REPORT OF THE COTTONSEED TREATMENT COMMITTEE FOR 2010

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Abstract

The 2010 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 2010. The results from the 14 locations where stand data were collected for the 2010 National Cottonseed Treatment Program indicated that seed treatment fungicides improved stands of cotton compared to a nontreated control for 29% of the sites (4 sites). Four of the eight nominated fungicide combinations improved stands over the nontreated seed at all four sites where a stand improvement was observed. In addition, four of the nominated treatment combinations improved stands over the historical standard fungicide seed treatment at two of these four sites. *Pythium* was identified as an important component of the seedling disease complex at two sites and *Rhizoctonia solani* was

identified as an important component of the seedling disease complex at three sites where a fungicide response was found. The root disease index was positively correlated with the soil populations of *T. basicola* 0.71 (P=0.0064) and soil populations of R. *solani* 0.61 (P=0.0284). The National Cottonseed Treatment Program documents the importance of fungicide seed treatments and the advances in fungicide efficacy.

Introduction

The 2010 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 2010 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 and PCNB were included to aid in determining the importance of *Pythium* spp. and *Rhizoctonia solani*, respectively. In 2010, an additional Baytan 30 + Allegiance + Vortex FL treatment without an insecticide seed treatment was included to examine the importance of seed treatment insecticides on stand establishment and seedling diseases. 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 2010 National Cottonseed Treatment Program.

Materials and Methods

Fungicide treatment

Acid-delinted seed of *Gossypium hirsutum* L. cv 'DP 0935 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), Gaucho Grande (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, 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^{o}C.

Field experiments

Fifteen field experiments were conducted by 13 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 7. The stand counts used in the analyses were taken from 26 to 41 days after planting, average 31 days, depending on the location. At the time of the stand counts, cooperators also took photographs of plants from plots planted with seed not having a seed treatment insecticide. A soil sample and seedling sample from plots containing nontreated seed were taken from 26 to 41 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, 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

Table 1. Fungicides, formulations and active ingredients included in the 2010 National Cottonseed Treatment Program

Common or registered name[1]	Formulation	Active ingredient (%)
A16148C		Bayer CropScience
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
BAYTAN 30 (Triadimenol)	Flowable	30% Beta-(4-Chlorophenoxy)-alpha-(1,1-dimethylethyl)-1*H*-1,2,4-triazole-1-ethanol
BION (Acibenzolar-S-methyl)	Liquid	42% 1,2,3-benzothiadiazole-7-thiocarboxylic acid S-methyl ester
DYNASTY CST (Azoxystrobin)	Liquid	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
MAXIM 4FS (Fludioxonil)	Liquid	40.3% 4-(2,2-difluoro- 1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile
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
SYSTHANE (Myclobutanil)	Flowable	40% A-butyl-a-(4-chlorophenyl)-1*H*-1,2,4-triazole-1-propanenitrile
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 2010 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)	4/13	5/13	5/13	5	10	40	21(17)
J. Barham	Rohwer, AR	(AR1)	4/22	5/18	5/18	4	40	120	20(16)
A. Beach	Keiser,AR	(AR2)	4/15	5/19	5/19	6	24	100	20(16)
C. Rothrock	Judd Hill, AR	(AR4)	4/29	5/26	5/26	7	50	250	20(16)
R. Kemerait	Tifton, GA	(GA)	4/16	5/14	5/14	4	50	150	21(16)
P. Colyer	Bossier City, LA	(LA1)	4/15	5/17	5/17	4	25	100	20(16)
B. Padgett	Winnsboro, LA	(LA2)	4/19	5/19	5/19	5	25	100	21(16)
G. Lawrence	Mississippi State, MS	(MS1)	4/20	5/24	5/24	5	40	125	21(16)
T. Kelly	Tipton, OK	(OK1)	5/7	6/4	6/4	4	20	100	21(16)
R. Thacker	Altus, OK	(OK2)	5/7	6/4	6/4	4	20	100	20(14)
M. Bayles	Perkins, OK	(OK3)	5/11	6/21	6/21	4	20	100	19(15)
J. Woodward	Halfway, TX	(TX2)	5/3	6/7	6/2	4	35	140	20(19)[2]
J. Woodward	Quaker, TX	(TX10)	4/28	6/6	5/31	4	35	140	16(15)[2]
P. Phipps	Suffolk, VA	(VA)	4/22	5/26	5/24	4	60	180	20(16)

[1] Mean (Minimum) 4" soil temperature; 3-day average following planting.
[2] Mean (Minimum) 6" soil temperature; 3-day average following planting.

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.

Soil samples were assayed for populations of *Rhizoctonia* species by using the tootpick baitng method (Paulitz and Schroeder, 2005) using 6 toothpicks per sample 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 (oven dry weight) 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 80% to 97% for the cultivar DP 0935 B2RF, with an average germination of 89%. Two of the nominated treatments reduced seed germination in the rolled germination paper assay compared to no fungicide seed treatment; Baytan 30 + Allegiance FL + Vortex FL + SP1020 and RTU-PCNB. For the 14 locations in the 2010 National Cottonseed Treatment Program reporting stand data, 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 14 sites (Table 4). This frequency of response, 29%, is 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, suggesting factors other than seedling diseases were important in 2010 in stand establishment. The Allegiance treatment increased stands compared to the nontreated control at 2 of these 4 sites having a significant response (LA1, TX10), indicating the importance of *Pythium* spp. in stand establishment at these sites. At 3 of these 4 sites, the PCNB treatment increased stands over the nontreated control (AL, TX10, VA), indicating *Rhizoctonia solani* was a major factor in stand establishment at these sites in 2010. The Vitavax-PCNB + Allegiance historical standard fungicide treatment increased stands compared to the nontreated control at 3 of the 4 sites (AL, LA1, TX10). The nominated treatments increased stands over the nontreated control from 75% of the sites (3 of 4 sites) to 100% of the sites (4 of 4 sites) depending on the treatment. The treatments giving an increase in stand compared to the nontreated control at all 4 sites where a stand response was found were Baytan 30 + Allegiance FL + Vortex FL + SP1020, Apron XL + Maxim 4FS + Systhane 40WP + Dynasty CST +

Table 3. Mean squares for combined analysis of variance across locations, 2010 National Cottonseed Treatment Program.

Source	Degrees of freedom	Mean squares
Location	13	23212*[1]
Replication(Location)	49	363*
Treatment	13	967*
Location*treatment	169	184**
Error	635	127

[1] Significant *F*-test; * $P<0.0001$ or ** $P=0.0007$.

Table 4. Cotton seedling stands for locations of the 2010 National Cottonseed Treatment Program.

		Plant stand (%)														
Treatment	Rate (oz/cwt)	AL	AR1	AR2	AR4	GA	LA1	LA2	MS1	OK1	OK2	OK3	TX2	TX10	VA	Mean
Baytan 30 + Allegiance FL + Vortex FL + SP1020	0.75 + 1.5 + 0.08 + 0.32	70	64	77	44	56	46	63	73	85	78	12	63	77	67	62
Apron XL + Maxim 4FS + Systhane 40WP + A16148C	0.64 + 0.04 + 0.84 + 0.5	69	88	71	33	58	51	70	82	76	70	9	60	77	54	62
WECO 100 + Nu-Flow M HF + Apron XL + Nusan 30 EC	4.0 + 4.0 + 0.32 + 2.0	71	72	65	46	64	36	64	75	82	69	11	60	80	56	61
Baytan 30 + Allegiance FL + Vortex FL	0.5 + 0.75 + 0.08	68	71	79	38	50	47	62	74	86	77	10	58	74	56	61
Apron XL + Maxim 4FS + Systhane 40WP + Dynasty CST + Bion	0.64+ 0.04+ 0.84+ 4.0+ 0.03	66	71	79	34	70	51	58	80	78	61	6	61	71	62	61
Apron XL + Maxim 4FS + Systhane 40WP + Dynasty CST	0.64 + 0.04 + 0.84 + 4.0	74	68	65	43	59	46	61	75	80	81	6	48	73	61	60
WECO 100 + Nu-Flow M HF + Apron XL + Nusan 30	4.0 + 1.75 + 0.32 + 2.0	72	68	64	42	48	46	58	79	86	68	8	55	76	59	59
WECO 100 + Nu-Flow M HF + Apron XL + Nusan 30 EC + WECO 1090	4.0 + 4.0 + 0.32 + 2.0 + 0.2	80	73	69	34	44	35	58	80	80	74	6	47	82	53	58
Baytan 30 + Allegiance FL + Vortex FL (w/o Insecticide)	0.5 + 0.75 + 0.08	80	67	77	40	63	53	62	77	83	65	5	59	79	58	62
RTU Baytan Thiram + Allegiance FL	3.0 + 0.75	65	79	81	42	54	48	57	81	84	68	8	63	80	67	63
Vitavax-PCNB + Allegiance FL	6.0 + 0.75	52	69	78	40	52	36	62	80	81	72	16	47	78	49	58
Allegiance FL	1.5	42	76	78	33	53	40	63	81	82	78	13	48	76	49	58
RTU-PCNB	14.5	52	70	58	35	64	17	58	76	82	67	6	44	81	61	55
Nontreated	---	32	61	70	26	37	24	58	65	77	73	6	39	57	45	48
Location average		64	71	72	38	55	41	61	77	81	72	9	54	76	57	
Coefficient of Variation (%)		20.2	15.6	18.8	34.5	28.1	18.7	20.6	15.5	8.3	11.8	79.7	22.2	10.4	14.4	
LSD (P=0.05)		16.3	NS	NS	NS	NS	11.0	NS	NS	NS	NS	NS	NS	11.2	11.7	

Bion, Apron XL + Maxim 4FS + Systhane 40WP + Dynasty CST, and WECO 100 + Nu-Flow M HF + Apron XL +Nusan 30. At 3 of the 4 sites where a response was found (AL, LA1, VA), some of the nominated fungicide treatments performed significantly better than the historical standard fungicide treatment Vitavax-PCNB + Allegiance. Baytan 30 + Allegiance FL + Vortex FL + SP1020, Apron XL + Maxim 4FS + Systhane 40WP + A16148C, Apron XL + Maxim 4FS + Systhane 40WP + Dynasty CST + Bion, and Apron XL + Maxim 4FS + Systhane 40WP + Dynasty CST increased stands significantly compared to the historical standard at two of the three sites where this increase in stands was found. The number of nominated 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 AL, LA1, and TXQ sites.

Seedling development across the sites at the time of disease assessment and isolation ranged from 2.0 nodes to 7.5 nodes (Table 5). Thrips damage was detected at all sites evaluated and ranged from slight leaf distortion (1) to severe damage with leaves not expanding (3). However, no significant differences in stand were found for seed treatment insecticide using Baytan 30 + Allegiance FL + Vortex FL treated seed for the four sites with a seed treatment response. Hypocotyl disease indices ranged from 2.1 at the OK1 and TX10 sites to 3.4 at the AR1 site, average 2.6 (Table 5). Root disease indices ranged from 2.1 for the LA1 and TX10 sites to 4.5 for the AL site, average 2.9. *Rhizoctonia solani* was isolated from seedlings from the nontreated plots at all 14 locations (Table 5). *R. solani* was isolated from 20% or greater of the seedlings at 5 locations (GA, LA1, LA2, TX10, VA). *Pythium* spp. were isolated from seedlings at all locations (Table 5). Isolation frequencies for *Pythium* spp. on amended water agar were 20% or greater for 2 sites (AR2, VA). 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 11 of the 14 locations on the modified TB-CEN medium (Table 5). *T. basicola* was isolated from 20% or greater of the seedlings for the AL, AR1, AR4, OK2 and TX2 sites. *Fusarium* spp. were isolated from seedlings at all locations (Table 5). Isolation frequencies for *Fusarium* spp. ranged from 43% to 100%.

Table 5. Disease ratings and isolation frequencies of seedling pathogen groups for the 2010 National Cottonseed Treatment Program locations.

					Isolation frequency (%)[1]			
			Disease Index		*Rhizoctonia*	*Pythium*	*Thielaviopsis*	*Fusarium*
Location	Nodes[2]	Thrips[3]	Hyp.[4]	Root[5]	*solani*	spp.	*basicola*	spp.
AL	2.0	---	2.6	4.5	10	12 (38)[6]	24	92
AR1	3.7	3	3.4	4.0	16	10 (11)	100	52
AR2	4.0	1	2.9	2.8	6	28 (86)	0	70
AR4	4.3	1	2.7	2.9	18	10 (52)	100	58
GA	5.0	---	2.8	3.4	24	18 (76)	6	92
LA1	5.0	---	2.8	2.1	36	2 (42)	5	66
LA2	3.3	1	2.4	2.5	36	10 (25)	8	52
MS1	4.3	---	2.7	3.6	6	14 (83)	2	43
OK1	4.7	2	2.1	2.3	2	6 (8)	4	96
OK2	4.0	3	2.4	2.8	2	8 (26)	40	94
OK3	7.5	---	2.2	2.5	2	2 (21)	0	100
TX2	4.3	---	2.5	3.3	12	8 (18)	92	96
TX10	6.7	---	2.1	2.1	20	8 (40)	14	66
VA	4.0	2	2.6	2.4	22	22 (44)	4	74

[1] Isolation frequency is based on approximately 50 seedlings per location.
[2] Nodes based on five seedlings per location.
[3] Thrips rating; 0=no damage, 1=slight leaf distortion, 2= severe leaf distortion and cupping, 3=severe damage with leaves not expanding. Not evaluated, ---.
[4] Hypocotyl index; 1=no symptoms, 2=few pinpoint lesions or diffuse discolored areas, 3=distinct necrotic lesion, 4=girdling lesion, and 5=seedling dead.
[5] 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.
[6] Isolation frequency in parentheses from P_5ARP.

Soil populations of *R. solani* were detected for only 6 of the 13 soils assayed, average 3.1 CFU/ cm^3 of soil (Table 6). *Pythium* spp. were detected in soil at all but two sites for the soils assayed, range 17 to 428 CFU/g of soil. *T. basicola* was detected in 4 of the 13 soils assayed, range from 19 to 951 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, 0.54 (P=0.0482). The root disease index was positively correlated with the soil populations of *T. basicola* 0.71 (P=0.0064) and soil populations of R. *solani* 0.61 (P=0.0284).

Table 6. Soil populations of selected soilborne genera from sites in the 2010 National Cottonseed Treatment Program.

Location	*Rhizoctonia solani*	*Pythium* spp.	*Thielaviopsis basicola*
	CFU[1]/cm^3	CFU/g	CFU/g
AL	4.4	116	951
AR1	NA[2]	NA	NA
AR2	ND[3]	18	0
AR4	4.4	182	204
GA	ND	194	0
LA1	1.1	268	0
LA2	2.2	428	0
MS1	2.2	60	0
OK1	ND	ND	0
OK2	ND	17	166
OK3	ND	26	0
TX2	4.4	113	19
TX10	ND	103	0
VA	ND	ND	0

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

Summary

The results from the 14 locations where stand data were collected for the 2010 National Cottonseed Treatment Program indicated that seed treatment fungicides improved stands of cotton compared to a nontreated control for 29% of the sites (4 sites). Four of the eight nominated fungicide combinations improved stands over the nontreated seed at all four sites where a stand improvement was observed. In addition, four of the nominated treatment combinations improved stands over the historical standard fungicide seed treatment at two of these four sites. The root disease index was positively correlated with the soil populations of *T. basicola* 0.71 (P=0.0064) and soil populations of R. *solani* 0.61 (P=0.0284).

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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