Comparison of Isolates of *Sporidesmium sclerotivorum* in Vitro and in Soil for Potential as Active Agents in Microbial Pesticides

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ABSTRACT

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Thirty isolates of *Sporidesmium sclerotivorum* varied greatly in their growth habits, amount of growth on solid and in liquid media, and their production of macroconidia. The amount of growth in vitro had no effect on the ability of the isolates to parasitize sclerotia of plant pathogens in soil. In soil, six of eight isolates infected sclerotia of *Sclerotinia minor* and *Sclerotium cepivorum* caused their destruction, and produced macroconidia about equally. Two isolates that grew well in vitro were ineffective in parasitizing sclerotia of *Sclerotinia minor* and *Sclerotium cepivorum* in soil. A method for the evaluation of isolates of *S. sclerotivorum* is presented.

Additional key words: biological control, hyperparasite, mycoparasite.

Sporidesmium sclerotivorum Uecker, Ayers & Adams is an effective mycoparasite of sclerotia of at least five economically important plant pathogens (4). A single application of this mycoparasite has been shown to effectively control lettuce drop caused by *Sclerotinia minor* Jagger for three or more successive crops (6,10). The persistent nature of the mycoparasite is due, in part, to its ability to produce about 15,000 macroconidia in soil for each sclerotium parasitized (5). The mycoparasite has been shown to be involved in natural biological control of diseases caused by *Sclerotinia sclerotiorum* (Lib.) de Bary and *Sclerotium cepivorum* Berk. (3). It has also been shown to be widely distributed in the United States and elsewhere in the world (3,7).

During studies on the world distribution of *S. sclerotivorum*, approximately 30 isolates were successfully obtained in pure culture. The growth habit, rate of growth, and sporulation of some isolates were different from those of isolates used in previous studies (8,9).

The present study was performed to compare the 30 isolates of *S. sclerotivorum* for their ability to grow in a liquid medium and sporulate on a commercial production medium, to infect and destroy sclerotia of two host plant pathogens in soil, and to produce macroconidia in soil. The purpose of the work was to develop a laboratory basis for evaluating the potential usefulness and effectiveness of isolates of *S. sclerotivorum* before field testing.

MATERIALS AND METHODS

All isolates of *S. sclerotivorum* were obtained from soil samples baited with sclerotia of host fungi (4). Isolates CS-29 to 33 were collected from a single Wisconsin soil baited with sclerotia of *Sclerotium cepivorum* by E. Valdes (University of Guelph, Guelph, Ontario, Canada). All remaining isolates were from soil samples baited with sclerotia of *Sclerotinia minor* by W. A. Ayers. Isolates CS-16, 17, 18, 24, 25, and 27 were isolated from organic (muck) soils. All other isolates were from mineral soils of various textures. The origin of the isolates is indicated in Table 1.

Fourteen isolates were grown on SM-4 agar medium (8) to determine the variability of their growth habits. All 30 isolates were evaluated for amount of growth in liquid culture at 25 C for 28 days. The isolates were grown in 12.5 ml of SM-4 medium (8) in 250-ml Erlenmeyer flasks capped with aluminum foil (four flasks per isolate). All media were autoclaved at 121 C for 15 min. The flasks were seeded with a small piece of SM-4 agar containing mycelium of the fungus. Mycelial mats were collected by vacuum filtration on tared filter paper, rinsed with water, dried overnight at 95 C, and weighed.

The isolates that yielded 20 mg or more of growth in the previous experiments were evaluated for production of macroconidia by a potential commercial production method. Medium SM-4 was added to vermiculite, Grade 2 (Grace Horticultural Products, W. R. Grace & Co., Cambridge, MA) (15 g of vermiculite + 85 ml of SM-4) in 250-ml flasks, sterilized, and seeded with fragmented mycelial mats of the various isolates. The flasks (four per isolate) were incubated at room temperature (20–23 C). After 15 wk of incubation, the contents of each flask were mixed, and a 5-g sample was assayed for the number of macroconidia. The sample was diluted with 50 ml of water, stirred on a magnetic stirrer for 90 sec and filtered through a 0.13-mm filter. The number of macroconidia in the filtrate was determined with a four-cell Speirs Levy Eosinphil counter (Mausser, Philadelphia, PA). Each estimate of the number of macroconidia in a sample was based on the number of spores in 40 1-mm squares on the counter.

Two fast-growing isolates (CS-1 and 5) and two slow-growing isolates (CS-13 and 25) were compared for their activity in soil. Soil containing about 2,000 sclerotia of *Sclerotinia minor* per 100 g of soil was prepared (5) and 100-g quantities put in 250-ml beakers that were covered with plastic film. Macroconidia of *S. sclerotivorum* were mixed into the soil in each beaker at the rate of 1,000 spores per gram of soil. The soil used in this experiment was a Norfolk sandy loam containing 2.1% organic matter with a pH of 6.4. The soil was screened (3-mm pore size) and kept moist until used. The moisture content of the soil in this experiment was 10%. At weekly intervals, the beakers were weighed, lost water was restored, the soil was mixed, and a 5-g sample taken for assay. Each sample was assayed for inoculum density of sclerotia of *Sclerotinia minor* (2), number of sclerotia infected by *S. sclerotivorum* (5), number of macroconidia of the mycoparasite produced in the soil (5), and germinability of the macroconidia. Germinability of the spores was determined by applying 1 ml of the soil solution containing spores on a 5-μm pore size membrane filter. The filter was then placed on the surface of saturated soil containing about

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1.4×10^3 sclerotia per 100 g of soil. After 7 days of incubation at 25 C, the filters were stained (1) and germinability was determined.

Isolates CS-1, 5, 7, 8, 9, 18, 24, and 25 were evaluated in Norfolk sandy loam for infection and destruction of sclerotia of *Sclerotinia minor* and *Sclerotium cepivorum* mixed together in the same soil. The experiment was performed as outlined above for the slow and fast isolates. Macroconidia of *S. sclerotiorum* obtained from SM-4 agar cultures were added to soil at the rate of 1,000 spores per gram of soil.

Isolates CS-5, 8, and 9 were also evaluated in Norfolk sandy loam for infection and destruction of sclerotia of *Sclerotinia minor* and *Sclerotium cepivorum*. In this experiment, the sclerotia of the two host fungi were not combined in the same soil but kept separate. Macroconidia of *S. sclerotivorum* used to infest these soils were obtained from SM-4 vermiculite medium and were added at the rate of 500 spores per gram of soil. In all soil experiments, there were four replications of the treatments.

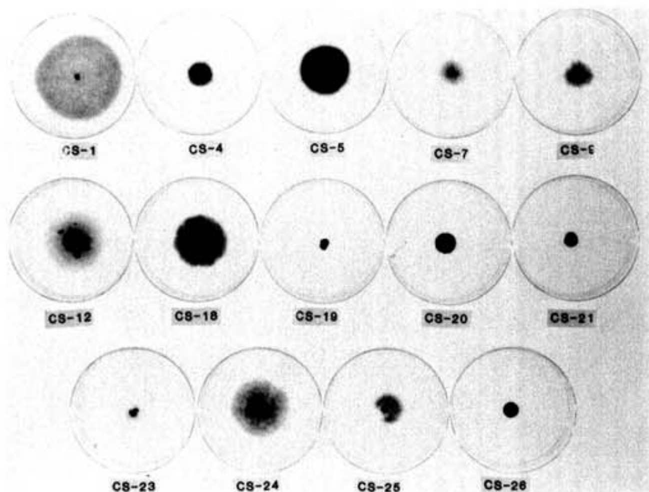

Fig. 1. Growth of single spore isolates of *Sporidesmium sclerotivorum* on SM-4 medium after 28 days incubation at 25 C.

RESULTS

Growth habits of the isolates varied greatly on solid SM-4 medium (Fig. 1). For example, growth of isolate CS-1 was somewhat fluffy with aerial hyphae and the colony spread across most of the plate. Growth of isolates CS-19, 20, 21, and 26 was very dense and compact with very little spreading of the colony. Isolate CS-23 grew very little. Other isolates were intermediate in their growth habit.

The amount of mycelial growth of 30 isolates of *S. sclerotivorum* in liquid culture in two experiments varied from 2 to 65 mg per flask in 28 days at 25 C (Table 1). When the results of the two experiments were averaged, the amount of growth varied from 7 to 57 mg. With two exceptions (CS-4 and 5), the results of the two experiments were fairly consistent. As when grown on a solid substrate, growth of some isolates in liquid culture was diffuse, whereas other isolates grew as dense compact colonies.

The isolates that averaged 20 mg or more of growth in liquid culture were evaluated further for production of macroconidia on SM-4 vermiculite medium. These isolates varied in the production of macroconidia from 0 to 2.2×10^6 spores per gram (fresh weight) of vermiculite medium (Table 1). Isolate CS-8 produced significantly more spores [2.5 times as many macroconidia as as the next best isolate (CS-9)] than any of the other isolates tested.

In soil there were no significant differences among the fast- and slow-growing isolates (Fig. 2 and Table 1). Isolates CS-1, 13, and 25 infected sclerotia at about the same rate, whereas CS-5 infected sclerotia at a slower rate (Fig. 2A). However, all four isolates destroyed the sclerotia of *Sclerotinia minor* in soil at essentially the same rate (Fig. 2B). All four isolates produced macroconidia in soil

TABLE 1. Amount of mycelial growth of isolates of *Sporidesmium sclerotivorum* in liquid SM-4 medium (pH 5.4) at 25 C for 28 days and production of macroconidia on vermiculite saturated with SM-4 medium after 15 wk at 21–23 C

Isolate (CS-) no.	Origin of isolate	Mycelial dry wt (mg)			Spore production on vermiculite medium ($\times 10^3$)
		Exp. 1	Exp. 2	Average	
1	Maryland (Beltsville)	62 a[x]	53 a	57	3 c
8	New Jersey (Fairton)	57 a	54 a	56	2,216 a
32	Wisconsin (Madison)	42 b	57 a	49	28 c
5	Oregon (Herminston)	65 a	22 h–k	43	415 bc
15	California (Salinas)	...[y]	43 b	...	14 c
22	Australia (Tasmania)	39 b	40 bc	39	8 c
19	Australia (Victoria)	37 b	31 ef	34	29 c
31	Wisconsin (Madison)	36 b	30 e–g	33	0 c
7	New Jersey (Landisville)	24 c	40 b–d	32	126 c
20	Japan (Hokkaido)	36 b	24 g–j	30	0 c
9	Mexico (Mexico)	21 c	34 cd	27	875 b
4	Oregon (Hermiston)	2 d	52 a	27	24 c
24	Canada (Quebec)	20 c	28 e–h	24	7 c
12	Canada (Manitoba)	21 c	27 e–h	24	113 c
26	Norway (Vestfold)	24 c	20 i–k	22	4 c
18	Canada (Ontario)	24 c	19 i–k	22	7 c
21	Finland (Helsinki)	24 c	18 i–k	21	32 c
28	Australia (Tasmania)	21 c	18 i–k	19	...
16	New York (Oswego)	10 d	25 f–i	17	...
27	New York (Oswego)	...	19 i–k	...	...
2	New Jersey (Vineland)	10 d	19 i–k	15	...
33	Wisconsin (Madison)	...	15 kl	...	...
13	New Jersey (Fairton)	8 d	18 i–k	13	...
29	Wisconsin (Madison)	9 d	17 j–l	13	...
11	Canada (Saskatchewan)	4 d	22 h–j	13	...
17	New York (Oswego)	...	15 kl	...	...
30	Wisconsin (Madison)	...	15 kl	...	...
25	Canada (Quebec)	6 d	15 kl	10	...
14	New Jersey (Belvidere)	...	10 l	...	...
23	Wisconsin (Madison)	2 d	12 kl	7	...

[x] Values in a column followed by a common letter are not significantly ($P<0.01$) different as determined by Duncan's multiple range test.
[y] Not determined.

at about the same rate (Fig. 2C), although there were significant differences in the final numbers of spores produced (Table 2). The germinability of these spores was similar (82–86%) for isolates CS-5, 13, and 25 and significantly less (42–54%) for isolate CS-1. At weeks 4–8 (Fig. 2), quantities of mycelium of *S. sclerotivorum* could be seen on the assay sieves. At week 6, an attempt was made to isolate this mycelium from each of the soil samples and determine its dry weight. This attempt failed because the mycelium could not be completely separated from fragments of sclerotia, organic matter, and sand grains. However, at weeks 6 and 7, a visual estimate made by two individuals could not detect differences in the amount of mycelium produced by the four isolates.

There were differences among eight isolates of *S. sclerotivorum* in their ability to infect and destroy sclerotia of *Sclerotinia minor* and *Sclerotium cepivorum* in soil (Table 3). Infection of sclerotia of *Sclerotinia minor* reached higher levels than that of sclerotia of *Sclerotium cepivorum*. However, rate of decline in survival of sclerotia of both plant pathogens was similar for isolates CS-1, 5, 7, 18, 24, and 25. Isolates CS-8 and 9 infected sclerotia of the two plant pathogens very poorly (0–21% at 6 wk) and did not adversely affect the survival of the sclerotia at 10 wk (Table 3).

In the experiment in which isolates CS-5, 8, and 9 were compared, the sclerotia of *Sclerotinia minor* and *Sclerotium cepivorum* were in soil in separate beakers. Under these conditions, isolate CS-5 infected nearly all of the sclerotia of *Sclerotinia minor* within 4 wk and reduced their survival to near zero in 9 wk. However, infection and destruction of sclerotia of *Sclerotium cepivorum* by isolate CS-5 occurred very slowly with 6% infection and 66% survival at 10 wk. Infection and survival of sclerotia of *Sclerotinia minor* by isolates CS-8 and 9 progressed very slowly with 69 and 4% infection and 70 and 72% survival at 10 wk by CS-8 and CS-9, respectively. Neither isolate infected sclerotia of *Sclerotium cepivorum* within 10 wk.

TABLE 2. Production and germinability of two fast-(CS-1 and 5) and two slow-(CS-13 and 25) growing isolates *Sporidesmium sclerotivorum* in soil infested with sclerotia of *Sclerotinia minor*

Isolate	Spores/g of soil ($\times 10^3$) at 8 wk	Spores/g of soil ($\times 10^3$) at 9.5 wk	Germinability (%) at 8 wk	Germinability (%) at 9.5 wk
CS-1	483 ab[x]	482 ab	42 a	54 a
CS-5	367 a	367 b	84 b	85 b
CS-13	386 ab	341 b	86 b	82 b
CS-25	709 a	731 a	85 b	83 b

[x]Values in a column followed by a common letter are not significantly ($P < 0.01$) different as determined by Duncan's multiple range test.

TABLE 3. Infection of sclerotia of *Sclerotinia minor* and *Sclerotium cepivorum* in soil by various isolates of *Sporidesmium sclerotivorum*, survival of the sclerotia, and production of macroconidia of *S. sclerotivorum* in soil

Isolate	Infection (%) at 6 wk *S. minor*	Infection (%) at 6 wk *S. cepivorum*	Sclerotia/5 g of soil at 10 wk *S. minor*	Sclerotia/5 g of soil at 10 wk *S. cepivorum*	Macroconidia per g of soil at 16 wk ($\times 10^3$)
CS-25	100 a[x]	71 a	3 a	7 a	279 a
CS-7	99 a	48 bc	8 a	12 a	278 a
CS-18	99 a	65 abc	3 a	6 a	232 ab
CS-24	100 a	74 a	2 a	9 a	226 ab
CS-5	98 a	40 c	8 a	4 a	216 b
CS-1	86 a	35 c	6 a	7 a	132 c
CS-9	21 b	2 d	35 b	31 b	166 bc
CS-8	1 c	0 d	35 b	37 b	...[y]
Control	0 c	0 d	40 b	34 b	...

[x]Values in a column followed by a common letter are not significantly ($P < 0.01$) different as determined by Duncan's multiple range test.
[y]Not determined.

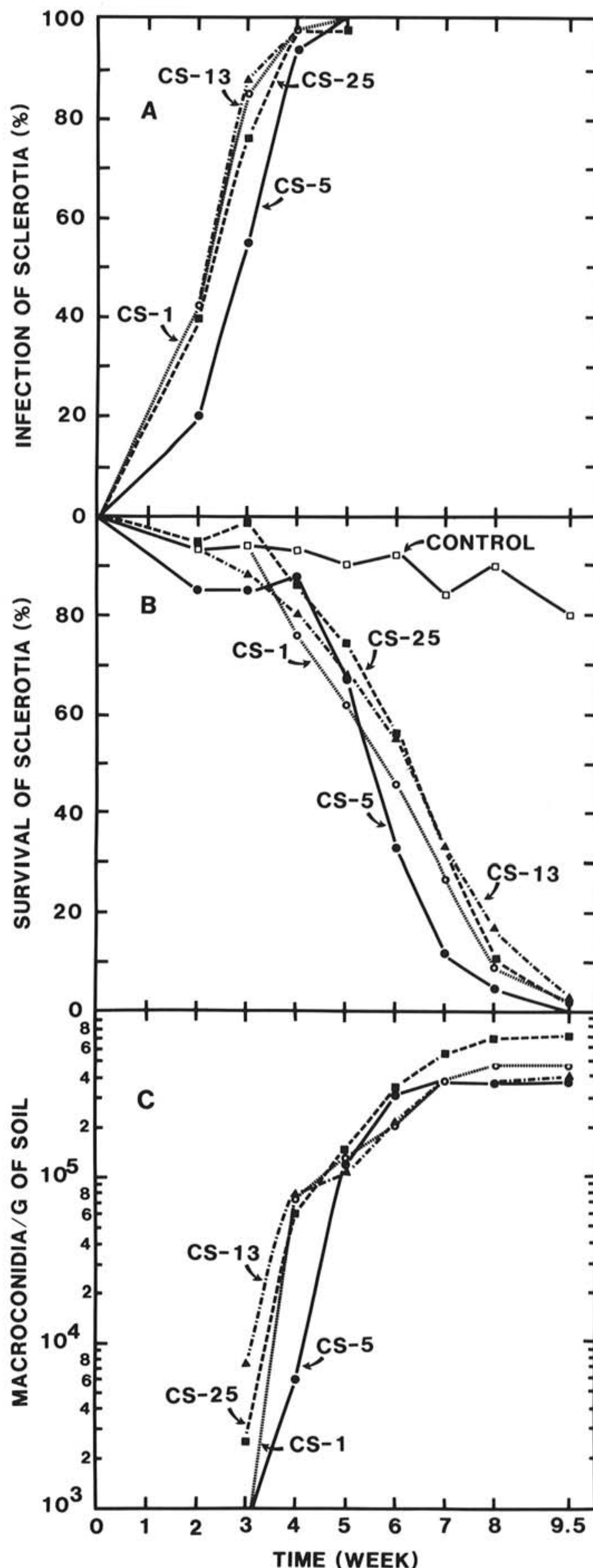

Fig. 2. Comparison of two 'fast' (CS-1 and 5) and two 'slow' (CS-13 and 25) growing isolates of *Sporidesmium sclerotivorum* in natural soil for: **A,** Infection of sclerotia of *Sclerotinia minor*; **B,** Survival of sclerotia; and **C,** production of macroconidia of *S. sclerotivorum*.

DISCUSSION

The purpose of this study was to determine the best isolate of *S. sclerotivorum* for further research to develop a practical microbial pesticide. It was also hoped that the research would lead to a more efficient method for the evaluation of future isolates of the mycoparasite. To identify the best isolate for biological control, one has to consider how the agent will be grown under commercial conditions, how it will be used in the field, and the ability of the isolates to destroy sclerotia of the plant pathogen in soil.

Isolates of *S. sclerotivorum* varied greatly in their growth habits and amount of growth on solid and in liquid medium SM-4. This medium was originally developed for isolates CS-1 and 5. Thus, it is not surprising that these two isolates grew best in this medium. In previous studies (P. B. Adams, *unpublished*), isolate CS-5 (considered an excellent spore producer) normally produced about $4\text{–}5 \times 10^5$ macroconidia per gram of SM-4 vermiculite medium as it did in this study. Isolate CS-8 produced almost 2.5 times that number of macroconidia.

It would appear that distinct biotypes of *S. sclerotivorum* exist together in the same field. All six of the Wisconsin isolates were obtained from various soil samples collected from the same field in Madison. This field was known to be infested with sclerotia of *Sclerotinia sclerotiorum*. In the growth study (Table 1), these isolates segregated into three growth groups as determined by the Duncan's multiple range test (CS-32; CS-31; and CS-23, 29, 30 and 33). This suggests that, based on growth, the field contained at least three distinct biotypes of *S. sclerotivorum*.

The amount of growth in liquid culture by the isolates had no relationship to their ability to infect and destroy sclerotia of the plant pathogens in soil. In fact, of the eight isolates of *S. sclerotivorum* evaluated in soil, no real differences could be detected in the ability of six isolates to infect and destroy sclerotia. Two isolates, CS-8 and 9, were markedly inferior to the other isolates in their abilities to infect and destroy sclerotia of *Sclerotinia minor* and *Sclerotium cepivorum*. Both isolates grew well on liquid media and were the highest ranking isolates in the production of macroconidia on the vermiculite medium (Table 1). However, the ineffectiveness of these two isolates was confirmed in a second experiment in which they were compared to isolate CS-5.

After *S. sclerotiorum* infects a sclerotium in soil, hyphae of the mycoparasite grow out into the soil where they produce new macroconidia. The amount of growth and subsequent sporulation per unit volume of soil is directly proportional to the number of host sclerotia in the soil (5). In the experiment in which two fast- and two slow-growing isolates were compared in soil (Table 2 and Fig. 2), the amount of mycelium in the soil samples was visually estimated to be the same for the four CS isolates. This would indicate that live sclerotia of host fungi are a more complete medium for growth of *S. sclerotivorum* than the SM-4 medium. Dead sclerotia of host fungi are a poor substrate for the mycoparasite (7).

A potential isolate for biological control must be effective in parasitizing sclerotia of plant pathogens in soil as well as producing large numbers of macroconidia on a production medium. It is necessary to evaluate each isolate in soil for infection and destruction of sclerotia of both *Sclerotinia minor* and *Sclerotium cepivorum* and compare the results to a standard isolate such as CS-5.

Isolates CS-1, 5, 7, 18, 24, and 25 were excellent at causing infection and destruction of sclerotia of both *Sclerotinia minor* and *Sclerotium cepivorum* in soil (Table 3). Of these isolates, only isolates CS-5 and 7 grew well on a liquid medium and produced spores on the vermiculite medium (Table 1). Although isolates CS-8 and 9 grew well and produced many macroconidia in vitro, they were poor mycoparasites of sclerotia in soil. Thus, isolates CS-5 and 7 are the isolates that should be evaluated further in the laboratory and field as active agents in microbial pesticides.

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