Biological Control of Sclerotinia Lettuce Drop in the Field by *Sporidesmium sclerotivorum*

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ABSTRACT

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Sporidesmium sclerotivorum, a mycoparasite of *Sclerotinia* spp., was evaluated under field conditions as a biological control agent for lettuce drop caused by *Sclerotinia minor*. The mycoparasite was applied to the field plots in May 1978 at 0, 1, 10, 100, and 1,000 conidia per gram (c/g) of soil. During the summer of 1978, *S. sclerotivorum* caused a 75–95% reduction in the numbers of sclerotia of *S. minor* in plots that had received 100 and 1,000 c/g of soil, respectively, compared to only 25% in the control. Disease control in these plots for four consecutive lettuce crops during the spring and fall of 1979 and 1980 varied from 40 to 83% compared with the disease incidence in the untreated plots. The mycoparasite became established in the field plots and caused infection and subsequent destruction of sclerotia produced on the diseased lettuce. *S. sclerotivorum* has the potential to be a useful biological control agent for lettuce drop.

Since its discovery and description in 1978 (8), *Sporidesmium sclerotivorum* Uecker, Ayers, & Adams was shown to be a mycoparasite of sclerotia of *Sclerotinia sclerotiorum* (Lib.) deBary, *S. minor* Jagger, and *Sclerotium cepivorum* Berk. (5). This dematiaceous hyphomycete was highly infectious to sclerotia of these plant pathogens in soil and caused near complete destruction of the sclerotia within 10 wk in laboratory experiments. It also displayed the unusual ability to grow through soil and produce many conidia along its network of hyphae (5,6). The mycoparasite seems to be nationwide in its distribution and is associated with the natural decline of sclerotia of *Sclerotinia* spp. and *S. cepivorum* in some fields (3). In laboratory studies of field soils, *S. sclerotivorum* infected sclerotia and caused their destruction in a variety of soil textures at temperatures of 15–25 C in soils ranging in pH from 5.5 to 7.5 and at soil water potentials of −8 bars or higher (2). *S. sclerotivorum* has many of the attributes of an ideal biological control agent for diseases caused by *Sclerotinia* spp. and *S. cepivorum*.

This study was undertaken to determine if *S. sclerotivorum* would infect sclerotia of *S. minor* and cause their destruction under field conditions and to determine if this reduction in survival of the sclerotia is sufficient to cause a reduction in the severity of lettuce drop.

MATERIALS AND METHODS

The inoculum density of sclerotia of *S. minor* in soil samples was determined periodically by a wet-sieving technique described previously (1). To determine whether sclerotia of *S. minor* isolated from soil were infected with *S. sclerotivorum*, the sclerotia were plated on moist filter paper in petri dishes and incubated for 2 wk at 25 C. Infected sclerotia were readily detected by the presence of the characteristic conidia of the mycoparasite (3,5,7). *S. sclerotivorum* was detected in soil samples by a baiting technique with sclerotia of *S. minor* as described previously (3).

A field test at the Beltsville Agricultural Research Center was set up to evaluate *S. sclerotivorum* as a biological control agent for Sclerotinia lettuce drop. The soil in this field was Elkton silt loam with an initial pH of 6.2. This field was not naturally infested with the pathogen, *S. minor*, and appeared to be free of *S. sclerotivorum* as determined by the baiting technique (3). The area of the field was divided into 25 plots, each 3 × 3 m, with five plots to a side in a latin square design. Each plot was separated by a 2-m grass border to reduce interplot interference. Romaine lettuce seedlings (*Lactuca sativa* L. 'Paris Island Cos'), were transplanted in September 1977, and each plant was inoculated in early October with *S. minor* (isolate Ss-13) grown on autoclaved oat kernels. By late October, all plants exhibited typical symptoms of lettuce drop and developed numerous sclerotia. The crop debris was left undisturbed over the winter so that sclerotia could continue to develop. In early March 1978, the crop debris along with the sclerotia were rototilled into the soil to a depth of about 15 cm. Romaine lettuce seedlings were immediately transplanted into each of the plots, 100 seedlings per plot, and allowed to grow without further inoculation. This crop exhibited from 85 to 100% lettuce drop in each of the plots by late May 1978. The inoculum density of *S. minor* in each of the plots at this time was 21.3 ± 6.5 sclerotia per 100 g of soil. This artificially introduced, but field-developed, infestation of sclerotia of *S. minor* served as the inoculum source for the field experiment.

The *S. sclerotivorum* (isolate CS-1) was grown on nonsterile moist quartz sand containing live sclerotia of *S. minor* (1% w/w) in plastic mixing pans (5,6). The pans containing the fungus growing in the medium were placed in plastic bags to reduce water loss, and incubated at room temperature (20–25 C) for 8 wk. The mycoparasite inoculum was added to the plots as the sand-sclerotium medium on which it was grown at the rate of 0.02, 0.21, 2.08, and 20.82 kg per plot and mixed into the soil. These rates were the equivalent to 1, 10, 100, and 1,000 conidia (macroconidia) of *S. sclerotivorum* per gram of soil, respectively.

Inoculum of *S. sclerotivorum* was applied to the plots on 23 May 1978. The plots were rototilled two times, once in each direction, to a depth of 15 cm. After tillage, and at 4–5 wk intervals thereafter, throughout the field test, soil samples (10 subsamples per plot) were taken from each plot to assay for the inoculum density of *S. minor* and to determine the percentage of sclerotia infected with *S. sclerotivorum*. The soil in the plots was rototilled thereafter at 2-wk intervals, weather permitting, to prevent the establishment of weeds. To reduce the spread of *S. sclerotivorum* from plot to plot, they were always rototilled in the following order: control, 1, 10, 100, and 1,000 c/g of soil.

Romaine lettuce seedlings were transplanted in the plots (100 seedlings per plot) on 19 March 1979. The number of plants with symptoms of lettuce drop was recorded in each of the plots from two to seven times a wk from planting to harvest. The crop was harvested on 24 May 1979. During the summer of 1979, the plots were kept fallow by rototilling the plots at 2- to 3-wk intervals. On 4

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September 1979, a second planting of Romaine lettuce seedlings was made. This crop was harvested on 29 October 1979. On 17 April 1980, a third crop of lettuce was planted. This crop was harvested on 12 June 1980. During the summer of 1980, the plots were again rototilled at 2- to 3-wk intervals. A fourth crop of lettuce was planted on 9 September 1980 and harvested on 20 November 1980. All crops were planted with seedlings grown in peat pellets in the greenhouse. No pesticides were applied to the field plots during this field test. So that results from the four crops could be compared, all the disease severity data were transformed to percent disease control (I. F. Brown, *personal communication*) by the following equation:

$$\left[\begin{matrix}\%\text{ Disease}\\ \text{control}\end{matrix}\right] = \frac{\left[\begin{matrix}\text{Average \% disease}\\ \text{in control plots}\end{matrix}\right] - \left[\begin{matrix}\text{Average \% disease}\\ \text{in treatment plots}\end{matrix}\right]}{[\text{Average \% disease in control plots}]} \times 100.$$

RESULTS

Infection and survival of sclerotia. The inoculum density of sclerotia of *S. minor* decreased significantly more than in other treatments in plots treated with the two highest rates of *S. sclerotivorum* during the summer of 1978 (Fig. 1). From an initial average inoculum density of 22–24 sclerotia per 100 grams of soil on 23 May, the numbers of sclerotia decreased to 5.2 and 1.4 sclerotia/100 g of soil in plots treated with 100 and 1,000 c/g, respectively, by 6 November 1978. The numbers of sclerotia in untreated control plots also decreased, but reached a low of about 12.6 during this period (Fig. 1). The survival of sclerotia in plots treated with 1 c/g (not shown in Fig. 1) and 10 c/g did not differ significantly from that in the control plots. The inoculum density fluctuated throughout the 2.5-yr period of the field test. In general, it increased following the incorporation of the crop debris after harvest and decreased between successive crops. The inoculum density of *S. minor* remained relatively low in the plots treated with the two highest rates of *S. sclerotivorum*. In October 1980, the inoculum density of *S. minor* in all plots had declined to less than 25% of those at the beginning of the field test. This decline in the inoculum density in the plots treated with 0, 1, or 10 c/g occurred during the summer of 1980 (Fig. 1).

In the plots where there was no significant decline in numbers of sclerotia in 1978, less than 10% of the sclerotia were infected with *S. sclerotivorum*. However, where there was a significant decline in numbers of sclerotia in the plots in the summer of 1978, there was a significant number (25–75%) of sclerotia infected with *S.*

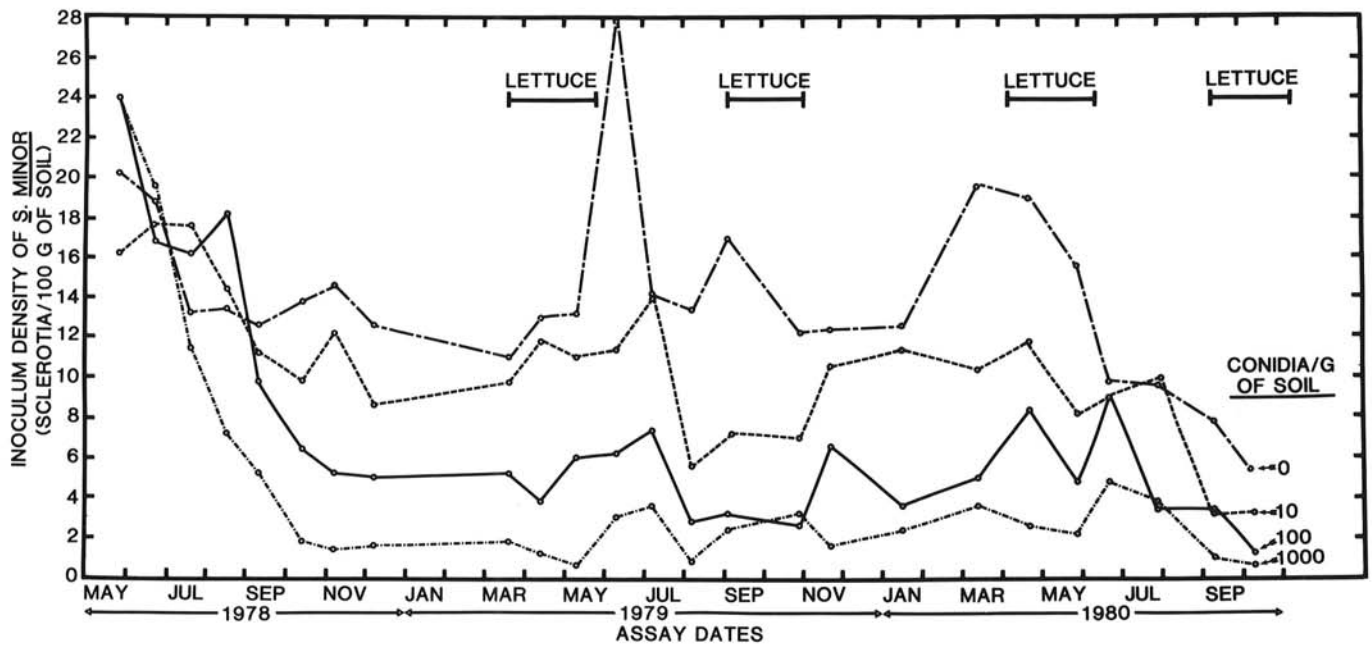

Fig. 1. Survival of sclerotia of *Sclerotinia minor* in field plots treated with various rates of the mycoparasite, *Sporidesmium sclerotivorum*.

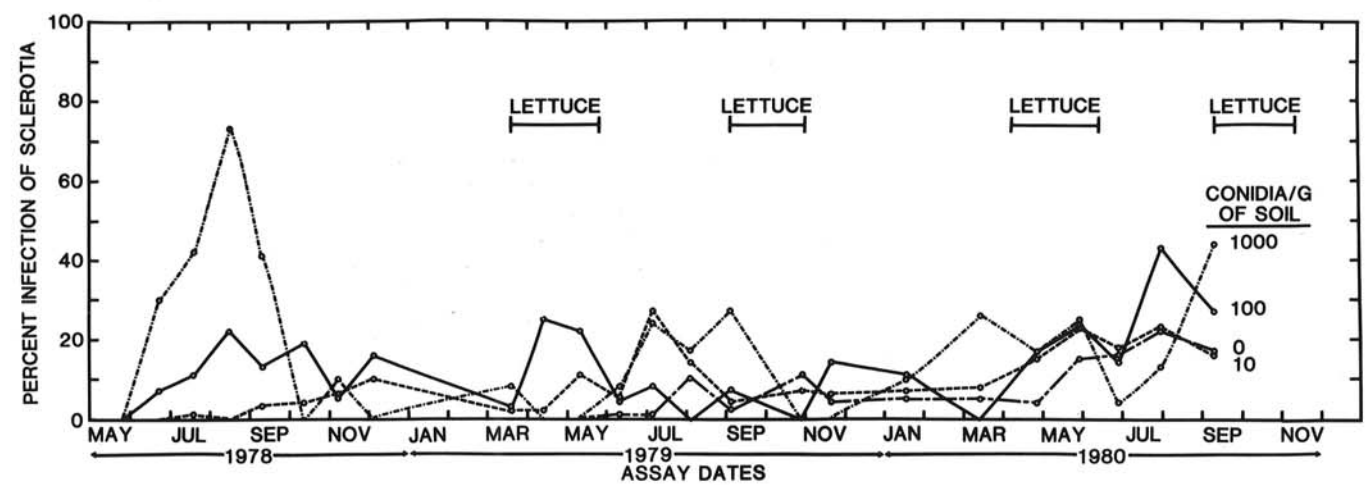

Fig. 2. Infection of sclerotia of *Sclerotinia minor* in field plots artificially infested with various population levels of the mycoparasite, *Sporidesmium sclerotivorum*.

sclerotivorum (Fig. 2). Much of the decline in numbers of sclerotia obtained in July and August of 1978, 1979, and 1980 was associated with mycoparasitic activity by *S. sclerotivorum* at these assay periods. In fact, mycelium and spores of *S. sclerotivorum* were observed microscopically on the assay sieves from soil samples in which there was a decline in numbers of sclerotia.

The soil samples collected in May and October 1978, March 1979, and August 1980 were assayed for the presence of *S. sclerotivorum* by the baiting technique (3). The results of these assays (Table 1) indicated that the mycoparasite survived the winter of 1978–1979 at about the same population density as the previous fall. In May 1978, *S. sclerotivorum* was not detected in the soil samples taken from the control plots with the baiting technique. *S. sclerotivorum* was in the control plots at a relatively low level in October 1978. It was found in four of the five control plots. By August 1980, *S. sclerotivorum* was detected in all five control plots at a level equal to that in the plots that had been treated with 1,000 c/g of soil.

Biological control of lettuce drop. Lettuce drop was severe in the spring of 1979; 65% diseased in the control plots. There was significantly less disease ($P = 0.05$) in the plots that received 10, 100, or 1,000 c/g of soil than in the control plots (Table 2). In the fall 1979 crop, there was only 30% disease in the control plots. In the fall of 1979, the plots received 35.4 cm (13.95 in.) of rainfall. Thus, during much of the growing season the soil was at or near field capacity, and on several occasions, was flooded. Under these conditions *S. sclerotivorum* caused a significant reduction in disease incidence in plots that had received 100 and 1,000 c/g of soil. High incidence of lettuce drop was again observed in the control plots in the spring 1980 crop. As in previous crops, *S. sclerotivorum* provided significant disease control at the rates of 100 and 1,000 c/g of soil. Disease incidence in the control plots of the fall 1980 crop was relatively low. No significant differences in disease incidence could be demonstrated between the various treatments or the untreated control. In all four crops, there were no significant differences in disease incidence ($P = 0.05$) between replications.

DISCUSSION

The results of the field test demonstrated conclusively that a soil treatment with the mycoparasite *S. sclerotivorum* caused a reduction in the inoculum density of *S. minor* and resulted in a significant control of lettuce drop. A single application of the mycoparasite at the rates of 100 and 1,000 c/g of soil provided disease control ranging from 40 to 83% in four successive crops over a 2-yr period compared to that in untreated plots. Mycoparasitic activity of the biological control agent was correlated with the reduction in the numbers of sclerotia of the plant pathogen. This is one of the few examples of successful biological control in the field for which the mechanism of the control has been established.

Evidence for destructive mycoparasitism as the mechanism of disease control was obtained by two approaches. First, the numbers of sclerotia in the soil were monitored throughout the field test. Following incorporation of *S. sclerotivorum* into the soil at the two highest rates, the numbers of sclerotia decreased to relatively low levels after 4 mo and remained low throughout the field test (Fig. 1). Many of the sclerotia retrieved in these treatments were parasitized by *S. sclerotivorum* (Fig. 2). Second, the continued presence and activity of the mycoparasite in the field-soil samples during the span of the field test was demonstrated by the baiting technique with fresh sclerotia (Table 1). Thus, *S. sclerotivorum* became established in the field plots and actually increased in numbers of infective units during the field test. In related laboratory experiments (*unpublished*) the mycoparasite increased from 1,000 to 100,000 c/g of soil in 12 wk when added to soil containing sclerotia of *S. minor*.

Although the numbers of sclerotia in field plots treated with 100 and 1,000 conidia of *S. sclerotivorum* per gram of soil remained relatively low in comparison to those in the untreated control plots throughout the field test, the numbers generally increased slightly following each crop, and then declined (Fig. 1). These increases can be attributed to the production of new sclerotia on diseased lettuce plants that became incorporated into the soil. The subsequent decline was due to the destructive mycoparasitic activity of *S. sclerotivorum*. The destructive activity of *S. sclerotivorum* to sclerotia of *S. minor* in soil was firmly established (2,5).

At the beginning of the field test, *S. sclerotivorum* was not detectable in the control plots. However, it was detected at low levels by the baiting technique in pooled soil samples taken on October 1978 and March 1979, and at high levels in the August 1980 samples (Table 1). It was also detected on field-formed sclerotia retrieved from the untreated control plots in June 1979 and the percentage of parasitized natural sclerotia became progressively greater with time (Fig. 2). Thus, the control plot had either become contaminated by soil from plots treated with *S. sclerotivorum*, or the mycoparasite developed from undetectable levels at the start to significant levels through its parasitic activity on the sclerotia. Great care was taken to reduce interplot interference by the use of grass border strips and by cultivating, rototilling, and sampling in a manner to reduce the possibility of contamination. We suspect that *S. sclerotivorum* in the control plots developed from an initially very low inoculum level, although contamination cannot be ruled out. In either event, the development of *S. sclerotivorum* in the control plots underlines its natural ability to increase with time when sclerotia are present in the soil.

The amount of disease in the spring lettuce crops in the control plots was consistently greater than that in the fall crops (Table 2).

TABLE 1. The presence of *Sporidesmium sclerotivorum* in the soil of the lettuce drop field test at four selected assay periods as determined by baiting with sclerotia of *Sclerotinia minor*

S. sclerotivorum treatment (conidia/g of soil)	Infection (%)[a] by *S. sclerotivorum* of *S. minor* sclerotia added to soil samples collected in: May 1978	October 1978	March 1979	August 1980
0	0 A	14 A	19 A	90 A
1	1 A	61 B	62 B	83 AB
10	0 A	92 C	74 B	79 B
100	12 A	99 C	92 B	79 B
1,000	52 B	98 C	95 B	85 AB

[a] Values in the same column not followed by the same letter are significantly different ($P = 0.05$).

TABLE 2. Disease incidence and biological control of Sclerotinia lettuce drop with *Sporidesmium sclerotivorum*[a]

Conidia of *S. sclerotivorum* (no./g of soil)[a]	Spring 1979		Fall 1979		Spring 1980		Fall 1980	
	Plants with symptoms (%)	Disease control (%)	Plants with symptoms (%)	Disease control (%)	Plants with symptoms (%)	Disease control (%)	Plants with symptoms (%)	Disease control (%)
0	66 A[b]	0	30 A[b]	0	64 A[b]	0	24 A[b]	0
1	62 A	6	31 A	−5	60 AB	6	20 A	15
10	47 B	28	27 AB	9	49 BC	23	15 A	38
100	39 C	40	13 BC	55	36 C	43	10 A	59
1,000	24 D	63	5 C	83	22 D	65	6 A	75

[a] *S. sclerotivorum* was applied to the field plot once in May 1978.
[b] Values not followed by the same letter are significantly different ($P = 0.05$).

The low level of disease in the 1979 fall crop can be attributed to excessive rainfall during the growing season. High levels of soil moisture have been shown to reduce disease incidence (4). The low level of disease in the 1980 fall crop was likely due to the low inoculum density of *S. minor* brought about the parasitic activity of *S. sclerotivorum* that had become established therein.

The extent of disease control by the biological control agent was variable, but substantial and statistically significant in plots that had received inoculum at 100 or 1,000 c/g of soil application rate (Table 2). Less but significant disease control (23–28%) also resulted from the 10 c/g treatment in the two spring crops. It is possible that an increased level of disease control could be obtained by the planting of a single lettuce crop each year. This would allow the mycoparasite more time under favorable conditions of temperature and moisture to destroy the sclerotia produced on the previous crop. Also, the application rate used was based on the amount of *S. sclerotivorum* mixed into the plots to a 15-cm depth. It is possible that a greater efficiency could be achieved by other methods of application and at lower rates.

Some of the management practices used in this field test may be impractical on a cost-benefit basis. However, the main purpose of this field test was to determine whether *S. sclerotivorum* could be established in the soil, cause destruction of *S. minor*, and provide disease control under field conditions. Based on these results, we believe that this mycoparasite, if properly managed, has high potential as a biological control agent for lettuce drop, and possibly, for other plant diseases caused by sclerotial fungi susceptible to infection by *S. sclerotivorum*. A U.S. patent was recently issued for the use of *S. sclerotivorum* as a biological control agent (7).

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