Efficacy of Trichoderma harzianum as a Biocontrol for Sclerotium rolfsii

Homer D. Wells, Durham K. Bell, and Casimir A. Jaworski

Research Plant Pathologist, Plant Science Research Division, ARS, USDA; Assistant Professor of Plant Pathology, University of Georgia Coastal Plain Experiment Station; and Research Soil Scientist, Plant Science Research Division, ARS, USDA, respectively, Tifton 31794.

Cooperative investigations of the Plant Science Research Division, ARS, USDA, and the University of Georgia College of Agriculture Experiment Stations, Coastal Plain Station, Tifton.

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ABSTRACT

Trichoderma harzianum isolated from diseased sclerotia of *Sclerotium rolfsii* was pathogenic to *S. rolfsii, Sclerotinia trifoliorum*, and *Botrytis cinerea* in agar culture, but was innocuous to *Rhizoctonia solani, Pythium aphanidermatum*, and *P. myriotylum*. Under greenhouse conditions, *T. harzianum* effectively controlled *S. rolfsii* on blue lupines, tomatoes, and peanuts. Under natural field conditions, one to three applications of *T. harzianum* inoculum applied over the plants onto the soil surface was highly effective in reducing *S. rolfsii* damage to tomato transplants.

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Additional key words: antagonism, white mold, southern blight, and hyperparasitism.

In late April 1970, *Sclerotium rolfsii* Sacc. caused extensive damage to blue lupine (*Lupinus angustifolius* L.) seed fields in Georgia and Florida. While inspecting a severely damaged blue lupine seed field at the University of Florida, Gainesville, we noted that many of the sclerotia were lighter-colored than usual, and disintegrated readily when pressed between the fingers. A species of *Trichoderma* was isolated from these sclerotia and identified by Richard Hanlin, University of Georgia, Athens, as *Trichoderma harzianum* Rifai.

Due to taxonomic uncertainty existing in the genus *Trichoderma* (2, 7, 8, 9, 11, 12, 13), we were not able to determine with certainty if *T. harzianum* had previously been reported to be antagonistic to *S. rolfsii*. In two recent papers, Dennis & Webster (4, 5) reported some activity of *T. harzianum* against certain fungi, but *S. rolfsii* was not included in their studies.

This paper contains a description of our isolate (1970-3A) of *T. harzianum*, its activity against select microorganisms grown in artificial culture, and the efficacy of the organism as a biocontrol for *S. rolfsii* on selected crops in the greenhouse and on tomato, *Lycopersicon esculentum* Mill., in the field.

MATERIALS AND METHODS.—Diseased sclerotia of *S. rolfsii* were placed on 20% V-8 juice-2.5% agar (V8A) in petri dishes without surface sterilization or after surface sterilization by a quick dip in a 0.53% solution (w/v) of sodium hypochlorite. Cultural characteristics of *T. harzianum* isolate 1970-3A were determined on V8A and 2% water agar (WA) at ambient temperatures under diffuse light in the laboratory. The description of *T. harzianum* is

based on observations and averages of 50 measurements from 10- to 12-day-old cultures grown on V8A and WA.

Medium for growing inocula of *T. harzianum* and *S. rolfsii* was prepared by mixing 1 g of ground annual ryegrass (*Lolium multiflorum* Lam.) seed, 10 g Tifton sandy loam (sifted through a 2-mm mesh screen), and 2 ml H_2O (RSM). The medium was placed in Fernbach flasks, plugged, and autoclaved 1 hr at 120 C on 2 successive days. Flasks containing autoclaved RSM were seeded with *T. harzianum* or *S. rolfsii* and shaken daily for 10 days. Just before use as inoculum, cultures were comminuted with an equal amount of fresh RSM (RSMI).

RESULTS.—*The fungus.*—*Trichoderma harzianum* was the only fungus isolated from the diseased *S. rolfsii* sclerotia. On V8A, *T. harzianum* isolate 1970-3A grows rapidly, covering the culture medium in a 100-mm petri dish in 4 days. Cultures are at first white and cottony. When grown under alternating light and dark, they become zonate, with alternating narrow bands that are whitish, and wide bands that are dark green. When grown under constant light, they are uniformly dark green. Cultures are nonodoriferous on V8A. Chlamydospores are intercalary and/or terminal, cylindric to globose, and average 6.9 μ in diam. Single phialides arise laterally on the conidiophores and in clusters of 2-4, terminally. They are hyaline, smooth, ampuliform, and average 6.6 X 3.7 μ. Phialispores arise by budding from the tips of the phialides, and occur in chains in mucous aggregates. They are smooth, subhyaline, mostly subglobose, and average 3.2 X 2.7 μ (Fig. 1).

Antagonism in culture.—In laboratory tests, 5-mm-diam discs of *T. harzianum* isolate 1970-3A were removed from the edge of 4- to 6-day-old cultures and placed on one side of a 100-mm petri dish containing V8A. Similar discs of the suscept host grown in the same manner were placed on the opposite side of the petri dish. Suscept hosts included: *S. rolfsii* from blue lupine, peanut (*Arachis hypogaea* L.), *Desmodium tortuosum* (Sw.) DC., *Dolichos lablab* L., and snapbean (*Phaseolus vulgaris* L.); *Rhizoctonia solani* Kuehn from blue lupine and Southern pea [*Vigna sinensis* (Torner) Savi]; *Botrytis cinerea* (Pers.) Fr. from blue lupine and vetch (*Vicia atropurpurea* Desf.); *Sclerotinia trifoliorum* Eriks. from vetch; *Pythium aphanidermatum* (Edson) Fitzp. from bentgrass (*Agrostis palustris* Huds.), tomato, and snapbean; and *P. myriotylum* Drechs. from snapbean. Each treatment was replicated a minimum of 4 times. Cultures were observed daily, and notes taken on compatibility, antagonism, or pathogenicity to suscept fungal hosts.

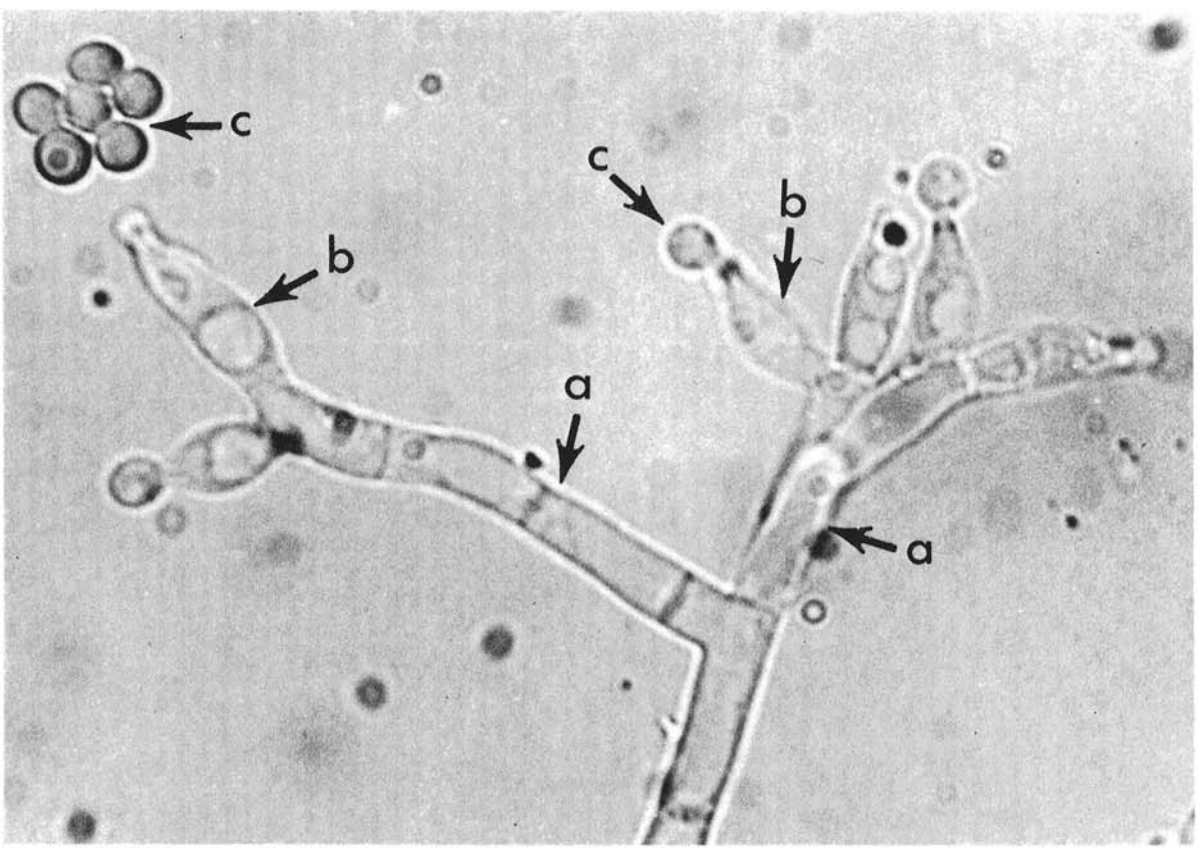

Fig. 1. Photomicrograph of (a) conidiophore with (b) philiades and (c) philiaspores of *Trichoderma harzianum*.

On V8A in petri dishes,isolate 1970-3A of *T. harzianum* had no apparent effect on any isolate of *R. solani, P. aphanidermatum*, or *P. myriotylum*. These fungi grew together with *T. harzianum* without showing any zone of demarcation. After colonies met, the mycelium from each colony grew into areas that had already been occupied by the fungus placed on the opposite side of the dish. In contrast, *S. rolfsii, S. trifoliorum*, and *B. cinerea* only grew until they met the leading edge of the *T. harzianum* isolate, at which time growth ceased and the colonies were soon overrun with *T. harzianum*. No sclerotia were produced, and attempts to reisolate the test hosts from areas where it had been growing vigorously resulted in the recovery of *T. harzianum* alone. This appeared to be adequate proof that *T. harzianum* is capable of attacking and killing *S. rolfsii, S. trifoliorum*, and *B. cinerea* in culture.

Greenhouse biocontrol tests.—Topcrop tomato, Starr peanut, and Rancher blue lupine plants were established in a Tifton sandy loam and vermiculite (4:1, v/v) potting mixture in 18-liter metal bucket-type containers. Plants were fertilized and watered as needed to keep them growing vigorously for 8 weeks. After plants became well established, they were thinned to two each per container with the exception of blue lupine, where ca. 12 were in each container. At the end of 8 weeks, the tomato plants were clipped to a height of ca. 20 cm, and treatments were applied as follows on the three crops: (i) 10 g of *T. harzianum* RSMI/container sprinkled over soil surface; (ii) 10 g of *S. rolfsii* RSMI/container; (iii) 10 g of *T. harzianum* RSMI plus 10 g of *S. rolfsii* RSMI/container; and (iv) a nontreated control. All treatments were replicated 6 times. After treatments were applied, the soil was kept moist. An effort was made to keep greenhouse temperatures between 26 and 32 C. Observations were made at frequent intervals on the progress of *S. rolfsii*, and at the end of 3 weeks after treatment, plants were harvested and rated for incidence of necrosis caused by *S. rolfsii*.

Trichoderma harzianum alone or combined with *S. rolfsii* did not affect any test plants. In containers receiving only *S. rolfsii*, the fungus grew and produced sclerotia profusely on the soil surface, and plants of all host species were killed or infected (Table 1). Mycelium and sclerotia of *S. rolfsii* were not evident in containers receiving both fungi or *T. harzianum* alone. Some necrosis or killing of blue lupine plants occurred in all treatments, and at the time of harvest plants were too badly decomposed to determine if *S. rolfsii* was involved. The percentage of healthy plants with treatments *T. harzianum, T. harzianum* + *S. rolfsii*, and nontreated controls were, however, approximately equal, and were at least 80% higher than with *S. rolfsii* alone. The results with peanut and tomato demonstrated that *T. harzianum* RSMI (isolate 1970-3A) provided 100% biocontrol of *S. rolfsii*. These data were not analyzed statistically because unequal variances associated with no variability (100% healthy plants) in three of the treatments on tomato and peanut would have invalidated an analysis of variance. The differences were, however, sufficiently great to warrant the assumption that they were real and significant.

TABLE 1. Efficacy of *Trichoderma harzianum* applied on the soil surface around plants as a biocontrol for *Sclerotium rolfsii* in 18-liter metal bucket-type containers under greenhouse conditions in the spring of 1971[a]

Treatment	Percentage healthy plants		
	Blue lupine	Tomato	Peanuts
Noninoculated, nontreated	89	100	100
T. harzianum only	91	100	100
S. rolfsii only	7	33	25
T. harzianum + *S. rolfsii*	88	100	100

[a] Inocula of each fungus was prepared by growing it on a mixture of 1 part ground annual ryegrass seed and 10 parts soil for 10 days, and comminuted with an equal amount of fresh media just prior to application. Inocula was applied at rate of 10 g/container.

Biocontrol in the field.—On 20 April 1971, Urbana tomatoes were seeded in a field of Stilson loamy sand where *S. rolfsii* had been a serious problem during the 1970 tomato transplant production season. Tomatoes were seeded at ca. 1 cm depth at a rate of ca. 160 seed/m of row, rows 35 cm on centers, four rows/bed, and beds on 185-cm centers. Fertilization consisted of 800 kg/hectare of 5-4.3-4 (NPK) in 7-cm bands, 2 cm below the seed level, and 100 kg/hectare of ammonium nitrate broadcast 3 weeks after seeding. Soil pH at time of seeding was 5.4. Generally accepted cultural practices for tomato transplant production were followed in irrigation and herbicide (diaphenamid), insecticide (sevin), and fungicide (zineb) applications. In one experiment, treatments consisted of applying *T. harzianum* RSMI by hand at rates of 1.5 g/cm of row over a ca. 10 cm-wide band on different dates as listed in Table 2, and a nontreated control. Plants were ca. 4 cm tall at the first application. Plots were one bed wide and 300 cm long. All treatments were replicated 5 times in a randomized block design. Disease ratings were made periodically until 6 July, when all plants from two randomly selected 150 cm-long sections of row from each plot were pulled and rated for the presence of root or stem rot caused by *S. rolfsii*. Disease ratings and percentage of healthy plants were analyzed statistically.

In *T. harzianum* RSMI-treated plots, the soil surface took on a white, cottony appearance in 2 to 4 days after treatment. Later, 6 to 10 days after treatment, the soil surface in these areas became a dark green.

Nontreated plots in the first experiment had the highest disease ratings and the lowest yield of healthy plants (Table 2). The averages of these differences as compared to any *T. harzianum* treatment were highly significant. One application of *T. harzianum* increased the percentage of healthy plants from 21.9 to 90.0. Plots receiving three applications (4, 13, and 24 May) produced 99.5% healthy plants. Visual comparison between *T. harzianum*-treated plots and nontreated controls was striking (Fig. 2).

TABLE 2. Efficacy of *Trichoderma harzianum* applied on the soil surface around tomato seedlings on different dates as a biocontrol of *Sclerotium rolfsii* under field conditions during the spring of 1971[a]

Treatment dates	Average disease ratings[b]			Percentage disease-free plants at harvest[c]
	14 June	23 June	30 June	
Nontreated	2.0	4.4	4.2	21.9
4 May	0.0	0.4	0.8	91.4
13 May	0.0	0.0	0.2	95.6
24 May	0.0	0.0	0.6	91.8
4 and 13 May	0.0	0.0	0.6	94.3
4 and 24 May	0.0	0.0	0.6	90.0
13 and 24 May	0.0	0.0	0.0	97.2
4, 13, and 24 May	0.0	0.0	0.0	99.5
LSD .05		0.6	0.6	8.6
LSD .01		0.9	0.9	11.6

[a] *Trichoderma harzianum* inoculum was prepared by growing it on a mixture of 1 part ground annual ryegrass seed and 10 parts Tifton sandy loam for 10 days, and comminuted with an equal amount of fresh media just prior to application. Planted 20 April, harvested 6 July.

[b] 0 = no visible symptoms or signs of disease; 5 = extensive necrosis or killing.

[c] Total number of plants from 300 cm of row.

In a second field experiment, variables consisted of nontreated versus *T. harzianum*-treated plots of tomato seedlings, nonclipped versus clipped, and treated 1 week before and 1 week after being clipped. Clipping consisted of cutting plants back to ca. 20 cm height as is customarily done by transplant growers who wish to hold plants for a later market. *Trichoderma harzianum* RSMI was applied at the same rate as in the first test. Plots that were nonclipped and plots that were treated with *T. harzianum* before being clipped were treated on 28 May. Plots that were *T. harzianum* RSMI-treated after being clipped were treated 2 June. Plots treated after being clipped were clipped on 28 May. Plots treated

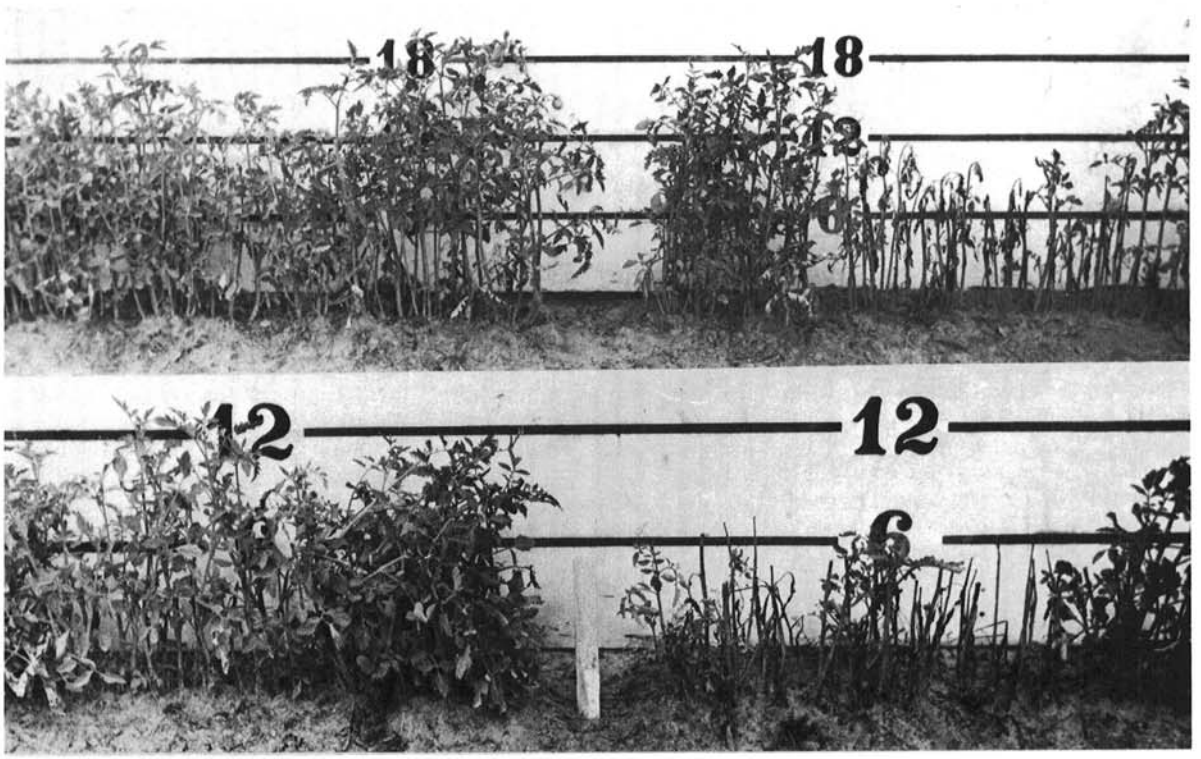

Fig. 2. Biocontrol of *Sclerotium rolfsii* on tomato transplants in the field with *Trichoderma harzianum*. Above: (left) one application of *T. harzianum*; (right) nontreated control. Below: (left) *T. harzianum* applied before clipping; (right) clipped, no *T. harzianum*.

TABLE 3. Efficacy of *Trichoderma harzianum* applied on the soil surface around tomato seedlings before and after clipping and without clipping as a biocontrol for *Sclerotium rolfsii* under field conditions in the spring of 1971[a]

Type of treatments	Average disease ratings[b]			Percentage disease-free plants at harvest[c]
	14 June	23 June	30 June	
Nontreated, nonclipped	2.7	4.3	4.8	46.2
Nontreated, clipped	1.3	2.7	4.2	39.5
Treated, nonclipped	0.3	0.7	0.8	88.8
Treated before clipped	0.0	0.2	0.3	94.0
Treated after clipped	0.3	0.2	0.3	93.7
LSD .05	1.2	1.4	0.9	15.9
LSD .01	1.7	1.9	1.2	21.6

[a] *Trichoderma harzianum* inoculum was prepared by growing it on a mixture of 1 part ground annual ryegrass seed and 10 parts Tifton sandy loam for 10 days, and comminuted with an equal amount of fresh media just prior to application. Planted 20 April, harvested 2 July.

[b] 0 = no visible symptoms or signs of disease; 5 = extensive necrosis or killing.

[c] Total number of plants from 300 cm of row.

before being clipped were clipped on 2 June. Plots were one bed wide and 150 cm in length. These treatments were replicated 5 times in a randomized block design. *Sclerotium rolfsii* disease ratings were made periodically until 2 July, when all plants from two randomly selected 150 cm-long sections of row from each plot were pulled and rated for presence or absence of root or stem rot. Disease ratings and percentage of healthy plants were analyzed statistically.

In the second field experiment, clipped and nonclipped plots not treated with *T. harzianum* averaged 39.5 and 46.2% disease-free plants, respectively (Table 3). All plots that received *T. harzianum* yielded at least 88.8% healthy plants. The clipping factor was not significant. There was no evidence that *T. harzianum* was harmful to tomato plants.

DISCUSSION.—Those who have studied antagonism of *Trichoderma* in the past have usually named but not described the fungus with which they were working. Further confusion has arisen because of general disagreement among taxonomists in the classification of species of *Trichoderma* (2, 7, 11, 12). We have elected, therefore, to accept the most recent classification by Rifai (11), and in most essential respects, our fungus agrees with his concept of *T. harzianum* aggregate. Therefore, we feel that until the taxonomy of *Trichoderma* is uniform and well accepted, a sufficiently complete description of a fungus under discussion should be made for convenient identification with any system.

Isolate 1970-3A of *T. harzianum* was pathogenic to *S. rolfsii, S. trifoliorum*, and *B. cinerea*, but was innocuous to *R. solani, P. aphanidermatum*, and *P. myriotylum*. In view of demonstrated antagonism of *Trichoderma* against species of *Rhizoctonia* and *Pythium* (3, 10, 12, 13, 14) we were surprised that our isolate, 1970-3A, had no effect on these organisms. Species or strains of *Trichoderma* may be differentially selective against different fungi, however. Dennis & Webster (4, 5) demonstrated such differences both within and between species of *Trichoderma*. A concerted effort should be made to obtain isolates that are highly active against individual plant pathogens.

Our success with *T. harzianum* as a biocontrol for *S. rolfsii* may be partially due to our method of culturing, preparing, and applying the *T. harzianum* RSMI. We did not have sufficient information to determine if *T. harzianum* spores were capable of germinating and attacking *S. rolfsii* directly. We, therefore, elected to blend the RSM culture of *T. harzianum* with an equal amount of new media just before use to insure an adequate food base for vigorous growth. There is usually some leaf drop from dense stands of tomato seedlings. *Sclerotium rolfsii* and other weak parasites could colonize such material in advance of *T. harzianum*, as outlined by Garrett (6). Thus, a fresh supply of a readily usable food base for *T. harzianum* may have contributed to *S. rolfsii* control. We did not attempt to alter the soil microflora per se by incorporating inoculum or amendments into the soil, but only to overwhelm temporarily the infection court with *T. harzianum* and a fresh food base. The earliest treatment (4 May) demonstrated that the technique was successful by providing control until the termination date (6 July). If economical methods can be worked out for producing and applying the biocontrol agent, this could be a significant advance.

Investigations are continuing using *T. harzianum* as a potential biocontrol agent of *S. rolfsii* in peanut, where it annually causes monetary losses exceeding $8,000,000 in Georgia alone (J. F. McGill & S. S. Thompson, Univ. Ga. Extension Service, *personal communication*). We are also planning biocontrol tests on a number of additional crops that annually suffer economic losses from *S. rolfsii* (1).

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