Field Evaluations of Bacterial Inoculants to Control Seedling Disease Pathogens on Cotton

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ABSTRACT

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Through two seasons of field evaluations, selected bacterial strains performed as well as commercial fungicides in repressing seedling disease pathogens (*Rhizoctonia solani* and *Pythium ultimum*) on cotton. While certain strains did effectively control seedling disease at several locations, there was a lack of consistency among locations and between years, and the performance of an individual strain could not be related to either disease-pressure estimates or those environmental factors that were measured (soil moisture and temperature). Improved seedling stand counts by the introduced strains were not correlated with improved cotton yields in either year of the tests. For two bacterial strains, both in-furrow granular and in-furrow liquid (spray) inoculants produced plant stands that were superior to those obtained with seed-applied inoculants.

Cotton seedling disease, caused primarily by *Pythium ultimum* Trow and *Rhizoctonia solani* Kühn, is responsible for substantial annual stand damage to cotton (4). Yield-loss estimates over a 35-yr period ranged from 1.0 to 6.5% annually, and seedling disease continues to be a problem in stand establishment even with the currently recommended fungicides (3,4). While effective fungicides are available, the future of many compounds is uncertain because of concerns about exposure risks, toxicity, and residue persistence (3). Recently, biological control has been considered as a serious alternative (8), and both bacteria and fungi have been examined for their ability to control root diseases in a number of crops (2,7–11,15,18).

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This research was conducted from 1982 to 1985 as part of a crop biotechnology program within Allied Corporation. The strain collection was obtained by Virginia Polytechnic Institute and State University in 1986, and the confidentiality agreement ended in 1991.

From the cotton rhizosphere, agar and greenhouse in planta assays identified promising bacterial strains for the suppression of seedling pathogens (9). The best strains were chosen for the field tests described in this study. The objectives were to evaluate the most promising bacterial strains from the greenhouse screening program for their effectiveness as biological disease-control agents in the field, and to examine the effects of three application systems on strain performance in pathogen suppression.

MATERIALS AND METHODS

Source of biocontrol strains. The bacterial strains were obtained from the rhizosphere of cotton plants collected in various locations in the southern United States. The media and isolation procedures have been described (9), and the most effective strains from an in planta greenhouse screen were selected for field tests.

Preparation of inoculants. A culture in 10% trypticase soy broth (TSB, [TSA for agar]) was prepared for each bacterial strain and incubated on a shaker for 24–48 hr at 30 C. Results based on platings on TSA were 10^8 to 10^9 cfu/ml. TSB cultures (100 ml) were centrifuged and washed once in 50 ml of sterile phosphate buffer (0.1 M Na_2HPO_4), pH 7.0, containing 0.1% Bacto Peptone (Difco), and resuspended in 10 ml of sterile buffer to produce suspensions of 10^9 to 10^{10} cfu/ml. For direct treatment of cotton seed, each bacterial suspension was applied in several volumes to preneutralized, sterile, class 3 peat, (sterilized by gamma irradiation and provided by the Nitragin Co., Milwaukee, Wisconsin). Preneutralization was accomplished by mixing fine-grade calcium carbonate with the peat, wetting the samples, and measuring the pH until it reached at least 6.8. The inoculated peat (35% moisture w/w) was incubated at 30 C for 3 wk, and plate counts on TSA and King's medium B (9) were obtained by dilution plating prior to shipping the inoculants to the field sites. The peat-based inoculants were stored at 4 C for 2–3 wk and were applied to cotton seeds at a rate of 110 g/kg seed within 48 hr before planting. Methylcellulose (1%, Sigma Chemical Co.) was added as a sticker at a rate of 55 ml/kg seed. The inoculated seed was

air-dried at room temperature (25 C) for 2–3 hr, then refrigerated (4 C) in airtight plastic containers until planted.

For granular in-furrow application, a bacterial suspension was prepared as described above, centrifuged, resuspended in buffer, and applied as several volumes to nonsterile, preneutralized, class 1 peat. For field application of granular peat inoculants, bacterial suspensions were prepared, and the inoculated peat was examined as described above. The granular inoculants were shipped in insulated containers to the field sites, refrigerated (4 C) in airtight containers until use, and applied to the row at 2.3 g/m (22.4 kg/ha) using Gandy applicators that placed the granules directly into the planting furrow, in contact with the seeds.

For liquid in-furrow application, bacteria were centrifuged and resuspended in buffer as described to provide 10^{10} cfu/ml. The liquid inoculants were shipped to the field sites in insulated containers and refrigerated until use. The suspension was applied into the planting row at a rate of 14.1 ml/m (140.3 L/ha) using CO_2-pressurized spray tanks attached to the planter.

The MS-97 and BD4-13 inoculants were tested immediately prior to shipping, and all inoculant materials were found to contain at least 10^9 cfu/g or ml. Leftover inoculants were returned to the laboratory as quickly as possible for testing (usually within one week). Both the seed coat and the liquid inoculants yielded 10^8–10^9 cfu/g or ml of inoculum, while the granular peat yielded at least 10^7 cfu/g.

1984 Field trials. The 1984 strain-evaluation trials were conducted at six locations (one in Arkansas, three in Mississippi, and two in Texas) and included 15 treatments: a nontreated control, a fungicide, 10 bacterial strains, and three strain combinations. All inoculants were applied as peat-based seed treatments. The selection of cotton cultivars and fungicides, crop management, and the taking of stand counts and root ratings were the responsibility of each local cooperator, although recommended procedures were provided in a field-test protocol. Fungicides applied included etridiazole + quintozene (Terra-Coat L 205, 7.5 ml/kg of seed) plus etridiazole (Terraclor Super X 12.5G, 11.2 kg/ha) for the Arkansas and Mississippi sites, and chloroneb (Demosan 65W, 6.25 ml/kg of seed) plus Captan 400-S (1.25 ml/kg of seed) for both Texas sites. A randomized complete block design with at least five replications was used in all cases, and most plots were 4 row × 50.5 m. Seedling counts and root ratings were determined at 6 wk. Root ratings were obtained by removing selected plants (as described in the field-test protocol) and visually inspecting for *Pythium* root lesions and *Rhizoctonia* lesions and discoloration on the plant stem (9). Soil temperature and moisture levels were requested both at planting and at 6 wk when plant stands were determined. The plots at all locations were harvested for yield.

1985 Field trials. *Strain-evaluation trials.* The 1985 field trials were conducted at eight locations (one each in Arkansas, California, Georgia, and Louisiana, and two each in Mississippi and Texas) and included 15 treatments: a nontreated control, a fungicide, the 10 most promising strains from the 1984–85 greenhouse evaluations, strains MS-97 and BD4-13 (the best 1984 field strains), and a combination of MS-97 and BD4-13 (inoculated separately into the peat). All inoculants were applied as peat-based seed treatments, and the tests were conducted as described for the 1984 field trials. Fungicides for Georgia and Louisiana were the same as those used at the Arkansas and Mississippi sites in 1984, and metalaxyl (Ridomil PC-11G, 11.2 kg/ha) was used in California. Yields were determined at all sites.

Application method trials. The two strains that demonstrated the most consistent control of cotton seedling disease in the 1984 field studies (MS-97 and BD4-13) were also evaluated at all eight sites in a series of tests examining the method of application. Strains MS-97 and BD4-13 and their combination were applied as a peat-based seed treatment, as a granular peat inoculant placed in the seed furrow, and as a suspension in phosphate buffer sprayed into the furrow. The in-furrow treatments were applied only to fungicide-treated seed (Terra-Coat L 205, 7.5 ml/kg), while the treatments applied directly to the seed included both nontreated and fungicide-treated seed. Since pretreating cotton seed with fungicides (to reduce seed loss during storage) is the accepted practice, such seed was used in these tests to be more agronomically realistic. One set of treatments to non-fungicide-treated seed was included to allow some assessment of biological disease control if low disease pressure occurred at any one site. Controls included both nontreated and fungicide-treated seed without inoculants. Yields were not obtained.

Statistical analysis. Results from each field site were analyzed independently by year and location because of differences in environmental conditions and site management. Analysis of variance (ANOVA) of treatment means was performed with the general linear model (GLM) procedure, and mean separations were obtained by Duncan's multiple range test when the overall F test was significant at $P > 0.05$. The application method test was analyzed by orthogonal contrast with the F test at significance levels of 0.05 and 0.01.

RESULTS

Source of biocontrol strains. Approximately 1,000 strains were evaluated in the greenhouse during the winter of 1983–84 (9). The 10 most effective strains were field tested in 1984, and the two best-performing strains were retested in the field in 1985 (Table 1). The 10 additional strains field tested in 1985 were selected from another set of 1,600 strains that were screened in the greenhouse during the winter of 1984–85. All strains field tested in 1984 and 1985 were identified as *Pseudomonas* species (9, Table 1).

1984 Field trials. *Strain evaluations: plant stand.* The bacterial seed treatment MS-97 and the combined treatment of AD4-34 + MS-112 produced increased plant stands compared to the nontreated control at five of the six field sites (Table 2). The bacterial seed treatment BD4-13 increased stands at four sites. The fungicide treatment increased plant stands at three of six sites. Of the remaining 1984 strains, five (UR-24, AD4-37, AD4-34, RAL-3, and RSC-6) produced increased stands at three sites each, while three strains (AD8-27, MS-19, and MS-112)

Table 1. Biocontrol *Pseudomonas* strains used in field tests

Strain code	Years tested	Species	State isolated from
MS-97	1984, 1985	*fluorescens*	Mississippi
AD4-37	1984	*fluorescens*	Arkansas
BD4-13	1984, 1985	*fluorescens*	Georgia
AD4-34	1984	not determined[a]	Alabama
AD8-27	1984	*cepacia*	Alabama
MS-19	1984	not determined	Mississippi
MS-112	1984	not determined	Mississippi
RAL-3	1984	not determined	Alabama
RSC-6	1984	not determined	South Carolina
AC4-46	1985	not determined	Mississippi
AC4-40	1985	not determined	Texas
AC4-69	1985	not determined	Georgia
AC4-39	1985	not determined	Texas
AC4-75	1985	*cepacia*	Arkansas
AC4-65	1985	*cepacia*	Georgia
AC4-25	1985	*fluorescens*	Georgia
AC4-91	1985	*fluorescens*	Mississippi
AC4-97	1985	*fluorescens*	Mississippi
AC4-105	1985	*fluorescens*	South Carolina

[a] Identification as a *Pseudomonas* sp. uncertain.

increased stands at two sites. One combination (UR-24 + AD4-34) had no effect at any site, and the remaining combination (UR-24 + MS-112) increased stands at three of six sites. For all sites combined, 38 biological treatments increased stands; for each individual site the number ranged from two at MS3 to 10 at TX1.

Evaluations of environmental conditions, disease ratings, and plant stands by cooperators during the seedling period provided the basis for disease-pressure estimates that were reported to be high at both Texas locations and moderate at the Arkansas and the three Mississippi sites. Plant-disease ratings (*unpublished*) generally supported the plant stands as an indicator of disease presssure, but their usefulness was limited by the cooperators's variations from the recommended disease-rating procedure. Stand increases for all biological treatments ranged from 18% (RAL-3 at MS3) to 43% (AD8-27 at TX1, MS-19 at TX2, and MS-112 at MS2). No treatments produced stands that were lower than the nontreated control (Table 2).

Strain evaluations: cotton yields. Cotton yields were increased (above the nontreated control) at three sites for three strains (AD8-27, MS-19, and RAL-3), at two sites for three strains, and at one site for four strains (Table 3). The fungicide treatment was associated with increased yields at two sites, while two strain-combination treatments increased yields at one site, and a third produced no increased yield. For all sites combined, 21 biological treatments increased yields; for each individual site the number ranged from one at TX1 to six at TX2. There was no correlation between stand counts and cotton yields for any of the biological treatments or the fungicide at any site in the 1984 trials.

Table 2. Cotton-stand counts (% of nontreated control) for the 1984 strain-evaluation tests[a,b]

Treatment	AR	MS1	MS2	MS3	TX1	TX2
Fungicide	97	136*	128*	139*	111	108
UR-24	121*	124	134*	106	135*	116
MS-97	114	128*	127*	124*	126*	121*
AD4-37	119*	103	116	97	137*	126*
BD4-13	132*	132*	96	92	122*	138*
AD4-34	121*	116	101	89	131*	131*
AD8-27	126*	105	107	106	143*	105
MS-19	105	112	123	101	138*	142*
MS-112	98	130*	142*	105	120	98
RAL-3	130*	99	129*	118*	108	95
RSC-6	127*	113	101	112	129*	126*
UR-24 + MS-112	94	133*	98	96	124*	130*
UR-24 + AD4-34	89	106	91	103	113	112
AD4-34 + MS-112	121*	125*	132*	110	127*	127*
LSD 0.05	18	21	26	14	23	20

[a] Sites were AR = Marianna, Arkansas; MS1 = Starkville, Mississippi; MS2 = Stoneville, Mississippi; MS3 = Lexington, Mississippi; and TX1 and TX2 = College Station, Texas. Cultivars were Stoneville 213 at AR and MS sites, Tamcot SP215 and TX1, and Tamcot CAMD-E at TX2. Fungicides were etridiazole + quintozene (Terra-Coat L 205, 7.5 ml/kg seed) plus etridiazole (Terraclor Super X 12.5G, 11.2 kg/ha) for AR and MS sites, and chloroneb (Demosan 65W, 6.25 ml/kg seed) plus Captan 400-S (1.25 ml/kg seed) for both TX sites.

[b] * Indicates a stand greater than the nontreated control.

Table 3. Cotton yields (kg/ha) for the 1984 strain-evaluation tests[a,b]

Treatment	AR	MS1	MS2	MS3	TX1	TX2
Nontreated	768	796	1008	729	377	442
Fungicide	778	840*	997	749	479*	477
UR-24	768	829	983	741	393	496*
MS-97	817	91	1210*	786*	389	445
AD4-37	700	744	1031	697	410	481*
BD4-13	797	770	1007	721	435*	469
AD4-34	797	795	1003	708	413	495*
AD8-27	827*	793	1145*	648	407	483*
MS-19	856*	835*	1081*	731	376	479
MS-112	865*	808	965	691	344	488*
RAL-3	856*	860*	910	830*	388	415
RSC-6	846*	882*	962	712	382	459
UR-24 + MS-112	749	814	869	695	412	472
UR-24 + AD4-34	758	806	961	768*	376	471
AD4-34 + MS-112	807	819	1016	700	409	492*
LSD 0.05	52	36	48	33	41	39

[a] Sites were AR = Marianna, Arkansas; MS1 = Starkville, Mississippi; MS2 = Stoneville, Mississippi; MS3 = Lexington, Mississippi; and TX1 and TX2 = College Station, Texas. Cultivars were Stoneville 213 at AR and MS sites, Tamcot SP215 at TX1, and Tamcot CAMD-E at TX2. Fungicides were etridiazole + quintozene (Terra-Coat L 205, 7.5 ml/kg seed) plus etridiazole (Terraclor Super X 12.5G, 11.2 kg/ha) for AR and MS sites, and chloroneb (Demosan 65W, 6.25 ml/kg seed) plus Captan 400-S (1.25 ml/kg seed) for both TX sites.

[b] * Indicates a yield greater than the nontreated control.

1985 Field trials. *Strain evaluations: plant stand.* Increased plant stands were obtained at five of eight sites for two treatments: the fungicide and the 1985 strain AC-46 (Table 4). Of the nine remaining strains, four (AC-39, AC-40, AC-91, and AC-97) increased stands at three sites, three (AC-25, AC-65, and AC-75) at two sites, and one (AC-69) at one site; one (AC-105) did not increase stands at any site. The best 1984 strains improved stands at only one (MS-97 and MS-97 + BD4-13) and two (BD4-13) sites. For all sites combined, 28 biological treatments increased plant stands; for each individual site the number ranged from none at MS2 to seven at CA and LA.

Cooperator estimates of disease pressure during the seedling period were reported as moderate at CA and LA, low at GA and MS1, and virtually absent at AR, MS2, TX1, and TX2. Despite variations in methods of determining plant-disease ratings (*unpublished*), these ratings also indicated the lack of disease pressure at most sites. Stand increases for biological treatments ranged from 14% (MS-97 at AR) to 49% (AC-46 at CA). Two biological treatments at AR (AC-39 and AC-105) produced stands lower than the nontreated control.

Strain evaluations: cotton yields. Increased yields were obtained at six sites for the fungicide treatment, five sites for one strain (BD4-13), four sites for two strains (AC-91 and AC-97), two sites for one combination (MS-97 + BD4-13), and one site for two strains (AC-46 and AC-69); there were no increased yields for the remaining seven strains (Table 5). On all sites combined, 17 biological treatments increased yields; for each individual site the number ranged from one at two locations (LA and MS1) to four at AR. There was no correlation between stand counts and cotton yields for any of the biological treatments, but an r value of 0.79 was obtained with the fungicide treatment.

Application method: plant stand. When each treatment was compared to the untreated check, plant stands increased at four sites for three treatments (fungicide-treated seed [TS] granular in-furrow BD4-13 and MS + BD, and TS liquid in-furrow BD4-13), three sites for two treatments (fungicide and TS liquid in-furrow MS + BD), two sites for two treatments (TS granular and liquid in-furrow MS-97), and one site for four treatments (all three TS seed-applied inoculants, and nontreated seed [US] seed-applied BD4-13); there were no increases for the remaining two US seed-applied inoculants (Table 6). For the combined

TS treatments, the inoculants were associated with increased stands at three sites for the three seed-applied inoculants, 10 sites for the three granular inoculants, and nine sites for the three liquid inoculants. The fungicide increased plant stands at three of eight sites, and only one of the three seed inoculants on US was associated with enhanced stands, and at just one location (AR). For all sites combined, 26 treatments increased plant stands; for each individual site the number of treatments ranged from none at GA and MS2 to 11 at AR. These tests were planted late (compared to the 1985 strain evaluations) because of the weather. The sporadic occurrence of seedling disease because of late planting was reflected in 11 of 26 increased plant-stand responses at one location (AR) and three or fewer stand responses at five locations (CA [1], GA [0], MS2 [0], TX1 [2], and TX2 [3]). Stand counts were correlated with both the granular and the liquid formulations ($r = 0.93$ and $r = 0.82$, respectively) but not with seed coating.

For all sites combined, the granular and liquid in-furrow applications of BD4-13 and MS + BD were superior to their respective seed applications on both treated and nontreated seed (Table 7). For strain MS-97, results were the same for all application methods. The fungicide treatment was superior to all seed-applied biologicals and equivalent to the granular and liquid in-furrow biologicals. One biological (MS + BD), applied as an in-furrow granule, was superior to the fungicide treatment.

DISCUSSION

The most important consideration in the control of seedling disease on cotton is to prevent stand losses severe enough to reduce yields. Even moderate stand losses generally will not reduce yields, because individual plants grow rapidly and compensate for variations in stand density (3). The 1984 strain evaluations illustrated cotton's "elasticity": at only a few sites were treatments that improved cotton stands associated with increased yields (Tables 2 and 3). Equivalent yields were often obtained from treatments that did not improve stands, because the unimproved stands produced fewer but larger plants. This same trend was observed during the 1985 tests where, of the five best strains, only two (AC-91 and AC-97) improved cotton yields at more than two sites (Tables 4 and 5).

It was difficult to detect consistent performance in the treatments tested both years. BD4-13 and MS-97 were clearly the best strains in 1984 (increased stand counts in nine of 12 tests), but were quite poor in 1985 (increased stands in only three of 16 tests). In 1984 the fungicides did not increase stands at the sites with the greatest disease pressure, but in 1985 they performed well at five of eight sites, including those with the highest disease pressure. The fungicides were also correlated with yield enhancement.

The poor response to many bacterial-strain treatments at some field sites during both seasons can probably be attributed to a variety of factors, including variability in local environmental conditions, although the two moisture and temperature measurements obtained for each site were not sufficient to explain strain performance. Although stand improvements and subsequent yield increases were not consistently observed for the bacterial inoculants, similar results were reported for chemical treatments (3,4). The cotton cultivar, its interactions with the weather and indigenous microorganisms, and soil physical and chemical properties will all influence the performance of an introduced biological control agent (13,14). The effects of soil properties and moisture status on root colonization, and pathogen suppression by the added biocontrol strain may be the most important considerations in strain performance (1,3,4,6,12,15,16).

While the collection and evaluation of the strains for these field tests did not focus on *Pseudomonas* species (9), the identification of all 20 strains as pseudomonads further indicates the utility of

Table 4. Cotton-stand counts (% of nontreated control) for the 1985 strain-evaluation tests[a,b]

Treatment	AR	CA	GA	LA	MS1	MS2	TX1	TX2
Fungicide	112*	158*	114	163*	125*	112	108	118*
MS-97	114*	113	104	111	102	91	105	94
BD4-13	95	122	105	122	116*	94	110	121*
MS-97 + BD4-13	94	124	107	102	104	90	117*	92
AC-25	88	125*	116	122	116*	89	108	87
AC-39	86	141*	117	130*	118*	87	113	103
AC-40	88	143*	111	124*	104	106	113	119*
AC-46	101	149*	126*	124*	118*	96	117*	92
AC-65	94	110	132*	125*	111	88	105	106
AC-69	96	113	113	102	111	102	117*	100
AC-75	91	127*	105	125*	102	90	98	86
AC-91	90	133*	123*	123*	112	94	115	110
AC-97	95	138*	123*	123*	112	94	115	110
AC-105	80	114	107	87	109	104	112	97
LSD 0.05	12	25	22	23	14	23	16	18

[a] Sites were AR = Marianna, Arkansas; CA = Shafter, California; GA = Athens, Georgia; LA = Cheneyville, Louisiana; MS1 = Starkville, Mississippi; MS2 = Stoneville, Mississippi; and TX1 and TX2 = College Station, Texas. Cultivars were Stoneville 825 at AR, GA, and MS1; Acala SJ2 at CA; DPL-41 at LA; Miscot at MS2; Tamcot SP215 at TX1; and Tamcot CAMD-E at TX2. Fungicides were etridiazole + quintozene (Terra-Coat L 205, 7.5 ml/kg seed) plus etridiazole (Terraclor Super X 12.5G, 11.2 kg/ha) for AR, GA, LA, and MS sites; metalaxyl (Ridomil PC-11G, 11.2 kg/ha) for CA; and chloroneb (Demosan 65W, 6.25 ml/kg seed) plus Captan 400-S (1.25 ml/kg seed) for both TX sites.

[b] * Indicates a stand greater than the nontreated control.

Table 5. Cotton yields (kg/ha) for the 1985 strain-evaluation tests[a,b]

Treatment	AR	CA	GA	LA	MS1	MS2	TX1	TX2
Untreated	752	902	621	653	809	762	507	581
Fungicide	840*	1135*	670	726*	964*	858*	529	634*
MS-97	748	947	611	642	867	810	408	565
BD4-13	827*	1023*	686*	704	765	766	571*	658*
MS-97 + BD4-13	763	972*	643	637	825	816*	521	593
AC-25	751	826	651	663	756	772	463	584
AC-39	736	905	594	714	816	745	486	529
AC-40	801	921	642	619	783	747	481	611
AC-46	823*	946	581	718	832	782	494	573
AC-65	778	932	608	679	774	796	516	609
AC-69	819*	874	651	638	853	761	546	581
AC-75	784	863	597	654	768	808	539	612
AC-91	795	905	675	731*	901*	821*	491	647*
AC-97	812*	1103*	681*	720	846	784	563*	595
AC-105	804	918	663	667	821	794	532	627
LSD 0.05	56	63	51	73	68	54	49	61

[a] Sites were AR = Marianna, Arkansas; CA = Shafter, California; GA = Athens, Georgia; LA = Cheneyville, Louisiana; MS1 = Starkville, Mississippi; MS2 = Stoneville, Mississippi; and TX1 and TX2 = College Station, Texas. Cultivars were Stoneville 825 at AR, GA, and MS1; Acala SJ2 at CA; DPL-41 at LA; Miscot at MS2; Tamcot SP215 at TX1; and Tamcot CAMD-E at TX2. Fungicides were etridiazole + quintozene (Terra-Coat L 205, 7.5 ml/kg seed) plus etridiazole (Terraclor Super X 12.5G, 11.2 kg/ha) for AR, GA, LA, and MS sites; metalaxyl (Ridomil PC-11G, 11.2 kg/ha) for CA; and chloroneb (Demosan 65W, 6.25 ml/kg seed) plus Captan 400-S (1.25 ml/kg seed) for both TX sites.

[b] * Indicates a yield greater than the nontreated control.

this genus as a source of antifungal mechanisms (11,14,17). One strain (BD4-13) was developed as a product for controlling seedling disease on cotton and marketed as "Dagger" by Ecogen, Inc., from 1986 to 1988.

The application method tests indicated that in-furrow inoculants, both granular and liquid, were superior to seed coating (Table 6). In-furrow application has one major advantage over seed coating: much greater quantities of inoculant can be added to the seedbed. Seed bacterization must consider compatibility with seed-coat fungicides (generally used), and seed coated with inoculants must be planted immediately to prevent drying and rapid cell death. In-furrow application of inoculants avoids the seed-coating process by the producer, and seedbed inoculants can be used with delivery equipment commonly employed by cotton growers.

Effective control of seedling disease by certain bacterial strains indicates that the strains do possess some type of mechanism(s) for repressing the seedling disease pathogens. Once specific mechanisms have been identified, it may be possible to exploit them to improve strain performance (11,12). There are at least three possible research approaches to enhancing performance: 1) genetic manipulation to combine multiple repression mechanisms in one strain, 2) derepression of an antifungal mechanism(s) in a strain to increase activity, and 3) development of transformed cotton lines that contain the fungal repression mechanisms. The first two approaches will require the choice of strains that are superior root/soil colonizers (5), and the development of inoculant formulations that protect both strain viability and physiological competence (8).

Table 6. Cotton-stand counts (% of nontreated control) for the 1985 carrier-development test[a,b]

Treatment[c] Strain	AR	CA	GA	LA	MS1	MS2	TX1	TX2
TS, fungicide[d]	169*	107	88	133*	125*	87	107	111
US, seed-applied								
MS-97	114	100	77	71	80	79	100	88
BD4-13	130*	90	93	89	78	77	90	90
MS + BD	118	78	91	91	86	80	78	86
TS, seed-applied								
MS-97	129*	98	83	77	91	103	98	97
BD4-13	132*	77	89	71	79	110	77	94
MS + BD	138*	81	83	72	98	97	81	95
TS, granular in-furrow								
MS-97	161*	82	96	106	114*	97	82	107
BD4-13	126*	97	99	127*	116*	106	97	124*
MS + BD	191*	117*	100	97	118*	104	117*	118*
TS, liquid in-furrow								
MS-97	148*	104	105	116*	92	97	104	107
BD4-13	174*	116*	103	114*	94	101	116*	112
MS + BD	135*	100	97	111*	97	108	100	121*
LSD 0.05	26	15	9	10	14	25	17	13

[a] Sites were AR = Marianna, Arkansas; CA = Shafter, California; GA = Athens, Georgia; LA = Cheneyville, Louisiana; MS1 = Starkville, Mississippi; MS2 = Stoneville, Mississippi; and TX1 and TX2 = College Station, Texas. Cultivars were Stoneville 825 at AR, GA, and MS1; Acala SJ2 at CA; DPL-41 at LA; Miscot at MS2; Tamcot SP215 at TX1; and Tamcot CAMD-E at TX2.

[b] * Indicates a stand greater than the nontreated control.

[c] TS = fungicide-treated seed, US = nontreated seed. All TS received etridiazole + quintozene (Terra-Coat L 205) at 7.5 ml/kg seed.

[d] Etridiazole + quintozene plus etridiazole (Terraclor Super X 12.5G, 11.2 kg/ha) for AR, GA, LA, and MS sites; metalaxyl (Ridomil PC-11G, 11.2 kg/ha) for CA; and chloroneb (Demosan 65W, 6.25 ml/kg seed) plus Captan 400-S (1.25 ml/kg seed) for both TX sites.

Table 7. Orthogonal contrast analysis of treatments in application method test[a]

Treatment[b]	MS-97	BD4-13	MS-97 + BD4-13
US Seed App vs. TS Seed App	NS	NS	NS
US Seed App vs. TS Gran	NS	**	**
US Seed App vs. TS Lqd	NS	**	*
US Seed App vs. TS Fung	*	*	*
TS Seed App vs. TS Gran	NS	**	**
TS Seed App vs. TS Lqd	NS	**	*
TS Seed App vs. TS Fung	*	*	*
TS Gran vs. TS Lqd	NS	NS	NS
TS Gran vs. TS Fung	NS	NS	*
TS Lqd vs. TS Fung	NS	NS	NS

[a] * = F test significant at 0.05, ** = F test significant at 0.01, and NS = not significant.

[b] US = nontreated seed; TS = seed treated with etridiazole + quintozene (Terra-Coat L 205, 7.5 ml/kg seed); Seed App = peat-based biologicals applied on seed at 110 g/kg; Gran = granular, peat-based biologicals applied in furrow at 2.3 g/m; Lqd = liquid biologicals applied in furrow at 14.1 ml/m; and Fung = fungicide applied. All inoculants contained at least 10^9 cfu/g or ml.

LITERATURE CITED

1. Bashan, Y. 1986. Enhancement of wheat root colonization and plant development by *Azospirillum brasilense* Cd. following temporary depression of rhizosphere microflora. Appl. Environ. Microbiol. 51:1067-1070.
2. Becker, J. O., Hepfer, C. A., Yuen, G. Y., Van Gundy, S. D., Schroth, M. N., Hancock, J. G., Weinhold, A. R., and Bowman, T. 1990. Effect of rhizobacteria and metham-sodium on growth and root microflora of celery cultivars. Phytopathology 80:206-211.
3. Bell, A. 1984. Cotton protection practices in the USA and world. Pages 288-309 in: Sect. B: Diseases. R. J. Kohel and C. F. Lewis, eds. Cotton. Am. Soc. Agron., Monogr. 24.
4. Brown, J. M., and Nelson, T. C., eds. 1987. Cotton Disease Council report. Pages 2-57 in: Proc. Beltwide Cotton Prod. Res. Conf., National Cotton Council of America, Memphis, TN.
5. Chao, W. L., Nelson, E. B., Harman, G. E., and Hoch, H. C. 1986. Colonization of the rhizosphere by biological control agents applied to seeds. Phytopathology 76:60-65.
6. De Freitas, J. R., and Germida, J. J. 1990. Plant growth promoting rhizobacteria for winter wheat. Can. J. Microbiol. 36:265-272.
7. Dupler, M., and Baker, R. 1984. Survival of *Pseudomonas putida*, a biological control agent, in soil. Phytopathology 74:195-200.
8. Gould, W. D. 1990. Biological control of plant root diseases by bacteria. Pages 287-317 in: Biotechnology of Plant-Microbe Interactions. J. P. Nakas and C. Hagedorn, eds. McGraw-Hill Publishing Company, New York.
9. Hagedorn, C., Gould, W. D., and Bardinelli, T. R. 1989. Rhizobacteria of cotton and their repression of seedling disease pathogens. Appl. Environ. Microbiol. 55:2793-2797.
10. Harman, G. E., Chet, I., and Baker, R. 1980. *Trichoderma hamatum* effects on seed and seedling disease induced in radish and pea by *Pythium* spp. or *Rhizoctonia solani*. Phytopathology 70:1167-1172.
11. Howell, C. R., and Stipanovic, R. D. 1979. Control of *Rhizoctonia solani* on cotton seedlings with *Pseudomonas fluorescens* and with an antibiotic produced by the bacterium. Phytopathology 69:480-482.
12. Howell, C. R., and Stipanovic, R. D. 1980. Suppression of *Pythium ultimum*-induced damping-off of cotton seedlings by *Pseudomonas fluorescens* and its antibiotic, pyoluteorin. Phytopathology 70:712-715.
13. Miller, H. J., Liljeroth, E., Henken, G., and Van Veen, J. A. 1990. Fluctuations in the fluorescent pseudomonad and actinomycete populations of rhizosphere and rhizoplane during the growth of spring wheat. Can. J. Microbiol. 36:254-258.
14. Nesmith, W. C., and Jenkins, S. F., Jr. 1985. Influence of antagonists and controlled matric potential on the survival of *Pseudomonas solanacearum* in four North Carolina soils. Phytopathology 75:1182-1187.
15. Paulitz, T. C., Park, C. S., and Baker, R. 1987. Biological control of Fusarium wilt of cucumber with nonpathogenic isolates of *Fusarium oxysporum*. Can. J. Microbiol. 33:349-353.
16. Weller, D. M., and Cook, R. J. 1983. Suppression of take-all of wheat by seed treatments with fluorescent pseudomonads. Phytopathology 73:463-469.
17. West, A. W., Burges, H. D., Dixon, T. J., and Wyborn, C. H. 1985. Survival of *Bacillus thuringiensis* and *Bacillus cereus* spore inocula in soil: Effects of pH, moisture, nutrient availability and indigenous microorganisms. Soil Biol. Biochem. 17:657-665.
18. Yuen, G. Y., Schroth, M. N., and McCain, A. H. 1985. Reduction of Fusarium wilt of carnation with suppressive soils and antagonistic bacteria. Plant Dis. 69:1071-1075.