REPORT OF THE COTTONSEED TREATMENT COMMITTEE FOR 2011

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Abstract

The 2011 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. Six

fungicide seed treatments were nominated by chemical industry representatives for evaluation in 2011. The results from the 13 locations where stand data were collected for the 2011 National Cottonseed Treatment Program indicated that seed treatment fungicides improved stands of cotton compared to a non-treated control for 38% of the locations (5 locations). All of the six nominated fungicide combinations improved stands over the non-treated seed at all five locations where a stand improvement was observed. In addition at one of the five locations, two of the nominated treatment combinations improved stands over the historical standard fungicide seed treatment. *Pythium* was identified as an important component of the seedling disease complex at three locations and *Rhizoctonia solani* was identified as an important component of the seedling disease complex at four locations where a fungicide response was found. Stand for the non-treated control was negatively correlated with the hypocotyl disease index, -.60 (P=0.0389), isolation of *Pythium* on P_5ARP, -0.74 (P=0.0220), and soil population of *Rhizoctonia solani*, -0.71 (P=0.0225).

Introduction

The 2011 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. Six fungicide seed treatments were nominated by chemical industry representatives for evaluation in the 2011 National Cottonseed Treatment Program. Two standard fungicide treatments, Vitavax-PCNB + Allegiance, the historical standard, and RTU Baytan Thiram + Allegiance FL, and a non-treated 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 2011, 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. Disease ratings and pathogen isolations for seedlings and soil populations of selected soilborne genera were conducted by collecting seedlings and soil from the non-treated control plots at each location. Soil temperature and water, plant development, and thrips' damage data also were collected for sites for the 2011 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), Cruiser 5FS (9oz/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 5FS and dye also were applied to the non-treated 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 non-treated seed by rolling seed in moistened germination paper and incubating for 3 days at 30°C.

Field experiments

Fifteen field experiments were conducted by 12 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 9. The stand counts used in the analyses were taken from14 to 41 days after planting, average 30 days, depending on the location. At the time of the stand counts, cooperators also took data on plants from plots planted with seed not having a seed treatment insecticide and the corresponding fungicide treatment. The thrips rating scale was 0=no damage, 1=slight leaf distortion, 2= severe leaf distortion and cupping, and 3=severe damage with leaves not expanding. A soil sample and seedling sample from plots containing non-treated seed were taken from 14 to 41 days after planting, average 31 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, 5=51-75% of the root system discolored, and 6>75% of the root system.

Table 1. Fungicides, formulations and active ingredients included in the 2011 National Cottonseed Treatment Program.

Common or registered name[1]	Formulation	Active ingredient (%)
A16148C		Bayer CropScience
ALLEGIANCE (Metalaxyl)	Flowable	28.35% *N*-(2,6-dimethylphenyl) (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-methylester
DYNASTY 100FS (Azoxystrobin)	Liquid	9.6% Methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate
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
EMERION (Penflufen)	Flowable	22.68% 2′-[(*RS*)-1,3-dimethylbutyl]-5-fluoro-1,3-dimethylpyrazole-4-carboxanilide
MAXIM 4FS (Fludioxonil)	Liquid	40.3% 4-(2,2-difluoro- 1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile
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
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

[1] Registered chemical name, all capital letters.

Table 2. List of cooperators and procedures for locations in the 2011 National Cottonseed Treatment Program.

Cooperator	Location		Date Planted	Date Sampled	Date Counted	Reps.	Row feet counted	Seed planted	Soil temperature[1]
K. Lawrence	Auburn, AL	(AL)	4/19	5/25	5/25	5	10	100	20(18)
J. Barham	Rohwer, AR	(AR1)	4/19	5/17	5/17	4	50	150	19(18)
A. Beach	Keiser,AR	(AR2)	5/11	6/2	6/2	6	20	100	22(20)
C. Rothrock	Judd Hill, AR	(AR4)	5/10	6/9	6/9	9	50	250	25(22)
R. Kemerait	Tifton, GA	(GA)	4/22	5/6	5/6	5	25	150	24 (NA)
P. Colyer	Bossier City, LA	(LA1)	4/7	5/9	5/9	6	25	100	23(20)
B. Padgett	Winnsboro, LA	(LA2)	4/13	5/16	5/13	5	25	100	20(16)
G. Lawrence	Mississippi State, MS	(MS1)	5/6	6/12	6/6	5	40	160	23(16)
G. L. Sciumbato	Stoneville, MS	(MS2A)	4/8	---	5/9	4	45	180	NA
G. L. Sciumbato	Stoneville, MS	(MS2B)	4/18	5/25	5/17	4	45	180	36(21)[2]
M. Bayles	Perkins, OK	(OK3)	5/11	6/21	6/21	4	20	100	15(11)
J. Woodward	Quaker, TX	(TX10)	5/5	6/2	6/2	4	35	140	(NA)
P. Phipps	Suffolk, VA	(VA)	4/14	5/16	5/16	4	60	180 .	17(12)

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

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.

Soil samples were assayed for populations of *Rhizoctonia* species by using the toothpick-baiting-method (Paulitz and Schroeder, 2005) using 9 toothpicks per sample (6 toothpicks AR1) and Rhizoctonia populations were quantified on the Rhizoctonia selective medium TS (Spurlock et al., 2011). 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 89% for the cultivar DP 0935 B2RF, with an average germination of 84%. No differences in seed germination in the rolled germination paper assay were found among seed treatments. For the 13 trials in the 2011 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 7 of the 13 locations (Table 4). However, significant differences in treatment response compared to the non-treated control were only found for 5 of these locations. This frequency of response, 38%, is slightly lower than most years when stands from over 50% of the locations 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 2011 in stand establishment. The Allegiance treatment increased stands compared to the non-treated control at 3 of these 5 locations having a significant response (AR2, AR4, LA2), indicating the importance of *Pythium* spp. in stand establishment at these locations. At 4 of these 5 locations, the PCNB treatment increased stands over the non-treated control (AR2, AR4, GA, LA1), indicating *Rhizoctonia solani* was a major factor in stand establishment at these locations in 2011. The Vitavax-PCNB + Allegiance historical standard fungicide treatment increased stands compared to the non-treated control at 5 of 5

Table 3. Mean squares for the combined analysis of variance across location, 2011 National Cottonseed Treatment Program

Source	Degrees of freedom	Mean squares
Location	12	18751*[1]
Replication (Location)	52	145*
Treatment	11	958*
Location*treatment	132	185*
Error	56	64

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

Table 4. Cotton seedling stands for locations of the 2011 National Cottonseed Treatment Program.

		Plant stand (%)													
Treatment	Rate (oz/cwt)	AL	AR1	AR2	AR4	GA	LA1	LA2	MS1	MS2A	MS2B	OK3	TX10	VA	Mean
Apron XL + Maxim 4FS + Systhane 40WP + A16148C + Dynasty 100FS	0.32 + 0.08 + 0.84 + 0.32 + 1.53	23	80	71	83	83	82	79	75	86	48	85	79	58	73
Baytan 30 + Allegiance FL + Vortex FL + Emerion	0.75 + 1.5 + 0.08 + 0.32	22	75	66	82	70	79	81	72	78	52	81	82	64	70
Apron XL + Maxim 4FS + Systhane 40WP + Dynasty CST	0.32 + 0.08 + 0.84 + 4.13	10	76	66	83	78	80	82	71	77	50	88	78	62	70
Apron XL + Maxim 4FS + Systhane 40WP + Dynasty CST + Bion	0.32 + 0.08 + 0.84 + 4.13 + 0.03	16	64	68	80	84	80	82	57	86	59	82	78	60	70
Baytan 30 + Allegiance FL + Vortex FL	0.5 + 0.75 + 0.08	17	68	70	82	68	80	74	66	78	55	80	72	64	68
Baytan 30 + Allegiance FL+ Vortex FL (w/o Insecticide)	0.5 + 0.75 + 0.08	7	66	66	80	76	74	75	77	73	43	83	81	58	67
Apron XL + Maxim 4FS + Systhane 40WP	0.32 + 0.08 + 0.84	10	69	58	76	77	75	81	68	76	56	75	71	62	66
RTU BaytanThiram + Allegiance FL	3.0 + 0.75	19	72	66	82	66	76	76	68	79	41	89	71	56	67
Vitavax-PCNB + Allegiance FL	6.0 + 0.75	14	68	66	80	74	77	82	78	78	52	84	80	54	69
RTU-PCNB	14.5	22	69	55	77	86	82	69	79	82	49	84	71	64	69
Allegiance FL	1.5	9	74	56	76	45	68	82	84	61	39	77	73	57	62
Non-treated	---	28	61	39	69	46	64	62	76	63	29	86	65	54	57
Location average		16	70	62	79	71	76	77	72	77	48	83	75	59	
Coefficient of Variation (%)		37.7	14.3	10	6.6	7.6	9.9	8.7	10.7	15.8	27.7	12.8	10	12.2	
LSD (P=0.05)		7.8	NS	6.8	5.1	6.9	8.7	8.7	9.9	NS	NS	NS	NS	NS	

locations, as did the treatment RTU BaytanThiram + Allegiance FL. All the nominated treatments increased stands over the non-treated control for all of the five locations where a significant stand response was observed. At 1 of the 5 locations where a response was found (GA), two of the nominated fungicide treatments performed significantly better than the historical standard fungicide treatment Vitavax-PCNB + Allegiance. These treatments were Apron XL + Maxim 4FS + Systhane 40WP + A16148C + Dynasty 100FS and Apron XL + Maxim 4FS + Systhane 40WP + Dynasty CST + Bion. Stand response for the fungicide combination Baytan 30 + Allegiance FL + Vortex FL did not differ with or without the insecticide seed treatment for four of these five locations with a fungicide response. However for two locations with a significant *F*-test, one location (AL) had reduced stands with the insecticide seed treatment and one location (GA) had increased stands for the insecticide seed treatment.

Seedling development across the locations at the time of disease assessment and isolation ranged from 2.0 nodes to 9.0 nodes (Table 5). Thrips damage was detected at all locations evaluated and ranged from slight leaf distortion (0.5) to severe damage with leaves not expanding (3.0) for the treatment not receiving an insecticide. The seed treatment insecticide reduced thrips damage, except for two of the locations which had severe damage for treatments with and without the seed treatment insecticide. Hypocotyl disease indices ranged from 2.0 at the GA, OK3 and TX10 locations to 3.0 at the AR2 location, average 2.6 (Table 5). Root disease indices ranged from 2.0 for the GA, MS1 and OK3 locations to 4.6 for the AL location, average 2.9. *Rhizoctonia solani* was isolated from seedlings from the non-treated plots for 9 of the 12 locations (Table 5). *Rhizoctonia solani* was isolated from 20% or greater of the seedlings at 5 locations (AR1, LA1, LA2, MS2B, VA). *Pythium* spp. were isolated from seedlings at all locations (Table 5). Isolation frequencies for *Pythium* spp. on amended water agar were greater than 20% for 3 locations (AR1, AR2, LA2). Isolation frequencies increased dramatically by plating roots without surface disinfestation on the selective medium P_5ARP (Table 5). *Thielaviopsis basicola* was isolated from seedlings at 4 of the 12 locations on the modified TB-CEN medium (Table 5). *Thielaviopsis basicola* was isolated from 20% or greater of the seedlings for the AR4, MS2B, and TX10 locations. *Fusarium* spp. were isolated from seedlings at all locations (Table 5). Isolation frequencies for *Fusarium* spp. ranged from 42% to 92%.

Table 5. Disease ratings and isolation frequencies of seedling pathogen groups for the 2011 National Cottonseed Treatment Program locations.

		Thrips rating[1]		Disease index		Isolation frequency (%)[2]			
		Insecticide				*Rhizoctonia*	*Pythium*	*Thielaviopsis*	*Fusarium*
Location	Nodes[3]	with	without	Hyp.[4]	Root[5]	*solani*	spp.	*basicola*	spp.
AL	3.7	2.3	2.8	2.6	4.6	8	4	0	92
AR1	2.0	NA	NA	2.5	3.4	20	48(92)[6]	0	74
AR2	3.7	NA	NA	3.0	4.0	0	22(96)	0	86
AR4	6.0	1.0	2.0	2.1	2.1	18	10(10)	100	60
GA	9.0	NA	NA	2.0	2.0	0	2	0	92
LA1	5.3	2.0	3.0	2.7	3.6	22	12(70)	0	82
LA2	5.3	1.2	2.2	2.6	3.7	54	22(100)	12	42
MS1	6.0	3.0	3.0	2.2	2.0	2	6(44)	0	59
MS2B	6.0	3.0	3.0	2.7	2.3	57	6	20	84
OK3	7.3	0.25	0.5	2.0	2.0	0	8(11)	0	90
TX10	5.0	1.1	1.3	2.0	2.1	6	2(94)	50	80
VA	2.7	1.3	1.0	2.6	2.5	46	12(98)	0	78

[1] Thrips rating; 0=no damage, 1=slight leaf distortion, 2= severe leaf distortion and cupping, 3=severe damage with leaves not expanding. NA = not available.

[2] Isolation frequency is based on approximately 50 seedlings per location.

[3] Nodes based on five seedlings per location.

[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, 5=51-75% of the root system discolored, and 6>75% of the root system discolored.

[6] Isolation frequency in parentheses from P_5ARP.

Soil populations of *R. solani* were detected for 7 of the 10 soils assayed, range 1.5 to 6 CFU/cm^3 of soil (Table 6). *Pythium* spp. were detected in soil at all but one location for the soils assayed, range 16 to 313 CFU/g of soil. *T. basicola* was detected in 3 of the 10 soils assayed, range 12 to 204 CFU/g soil.

Stand for the non-treated control was negatively correlated with the hypocotyl disease index, -0.60 (*P*=0.0389), isolation of *Pythium* on P_5ARP, -0.74 (*P*=0.0220), and soil populations of *Rhizoctonia solani*, -0.71 (*P*=0.0225). Soil populations of *Pythium* spp. were positively correlated with isolation of *Pythium* spp. from seedlings, 0.92 (*P*=0.0001). The hypocotyl disease index was positively correlated with the root disease index, 0.75 (*P*=0.0047).

Table 6. Soil populations of selected soilborne genera from sites in the 2011 National Cottonseed Treatment Program.

Location	*Rhizoctonia solani*	*Pythium* spp.	*Thielaviopsis basicola*
	CFU[1]/cm^3	CFU/g	CFU/g
AL	6.0	53	204
AR1	ND[3]	313	0
AR2	NA[2]	NA	NA
AR4	3.0	88	183
GA	1.5	16	0
LA1	1.5	145	0
LA2	3.0	134	0
MS1	1.5	32	0
MS2B	NA	NA	NA
OK3	ND	ND	0
TX10	ND	67	12
VA	3.0	64	0

[1] Colony forming units.
[2] Information not available.
[3] Populations not detected in soil sample; less than approximately 0.5CFU/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 13 locations where stand data were collected for the 2011 National Cottonseed Treatment Program indicated that seed treatment fungicides improved stands of cotton compared to a non-treated control for 38% of the locations (5 locations). All six nominated fungicide combinations improved stands over the non-treated seed at all five locations where a stand improvement was observed. In addition, two of the nominated treatment combinations improved stands over the historical standard fungicide seed treatment at one of these five locations. The insecticide seed treatment increased stands of the corresponding fungicide seed treatment without a seed treatment insecticide for one of the locations. Stand for the non-treated control was negatively correlated with the hypocotyl disease index, -0.60 (*P*=0.0389), isolation of *Pythium* on P_5ARP, -0.74 (*P*=0.0220), and soil populations of *Rhizoctonia solani*, -0.71 (*P*=0.0225).

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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