In Vitro Antagonism of *Trichoderma* species Against Six Fungal Plant Pathogens

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ABSTRACT

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Antagonistic activities of 77 isolates of *Trichoderma*, primarily *T. harzianum*, against representative isolates of *Sclerotium rolfsii*, *Ceratobasidium cornigerum*, *Phytophthora parasitica* f. *nicotianae*, *Pythium aphanidermatum*, *P. myriotylum*, and anastomosis groups 1, 2, 3, and 4 of *Rhizoctonia solani* were compared in vitro on 20% V-8 juice agar. Each pathogen isolate was antagonized by one or more isolates of *Trichoderma*. However, the greatest difference was in *Trichoderma* × pathogen isolate interaction, indicating that a single isolate of *Trichoderma* can be highly effective against an isolate of a pathogen species, but may have only minimal effects on other isolates of the same species. This was especially evident in interactions with *R. solani*.

Additional key words: biological disease control, soilborne diseases.

During the past decade biological control of soilborne plant pathogens was demonstrated with organic soil amendments containing *Trichoderma* spp. (1,2,9,10–13,15,17,18). We screened numerous isolates of *T. harzianum* Rifai with varying degrees of antagonism in vitro against *Ceratobasidium cornigerum* (Bourd.) Rogers, *Rhizoctonia solani* Kühn (AG-1, 2, 3, and 4), *Sclerotium rolfsii* Sacc., *Pythium aphanidermatum* (Eds.) Fitzp., and *Phytophthora parasitica* f. *nicotianae* (Breda de Haan) Tucker. Preliminary results indicated that an isolate of *T. harzianum* antagonistic against one pathogen was often innocuous against others in culture (3,16). Other workers reported similar phenomena (2,4,5,8,13,14).

To determine the potential antagonistic variation of isolates of *Trichoderma* to the pathogens and varying susceptibility of pathogens to *Trichoderma* in vitro, isolates were compared on a medium and at temperatures where both *Trichoderma* and the pathogens grow well in the laboratory. Most sites where biological control may be active in nature are not likely to supply nutrients where the potential antagonist or plant pathogen can grow well, and temperatures during different seasons may be more favorable for the antagonist or pathogen. Thus, results from these studies were not expected to be necessarily related to the degree of biological control that may be observed in the field, but should reflect the capacities and genetic variability of the isolates of *Trichoderma* as antagonists and of the various plant pathogens to resist antagonism. A preliminary report has been published (16).

MATERIALS AND METHODS

Seventy-seven isolates of *Trichoderma* (mostly *T. harzianum*) isolated primarily from the Tifton, GA, area were evaluated as antagonists in vitro against the following plant pathogens: *Sclerotium rolfsii* and *Rhizoctonia solani* anastomosis groups (AG 1, 2, 3, and 4 (three isolates each), *Ceratobasidium cornigerum* anastomosis group (CAG) 2 (two isolates), and *Phytophthora parasitica* f. *nicotianae*, *Pythium aphanidermatum* and *P. myriotylum* Drechs. (one isolate each). All pathogens were compared against at least 32 of the 77 isolates of *Trichoderma*. All pairings were replicated five times. Thirty-three isolates of *Trichoderma* had been previously evaluated, some of which had been selected for activity against *S. rolfsii* and *R. solani*, and 44 of the isolates had not previously been evaluated.

In vitro comparisons consisted of removing 4-mm-diameter disks from the edge of expanding colonies grown on 20% V-8 juice agar medium (V-8 AM = 200 ml unstrained V-8 juice, 15 ml 0.1 N NaOH, 785 ml deionized water and 26 g agar) in petri dishes. The paired isolates of *Trichoderma* and plant pathogens were placed on opposite sides of 8.57 × 1.5 cm (ID) Optilux plastic petri dishes containing ~20 ml of V-8 AM. Isolates of *R. solani* and *C. cornigerum* were placed on the agar 24 hr before the *Trichoderma*. All other pathogen isolates were started simultaneously with *Trichoderma*.

Paired cultures were incubated on a laboratory bench at ambient room temperature of ~26 C under ~2,800 lux constant "daylight" fluorescent light for 5 days, then scored for degree of antagonism on a scale of classes 1–5: class 1 = *Trichoderma* completely overgrew the pathogen and covered the entire medium surface, class 2 = *Trichoderma* overgrew at least two-thirds of the medium surface, class 3 = *Trichoderma* and the pathogen each colonized approximately one-half of the medium surface (more than one-third and less than two-thirds) and neither organism appeared to dominate the other, class 4 = the pathogen colonized at least two-thirds of the medium surface and appeared to withstand encroachment by *Trichoderma*, and class 5 = the pathogen completely overgrew the *Trichoderma* and occupied the entire medium surface. Paired cultures were observed for a total of 9 days before being discarded. We considered an isolate of *Trichoderma* to be antagonistic to the pathogen if the mean score for a given comparison (when rounded to the nearest whole class number) was $\leqslant 2$, but not highly antagonistic if the number was $\geqslant 3$. Selected cultures from pairings of *Trichoderma* × pathogen resulting in the different antagonism classes were viewed microscopically to determine the approximate state of the pathogen thalli after 9 days. This system appeared adequate only for determining the viability of *S. rolfsii*. The other pathogens were evaluated for viability in various classes by assay tests described below.

All isolates of *S. rolfsii* produced sclerotia and rhizomorphs abundantly on V-8 AM in pure culture, and the absence or disintegration of these structures during 9 days of exposure to *Trichoderma* was assumed to indicate death of *S. rolfsii*.

Viability of *P. parasitica* f. *nicotianae* was determined by bioassay. Five pairings from class 1 and class 5 (Fig. 1B) were selected, and the original pathogen inoculum disks were placed

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against the stem of a susceptible 'Hicks'-type tobacco plant entering the first true leaf growth stage. Plants were grown in vermiculite previously autoclaved at 121 C for 1 hr. Plants were grown for 12 days. Absence of disease symptoms was presumed to indicate death of the isolate of *Phytophthora*. Pure culture disks of *Phytophthora* were used to inoculate Hicks tobacco plants as controls.

Viability of *Pythium* spp., *C. cornigerum* and *R. solani* after exposure to *Trichoderma* for 9 days was determined as follows: with *P. aphanidermatum*, *P. myriotylum*, *C. cornigerum* CAG-2, and *R. solani* AG-1, 2, 3, and 4, five pairings were selected from each of the antagonism classes in Fig. 1C,D,F,G,H, and I; and the original inoculum disks were placed on separate selective media for *Pythium* (7) and *Rhizoctonia* (6). The Rhizoctonia medium was also used for *C. cornigerum*. Pure cultures of *Pythium* spp., *C. cornigerum* and the *Rhizoctonia* AGs were used as controls on the selective media. If there was no growth of the plant pathogens after 4 days, death was assumed.

Antagonism class ratings were subjected to statistical analysis. Means squares were subdivided into orthogonal components to analyze factors contributing to significant differences for means squares and variance ratios (Fisher's F-values) in antagonism classes 1–5 and interactions. Since interactions at all levels were highly significant, data are also presented as percentages of isolates of *Trichoderma* in the various antagonism classes (means rounded to nearest whole numbers) for the different pathogens.

RESULTS

There were differences ($P = 0.01$) in the average antagonistic abilities of isolates of *Trichoderma* against the array of plant pathogens (Table 1). There was also a significant difference in the

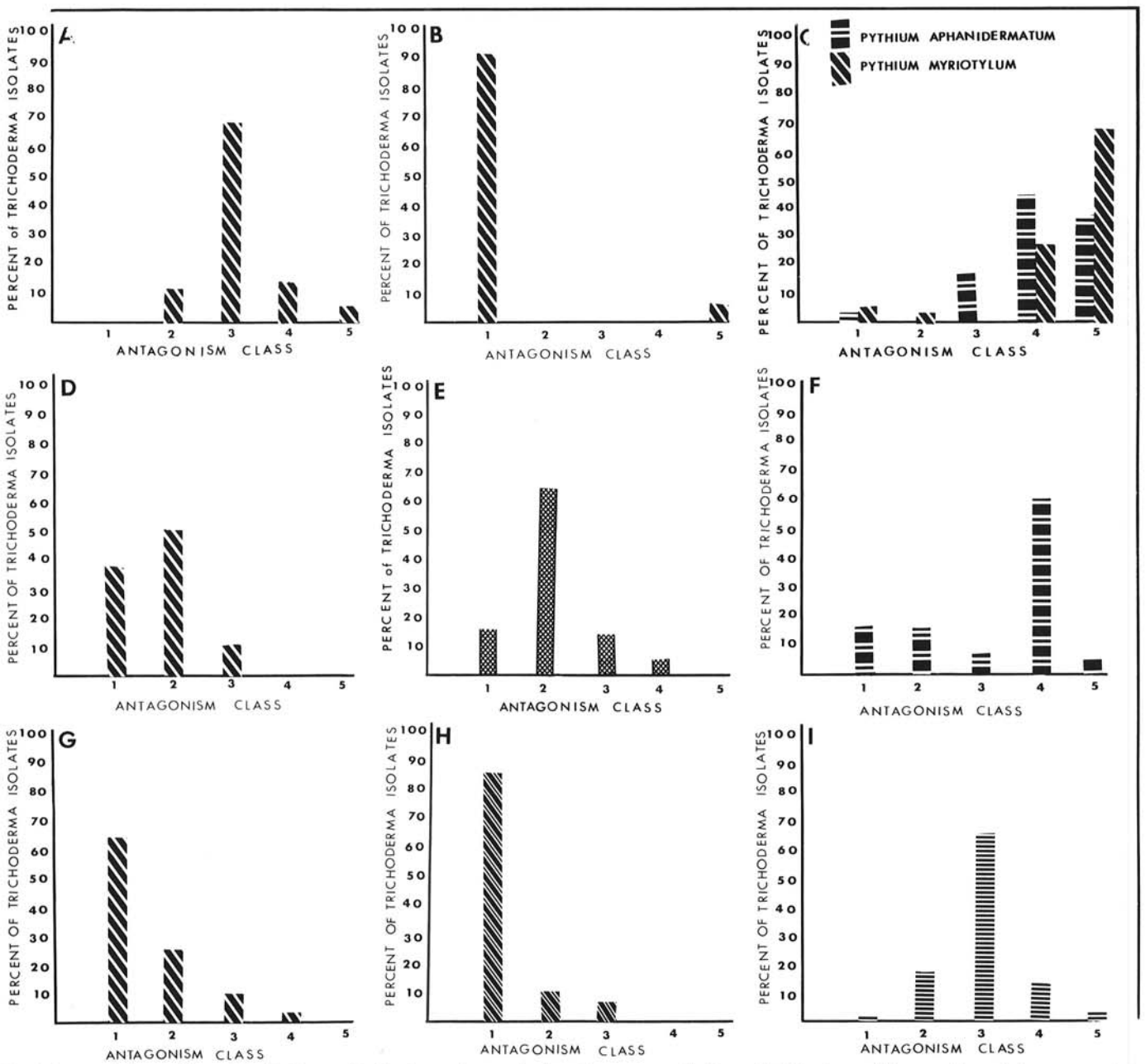

Fig. 1. Antagonistic reactions of *Trichoderma* with six plant pathogens in vitro. Percentages of isolates of *Trichoderma* with means (rounded to nearest whole number) in antagonism classes 1–5: 1 = *Trichoderma* completely overgrew the pathogen and covered the entire medium surface; 2 = *Trichoderma* overgrew at least two-thirds of the medium surface; 3 = *Trichoderma* and the pathogen each colonized approximately one-half of the medium surface and neither organism dominated the other; 4 = the pathogen colonized at least two-thirds of the medium surface and withstood encroachment by *Trichoderma*; and 5 = the pathogen completely dominated *Trichoderma*, overgrew it, and occupied the entire medium surface. *Trichoderma* paired with: **A,** *Sclerotium rolfsii*; **B,** *Phytophthora parasitica* f. *nicotianae*; **C,** *Pythium aphanidermatum* and *P. myriotylum*; **D,** *Ceratobasidium cornigerum*; **E,** *Rhizoctonia solani* averaged over AG-1, 2, 3, and 4; **F,** *Rhizoctonia solani* AG-1; **G,** *Rhizoctonia solani* AG-2; **H,** *Rhizoctonia solani* AG-3; and **I,** *Rhizoctonia solani* AG-4.

way pathogens reacted to *Trichoderma*. With *R. solani*, significant differences in susceptibility to antagonism occurred between AGs and between isolates within an AG. Interactions between isolates of *Trichoderma* with pathogen isolates, pathogen species, *R. solani* AGs and isolates of *R. solani* within an AG were also significant.

Antagonism was not apparent against *S. rolfsii* for 88% of the *Trichoderma* isolates (rating ≥ 3) and only 12% had a high level of antagonism (rating ≤ 2) (Fig. 1A). All antagonistic isolates had been previously selected for activity against *S. rolfsii* representing the evaluation of $>1{,}000$ isolates during the past 8 yr. Ninety-six percent of the isolates of *Trichoderma* were antagonistic to *P. parasitica* f. *nicotianae* (Fig. 1B). Only 2 and 9% of the isolates of *Trichoderma* were highly antagonistic against *P. aphanidermatum* and *P. myriotylum*, respectively, with most of the isolates of *Trichoderma* rating ≥ 4 (Fig. 1C). Eighty-eight percent of the isolates of *Trichoderma* were antagonistic against *C. cornigerum* and the remaining isolates rated ≤ 4 (Fig. 1D). When antagonistic classes were averaged over AGs for *R. solani* (Fig. 1E), 85% of the isolates of *Trichoderma* were antagonistic and only 15% appeared to be nonantagonistic against *R. solani*. However, only 20 and 33% of the isolates were antagonistic against AG-4 (Fig. 1I) and AG-1 (Fig. 1F), respectively, but 91 and 95% of the isolates were antagonistic to AG-2 (Fig. 1G) and AG-3 (Fig. 1H), respectively. Also, individual isolates of *Trichoderma* that were antagonistic to one isolate within an AG were nonantagonistic to one or two of the other isolates with the same AG or to other isolates in other AGs. This difference is reflected by the large F-values for isolates of *Trichoderma* × isolates of *R. solani* within AGs (Table 1).

After 9 days, either no sclerotia and rhizomorphs had been produced by *S. rolfsii* rated in antagonism class 2 or these structures were partially to completely disintegrated. Those in class 3 produced sclerotia and rhizomorphs, but in smaller numbers than by those in classes 4 and 5. There was partial disintegration of rhizomorphs contacting the leading edge of the thallus of *Trichoderma* rated in class 3, but the rhizomorphs and sclerotia behind the contact zone were intact and sclerotia were firm. There was no microscopic evidence of viable mycelium of *S. rolfsii* in class 1.

Pure cultures of *P. parasitica* f. *nicotianae* and cultures from antagonism class 5 produced disease symptoms on inoculated Hicks tobacco plants within 3 days. Two cultures in class 1 did not induce disease during 12 days, and with three cultures disease symptoms developed 6–10 days after inoculation.

Pure cultures of *P. aphanidermatum* and *P. myriotylum* grew on the Pythium medium (7) after 1 day. Cultures of both *Pythium* spp. from antagonism class 1 produced no growth on the medium during 4 days. Two cultures of *P. myriotylum* rated in class 2 grew by the third day and three did not grow during 4 days. All cultures of the *Pythium* spp. in classes 3, 4, and 5 grew on the selective medium after 1 day.

Pure cultures of both isolates of *C. cornigerum* CAG-2 grew on the Rhizoctonia medium (6) after 1 day. Cultures of both isolates of *Ceratobasidium* from antagonism classes 1 and 2 did not grow within the 4-day incubation period. All cultures in class 3 grew after 2 days.

Pure cultures of all isolates of *R. solani* AG-1, 2, 3, and 4 grew on the Rhizoctonia medium (6) after 1 day. In antagonism class 1, there was no growth of *Rhizoctonia* on the selective medium within 4 days. There was no growth of *R. solani* AG-2 and 3 in antagonism class 2 within 4 days; however, in antagonism class 2, all cultures of one AG-1 isolate grew the second day. These isolates had formed abundant sclerotia atop the original inoculum disks. In all antagonism classes ≥ 3, there was growth of all isolates of *R. solani* on the selective medium after 2 days.

TABLE 1. Antagonism between *Trichoderma* and plant pathogenic fungi. Variance ratios and significance of Fisher's F-values for antagonism class ratings for the indicated variables as determined by analysis of variance

Variables	Degrees of freedom	Fisher's F-values[a]
Main Effects		
Trichoderma isolates	76	281**
Plant pathogen isolates	19	2,647**
Plant pathogen species	5	5,587**
Rhizoctonia solani AGs	3	1,193**
R. solani isolates within AGs	6	2,135**
Interactions		
Trichoderma isolates × plant pathogen isolates	1,140	5.19**
Trichoderma isolates × plant pathogen species	380	2.65**
Trichoderma isolates × *R. solani* AGs	608	35.59**
Trichoderma isolates × *R. solani* isolates within AGs	560	23.45**
Error	3,140	

[a]** Following an F-value indicates statistical significance, $P \leq 0.01$.

DISCUSSION

We realize that in vitro screening with our arbritary rating system for biological antagonists effective against soilborne plant pathogens is a simplistic approach to understanding a small sector of biological systems in disease control. However, controlling a large sector of the environment, excluding other soil microflora and supplying a uniform food base, temperature, moisture, and light should yield useful information on the degree of antagonistic variability within *Trichoderma* and the diversity of ability among soilborne pathogens to resist antagonism.

There were significant differences in average responses to antagonism by the 77 isolates of *Trichoderma* between the different genera and species of pathogens as shown in both Table 1 and Fig. 1. Also, there were significant differences in average antagonistic abilities of the isolates of *Trichoderma* when averaged over pathogens. This was expected because our isolate of *T. harzianum* was highly effective in vitro and in the field against *S. rolfsii* (17) but was ineffective in vitro against *R. solani*. However, within the limited parameters studied, the high level of significant interactions observed (Table 1) indicates that several genes of both the antagonist and pathogen must be involved in regulating the different levels of antagonism observed in the study. If these and other genetic factors interact with the environment, the likelihood of finding a specific biological antagonist that has wide adaptability is remote. Therefore, it may be more prudent to search for biological antagonists against specific diseases and evaluate blends of antagonists for wider applications.

LITERATURE CITED

1. Backman, P. A., and Rodriguez-Kabana, R. 1975. A system for the growth and delivery of biological control agents to the soil. Phytopathology 65:819-821.
2. Baker, K. F., and Cook, R. J. 1974. Biological Control of Plant Pathogens. W. H. Freeman, San Francisco. 433 pp.
3. Bell, D. K., and Wells, H. D. 1977. Reactions of *Rhizoctonia solani* and *Ceratobasidium* sp. to *Trichoderma* sp. in vitro. (Abstr.) Proc. Am. Phytopathol. Soc. 4:167.
4. Dennis, C., and Webster, J. 1971. Antagonistic properties of species-groups of *Trichoderma*. I. Production of non-volatile antibiotics. Trans. Br. Mycol. Soc. 57:25-29.
5. Dennis, C., and Webster, J. 1971. Antagonistic properties of species-groups of *Trichoderma*. II. Production of volatile antibiotics. Trans. Br. Mycol. Soc. 57:41-48.
6. Flowers, R. A. 1976. A selective media for isolation of *Rhizoctonia* from soil and plant tissue. (Abstr.) Proc. Am. Phytopathol. Soc. 3:219.
7. Flowers, R. A., and Littrell, R. H. 1972. Oospore germination of *Pythium aphanidermatum* as affected by casein, gallic acid and pH levels in a selective agar medium. (Abstr.) Phytopathology 62:757.
8. Greer, J. E. 1978. Antagonistic reactions of *Trichoderma harzianum* toward *Rhizoctonia solani* and *Sclerotium rolfsii*. M.S. thesis. University of Georgia, Athens. 101 pp.
9. Hadar, Y., Chet, I., and Henis, Y. 1979. Biological control of *Rhizoctonia solani* damping-off with wheat bran culture of *Trichoderma harzianum*. Phytopathology 69:64-68.
10. Henis, Y., Ghaffar, A., and Baker, R. 1978. Integrated control of Rhizoctonia solani damping-off of radish: effect of successive planting,

PCNB, and *Trichoderma harzianum* on pathogen and disease. Phytopathology 68:900-907.
11. Henis, Y., Ghaffar, A., and Baker, R. 1979. Induction in soils of suppressiveness to *Rhizoctonia solani*. Phytopathology 69:1164-1169.
12. Kelley, W. D. 1976. Evaluation of *Trichoderma harzianum* impregnated clay granules as a biocontrol for *Phytophthora cinnamomi* causing damping-off of pine seedlings. Phytopathology 66:1023-1027.
13. Papavizas, G. C., and Lumsden, R. D. 1980. Biological control of soilborne fungal propagules. Annu. Rev. Phytopathol. 18:389-413.
14. Tansomo, A., and Dennis, C. 1978. Effect of temperature on antagonistic properties of *Trichoderma* species. Trans. Br. Mycol. Soc. 71:469-474.
15. Wells, H. D., and Bell, D. K. 1974. Antagonism of *Trichoderma* isolates to *Sclerotium rolfsii* in vitro. (Abstr.) Proc. Am. Phytopathol. Soc. 1:168.
16. Wells, H. D., and Bell, D. K. 1979. Variable antagonistic reaction in vitro of *Trichoderma harzianum* against several pathogens. (Abstr.) Phytopathology 69:1048-1049.
17. Wells, H. D., Bell, D. K., and Jaworski, C. A. 1972. Efficacy of *Trichoderma harzianum* as a biocontrol of *Sclerotium rolfsii*. Phytopathology 62:442-447.
18. Wijetunga, C., and Baker, R. 1979. Modeling of phenomena associated with soil suppressive to *R. solani*. Phytopathology 69:1287-1293.