16 cm) with quartz sand between the walls of the beaker and the pot. After a 4-week growing period in a growth chamber at a constant temperature of 15 C, or in the soil temperature tanks, the seedlings were harvested and disease severity data recorded as the percentage of plants in each pot infested with *S. cepivorum*. In all experiments, there were at least five replications, and each experiment was performed at least twice.

**RESULTS.—Effect of soil temperature and pH on *Sclerotium* germination and white rot severity.**—Germination in autoclaved soil at 20 C after 24 hr was 5%; at 24 hr, about 40%; and at 72 hr, 60%. We terminated all germination experiments after a 72-hr incubation period because it became difficult to distinguish between *S. cepivorum* hyphae and hyphae of other fungi. The optimum temperature for germination of sclerotia was ca. 20 C, with an optimum range between 15 and 25 C (Fig. 1-A). The optimum temperature for

disease development was ca. 15 C, with an optimum range from 10 C to ca. 18 C.

Germination of sclerotia at 20 C in autoclaved soil at pH 4.8 was 12% at 24 hr, 60% at 48 hr, and 87% at 72 hr. The optimum pH for germination was ca. 4.8, with substantial germination (greater than 50%) occurring at all pH levels tested (Fig. 1-B). The optimum pH for disease development was ca. 6.1, with an optimum range from about 5.4 to approximately a level greater than 7.8.

An experiment was performed to see whether the pH optimum values for germination of sclerotia on agar approximated those observed in autoclaved soil. Batches of PCA were adjusted after autoclaving to pH 3.4, 4.3, 4.8, 5.3, 5.8, 6.3, 6.6, 7.3, and 8.1 with sterile NaOH or HCl. Sclerotia of J-11 were plated out, and the dishes incubated at 20 C. The optimum pH for germination on PCA was 5.3 at 48, 72, and 96 hr after plating (Fig. 1-C). Per cent germination fell off sharply at pH values above or below 5.2.

Because Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> · 18H<sub>2</sub>O was used to lower the soil pH, we performed experiments to determine whether disease reduction with low pH was due to the Al ion or pH per se. When the pH of the soil was lowered from 5.4 to 4.3 with 1 N H<sub>2</sub>SO<sub>4</sub>, there was a 50% reduction in disease severity. However, when we added AlCl<sub>3</sub> · 6H<sub>2</sub>O to the soil at rates up to 800 ppm Al, we could observe no reduction in disease severity.

**Inoculum density and disease severity.**—Twelve isolates of *S. cepivorum* from various parts of the world were tested individually for pathogenicity on Yellow Globe Danvers onion. All isolates were added to non-infested soil at 25 sclerotia/g of soil, onion seedlings were transplanted immediately, and the plants were grown in a growth chamber at 15 ± 1 C. The twelve isolates ranged from weakly pathogenic to highly pathogenic (Table 1). Most of the isolates tested were very pathogenic, causing more than 75% infection at 25 sclerotia/g. The origin of the isolates did not seem to determine the degree of their pathogenicity.

The effect of various concentrations of sclerotia per gram of soil on white rot severity was also tested. Isolates D-1-e, J-11, and J-18 were selected for this study, and their final concentrations of sclerotia were adjusted to equivalents of 200, 100, 75, 50, 25, and 5 sclerotia/g of air-dry soil.

The minimum number of sclerotia of J-11 and J-18 per gram of soil for 90% infection under the environmental conditions used was 100 sclerotia/g of soil when the indicator host was transplanted immediately after incorporation of inoculum into the soil (Fig. 1-D). Infection by D-1-e did not reach 100%, even at 200 sclerotia/g. With all three isolates, as few as 5 sclerotia/g of dry soil were required to obtain at least 20% infection, and 25 sclerotia/g of dry soil were required to obtain over 50% infection.

A similar inoculum density experiment was performed in which the sclerotia of J-11 were added to air-dried natural field soil. The results were not significantly different from those illustrated in Fig. 1-D.

**Susceptibility of various *Allium* spp. to *S. cepivorum*.**

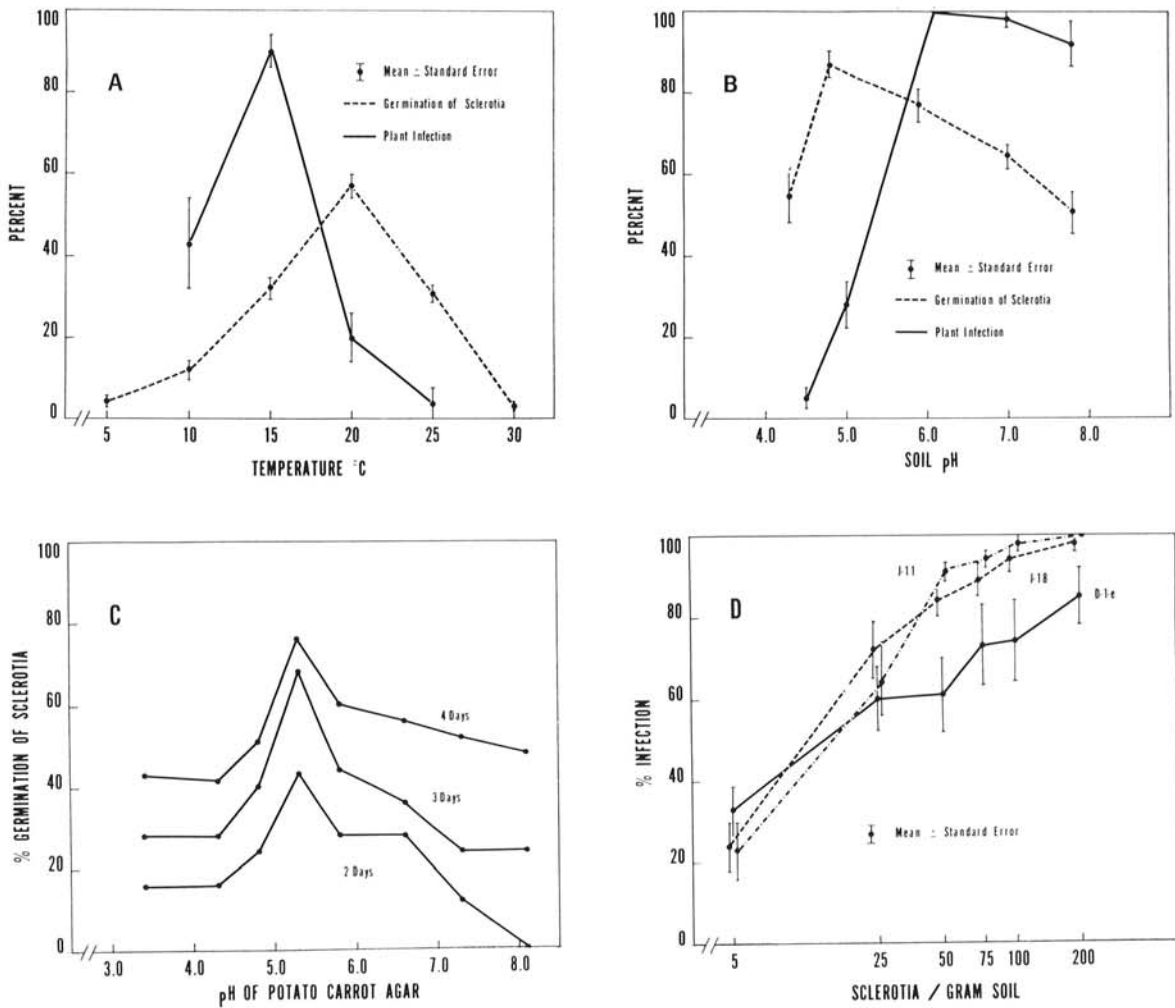


Fig. 1. A) Effect of soil temperature on germination of sclerotia of *Sclerotium cepivorum* and on onion white rot severity. B) Effect of soil pH on germination of sclerotia of *S. cepivorum* and on onion white rot severity. C) Effect of pH of potato carrot agar on germination of sclerotia. D) Effect of inoculum density of three isolates of *S. cepivorum* on onion white rot severity.

The following *Allium* spp. were evaluated for susceptibility to isolate J-11: *A. ampeloprasum* L., *A. caeruleum* Pallas, *A. cernuum* Roth, *A. ferreri* Stearn, *A. fistulosum* L., *A. fuscoviolaceum* Tom., *A. galanthum* Kar. & Kir., *A. longicuspis* Regel, *A. odorum* L., *A. pulchellum* G. Don, *A. ramosum* L., *A. roylei* Stearn, *A. sativum* L., *A. schoenoprasum* L., *A. senescens* L., and *A. tuberosum* Rottler ex Sprengel. The following cultivars of *A. cepa* were included in the experiment for comparison: Evergreen Bunching, Sweet Spanish, White Skins, and Yellow Globe Danvers. The soil for this experiment was uniformly infested with an unknown number of sclerotia. Of these species and cultivars in the first experiment, only *A. caeruleum* and *A. fuscoviolaceum* had less than 50% infection. In subsequent repetitions of the experiments with these two species, *A. fuscoviolaceum* failed to resist infection, whereas *A. caeruleum* generally resisted infection.

**DISCUSSION.**—Sclerotia of *S. cepivorum* germinate

in two different ways, depending on the environmental conditions. On agar or in autoclaved soil, hyphae emerge from the sclerotium within a period of about 24-48 hr (6). In contrast, hyphal plug germination occurs in natural soil and requires an incubation period of at least 2 weeks (5). We have never observed the hyphal plug type of germination; therefore, our results on germination refer to the hyphal type of germination.

Coley-Smith (5) determined the effect of soil pH on hyphal plug germination in three different natural soils. Germination was 20, 65, and 95% in soils with pH values of 4.8, 6.0, and 7.6, respectively. Our data (Fig. 2-B), however, are not in agreement with those of Coley-Smith. The discrepancy may be due to the fact that we actually studied hyphal germination in a single soil adjusted to various pH levels, whereas he studied hyphal plug germination in three different soils varying in pH values.

Harrison (7) reported that a strain of *S. cepivorum*

from the Colac District of Victoria, Australia, grew best in acid soils of pH 5.0-6.5, and was restricted in alkaline soils of pH 7.5 and above. He further stated that white rot had not been reported from natural limestone areas of Victoria, which had a pH of 8.0 or more. Our results (Fig. 1-B), however, indicate that white rot can be severe in soils with pH values from 5.5 to at least 7.8. In the state of Washington, *S. cepivorum* causes severe losses in fields with a soil pH ranging from 6.8 to 7.7 (C. W. Stambaugh, *personal communication*). In addition, Asthana (3) and Tims (12) reported that growth of *S. cepivorum* occurred in culture at pH levels ranging from 2.0 to ca. 9.0.

Little is known about the inoculum levels required to obtain a given amount of disease with many soil-borne diseases; and this is particularly true of onion white rot. Our data on inoculum density (Fig. 1-D) show that even five sclerotia/g of soil may cause an appreciable amount of disease. An approximate 20% infection of onions in the field would undoubtedly result in appreciable economic loss. McCain (10) isolated four to eight sclerotia of *S. cepivorum* from 50 g of naturally infested soil. However, he did not indicate how severe white rot was in that particular soil sample. With the exception of McCain's work, no other data are available on the inoculum density of naturally infested commercial fields in the onion-growing areas of the United States. Except for the selective indicator host method and McCain's wet sieving method (10), no other methods or selective media are known for evaluating inoculum density in the field.

No resistance was encountered in various *Allium* spp. to *S. cepivorum*. Only *A. caeruleum* appeared to possess some resistance to isolate J-11. Further testing with other isolates under field conditions is desired.

## LITERATURE CITED

- ADAMS, P. B., & G. C. PAPAIVIZAS. 1970. Onion white rot caused by *Sclerotium cepivorum* as affected by soil temperature, pH, and inoculum density. *Phytopathology* 60:1281 (Abstr.).
- ALDRICH, R. A., & P. E. NELSON. 1969. Equipment for aerated steam treatment of small quantities of soil and soil mixes. *Plant Dis. Repr.* 53:784-788.
- ASTHANA, R. P. 1947. Studies on sclerotium-forming fungi. I. *Sclerotium cepivorum* Berk. and *S. tuliparum* Klebahn. Part 1. Cultural studies. *Indian Acad. Sci. Proc.* 26 Sect. B:93-107.
- ASTHANA, R. P. 1947. Studies on sclerotium-forming fungi. I. *Sclerotium cepivorum* Berk. and *S. tuliparum* Klebahn. Part 2. Symptoms, mode of infection and host range. *Indian Acad. Sci. Proc.* 26 Sect. B:108-116.
- COLEY-SMITH, J. R. 1960. Studies on the biology of *Sclerotium cepivorum* Berk. IV. Germination of sclerotia. *Ann. Appl. Biol.* 48:8-18.
- COLEY-SMITH, J. R., J. E. KING, D. J. DICKINSON, & R. W. HOLT. 1967. Germination of sclerotia of *Sclerotium cepivorum* Berk. under aseptic conditions. *Ann. Appl. Biol.* 60:109-115.
- HARRISON, D. E. 1954. White rot of onions in Victoria. *J. Dep. Agr. Victoria* 52:510-520, 525.
- HOAGLAND, D. R., & D. I. ARNON. 1950. The water-culture method of growing plants without soil. *Calif. Agr. Exp. Sta. Circ.* 347. 32 p.
- LOCKE, S. B. 1968. Experimental control of onion white rot by means of soil chemicals. *Plant Dis. Repr.* 52:272-276.
- MCCAIN, A. H. 1967. Quantitative recovery of sclerotia of *Sclerotium cepivorum* from field soil. *Phytopathology* 57:1007 (Abstr.).
- TIMS, E. C. 1942. White rot of *Allium* in Louisiana. *Plant Dis. Repr.* 26:219-221.
- TIMS, E. C. 1948. White rot of shallot. *Phytopathology* 38:378-394.
- WALKER, J. C. 1924. White rot of *Allium* in Europe and America. *Phytopathology* 14:315-322.
- WALKER, J. C. 1926. The influence of soil temperature and soil moisture upon white rot of *Allium*. *Phytopathology* 16:697-710.