

Factors Affecting Parasitic Activity of *Sporidesmium sclerotivorum* on Sclerotia of *Sclerotinia minor* in Soil

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ABSTRACT

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Laboratory experiments indicated that the newly described mycoparasite *Sporidesmium sclerotivorum* may be a useful agent for the control of plant diseases caused by *Sclerotinia minor*. In field soils to which sclerotia of *S. minor* and inoculum of *S. sclerotivorum* were added, infection of the sclerotia by the mycoparasite began within 2 wk, and the sclerotia were completely destroyed within about 10 wk under appropriate environmental

conditions. The optimum conditions for infection and decay of sclerotia in soil were 20–25 C, pH 5.5–7.5, and soil water potentials of –8 bars and higher. Infection and decay of sclerotia occurred after a sand-sclerotia culture of *S. sclerotivorum* was added to soils at rates as low as 2×10^3 spores per 100 g of soil.

Recently, a new species of *Sporidesmium* was described (5) as a mycoparasite of sclerotia of *Sclerotinia sclerotiorum* (Lib.) de Bary [*Whetzelinia sclerotiorum* (Lib.) Korf & Dumont]. Proof of its parasitic activity on sclerotia of *S. sclerotiorum*, *S. minor* Jagger, and *Sclerotium cepivorum* Berk. was subsequently reported (3) and it was suggested that this mycoparasite, designated as *Sporidesmium sclerotivorum* Uecker, Ayers, and Adams (5), may play a role in the natural decline of sclerotia in field soils. The mycoparasite could be introduced into *Sporidesmium*-free soil infested with sclerotia of *S. minor* and cause infection and complete decay of the sclerotia (3). These results suggested that *S. sclerotivorum* might be a useful agent for biological control of plant diseases caused by species of *Sclerotinia* and *Sclerotium cepivorum*.

More recently, it was reported (4) that macroconidia of *S. sclerotivorum* germinated in soil at distances up to 9 mm from a single sclerotium of *S. minor*. Germination of macroconidia on an agar medium was favored when the pH of the medium was between 5 and 7.

A prerequisite to field evaluation of a potential biological control agent is a knowledge of the factors that affect its activity in soil. Thus, laboratory experiments were designed to determine the effects of soil temperature, soil moisture, pH, and inoculum concentration of the mycoparasite on infection and destruction of sclerotia of *S. minor* Jagger by *S. sclerotivorum* in soil.

MATERIALS AND METHODS

The isolates of *Sporidesmium sclerotivorum* and *Sclerotinia minor* were the same as those used in our previous studies (1,3–5).

S. sclerotivorum was cultured on nonsterile moist quartz sand containing sclerotia of *S. minor* (1% by weight) freshly collected from aseptically grown sterilized oat cultures (1) in culture dishes. The *Sporidesmium* cultures were grown for at least 4 wk and produced 200,000 macroconidia per gram sand-sclerotia medium. Sand-sclerotia cultures of *Sporidesmium* were added (1%, w/w) to 100 g of soil in 250-ml beakers in all experiments, unless otherwise indicated.

Four field soils were used in these studies: Rumford loamy sand

with (1.95% organic matter, pH 6.4) from the field in Beltsville, MD, in which *S. sclerotivorum* originally was discovered; Elkton silt loam (3.7% organic matter, pH 6.8) also from Beltsville, but from a field in which *S. sclerotivorum* rarely had been detected; Norfolk sandy loam (1.62% organic matter, pH 5.7) from a peanut field near Courtland, VA, with a history of *Sclerotinia* blight, but with no detectable *S. sclerotivorum*; and a similar *S. sclerotivorum*-free Norfolk sandy loam from a field in Salisbury, MD.

Sclerotia of *S. minor* were obtained from 5-wk-old cultures grown on sterilized oats (1). The cultures were wet sieved on nested 2 mm- and 355- μ m sieves and the sclerotia collected on the latter sieve were used to infest the soils for each experiment. Fresh *S. sclerotiorum* sclerotia (not dried) were added (1%, w/w) to the soil; inoculum densities ranged 5–18 sclerotia per gram of soil, depending on the size of the sclerotia.

All experiments were carried out in beakers covered with plastic film to prevent loss of water from the soil. Each experiment included four replications, and each experiment was repeated at least once with a different soil. The soil pH was adjusted either with $\text{Ca}(\text{OH})_2$ (hydrated lime) or $\text{Al}_2(\text{SO}_4)_3 \cdot 18 \text{H}_2\text{O}$ (aluminum sulfate) 8–12 wk prior to the experiment. Except for the temperature experiments, all experiments were incubated at 25 C. Controls for each experiment consisted of soils infested with sclerotia but lacking added *Sporidesmium* inoculum.

At the beginning of each experiment, and at 2-wk intervals thereafter, the inoculum concentration of *S. minor* in the soils was determined by collecting the sclerotia from weighed soil samples on 355- μ m sieves (2). Either 25 or 50 sclerotia were placed on moist filter paper in 9-cm-diameter petri dishes (25 sclerotia per dish), and the dishes were incubated for 2 wk at 25 C to determine the percentage of sclerotia infected by *S. sclerotivorum* (2). Infection was determined by the appearance of typical macroconidia of the mycoparasite over the surface of the sclerotia. With this procedure we monitored the percentage of *S. sclerotivorum*-infected sclerotia and the percentage of surviving *S. minor* sclerotia during the course of each experiment. Percentage survival of sclerotia in a given treatment was calculated as the percentage of that in the *Sporidesmium*-free control for that treatment at each assay period.

In most experiments, laboratory-produced sclerotia of *S. minor* and the inoculum of *S. sclerotivorum* were added to the soil at the same time. In other experiments the sclerotia were added to the soil 6–8 wk prior to the addition of the *S. sclerotivorum* inoculum. This

was done in an attempt to acclimatize the sclerotia to soil conditions and to render them more like natural sclerotia.

Soil samples were taken at the beginning, during, and at the end of each experiment for pH determinations. The pH was determined 1 hr after adding distilled water to the soil samples at soil:water ratios of 1:2.5 (v/v).

A soil moisture curve was determined for the soils at various moisture levels with a Wescor HR-33T Dew Point Microvoltmeter (Wescor, Inc., Logan, UT 84321). Water potential of the soil was measured by the dew point method.

RESULTS

Temperature effects. Experiments on the infection of *S. minor* sclerotia by *S. sclerotivorum* and the survival of these sclerotia were carried out in Elkton silt loam, Norfolk sandy loam, and Rumford loamy sand at 10, 15, 20, 25, and 30 C all with similar results. In these experiments, infection of sclerotia of *S. minor* by *S. sclerotivorum* began within 4 wk and increased to about 80–90% within 6 wk at 20–25 C (Fig. 1). Decline in survival of the *S. minor* sclerotia corresponded with increase in infection (Fig. 1). Survival of sclerotia declined slightly in soil incubated at 30 C even though infections by *S. sclerotivorum* rarely were observed. We assume that this decline was due to the action of other microorganisms frequently observed on the sclerotia. When about 70–80% of the sclerotia were infected with *S. sclerotivorum*, the soil mass in the beakers was held together by the brown-pigmented mycelium of the mycoparasite, a phenomenon that also was observed in other experiments.

Soil pH effects. The effect of soil pH on infection and viability of sclerotia of *S. minor* by *S. sclerotivorum* was similar in Elkton silt loam, Norfolk sandy loam, and Rumford loamy sand. Within 2 wk, a significant number of sclerotia were infected by the mycoparasite at pH levels between 5.5 and 7.0. At 6 wk, over 75% of the sclerotia in soil at pH 5.5–6.5 were infected with *S. sclerotivorum* (Fig. 2). Little or no infection occurred below pH 5.0. Decline in survival of sclerotia lagged behind infection by about 2 wk.

The pH values of the soils in these experiments remained fairly constant during the course of the experiment. The exception to this was the soil sample adjusted to pH 8.7, which dropped to pH 8.3.

Soil moisture effects. The effects of soil moisture on infection and survival of sclerotia of *S. minor* by *S. sclerotivorum* was studied in Elkton silt loam, Norfolk sandy loam, and Rumford loamy sand. Results in these soils were similar in that *S. sclerotivorum* readily infected sclerotia of *S. minor* at water potentials of –8 bars and higher in all experiments. Infection and decline in survival of sclerotia due to *S. sclerotivorum* was favored by high soil moistures (Fig. 3).

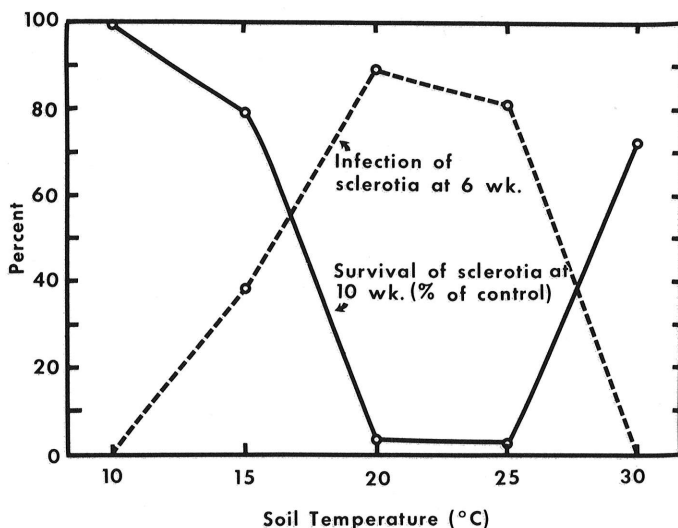


Fig. 1. Effect of soil temperature on infection by *Sporidesmium sclerotivorum* and survival of sclerotia of *Sclerotinia minor* in soil incubated for 6 and 10 wk.

Inoculum concentration effects. *Sporidesmium sclerotivorum* was added to soil in sand-sclerotia medium. This medium usually allowed the production of about 2×10^5 macroconidia per gram of medium. On this basis, the concentrations of the *S. sclerotivorum* inoculum were 0.2×10^3 , 2×10^4 , 2×10^5 , and 2×10^6 macroconidia per 100 g of soil. Results obtained in Norfolk sandy loam and Elkton silt loam were similar in that infection of sclerotia occurred at the lowest inoculum level tested (Fig. 4). As the inoculum concentration was increased to 2×10^6 , infection and decline in survival of the sclerotia occurred more rapidly.

Susceptibility of "naturalized" sclerotia. In the foregoing experiments, sclerotia of *S. minor* and the inoculum of *S. sclerotivorum* were added to soil at the same time. In similar experiments, sclerotia were added to the soil 6–8 wk prior to the addition of the *S. sclerotivorum* in an attempt to render them more like natural sclerotia. Results of these experiments did not reveal substantial differences between the susceptibility of fresh and "naturalized" sclerotia. For example, in the temperature experiment in which sclerotia of *S. minor* were added to the soil

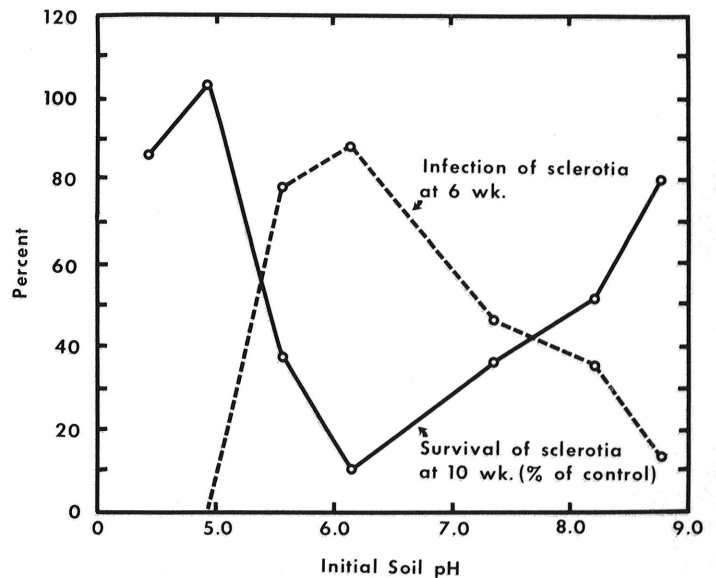


Fig. 2. Effect of soil pH on infection by *Sporidesmium sclerotivorum* and survival of sclerotia of *Sclerotinia minor* in soil incubated at 25 C for 6 and 10 wk.

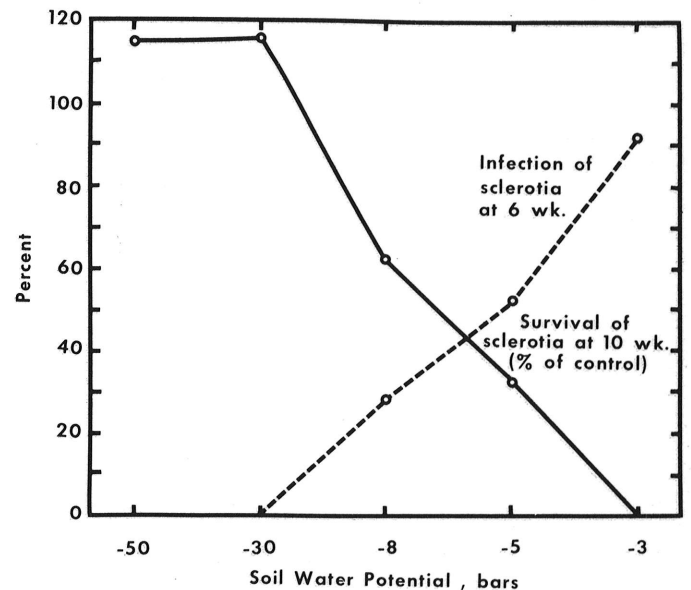


Fig. 3. Effect of soil moisture on infection by *Sporidesmium sclerotivorum* and survival of sclerotia of *Sclerotinia minor* in soil incubated at 25 C for 6 and 10 wk.

with the *S. sclerotivorum* inoculum, infection of the sclerotia at 6 wk was 76% and survival of the sclerotia at 10 wk was 4%. When sclerotia of *S. minor* was added to the soil 6 wk prior to the addition of *S. sclerotivorum* inoculum, infection of the sclerotia was 81% and survival was 2% at 6 and 10 wk, respectively.

DISCUSSION

In the experiments presented above the initial inoculum densities of *S. minor* were 500–1,800 sclerotia per 100 g of soil. The natural inoculum densities of this fungus appear to range from less than 1 to as high as 100 sclerotia per 100 g of soil (2). The reason for using such a high initial inoculum density of *S. minor* was to obtain a sufficient number of sclerotia to determine the percentage of *S. sclerotivorum*-infected sclerotia at each assay period. To conduct these experiments at inoculum densities of 25–50 sclerotia per 100 g soil would have required prohibitively large quantities of soil and

incubators of a size not available in most laboratories. Thus, although the sclerotial concentrations used were unnaturally high, the results of the experiments revealed the environmental conditions favoring the mycoparasite.

The ideal mycoparasitic biological control agent should be active over a broad temperature range, about 10–30 C; be active over the entire agricultural pH range, pH 5–8; be active over a broad soil-moisture range; be effective when applied to fields at “low” rates; be able to move through soil and to propagate itself; and be able to attack its host in the presence of other soil organisms. *S. sclerotivorum* is very active at 20 C and 25 C, less active at 15 C, and exhibits little or no activity at 10 C and 30 C (Fig. 1), thus, it rates only fair in this attribute. It is very active in soil over the pH range of 5.5–7.0 (Fig. 2), and thus should be active in soil within the normal pH range of many agricultural soils. *S. sclerotivorum* is very active at soil moistures above –8 bars (Fig. 3). When added to soils at the rate of 2,000 spores per 100 g of soil, *S. sclerotivorum* infected sclerotia of *S. sclerotiorum* within 4 wk (Fig. 4). *S. sclerotivorum* appears to be equally active in the several soil types examined (sandy loam, loamy sand, and silt loam). In addition, *S. sclerotivorum* appears to infect fresh as well as “naturalized” sclerotia equally well. These laboratory results, coupled with the ability of the mycoparasite to spread through soil (3) suggest that *S. sclerotivorum* has unusual potential as an applied biological control agent against diseases caused by *S. minor* and possibly other sclerotia-producing plant pathogens.

LITERATURE CITED

- ADAMS, P. B. 1975. Factors affecting survival of *Sclerotinia sclerotiorum* in soil. *Plant Dis. Rep.* 59:599-603.
- ADAMS, P. B. 1979. A rapid method for quantitative isolation of sclerotia of *Sclerotinia minor* and *Sclerotium cepivorum* from soil. *Plant Dis. Rep.* 63:349-351.
- AYERS, W. A., and P. B. ADAMS. 1979. Mycoparasitism of sclerotia of *Sclerotinia* and *Sclerotium* species by *Sporidesmium sclerotivorum*. *Can. J. Microbiol.* 25:17-23.
- AYERS, W. A., and P. B. ADAMS. 1979. Factors affecting germination, mycoparasitism and survival of *Sporidesmium sclerotivorum*. *Can. J. Microbiol.* 25:1021-1026.
- UECKER, F. A., W. A. AYERS, and P. B. ADAMS. 1978. A new hyphomycete on sclerotia of *Sclerotinia sclerotiorum*. *Mycotaxon* 7:275-282.

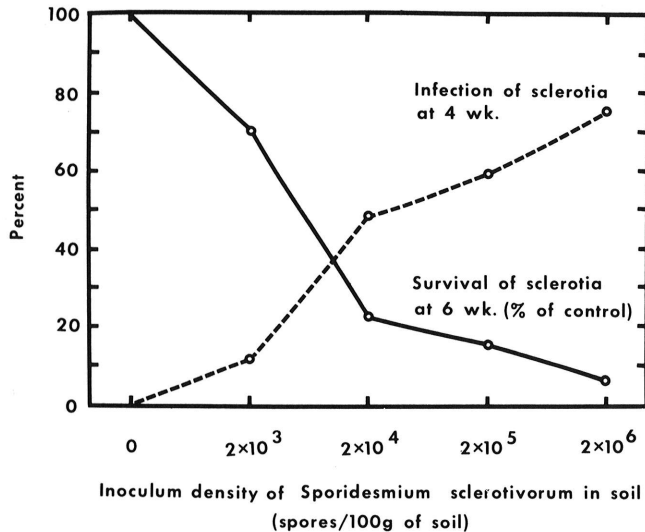


Fig. 4. Effect of concentration of *Sporidesmium sclerotivorum* inoculum added to Elkton silt loam on infection and survival of sclerotia of *Sclerotinia minor* after 6 and 10 wk of incubation at 25 C.