REPORT OF THE COTTONSEED TREATMENT COMMITTEE FOR 2014

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Abstract

The 2014 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. Seven fungicide seed treatments were nominated by chemical industry representatives for evaluation in 2014. The results from the 13 locations where stand data were collected for the 2014 National Cottonseed Treatment Program indicated that seed treatment fungicides improved stands of cotton compared to the nontreated control for 31% of the locations (4 locations). Two of the seven nominated seed treatments increased stand compared to the nontreated control at three locations where a stand response was observed. In addition, all but two of the nominated treatment combinations improved stands over the historical standard fungicide seed treatment Vitavax-PCNB + Allegiance FL at one of these four locations. Stand for the nontreated seed was negatively correlated with the hypocotyl disease index, -0.63 (P=0.0211). Isolation of *Thielaviopsis basicola* was positively correlated with soil populations of *Thielaviopsis basicola,* 0.99 (P=<0.0001).

Introduction

The 2014 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. Seven fungicide seed treatments were nominated by chemical industry representatives for evaluation in the 2014 National Cottonseed Treatment Program. Two historical standard fungicide treatments, Vitavax-PCNB + Allegiance and RTU Baytan-Thiram + Allegiance, 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. 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 2014 National Cottonseed Treatment Program.

Materials and Methods

Fungicide treatment

Acid-delinted seed of *Gossypium hirsutum* L. cv 'DP 1044 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), and dye (Color Coat Red, 1 oz/cwt) (Syngenta Crop Protection) and Gaucho 600 (12.8 oz/cwt) (Bayer CropScience) in water at a rate of 2.75% (RTU-PCNB 2.86%) liquid to seed weight (w/w). Water, $CaCO_3$, polymer, Gaucho 600, 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 provided 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

Data from the 13 field experiments reported 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 8. The stand counts used in the analyses were taken from 19 to 47 days after planting, average 31 days, depending on the location. A soil sample and seedling sample from plots containing nontreated seed were taken from 27 to 64 days after planting, average 38 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 were 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 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.*

Soil samples were assayed for populations of *Rhizoctonia* spp. by using the toothpick-baiting-method (Paulitz and Schroeder, 2005) using 9 toothpicks per sample and *Rhizoctonia* populations were quantified on the Rhizoctonia selective medium TS (Spurlock et al., 2011). Soil populations of *Pythium* spp. and *Thielaviopsis 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 (Jeffers and Martin, 1986) and *Thielaviopsis basicola* populations were quantified using the pour-plate method with the modified selective medium TB-CEN.

Table 1. Fungicides, formulations and active ingredients included in the 2014 National Cottonseed Treatment Program.

Common or registered name[1]	Formulation	Active ingredient (%)
A9382A		Syngenta Crop Protection
A9625C		Syngenta Crop Protection
A16148C		Syngenta Crop Protection
ALLEGIANCE FL (Metalaxyl)	Flowable	28.35% *N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl) alanine methyl ester
APRON XL 3LS (Mefenoxam)	Liquid	33.3% (R,S)-2-{(2,6-dimethylphenyl)-methoxyacetylamino}-propionic acid 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
EVERGOL ENERGY (Penflufen)	Flowable	3.59% N-[2-(1,3-dimethylbutyl)phenyl]-5-fluoro-1,3-dimethyl-1Hpyrazole-4-carboxamide
(Prothioconazole)		7.18%2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1H-1,2,4-triazole-3-thione
(Metalaxyl)		5.74% *N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl) alanine methyl ester
EVERGOL PRIME (Penflufen)	Flowable	22.4% N-[2-(1,3-dimethylbutyl)phenyl]-5-fluoro-1,3-dimethyl-1Hpyrazole-4-carboxamide
EVERGOL XTEND (Penflufen)	Flowable	13.3% N-[2-(1,3-dimethylbutyl)phenyl]-5-fluoro-1,3-dimethyl-1Hpyrazole-4-carboxamide
(Trifloxystrobin)		13.3% methyl (E)-methoxyimino-{(E)-α-[1-(α,α,α-trifluoro-m-tolyl)ethylideneaminooxy]-o-tolyl}acetate
MAXIM 4FS (Fludioxonil)	Liquid	40.3% 4-(2,2-difluoro- 1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile
RIZOLEX (Tolclofos-methyl)	Flowable	40-44% Phosphorothioic acid, O-(2,6-dichloro-4-methylphenyl) O,O-dimethyl phosphorothioate
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
SPERA 240FS (Myclobutanil)	Flowable	22.37% A-butyl-a-(4-chlorophenyl)-1*H*-1,2,4-triazole-1-propanenitrile
SYSTHANE 40WSP (Myclobutanil)	Powder	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 (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 2014 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)	4/24	5/28	5/28	5	25	100	23(7)
J. Barham	Hope, AR	(AR1)	5/6	6/2	6/2	4	40	120	23(19)
A. Beach	Keiser,AR	(AR2)	4/24	5/24	5/24	6	20	100	20(14)
C. Rothrock	Judd Hill, AR	(AR4)	5/6	6/4	6/4	6	50	250	23(20)
R. Kemerait	Tifton, GA	(GA)	5/13	6/19	6/12	4	50	150	22([2])
P. Colyer	Bossier City, LA	(LA1)	4/23	5/27	5/27	8	25	100	20(20)[3]
T. Price	Winnsboro, LA	(LA2)	4/21	5/19	5/19	5	20	100	22(16)
G. Lawrence	Mississippi State, MS	(MS1)	4/24	5/26	6/1	5	40	160	21(18)
M. Bayles	Perkins, OK	(OK3)	5/23	7/9	7/9	4	20	100	25(20)
H. Kelly	Jackson, TN	(TN)	4/23	5/28	5/28	4	60	240	19(14)
J. Woodward	Halfway, TX	(TX10)	5/6	7/9	5/29	4	70	280	17(16)
J. Woodward	Quaker, TX	(TX10)	5/9	7/9	5/28	4	140	560	24(22)[4]
H. Mehl	Suffolk, VA	(VA)	5/5	6/4	6/4	4	60	240	20(15)

[1] Mean (Minimum) 4" soil temperature; 3-day average following planting.
[2] Not Available
[3] 6" depth
[4] 8" depth

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 96% to 99%, with an average germination of 98%. For the 13 trials in the 2014 National Cottonseed Treatment Program reporting stand data, there were significant location, treatment, and location × treatment effects (Table 3), indicating that the treatment response was dependent on the environment or pathogen pressures for a particular location.

Significant increases in stands for a fungicide treatment compared to the nontreated control were only found for 4 of the 13 locations (Table 4). This frequency of response, 31%, is lower than in past years. 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 at some locations in 2014 in stand establishment. The Allegiance treatment increased stands compared to the nontreated control in 0 of these 4 locations having a significant response, indicating *Pythium* spp. as a group was not limiting stand establishment in 2014. At 2 of these 4 locations (LA1 and MS1), the PCNB treatment increased stands over the nontreated control, indicating *Rhizoctonia solani* was a major factor in stand establishment at these locations in 2014. Vitavax-PCNB + Allegiance, the historical standard fungicide treatment, increased stands compared to the nontreated control at 2 of these 4 locations having a fungicide seed treatment response (LA1 and MS1). The RTU BaytanThiram + Allegiance FL standard treatment increased stands at 3 of the 4 locations having a fungicide response (AR2, LA1, and MS1). The nominated products increased stand for 2 or 3 locations of the 4 locations where a response was found. The nominated treatments that increased stands over the nontreated control for 3 of the 4 locations where a significant stand response was observed were Vortex + Allegiance + Spera + Evergol Prime and Apron XL + Maxim + Systhane + Dynasty CST + A9625C + A16148C + A9382A. Five of the 7 nominated treatments significantly increased stands compared to the historical standard fungicide treatment Vitavax-PCNB + Allegiance.

Seedling development across the locations at the time of disease assessment and isolation ranged from 3.7 nodes to 14.0 nodes (Table 5). Hypocotyl disease indices ranged from 2.0 at TXQ to 2.7 at the AR1 location, average 2.3 (Table 5). Root disease indices ranged from 1.9 for the TN location to 3.2 for the LA2 location, average 2.5. *Rhizoctonia solani* was isolated from seedlings from the nontreated plots for 11 of the 13 locations (Table 5).

Table 3. Mean squares for the combined analysis of variance across locations, 2014 National Cottonseed Treatment Program.

Source	Degrees of freedom	Mean squares[1]
Location	12	20779*
Replication(Location)	51	192*
Treatment	11	554*
Location*treatment	132	114**
Error	561	88

[1] Significant *F*-test; * $P<0.0001$ or ** $P=0.0240$.

Table 4. Cotton seedling stands for locations of the 2014 National Cottonseed Treatment Program.

		Plant stand (%)													
Treatment	Rate (oz/cwt)	AL	AR1	AR2	AR4	GA	LA1	LA2	MS1	OK3	TN	TXH	TXQ	VA	Mean
Vortex + Allegiance + Spera + Evergol Prime	0.08+0.75+1.8 +0.32	78	25	75	71	89	90	88	80	67	65	43	73	84	71
Apron XL + Maxim + Systhane + Dynasty CST	7.5+2.5+21.0 g ai/100kg seed +0.03 mg ai/seed	78	29	61	68	80	89	87	75	70	63	48	75	84	70
Vortex + Allegiance + Spera + Evergol Prime + Rizolex	0.08+0.75+1.8 +0.32+1.5	79	25	58	75	84	90	89	83	64	60	39	72	77	69
Vortex + Allegiance + Spera + Evergol Prime + Evergol Energy	0.08+0.75+1.8 +0.32+2.0	78	24	68	70	83	90	87	71	73	65	36	76	74	69
Apron XL + Maxim + Systhane + Dynasty CST + A9625C + A16148C + A9382A	7.5+2.5+21.0 g ai/100kg seed +0.03 mg ai/seed+1.0+7.5 +11.0 g ai/100kg seed	80	17	55	73	82	91	90	74	65	54	34	74	75	66
Vortex + Allegiance + Spera + Evergol Prime + Evergol Xtend	0.08+0.75+1.8 +0.32+0.5	72	15	71	72	83	89	86	70	59	63	43	64	74	66
Apron XL + Maxim + Systhane + Dynasty CST + A9625C + A16148C + A9382A	7.5+2.5+21.0 g ai/100kg seed +0.03 mg ai/seed+1.0 +10.0+11.0 g ai/100kg seed	67	20	55	67	82	90	81	84	63	55	39	69	75	65
RTU BaytanThiram + Allegiance	3.0+0.75	73	30	70	67	86	89	88	82	74	64	38	64	78	70
Vitavax-PCNB + Allegiance	6.0+0.75	63	28	61	71	81	87	85	73	64	60	38	73	76	66
RTU-PCNB	14.5	60	13	48	59	86	88	88	82	78	45	34	70	69	63
Allegiance	1.5	64	21	59	65	79	75	86	65	62	56	38	71	69	62
Nontreated	---	65	19	50	66	81	80	88	60	66	52	39	73	79	63
Location average		71	22	61	69	83	87	87	75	67	59	39	71	76	
Coefficient of Variation (%)		15.7	57.2	20.6	13.9	7.1	6.8	6.9	13.5	17.3	16.1	18.5	11.8	10.1	
LSD (P=0.05)		14.3	NS	14.5	NS	NS	5.9	NS	12.9	NS	NS	NS	NS	NS	

Rhizoctonia solani was isolated from at least 20% of the seedlings for the LA2, MS1, and VA locations. *Pythium* spp. were isolated from seedlings from 12 of 13 locations (Table 5). *Pythium* was isolated from 20% of the seedling for the AR2 site. Isolation frequencies for *Pythium* spp. increased dramatically by plating roots without surface disinfestation on the selective medium P_5ARP (Table 5). *Thielaviopsis basicola* was isolated from seedlings at 3 of the 13 locations using the modified TB-CEN medium (Table 5). *Thielaviopsis basicola* was isolated from greater than 40% of the seedlings for the AR4 and TXH locations. *Fusarium* spp. were isolated from seedlings at all 13 locations (Table 5). Isolation frequencies for *Fusarium* spp. ranged from 60 to 98%.

Table 5. Disease ratings and isolation frequencies of seedling pathogen groups for the 2014 National Cottonseed Treatment Program locations.

		Disease index		Isolation frequency (%)[1]			
Location	Nodes[2]	Hyp.[3]	Root[4]	*Rhizoctonia solani*	*Pythium* spp.	*Thielaviopsis basicola*	*Fusarium* spp.
AL	5.3	2.5	2.6	4	8 (83)[5]	0	96
AR1	4.0	2.7	3.0	16	10 (98)	0	98
AR2	4.0	2.6	2.4	16	20 (84)	0	80
AR4	4.3	2.3	2.1	4	4 (90)	98	90
GA	4.7	2.1	2.9	2	12 (68)	0	88
LA1	4.3	2.5	2.4	14	18 (100)	0	78
LA2	3.7	2.1	3.2	26	12 (78)	0	98
MS1	4.7	2.5	2.1	28	6 (86)	2	60
OK3	4.0	2.4	2.6	2	4 (2)	0	96
TN	4.3	2.1	1.9	14	0 (100)	0	82
TXH	9.5	2.4	2.2	0	2 (19)	48	96
TXQ	14.0	2.0	2.0	0	8 (44)	0	92
VA	4.7	2.1	2.8	20	6 (100)	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, 5=51-75% of the root system discolored, and 6>75% of the root system discolored.
[5] Isolation frequency in parentheses from P_5ARP.

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Rhizoctonia solani was detected in soil for 6 of the 13 soils assayed, range 0.7 to 4.2 propagules/100 cm^3 of soil (Table 6). *Pythium* spp. were detected in soil at 5 locations for the 9 soils assayed, range 17 to 158 CFU/g of soil (Table 6). *Thielaviopsis basicola* was detected in 2 of the 13 soils assayed, range 31 to 69 CFU/g soil (Table 6). Stand for the nontreated seed was negatively correlated with the hypocotyl disease index, -0.63 (P=0.0211). Isolation of *Thielaviopsis basicola* was positively correlated with soil populations of *Thielaviopsis basicola,* 0.99 (P=<0.0001).

Summary

The results from the 13 locations where stand data were collected for the 2014 National Cottonseed Treatment Program indicated that seed treatment fungicides improved stands of cotton compared to a nontreated control for 31% of the locations (4 locations). Two of the seven nominated seed treatments increased stand compared to the nontreated control at three of the four locations where a stand response was observed. In addition, all but two of the nominated treatment combinations improved stands over the historical standard fungicide seed treatment Vitavax-PCNB + Allegiance at one of these four locations. Stand for the nontreated seed was negatively correlated with the hypocotyl disease index, -0.63 (P=0.0211). Isolation of *Thielaviopsis basicola* was positively correlated with soil populations of *Thielaviopsis basicola,* 0.99 (P=<0.0001).

Table 6. Soil populations of selected soilborne genera from sites in the 2014 National Cottonseed Treatment Program.

Location	*Rhizoctonia solani*	*Pythium* spp.	*Thielaviopsis basicola*
	CFU[1]/100cm^3	CFU/g	CFU/g
AL	0[2]	---[3]	0
AR1	0	---	0
AR2	4.2	16.5	0
AR4	0.7	157.5	69
GA	0	29.6	0
LA1	0.7	32.0	0
LA2	0.7	67.5	0
MS1	1.4	0.0	0
OK3	0	---	0
TN	0	---	0
TX-H	0	0.0	31
TX-Q	0.7	0.0	0
VA	0	0.0	0

[1] Colony forming units.
[2] Populations not detected in soil sample; less than approximately 0.4 CFU/100 cm^3 of soil for *Rhizoctonia solani*, 8 CFU/g of soil for *Pythium* spp. and 0.5 CFU/g of soil for *Thielaviopsis basicola.*
[3] Information not available.

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 System Division of Agriculture nor does it imply registration under FIFRA.

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