Induction of Terpenoid Synthesis in Cotton Roots and Control of *Rhizoctonia solani* by Seed Treatment with *Trichoderma virens*

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ABSTRACT

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Research on the mechanisms employed by the biocontrol agent *Trichoderma virens* to suppress cotton (*Gossypium hirsutum*) seedling disease incited by *Rhizoctonia solani* has shown that mycoparasitism and antibiotic production are not major contributors to successful biological control. In this study, we examined the possibility that seed treatment with *T. virens* stimulates defense responses, as indicated by the synthesis of terpenoids in cotton roots. We also examined the role of these terpenoid compounds in disease control. Analysis of extracts of cotton roots and hypocotyls grown from *T. virens*-treated seed showed that terpenoid synthesis and peroxidase activity were increased in the roots of treated plants, but not in the hypocotyls of these plants or in the untreated controls. Bioassay of the terpenoids for toxicity to *R. solani* showed that the pathway intermediates desoxyhemigossypol (dHG) and hemigossypol (HG) were strongly inhibitory to the pathogen, while the final product gossypol (G) was toxic only at a much higher concentration. Strains of *T. virens* and *T. koningii* were much more resistant to HG than was *R. solani*, and they thoroughly colonized the cotton roots. A comparison of biocontrol efficacy and induction of terpenoid synthesis in cotton roots by strains of *T. virens*, *T. koningii*, *T. harzianum*, and protoplast fusants indicated that there was a strong correlation (+0.89) between these two phenomena. It, therefore, appears that induction of defense response, particularly terpenoid synthesis, in cotton roots by *T. virens* may be an important mechanism in the biological control by this fungus of *R. solani*-incited cotton seedling disease.

Additional keywords: induced resistance.

"Q" strains of the biocontrol fungus *Trichoderma* (*Gliocladium*) *virens* (Miller, Giddens & Foster) Arx are effective biocontrol agents of *Rhizoctonia solani* Kühn-induced cotton seedling disease in both greenhouse and field studies (6,9,12). An operative mechanism in the biological control of *Pythium ultimum*-incited seedling diseases by *T. virens* "Q" and "P" strains appears to be antibiosis (8,10,14), because effective strains of the biocontrol agent produce gliotoxin or gliovirin, potent inhibitors of oomycetes, and mutant strains deficient for production of these compounds show reduced effectiveness as biocontrol agents (10,18). The mechanisms involved in the biological control of *R. solani* by "Q" strains, however, remain obscure. Assay of biocontrol activity following elimination of mycoparasitic activity (7) and antibiotic (gliotoxin) production (10) by ultraviolet mutagenesis has shown that mutants are as suppressive as the parent strains. These mechanisms, therefore, do not appear to be of primary importance in the biocontrol process. This leaves competition on the rhizoplane or in the rhizosphere, enzyme production, and host resistance induction as possible mechanisms in disease control. Previous work (C. R. Howell, *unpublished data*) has shown that some *Trichoderma* spp. that are highly competitive on the cotton root system are poor biocontrol agents; therefore, competition probably is not a primary factor in biocontrol activity.

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The objective of this study was to test the hypothesis that suppression of *R. solani*-incited cotton seedling disease by *T. virens* is the result, in part, of induction of resistance mechanisms in the cotton host. Cotton responds to pathogen attack by increased synthesis of terpenoid compounds (2,13,21), and terpenoids are known to be toxic to certain pathogens (15,21); therefore, we used this as the main indicator of induction of host defense response. It also was our objective to ascertain whether cotton responds to exposure to *T. virens* by increased terpenoid synthesis, to determine the relative toxicities of terpenoids to *R. solani* and *T. virens*, and to correlate the capacity of given strains to stimulate the synthesis of terpenoid compounds in cotton roots with their relative ability to induce resistance to seedling disease in cotton.

MATERIALS AND METHODS

Parent and mutant strains of *T. virens* and other *Trichoderma* spp. were stored as conidia in 25% glycerol at –70°C until used. Protoplast fusants were stored on sterile filter paper as mycelium at –20°C. Prior to use, conidia or mycelia were transferred to potato dextrose agar (Difco Laboratories, Detroit) plates containing 50 µg of rifampicin per ml (rifPDA), and colonies developing from them were used as a source of inoculum. The *R. solani* strain used in this study was isolated from a diseased cotton seedling on rifPDA. Dry, granular preparations of *Trichoderma* spp. were made by liquid shake culture (G 10 gyratory shaker; New Brunswick Scientific Co. Inc., New Brunswick, NJ) of the fungus at 27°C for 7 days in a medium consisting of 5% ground wheat bran and 1% ground peat moss adjusted to pH 4.0 with HCl. The cultures were centrifuged (C4-12; Jouan, Inc., Winchester, VA) and the pellets were air-dried and ground to a ≥500-µm particle size. The preparations were stored at 5°C until used.

The parent, mutant, and fusant strains of *Trichoderma* spp. used in this work were chosen because of their variability in antibiotic

production, mycoparasitism, and suppression of cotton seedling disease incited by *R. solani*. Strains G-6 and G-11 of *T. virens* are effective biocontrol agents that produce gliotoxin and parasitize the hyphae of *R. solani*, while strain G-4 of *T. virens* is a less effective biocontrol agent that parasitizes *R. solani* but does not produce gliotoxin (12). Mutant strains G6-5 and G6-4 of *T. virens* are both deficient for gliotoxin production and mycoparasitism, but G6-5 is an effective biocontrol agent of cotton seedling disease, while G6-4 is not (C. R. Howell, *unpublished data*). The *T. harzianum* strain TH-23 and the *T. koningii* strain TK-7 are the parents, along with G-6, of fusants GTH-34 and GTK-53 and GTK-56, respectively. GTH-34 is an effective biocontrol strain, while GTK-53 and GTK-56 are moderate to low performers as biocontrol agents (C. R. Howell, *unpublished data*).

R. solani AG-4 inoculum was grown as mycelial mats in potato dextrose broth (PDB) still cultures, incubated at 27°C for 5 days. The mats were then aseptically washed, weighed, and macerated in a Waring blender (model 33BL79; Dynamics Corp., New Hartford, CT) for 1 min. Inoculum was applied as a 0.0125% (wt/vol) mycelial fragment suspension. *R. solani* inoculum used in the soil flat tests was prepared as previously described (11).

Effect of seed treatment on cotton seedling disease symptoms in sterile vermiculite. Seed of cotton (*Gossypium hirsutum*) cv. Stoneville 213 were coated with latex sticker (Rhoplex B15J; Rohm and Haas, Philadelphia, PA) and dry, granular preparations of the effective *T. virens* biocontrol strain G-6 or ineffective mutant G6-4. The control was coated with a sterile preparation of wheat bran plus peat moss (WB+PM). Treated seed were planted in 96-cavity seedling trays containing sterile moist vermiculite, with 10 seeds per treatment, and each treatment was replicated six times. The plantings were incubated at 27°C for 2 days; 10-ml aliquots of the 0.0125% suspension of *R. solani* mycelial fragments were then added to each cavity for one half of the seedlings in each treatment. After a further 2 days of incubation, the seedlings in each treatment were harvested and examined for symptoms of disease.

Induction of terpenoids in cotton roots from *T. virens*- and *R. solani*-treated seed. 'Stoneville 213' seed coated with latex sticker and dry, granular preparations of *T. virens* strain G-6 or WB+PM control were planted in sterile vermiculite seedling trays and incubated as described above. After 3 days, one half of the cavities in each treatment were drenched with 10-ml aliquots of the 0.0125% *R. solani* fragment suspension. After a further 24 h of incubation, the seedlings were harvested and rinsed free of vermiculite, after which the roots were excised from the plants. The top 4 cm of the roots in each replication was weighed, cut into 5-mm sections, and soaked for 24 h in a volume of 90% acetone, 9.9% water, and 0.1% ascorbic acid (3 ml/g of tissue). The acetone extracts were then drawn off with a pipette and subjected to high-performance liquid chromatography (HPLC) analysis as previously described (21). The HPLC system consisted of a model 1090 liquid chromatograph (Hewlett-Packard Co., Atlanta, GA) with a diode array detector set at 235 nm. The solvents, methanol (0.07% H_3PO_4) and H_2O (0.07% H_3PO_4), were delivered using a gradient starting with 80% H_2O, changing to 40% H_2O after 5 min, and ending at 13% H_2O after 22 min. The flow rate was 1.25 ml/min. The hypocotyls from seedlings treated with strain G-6 or WB+PM control were also extracted and analyzed as described above. All of the above treatments were replicated three times, and the experiment was repeated.

Seed of cotton cvs. Deltapine 50, Deltapine 5409, Coker 312, Acala Maxxa, and Rowden were treated with latex sticker and coated with dry, granular preparations of *T. virens* strain G-6 or WB+PM control. The treated seed were then planted in sterile, moist vermiculite in seedling trays, harvested after 4 days, and extracted and analyzed for terpenoids by HPLC as described above.

Dry, granular WB+PM preparations of the *T. virens*, *T. koningii*, *T. harzianum*, and protoplast fusant strains to be used in disease assays were coated on cotton seed with latex sticker. The treated seed were planted in sterile vermiculite seedling trays, incubated, extracted, and analyzed for terpenoid synthesis as described above.

Toxicity of induced cotton terpenoids to *R. solani*, *T. virens*, and *T. koningii*. Authentic samples of the cotton terpenoids desoxyhemigossypol (dHG), hemigossypol (HG), and gossypol (G) were dissolved in dimethyl sulfoxide (DMSO), and aliquots were added to a buffered nutrient solution (15) to make final concentrations of 0 to 35 μg ml^{-1} at 5-μg-per-ml intervals. The final DMSO concentration in each was 4%. The buffered nutrient solution contained mycelial fragments of *R. solani*, *T. virens*, or *T. koningii* strains prepared by macerating 5-day-old mycelial mats, grown in PDB still cultures, in sterile water with a Waring blender. The macerate was then centrifuged (C4-12; Jouan, Inc.) at 2,000 × *g* for 10 min, and 0.5 g of the pellet was resuspended in 100 ml of the buffered nutrient solution. Aliquots (0.08 ml) of the terpenoid and DMSO mixtures were added to 1.92-ml lots of the mycelial fragment suspension, 0.4-ml aliquots of the treatments were dispensed into four replicate wells of 24-well tissue culture plates, and the plates were incubated for 24 h at 27°C. The contents of the wells were then spread on the surface of rifPDA plates, incubated at 27°C for 24 h, and observed for the presence of mycelial growth.

Biocontrol efficacy of *Trichoderma* spp. against cotton seedling disease incited by *R. solani*. Seed of cotton cv. Stoneville 213 were treated with latex sticker and dry, granular WB+PM preparations of *T. virens* parent and mutant strains G-4, G-6, G-11, G6-5, and G6-4; *T. koningii* strain TK-7; *T. harzianum* strain TH-23; and protoplast fusant strains GTK-53, GTK-56, and GTH-34 and planted in flats containing cotton field soil (Lufkin fine sandy loam). Control seed were treated with latex and sterile WB+PM alone. Seed were planted in 10-seed rows with three replicate rows for each treatment, and the open furrows were evenly sprinkled with 0.1 g of *R. solani* inoculum (dry granules of a millet culture diluted 1:9 with ground sterile vermiculite) per row prior to closure. The flats were watered each day and incubated for 10 days in a growth chamber at 25°C with a 14-h photoperiod. Counts of surviving seedlings were made in each treatment in order to calculate percent damping-off. Treatments were arranged in a completely randomized design. Biocontrol efficacy was calculated as 100 to 0% damping-off. The experiment was repeated twice.

Colonization and penetration of cotton roots by *Trichoderma* spp. Seeds of cotton cv. Stoneville 213 were coated with latex sticker and dry, granular preparation of *T. virens* strains G-6 and G6-4, *T. koningii* strain TK-7, and the protoplast fusant of G-6 and TK-7, GTK-56. Seed treated with latex and sterile WB+PM served as a control. The treated seed were planted in sterile, moist vermiculite in seedling trays as described above and incubated for 6 days at 27°C. The seedling roots from part of each treatment then had the vermiculite removed manually with a soft brush, and the roots were cut into 5-cm sections and plated on rifPDA. Roots from these same treatments also were washed thoroughly under a stream of water purified by reverse osmosis, surface-sterilized with a solution of 20% Clorox and 10% ethanol for 1 min, rinsed with sterile water, and plated in 5-cm sections on rifPDA.

Production of terpenoid synthesis inducers in liquid cultures of *T. virens*. Supernates obtained from centrifugation (C4-12; Jouan, Inc.) of 6-day-old WB+PM shake cultures of effective strain G-6, noneffective strain G6-4, and WB+PM control were filter-sterilized by passage through 0.2-μm sterile filters (Sterile Acrodisc; A. Daigger & Co., Inc., Lincolnshire, IL). The filtrates were applied to the radicles of germinated cv. Deltapine 50 seed by pipetting 3 ml of solution onto the radicles of 12 seeds when they were 2 cm in length. After 2 days of incubation on moist filter paper in sterile petri dishes at 27°C, the radicles were excised, extracted, and analyzed for terpenoid concentration as previously described.

Stimulation of peroxidase activity. 'Deltapine 50' seed, coated with latex and G-6 or WB+PM as previously described, were planted in sterile vermiculite and harvested after 6 days of incubation. Six replicate 0.55-g samples of roots or hypocotyls were

each ground separately with a glass tissue grinder (Ace Glass Inc., Vineland, NJ) in 1-ml aliquots of 0.1 M phosphate buffer (pH 6.0) and centrifuged (Eppendorf 5415; Brinkmann Instruments, Inc., Westbury, NY) at 10,000 × *g* for 5 min The supernates were assayed for peroxidase activity by the guiacol assay as previously described (17).

Statistics. All experiments were repeated at least once, with similar results, and the data from the last trials are presented here. Statistics were performed using general linear models (version 6; SAS Institute, Cary, NC) or the *t* test of correlation functions of EXCEL (Microsoft Corp., Bothell, WA).

RESULTS

Induction of host resistance and terpenoid synthesis. Of those seedlings grown in vermiculite and infested with *R. solani*, only those from seed treated with *T. virens* G-6 preparations were symptomless. Seedlings from seed coated with the mutant G6-4 or control preparations produced stunted and necrotic radicles. These differences in symptomatology were similar to those observed in nonsterile cotton field soil infested with *R. solani* in previous studies (C. R. Howell, *unpublished data*).

HPLC analyses of seedling radicle extracts for terpenoid content (Table 1) showed that only low levels of dHG, HG, and G were present in radicles from seed treated with WB+PM control preparation. Terpenoid concentrations in radicles from control seed infested with *R. solani* for 24 h were not significantly elevated when compared with those of the uninfested controls. Radicles from seed treated with *T. virens* strain G-6, however, contained significantly higher levels of dHG, HG, and G than did the control, while radicles from seed treated with G-6 and infested with *R. solani* contained significantly higher concentrations of dHG and HG than did those treated with G-6 alone (Table 1). Unlike the radicles, hypocotyls from seed treated with G-6 had significantly elevated levels only for dHG compared with that of the control. HG or G were not significantly different (data not shown).

Analysis of the terpenoid content of radicles from G-6-treated and nontreated seed of cotton cvs. Deltapine 50, Deltapine 5409, Coker 312, Acala Maxxa, and Rowden showed that all varieties, except Deltapine 5409 with dHG, responded to treatment with G-6 by synthesizing significantly higher amounts of dHG, HG, and G than did the nontreated controls (Table 2).

Effect of terpenoids on growth of *R. solani* and *Trichoderma* spp. Bioassay of the terpenoids induced in *T. virens*-treated cotton radicles for toxicity to *R. solani* (Table 3) showed that dHG was the most toxic to *R. solani* (100% lethal dose = 5 μg ml^{-1}), followed by HG (10 μg ml^{-1}). G was much less toxic than its precursors, because it did not extensively inhibit growth until 30 μg ml^{-1}, and it did not give 100% kill at any level tested. *T. virens* strain G-6 was much more resistant to the terpenoid HG than was *R. solani*. HG only achieved significant inhibition of *T. virens* at 25 μg ml^{-1} and complete kill at 35 μg ml^{-1}. Similar to *T.* virens, *T. koningii* strain TK-7 was significantly inhibited at 25 μg of HG per ml, but complete kill did not occur at any level tested.

Effect of terpenoid stimulation on biocontrol activity. Assay of the biocontrol efficacy of strains of *T. virens*, *T. koningii*, *T. harzianum*, and protoplast fusants (Table 4) in *R. solani*-infested cotton field soil flats showed that *T. virens* strains G-6, G-11, G6-5, G-4, and the protoplast fusant GTH-34 significantly reduced seedling damping-off. The *T. harzianum* strain TH-23 and the protoplast fusants GTK-53 and GTK-56 partially reduced seedling damping-off, while the *T. koningii* strain TK-7, the *T. virens* mutant strain G6-4, and WB+PM control did not control damping-off.

A comparison of the terpenoid concentrations in extracts of cotton radicles treated with *Trichoderma* strains showing strong (≥60%), weak (40 to 50%), and no (≤20%) biocontrol activity (Table 4) showed that the effective biocontrol strains stimulated terpenoid production the most, the weak biocontrol strains stimulated an intermediate level, and the ineffective strains stimulated the least. The correlation coefficient calculated with EXCEL for the relationship between disease suppression and terpenoid induction by the 10 strains tested was $r = +0.89$, and this was determined to be significant ($\propto$ = 0.01) by the *t* test for $\rho = 0$.

Examination of the root segments from seedlings treated with biocontrol effective G-6, ineffective TK-7, and an intermediate fusant GTK-56 showed that all strains colonized the entire root system. Those root systems that were surface-sterilized were also colonized in their entirety. The control root systems showed no evidence of *Trichoderma* infestation.

Effect of culture filtrates on induction of terpenoid synthesis and of seed treatment on peroxidase activity. Bioassay of the

TABLE 1. Effect of *Trichoderma virens* on terpenoid concentrations in cotton roots inoculated with *Rhizoctonia solani*

Treatment	Terpenoids (μg/g of tissue)[y]		
	HG	dHG	G
NT control[z]	1.58 a	1.75 a	33.30 a
R. solani	4.00 a	3.33 a	29.00 a
T. virens	29.30 b	13.97 b	109.26 b
T. virens + *R. solani*	39.78 c	22.62 c	94.84 b

[y] HG = hemigossypol, dHG = desoxyhemigossypol, and G = gossypol; these terpenoids are found in extracts of cotton seedling roots. Means within a column followed by different letters are significantly different according to the protected least significant difference test at $\propto$ = 0.05 using general linear models (SAS Institute, Cary, NC).
[z] NT = nontreated.

TABLE 2. Effect of seed treatment with *Trichoderma virens* on terpenoid levels in seedling radicles of five cotton cultivars

Cotton cultivars[y]	Terpenoids (μg/g of tissue)[z]		
	HG	dHG	G
Deltapine 5409-C	17.30	7.20	242.98
Deltapine 5409-T	42.29**	9.33	533.99**
Coker 312-C	7.57	10.19	80.35
Coker 312-T	23.87**	22.89**	253.23**
Deltapine 50-C	10.57	11.53	103.65
Deltapine 50-T	39.98**	36.16**	293.80**
Acala Maxxa-C	13.44	9.90	263.93
Acala Maxxa-T	31.97**	16.15**	349.26*
Rowden-C	19.34	10.27	223.53
Rowden-T	51.36**	22.20**	362.00*

[y] C = control seed coated with sterile wheat bran plus peat moss; T = seed coated with *T. virens* wheat bran plus peat moss preparation.
[z] Numbers given are average concentrations. HG, dHG, and G are the terpenoids hemigossypol, desoxyhemigossypol, and gossypol, respectively. Treatment means are significantly different from the control means as assessed by Student's *t* test; * and ** indicate $\propto$ = 0.05 and 0.01, respectively.

TABLE 3. Effects of terpenoids from cotton roots on the growth of *Rhizoctonia solani* and of hemigossypol on strains of *Trichoderma virens* and *T. koningii*[z]

Concentration (μg ml^{-1})	dHG	HG	Tv	Tk	G
1	+	+	+	+	+
2.5	+	+	+	+	+
5	–	+	+	+	+
7.5	–	+	+	+	+
10	–	–	+	+	+
12.5	–	–	+	+	+
15	–	–	+	+	+
20	–	–	+	+	+
25	–	–	+–	+–	+
30	–	–	+–	+–	+–
35	–	–	–	+–	+–

[z] dHG, HG, and G are the cotton terpenoids desoxyhemigossypol, hemigossypol, and gossypol, respectively. Tv = *T. virens* strain G-6 and Tk = *T. koningii* strain TK-7; + = mycelial growth after 24 h of incubation, – = no mycelial growth, and +– = mycelial growth inhibited.

filtrate from WB+PM shake cultures of strain G-6 for induction of terpenoid synthesis in cotton radicles showed that application of the filtrate stimulated terpenoid synthesis. Radicles from WB+PM-treated seedlings produced an average of 4.36 µg of dHG per ml, 14.89 µg of HG of ml, and 133.2 µg of G per ml, while those treated with G-6 filtrate produced 26.16, 90.58, and 250.8 µg ml^{-1}, respectively (differences are statistically significant at the 95% probability level). Radicles treated with G6-4 filtrate produced only 3.93 µg of dHG of ml, 10.11 µg of HG per ml, and 129.2 µg of G per ml.

Peroxidase activity in hypocotyls from control and G-6-treated seed was not significantly different ($P = 0.383$), but there was significantly ($P = 0.006$) greater activity from roots of G-6-treated seed than there was from roots developed from WB+PM-treated seed.

DISCUSSION

Efficient means of screening for effective biocontrol agents, and the development of strategies to optimize their use, require detailed knowledge of the mechanisms employed by the agents to effect disease control. In recent years, research on the mechanisms employed by *Trichoderma* spp. for disease control has centered on the role of protease and chitinase in the biocontrol process (4,5, 19). Chitinase has also been implicated in the biological control of cotton seedling disease by *T. virens* (1). More recently, research emphasis has been expanded to include induction of defense responses in the host plant by *T. harzianum* (3,20) and *Penicillium janczewskii* on foliar diseases of cotton and melons (16). The data presented in this paper indicate that treatment of cotton seed with biocontrol strains of *T. virens* triggers plant defense responses in the developing seedling radicles. A part of this response appears to be stimulation of terpenoid synthesis in the root system, that portion of the plant that has been colonized by the biocontrol agent. The data indicate that terpenoid synthesis by the host plant is in response to colonization and penetration of the epidermis and outer cortical tissues of the root by *T. virens* and production of the terpenoid stimulating factor or factors. The fungus is easily cultured from surface-sterilized roots of treated cotton seedlings, and a similar induction of resistance by penetration of the outer layers of cucumber roots by *T. harzianum* was recently demonstrated by Yedidia et al. (20). A further indication that host resistance may be induced by *T. virens* is the increase in peroxidase activity in cotton roots treated with an effective strain. Together with stimulation of terpenoid synthesis, this suggests that general host resistance is induced.

TABLE 4. Induction of terpenoid synthesis in cotton roots by *Trichoderma* spp. and their efficacy as biocontrol agents of *Rhizoctonia solani*-induced cotton seedling disease

Treatment[y]	Terpenoids (µg/g root)[z]				Disease (%)
	HG	dHG	G	Total	
NT control	10.0 gh	5.4 de	42.9 de	58.3 de	80 de
TK-7	19.2 d–f	7.4 bc	40.2 de	66.8 de	90 e
G6-4	14.1 e–g	7.0 cd	48.0 de	69.0 de	80 de
GTK-56	13.6 f–h	4.6 e	47.9 de	66.1 de	67 c–e
TH-23	19.9 de	7.2 c	49.1 de	76.2 d	50 a–d
GTK-53	34.0 a	11.4 a	60.0 d	105.4 c	50 a–d
G-4	21.5 cd	8.0 bc	83.1 c	112.6 bc	40 a–c
G-6	27.2 bc	8.9 b	107.2 ab	143.3 a	37 a–c
G-11	32.0 ab	8.0 bc	121.0 a	161.0 a	30 ab
GTH-34	33.6 a	7.7 bc	93.4 bc	134.6 ab	30 ab
G6-5	33.5 a	11.5 a	94.4 bc	139.4 ab	17 a

[y] NT = nontreated. G-4, G-6, and G-11 are *T. virens* strains; G6-4 and G6-5 are mutant strains of G-6. TK-7 is a *T. koningii* strain, and TH-23 is a *T. harzianum* strain. GTK-53 and GTK-56 are protoplast fusants of G-6 and TK-7. GTH-34 is a protoplast fusant of G-6 and TH-23.

[z] HG, dHG, and G are the cotton terpenoids hemigossypol, desoxyhemigossypol, and gossypol, respectively. Means within a column followed by different letters are significantly different according to the protected least significant difference test at $\propto = 0.05$ using general linear models (SAS Institute, Cary, NC).

The intermediates, dHG and HG, in the gossypol pathway are highly toxic to the seedling disease pathogen, *R. solani*, and even the end product, gossypol, is inhibitory to the pathogen at higher concentrations. The presence of elevated terpenoid concentrations in induced seedling roots might well prevent subsequent development of pathogen hyphae attacking the root. The effective *T. virens* biocontrol strain G-6 is much more resistant to HG than is the pathogen, and it is present at the earliest stage of radicle development, when terpenoid concentrations are very low. Therefore, its colonization of the developing seedling root may proceed unimpeded.

A comparison of the biocontrol efficacies of *T. virens*, *T. koningii*, *T. harzianum*, and protoplast fusant strains with their capacity to induce terpenoid synthesis in treated seedling roots indicates that there is a strong correlation between biocontrol efficacy and induction of terpenoid synthesis in cotton roots. Although biological control is almost certainly the culmination of many factors, a major mechanism in the biological control by *T. virens* of cotton seedling damping-off incited by *R. solani* appears to be the induction of host resistance, as indicated here by peroxidase activity and terpenoid synthesis, in seedling roots by the biocontrol agent prior to attack by the pathogen. Terpenoid synthesis is even further enhanced by subsequent pathogen attack. Penetrating pathogen hyphae are, therefore, met by increased concentrations of bioactive compounds, and subsequent development of the fungus is suppressed.

Assay of *T. virens* culture filtrate for terpenoid induction indicates that the inducer or inducers are synthesized by the fungus and released to the environment. Efforts are currently underway to isolate and characterize these inducers.

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Erratum

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In the manuscript entitled "Induction of Terpenoid Synthesis in Cotton Roots and Control of *Rhizoctonia solani* by Seed Treatment with *Trichoderma virens*" by C. R. Howell, L. E. Hanson, R. D. Stipanovic, and L. S. Puckhaber (Phytopathology 90:248-252), the author M. H. Wheeler was inadvertantly left off.