Sporidesmium sclerotivorum: Distribution and Function in Natural Biological Control of Sclerotial Fungi

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ABSTRACT

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The mycoparasite *Sporidesmium sclerotivorum* was detected in soil samples from fields in Arizona, California, Louisiana, Maryland, Michigan, New Jersey, New York, North Carolina, Oregon, and Washington. In a field test with natural populations of *S. sclerotivorum* and *Sclerotinia minor*, the mycoparasite apparently was responsible for the decline in the numbers of sclerotia of *S. minor*. A second field test showed that *S. sclerotivorum* applied to soil at the rate of 100 spores per gram of soil was responsible for a similar decline in the survival of sclerotia. Evidence is presented that indicates that *S. sclerotivorum* was responsible for the natural decline of sclerotia of *Sclerotinia sclerotiorum* and *Sclerotium cepivorum* in a number of field soils.

Additional key words: Sclerotinia minor, Sclerotium cepivorum.

Sclerotia are the principal survival structures of many soilborne fungi (3,6). Depending upon the species in question, sclerotia of *Sclerotinia* spp. survive in soil for 4–8 yr (3). Coley-Smith (5) shows that more than 90% of the sclerotia of *Sclerotium cepivorum* Berk., the onion white rot pathogen, survives in natural soil for 4 yr and that soil pH, soil nutrient status, or inorganic supplements to the soil do not reduce survival. Adams and Ayers (3) suggested that the biological component of the soil most significantly affects survival of sclerotia of *Sclerotinia* spp. *Coniothyrium minitans* Campbell and certain isolates of *Trichoderma* species appear to be firmly established as mycoparasites of sclerotia of *Sclerotinia* spp. and in some soils may be responsible for natural destruction of

sclerotia (3).

Recently, a new mycoparasite of sclerotia of *Sclerotinia* spp. was described (7) and named *Sporidesmium sclerotivorum* Uecker, Ayers, and Adams. Subsequently, sclerotia of *S. cepivorum* were found to be parasitized by *S. sclerotivorum* (4). Introduction of this mycoparasite into natural soil resulted in a 95% reduction of the inoculum density of *Sclerotinia minor* Jagger (4) within 10 wk. An unusual property of *S. sclerotivorum* was its ability to grow through soil from one sclerotium to another, producing many new condidia throughout the soil mass.

This study was conducted to determine the geographical distribution of *S. sclerotivorum* in soils of the United States and to determine whether it is a factor in the natural decline in survival of sclerotia of *S. cepivorum* and species of *Sclerotinia.*

MATERIALS AND METHODS

S. sclerotivorum was detected in soil samples by a simple baiting technique. Sclerotia of *S. minor*, sieved from autoclaved oat cultures (4), were mixed into the soils (2 g/ 100 g of soil), which were then moistened to −8 bars or greater in 250-ml beakers. The beakers were covered with plastic film to reduce moisture loss and incubated at 20–25 C. After 4 wk, sclerotia were retrieved from the soils by wet sieving (2) and were distributed over the surface of moistened filter paper in two 9-cm-diameter petri dishes (25 sclerotia per dish). After two additional wk at 25 C, the sclerotia were examined microscopically for the macroconidia and hyphae of *S. sclerotivorum* characteristic of infection. Results were recorded as the percentage of total sclerotia infected. If the assay was negative after the sclerotia had been in soil 4 wk, the assay was continued for an additional 4 wk.

In an attempt to relate infection of sclerotia and inoculum density of the mycoparasite by the baiting technique, aqueous suspensions of macroconidia of *S. sclerotivorum* at concentrations of 10,000, 1,000, 100, and 10 macroconidia per milliliter were prepared. To 100 g of moist field soil in 250-ml beakers, 1 ml of each spore concentration and 2 g of sclerotia of *S. minor* were added separately. There were four replications in this experiment. After 4 wk at 25 C, the percentage of sclerotia infected by *S. sclerotivorum* was determined as described above.

The soil samples furnished by farmers, graduate students, and plant pathologists acknowledged above were partially air-dried so that they could be sieved through a 2-mm screen to remove stones and other large particles. Subsamples of 100 g were assayed for *S. sclerotivorum* by the baiting technique.

An experiment was performed in the field at Beltsville, MD, to determine the effect of a natural infestation of *S. sclerotivorum* on a natural population of *S. minor* sclerotia. The soil in this field, Rumford loamy sand, contained 1.95% organic matter and had a pH of 6.4. This field was the one in which *S. sclerotivorum* was first detected and from which it was first isolated in 1976 (7). In October 1977, four plots in the field planted to lettuce developed 35–65% lettuce drop. These plots in March 1978 contained sclerotia of *S. minor* naturally produced on diseased lettuce plants (11–29 sclerotia per 100 g of soil) as well as the mycoparasites at unknown densities.

In March 1978, these four plots (1.5 × 3 m) were rototilled and left fallow. At 4- to 5-wk intervals, 10 subsamples were collected and pooled from each plot, and the pooled samples were assayed for the inoculum density of *Sclerotinia minor* (2). The retrieved sclerotia were counted to determine survival and were plated out on moist filter paper and examined for infection by *S. sclerotivorum.* The results were expressed as percentage survival based on the initial sclerotial inoculum density in the soil in March 1978, and the percentage of sclerotia infected by the mycoparasite. Each plot was rototilled periodically to inhibit the growth of weeds. Control plots (plots with natural populations of *S. minor*, but no *S. sclerotivorum*) were not available for this field study.

In another field at Beltsville, an experiment was performed to determine whether *S. sclerotivorum* could be introduced into the field and reduce the number of sclerotia of *S. minor.* In the fall of 1977, 3- × 3-m plots were established and 100 lettuce seedlings were planted in each plot. As the crop approached maturity each plant was inoculated with *S. minor* grown on autoclaved oat seeds. The diseased plants were left on the plots over the winter of 1977-1978 to increase the number of sclerotia produced on the plant tissue. In the spring of 1978 each plot was rototilled and again planted to lettuce seedlings. By mid-May 1978 each plot had 85–100% lettuce drop. On 24 May 1978, *S. sclerotivorum* grown on a sand-sclerotia medium (4) was rototilled into five plots to a depth of 15 cm at a rate to provide 100 macroconidia of the mycoparasite per gram of soil; five other plots were left untreated to serve as controls. Immediately after adding the mycoparasite and at 4-wk intervals thereafter, soil samples were taken from each plot and brought to the laboratory for analysis. The samples were assayed for the inoculum density of *S. minor* (2) and for the number of sclerotia infected with *S. sclerotivorum.* The field plots were rototilled at about 2-wk intervals to prevent the establishment of weeds during the summer of 1978. The soil in this field was Elkton silt loam with 3.7% organic matter and a pH of 6.2.

A number of fields in southern New Jersey were assayed for the inoculum density of sclerotia of *S. cepivorum* for a number of years. The inoculum density was determined by a wet-sieving method previously described (2). At certain assay periods these fields were also assayed for the presence of *S. sclerotivorum* by the baiting technique with sclerotia of *S. minor.*

TABLE 1. Distribution of *Sporidesmium sclerotivorum* in soil samples from selected fields in various states as determined by a baiting method

State	No. of fields sampled	No. of fields with *S. sclerotivorum*[a]	Recent crop history of fields sampled
Arizona	1	1	Lettuce
California	11	4	Lettuce, onion
Louisiana	1	1	Sugarcane, soybean
Maryland	18	9	Soybean, corn, sesame, tomato, turf
Michigan	1	1	Bean
Nebraska	3	0	Bean
New Jersey	17	11	Lettuce, onion
New York	3	1	Lettuce
North Carolina	1	1	Alfalfa
North Dakota	1	0	Bean
Oregon	5	4	Potato
Virginia	2	0	Corn, peanut
Washington	9	1	Potato, mint, onion, sunflower
Wisconsin	1	0	Soybean

[a] *S. sclerotivorum* was detected by a baiting technique with sclerotia of *Sclerotinia minor* recovered from soil after 4 and 8 wk.

RESULTS

Effect of macroconidia concentration on infection of sclerotia. Application of *S. sclerotivorum* to *Sporidesmium*-free soil in the laboratory at 10 and 100 macroconidia per gram of soil resulted in 80.5 ± 14.1, and 98.5 ± 1.0% infection, respectively, of added sclerotia after 4 wk. At the low concentrations, one-tenth or one, macroconidium per gram of soil, only one sclerotium became infected during 4 wk in soil. The results suggest that *S. sclerotivorum* can be detected in soils at levels as low as 10 macroconidia per gram of soil. There was a general trend indicating that the percentage of sclerotia infected increased with the concentration of macroconidia.

Distibution of *S. sclerotivorum*. *S. sclerotivorum* was detected in soils from 10 of 14 states in a limited survey of soil samples sent to our laboratory (Table 1). About 46% of the soils from the fields assayed by baiting with sclerotia of *S. minor* gave positive evidence for the presence of the mycoparasite. Most of the fields sampled had a cropping history of hosts of *Sclerotinia* spp. or *S. cepivorum*.

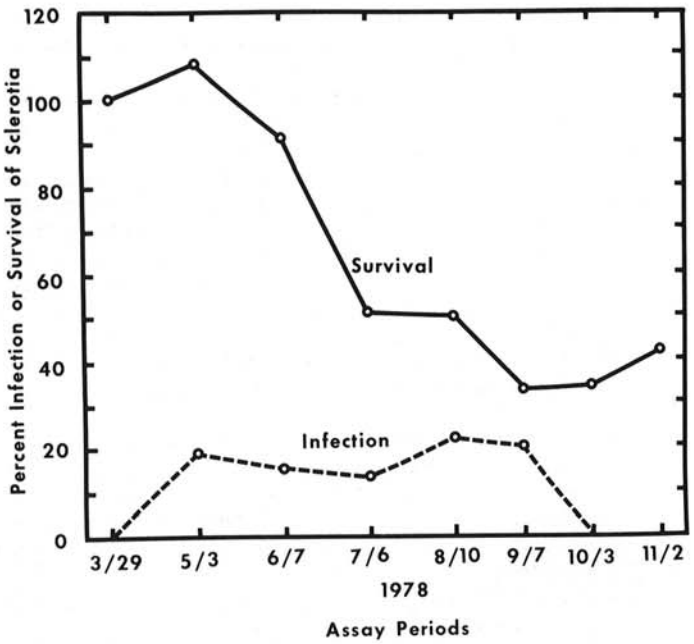

Fig. 1. The effect of a natural population of *Sporidesmium sclerotivorum* on infection of sclerotia of *Sclerotinia minor* and their subsequent survival in the field.

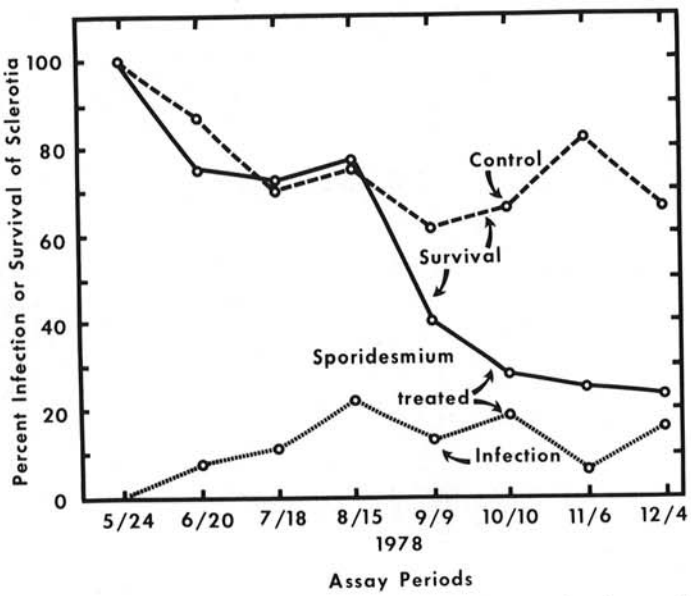

Fig. 2. The effect of *Sporidesmium sclerotivorum* added to soil at the rate of 100 macroconidia per gram of soil on infection and survival of sclerotia of *Sclerotinia minor* in the field.

Effect of *S. sclerotivorum* on survival of sclerotia. In a field experiment designed to determine the effect of a natural *S. sclerotivorum* population on the survival of sclerotia of *S. minor*, recovery of sclerotia of *S. minor* declined from about 90% in June to about 30% in September, for about a 65% reduction in the inoculum density of the plant pathogen (Fig. 1). At each assay period during the summer of 1978 from 13 to 22% of the sclerotia were infected with *S. sclerotivorum*. *S. sclerotivorum* was active in the field from early May to early September, as indicated by the assays, where infected sclerotia were obtained.

In the field test in which *S. sclerotivorum* was added to the field plots the inoculum density of *S. minor* ranged from 12 to 37 sclerotia/100 g of soil in May 1978. By October, the survival of the sclerotia in the plots treated with *S. sclerotivorum* declined to less than 30%, whereas that in the control plots was greater than 65% (Fig. 2). Infection of the sclerotia in the treated plots during the summer varied between 0 and about 20% (Fig. 2). None of the sclerotia in the control plots were found to be infected with *S. sclerotivorum*.

The presence of *S. sclerotivorum* in soil was correlated with a decline in the numbers of sclerotia of *S. cepivorum* in fields PT-44, R-19, and PT-46 in southern New Jersey (Table 2). Some of these fields, used to grow fall-planted *Allium* spp., were assayed at different times for inoculum density of *S. cepivorum* and for white rot disease severity. In the soils from these fields, infection of *S. minor* sclerotia by *S. sclerotivorum* as determined by the baiting technique after 4 wk, was 100, 40, and 6%, respectively.

In field PT-45 there was a reduction in the inoculum density of *S. cepivorum*, and the mycoparasite was detected in this soil at the 8-wk assay. In fields PT-11 and PT-47 there was either little change or an increase in the inoculum density of *S. cepivorum* and *S. sclerotivorum* was not detected at either 4 or 8 wk.

TABLE 2. Inoculum density of *Sclerotium cepivorum*, and presence or absence of *Sporidesmium sclerotivorum* in soil from fields in southern New Jersey

Field	Date	Inoculum density (sclerotia/100 g of soil)	Presence of [a] *S. sclerotivorum*
PT-44	Apr. 1978	26	
	May 1978		+
	Sept. 1978	0	
R-19	May 1976		
	June 1976	43	
	May 1978	20	+
	Oct. 1978	4	
PT-46	Apr. 1978	26	
	May 1978		
	Sept. 1978	0	
	Oct. 1978		+
PT-45	Apr. 1978	34	
	May 1978		+
	Sept. 1978	3	
PT-11	Oct. 1977	2	
	Apr. 1978		
	Sept. 1978	14	
	Oct. 1978		−
PT-47	Apr. 1978	10	
	May 1978		−
	Sept. 1978	6	
	Oct. 1978		−

[a] Presence (+) or absence (−) of *S. sclerotivorum* as detected by a baiting technique with sclerotia of *Sclerotinia minor* recovered from soil after 4 and 8 wk.

Soil samples from several potato fields in Oregon were tested for *Sporidesmium*. These fields were put into agricultural production within the past 3–4 yr with center-pivot irrigation. In the four field soils that had given rise to moderate to severe stem rot (*Sclerotinia sclerotiorum*), infection of the sclerotia by *S. sclerotivorum*, as determined by the baiting technique, was 16, 6, 0, and 0%, whereas in the field that had little stem rot it was 92%.

DISCUSSION

The baiting technique was useful for determining the presence of *S. sclerotivorum* at population levels as low as 10 macroconidia per gram of soil. In addition, a soil in which infection of *S. minor* sclerotia by *S. sclerotivorum* after a 4-wk incubation period is 90% should have a higher population of the mycoparasite than a soil in which infection of sclerotia after a 4-wk period is only 10%.

The distribution of *S. sclerotivorum* in the United States appears to be widespread. The results indicated that *S. sclerotivorum* was present in many areas and could survive a wide variety of soil conditions. The crop history of the soils appeared to have little influence on the presence of the mycoparasite, perhaps in part because of the extensive host range of *Sclerotinia* spp. (3).

The mycoparasite was not detected in all soils assayed from a given state. For example, it was found in soils from nine of the 18 fields tested at the Beltsville Agricultural Research Center. Because *S. sclerotivorum* was found in fields in most regions of the United States and because only a limited number of soil samples were tested, we suspect that it may be even more widely distributed in the United States than is indicated in Table 1.

The results of the field test at Beltsville (Fig. 1) indicate that the natural level of *S. sclerotivorum* was sufficient to substantially reduce the inoculum density of *S. minor*. We cannot be certain that all of this decline in the number of *S. minor* sclerotia was due to the effects of *S. sclerotivorum*, since suitable control plots were not available. However, the extent of infection of the sclerotia by *S. sclerotivorum* from May through September strongly suggested that the mycoparasite was largely responsible.

In 1975, Adams (1) reported a similar decline in survival of *S. minor* in this same field during the summer of 1973. In that paper it was suggested that the decline in the inoculum denisty of *S. minor* was probably due to the detrimental effects of drying and remoistening of the soil. Because of the results reported herein, and the proven ability of *S. sclerotivorum* to completely destroy the pathogen in soil (4), we now suggest that the reduction in the inoculum density reported in that paper was most likely due to the then unknown *S. sclerotivorum*.

In the field test in which *S. sclerotivorum* was added to *S. minor*-infested soil, control plots were included in the experiment. The results of this field test were similar to those of the other field test and substantiate those results.

The information obtained from soils from six fields on two farms in New Jersey add more evidence that *S. sclerotivorum* is a major factor in the natural decline of sclerotia in soil. These soils were naturally infested with various levels of sclerotia of *S. cepivorum*. We previously showed that *S. sclerotivorum* could infect sclerotia of *Sclerotium cepivorum* (4).

The few reports available concerning the survival of *S. cepivorum* sclerotia in soil suggest that these sclerotia generally survive for 4 yr or more (5), yet in field R-19 (Table 2) there was a 90% reduction in the inoculum density of *S. cepivorum* and *S. sclerotivorum* was present. In a nearby field (PT-11) (less than 200 m distant) *S. sclerotivorum* was absent and the inoculum density increased from two to 14 sclerotia per 100 g of soil.

On another farm, in the three fields that contained *S. sclerotivorum* (PT-44, -45, -46) the inoculum density of *S. cepivorum* declined by 90–100%. In the fourth field (PT-47), within 50 m of the other three fields, *S. sclerotivorum* could not be detected and the inoculum density of *S. cepivorum* declined by only 40%.

S. sclerotiorum also has been shown to be a host for *S. sclerotivorum* (4). The data obtained from the five soil samples from Oregon potato fields suggest that *S. sclerotivorum* also may be involved in the natural decline of sclerotia of this *Sclerotinia* species.

The information obtained from the fields in New Jersey and Oregon as well as the results from the two field tests in Beltsville suggest that *S. sclerotivorum* is a major factor in the natural decline of *S. cepivorum* and *Sclerotinia* species. Other organisms may also be involved, either directly, as primary mycoparasites, or indirectly, as secondary microorganisms (3).

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