Ecology and Epidemiology

Comparison of Antagonists of *Sclerotinia* Species

P. B. Adams

Biocontrol of Plant Diseases Laboratory, Plant Science Institute, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705.

I am grateful to C. J. Tate for technical assistance and A. S. Paau, J. J. Marois, and R. Baker for critical review of the manuscript.
Accepted for publication 24 July 1989 (submitted for electronic processing).

ABSTRACT

Adams, P. B. 1989. Comparison of antagonists of *Sclerotinia* species. Phytopathology 79:1345-1347.

Eight reported antagonists of *Sclerotinia* spp. were evaluated for their ability to destroy sclerotia of *Sclerotinia minor* in soil. When sclerotia of *S. minor* were soaked in concentrated spore suspensions of the antagonists for 3 hr and added to moist natural soil, *Teratosperma oligocladum* caused a 94% reduction in the inoculum density of *S. minor* within 10 wk. *Dictyosporium elegans* and *Coniothyrium minitans* were intermediate in reducing the number of sclerotia after 30 wk, whereas *Penicillium citrinum*, *Talaromyces flavus*, a *Trichoderma* sp., and *Gliocladium virens* were ineffective. When sclerotia of *Sclerotinia sclerotiorum* were soaked in concentrated spore suspensions of the antagonists for 3 days and then added to moist natural soil infested with sclerotia of *S. minor*, only *T. oligocladum* reduced the inoculum density of *S. minor*. When various concentrations of spores of the antagonists were added to moist natural soil infested with sclerotia of *S. minor*, only *Sporidesmium sclerotivorum* and *T. oligocladum* reduced the inoculum density of *S. minor*. These two mycoparasites were effective at rates of 10–14 spores per gram of soil, whereas the other antagonists were ineffective at rates as high as 10^6–10^7 spores per gram of soil. It was concluded that *S. sclerotivorum* and *T. oligocladum* are aggressive mycoparasites, whereas the other antagonists are passive mycoparasites.

Additional keywords: biological control, hyperparasites.

In 1947, Campbell (8) isolated and described *Coniothyrium minitans* Campbell as the first reported mycoparasite of sclerotia of *Sclerotinia* spp. During the succeeding 40 yr, 45 more fungi, two bacteria, two insects, a mite, and a snail were reported to be parasites, predators, or antagonists of sclerotia of *Sclerotinia* spp. In the past 30 yr, a few of these organisms, primarily fungi, have been evaluated in the laboratory or in the field for their ability to suppress plant diseases caused by *Sclerotinia* spp. These potential biocontrol agents have proven to be less than spectacular in disease control or have been required in large amounts to obtain disease suppression.

Other than *Sporidesmium sclerotivorum* Uecker, Ayers, & Adams and *Teratosperma oligocladum* Uecker, Ayers, & Adams, none of the potentially antagonistic fungi were compared with one another in soil for their antagonistic activity under standard conditions. The purpose of this investigation was to compare the biocontrol potential of several antagonists of *Sclerotinia* spp. under varied conditions to provide a better understanding of the characteristics required for a good biocontrol agent.

MATERIALS AND METHODS

Isolates of antagonistic fungi were obtained from various sources. *Dictyosporium elegans* Corda and *C. minitans* were supplied by T. A. McCredie, Nedlands, Western Australia, Australia; *Talaromyces flavus* (Klocker) Stolk & Sampson were supplied by H. C. Huang, Lethbridge, Alberta, Canada; and *Penicillium citrinum* Thom was contributed by H. Melouk, Stillwater, OK. An unidentified *Trichoderma* sp. was isolated by the author from a sclerotium of *Sclerotinia minor* Jagger added to a soil sample collected near Chinhae, South Korea by K. Kim, Beltsville, MD. *Gliocladium virens* Miller et al, *S. sclerotivorum*, and *T. oligo-*

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 1989.

cladum were obtained from the collection of the Biocontrol of Plant Diseases Laboratory, Beltsville, MD. Sclerotia of *S. minor* and *S. sclerotiorum* were used as hosts for the antagonists and were obtained from the Laboratory collection.

The soil used for all the experiments was Norfolk sandy loam, collected from a field near Salisbury, MD. The pH was adjusted to 7.0 with hydrated lime. Soil moisture content for each experiment was adjusted to approximately −0.1 bar matric potential.

Three types of experiments were conducted to compare the ability of different antagonists to degrade sclerotia of *S. minor* in soil. In the first experiment, sclerotia were soaked in concentrated water suspensions of spores of the antagonists (Table 1) for 3 hr at room temperature, and 2 g of infested sclerotia were added to 100 g of Norfolk sandy loam. The soils (100-g quantities in 250-ml beakers) were incubated at room temperature and assayed in 5-g samples for the number of sclerotia at various intervals up to 30 wk (1,3). This experiment indicated which antagonists could germinate on sclerotia in soil, infect the sclerotia, and cause their decay. In the second experiment, sclerotia of *S. sclerotiorum* were soaked in similar spore suspensions of the antagonists for 3 days. Six infested sclerotia of *S. sclerotiorum* were then mixed with 100-g quantities of soil infested with sclerotia of *S. minor* (1,000 sclerotia per 100 g of soil). These soils were assayed periodically for survival of sclerotia (sclerotia per 5 g of soil) of *S. minor*. This experiment was designed to indicate which antagonists could grow through soil from an infected sclerotium to healthy sclerotia. In the third type of experiment, conducted separately for each antagonist, spores of the antagonists in water were mixed into soil containing sclerotia of *S. minor*. Based on preliminary experiments, spore concentrations varied with the antagonist. With *G. virens*, they varied from 0 to 9.6×10^5 spores per gram of soil, whereas with *T. oligocladum* the concentrations varied from 0 to 10 spores per gram of soil. Soils were assayed periodically for the survival of the sclerotia, and the antagonists were compared for their ability to germinate in soil, infect and destroy sclerotia, and grow through soil and infect healthy sclerotia.

In some assays of each experiment, up to 50 sclerotia were placed on moist filter paper and observed for 2 wk to determine whether they were infected by the mycoparasites. Sclerotia were considered infected when growth of the mycoparasites could be observed on or around the sclerotia. In addition, in some assays sclerotia were surface-disinfested in 0.5% sodium hypochlorite for 5 min, rinsed in sterile water, and placed on potato-dextrose agar to determine their viability (2). Survival of sclerotia in soil containing antagonists was compared with survival in the untreated control soils without antagonists. Survival of sclerotia in control soils remained high (90%) during the 30-wk period of each experiment. Four replicates were used in each experiment, and experiments were repeated.

RESULTS

In the first experiment, *C. minitans*, *D. elegans*, and *T. oligocladum* caused a significant reduction in the survival of sclerotia in soil (Table 1). *T. oligocladum* was far superior to the other fungi tested in reducing survival of sclerotia. Within 10 wk, survival of sclerotia was reduced to 6% (Table 1). *C. minitans* and *D. elegans* were intermediate in their ability to reduce survival of the sclerotia over the 30-wk period. *P. citrinum*, *G. virens*, *T. flavus*, and *Trichoderma* sp. were ineffective in reducing sclerotial survival.

In the second experiment, only *T. oligocladum* was effective in reducing sclerotial survival of *S. minor* (Table 2).

In the third type of experiment, *S. sclerotivorum* and *T. oligocladum* caused a marked reduction in sclerotial survival of *S. minor* (Table 3). Ninety percent of the retrieved sclerotia in the various treatments were viable. Infection of sclerotia by *S. sclerotivorum* and *T. oligocladum* was consistent and increased with time. Infection was correlated with a decline in the number of sclerotia recovered from the soil. The percentage of sclerotia infected by the other antagonists was inconsistent in the first few weeks of the experiments and declined to almost zero in the latter stages of the experiments.

DISCUSSION

Eight fungi reported to be antagonistic to sclerotia of *Sclerotinia* spp. were evaluated in soil in three different ways. The experiments were designed to determine some of the attributes of antagonists important in biological control of soilborne plant pathogens.

P. citrinum, *T. flavus*, *G. virens*, and *Trichoderma* sp. failed to reduce the survival of sclerotia of *S. minor* in each of three experiments. When sclerotia of *S. minor* were soaked in spore suspensions of *D. elegans*, *C. minitans*, or *T. oligocladum* and added to soil, the number of sclerotia declined. Only *S. sclerotivorum* and *T. oligocladum* were capable of causing a marked reduction in the survival of *S. minor* when spores of the antagonists were added to soil infested with *S. minor*. These two mycoparasites were active when applied at rates as low as 10–14 spores per gram of soil, whereas the other antagonists were ineffective at rates as high as 10^6–10^7 spores per gram of soil. Therefore, these two fungi were superior to the other antagonists as potential biocontrol agents.

S. sclerotivorum was not included in the first two series of experiments because previously published reports (3,5,6) indicated that *S. sclerotivorum* and *T. oligocladum* behaved in soil in a similar manner. *S. sclerotivorum* was included in the third series of experiments, the most stringent of the three, so that it could be compared with the other seven antagonists.

Why are *S. sclerotivorum* and *T. oligocladum* superior to the other antagonists? The macroconidia of these two fungi were able to germinate in soil when they were adjacent to sclerotia of *Sclerotinia* spp. The germinated macroconidia were able to infect the sclerotia, cause their decay, and, in the process, grow out into the soil mass and infect healthy sclerotia (1,3,5,6). Hyphae of these two fungi were observed in the soil at the times the soils were assayed for sclerotia (1). The infected sclerotia served as a food base, protected from other microorganisms, from which the mycoparasite could grow out into the soil mass. Thus, both *S. sclerotivorum* and *T. oligocladum* are aggressive mycoparasites in that they were able to grow through natural soil from infected

TABLE 1. Survival of sclerotia of *Sclerotinia minor* in soil when the sclerotia were soaked in spore suspensions of antagonists for 3 hr before being mixed with soil

Antagonist	Concentration of spore suspension (spores/ml)	Percent survival of sclerotia at[a]		
		10 wk	20 wk	30 wk
Penicillium citrinum	22.8×10^6	100 a[b]	101 a[b]	98 ab[b]
Dictyosporium elegans	1.1×10^3	64 b	59 d	79 c
Coniothyrium minitans	2.0×10^4	79 ab	67 cd	101 bc
Talaromyces flavus	2.2×10^7	84 ab	89 abc	76 abc
Trichoderma sp.	6.0×10^7	99 a	94 ab	69 a
Gliocladium virens	4.6×10^6	78 ab	75 bcd	64 abc
Terotosperma oligocladum	<10	6 c	0 e	...

[a]Survival expressed as percentage of the sclerotia surviving in untreated control soil without antagonists.
[b]Values in a column followed by a common letter are not statistically different ($P = 0.01$) as determined by the Duncan's multiple range test.

TABLE 2. Survival of sclerotia of *Sclerotinia minor* in soil when sclerotia of *Sclerotinia sclerotiorum* were previously soaked in spore suspensions of antagonists for 3 days before mixing in soil

Antagonist	Percent survival of sclerotia of *S. minor* at[a]		
	8 wk	16 wk	30 wk
Penicillium citrinum	97 a[b]	93 a[b]	70 a[b]
Dictyosporium elegans	106 a	79 a	74 a
Coniothyrium minitans	99 a	82 a	77 a
Talaromyces flavus	105 a	98 a	80 a
Trichoderma sp.	88 a	105 a	91 a
Gliocladium virens	88 a	89 a	88 a
Teratosperma oligocladum	72 a	16 b	0 b

[a]Percent of the sclerotia of *S. minor* surviving in untreated soil without antagonists.
[b]Values in a column followed by a common letter are not statistically different ($P = 0.01$) as determined by Duncan's multiple range test.

TABLE 3. Survival of sclerotia of *Sclerotinia minor* in soil when spores of various antagonists were added to the soil at various rates of application

Antagonist	Spore type	Population density (spores/g of soil)	Percent survival of sclerotia at[a]		
			10 wk	20 wk	30 wk
Penicillium citrinum	Conidia	1.0×10^7	97	100	100
Dictosporium elegans	Conidia	9.1×10^3	96	92	99
Coniothyrium minitans	Conidia	1.2×10^5	97	78	96
Talaromyces flavus	Ascospore	3.8×10^6	97	88	92
Trichoderma	Conidia	3.4×10^6	87	83	88
Gliocladium virens	Conidia	9.6×10^5	83	98	78
Teratosperma oligocladum	Conidia	10	22	1	0
Sporidesmium sclerotivorum	Conidia	14	58	0	0

[a]Percent of sclerotia of *S. minor* surviving in untreated control soil without antagonists.

sclerotia to healthy sclerotia and cause their decay.

C. minitans, *D. elegans*, *G. virens*, *T. flavus*, and *Trichoderma* spp. are all known to parasitize sclerotia of *Sclerotinia* spp. (4,7–12). However, these fungi are passive mycoparasites because they do not grow from infected sclerotia through soil to infect healthy sclerotia (Table 2). Apparently, these fungi cannot utilize sclerotia as a food base from which to grow out into soil and infect healthy sclerotia. To remedy this deficiency, some researchers have added these mycoparasites to soil with a food base (7). Such a food base is not protected from numerous microorganisms that can utilize it, and, thus, nutrients in the food base are rapidly depleted. Once the food base has been depleted of nutrients, the biocontrol agent becomes quiescent.

Davet (9) worked with 20 isolates of *Trichoderma* spp. and concluded that the parasitic activity in a nonsterile soil varied positively with their competitive saprophytic ability. The present study tends to confirm Davet's conclusion. Thus, not only must a mycoparasite be able to infect and destroy a plant pathogen, but it must also be able to grow through natural soil and infect healthy propagules of the pathogen. That is, it must be an aggressive mycoparasite as opposed to a passive mycoparasite.

LITERATURE CITED

1. Adams, P. B. 1987. Comparison of isolates of *Sporidesmium sclerotivorum* in vitro and in soil for potential as active agents in microbial pesticides. Phytopathology 77:575-578.
2. Adams, P. B., Johnston, S. A., Krikun, J., and Carpenter, H. E. 1983. Application of methan sodium by sprinkler irrigation to control lettuce drop caused by *Sclerotinia minor*. Plant Dis. 67:24-26.
3. Adams, P. B., Marois, J. J., and Ayers, W. A. 1984. Population dynamics of the mycoparasite, *Sporidesmium sclerotivorum*, and its host, *Sclerotinia minor*, in soil. Soil Biol. Biochem. 16:627-633.
4. Artiques, M., and Davet, P. 1984. Comparaison des aptitudes parasitaires du clones de *Trichoderma* vis-à-vis de quelques champignons à sclerotes. Soil Biol. Biochem. 16:413-417.
5. Ayers, W. A., and Adams, P. B. 1981. Mycoparasitism of sclerotial fungi by *Teratosperma oligocladum*. Can. J. Microbiol. 27:886-892.
6. Ayers, W. A., and Adams, P. B. 1985. Interaction of *Laterispora brevirama* and the mycoparasites *Sporidesmium sclerotivorum* and *Teratosperma oligocladum*. Can. J. Microbiol. 31:786-792.
7. Backman, P. A., and Rodriquez-Kabana, R. 1985. A system for the growth and delivery of biological control agents to the soil. Phytopathology 65:819-821.
8. Campbell, W. A. 1947. A new species of *Coniothyrium* parasitic on sclerotia. Mycologia 39:190-195.
9. Davet, P. 1986. Activité parasitaire des *Trichoderma* vis-à-vis des champignons à sclerotes; corrélation avec aptitude à la compétition dans un sol nonsterile. Agronomie (Paris) 6:863-867.
10. McCredie, T. A., and Sivasithamparam, K. 1985. Fungi mycoparasitic on sclerotia of *Sclerotinia sclerotiorum* in some western Australian soils. Trans. Br. Mycol. Soc. 84:736-739.
11. McLaren, D. L., Huang, H. C., and Rimmer, S. R. 1986. Hyperparasitism of *Sclerotinia sclerotiorum* by *Talaromyces flavus*. Can. J. Plant Pathol. 8:43-48.
12. Tu, J. C. 1980. *Gliocladium virens*, a destructive mycoparasite of *Sclerotinia sclerotiorum*. Phytopathology 70:670-674.