Susceptibility of Selected Cotton Cultivars to Seedling Disease Pathogens and Benefits of Chemical Seed Treatments

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ABSTRACT

Wang, H., and Davis, R. M. 1997. Susceptibility of selected cotton cultivars to seedling disease pathogens and benefits of chemical seed treatments. *Plant Dis.* 81:1085-1088.

The susceptibility of 12 Upland cotton cultivars to three soilborne fungi, *Pythium ultimum*, *Rhizoctonia solani*, and *Thielaviopsis basicola*, was evaluated in greenhouse experiments. Based on symptom development and seedling survival, cultivars highly resistant to *P. ultimum* included Delta Pine (DP) 6166, Prema, DP 6100, and Maxxa. A relatively low incidence of pre-emergence damping-off caused by *R. solani* occurred in ChemBred 7, DP 6100, and Royale, although all cultivars subsequently suffered significant post-emergence damping-off. All cultivars were equally susceptible to *T. basicola*. In both greenhouse and field evaluations, there were no benefits of metalaxyl seed treatment in *Pythium*-resistant cultivars. Seed treatment with carboxin-pentachloronitrobenzene for the control of *Rhizoctonia*-induced damping-off resulted in stand increases in all 12 cultivars in greenhouse tests and in 3 of 6 cultivars in field trials.

Seedling diseases cause the largest yield losses of any cotton disease in the United States. In 1995, the estimated nationwide reduction in cotton yields from seedling diseases was 828,199 bales (1). In California, seedling diseases caused an estimated loss of 81,994 bales in 1995, and similar lint losses have occurred in previous years (5).

The complex of pathogens associated with cotton seedling diseases, including *Pythium* spp., *Rhizoctonia solani* Kühn (teleomorph: *Thanatephorus cucumeris* (A. B. Frank) Donk), and *Thielaviopsis basicola* (Berk. & Broome) Ferraris, confounds seedling disease control (6). Generally, appropriate fungicide seed treatments are the most effective control of seedling diseases (15). In California, virtually all cotton seeds are treated with at least 2 fungicides for protection from seedling diseases caused by *Pythium* spp. and *R. solani* (9). Recently, fungicides have been registered that reduce black root rot caused by *T. basicola*, and many acres are now planted with seeds treated with 3 or more fungicides.

Resistance is potentially the most economical method to manage seedling diseases because fungicide seed treatments could then be reduced or eliminated (7). Hefner (11) found that 1 of 10 cotton lines had both good emergence and good survival when challenged with *R. solani*. Gar-

ber et al. (8) evaluated the susceptibility of 7 cultivars to seedling disease and, although some cultivars were relatively tolerant to *P. ultimum*, none of the cultivars provided adequate stands without fungicide seed treatments.

Within the last several years, a number of new cotton cultivars have been introduced into California. The objective of this study was to evaluate the susceptibility of these new cultivars to infection by several soilborne pathogens, including *Pythium ultimum*, *R. solani*, and *T. basicola*. In addition, the benefits of individual fungicide seed treatments for the control of *Pythium*- and *Rhizoctonia*-induced seedling diseases were evaluated in greenhouse and field tests.

MATERIALS AND METHODS

Greenhouse evaluation of cultivar susceptibility to seedling diseases. Twelve cultivars of Upland cotton (*Gossypium hirsutum* L.), including ChemBred (CB) 7, CB 305, Delta Pine (DP) 6100, DP 6166, Germain's Seeds (GC) 510, GC 610, GC 702, GC 717, Maxxa, Prema, Royale, and SJ-2, were evaluated for susceptibility to *P. ultimum* (isolate from soil from the Shafter Cotton Research Station in Kern County, California), *R. solani* AG-4 (isolate from a cotton seedling from the Kearney Agricultural Center in Tulare County, California), and *T. basicola* (original isolate from R. J. Wakeman, University of California [UC], Davis) under varied population densities in the greenhouse.

Inocula of *P. ultimum* and *T. basicola* were produced on potato-dextrose agar (PDA) plates, while inoculum of *R. solani* was produced on PDA plates amended with wheat bran (3 to 4 g/liter). Two-week-

old plates of *P. ultimum* and *R. solani* were blended in water for 2 to 3 min in a Waring Commercial Blender (Dynamics Corporation of America, New Hartford, CT) at low speed, and mixed well with UC potting mix (1:1, peat:sand). Inoculum density of *P. ultimum* was determined by soil dilution plating onto a semiselective agar (14), while the inoculum density of *R. solani* was determined by placing soil plugs of known weight on a selective medium (13). Phialoconidia of *T. basicola* were counted with a hemacytometer under the microscope, suspended in water, and added to UC potting mix for the desired final concentrations.

Inoculum densities were adjusted to 30 and 150 CFU of *P. ultimum*/g potting mix, 1, 5, and 9 CFU of *R. solani*/100 g potting mix, and 75 and 150 conidia of *T. basicola*/g potting mix. The seed of all cultivars had germination rates of 95 to 100% when incubated 7 days at 18°C, then 7 days at 25°C. Seeds of all the cultivars were planted in a metal flat (50 by 35 by 8 cm) as a replication to ensure all cultivars were under the same pathogen pressure. The cultivars were randomized in the flats, which in turn were randomized on a greenhouse bench. Ten seeds from each cultivar were planted 2 cm deep in a row in each flat. Flats were watered daily. Each trial consisted of four replications. Temperature in the greenhouse ranged from 10 to 20°C. Pre-emergence damping-off and final stands were determined 3 to 4 weeks after planting in *Pythium*- and *Rhizoctonia*-infested soil. The susceptibility of 3 cultivars (Maxxa, Royale, and SJ - 2) to 3 isolates each of *P. ultimum* or *R. solani* was also evaluated in the greenhouse. All experiments were repeated once.

In evaluations of cotton susceptibility to black root rot caused by *T. basicola*, the incidence of black root rot (percentage of seedlings with black root rot symptoms) was recorded, and disease severity was measured according to the following scale: 0 = no discoloration; 1 = slight discoloration of hypocotyl or one small lesion; 2 = general discoloration of hypocotyl; 3 = black discoloration of hypocotyl without loss of cortical tissue; and 4 = constricted black hypocotyl with few or no healthy lateral roots and loss of cortical tissue.

Greenhouse evaluation of seed treatment benefits. The benefit of fungicide seed treatments against *P. ultimum* or *R. solani* AG-4 on the above 12 Upland cotton cultivars was evaluated. Seeds were

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Accepted for publication 16 June 1997.

Table 1. Susceptibility of selected cotton cultivars to seedling disease caused by *Pythium ultimum* or *Rhizoctonia solani*

Cultivar	Pre-emergence damping-off (%) ^y		Final stand (10 seeds) ^y	
	<i>P. ultimum</i>	<i>R. solani</i>	<i>P. ultimum</i>	<i>R. solani</i>
GC 717	100.0 a ^z	35.0 bc	0.0 c	5.3 ab
GC 510	95.0 a	41.7 ab	0.0 c	4.7 ab
GC 702	92.5 ab	38.3 abc	0.4 c	4.8 ab
GC 610	91.3 ab	29.2 bc	0.0 c	5.9 a
CB 305	82.5 ab	40.0 ab	1.3 c	4.3 abcd
Royale	75.0 b	25.0 c	1.0 c	5.4 ab
CB 7	47.5 c	24.2 c	3.9 b	4.5 ab
SJ - 2	41.3 c	43.3 ab	4.0 b	2.7 d
Maxxa	18.8 d	50.0 a	7.6 a	2.8 cd
DP 6100	13.8 d	24.2 c	8.3 a	5.7 a
Prema	7.5 d	37.5 abc	9.0 a	3.8 bcd
DP 6166	5.0 d	32.5 bc	9.3 a	4.8 ab
Inoculum level				
Low	49.6 b ^z	21.7 b	4.2 a	5.7 a
Medium	62.1 a	26.5 b	3.2 b	5.5 a
High		57.1 a		2.4 b

^y Values are means of four replications in each of 2 inoculum levels of *P. ultimum* (30 and 150 CFU/g potting mix), or in each of 3 inoculum levels of *R. solani* (1, 5, and 9 CFU/100 g potting mix).

^z Means in a column followed by a common letter are not significantly different according to least significant difference at $P \leq 0.05$.

treated in a Hege Seed Treater (Hege Equipment Inc., Colwich, KS) with metalaxyl (Apron FL) at 0.31 g a.i./kg seed for the selective control of *P. ultimum* or with carboxin-pentachloronitrobenzene (PCNB; Vitavax-PCNB) at 0.92 g a.i. each/kg of seed for the selective control of *R. solani*. Twenty treated and nontreated seeds were planted with three replications in flats filled with *Pythium*- or *Rhizoctonia*-infested potting mix at inoculum densities of 150 CFU/g or 9 CFU/100 g, respectively. Pre-emergence damping-off and final stand data were collected 3 to 4 weeks after planting. The experiment was repeated once.

Field evaluation of seed treatment benefits. Three field experiments were conducted in 1995 and 1996 in the San Joaquin Valley, the primary cotton growing region in California. In 1995, field experiments were located at the West Side Field Station and the Kearney Agricultural Center. The same experiment was repeated at the Shafter Cotton Research Station in 1996. Selected cotton cultivars (GC 510, GC 710, Maxxa, Prema, Royale, and SJ-2) were planted at all field sites. The experiment included 4 seed treatments: (i) metalaxyl, 0.31 g a.i./ kg seed; (ii) carboxin-PCNB, 0.92 g a.i. each/kg seed; (iii) metalaxyl + carboxin-PCNB, 0.31 g a.i./ kg + 0.92 g a.i. each/kg seed; and (iv) nontreated seeds. Treatments were arranged in a randomized complete block design and replicated four times. Each plot consisted of one 7.6-meter-long row planted with 120 seeds. Seedling stands in each plot were counted approximately 4 weeks after planting.

To measure the population densities of *Pythium* spp., *R. solani*, and *T. basicola* in experimental field sites, randomized soil samples (each consisting of 8 pooled subsamples in 1995 and 3 pooled subsamples

in 1996) were collected at planting across each replication with a 2.54-cm-diameter soil sampler (0 to 20 cm deep). Soil population densities of *Pythium* spp. were determined by the soil dilution method described above, and species were identified according to van der Plaats-Niterink (16). Population densities of *R. solani* in the soil were obtained by a soil sieving method (17). Isolates of *R. solani* were further assigned to anastomosis groups using the cellophane method by pairing the isolates with "tester" strains (from E. E. Butler, UC Davis) and observing hyphal fusion (3,4). A selective medium, *T. basicola*-carotetridiazol-nystatin-pentachloronitrobenzene (TB-CENP) medium, was used to isolate and quantify *T. basicola* from the soil (12).

Data analysis. Data from the greenhouse tests and the field trials were analyzed by analysis of variance, least significant difference (LSD) mean separation, and homogeneity tests for combining trials with the Statistical Analysis System 6.04 (SAS Institute, Cary, NC).

RESULTS

Cultivar susceptibility. Since the relative performance of the cultivars was the same in each trial, the results of one experiment are presented (Table 1). There were no interactions between cultivar and trial, or between inoculum level and trial. Among the 12 Upland cultivars, DP 6166, Prema, DP 6100, and Maxxa were relatively resistant to *Pythium*-induced damping-off at the two inoculum levels, 30 and 150 CFU/g potting mix (the results of the two levels were combined since there were no interactions between cultivar performance and inoculation levels). Pre-emergence damping-off in these relatively resistant cultivars was less than 20%. Final stand establishment was also greatest among these 4 cultivars. Cultivars GC 717,

702, 610, and 510, CB 305, and Royale were among the most susceptible cultivars to *Pythium* infection (the incidence of pre-emergence damping-off ranged from 71.3 to 100%). Two cultivars, CB 7 and SJ-2, were considered to have intermediate levels of resistance to *P. ultimum*. Among all cultivars, the higher inoculum density of *P. ultimum* (150 CFU/g) caused 12.5% more pre-emergence damping-off than the lower inoculum density (30 CFU/g).

Maxxa was the most susceptible cultivar to *R. solani*; at least 50% of the seeds failed to emerge when the results from the 3 inoculum densities were combined (there were no interactions between cultivar performance and inoculum levels; Table 1). The cultivars DP 6100, CB 7, and Royale were among the most resistant cultivars to *R. solani*, although the differences in performance among the cultivars were generally small. Pre-emergence damping-off was positively correlated ($r = 0.772$, $P < 0.001$) and the final stand was negatively correlated ($r = -0.652$, $P < 0.001$) with increasing inoculum levels.

Virulence among the 3 isolates of *P. ultimum* or *R. solani* varied, but the 3 cotton cultivars Maxxa, SJ-2, and Royale reacted similarly to all isolates. The incidence of *Pythium*-induced damping-off in Maxxa was 25% when the results of 3 isolates were combined, compared with 55.9% in SJ-2 and 82.5% in Royale. In *Rhizoctonia*-infested soil, the incidence of damping-off in Maxxa was 57.1% when the results of 3 isolates were combined, compared with 52.9% in SJ - 2 and 38.8% in Royale.

No significant differences in black root rot incidence or disease severity caused by *T. basicola* were found among the 12 cultivars. All cultivars were considered highly susceptible. Symptoms of black root rot were observed on 61 to 84% of the seedlings of all cultivars in the combined results from the 2 inoculum densities, while the disease severity index was 0.77 to 1.09 (data not presented). There were no significant interactions between cultivars and the 2 inoculum densities.

Benefits of fungicide seed treatment.

The results of the two trials were combined since there were no significant interactions among treatment, trial, and cultivars. In *Pythium*-infested soil, the relatively susceptible cultivars benefited the most from the metalaxyl seed treatment (Table 2). For example, in GC 717, a relatively susceptible cultivar, the seed treatment decreased pre-emergence damping-off 87.5% and increased the final stand 86.0% relative to the controls. Among resistant cultivars, the benefits of the seed treatment were small, and the effect of the fungicide treatment on stand establishment was limited. In 4 cultivars (Prema, DP 6100, Maxxa, and SJ-2), there were no significant benefits of the metalaxyl seed treatment. In *Rhizoctonia*-infested soil, the carboxin-PCNB seed treatment significantly ($P \leq 0.05$) reduced

pre-emergence damping-off in all cultivars (Table 2). Stands of all cultivars were increased from 33.5 to 77.5% relative to the controls by the carboxin-PCNB treatment.

Field evaluation of fungicide seed treatments. The benefits of seed treatments in the field varied among seed treatments and cultivars (Table 3). The results from the 3 field sites were combined since there was no significant interaction between seed treatment and field site. The metalaxyl treatment did not significantly increase stands in 2 of the *Pythium*-resistant cultivars, Maxxa and Prema. However, final stands of Royale, a cultivar highly susceptible to *Pythium*, were significantly improved by the metalaxyl seed treatment. Seed treatment with carboxin-PCNB alone increased stands of Maxxa, Royale, and GC 510. The combination of metalaxyl with carboxin-PCNB did not generally improve the performance of carboxin-PCNB alone, although there was a beneficial effect in cultivar GC 702.

Populations of *Pythium* spp. were generally high (300 to 390 CFU/g dry soil) in all 3 field sites, and populations of *R. solani* were relatively low (1.5 to 2.8 CFU/100 g dry soil). *T. basicola* was not detected in any field site. *P. ultimum* represented 60 to 70% of *Pythium* isolates. *P. irregulare* and *P. oligandrum* were isolated occasionally.

DISCUSSION

The commercial cotton cultivars included in this study varied in response to the seedling disease pathogens. Different responses to seedling pathogens among cotton cultivars also were reported by Bush et al. (2), Garber et al. (8), and Hefner (11). Several cultivars in this study were relatively resistant to *P. ultimum*, but none were appreciably resistant to *R. solani* or *T. basicola*. Maxxa, the cotton cultivar currently grown on more hectares than any other in California, was resistant to *P. ultimum*, but was among the most susceptible cultivar to *R. solani*. Prema and 2 DP cultivars, 6100 and 6166, were also highly resistant to *P. ultimum* infection. Four GC cultivars (510, 610, 702, and 717), CB 305, and Royale were very susceptible to *P. ultimum*, while SJ-2 and CB 7 were considered to have an intermediate level of resistance to *P. ultimum*. Cotton cultivars responded similarly to various inoculum levels of *P. ultimum* or *R. solani*, and to the 3 isolates of *P. ultimum* or *R. solani*.

Black root rot is a disease of increasing importance in cotton disease management in California (12), even though *T. basicola* was not recovered from the 3 field sites in this study. All the cultivars included here responded similarly to *T. basicola* and were considered susceptible.

Seedling diseases are largely controlled by fungicide seed treatments. Each year, numerous fungicides are tested nationwide for efficacy in controlling seedling diseases. The performance of seed treatments are often dependent on the populations of soilborne pathogens (10). Likewise, the susceptibility of various cultivars also affects the benefits of seed treatments. Cultivars highly resistant to *Pythium* infection (Prema, DP 6100, DP 6166, and Maxxa) benefited little from the metalaxyl seed treatment in both greenhouse and field tests. Two cultivars (GC 510 and 702), highly susceptible to *Pythium* infection in greenhouse tests, also failed to benefit from metalaxyl in the field, possibly due to relatively lower inoculum pressure and increased microbial interactions in field soil than potting mix. In contrast, all cultivars benefited from the carboxin-PCNB seed treatment, consistent with their relatively high level of susceptibility to *R. solani*. Consequently, eliminating fungicides with specific activity against *Pythium* spp. may be feasible when using resistant cultivars, but there is no evidence that the use of fungicides with specific activity against *R. solani* or *T. basicola* could be altered in fields where these diseases occur.

Table 2. Benefits of fungicide seed treatments for the specific control of *Pythium*- or *Rhizoctonia*-induced damping-off of Upland cotton*

Cultivar	Decrease in pre-emergence damping-off (%) ^y		Increase in stands (%) ^y	
	<i>P. ultimum</i>	<i>R. solani</i>	<i>P. ultimum</i>	<i>R. solani</i>
GC 717	87.5 ^z	46.6	86.0	33.5
GC 510	26.7	39.2	30.0	34.0
GC 702	80.8	40.0	86.5	40.0
GC 610	84.2	46.6	76.5	45.5
CB 305	35.8	47.5	39.5	39.0
Royale	41.6	52.5	51.0	62.5
CB 7	43.3	75.0	50.0	73.0
SJ - 2	5.0	55.0	14.0	59.0
Maxxa	5.8	72.5	12.5	73.5
DP 6100	5.0	80.0	25.0	77.5
Prema	5.9	60.0	6.5	68.5
DP 6166	9.2	66.7	22.5	59.0
Least significant difference, <i>P</i> = 0.05	8.2	10.1	9.0	9.6

* Fungicide seed treatments were metalaxyl at 0.31 g a.i./kg seed for *Pythium* control and carboxin-pentachloronitrobenzene at 0.92 g a.i. each/kg seed for *Rhizoctonia* control. The inoculum densities of *P. ultimum* and *R. solani* were 150 CFU/g and 9 CFU/100 g potting mix, respectively.

^y Relative to pre-emergence damping-off or stands in nontreated control.

^z Values are means of two identical trials with three replications each; 20 seeds were planted in each treatment.

Table 3. Effects of fungicide seed treatment on cotton stand establishment in field trials, 1995-1996

	Final stand (120 seeds/plot) ^z					
Treatment	GC 510	GC 702	Royale	SJ-2	Maxxa	Prema
Nontreated	77.7	79.8	65.9	79.7	74.8	92.8
Metalaxyl	79.3	82.3	80.3	88.6	79.3	89.4
Carboxin-pentachloronitrobenzene	84.7	86.0	88.0	88.7	85.3	97.3
Combination	90.1	96.7	94.0	89.8	90.3	98.9
Least significant difference, <i>P</i> = 0.05	6.6	9.7	6.2	NS	9.4	5.9

^z Values are means of three field trials with four replications each. NS = not significant.

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