Economical Biological Control of Sclerotinia Lettuce Drop by *Sporidesmium sclerotivorum*

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We acknowledge the able technical assistance of C. J. Tate. We thank Ralph Baker, James Locke, and Alan Paau for their critical reviews of the manuscript.

Accepted for publication 30 April 1990 (submitted for electronic processing).

ABSTRACT

Adams, P. B., and Fravel, D. R. 1990. Economical biological control of Sclerotinia lettuce drop by *Sporidesmium sclerotivorum*. Phytopathology 80:1120-1124.

In a field trial, lettuce plants infected with *Sclerotinia minor* were treated with a preparation of *Sporidesmium sclerotivorum* at rates of 0, 0.2, 2, or 20 kg/ha of *Sporidesmium* product in May 1987. From the fall of 1987 to the fall of 1989, five successive lettuce crops were grown in this field with no additional application of the biocontrol agent. Disease incidence was significantly lower than the nontreated plots at the highest, two highest, and all three rates in the first, second, and third crop, respectively. In the third crop, 53, 68, and 72% disease control was obtained at the 0.2, 2, and 20 kg/ha rates, respectively. There were no differences in disease incidence among treatments in the last two crops due to an increase in indigenous populations of *Sporidesmium* in the nontreated plots. In October 1987, population densities of *S. minor* were significantly lower in plots treated with 2 or 20 kg/ha than in nontreated plots or plots treated with 0.2 kg/ha. By 1989, populations of *S. minor* in all treatments were low due to activity of the biocontrol agent. Estimated cost of the three rates of application is $2, 20, and 200/ha, respectively.

One obstacle to the widespread adoption of biocontrol in agricultural production systems is the extremely high application rates necessary to achieve disease control. Application rates of 0.5–15% for soil amendments are commonly reported (13,16). Based on a 30-cm furrow slice, this would require an application of 22,400–672,000 kg/ha of a biocontrol preparation! Clearly this is not feasible either physically or economically. Effective disease control at acceptable application rates (up to 15 kg/ha) is imperative to the practical use of biocontrol in field production systems.

We hypothesized that certain plant pathogen-antagonist combinations should be good candidates for biological control at low rates of application for several reasons. First, the antagonist should be reproductively dependent on propagules of the plant pathogen. Second, the plant pathogen should occur in an aggregate distribution. Third, the antagonist should be capable of secondary spread from pathogen propagules within the aggregate. Thus, growth and proliferation of the antagonist with concomitant destruction of the pathogen propagules depend on the density of the pathogen propagules. Finally, the propagules of the plant pathogen must be accessible for application of the antagonist at a stage(s) of the disease cycle. Whenever these criteria are met, persistent biocontrol should be achieved at low rates. However, other criteria may be important in exploiting other pathogen-antagonist systems.

Mycoparasites affect biocontrol through destruction of pathogen inoculum. Monocyclic diseases are amenable to control by reduction of initial inoculum. Further, soilborne, monocyclic pathogens are usually spatially aggregated, thus facilitating new infections by the mycoparasite. Based on these epidemiological characteristics, *Sporidesmium sclerotivorum* Uecker, Ayers & Adams was selected as a model system for the control of *Sclerotinia minor* Jagger. *S. sclerotivorum*, an obligate mycoparasite of sclerotia of *Sclerotinia* spp., is a persistent biocontrol agent in soil, and it reproduces on host sclerotia (7,8). It controls lettuce drop caused by *S. minor* in the field when added to soil at a rate of 2,300–23,000 kg/ha (7,18).

MATERIALS AND METHODS

Establishment of field plots. An area at the Beltsville Agricultural Research Center that had been in turfgrass for at least 10 yr was selected. In August 1986, plots 3 × 3 m were established with 3 m of undisturbed turf between adjacent plots. The turf in each plot was removed and the soil rototilled twice in each of two perpendicular directions. To assess indigenous populations of *S. sclerotivorum*, soil samples were processed as described below. Two grams of fresh sclerotia of *S. minor* was mixed into 100 g of moist soil from each plot. Soils were incubated under ambient conditions in 250-ml beakers covered with plastic. At intervals up to 72 wk, 5-g subsamples from each of the 20 beakers were assayed for the presence of the mycoparasite (6).

Plots were infested with *S. minor* as follows. In early September 1986, 100 romaine lettuce seedlings, *Latuca sativa* L. 'Parris Island Cos', were transplanted into each of the plots. In late October 1986, each plant in each of the plots was inoculated with oat grains infested with *S. minor*. Within a few weeks all plants exhibited typical symptoms and signs of lettuce drop. In early December 1986, the diseased lettuce plants were rototilled into the soil to a depth of 15 cm. In March 1987 soil samples were collected from each of the 20 plots. The samples were air-dried sufficiently to pass a 2-mm screen, and 100-g subsamples were assayed to determine the number of sclerotia (1). Inoculum density of *S. minor* ranged from 54 to 122 sclerotia/100 g of soil, with a mean of 79.

Preparation and application of the mycoparasite. *S. sclerotivorum* was grown on vermiculite saturated with SM-4 medium (85 ml of medium and 15 g of vermiculite) (11). The vermiculite, containing mycelium and spores, was comminuted in a blender to pass through a 0.6-mm screen. Inoculum in water was applied to the plots as a spray (Solo jet pack sprayer, Solo, Inc., Newport News, VA) on mature diseased lettuce plants on 8 May 1987 at 0.2, 2, or 20 kg/ha. The four treatments were arranged in a randomized complete block design and replicated five times. These rates are equivalent to 2, 20, or 200 macroconidia/cm^2 or 0.08, 0.8, or 8 macroconidia/g of soil. Immediately after application of the mycoparasite, the plots were rototilled to incorporate the diseased lettuce debris thoroughly into the soil. After incorporation, the plots were sprinkler irrigated with 31 mm of water.

Lettuce crops. Lettuce was grown in the plots for five consecutive crops from fall 1987 through fall 1989. In the three fall and two spring crops, romaine lettuce seedlings were grown in the greenhouse and transplanted into the field. In the spring only, lettuce seedlings were maintained in outdoor cold frames for about

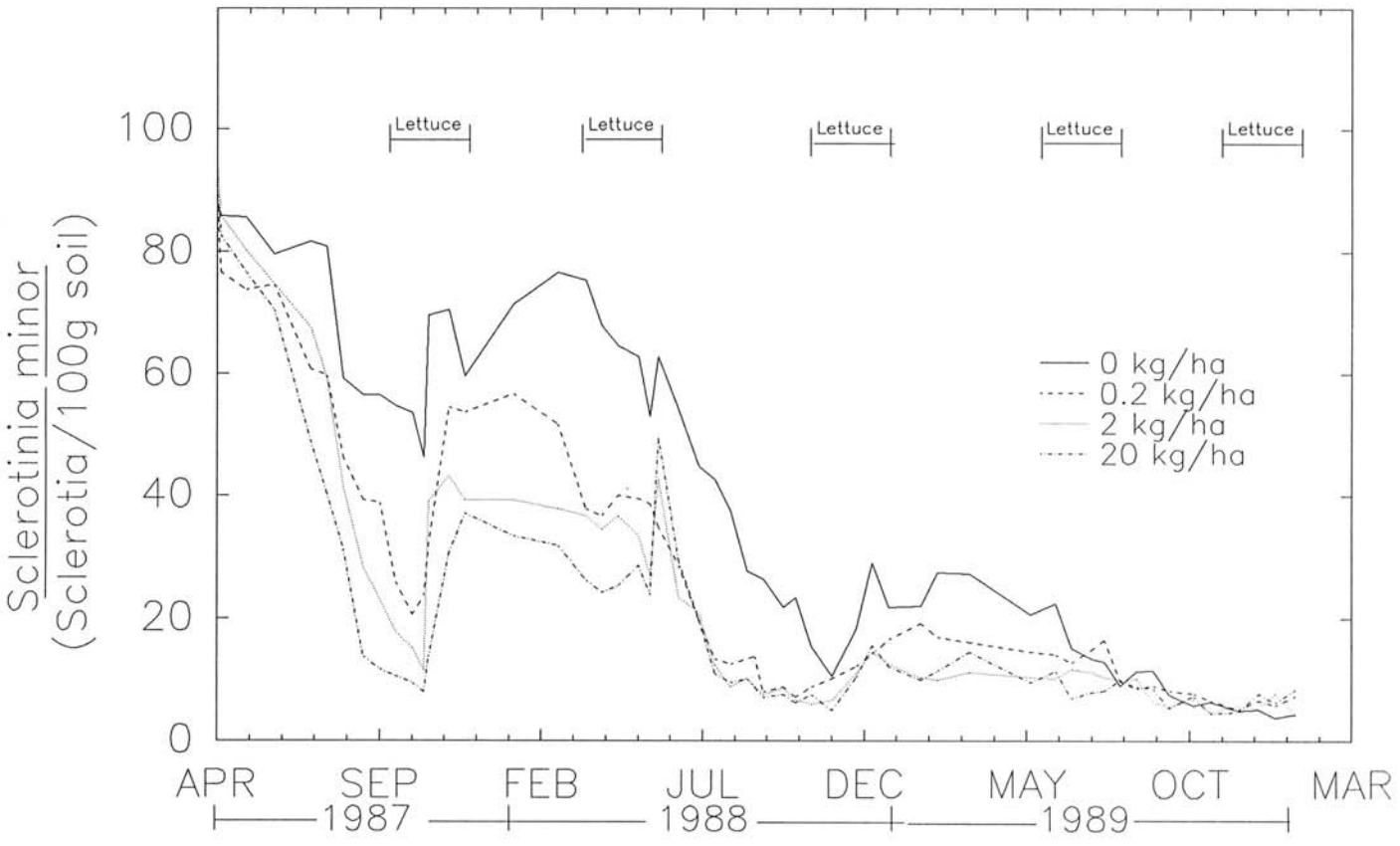

Fig 1. Survival of sclerotia of *Sclerotinia minor* in field plots treated with various rates of the mycoparasite *Sporidesmium sclerotivorum*.

1 wk before transplanting into the field. One hundred seedlings were planted in each plot in 10 rows of 10 plants with 30 cm between both rows and plants. At harvest all apparently healthy plants were harvested and the remaining crop debris and diseased plants were rototilled into the soil.

Population dynamics of *S. sclerotivorum*. From application of the mycoparasite (May 1987) until the conclusion of the field trial (November 1989), soil samples were collected at approximately 2-wk intervals. Plots were rototilled prior to collecting samples, unless lettuce plants were in the plots. From each of the 20 plots, 10 2-cm core samples were taken to a depth of 15 cm and bulked (600–800 g/plot). The 20 samples were partially air dried and sieved through a 2-mm screen. Two 50-g portions of each sample were assayed for population densities of sclerotia of *S. minor* in the soil as previously described (1). All sclerotia (up to 50) were placed on moist filter paper in 9-cm petri dishes (25 sclerotia/dish) to determine the percentage of sclerotia infected by *S. sclerotivorum* (6). Soil samples also were assayed for the number of macroconidia of the mycoparasite in the soil from November 1987 (25 wk after the application of *S. sclerotivorum*) through November 1989 (8).

Throughout the field trial, minimum and maximum air temperature and rainfall were recorded at a standard weather station. Irrigation frequency and amounts were also recorded.

Data analysis. For each sampling date, treatment means of pathogen and mycoparasite populations, and disease incidence were each compared to the nontreated control with Fisher's least significant difference in SAS (Statistical Analysis System, Cary, NC).

RESULTS

Pathogen population densities. The mean population of *S. minor* on 5 May 1987, when *S. sclerotivorum* was applied, was 88.9 sclerotia/100 g of soil. The population density of *S. minor* declined slowly but not significantly ($P = 0.05$) in all treatments by June 1987 (Fig. 1). After this date, inoculum density of *S. minor* declined more rapidly in the plots treated with *S. sclerotivorum* than in the nontreated plots (Fig. 1). During the summer of 1987, infection of sclerotia of *S. minor* by *S. sclerotivorum* reached a maximum 36% in plots treated with 20 kg/ha of *S. sclerotivorum* (Fig. 2). When lettuce was planted in September 1987, population densities of *S. minor* in the plots treated with 2 or 20 kg/ha *S. sclerotivorum* were significantly ($P = 0.01$) less than those in the nontreated plots (Fig. 1). After the debris from the fall 1987 crop was rototilled into the plots, the population of *S. minor* increased in all treatments and remained high through the winter months. During the summer of 1988, the pathogen population density declined in all of the treatments. At this time, infection of sclerotia of *S. minor* by *S. sclerotivorum* was as high as 51%. Further, in August 1988, 45% of sclerotia of *S. minor* recovered from the nontreated control were infected with *S. sclerotivorum*. When the refuse of the 1988 fall crop was rototilled into the soil, there was a nonsignificant ($P = 0.05$) increase in the population density of *S. minor*. During the winter of 1988–1989, the population density of *S. minor* was below 30 sclerotia/100 g in all of the treatments. There were no significant differences in population densities of *S. minor* in the various treatments from this time to the end of the experiment ($P = 0.05$).

Disease incidence. During fall 1987, only *S. sclerotivorum* at 20 kg/ha reduced the incidence of lettuce drop compared to the nontreated control (Table 1). In the spring of 1988, both the 20 and 2 kg/ha treatments had significantly lower disease incidence than the nontreated control, although the incidence was high (>98%) in all treatments (Table 1). In the fall 1988 crop, the incidence of lettuce drop was significantly less in all the treatments of *S. sclerotivorum* than in the nontreated control. The average disease control in the 0.2, 2, and 20 kg/ha treatments was 53, 68, and 72%, respectively. In the spring and fall crops of 1989 there were no significant differences in the incidence of lettuce drop among the treatments of *S. sclerotivorum* compared to the nontreated control.

Population of *S. sclerotivorum*. During the summer of 1987,

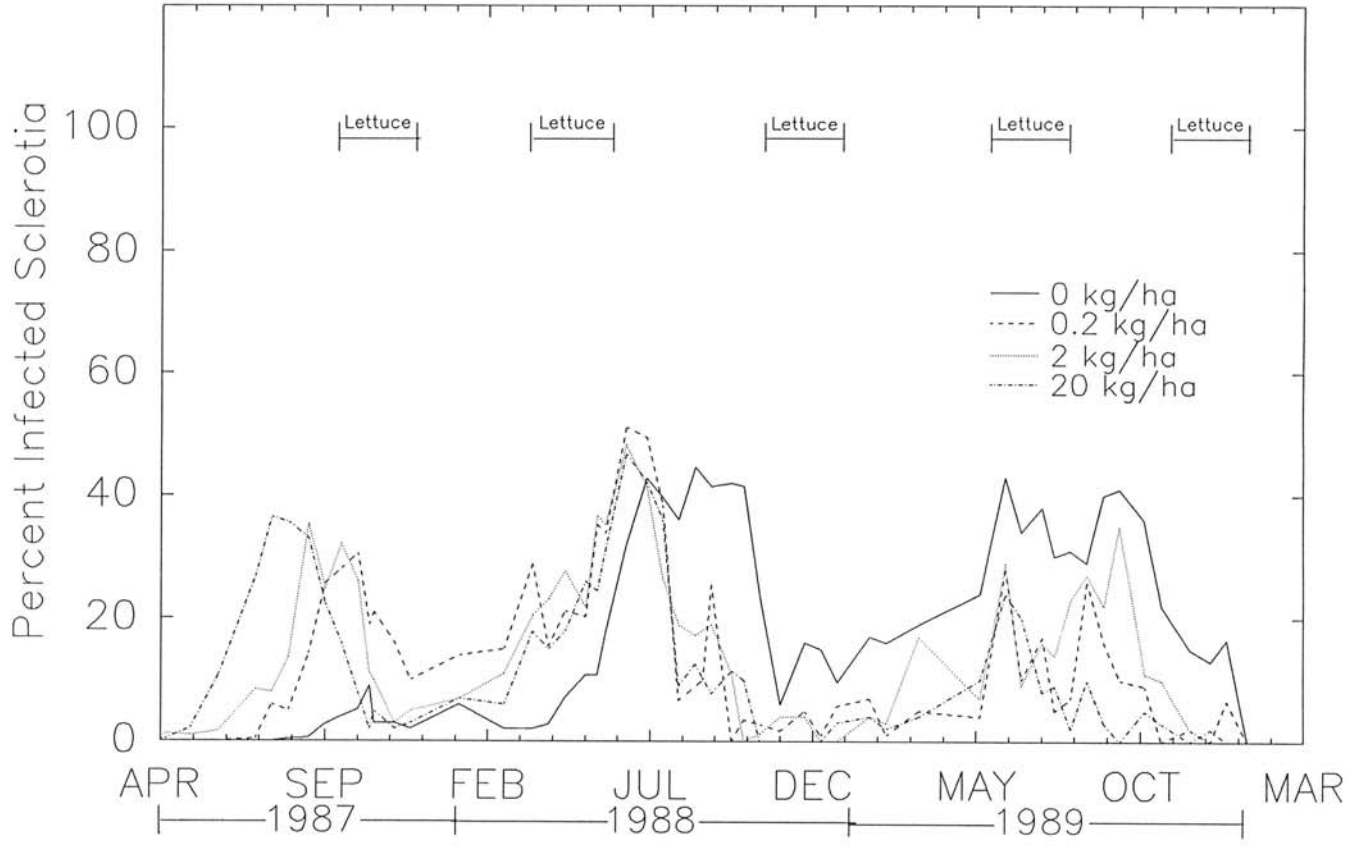

Fig 2. Infection of sclerotia of *Sclerotinia minor* in field plots artificially infested with various rates of *Sporidesmium sclerotivorum*.

macroconidia of *S. sclerotivorum* were recovered only in soil samples from the 20 kg/ha treatment. During the fall of 1987, there was an increase in the population of the mycoparasite in all treatments. Populations of *S. sclerotivorum* in all mycoparasite treatments varied from 500 to 5,500 macroconidia/g of soil during the summer of 1988. In July, 1988 the mycoparasite was consistently detected in the nontreated control at populations that subsequently varied from 500 to 2,000 macroconidia/g of soil.

DISCUSSION

Biocontrol of lettuce drop was achieved with *S. sclerotivorum* at rates as low as 0.2 kg/ha. In a previous study, 2,300 kg/ha was required for disease control (7). Research on the growth of *S. sclerotivorum* in culture (11), activity of the mycoparasite in natural soil (3,8), production of sclerotia of *S. minor* on lettuce, and the subsequent spatial arrangement of these sclerotia in the field (2) made possible strategies for increasing the efficiency of the biocontrol agent so that this substantial reduction in application rate was possible.

With both biological and chemical control agents, one of the major problems in the control of soilborne plant pathogens is establishing contact between the control agent and the pathogen propagules within the soil mass. To overcome this difficulty, the characteristics of the plant pathogen and the attributes of the mycoparasite must be used (3,4,9,10). The dynamics of this system permitted us to apply a small amount of *S. sclerotivorum* over the surface of diseased plants, resulting in a high percentage of macroconidia of the mycoparasite contacting sclerotia of *S. minor*. When the diseased plants, sclerotia, and *S. sclerotivorum* were disked into the soil, macroconidia of the antagonist were in a position to infect sclerotia of the pathogen. After infection of the sclerotia, *S. sclerotivorum* has the potential to grow in the soil mass for a distance of 3 cm (Ayers and Adams, *unpublished*) where it may come in contact and infect other sclerotia. The aggregated spatial arrangement of *S. minor* (2,14) aided in progress of destruction of the plant pathogen. If only one or two sclerotia in each aggregate become infected initially, subsequent secondary spread of the mycoparasite should, with time, destroy nearly all of the sclerotia in the aggregate. In the process, *S. sclerotivorum* should produce many new macroconidia and increase its population density (8). Biocontrol in this field trial was successful when *S. sclerotivorum* was added to soil at the rate equivalent to 0.08 spores/g of soil. One hundred spores/g of soil were required in a previous field test (7). By utilizing the natural attributes of the biocontrol agent with knowledge of where the pathogen produces sclerotia and their subsequent distribution in soil, the amount of the biocontrol agent required for control was reduced by a factor of about 12,000.

S. sclerotivorum at 20 kg/ha destroyed enough sclerotia of *S. minor* during the summer of 1987 to provide 36% control of lettuce drop. Sufficient sclerotia were produced on the diseased lettuce plants to cause an increase in the pathogen population by November 1987. The pathogen population remained high during the winter months of 1987–1988, presumably because *S. sclerotivorum* is inactive at low temperatures (5). Thus, in the spring 1988 crop, disease incidence was high (>98%) in all treatments, even though the 2 and 20 kg/ha rates showed statistically less disease than the nontreated control. This level of control would not, however, be acceptable to a grower. During the summer of 1988, *S. sclerotivorum* caused a significant ($P = 0.05$) reduction in the population of the pathogen in all of the treated plots. The reduction in the pathogen population was sufficient to suppress ($P = 0.05$) disease incidence in all treatments compared to that of the nontreated control in the fall of 1988 (Table 1).

During September 1987, soil assays revealed the presence of *S. sclerotivorum* in the nontreated control. Infection of sclerotia by the mycoparasite was at a low level (0–10%) and remained low until May 1988. By late June 1988, the percentage of infected sclerotia in the nontreated control increased to 40%. During the summer of 1988 the inoculum density of *S. minor* declined to a low level. By November 1988 there were no significant ($P = 0.05$) differences in the inoculum densities among the various treatments, including the nontreated control. The mycoparasite kept the inoculum density of *S. minor* in all treatments low during 1989, and, thus, there was no significant ($P = 0.05$) difference in the amount of lettuce drop in treatments of *S. sclerotivorum* compared to the control in the spring or fall crops in 1989.

This field was chosen for the trial because it was thought to contain no indigenous *S. sclerotivorum*. Soil samples collected from each plot in August 1986, baited with sclerotia of *S. minor* and assayed at intervals up to 72 wk indicated that there was no indigenous *S. sclerotivorum* in the field. Contamination of the nontreated plots from the treated ones was considered unlikely since there were 3-m turf borders around each plot. Whenever work was done in the plots, the control plots were done first. Wildlife observed in or around the plots at various times might have carried *S. sclerotivorum*-infested soil from some of the treated plots to nontreated plots. Contamination by wildlife was not considered likely because in May 1988 *Laterispora brevirama* Uecker, Ayers & Adams also was detected on sclerotia of *S. minor* retrieved from some of the plots. By the completion of the field trial, *L. brevirama* had been detected on sclerotia retrieved from 16 of the 20 plots including all five of the nontreated plots. *L. brevirama* is a known mycoparasite of the mycoparasites *S. sclerotivorum* and *Teratosperma oligocladum* Uecker, Ayers & Adams and is found in nature only in association with one of its host fungi (12). Thus, it is likely that the field had a very low natural population of *S. sclerotivorum* which was not detected at the start of the experiment.

The rates of application of *S. sclerotivorum* in Table 1 are based on the production of 10^6 macroconidia/g of fresh medium. This semidefined liquid medium, SM-4 (11) was added to vermiculite. The estimated cost, in laboratory quantities, of the ingredients per kilogram of medium is approximately $0.55. This estimate does not include the cost of labor, facilities, packaging, storage, shipment, advertising, overhead, and profit. However, we estimate that the cost of the finished commercial product containing *S. sclerotivorum* would be approximately $10/kg. The cost of the biocontrol material at the rates of applied would vary from $2 to 200/ha. In this field test, disease control was obtained at all rates of application of *S. sclerotivorum*. Thus, disease control was obtained at a cost of as little as $2/ha! In New Jersey, the average annual loss to lettuce drop is 10% (17). The mean value to the grower of romaine lettuce in New Jersey in 1988 was $0.20/plant. A lettuce field contains approximately 54,000 plants/ha. Hence, a 10% disease loss represents $1,089.18/ha, and 50% disease control would save the grower $544.59/ha. In New Jersey, growers use two to three applications of a fungicide to control lettuce drop. These chemical treatments provide about 50–95% disease control (15,19). Since the expected savings are greater than the anticipated loss, application of *S. sclerotivorum* is economically beneficial.

Exploitation of characteristics of both the antagonist and the plant pathogen provided biocontrol at practical application rates. Epidemiology of lettuce drop permitted a strategy of reducing initial inoculum rather than the commonly used strategy of protecting the infection court. Incorporation of the antagonist

TABLE 1. Incidence of lettuce drop caused by *Sclerotinia minor* in plots treated with *Sporidesmium sclerotivorum* on 8 May 1987

Rate of application (kg/ha)	Percent lettuce drop				
	Fall 1987	Spring 1988	Fall 1988	Spring 1989	Fall 1989
0	85.4	99.8	72.6	85.8	26.8
0.2	84.2	99.4	34.4*	69.8	30.8
2.0	74.6	98.6*	23.0*	71.6	18.6
20.0	54.2*[a]	98.4*	20.2*	69.4	19.4
LSD ($P = 0.05$)	19.8	1.2	33.4	19.8	17.8

[a]Values marked with an asterisk differ significantly from the 0 kg/ha treatment within each column.

into the soil is often used in biocontrol of soilborne pathogens. We applied the antagonist while there was easy access to some of the pathogen propagules, i.e., while they were above ground. Knowledge of the aggregated distribution of the pathogen propagules and a thorough understanding of the life cycle of the mycoparasite made biocontrol at low application rates of the antagonist possible.

We reasoned that a certain set of characteristics of a plant pathogen-antagonist system should result in biological control at low rates of application of the antagonist. Extensive knowledge of other systems may permit similar successes with different pathogen-antagonist systems in the future.

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