Pathogenicity of *Trichoderma* spp. on the sclerotia of *Sclerotinia sclerotiorum*

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One isolate of *Trichoderma viride*, 12 of *T. koningii*, 5 of *T. harzianum*, 11 of *T. pseudokoningii*, and 9 *Trichoderma* spp. obtained from 15 soils by baiting with the sclerotia of *Sclerotinia sclerotiorum* were tested for pathogenicity and aggressiveness *in vitro* and in field soil against the latter. Five isolates of *T. koningii*, two of *T. harzianum*, three of *T. pseudokoningii*, and one of an unidentified species killed 62–100% of the sclerotia within 25 days when tested *in vitro*. Two isolates of *T. koningii* killed 100% of the sclerotia within 7 days. One isolate of *T. koningii* killed 100% of the sclerotia within 60 days under field conditions, when soil was infested with 10^4, 10^6, or 10^8 conidia/g. The rate of decline was more rapid at inoculum level of 10^6 and 10^8 conidia/g of soil than at 10^4.

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Un isolat du *Trichoderma viride*, 12 du *T. koningii*, 5 du *T. harzianum*, 11 du *T. pseudokoningii* et 9 d'espèces de *Trichoderma* non identifiées ont été extraits de 15 sols en amorçant avec des sclérotes du *Sclerotinia sclerotiorum*. On a ensuite mesuré la pathénogénicité et l'aggressivité *in vitro* et au champ de ces isolats contre le *S. sclerotiorum*. Cinq isolats du *T. koningii*, deux du *T. harzianum*, trois du *T. pseudokoningii* et un isolat d'une espèce non identifiée ont provoqué la mort de 62 à 100% des sclérotes en 25 jours *in vitro*. Deux isolats du *T. koningii* ont tué 100% des sclérotes en 7 jours. Un isolat du *T. koningii* a provoqué la mort de 100% des sclérotes en 60 jours au champ après que le sol eut été infesté de 10^4, 10^6 et 10^8 conidies/g. Le taux de diminution des sclérotes était plus rapide avec les inoculums de 10^6 et 10^8 qu'avec ceux de 10^4 conidies/g de sol.

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Introduction

Sclerotia sclerotiorum (Lib.) de Bary, a fungal pathogen of vegetable crops, causes heavy yield losses in certain vegetable seed production fields of Minas Gerais, Brazil. On one particular farm, the population of sclerotia per kilogram of soil was exceptionally high (10–13). In this case crops are cultivated on leveled terraces prepared by removing up to 1.5 m of topsoil from hill slopes. Vegetable crops, especially snap bean, pea, lettuce, cauliflower, and cabbage, have been grown continuously for the last 15 years. Because losses from 1977 to 1979 were as high as 85–100%, the land is no longer being used for vegetable seed production. Fumigation and application of fungicide to soil failed to control the pathogen and therefore a search for biocontrol agents was initiated. The sandy soil in these fields is acidic (pH 5–5.6), has a low organic matter content, and poor water retention capacity. Therefore we have focused our attention on fast-growing fungal hyperparasites that can be propagated on a large scale and are able to tolerate acidic and relatively dry soils. Numerous *Trichoderma* species satisfy these requirements and are reported to be parasites of many sclerotial and non-sclerotial fungal plant pathogens. *Trichoderma harzianum* Rifai has been the fungus most often tested for the control of *Rhizoctonia solani* Kuhn and *Sclerotium rolfsii* Sacc. (Elad *et al.* 1980; Hadar *et al.* 1979; Henis *et al.* 1978; Wells *et al.* 1972). Huang (1980) showed the potential of *T. viride* Pers. ex S. F. Gray as a biocontrol agent of *S. sclerotiorum*.

The following study was done to determine the potential of *Trichoderma* spp. naturally occurring in the soils of Minas Gerais in reducing the inoculum density of *S. sclerotiorum*.

Materials and methods

Isolates of *Trichoderma* spp. were obtained from 15 soils collected from vegetable fields in different agroclimatic regions, with or without occurrence of the disease caused by *S. sclerotiorum*. Isolations were made by a baiting technique. After adjusting the soil moisture to about 30% of its moisture holding capacity, 25 sclerotia harvested from cornmeal culture were mixed with 500 g of soil and incubated at room temperature. After 25–30 days sclerotia were recovered, washed under running tap water for 15–20 min, surface sterilized in 1.7% sodium hypochlorite solution for 30 s and in 70% ethanol for 1 min, and then rinsed in sterile distilled water. Sclerotia were then placed on 1.5% water agar in petri plates and incubated at 20°C for 10 days. *Trichoderma* spp. and other fungi growing on the ungerminated sclerotia were isolated and maintained on potato–dextrose agar (PDA). The *Trichoderma* spp. were identified according to Rifai (1969).

For pathogenicity tests, a conidial suspension of each isolate of *Trichoderma* was prepared by washing the conidia from 5-day-old PDA cultures with sterile distilled water. Counts were adjusted to 10^6 conidia/mL. Surface-sterilized sclerotia

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were inoculated by immersion for 1 min in the conidial suspension. Control sclerotia were immersed in sterile distilled water. Sclerotia were then placed on sterile moist sand and incubated at 25°C for 25 days, washed in running tap water, surface sterilized, and plated on 1.5% water agar and incubated at 20°c for 15 days to check their viability.

The four isolates designated as Tk-2, Tk-5, Th-1, and Th-2 which killed 100% of the sclerotia in the above experiment were further tested for pathogenicity *in vitro* and in the soil. The *in vitro* test was done as described above except that the sclerotia were inoculated with suspensions of 10^4, 10^6, and 10^8 conidia/mL, and the incubation period was reduced to 7 or 15 days. The field test was done in the vegetable seed production farm described earlier. Three holes of 15-cm diameter, 20 cm deep, and 2 m apart were dug in the field. The soil obtained from the upper 10 cm of each hole was collected separately from the lower 10 cm. Each portion was infested with 10 sclerotia of *S. sclerotiorum*. The infested soil from the lower portion was placed in the bottom part of a nylon mesh (1-mm) cylinder, without adding *Trichoderma* (hence hereafter referred to as the bottom noninfested layer (BNL), whereas the soil from upper portion was infested with an isolate of *Trichoderma* at the rate of 10^4, 10^6, or 10^8 conidia/g of soil, before placing over the BNL. This top layer (hereafter referred to as upper infested layer (UIL)), was separated from BNL by a meshed nylon sheet. The soil-filled cylinders were put back into the corresponding holes. After 20, 40, or 60 days, sclerotia were recovered from the BNL and UIL and their viability was tested on water agar as described earlier. The pathogenic isolates used in this study are stored on colonized sclerotia kept on silica gel in a refrigerator.

The culture experiments included three replicates and were repeated twice. The field experiment was conducted twice at about 1-month intervals at two different sites in the same field. The data from *in vitro* studies were analysed statistically using one-way analysis of variance. The field-study data were analysed using arc-sine transformation. Initial analysis of variance was done separately on each isolate using three-way factorial ANOVA (inoculum concentration × days of incubation × soil layer). Since the viability of sclerotia in BNL and UIL was not significantly different and no interaction of soil layer and other factors was significant, the values from both layers were averaged and data analysed using two-way factorial ANOVA. Tukey's *w* procedure was applied to test the difference between means.

Results

Of the 38 isolates of *Trichoderma* tested *in vitro*, 5 of *T. koningii*, 2 of *T. harzianum*, 3 of *T. pseudokoningii* Rifai, and 1 of an unidentified species killed 62–100% of the sclerotia (Table 1). One isolate of *T. viride*, seven of *T. koningii*, three of *T. harzianum*, eight of *T. pseudokoningii*, and eight of unidentified species did not affect the germinability of the sclerotia. The isolates which killed the sclerotia grew profusely on them and caused a soft rot. In most cases the pathogenicity of isolates within the species varied significantly. For instance two isolates of *T. koningii*, Tk-2 and Tk-5, killed 100% of the sclerotia, Tk-4 killed only 62%, whereas the other isolates did not affect sclerotium germination. Similarly 2 isolates of *T. harzianum* killed all the sclerotia but the other 3 did not, whereas only 3 of the 11 isolates of *T. pseudokoningii* were relatively aggressive. Inoculations of sclerotia with isolates Th-1, Th-2, Tk-2, and Tk-5 at 10^4 conidial/mL killed 88–93% sclerotia within 7 days and 100% within 15 days; when inoculations were made with 10^6 or 10^8 conidia/mL all sclerotia were killed within 7 days.

TABLE 1. Germination percentage of sclerotia of *Sclerotinia sclerotiorum* inoculated by immersion in conidial suspension of the respective isolate of *Trichoderma* spp.*

Isolate of *Trichoderma*		Germination (%)†
Trichoderma koningii	(Tk-1)	15*b*‡
T. koningii	(Tk-2)	0*a*
T. koningii	(Tk-3)	24*c*
T. koningii	(Tk-4)	38*d*
T. koningii	(Tk-5)	0*a*
T. harzianum	(Th-1)	0*a*
T. harzianum	(Th-2)	0*a*
T. pseudokoningii	(Tp-1)	28*c*
T. pseudokoningii	(Tp-3)	22*c*
Trichoderma sp.		24*c*
Control		100*e*

*Inoculated sclerotia were incubated at 25°C for 25 days. Germination tested on water agar at 20°C.

†Average of three replications with 50 sclerotia in each replication.

‡Numbers followed by the same letter are not statistically different as determined by Tukey's *w* procedure.

The viability of sclerotia in soil infested with isolates Tk-2, Tk-5, Th-1, and Th-2 declined significantly at the same rate in UIL and BNL. In general this reduction was related to the inoculum concentration of the antagonist and the time of the incubation period, with a higher number of sclerotia being killed in a shorter period of time in soil infested with high concentrations of conidia (Table 2). Averaging the effects of days of incubation and isolates showed conidia concentration of 10^8 conidia/g soil to be more effective than 10^4 or 10^6/g. Similarly, averaging the effects of isolates and concentrations revealed that the number of live sclerotia decreased with increasing incubation period. However the increase in number of dead sclerotia with time and inoculum concentration was caused more rapidly by isolates of *T. koningii* than by those of *T. harzianum*. On an average both isolates of *T. koningii* were more virulent and aggressive than both isolates of *T. harzianum* in soil. Respective species of *Trichoderma* were recovered from all nongerminating and some germinating sclerotia.

TABLE 2. Percentage (arc sine) germination of sclerotia of *Sclerotinia sclerotiorum* recovered from field soil to a depth of 20 cm, of which the upper 10-cm layer was infested with conidia of *Trichoderma koningii* or *T. harzianum**

Conidia/g	Days of incubation	*T. koningii*†		*T. harzianum*	
		Tk-2	Tk-5	Th-1	Th-2
Control	20	69.2	69.2	69.2	69.2
	40	60.5	60.5	60.5	60.5
	60	54.5	54.5	54.5	54.5
10^4	20	45.0	45.0	45.0	48.9
	40	33.1	18.6	39.9	42.0
	60	17.2	0.0	35.1	34.8
10^6	20	34.5	39.8	44.1	44.8
	40	31.2	21.8	34.7	40.9
	60	14.4	10.5	35.1	30.0
10^8	20	31.0	17.0	36.5	43.9
	40	16.1	7.5	32.6	40.0
	60	7.5	0	31.1	30.6
Tukey's *w*, to compare:					
concn. within time		9.0	7.4	6.5	7.8
incubation period within concn.		7.1	6.1	5.3	6.3

*Average of three replications of 10 sclerotia in each. The initial statistical analysis showed no difference in the germination of sclerotia from upper 10-cm infested layer of soil and lower noninfested layer of soil; therefore the values from both layers were averaged and analysed using two-way factorial ANOVA.
†Numbers rounded to the nearest one decimal point.

Discussion

This study shows that some strains of *T. koningii*, *T. pseudokoningii*, and *T. harzianum* parasitize the sclerotia of *S. sclerotiorum*. This confirms or adds to the list of mycoparasites that show some promise for controlling soil-borne plant pathogens (Elad *et al.* 1980; Grinstein *et al.* 1979; Hadar *et al.* 1979; Harman *et al.* 1980; Henis *et al.* 1978; Huang 1978, 1980; Huang and Hoes 1976; Tu 1980; Uecker *et al.* 1978; Wells *et al.* 1972). However contrary to the reports by others (Jones and Watson 1969; Huang 1980) *T. viride* was not pathogenic to *S. sclerotiorum*. This discrepancy might be attributed to variation in the aggressiveness of this particular isolate as is shown for some isolates of *T. koningii* and *T. harzianum* in the present study. Such variation does not seem to have been reported previously. From the small number of isolates tested it is apparent that in soils of Minas Gerais, strains nonpathogenic to *S. sclerotiorum* are more common than pathogenic strains. Of the pathogenic isolates, those of *T. koningii* were more aggressive than isolates of other species.

Jones *et al.* (1974) reported that 1,3-β-glucanase is the key enzyme involved in the destruction of sclerotial walls by *T. viride*. The observation of soft rot caused by pathogenic isolates tested in our study suggests that some enzymatic mechanism may also be involved in the breakdown of the pseudoparenchymatous tissues of sclerotia.

The four isolates shown to be most virulent in culture trials reduced the viability of the sclerotia equally as well in the UIL as in the BNL of the soil. Destruction of sclerotia in the BNL indicates that lower layers in sandy soils can become infested with *Trichoderma* by conidia carried down with rain or irrigation water or by downward growth of mycelium. Since the number of dead sclerotia in the UIL and BNL of the soil was similar at each sampling time, conidial infection seems to be more probable.

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