REPORT OF THE COTTONSEED TREATMENT COMMITTEE FOR 2008

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Introduction

The 2008 National Cottonseed Treatment Program evaluated cotton seedling survival for a number of fungicide seed treatment combinations over diverse environmental conditions and populations of cotton seedling pathogens. Eight fungicide seed treatments were nominated by chemical industry representatives for evaluation in the 2008 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, and Argent were included to aid in determining the importance of *Pythium* spp., *Rhizoctonia solani*, and *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 2008 National Cottonseed Treatment Program.

Materials and Methods

Fungicide treatment

Acid-delinted seed of *Gossypium hirsutum* L. cv 'DP 161 B2RF' were provided by Delta and Pine Land Company, Scott, MS. DP 161 B2RF was planted at all locations. Fungicide treatments were mixed with $CaCO_3$ (7 oz/cwt), polymer (Secure 1 oz/cwt, Syngenta Inc.), Gaucho Grande (12.8 oz/cwt, Bayer CropScience), 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, Gaucho Grande, 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). However, stand data was only collected from 14 of these sites. 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 25 to 39 days after planting, average 30 days, depending on the location. A soil sample and seedling sample from plots containing nontreated seed were taken from 28 to 40 days after planting, average 33 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, Southwest 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 modified 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.

Table 1. Fungicides, formulations and active ingredients included in the 2008 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
BAYTAN 30 (Triadimenol)	Flowable	30% Beta-(4-Chlorophenoxy)-alpha-(1,1-dimethylethyl)-1*H*-1,2,4-triazole-1-ethanol
BION	Liquid	42% 1,2,3-Benzothiadiazole-7-carbothioic acid S-methyl ester
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	Flowable	
(Azoxystrobin)		8.3% Methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate
(Fludioxonil)		1.4% 4-(2,2-difluoro- 1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile
(Mefenoxam)		3.7% (R,S)-2-{(2,6-dimethylphenyl)-methoxyacetylamino}-propionic acid methyl ester
(Mycobutanil)		9.7% 1-H-1,2,4-Triazole-1-propanenitrile, alpha-butyl-alpha-(4-chlorophenyl)
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
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

[1] Registered chemical name, all capital letters.

Table 2. List of cooperators and procedures for sites used in the 2008 National Cottonseed Treatment Program

Cooperator	Location		Date Planted	Date Sampled	Date Counted	Reps.	counted (ft)	Seed planted	Soil temperature[1]
K. Lawrence	Auburn, AL	(AL)	4/15	5/13	5/13	5	25	40	16(11)
J. Barham	Hope, AR	(AR1)	4/21	5/21	5/21	5	46	184	22(19)
C. Rothrock	Judd Hill, AR	(AR4)	5/1	5/29	5/29	6	50	250	18(12)
R. Kemerait	Tifton, GA	(GA)	4/15	NA	5/12	4	50	150	NA
P. Colyer	Bossier City, LA	(LA1)	4/10	5/19	5/19	4	25	125	19(14)
B. Padgett	Winnsboro, LA	(LA2)	4/15	5/16	5/16	5	25	100	19(14)
J. Caceres	Mississippi State, MS	(MS1)	4/21	5/21	5/21	5	83	250	25(12)
G. Sciumbato	Stoneville, MS	(MS2)	4/21	5/20	5/19	4	45	180	24(22)
R. Thacker	Tipton, OK	(OK1)	5/1	6/2	6/2	4	20	100	22(14)
R. Thacker	Altus, OK	(OK2)	5/1	6/2	6/2	4	20	100	21(16)
L. Verhalen & M. Bayles	Perkins, OK	(OK3)	5/20	6/23	6/23	4	20	100	24(20)
J. Woodward	Halfway, TX	(TX2)	5/1	6/24	5/294	35.5	142	20(20)	
J. Woodward	Lubbock, TX	(TX7)	5/16	6/25	6/103	35.5	142	22(NA)	
P. Phipps	Suffolk, VA	(VA)	4/18	5/19	5/19	4	60	180	17(13)

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

Results and Discussion

Seed germination after seed treatment ranged from 83 to 88% for the cultivar DP 161 B2RF, with an average germination of 86%. No differences were found among treatments for seed germination. For the 2008 National Cottonseed Treatment Program, 14 sites reported data. For these 14 locations, there were significant location, treatment, and location x treatment effects (Table 3), indicating that the treatment response was dependent on the environment or pathogen pressures for a particular location. A significant difference among treatments was found for 6 of the 14 sites (Table 4). This frequency of response, 43%, is considerably lower than most years when stands from over 50% of the sites respond to fungicide use. The mean stand for a location was not related to locations where stands were increased by fungicide treatments. The generally low stands at many of the sites, including sites having no fungicide response, suggest factors other than seedling diseases were important in 2008 in stand establishment. The Allegiance treatment increased stands compared to the nontreated control at 1 of these 6 sites having a significant response (LA2), indicating the importance of *Pythium* spp. in stand establishment at this site. At 1 of these 6 sites (GA), the PCNB treatment increased stands over the nontreated control, indicating the importance of *Rhizoctonia solani* in stand establishment at this site. The Vitavax-PCNB + Allegiance standard fungicide treatment increased stands compared to the nontreated control at 2 of the 6 sites (GA, LA1). The nominated treatments increased stands over the nontreated control from 50% of the sites (3 of 6 sites) to 100% of the sites (6 of 6 sites) depending on the treatment. The treatment giving an increase in stand compared to the nontreated control at all 6 sites where a stand response was found was RTU Baytan Thiram + Allegiance FL + Dynasty CST + Bion. Two other treatments gave increased stands compared to the nontreated control in 5 of the 6 sites where a stand response was found; Baytan Thiram + Allegiance FL + Bion and Vortex + Allegiance FL + Trilex FL. At 5 of the 6 sites where a response was found (AR4, LA1, LA2, MS1, VA), some of the nominated fungicide treatments performed significantly better than the historical standard fungicide treatment, Vitavax-PCNB + Allegiance. RTU Baytan Thiram + Allegiance FL + Dynasty CST + Bion performed better than the historical standard at 3 of the 6 sites. The number of fungicide treatments significantly increasing stands over the nontreated control ranged from 4 of the 8 nominated treatments for VA to 8 of the 8 nominated treatments for the LA1 site.

Table 3. Mean squares for combined analysis of variance across locations, 2008 National Cottonseed Treatment Program.

Source	Degrees of freedom	Mean squares
Location	13	12192*
Replication(Location)	47	392*
Treatment	12	950*
Location*treatment	156	180*
Error	563	106

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

Table 4. Cotton seedling stands for locations of the 2008 National Cottonseed Treatment Program.

Treatment	Rate (oz/cwt)	Plant stand (%)														
		AL	AR1	AR4	GA	LA1	LA2	MS1	MS2	OK1	OK2	OK3	TX2	TX7	VA	Mean
RTU Baytan Thiram + Allegiance FL + Dynasty CST + Bion	3.0 + 0.75 + 3.95 + 0.6 gai/100kg	64	57	60	72	80	50	59	52	62	59	44	30	19	37	53
RTU Baytan Thiram + Allegiance FL + Dynasty Extreme	3.0 + 0.75 + 0.045 mgai/seed	76	58	56	67	83	35	59	54	63	74	40	33	19	31	53
RTU Baytan Thiram + Allegiance FL	3.0 + 0.75	59	65	58	60	84	37	62	54	61	65	52	23	20	40	53
RTU Baytan Thiram + Allegiance FL + Bion	3.0 + 0.75 + 0.6 gai/100kg	55	61	60	65	74	50	56	62	58	70	43	27	17	38	53
Baytan 30+ Allegiance FL + Vortex FL	0.5 + 0.75 + 0.17	69	59	57	58	74	43	63	54	62	69	45	27	13	33	52
Baytan 30 + Allegiance FL + Vortex FL	0.5 + 0.75 + 0.08	57	59	54	70	78	35	65	55	59	62	51	37	17	32	52
Baytan 30 + Allegiance FL + Vortex FL + Trilex FL	0.5 + 0.75 + 0.08 + 0.32	64	57	52	66	73	53	54	55	64	67	45	22	18	35	52
Vortex + Allegiance FL + Trilex FL	0.34 + 0.75 + 0.64	68	59	50	64	74	44	61	38	64	62	46	23	9	38	50
Vitavax-PCNB + Allegiance FL	6.0 + 0.75	70	60	48	64	64	27	53	51	58	69	51	30	13	29	49
RTU-PCNB	14.5	85	56	49	69	51	35	52	45	57	67	45	21	18	22	48
Allegiance FL	1.5	53	56	41	53	52	50	52	51	60	56	45	24	13	33	46
Argent	4.5	79	45	37	56	44	39	44	42	56	56	51	28	13	20	44
Nontreated	---	51	57	44	49	41	24	52	48	53	57	38	26	8	27	41
Location average		65	58	51	63	67	40	56	51	60	64	46	27	15	32	
Coefficient of Variation (%)		35	13	18	14	16	29	7	18	11	14	15	29	36	19	
LSD (P=0.05)		NS	NS*	10.9	12.8	14.9	15.0	5.2	NS*	NS	NS	NS	NS	NS	8.5	

[1] F-test significant at $P < 0.10$.

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Hypocotyl disease indices ranged from 2.0 at the MS2, OK1, and OK3 sites to 3.2 at the LA1 site, average 2.4 (Table 5). Root disease indices ranged from 2.0 for the MS2, OK2 and OK3 sites to 4.4 for the AL site, average 2.7. *Rhizoctonia solani* was isolated from seedlings from the nontreated plots at 11 of 13 locations (Table 5). *R. solani* was isolated from 20% or greater of the seedlings at 6 locations (AR4, LA1, MS1, OK1, OK3, TX2). *Pythium* spp. were isolated from seedlings at all locations (Table 5). Isolation frequencies for *Pythium* spp. on amended water agar was 20% or greater for only 1 site (LA1). Isolation frequencies were increased dramatically by plating roots without surface disinfestation on the selective medium P_5ARP, with most sites having greater than 20% recovery of *Pythium* spp. (Table 5). *Thielaviopsis basicola* was isolated from seedlings at 5 of the 13 locations on the modified TB-CEN medium (Table 5). *T. basicola* was isolated from 30% or greater of the seedlings for the AL, AR4, OK1, and TX2 sites. *Fusarium* spp. were isolated from seedlings at all locations (Table 5). Isolation frequencies for *Fusarium* spp. ranged from 49% to 96%.

Table 5. Disease ratings and isolation frequencies of seedling pathogen groups for the 2008 National Cottonseed Treatment Program locations.

		Disease Index		Isolation frequency (%)[1]			
				Rhizoctonia	*Pythium*	*Thielaviopsis*	*Fusarium*
Location	Nodes[2]	Hyp.[3]	Root[4]	*solani*	spp.	*basicola*	spp.
AL	2.0	2.4	4.4	9	9 (NA[6])[5]	91	80
AR1	1.0	2.4	3.6	2	18 (100)	0	76
AR4	NA	2.7	3.2	48	12 (NA)	94	60
GA	NA	NA	NA	NA	NA (NA)	NA	NA
LA1	1.3	3.2	3.2	50	22 (100)	0	62
LA2	4.0	2.2	3.1	14	8 (100)	0	86
MS1	2.3	3.1	3.2	22	2 (94)	0	49
MS2	8.0	2.0	2.0	5	5 (NA)	0	90
OK1	5.0	2.0	2.1	22	12 (45)	30	86
OK2	5.7	2.1	2.0	0	8 (76)	0	84
OK3	6.3	2.0	2.0	20	8 (26)	0	86
TX2	10.3	2.1	2.3	28	10 (9)	50	92
TX7	7.7	2.1	2.1	0	0 (8)	6	82
VA	2.0	2.4	2.3	2	2 (96)	0	96

[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

Soil populations of *R. solani* were detected for only 7 of the 13 soils assayed, range 1.2 to 14.0 CFU/100 g of soil (Table 6). *Pythium* spp. were detected in soil at all but one site for the soils assayed, range 18 to 515 CFU/g of soil. *T. basicola* was detected in 5 of the 13 soils assayed, ranging from 1 to 202 CFU/g soil. The root-knot nematode, *Meloidogyne incognita*, was detected in soil from two sites, OK1 and VA, and the reniform nematode, *Rotylenchulus reniformis*, was detected in soil samples from the two Mississippi sites.

The hypocotyl disease index was positively correlated with the root disease index, 0.57 (P=0.04) and the isolation frequency of *R. solani*, 0.58 (P=0.04). The root disease index was positively correlated with the isolation frequency of *Pythium* spp. on P_5ARP medium, 0.71 (P=0.02). The isolation frequency of *T. basicola* was positively correlated with the soil populations of *T. basicola,* 0.88 (P<0.0001) and negatively correlated with mean soil temperature, -0.56 (P=0.05)

Table 6. Soil populations of selected soilborne genera from sites in the 2008 National Cottonseed Treatment Program.

Location	*Rhizoctonia solani*	*Pythium* spp.	*Thielaviopsis basicola*
	CFU[1]/100g	CFU/g	CFU/g
AL	14.0	37	132
AR1	ND[2]	515	ND
AR4	11.7	125	202
GA	NA[3]	NA	NA
LA1	1.3	NA	ND
LA2	1.2	154	ND
MS1	2.5	78	ND
MS2	ND	NA	1
OK1	ND	18	ND
OK2	ND	ND	7
OK3	6.1	NA	ND
TX2	1.9	NA	19
TX7	ND	NA	ND
VA	ND	37	ND

[1] Colony forming units.
[2] Populations not detected in soil sample; less than approximately 0.3 CFU/100 g of soil for *R. solani,* 8 CFU/g of soil for *Pythium* spp., and 0.5 CFU/g of soil for *T. basicola.*
[3] Information not available.

Summary

The results from the 14 locations where stand data were collected for the 2008 National Cottonseed Treatment Program indicated that seed treatment fungicides improved stands of cotton compared to a nontreated control for 43% 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 hypocotyl disease index was positively correlated with the root disease index, 0.57 (P=0.04) and the isolation frequency of *R. solani*, 0.58 (P=0.04). The root disease index was positively correlated with the isolation frequency of *Pythium* spp. on P_5ARP medium, 0.71 (P=0.02).

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.

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