

Effect of Seed Quality and Combination Fungicide–*Trichoderma* spp. Seed Treatments on Pre- and Postemergence Damping-Off in Cotton

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ABSTRACT

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Good quality seeds of cotton cultivars often escaped pre-emergence damping-off incited by *Pythium* spp. and *Rhizopus oryzae*, and they were resistant to postemergence damping-off incited by *Rhizoctonia solani*. Poor quality seeds, however, were highly susceptible to both phases of seedling disease and required seed treatment in order to survive. Pre-emergence damping-off incited by *Pythium* spp. and *Rhizopus oryzae* could be controlled by seed treatment with biocontrol preparations of a number of *Trichoderma* spp., but these treatments were much less effective

in controlling postemergence disease incited by *Rhizoctonia solani*. Postemergence seedling disease can be controlled by fungicides, but they were much less effective in controlling the pre-emergence phase of the disease. Combination seed treatments of poor quality cotton seeds with fungicides and *Trichoderma* spp. preparations, followed by planting in pathogen-infested soil, indicated that this technique will control both phases of seedling disease. Seed treatment with either the fungicides or the biocontrol agents alone did not achieve this goal. The optimum combination treatment for disease control was that of chloroneb plus *Trichoderma* spp., followed by chloroneb plus metalaxyl (Deltacoat AD) plus *T. virens* strain G-6.

The principal inciters of pre-emergence damping-off of cotton seedlings are *Pythium* spp. and *Rhizopus oryzae* (16), and the principal inciter of postemergence damping-off is *Rhizoctonia solani* anastomosis group (AG)-4. The former phase occurs in cool or warm moist soils, while the latter occurs under warmer and dryer conditions and usually attacks the seedling hypocotyl (14). Strains of the biocontrol fungus *Trichoderma virens* belonging to the “Q” group give very effective control of pre-emergence damping-off of cotton seedlings incited by *Pythium ultimum*, *P. aphanidermatum*, and *Rhizopus oryzae* (16). The mechanisms employed by the fungus to affect disease control are induction of phytoalexins in seedling radicles (18) and metabolism of pathogen germination stimulants released by the seedling (16). Induction of phytoalexin synthesis in the cotton root is achieved through penetration of the epidermis and colonization of the root cortex by the fungus, concomitant with production of an inducer protein that stimulates synthesis of the fungitoxins hemigossypol and desoxyhemigossypol by the plant (13). This mechanism allows for protection of the pre-emerged seedling and the developing root. However, the seedling hypocotyl is not colonized by the biocontrol agent, and phytoalexin synthesis is not induced in that area of the plant, leaving it unprotected against post-emergence damping-off (18). The postemergence phase of cotton seedling disease can be successfully controlled with systemic fungicides (25), but these fungicides are much less efficient at controlling the pre-emergence phase. Metalaxyl effectively con-

trols disease incited by *Pythium* spp. (25), but it does not control disease incited by *Rhizopus oryzae*.

Combination treatments with *Trichoderma* spp. and fungicides to control plant diseases has been tried with other crops, using natural strains and those selected for fungicide resistance (5,9,10,21,23), as well as with cotton, where *T. virens* was used in combination with metalaxyl as a seed treatment (17). The fungicide fludioxonil has also been used in combination with bacteria, actinomycetes, and fungi to control Fusarium wilt of cyclamen (8), and *Rhizoctonia*-specific fungicides (pencycuron and flutamonil) and fungicides specific to oomycetes (cymoxanil or propanocarb) were used in combination with *Verticillium biguttatum* to control black scurf and tuber rot of potatoes (26).

There has been a good deal of work done on the relationship between cotton seed quality, seedling vigor and stand establishment (4,6,20). Much research has also gone into determining what environmental factors contribute to cotton seed quality and deterioration, including the role of microorganisms (7,11,12,22). Techniques for measuring cotton seed quality have also been thoroughly studied (2,15,24). However, relatively little has been done to establish a relationship between cotton seed quality and resistance or susceptibility to cotton seedling disease. The subject has been approached only in the broad sense of breeding cotton cultivars for multi-adversity resistance (3).

The purpose of this study was to determine the effect of seed quality on resistance or susceptibility to seedling disease, and to discern the disease control efficacy of various fungicides, *Trichoderma* spp., and *Trichoderma* species × fungicide combination seed treatments on poor quality cotton seed in soil infested with both pre- and postemergence damping-off pathogens. The goal was to identify the most effective means of controlling both phases of cotton seedling disease.

MATERIALS AND METHODS

Pathogens and soil. The soil in the cotton seedling disease plots at Texas A&M University is a Lufkin fine sandy loam, and it

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is infested with the seedling disease pathogens *Rhizopus oryzae*, *P. aphanidermatum*, and *P. ultimum* (16). However, there is currently very little *Rhizoctonia solani* inoculum in the soil. In order to infest the soil with this fungus, twice-autoclaved (121°C for 20 min) millet seed cultures (30 g in 60 ml of H₂O) were inoculated with 7-mm potato dextrose agar (PDA) plugs of *Rhizoctonia solani* AG-4. After 14 days at 25°C, cultures were air-dried under a positive pressure hood and ground into <425- μ m-diameter granules with a coffee grinder (Hamilton Beach/Proctor Silex Inc., Washington, NC). The granules were stored in sealed plastic bags at 5°C until used. Soil from the disease plots containing pre-emergence pathogens was infested with *Rhizoctonia solani* by thoroughly mixing in 0.2 g of inoculum per kg of soil. The soil was then spray-moistened with 80 ml of deionized water per kg of soil.

Assay of good and poor quality cotton seed lots for resistance to damping-off. Seed lots of different cotton cultivars that had been produced in different years and kept in storage for varying periods of time were gathered and planted in cotton field soil. Seed lots from the same cultivar showing good or poor emergence after 7 days incubation at 25°C were chosen for further investigation. Good and poor quality seed lots of cotton cvs. DP451 B/RR and SG747 obtained from Delta and Pineland Seed Company in Scott, MS, and Acala Maxxa obtained from California Planting Cotton Seed Distributors, Shafter, CA, were assayed for resistance to cotton seedling disease. Seeds of each seed lot were planted in test tubes containing 10 g each of moist Lufkin fine sandy loam that was heat-sterilized (121°C for 20 min), naturally infested with pre-emergence pathogens, or naturally infested with pre-emergence pathogens and amended with millet granules of *Rhizoctonia solani* as described previously. After 7 days incubation in a growth chamber at 25°C with a 12-h photoperiod, the numbers of surviving seedlings were counted.

Biological seed treatment component. The “Q” strain G-6 of *Trichoderma virens* (19) was incubated at 27°C with shaking (150 rpm) in 100-ml aliquots of deionized water containing 5% ground wheat bran and 1% ground peat moss, adjusted to pH 4.0. After 6 days the culture contents were centrifuged at 3,500 rpm for 10 min. The supernates were decanted, and the pellets were spread to air dry under a positive pressure hood for 24 h. The dried preparations were ground in a Retch grinder (Brinkman Instruments Inc., Westbury, NY) into 53- to 150- μ m-diameter granules and stored at 5°C until used.

Chemical seed treatment components and concentrations. The chemicals and their concentrations used as seed treatments were Baytan 30 fl (a.i. 30% triadimenol, 14.8 g/45.4 kg), Chloroneb 65 WP (147.9 g/45.4 kg), Deltacot AD (a.i. 30% chloroneb plus 3.5% metalaxyl, 347.8 g/45.4 kg), Dividend 32.8 fl (a.i. 32.8% difenoconazole, 29.6 g/45.4 kg), Flint (a.i. 50% trifloxystrobin, 2.22 g/45.4 kg), Maxim 42 fl (a.i. fludioxonil, 2.4 g/45.4 kg), Nuflow M 40 fl (a.i. 40% myclobutanil, 37 g/45.4 kg), Vitavax 34 fl (a.i. 34% carboxin, 88.8 g/45.4 kg), Vitavax plus PCNB, 17 fl plus 17 fl (a.i. 17% carboxin plus 17% pentachloronitrobenzene, 177.6 g/45.4 kg), and Vortex (a.i. ipconazole, trade secret, 2.7 g/45.4 kg). All of the fungicides, with the exception of Maxim, were obtained from Gustafson, Inc. (now Bayer Crop Science AG1), McKinney, TX. Maxim was obtained from Novartis Inc. (now Syngenta), Research Triangle Park, NC. The fungicides were applied to the seed in a volume of water equal to one tenth the weight of the seeds, and the seeds were air dried under the hood. The control was treated with water only.

Biological/chemical seed treatments. Seeds of a disease susceptible seed lot of cotton cv. SG747, nontreated or treated with fungicide, were coated with latex sticker (Rhoplex 15BJ, Rohm and Haas, Philadelphia, PA), and then air-dried biocontrol granules of G-6 were applied at a rate of 0.1 g/g of seed.

Assays of fungicide toxicity to strain G-6 in vitro. Aliquots of PDA containing rifampicin (50 μ g ml⁻¹) and the above listed

chemical fungicides, separately and in concentrations equivalent to those applied to seeds, were poured into petri dishes (10 ml per plate). After cooling, the plates were each inoculated with three 4-mm PDA plugs of *T. virens* strain G-6. The cultures were incubated at 27°C for 48 h, and colony diameters were measured.

Seeds of cotton cv. SG747 were treated with chloroneb plus G-6 preparation and planted in sterile soil tubes. After 3 days incubation at 25°C, the seeds were removed, examined under a dissecting microscope for signs of mycelial growth, and plated on PDA containing rifampicin (50 μ g ml⁻¹). After 72 h the plates were examined for evidence of *T. virens* growth and sporulation.

Assays of fungicides, *T. virens*, and combined seed treatments for disease control efficacy. Seeds of the disease susceptible seed lot of cotton cv. SG747 were planted in test tubes containing 10 g of moist field soil, with five tubes per replication and 15 tubes per treatment. The soil was naturally infested with *Rhizopus oryzae* and *Pythium* spp. alone (16) or it was amended with 0.2 g of *Rhizoctonia solani*-infested millet granules per kg of soil. The soils were planted with nontreated, fungicide-treated, G-6-treated, or fungicide plus G-6-treated seeds of cv. SG747. After 7 days incubation in a growth chamber at 25°C and with a 12-h photoperiod, the tubes were examined and the numbers of surviving seedlings were counted.

Production of other *Trichoderma* spp. strain preparations for disease control assays. *Trichoderma* spp. strains G-6, F19-4, G11-40V (*T. virens* “Q” strains), Tk-35 (*T. koningii*), T-22 (*T. harzianum*), and TKG-12 (*T. virens* \times *T. koningii* hybrid) were cultured in 500-ml flasks containing 20 g of vermiculite, 10 g of wheat bran, 2 g of peat moss, and 100 ml of dilute HCl (pH 1.57), after heat sterilization for 20 min at 121°C. The culture flasks were placed on their sides in a 27°C incubator and shaken every other day for 7 days. The contents were then removed, air dried under the hood overnight, and ground into granules (53 to 150 μ m) with a Proctor Silex grinding mill. The granules were stored in sealed plastic bags at 5°C until used. These preparations were used as seed treatments to compare their effectiveness with that of strain G-6 as biological control agents of cotton seedling diseases, alone or in combination with chloroneb.

Assay of chloroneb, *Trichoderma* strains, and combined seed treatments for disease control efficacy in soil infested with pre- and postemergence pathogens. Seeds of a disease susceptible seed lot of cotton cv. SG747 were treated with one of the following: chloroneb (147.9 g/45.4 kg), coated with latex sticker and one of the biocontrol preparations described above, or treated with a combination of chloroneb and biological control preparation. The treated seeds were planted in soil tubes containing 10 g of moist field soil naturally infested with pre-emergence pathogens and amended with *Rhizoctonia solani* as described previously. After 7 days incubation at 25°C, the tubes were examined and the numbers of surviving seedlings were counted.

Statistical analysis. All treatments in the experiments were replicated three times and the experiments were all repeated. The data shown are the results of single trials that were repeated with similar results. The data were analyzed using general linear models (version 6; SAS Institute, Cary, NC), and the data sets were analyzed individually.

RESULTS

Effect of seed quality on disease occurrence. When good and poor quality seeds of each cultivar were planted in sterile soil there were no significant differences in the numbers of surviving seedlings; survival was greater than 87% for all (Table 1).

However, when good and poor quality seeds were planted in soil infested with pre-emergence pathogens, the percent survival of seedlings from the good quality seeds of each cultivar was significantly greater than that from the poor quality seeds.

Seedling survival of good and poor quality seeds in soil amended with *Rhizoctonia solani* inoculum followed the same pattern as those in soil infested with only pre-emergence pathogens, except that seedling survival totals were considerably diminished for both good and poor quality seed lots. An exception to this pattern was observed with the excellent quality seed lot of cv. SG747. This seed lot maintained high levels of seedling survival even in soil infested with both pre- and postemergence pathogens.

Inhibitory effect of tested fungicides on *T. virens* strain G-6.

All of the fungicides assayed for toxicity to G-6 proved to be inhibitory to the fungus in vitro, with some less inhibitory than others. However, there was no positive correlation between fungicide resistance and disease control efficacy of the combined fungicide/biological treatments (data not shown).

Microscopic examination of chloroneb plus G-6-treated cotton seeds harvested from sterile soil tubes after 3 days incubation at 25°C gave evidence of fungal hyphae growing from the seed surface. After 72 h incubation at 25°C on PDA containing rifam-

picin, sporulating cultures of *T. virens* were observed growing from the seeds.

Assays of seed treatments for seedling disease control in soils infested with pre-emergence pathogens and amended or not amended with *R. solani*. The seedling survival data derived from planting nontreated, fungicide-treated, or G-6-treated SG747 seeds in soil naturally infested with *Rhizopus oryzae* and *Pythium* spp. showed that fungicide treatment of seeds planted in this soil resulted in little or no improvement in seedling stand over the nontreated control (0 to 13%). Seed treatment with preparations of the biocontrol strain G-6, however, gave 93% seedling survival in the pathogen-infested soil (Fig. 1).

The planting of SG747 seeds, nontreated, fungicide-treated, or G-6-treated, in naturally infested cotton field soil amended with *Rhizoctonia solani* inoculum produced somewhat different results. The percent survival of nontreated and fungicide-treated seeds remained at low levels, but survival of the G-6-treated seeds was reduced to 40% (Fig. 2).

The numbers of surviving seedlings from seeds planted in naturally infested soil amended with *Rhizoctonia solani* inoculum were vastly improved by some of the seed treatments with combinations of fungicides and the biocontrol agent preparation. Other combinations were no better than the individual treatments (Fig. 3). The most effective chemical combinations with strain G-6 were chloroneb (93%) and Deltacoat AD (80%), followed by Vitavax (60%) and Vitavax-PCNB (53%).

The results of the disease control assays of chloroneb, biological seed treatments, and combination of chloroneb plus biological seed treatments for efficacy in the control of pre- and post-emergence damping-off in cotton indicate that chloroneb in combination with any one of several biological control preparations gives effective disease control (Fig. 4). Neither chloroneb nor the biocontrol agents alone control all of the pathogens involved.

DISCUSSION

Seed quality is a very important factor in producing a good cotton stand (4). Seed lots of varying quality may all show good germination and subsequent growth in sterile soil. However, when

TABLE 1. Effect of seed quality on seedling survival of three cotton cultivars in sterile soil, soil naturally infested with pre-emergence pathogens, and naturally infested soil amended with *Rhizoctonia solani*

Seed lot	Seedling survival (%) ^x		
	SS ^y	NIS	NIS + Rs
Deltapine451 (G) ^z	100 a	60 b	20 b
Deltapine451 (P)	100 a	20 c	7 c
Acala Maxxa (G)	93 a	93 a	20 b
Acala Maxxa (P)	87 a	47 b	0 c
Suregrow747 (E)	93 a	87 a	73 a
Suregrow747 (P)	87 a	7 c	0 c

^x Means within a column followed by different letters are significantly different according to Fisher's protected least significant different test at $P = 0.05$ using general linear models (SAS Institute, Cary, NC). Fisher's least significant difference = 17%.

^y SS = sterile soil; NIS = naturally infested soil; NIS + Rs = naturally infested soil amended with *R. solani*.

^z (E) = excellent quality seed; (G) = good quality seed; (P) = poor quality seed. Seed quality designation was based on storage period and percent emergence in cotton field soil.

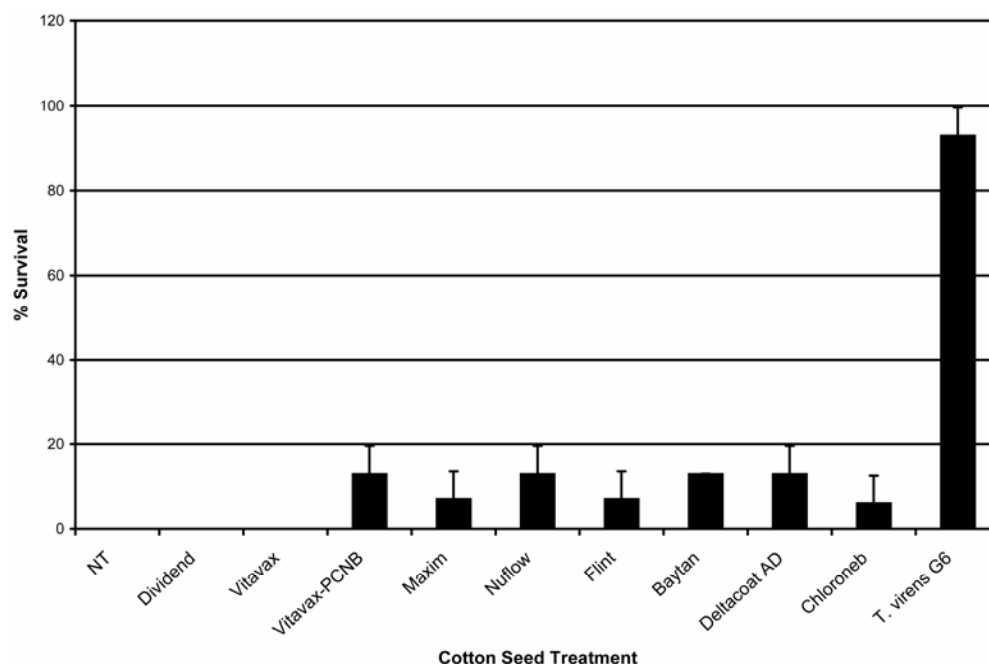


Fig. 1. Effect on cotton seedling survival of seed treatment with fungicides or *Trichoderma virens* strain G-6 in soil naturally infested with pre-emergence damping-off pathogens. The cotton cultivar was poor quality SG747. NT = nontreated. Values represent the mean \pm standard error of three replicates. Fisher's least significant difference = 17%.

planted in soil infested with seedling disease pathogens, the differences in seed quality often manifest themselves. Good quality cotton seeds may escape, or show a good deal of resistance to seedling disease as demonstrated by the results of this study with good and poor quality seed lots. These differences in disease susceptibility exhibited by good and poor quality seed lots most likely reflect differences in the kinds or amounts of pathogen germination stimulants that are released to the soil by the germinating seeds (1,16). Poor quality seeds are very susceptible to seedling diseases and may require a fungicide, a biological seed

treatment, or a combined seed treatment in order to survive. The appropriate treatment will depend on the kinds of pathogens present in the soil.

The results of this study indicate that in soils containing several different kinds of pre-emergence damping-off pathogens and a postemergence pathogen, seed treatment with a fungicide and a biocontrol agent are required to control cotton seedling diseases in poor quality cotton seed. In soils where only pre-emergence pathogens such as *Pythium* spp. and *Rhizopus oryzae* are present, the biocontrol agent strain G-6 of *T. vires* gave excellent disease

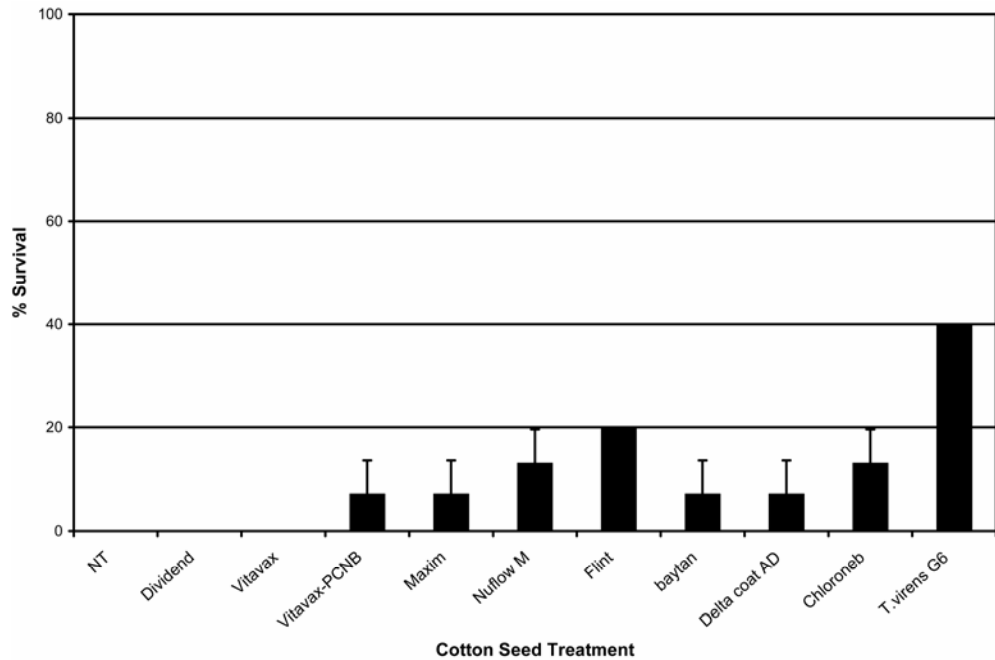


Fig. 2. Effect on cotton seedling survival of seed treatments with fungicides or *Trichoderma vires* strain G-6 in soil naturally infested with pre-emergence damping-off pathogens and amended with *Rhizoctonia solani*. The cotton cultivar was poor quality SG747. NT = nontreated. Values represent the mean \pm standard error of three replicates. Fisher's least significant difference = 14%.

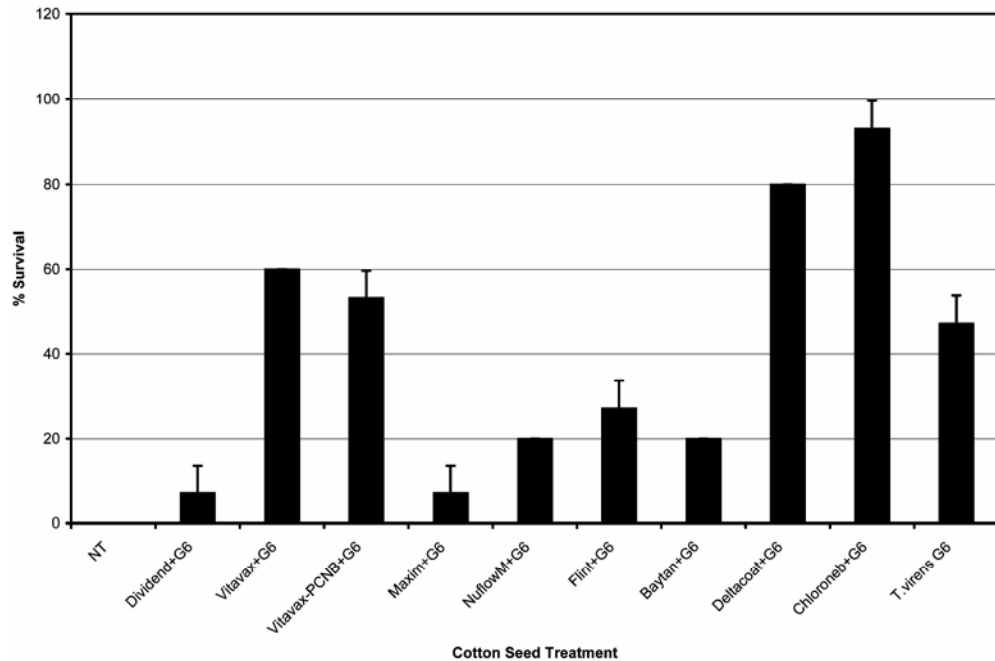


Fig. 3. Effect on cotton seedling survival of seed treatments with combinations of fungicides and *Trichoderma vires* strain G-6 in soil naturally infested with pre-emergence damping-off pathogens and amended with *Rhizoctonia solani*. The cotton cultivar was poor quality SG747. NT = nontreated. Values represent the mean \pm standard error of three replicates. Fisher's least significant difference = 17%.

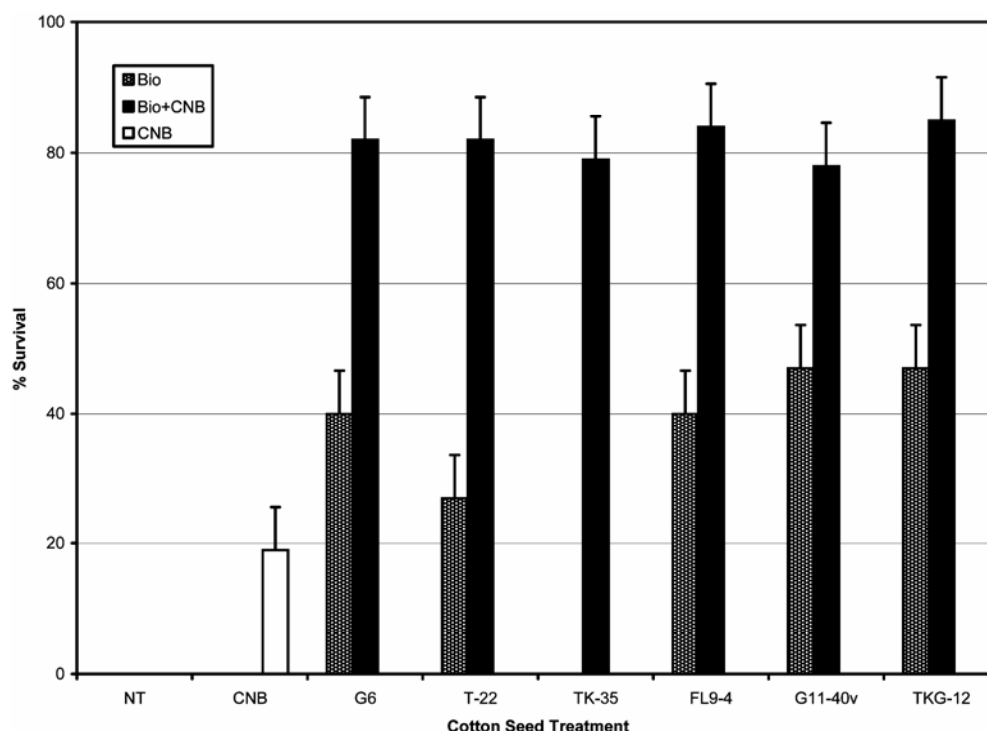


Fig. 4. Effect of Chloroneb, biological (*Trichoderma* spp.), and biological plus Chloroneb seed treatments on cotton seedling survival in soil naturally infested with pre-emergence damping-off pathogens and amended with *Rhizoctonia solani*. CNB = Chloroneb alone; Bio = biological alone; Bio + CNB = biological and Chloroneb in combination; G6 = *T. virens* Q strain; T-22 = *T. harzianum*; TK-35 = *T. koningii*; FL9-4 = *T. virens* Q strain; G11-40v = *T. virens* Q strain; and TKG-12 = *T. virens* × *T. koningii* hybrid. The cotton cultivar was poor quality SG747. NT = nontreated. Values represent the mean ± standard error of three replicates. Fisher's least significant difference = 14%.

control. Seed treatment with the fungicides used in this study did not give adequate control of cotton seedling disease when the seeds were planted in soil containing only pre-emergence pathogens or where both pre- and postemergence pathogens were present. In soil containing both pre- and postemergence pathogens, neither the fungicides nor the biocontrol agents alone gave adequate seedling disease control. This was because the fungicides, although effective in controlling *Rhizoctonia solani*, did not have a wide enough activity spectrum to control both *Rhizopus oryzae* and the *Pythium* spp. (25). Strain G-6 of *T. virens* controls the pre-emergence pathogens by metabolizing pathogen germination stimulants excreted by the seedling and by inducing phytoalexin synthesis in the roots (16,18). Induction of phytoalexin synthesis, however, does not extend to the hypocotyls, and that area is susceptible to postemergence damping-off. Combination seed treatments consisting of the biocontrol agent and a systemic fungicide can control both phases of cotton seedling disease. The biocontrol agent suppresses the activities of the pre-emergence pathogens, while the fungicide inhibits those of the post-emergence pathogen.

Although all of the fungicides used in this test were toxic to *T. virens* in vitro, and may have inhibited the activities of the biocontrol agent in vivo, much of the systemic chemical was likely absorbed by the germinating seedling, and the biocontrol agent was shielded from the remaining fungicide by the latex coating on the seed.

Optimum disease control in soil containing both pre- and post-emergence pathogens was achieved with a combination of chloroneb and any one of several *Trichoderma* spp., indicating that the mechanisms employed to control the pre-emergence phase of cotton seedling disease may be common within the genus. Delta-coat AD, in combination with *T. virens* strain G-6, was also effective in controlling cotton damping-off. However, the Delta-coat AD formulation is mostly chloroneb, and this may account for its success.

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