FIELD EFFICACY OF VIRIDIOL(-) MUTANTS OF *TRICHODERMA VIRENS* FOR BIOCONTROL OF COTTON SEEDLING DISEASES

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Abstract

Viridiol(-) mutants of the biocontrol agent *Trichoderma virens* can no longer synthesize the phytotoxin viridiol, but retain the capacity to produce antifungal antibiotics, act as mycoparasites, and control cotton seedling disease incited by *Rhizoctonia solani*. The use of biocontrol preparations containing viridiol (-) mutants virtually eliminated the phytotoxicity associated with treatment of cotton seed with high concentrations of parent strain preparations. The phytotoxic and biocontrol activities of parent and viridiol (-) strains were compared by coating Apron treated cotton seed with a latex sticker and air-dry, finely ground, preparations of the fungi. Field tests of treated seed in plots heavily infested with seedling disease pathogens at Shafter and Dos Palos, California showed that treatment with parent or some mutant strains of *T. virens* gave significantly better stands than nontreated or fungicide treated controls. With the exception of one strain, all seed treated with the biocontrol agents gave higher average lint yields than the nontreated or Apron treated controls, although the differences were not significant.

Introduction

The imperfect fungus *Trichoderma virens* has been shown in several instances to be an effective biocontrol agent of seedling diseases attacking cotton (Howell, 1982 & 1991; DeVay et al., 1992 & 1996; El-Zik et al., 1993). Among its positive characteristics are mycoparasitism, antibacterial and antifungal antibiotic production, tolerance of dry conditions, effective root colonization, and resistance to fungicides. A negative characteristic of the fungus is its production of the steroid compound viridiol, which acts as a potent phytotoxin when placed in close proximity to the growing tips of plant roots (Howell and Stipanovic, 1984). Viridiol production can be useful when employed as a herbicide for weed control, but it is most inconvenient when placed in the vicinity of developing crop plant roots. Because preparations of viridiol-deficient mutants of *T. virens* exhibit little or no phytotoxicity to young seedling roots when compared to parent strains (Howell and Stipanovic, 1995), it is conceivable that this difference might extend to the developing roots of older plants and result in yield enhancement. The purpose of this study was to determine the field efficacy of viridiol-deficient mutants as biocontrol agents of cotton seedling disease and to ascertain whether treatment with these strains resulted in increased lint yields.

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Materials and Methods

Cotton seed

Acid delinted seed of the cultivar Acala Maxxa, obtained from the Anderson Clayton Gin in Fresno, CA, was used in this study.

Biocontrol fungi

A viridiol-producing parent strain (G-6) and viridiol-deficient mutants (TV-108, 109, 110, 111, and 115) of *Trichoderma virens* in air-dried solids from 14 day shake wheat bran/peat moss cultures were used in this study.

Seed coatings

Acid delinted Acala Maxxa seed treated with Apron FL were coated with latex sticker (Rhoplex 15B, Rohm and Haas) and shaken with fine ground preparations of the strains listed above. Controls were nontreated or treated with Apron, latex, and groumd vermiculite.

Experimental plots

Heavily infested seedling disease plots at Shafter and at Dos Palos, California consisted of randomized complete block designs with 8 replications. At Shafter (1) treatments consisted of 100 seeds in 20 foot rows; at Shafter (2), 120 seeds in 28 foot rows; and at Dos Palos, 150 seeds in 30 foot rows. Lint yields at Shafter (2) were taken from 4 replicate 13 foot rows.

Data collection and analysis

Stand counts were made and percentage seedling survival calculated approximately one month after planting. Seed cotton in the treatment rows was hand picked. The data were subjected to analysis of variance, using the type III sums of squares method of the general linear models procedure of SAS.

Results and Discussion

A comparison of the seedling survival data between untreated and latex/Apron treated controls (Table 1) indicates that the test sites at Shafter were heavily infested with *Pythium* spp., as treatment with the fungicide gave very significant increases in seedling survival over the untreated control. Comparisons of seedling survival data between the

Apron treated control and those treated with Apron + *Trichoderma virens* strains showed, with few exceptions, that treatment with the biologicals gave further significant increases in seedling survival over the chemical control. This was probably due to suppression of *Rhizoctonia solani*-induced seedling disease. Altogether, combinations of a chemical seed treatment with biologicals that are not inhibited by it give highly significant increases in seedling survival over the untreated control.

When the seedling survival data from seed treated with parent (G-6) and viridiol-deficient mutant (108, 109, 110, 111, and 115) strains were compared (Table 1), there were no significant differences. This indicates that at this concentration of biocontrol inoculum on seed, viridiol-producing strains are having no serious adverse affect on seedling roots.

A comparison of the lint yields from control and *T. virens* treated plants (Table 1) showed, with the exception of TV-115, that those treated with biologicals consistently produced more cotton than the untreated or chemical treated controls. However, due to field variability and the ability of cotton plants to compensate in growth and yield for missing plants, the differences were not significant. The lack of significant differences in lint yield between treatments with viridiol-producing parent or viridiol-deficient mutant strains of *T. virens* indicates that loss of viridiol production does not confer any particular advantage on a strain with respect to its effect on lint yield.

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Table 1. Performance of seed treatments with *Trichoderma virens* for biocontrol of seedling diseases of cotton in California - 1996

Seed Treatments[1] (Acala Maxxa Seed)	Percentage Seedling Survival			Cotton Lint (lbs/acre) Shafter-2
	Dos Palos	Shafter - 1	Shafte r- 2	
1. Untreated control	59.2 a	16.0 a[2]	29.8 a	953.8 a
2. Latex/Apron control	61.9 b	62.3 b	63.4 b	994.5 a
3. *T. virens* isolate G-6	72.9 c	75.5 c	81.3 d	1200.5 a
4. *T. virens* isolate 108	71.5 c	63.0 b	73.3 c	1132.0 a
5. *T. virens* isolate 109	69.9 bc	76.1 c	81.6 d	1106.0 a
6. *T. virens* isolate 110	67.7 bc	69.4 bc	77.5 cd	1133.5 a
7. *T. virens* isolate 111	71.6 c	73.8 c	80.3 d	1078.8 a
8. *T. virens* isolate 115	62.3 b	73.0 c	80.4 d	898.8 a

[1] Except for treatment #1, all seed were coated with Apron FL @ 44 mg a.i./cwt seed. Propagules of the test fungi were applied to the seed with a latex sticker.
[2] The above treatments were tested using eight replications in a randomized block design for seedling stand and four replications for lint yield. Values in columns followed by the same letter are not significantly different (P=0.05).