Control of Cotton Seedling Damping-off in the Field by *Burkholderia* (*Pseudomonas*) *cepacia*

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ABSTRACT

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Four field trials were conducted in April 1995 and 1996 in Arizona to compare the effectiveness of: 1, a soil drench of isolate D1 of *Burkholderia (Pseudomonas) cepacia*; 2, isolate D1 barley meal formulation; 3, Deny seed treatment (a peat moss-based formulation of *B. cepacia*); 4, Deny soil drench; 5, Kodiak seed treatment (a formulation of *Bacillus subtilis*); 6, a mixture of three fungicides (metalaxyl, triadimenol, and thiram) seed treatment; and 7, a mixture of metalaxyl, triadimenol, thiram, and Kodiak seed treatment to increase cotton stand in the field. Except for D1, the other products are being marketed for the control of cotton seedling damping-off. Only D1 soil drench and a mixture of the three fungicides seed treatment increased cotton stand significantly ($P \leq 0.05$) in three of four field trials.

Additional keywords: biocontrol

Cotton seedlings are vulnerable to attack by a number of soilborne pathogens, including *Rhizoctonia solani* Kuhn (17). In Arizona, only *R. solani* and *Thielaviopsis basicola* are important pathogens (R. B. Hine and J. C. Silvertooth, *personal communication*). Pre- or post-emergence cotton seedling damping-off, caused by *R. solani*, can be quite serious in the United States (4) and often results in a substantial stand loss. Despite the effectiveness of fungicides, their widespread use has not eliminated cotton seedling damping-off caused by *R. solani* and other cotton seedling pathogens (1,2,3). In 1986, seedling diseases caused an estimated 2.2% loss to cotton in the United States (7). Moreover, the widespread use of chemicals has become a subject of public concern and scrutiny, mainly due to their potential harmful effect on non-target organisms, the development of resistant races of pathogens, and the possible carcinogenicity of some chemicals. Other problems include gradual elimination and phasing out of some available pesticides, and the reluctance of some chemical companies to develop and test new chemicals due to escalating development and registration costs. Thus, there is a need to examine the potential for non-chemical approaches to disease management.

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Biocontrol with beneficial microorganisms seems to be a promising approach to managing cotton seedling damping-off (8,9,10,12). A number of bacterial isolates collected from the cotton rhizosphere were as effective as commercial fungicides in suppressing seedling disease pathogens *R. solani* and *P. ultimum* on cotton in the field (9). However, results were not consistent among test locations and between years. *Rhizoctonia solani*-induced cotton seedling damping-off has also been suppressed by the biocontrol fungi *Trichoderma* spp. and *Gliocladium virens* in the field (12), and by *G. virens* (10), *Pseudomonas fluorescens* (11), and *Bacillus cereus* (16) in the greenhouse. *Burkholderia cepacia* (14), *Bacillus subtilis* (1), *Trichoderma* spp. (6), and nonpathogenic binucleate *Rhizoctonia* (18) have been reported to suppress *R. solani*-induced damping-off in other crops in the greenhouse.

An isolate of *B. cepacia* (D1), recovered from cotton bolls in Arizona, proved to be an extremely effective control agent against *Aspergillus flavus*-induced cotton boll decay in the field (15) and *R. solani*-induced cotton seedling damping-off in the greenhouse. The objective of the present study was to compare D1 with registered biological and chemical products for efficacy to increase cotton stand in the field.

MATERIALS AND METHODS

Preparation of *R. solani* inoculum. The fungal inoculum was prepared by wetting 1 kg barley seeds with 500 ml water, autoclaving at 15 psi for 60 min, infesting with *R. solani* (Anastomosis Group 4), and incubating at 25°C for 3 weeks. Inoculum was air-dried for 24 h, ground through a 3-mm sieve, and stored in a paper bag at 25 to 27°C in the laboratory. Eight g fungal inoculum were mixed with ca 200 g field soil, and the mixture was sprinkled by hand into the planting furrow shortly before sowing at 0.6 g fungal inoculum per linear m.

Test products. The following biological and chemical products were tested for efficacy to increase cotton stand in the field.

1. Isolate D1(B. cepacia) soil drench. An aqueous suspension (8 log CFU ml^{-1}) of the bacterium was prepared from 2-day-old King's Medium B (KMB) agar cultures 2 to 4 h before application to the field. The suspension was sprayed into the planting furrow at 30.6 ml per linear m shortly after sowing the cotton. The suspension penetrated into the soil ca 7.0 mm.

2. Isolate D1 barley meal formulation. Barley seed (1 kg) was ground through a 3-mm sieve, wetted with 500 ml water, and autoclaved at 15 psi for 60 min. The meal was then thoroughly mixed with 1.5 liters of an aqueous bacterial suspension (8.33 log CFU ml^{-1}) prepared from 24-h-old KMB agar cultures of D1 and incubated for 2 days at 25°C prior to field application. The barley meal formulation was mixed with an equal volume of field soil, then sprinkled into the planting furrow at a rate of 9 g barley meal formulation per linear m.

3. Deny seed treatment. A peat moss-based formulation of *B. cepacia* (CCT Corp., Carlsbad, CA) stored at 5°C prior to use, then mixed with cottonseed (3.1 g/kg seed) according to the manufacturer's recommendation shortly before application.

4. Deny soil drench. Fifteen ml liquid formulation of *B. cepacia* (Deny, CCT Corp.), stored at 5°C prior to use, was mixed with 1985 ml water, and the suspension sprayed into the planting furrow at 153 ml per linear m shortly after sowing the cotton, as recommended by the manufacturer.

5. Kodiak seed treatment. A biological control product containing an isolate of *Bacillus subtilis* (Gustafson Inc., Dallas, Texas), which is being used in combination with one or more fungicides for controlling cotton seedling damping-off, was applied to cottonseeds by Gustafson Inc.

6. A mixture of metalaxyl, triadimenol, and thiram seed treatment. The mixture of these three fungicides was applied to cottonseeds by Gustafson Inc.

7. A mixture of metalaxyl, triadimenol, thiram, and Kodiak seed treatment. The mixture was applied to cottonseeds by Gustafson Inc.

1995 field trials. Two field trials, with plots arranged in a randomized complete block experimental design with four replicates, were conducted at Safford, Arizona in April 1995. The loamy soil contained 14% clay, 36% silt, and 50% sand. Each trial included treatments 1, 2, 3, 5, 6, 7, and a non-treated control. Each replicate plot consisted of one 13-m-long planting bed. An 8-cm-wide, 3-cm-deep furrow at the crest of each planting bed was cut for the placement of 720 seeds of cultivar Deltapine 5415 and test products. The second experiment was performed like the first one, except that the soil was infested with the *R. solani* inoculum. This was done to increase the probability of occurrence of *R. solani*-induced damping-off in the field. The stand (the number of emerged plants in each plot) was determined 45 days after sowing. Two weeks after sowing, about 10 percent of the wilted seedlings in each plot were gently removed from the soil, brought to the laboratory, and tested for the presence of *R. solani*. Seedlings were visually examined for *R. solani*-induced symptoms, tissues from the advancing edge of lesions on roots and lower stems were placed on potato dextrose agar (PDA), and cultures were examined for the presence of the fungus 2 days after incubation at 30°C.

Another field trial was performed at Marana, Arizona in April 1995. The design of this trial was identical to that of the Safford trial described earlier. The loamy soil contained 14.4% clay, 35.5% silt, and 50.1% sand.

1996 field trials. Two field trials were conducted in April 1996 at Safford and Tucson, Arizona to test the efficacy of treatments 1, 3, 4, and 6. The design of these trials was identical to that of the 1995 Safford trial described earlier, except that the soil in the entire field was infested with the *R. solani* inoculum. The loamy soil at the Tucson site contained 15.1% clay, 33% silt, and 51.9% sand. The 1995 and 1996 field trials were furrow-irrigated and treated with insecticides, post-plant herbicides, and fertilizers according to production recommendations followed by cotton growers in the region.

The data from each of the four field trials were analyzed separately by analysis of variance and the least significant difference test (LSD) using Minitab (Minitab Inc., University Park, PA). For each of the 1995 trials, the data from the four blocks infested with *R. solani* and those from four non-infested blocks were analyzed separately.

RESULTS

Typical *R. solani*-induced damping-off symptoms were observed on all sampled seedlings (10 percent of wilted seedlings). Tissues from diseased roots and/or crowns of all sampled seedlings yielded *R. solani* when plated on PDA. Other cotton seedling pathogens, including *T. basicola* were not recovered.

The stand was generally lower in plots that received the *R. solani* inoculum than in those which did not receive the inoculum (Table 1).

The D1 soil drench was the only treatment that increased stand significantly ($P \leq 0.05$) relative to the non-treated control (not treated with biological or chemical products) at Safford in 1995 in both *R. solani*-infested and non-infested blocks (Table 1). In the 1995 Marana experiment, only the mixture of the three fungicides significantly ($P \leq 0.05$) increased cotton stand relative to the control in blocks infested with *R. solani*. In contrast, the D1 barley meal formulation resulted in a significant ($P \leq 0.05$) decrease in cotton stand in non-infested blocks compared to the control (Table 1). In 1996, only the D1 soil drench and the mixture of the three fungicides significantly ($P \leq 0.05$) increased cotton stand at both Safford and Tucson (Table 2).

The averages and ranges of soil temperature at 10 cm depth during the first 3 weeks after sowing at Safford and Marana in 1995, and at Safford and Tucson in 1996, were 18°C (14 to 20), 10°C (7 to 12), 27°C (23 to 29), and 20°C (18 to 23), respectively.

Table 1. The average cotton stand per linear m of planting bed in field trials conducted in April, 1995 at Marana and Safford, Arizona, comparing an isolate of *Burkholderia cepacia* (D1) with registered biological and chemical products for efficacy to reduce cotton seedling damping-off

	Marana		Safford	
Treatment	**Infested[y]**	**Non-infested**	**Infested**	**Non-infested**
D1 soil drench	9 bc[z]	17 ab	13 a	15 ab
D1 barley meal formulation	8 bc	8 c	8 ab	12 abc
Deny seed treatment	9 bc	15 abc	8 ab	11 abcd
Kodiak seed treatment	10 c	13 abc	5 b	8 cd
Metalaxyl, triadimenol, thiram, and kodiak seed treatment	13 ab	19 a	9 ab	9 bcd
Metalaxyl, triadimenol, and thiram seed treatment	18 a	15 abc	6 b	7 d
Non-treated control	9 bc	19 a	4 b	8 cd

[y] Infested with barley meal inoculum of *Rhizoctonia solani.*

[z] Means in each column followed by the same letter are not significantly different, least significant difference ($P = 0.05$).

Table 2. The average cotton stand per linear m of planting bed in field trials conducted in 1996 at Tucson and Safford, Arizona, comparing an isolate of *Burkholderia cepacia* (D1) with registered biological and chemical products for efficacy to reduce cotton seedling damping-off

Treatment	**Tucson**	**Safford**
D1 soil drench	14 a[z]	12 a
Deny soil drench	10 b	9 b
Deny seed treatment	10 b	7 b
Metalaxyl, triadimenol, and thiram seed treatment	14 a	14 a
Not-treated control	8 b	6 b

[z] Means in column followed by the same letter are not significantly different, least significant difference ($P = 0.05$).

DISCUSSION

Of the products tested, only the D1 soil drench and a commercial mixture of the three fungicides (metalaxyl, triadimenol, and thiram) increased cotton stands in three of four field trials. The D1 soil drench was as effective as the mixture of the three fungicides in three of the four trials. The two biological products, Deny and Kodiak, that were tested in their commercially available formulations failed to increase stands relative to the control in any of the trials. The lack of the activity of Deny and Kodiak compared to the D1 soil drench may have been due to differences in formulation or to the bacterial isolates used in the formulations. The increase in cotton stand mediated by the D1 soil drench is most likely due to a decrease in *R. solani*-induced cotton seedling damping-off because (i) cotton seedling diseases in Arizona are mainly caused by *R. solani* and *T. basicola* (R. B. Hine and J. C. Silvertooth, *personal communication*); (ii) in all field trials, the D1 soil drench and other products were tested in soil infested with *R. solani* inoculum; (iii) *R. solani* was the only pathogen recovered from the infected seedlings; and (iv) typical *R. solani*-induced damping-off symptoms were observed on all sampled seedlings.

To guard against the possibility of having a poor stand due to severe damping-off in these field trials, 720 seeds per plot were planted, which is between 2.3- and 8.2-fold higher than the levels used by Arizona farmers in commercial fields (J. C. Silvertooth, *personal communication*). Despite this, the average percentage of planted seeds that germinated and produced seedlings was relatively low: between 14 and

35% in the non-infested blocks in the 1995 trials, and between 8 and 16% in the infested blocks in the 1995 and 1996 trials.

Isolate D1 was effective only as a soil drench in the field. The barley meal formulation actually decreased cotton stand in non-infested blocks at Marana in 1995, perhaps by stimulating the development of *R. solani*.

A number of studies in the laboratory were performed prior to the field trials in order to determine optimal conditions for the activity of isolate D1 with respect to plant age, culture age of the bacterium, bacterial and fungal inoculum levels, fungal inoculum depth in the soil, and methods of delivery of fungal and bacterial inocula. The data obtained from these tests were carefully analyzed and used to select optimal protocols for the field trials.

Biocontrol fungi (12) and bacteria (9) have been shown to suppress seedling disease pathogens on cotton in the field. However, this study is unique because it measures the activity of isolate D1 against commercial biological products marketed to control cotton seedling damping-off. Information presented here is particularly useful for cotton growers in Arizona, where the *R. solani*-induced cotton seedling damping-off is important.

The mechanism of the D1-mediated increase in cotton stand is not known. Isolate D1 is known to inhibit the growth of *R. solani* in vitro and to produce antifungal compounds pyrrolnitrin and amino pyrrolnitrin (N. Mahoney, *personal communication*). Other isolates of *B. cepacia* also produce antibiotics (5,13). However, we have no evidence that antibiotic production by isolate D1 is involved in the increased cotton stand reported here.

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