REPORT OF THE COTTONSEED TREATMENT COMMITTEE FOR 2007

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Introduction

The 2007 National Cottonseed Treatment Program evaluated cotton seedling survival for a number of fungicide seed treatment combinations over diverse environmental conditions and levels and types of cotton seedling pathogens. Fourteen fungicide seed treatments were nominated by chemical industry representatives for evaluation in the 2007 National Cottonseed Treatment Program. A standard fungicide treatment, Vitavax-PCNB + Allegiance, and a nontreated control were included to assess efficacy of the nominations and seedling disease pressure. In addition, the fungicide

treatments Allegiance, PCNB, or Argent were included to aid in determining the importance of *Pythium* spp., *Rhizoctonia solani*, or *Fusarium* spp. and other pathogenic fungi, respectively. Disease ratings and pathogen isolations for seedlings and soil populations of selected soilborne genera were conducted by collecting seedlings and soil from the nontreated control plots at each location. Soil temperature and water and plant development data also were collected for sites for the 2007 National Cottonseed Treatment Program.

Materials and Methods

Fungicide treatment

Acid-delinted seed of *Gossypium hirsutum* L. cv 'DP 444 BG/RR' were provided by Delta and Pine Land Company, Scott, MS. DP444 BG/RR was planted at all locations. Fungicide treatments were mixed with $CaCO_3$ (7 oz/cwt), polymer (Secure 1 oz/cwt,, Syngenta Inc.), Cruiser 9 oz/cwt (Syngenta Inc.), and dye (Color Coat Blue 1 oz/cwt, Syngenta Inc) in water at a rate of 2.75% (RTU-PCNB 2.86%) liquid to seed weight (w/w). Water, $CaCO_3$, polymer, Cruiser, and dye also were applied to the nontreated seed treatment at the same rate. Treatments were applied to the cottonseed while the seed mixed in a Hege 11 Liquid Seed Treater. When two or more fungicides were applied, the fungicides were mixed and applied in a single application. The technical information for the fungicides is given in Table 1. Seed germination was evaluated for all treated and nontreated seed by rolling seed in moistened germination paper and incubating for 3 days at 30°C.

Field experiments

Fifteen field experiments were conducted by 14 cooperators across the U.S. Cotton Belt (Table 2). Each location utilized a randomized complete block experimental design, with the number of replications ranging from 4 to 6. The stand counts used in the analyses were taken from 27 to 36 days after planting, average 30 days, depending on the location. A soil sample and seedling sample from plots containing nontreated seed were taken from 27 to 49 days after planting, average 32 days, depending on the location. Soil and seedlings were placed in insulated packages with refrigerated cool packs and mailed overnight to the University of Arkansas for processing. A subsample of soil was sent to Dr. T. L. Kirkpatrick, Southeast Research and Extension Center, Hope, Arkansas, for determination of populations of plant parasitic nematodes. Soil temperature and moisture was monitored by burying a temperature sensor and a Watermark soil moisture sensor connected to a data logger (Spectrum Technologies, Inc., Plainfield, IL) 4" deep at planting.

Seedlings were evaluated for growth by recording the number of nodes from five arbitrarily selected seedlings and then the aboveground portions of all seedlings were removed and discarded. An average of 50 seedlings per location were then rinsed for 20 minutes in running tap water and rated for disease symptoms. The hypocotyl disease severity index was 1=no symptoms, 2=few pinpoint lesions or diffuse discolored areas, 3=distinct necrotic lesion, 4=girdling lesion, and 5=seedling dead. The root disease index was 1=no symptoms, 2=1-10% of the root system discolored, 3=11-25% of the root system discolored, 4=26-50% of the root system discolored, and 5>50% of the root system discolored. Seedlings were surface disinfested by immersion for 1.5 min in 0.5% NaClO, blotted dry in a paper towel, and plated on water agar (1.3%) amended with 10 mg and 250 mg of the antibiotics rifampicin and ampicillin, respectively, and 0.5 μl of the miticide Danitol (Valent Chemical Co.) per liter. Resulting colonies were transferred to PDA and identified to genus. Seedlings were subsequently transferred to the *Thielaviopsis* selective medium TB-CEN (Specht and Griffin, 1985), which was modified by adding Penicillin G (60 mg/L), to determine isolation frequency for *Thielaviopsis basicola*. An additional set of seedlings was plated on the selective medium P_5ARP (Jeffers and Martin, 1986) following a 20 minute water rinse as another method to examine the isolation frequency for *Pythium* species.

Soil samples were assayed for populations of *Rhizoctonia* species by using the multiple-pellet soil method (Henis et. al., 1978), and *Rhizoctonia* populations were quantified on a modified Ko and Hora medium (Ko and Hora, 1971). Soil populations of *Pythium* spp. and *T. basicola* were detected by diluting 25 g of soil in 0.2% water agar to a total volume of 250 ml and placing on a wrist action shaker for 20 minutes. *Pythium* spp. were quantified by the spread-plate method on the selective medium P_5ARP, and *T. basicola* populations were quantified using the pour-plate method with the selective medium TB-CEN.

Statistics

Data were analyzed by the GLM procedure using SAS (SAS Institute Inc., Cary NC). Percent stand was analyzed over locations and by location. Treatment means were separated by using a protected LSD at P=0.05. The Pearson-product

correlation method was used to examine the relationship among percent stand, disease, pathogen isolation frequency, and soil populations over locations.

Results and Discussion

After the seed were treated with the fungicide treatments, seed germination ranged from 91.5 to 95.5% for the cultivar DP 444 BG/RR, with an average germination of 93.6%. No differences were found among treatments for seed germination.

For the 2007 National Cottonseed Treatment Program, 15 sites reported data. For these 15 locations, there were significant location, treatment, and location x treatment effects (Table 3), indicating that the treatment response was dependent on the environmental or pathogen pressures for a particular location. A significant difference among treatments was found for 9 of the 15 sites (Table 4). The Allegiance treatment increased stands compared to the nontreated control at 3 of these 9 sites having a significant response (LA1, OK1, VA), indicating the importance of *Pythium* spp. in stand establishment at these sites. At 1 of these 9 sites (AR4), the PCNB treatment increased stands over the nontreated control, indicating the importance of *Rhizoctonia solani* in stand establishment at this site. The Argent treatment increased stands compared to the nontreated control in 4 of these 9 sites having a significant response (AR4, LA1, OK1, VA), indicating the importance of *Fusarium* spp. and other fungi in stand establishment at these sites. The Vitavax-PCNB + Allegiance standard fungicide treatment increased stands compared to the nontreated control at 3 of the 9 sites (AR2, AR4, LA1). The nominated treatments increased stands over the nontreated control from 33% of the sites (3 of 9 sites) to 100% of the sites (9 of 9 sites) depending on the treatment. The treatments giving an increase in stand compared to the nontreated control at all 9 sites where a stand response was found were Baytan 30 + Allegiance FL + Vortex FL (0.5 + 0.75 + 0.08) and Vortex + Allegiance FL+ Trilex FL. Four treatments gave increased stands compared to the nontreated control in 8 of the 9 sites where a stand response was found; Baytan 30 + Allegiance FL + Vortex FL+ Trilex FL, Dynasty Extreme–D, Baytan 30 + Allegiance FL + Vortex FL (0.25+0.75+0.342) and Dynasty CST. At 5 of the 9 sites where a response was found (AR4, MS1, OK1, OK2, VA), some of the nominated fungicide treatments performed significantly better than the historical standard fungicide treatment, Vitavax-PCNB + Allegiance. Both Baytan 30 + Allegiance FL + Vortex FL treatments and treatment ATM performed better than the historical standard at 3 of the 9 sites. The number of fungicide treatments significantly increasing stands over the nontreated control ranged from 3 of 14 nominated treatments for TX7 to 14 of the 14 nominated treatments for the LA1 site. The mean stand for a location was not related to locations where stands were increased by fungicide treatments.

Hypocotyl disease indices ranged from 2.0 at the AL site to 2.9 at the LA1 site, average 2.3 (Table 5). Root disease indices ranged from 1.9 for the MS1 site to 3.4 for the LA1 and TX8 sites, average 2.6. *Rhizoctonia solani* was isolated from seedlings from the nontreated plots at 14 of 15 locations (Table 5). *R. solani* was isolated from 20% or greater of the seedlings at 5 locations (AL, AR1, AR2, AR4, LA2, OK2). *Pythium* spp. were isolated from seedlings at all locations (Table 5). Isolation frequencies for *Pythium* spp. were 20% or greater for 4 sites (GA, LA1, OK1, OK3). Isolation frequencies were increased dramatically by plating roots without surface disinfestation on the selective medium P_5ARP, with all sites having 28% or greater recovery of *Pythium* spp. (Table 5). *Thielaviopsis basicola* was isolated from seedlings at 9 of the 15 locations on the modified TB-CEN medium (Table 5). *T. basicola* was isolated from 30% or greater of the seedlings for the AL, AR4, OK2, and TX8 sites. *Fusarium* spp. were isolated from seedlings at all locations (Table 5). Isolation frequencies for *Fusarium* spp. ranged from 72% to 95%.

Soil populations of *R. solani* were detected for only 4 of the 15 soils assayed, range 0.8 to 1.6 CFU/100 g of soil (Table 6). *Pythium* spp. were detected in soils at all sites assayed, range18 to 596 CFU/g of soil. *T. basicola* was detected in 10 of the 14 soils assayed, ranging from 1 to 215 CFU/g soil.

The percent stand for the nontreated seed treatment for the sites was negatively correlated with *Pythium* soil populations, –0.64 (P=0.03). The number of nodes on seedlings were negatively correlated with the hypocotyl disease index, -0.57 (P=0.03). The root disease index was positively correlated with *Pythium* isolation, 0.54 (P=0.04). *Rhizoctonia solani* isolation was positively correlated with *Rhizoctonia solani* populations, 0.68 (P=0.007), and *Thielaviopsis basicola* isolation was positively correlated with *Thielaviopsis basicola* populations, 0.60 (P=0.02).

Conclusions

The results from the 15 locations where data was collected for the 2007 National Cottonseed Treatment Program indicated that seed treatment fungicides improved stands of cotton compared to a nontreated control for 60% of the sites. Most of the nominated fungicide combinations improved stands over the nontreated seed at a majority of the sites where a response was found. Some treatment combinations improved stand over the nontreated seed at all of the locations where a response was found. In addition, many of the treatment combinations improved stands over the historical standard fungicide seed treatment at one or more sites. The percent stand for the nontreated seed treatment for the locations was negatively correlated with *Pythium* soil populations, –0.64 (P=0.03). The root disease index was positively correlated with *Pythium* isolation, 0.54 (P=0.04). The isolation frequencies for *Rhizoctonia solani* and *Thielaviopsis basicola* from seedlings were positively correlated with soil populations of these pathogens.

Disclaimer

This paper reports the results of research only. Mention of a pesticide in this paper does not constitute a recommendation by the University of Arkansas Division of Agriculture nor does it imply registration under FIFRA.

References

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Ko, W., and F. K. Hora. 1971. A selective medium for the quantitative determination of *Rhizoctonia solani* in soil. Phytopathology 61:707-710.

Specht, L. P., and G. J. Griffin. 1985. A selective medium for enumerating low populations of *Thielaviopsis basicola.* Can. J. Plant Pathol. 7:438-441.

Table 1. Fungicides, formulations and active ingredients included in the 2007 National Cottonseed Treatment Program

Common or registered name[1]	Formulation	Active ingredient (%)
ALLEGIANCE (Metalaxyl)	Flowable	28.35% *N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl) alanine methyl ester
ARGENT 30 (TCMTB)	Liquid	30.0% 2-(Thiocyanomethylthio)benzothiazole
ATM		Wilbur-Ellis Company
BAYTAN 30 (Triadimenol)	Flowable	30% Beta-(4-Chlorophenoxy)-alpha-(1,1-dimethylethyl)-1*H*-1,2,4-triazole-1-ethanol
DYNASTY CST (Azoxystrobin)	Flowable	6.64% Methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate
(Fludioxonil)		1.11% 4-(2,2-difluoro- 1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile
(Mefenoxam)		3.32% (R,S)-2-{(2,6-dimethylphenyl)-methoxyacetylamino}-propionic acid methyl ester
DYNASTY EXTREME		Syngenta Crop Protection
DYNASTY EXTREME D		Syngenta Crop Protection
RTU BAYTAN-Thiram	Flowable	5% Beta-(4-Chlorophenoxy)-alpha-(1,1-dimethylethyl)-1*H*-1,2,4-triazole-1-ethanol, 15.3% Tetramethylthiuram disulfide
RTU PCNB	Flowable	24% Pentachloronitrobenzene
TRILEX (Trifloxystrobin)	Flowable	22% Methyl (E)-methoxyimino-{(E)-alpha-[1-(alpha, alpha, alpha-trifluoro-m-tolyl) ethylideneaminooxyl]-o-tolyl}acetate
V10116		Valent USA Corporation
V10178		Valent USA Corporation
V10209		Valent USA Corporation
VITAVAX (Carboxin) – PCNB	Flowable	17% 5,6-dihydro-2-methyl-N-phenyl-1,4-oxathiin-3-carboxamide, 17% Pentachloronitrobenzene
VORTEX (Ipconazole)	Flowable	40.7% 2-[(4-chlorophenyl)methyl]-5-(1-methylethyl)-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol
WECO 0319		Wilbur-Ellis Company
WECO 3007		Wilbur-Ellis Company

[1] Registered chemical name, all capital letters.

Table 2. List of cooperators and procedures used in the 2007 National Cottonseed Treatment Program.

Cooperator	Location		Date Planted	Date Sampled	Date Counted	Reps.	Row length counted (ft)	Seed planted	Soil temperature[1]
K. Lawrence	Auburn, AL	(AL)	4/12	5/31	5/18	5	10	40	12(7)
J. Barham	Hope, AR	(AR1)	4/24	5/22	5/22	5	40	200	19(15)
A. Beach	Keiser, AR	(AR2)	4/19	5/21	5/21	6	25	100	21(19)
C. Rothrock	Judd Hill, AR	(AR4)	5/1	5/28	5/28	6	50	261	22(19)
R. Kemerait	Tifton, GA	(GA)	4/6	5/4	5/4	4	25	250	17(6)
P. Colyer	Bossier City, LA	(LA1)	4/11	5/9	5/8	4	25	100	17(13)
B. Padgett	Winnsboro, LA	(LA2)	4/19	5/21	5/21	5	25	100	20(16)
J. Caceres	Mississippi State, MS	(MS1)	4/9	5/9	5/9	5	80	240	15(9)
G. Sciumbato	Stoneville, MS	(MS2)	4/23	5/22	5/22	4	45	180	.
R. Thacker	Tipton, OK	(OK1)	5/2	6/4	6/4	4	20	100	24(18)
R. Thacker	Altus, OK	(OK2)	5/2	6/4	6/4	4	20	100	24(20)
L. Verhalen & M. Bayles	Perkins, OK	(OK3)	5/15	6/19	6/19	4	20	100	23(15)
J. Woodward	Lubbock, TX	(TX7)	4/27	5/31	5/254	34.5	138	20(14)	
G. Schuster	Lubbock, TX	(TX8)	5/18	6/15	6/154	50	150	19(14)[2]	
P. Phipps	Suffolk, VA	(VA)	4/18	5/17	5/17	4	60	180	14(10)

[1] Mean (Minimum) soil temperature; 3-day average following planting.
[2] 2" soil temperatures.

Table 3. Mean squares for combined analysis of variance across locations, 2007 National Cottonseed Treatment Program.

Source	Degrees of freedom	Mean squares
Location	14	20491*
Replication(Location)	53	73
Treatment	18	937*
Location*treatment	252	127*
Error	954	78

*=significant *F*-test, $P<0.0001$

Table 4. Cotton seedling stands for locations of the 2007 National Cottonseed Treatment Program.

		Plant stand (%)															
Treatment	Rate (oz/cwt)	AL	AR1	AR2	AR4	GA	LA1	LA2	MS1	MS2	OK1	OK2	OK3	TX7	TX8	VA	Mean
Baytan 30 + Allegiance FL + Vortex FL+ Trilex FL	0.5+0.75+0.08+0.64	66	34	81	83	85	85	56	80	79	90	87	71	77	86	58[2]	74
Dynasty Extreme -D	3.0+2	72	36	74	81	84	85	63	78	74	89	87	74	71	89	59	74
Baytan 30+ Allegiance FL + Vortex FL	0.25+0.75+0.342	70	35	76	82	83	84	50	74	78	90	90	78	80	84	59	73
Baytan 30 + Allegiance FL + Vortex FL	0.5+0.75+0.08	59	32	80	82	84	85	61	78	72	96	90	73	85	77	54	73
Vortex FL + Allegiance FL+ Trilex FL	0.342+0.75+0.64	64	35	70	93	84	82	50	72	71	96	89	62	87	80	58	72
Dynasty CST	3.95	54	36	70	83	88	88	57	73	75	89	87	73	83	77	64	72
Dynasty Extreme	3.0	64	35	77	83	80	87	57	66	67	91	90	78	81	72	51	71
RTU Baytan Thiram + Allegiance FL	3.0+0.75	61	34	74	82	82	80	40	74	73	84	87	69	82	83	60	70
ATM + WECO 0319	8.0+4.0	62	32	75	80	74	76	60	77	68	88	88	72	73	81	48	70
V10178 + V10116 + V10209	1.54+0.32+0.75	64	37	60	82	70	83	50	67	68	91	82	79	85	81	58[2]	70
V10178 + V10209	1.54+0.75	56	32	72	82	77	81	49	70	64	89	83	73	79	83	53	69
ATM	8.0	50	34	64	80	66	82	61	83	64	94	90	73	71	82	47	69
ATM + WECO 3007	8.0+0.5	56	34	79	75	75	84	42	77	70	87	81	70	70	85	38	68
ATM + WECO 3007 + WECO 0319	8.0+0.5+2.0	60	39	70	78	74	84	51	68	68	88[1]	88	58	72	78	39	67
Vitavax-PCNB + Allegiance FL	6.0+0.75	48	29	74	81	77	81	44	63	73	86	83	70	83	79	50	67
Allegiance FL	1.5	55	37	65	78	76	82	29	67	73	92	86	74	78	76	57	67
Argent	4.5	58	35	64	82	76	68	35	62	71	91	82	68	76	85	53	66
RTU-PCNB	14.5	50	32	58	80	68	63	31	54	74	80	84	75	77	79	50	63
Nontreated	---	49	34	55	75	69	58	27	60	57	81	80	65	75	73	44	59
Location average		59	34	70	81	78	80	48	71	70	89	86	71	78	81	53	69
Coefficient of Variation (%)		20.2	15.1	14.4	4.0	13.1	7.9	34.7	12.2	12.2	5.7	5.3	12.5	8.5	10.8	10.8	
LSD (P=0.05)		NS	NS	11.6	3.7	NS	9.0	21.0	10.9	NS	7.2	6.4	NS	9.4	NS	8.1	

[1] Treatment mean is significantly different from the nontreated control, even though the mean does not appear to be different as a result of rounding.

[2] Treatment mean is significantly different from the Vitavax-PCNB+ Allegiance FL treatment, even though the mean does not appear to be different as a result of rounding.

Table 5. Disease ratings and isolation frequencies of seedling pathogen groups for the 2007 National Cottonseed Treatment Program locations.

				Isolation frequency (%)[1]			
		Disease Index		*Rhizoctonia*	*Pythium*	*Thielaviopsis*	*Fusarium*
Location	Nodes[2]	Hyp.[3]	Root[4]	*solani*	spp.	*basicola*	spp.
AL	7.3	2.0	2.1	44	4 (46)[5]	94	86
AR1	4.0	2.1	2.3	24	18 (91)	2	86
AR2	4.3	2.3	2.6	36	18 (64)	2	86
AR4	4.3	2.2	2.7	42	4 (52)	98	72
GA	3.7	2.4	3.3	12	63 (NA)[6]	0	95
LA1	2.0	2.9	3.4	14	58 (100)	0	88
LA2	5.0	2.1	3.2	26	8 (56)	0	90
MS1	2.0	2.2	2.1	14	0 (68)	6	78
MS2	4.7	2.2	1.9	16	6 (76)	0	92
OK1	5.0	2.1	2.6	0	24 (100)	0	84
OK2	4.3	2.1	2.5	22	2 (42)	96	84
OK3	7.0	2.2	2.3	10	24 (46)	12	72
TX7	3.3	2.3	2.3	2	4 (NA)	6	94
TX8	NA[6]	2.4	3.4	4	6 (28)	30	86
VA	2.0	2.5	2.9	4	10 (82)	0	94

[1] Isolation frequency is based on approximately 50 seedlings per location.
[2] Nodes based on five seedlings per location.
[3] Hypocotyl index; 1=no symptoms, 2=few pinpoint lesions or diffuse discolored areas, 3=distinct necrotic lesion, 4=girdling lesion, and 5=seedling dead.
[4] Root index; 1=no symptoms, 2=1-10% of the root system discolored, 3=11-25% of the root system discolored, 4=26-50% of the root system discolored, and 5>50% of the root system discolored.
[5] Isolation frequency in parentheses from P_5ARP.
[6] Information not available

Table 6. Soil populations of selected soilborne genera from sites in the 2007 National Cottonseed Treatment Program.

Location	*Rhizoctonia solani*	*Pythium* spp.	*Thielaviopsis basicola*
	CFU[1]/100g	CFU/g	CFU/g
AL	NA[2]	NA	NA
AR1	ND[3]	596	1
AR2	1.1	74	ND
AR4	1.1	146	215
GA	ND	31	ND
LA1	0.8	281	22
LA2	1.6	234	ND
MS1	ND	82	20
MS2	ND	106	1
OK1	ND	77	ND
OK2	ND	18	8
OK3	ND	NA	120
TX7	ND	87	1
TX8	ND	NA	112
VA	ND	105	1

[1] Colony forming units.
[2] Information not available.
[3] Populations not detected in soil sample; less than approximately 0.3 CFU/100 g of soil for *R. solani,* and 0.5 CFU/g of soil for *T. basicola.*