REPORT OF THE COTTONSEED TREATMENT COMMITTEE FOR 2009

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Abstract

The 2009 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 2009. The results from the 12 locations where stand data were collected for the 2009 National Cottonseed Treatment Program indicated that seed treatment fungicides improved stands of cotton compared to a nontreated control for 33% of the sites. Most of the nominated fungicide combinations improved stands over the nontreated seed at two or more sites 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, isolation frequency of *Pythium* spp., and population of *T. basicola*. The root disease index

was positively correlated with the isolation frequency of *Pythium* spp. The mean low temperature was negatively correlated with average stand for the sites and isolation frequency of *Pythium* spp. The National Cottonseed Treatment Program documents the importance of fungicide seed treatments and the advances in fungicide efficacy.

Introduction

The 2009 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 2009 National Cottonseed Treatment Program. Two standard fungicide treatments, Vitavax-PCNB + Allegiance, the historical standard, and RTU Baytan Thiram + Allegiance FL, 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 2009 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. Fungicide treatments were mixed with $CaCO_3$ (7 oz/cwt), polymer (Secure 1 oz/cwt), Cruiser (9 oz/cwt), and dye (Color Coat Red, 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

Sixteen field experiments were conducted by 14 cooperators across the U.S. Cotton Belt (Table 2). However, analyses from only 12 sites are presented as a result of problems at the other locations. Each location utilized a randomized complete block experimental design, with the number of replications ranging from 4 to 8. The stand counts used in the analyses were taken from 28 to 42 days after planting, average 33 days, depending on the location. A soil sample and seedling sample from plots containing nontreated seed were taken from 28 to 42 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. Seedlings were then rinsed for 20 minutes in running tap water. Approximately 50 seedlings were rated for disease symptoms, 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. 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. 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) to examine the isolation frequency for *Pythium* species.

Table 1. Fungicides, formulations and active ingredients included in the 2009 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
APRON XL (Mefenoxam)	Liquid	33.3% (R,S)-2-{(2,6-dimethylphenyl)-methoxyacetylamino}-propionic acid 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	9.5% 1,2,3-benzothiadiazole-7-thiocarboxylic acid S-methyl ester
DYNASTY EXTREME	Flowable	
(Azoxystrobin)		8.33% Methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate
(Fludioxonil)		1.39% 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)
NU-FLOW M-HF (Myclobutanil)	Liquid	25% A-butyl-a-(4-chlorophenyl)-1*H*-1,2,4-triazole-1-propanenitrile
NUSAN 30 EC (TCMTB)	Liquid	30.0% 2-(Thiocyanomethylthio)benzothiazole
RTU BAYTAN-Thiram	Flowable	15.3% Tetramethylthiuram disulfide
(Triadimenol)		5% Beta-(4-Chlorophenoxy)-alpha-(1,1-dimethylethyl)-1*H*-1,2,4-triazole-1-ethanol,
RTU PCNB	Flowable	24% Pentachloronitrobenzene
SP 1020		Bayer CropScience
VITAVAX (Carboxin) – PCNB	Flowable	17% 5,6-dihydro-2-methyl-N-phenyl-1,4-oxathiin-3-carboxamide
		17% Pentachloronitrobenzene
VORTEX FL (Ipconazole)	Flowable	40.7% 2-[(4-chlorophenyl)methyl]-5-(1-methylethyl)-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol
WECO 100 (Flutolanil)	Flowable	25.0% N-[3-(1-methylethoxy)phenyl]-2-(trifluoromethyl)benzamide
WECO 1090		Wilbur-Ellis Company

[1] Registered chemical name, all capital letters.

Table 2. List of cooperators and procedures for sites used in the 2009 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)	4/23	5/21	5/21	4	10	40	18(16)
J. Barham	Rohwer, AR	(AR1)	4/24	5/28	5/28	4	80	240	17(13)
A. Beach	Keiser,AR	(AR2)	4/27	6/1	6/1	6	24	100	20(19)
C. Rothrock	Judd Hill, AR	(AR4)	5/18	6/16	6/16	8	42	250	23(15)
R. Kemerait	Tifton, GA	(GA)	4/20	5/20	5/20	4	25	100	19(NA)[3]
B. Padgett	Winnsboro, LA	(LA2)	4/22	5/20	5/20	5	25	100	23(14)
J. Caceres	Mississippi State, MS	(MS1)	4/23	6/4	6/4	5	80	240	23(18)
T. Kelly	Tipton, OK	(OK1)	5/19	6/26	6/26	4	20	100	23(17)
R. Thacker	Altus, OK	(OK2)	5/19	6/26	6/26	4	20	100	27(18)
M. Bayles	Perkins, OK	(OK3)	5/21	6/25	6/25	4	20	100	26(20)
J. Woodward	Station, TX	(TX9)	4/27	5/26	5/26	4	35.5	142	23(20)[2]
P. Phipps	Suffolk, VA	(VA)	4/27	5/27	5/27	4	60	180	21(15)

[1] Mean (Minimum) 4" soil temperature; 3-day average following planting.
[2] Mean (Minimum) 6" soil temperature; 3-day average following planting.
[3] Data not available.

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 soil temperature, percent stand, disease, pathogen isolation frequency, and soil populations over locations.

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 2009 National Cottonseed Treatment Program, 12 sites reported data. For these 12 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 4 of the 12 sites (Table 4). This frequency of response, 33%, 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 2009 in stand establishment. The Allegiance treatment increased stands compared to the nontreated control at 2 of these 4 sites having a significant response (AR1, AR2), indicating the importance of *Pythium* spp. in stand establishment at these sites. At 0 of these 4 sites the PCNB treatment increased stands over the nontreated control, indicating *Rhizoctonia solani* was not a major factor in stand establishment in 2009. Argent alone improved stands at 1 of 4 sites (AR2) suggesting *Fusarium* spp. or other fungi played a role in stand establishment. The Vitavax-PCNB + Allegiance standard fungicide treatment increased stands compared to the nontreated control at 2 of the 4 sites (AR1, MS1). The other treatments increased stands over the nontreated control from 25% of the sites (1 of 4 sites) to 100% of the sites (4 of 4 sites) depending on the treatment. The treatment giving an increase in stand compared to the nontreated control at all 4 sites where a stand response was found was RTU Baytan Thiram + Allegiance FL + Dynasty Extreme. Several treatments increased stands compared to the nontreated control in 3 of the 4 sites where a stand response was found; Baytan Thiram + Allegiance FL + Dynasty Extreme + Bion, Baytan Thiram + Allegiance FL, and Baytan Thiram + Allegiance FL + Bion. At 3 of the 4 sites where a response was found (AL, AR1, AR2), some

Table 3. Mean squares for combined analysis of variance across locations, 2009 National Cottonseed Treatment Program.

Source	Degrees of freedom	Mean squares
Location	11	23202*
Replication(Location)	44	270*
Treatment	13	566*
Location*treatment	143	180*
Error	564	105

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

Table 4. Cotton seedling stands for locations of the 2009 National Cottonseed Treatment Program.

		Plant stand (%)												
Treatment	Rate (oz/cwt)	AL	AR1	AR2	AR4	GA	LA2	MS1	OK1	OK2	OK3	TX9	VA	Mean
RTU Baytan Thiram + Allegiance FL + Dynasty Extreme + Bion	3.0 + 0.75 +3.0 + 0.03	69	98	42	70	97	53	60	74	70	39	46	59	64
WECO 100 + Nu-Flow M HF + Apron XL + Nusan 30 EC	4.0 + 1.75 + 0.32 + 2.0	50	94	45	71	92	53	62	80	68	43	38	61	63
RTU Baytan Thiram + Allegiance FL + Dynasty Extreme	3.0 + 0.75 +3.0	69	94	39	68	92	51	64	70	66	43	39	62	62
Baytan 30 + Allegiance FL + Vortex FL + SP1020	0.75 + 1.5 + 0.08 + 0.32	44	100	35	69	98	43	61	76	69	50	52	56	62
RTU Baytan Thiram + Allegiance FL + Bion	3.0 + 0.75 + 0.03	58	90	33	67	99	49	64	71	62	36	38	56	59
WECO 100 + Nu-Flow M HF + Apron XL + Nusan 30 EC	4.0 + 4.0 + 0.32 + 2.0	36	94	36	67	91	47	60	76	53	38	58	60	59
Baytan 30 + Allegiance FL + Vortex FL	0.5 + 0.75 + 0.08	49	85	45	70	100	42	58	72	68	30	32	52	59
WECO 100 + Nu-Flow M HF + Apron XL +Nusan 30 EC + WECO 1090	4.0 + 4.0 + 0.32 + 2.0 + 0.2	47	89	30	68	101	53	59	72	62	34	28	50	58
RTU Baytan Thiram + Allegiance FL	3.0 + 0.75	25	99	45	72	100	48	64	81	62	30	39	53	60
Vitavax-PCNB + Allegiance FL	6.0 + 0.75	36	83	26	64	102	39	64	71	63	34	36	57	56
Allegiance FL	1.5	64	81	34	71	88	45	52	68	62	32	34	53	57
Argent	4.5	58	75	36	68	90	44	50	77	59	30	36	55	57
RTU-PCNB	14.5	50	71	16	69	104	39	54	72	58	40	47	50	55
Nontreated	---	49	66	20	66	90	47	53	69	60	37	35	48	53
Location average		50	87	34	69	96	47	59	74	63	37	40	55	
Coefficient of Variation (%)		23.4	11.8	31.6	13.3	14.2	21.6	13.4	8.8	14.5	34.6	31.3	14.8	
LSD (P=0.05)		16.9	14.7	12.6	NS	NS	NS	10.0	NS	NS	NS	NS	NS	

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 4 sites. Baytan Thiram + Allegiance FL + Dynasty Extreme and Baytan Thiram + Allegiance FL performed better than the historical standard at 2 of 4 sites. The number of fungicide treatments significantly increasing stands over the nontreated control ranged from 2 of the 8 nominated treatments for AL and MS1 to 8 of the 8 nominated treatments for the AR1 site.

Seedling development across the sites at the time of disease assessment and isolation ranged from 2.0 nodes to 8.3 nodes. Hypocotyl disease indices ranged from 2.0 at the OK1 site to 3.1 at the AR1 site, average 2.5 (Table 5). Root disease indices ranged from 2.2 for the AR4 and MS1 sites to 5.0 for the AR1 site, average 3.1. *Rhizoctonia solani* was isolated from seedlings from the nontreated plots at 11 of 12 locations (Table 5). *R. solani* was isolated from 20% or greater of the seedlings at 4 locations (AL, AR4, LA2, VA). *Pythium* spp. were isolated from seedlings at 11 of the 12 locations (Table 5). Isolation frequencies for *Pythium* spp. on amended water agar was 20% or greater for 2 sites (AL, AR1). Isolation frequencies 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 3 of the 12 locations on the modified TB-CEN medium (Table 5). *T. basicola* was isolated from 80% or greater of the seedlings for all three sites. *Fusarium* spp. were isolated from seedlings at all locations (Table 5). Isolation frequencies for *Fusarium* spp. ranged from 46% to 100%.

Table 5. Disease ratings and isolation frequencies of seedling pathogen groups for the 2009 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	4.0	2.9	4.1	30	24 (28)[5]	80	98
AR1	2.0	3.1	5.0	10	38 (92)	100	74
AR2	4.3	3.0	2.9	14	12 (76)	0	80
AR4	4.7	2.2	2.2	30	6 (100)	94	76
GA	6.0	2.4	3.3	2	14 (60)	0	84
LA2	4.0	2.5	4.2	24	16 (86)	0	68
MS1	6.3	2.4	2.2	6	10 (76)	0	51
OK1	8.3	2.0	2.5	0	2 (16)	0	89
OK2	8.0	2.1	2.3	17	0 (14)	0	100
OK3	8.3	2.1	2.3	2	4 (33)	0	98
TX9	5.0	2.3	3.3	14	6 (31)	0	96
VA	4.0	2.5	3.1	40	14 (56)	0	84

[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.

Soil populations of *R. solani* were detected for only 2 of the 11 soils assayed, average 6.8 CFU/100 g of soil (Table 6). *Pythium* spp. were detected in soil at all but one site for the soils assayed, range 16 to 183 CFU/g of soil. *T. basicola* was detected in 4 of the 11 soils assayed, ranging from 50 to 282 CFU/g soil. The root-knot nematode, *Meloidogyne incognita*, was detected in soil from the Virginia site, and the reniform nematode, *Rotylenchulus reniformis*, was detected in the soil sample from the Mississippi site.

The hypocotyl disease index was positively correlated with the root disease index, 0.73 (P=0.0069), the isolation frequency of *Pythium* spp., 0.86 (P=0.0003), and the soil population of *T. basicola*, 0.64 (P=0.0335). The root disease index was positively correlated with the isolation frequency of *Pythium* spp., 0.89 (P=0.0001). The mean low temperature was negatively correlated with average stand for the sites, -0.64 (P=0.0329), and isolation

frequency of *Pythium* spp., -0.66 (0.0263). The isolation frequency of *T. basicola* and *Pythium* spp. on P_5ARP were positively correlated with their soil populations 0.89 (P=0.0002) and 0.86 (P=0.0008), respectively.

Table 6. Soil populations of selected soilborne genera from sites in the 2009 National Cottonseed Treatment Program.

Location	*Rhizoctonia solani*	*Pythium* spp.	*Thielaviopsis basicola*
	CFU[1]/100g	CFU/g	CFU/g
AL	5.7	16	282
AR1	ND[2]	167	172
AR2	NA[3]	NA	NA
AR4	ND	183	146
GA	ND	100	0
LA2	ND	67	0
MS1	8.0	117	0
OK1	ND	17	0
OK2	ND	17	50
OK3	NA	ND	0
TX9	ND	50	0
VA	ND	17	0

[1] Colony forming units.
[2] Populations not detected in soil sample; less than approximately 2.2, 1.0 for VA,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 12 locations where stand data were collected for the 2009 National Cottonseed Treatment Program indicated that seed treatment fungicides improved stands of cotton compared to a nontreated control for 33% of the sites. Most of the nominated fungicide combinations improved stands over the nontreated seed at two or more sites 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, 0.73, isolation frequency of *Pythium* spp., 0.86, and population of *T. basicola*, 0.64. The root disease index was positively correlated with the isolation frequency of *Pythium* spp., 0.89. The mean low temperature was negatively correlated with average stand for the sites, -0.64, and isolation frequency of *Pythium* spp., -0.66.

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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