REPORT OF THE COTTONSEED TREATMENT COMMITTEE FOR 2015

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Abstract

The 2015 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. Ten fungicide seed treatments were nominated by chemical industry representatives for evaluation in 2015. The results from the 13 locations where stand data were collected for the 2015 National Cottonseed Treatment Program indicated that seed treatment fungicides improved stands of cotton compared to the nontreated control for 38% of the locations (5 locations), with an additional 2 locations being significant at P=0.10. Three of the 10 nominated seed treatments increased stand compared to the nontreated control at 5 of the 5 locations where a stand response was observed. All but one of the nominated treatment combinations improved stands at 4 or more of the 5 locations where a stand response was found. In addition, all but one of the nominated treatments increased stand for at least one location compared to the historical standard fungicide seed treatment Vitavax-PCNB + Allegiance.

Introduction

The 2015 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. Ten fungicide seed treatments were nominated by chemical industry representatives for evaluation in the 2015 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 2015 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 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

Data from the 13 field experiments reported 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 18 to 55 days after planting, average 31 days, depending on the location. A soil sample and seedling sample from plots containing nontreated seed were taken from 18 to 89 days after planting, average 35 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) 10 cm (4 in.) 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* species 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

Table 1. Fungicides, formulations and active ingredients included in the 2015 National Cottonseed Treatment Program.

Common or registered name[1]	Formulation	Active ingredient (%)
A21204A (Azoxystrobin)	Flowable	6.7% 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)		6.7% (R,S)-2-{(2,6-dimethylphenyl)-methoxyacetylamino}-propionic acid methyl ester
(Sedaxane)		3.12% N-[2-[1,1′-bicyclopropyl]-2-ylphenyl]-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide
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
CCB-1 (Albaugh LLC)	Flowable	
CCB-2 (Albaugh LLC)	Flowable	
CCB-3 (Albaugh LLC)	Flowable	
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
L1979-A (Bayer CropScience)	Flowable	
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 2015 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)	5/5	6/2	6/2	5	25	100	24(16)
J. Barham	Hope, AR	(AR1)	5/7	6/9	6/9	4	40	120	24(22)
A. Beach	Keiser,AR	(AR2)	4/30	5/22	5/22	6	20	100	20(13)
C. Rothrock	Judd Hill, AR	(AR4)	5/6	6/6	6/6	5	50	250	24(21)
R. Kemerait	Tifton, GA	(GA)	5/28	6/15	6/15	5	50	150	---[2]
P. Colyer	Bossier City, LA	(LA1)	4/10	5/14	5/12	7	25	100	19(12)
P. Price	Winnsboro, LA	(LA2)	4/21	5/20	5/20	5	20	100	22(16)
G. Lawrence	Mississippi State, MS	(MS1)	4/27	5/27	5/27	5	40	160	19(16)
T. Allen	Stoneville, MS	(MS2)	5/5	6/5	6/5	4	80	320	28(20)
M. Bayles	Perkins, OK	(OK3)	6/5	7/30	7/30	4	20	100	29(25)
H. Kelly	Jackson, TN	(TN)	4/27	5/25	5/28	4	35	140	17(10)
J. Woodward	Quaker, TX	(TX10)	5/7	8/4	6/12	4	70	142	---
H. Mehl	Suffolk, VA	(VA)	5/4	6/2	6/1	4	60	270	21(17)

[1] Mean (Minimum) 10 cm (4 in.) soil temperature; 3-day average following planting.
[2] Not Available

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.

Statistics

Data were analyzed by the Mixed 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 92% to 96% for the cultivar DP 1044 B2RF, with an average germination of 94%. For the 13 trials in the 2015 National Cottonseed Treatment Program reporting stand data, there were significant location and treatment effects (Table 3). There was no significant treatment x location effect (P=0.0834) suggesting that in general the treatment response was independent of the environment or pathogen pressures for a particular location.

Significant increases in stands for a fungicide treatment compared to the nontreated control were found for 5 of the 13 locations, a frequency of response of 38% (Table 4). In addition, 2 locations, LA2 and OK3, had P-values of less than 0.10 suggesting a fungicide seed treatment response at these locations. 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 2015 in stand establishment. The Allegiance or PCNB treatments did not increase stands compared to the nontreated control at any of the 5 locations where a significant response was found indicating *Pythium* spp. or *Rhizoctonia solani* as individual pathogen groups were not the major factor in stand establishment at these locations in 2015. Vitavax-PCNB + Allegiance, the historical standard fungicide treatment, increased stands compared to the nontreated control at 2 of these 5 locations having a fungicide seed treatment response (AR2 and MS2). The RTU BaytanThiram + Allegiance FL standard treatment increased stands at 4 of the 5 locations having a fungicide response (AL, AR2, GA, and LA1). The nominated products increased stand for 3 of the 5 locations to 5 of the 5 locations where a response was found. The nominated treatments that increased stands over the nontreated control at all 5 locations were Albaugh CCB2, Vortex + Allegiance + Spera + Evergol Prime, and L1979-A + Allegiance + Spera + Evergol Prime. Nine of the 10 nominated treatments significantly increased stands compared to the historical standard fungicide treatment Vitavax-PCNB + Allegiance for at least one location. Nominations increasing stands over the historical standard fungicide treatment at 2 of the 5 locations were Albaugh CCB2, Vortex + Allegiance + Spera + Evergol Prime, Apron XL + Maxim + Systhane + A21204A, and Albaugh CCB1. Across locations all nominated treatment combinations increased stands over no fungicide seed treatment and the pathogen specific fungicide seed treatments.

Table 3. Mean squares for the combined analysis of variance across locations, 2015 National Cottonseed Treatment Program.

Source	Degrees of freedom	Mean squares[1]
Location	12	35192*
Replication(Location)	49	272*
Treatment	14	755*
Location*treatment	168	88**
Error	686	75

[1] Significant *F*-test; * $P<0.0001$ or ** $P=0.0834$.

Table 4. Cotton seedling stands for locations of the 2015 National Cottonseed Treatment Program.

		Plant stand (%)													
Treatment	Rate (oz/cwt)	AL	AR1	AR2	AR4	GA	LA1	LA2	MS1	MS2	OK3	TN	TXQ	VA	Mean
Albaugh CCB 2	6.65	92	63	67	80	20	79	81	87	82	56	78	32	59	67
Vortex + Allegiance + Spera + Evergol Prime	0.08 + 0.75 + 1.8 + 0.32	87	69	63	73	11	78	88	89	80	59	77	35	64	67
Albaugh CCB 3	3.78 + dry products	79	69	67	79	7	79	83	84	84	53	79	34	59	66
Vortex + Allegiance + Spera + Evergol Prime + Rizolex	0.08 + 0.75 + 1.8 + 0.32 + 1.5	84	63	65	74	16	76	85	89	81	50	78	34	60	66
L1979-A + Allegiance + Spera + Evergol Prime + Evergol Energy	0.3 + 0.75 + 1.8 + 0.32 + 2.0	83	71	68	77	13	77	77	82	90	52	75	32	59	66
L1979-A + Allegiance + Spera + Evergol Prime	0.3 + 0.75 + 1.8 + 0.32	86	66	66	80	7	73	74	89	84	62	82	30	61	66
Apron XL + Maxim + Systhane + A21204A	7.5 + 2. 5+ + 21.0[y] + 0.042[z]	85	67	73	80	18	72	77	88	81	44	81	32	51	65
L1979-A + Allegiance + Spera+ Evergol Prime	0.15 + 0.75 + 1.8 + 0.32	78	66	71	72	9	78	80	88	85	54	80	31	57	65
Albaugh CCB 1	4.04	86	70	63	77	11	72	80	86	82	44	79	28	60	65
Apron Xl 3 LS + Maxim 4 FS + Systhane 40 WP + Dynasty CST	7.5 + 2.5 + 21.0[y] + 0.03[z]	84	74	66	75	14	71	81	82	79	48	82	26	57	64
RTU BaytanThiram + Allegiance	3.0+0.75	80	73	69	74	9	78	78	85	76	62	75	28	54	65
Vitavax-PCNB + Allegiance	6.0+0.75	73	59	72	75	2	70	73	85	79	52	75	28	56	61
RTU-PCNB	14.5	74	60	58	70	2	67	63	91	75	40	72	27	62	58
Allegiance	1.5	68	57	59	68	2	56	78	82	68	45	83	27	54	57
Nontreated	---	66	59	53	69	2	66	71	87	67	34	84	26	52	56
Location average		80	66	65	75	10	73	78	86	80	50	79	30	58	64
Coefficient of Variation (%)		11.7	14.7	12.3	10.5	53.0	14.5	13.3	8.4	10.0	24.5	9.5	17.8	12.8	13.5
LSD (P=0.05)		11.9	NS	9.2	NS	6.4	11.2	NS	NS	11.3	NS	NS	NS	NS	2.4

Seedling development across the locations at the time of disease assessment and isolation ranged from 2.3 nodes to 11.7 nodes (Table 5). Hypocotyl disease indices ranged from 1.1 at OK3 to 3.1 at the LA1 location, average 2.3 (Table 5). Root disease indices ranged from 2.0 for the GA location to 4.7 for the LA2 location, average 3.3. *Rhizoctonia solani* was isolated from seedlings from the nontreated plots for 12 of the 13 locations (Table 5). *Rhizoctonia solani* was isolated from greater than 15% of the seedlings for the AR1 and MS1 locations. *Pythium* spp. were isolated from seedlings from 12 of 13 locations (Table 5). Pythium was isolated from 30% of the seedlings for the TX-Q 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 7 of the 13 locations using the modified TB-CEN medium (Table 5). *Thielaviopsis basicola* was isolated from greater than 80% of the seedlings for the AL, AR1 and AR4 locations. *Fusarium* spp. were isolated from seedlings at all 13 locations (Table 5). Isolation frequencies for *Fusarium* spp. ranged from 46 to 98%.

Table 5. Disease ratings and isolation frequencies of seedling pathogen groups for the 2015 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	3.7	2.5	4.1	6	14 (20)[5]	90	98
AR1	5.8	2.4	2.7	18	8 (---[6])	98	84
AR2	3.7	2.0	3.3	6	12 (64)	6	84
AR4	4.0	3.0	3.4	4	12 (98)	92	94
GA	10.3	2.0	2.0	0	10 (---)	0	80
LA1	3.3	3.1	4.3	2	2 (---)	0	56
LA2	2.3	2.6	4.7	4	14 (53)	0	94
MS1	7.0	2.2	2.1	27	0 (---)	6	76
MS2	6.0	2.2	2.4	10	10 (47)	4	88
OK3	10.0	1.1	2.7	6	12 (---)	0	46
TN	3.7	2.1	3.2	2	6 (64)	13	77
TXQ	11.7	---	---	10	30 (---)	0	94
VA	5.3	2.4	4.4	4	14 (70)	0	98

[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.
[6] Information not available.

Rhizoctonia solani was detection in soil for 6 of the 13 soils assayed, range 0.7 to 2.2 propagules/100 cm^3 of soil (Table 6). *Pythium* spp. were detected in soil at 9 locations for the 10 soils assayed, range 15 to 284 CFU/g of soil (Table 6). *Thielaviopsis basicola* was detected in 5 of the 13 soils assayed, range 7 to 83 CFU/g soil (Table 6). There was a trend for stand for the nontreated seed to be negatively correlated with minimal and mean soil temperature across sites, -0.55 and -0.59 (P=0.0781 and P=0.0546). Isolation of *Thielaviopsis basicola* was positively correlated with soil populations of *Thielaviopsis basicola,* 0.71 (P=0.0067).

Summary

The results from the 13 locations where stand data were collected for the 2015 National Cottonseed Treatment Program indicated that seed treatment fungicides improved stands of cotton compared to a nontreated control for 38% of the locations (5 locations), with an additional 2 locations being significant at P=0.10. Three of the 10 nominated seed treatments increased stand compared to the nontreated control at 5 of the 5 locations where a stand response was observed. All but one of the nominated treatment combinations improved stands at 4 or more of the 5 locations were a stand response was found. In addition, all but one of the nominated treatments increased stand for at least one location compared to the historical standard fungicide seed treatment Vitavax-PCNB + Allegiance.

Table 6. Soil populations of selected soilborne genera from sites in the 2015 National Cottonseed Treatment Program.

Location	*Rhizoctonia solani*	*Pythium* spp.	*Thielaviopsis basicola*
	CFU[1]/100cm^3	CFU/g	CFU/g
AL	2.2[2]	0	83
AR1	0	---[3]	31
AR2	0	15.3	0
AR4	0	30.6	7
GA	0	---	0
LA1	1.4	82.0	0
LA2	0	104.7	0
MS1	0	---	0
MS2	0.7	31.4	12
OK3	0	284.1	0
TN	1.4	32.4	8
TX-Q	0.7	46.4	0
VA	0.7	14.5	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. This work is supported in part by a USDA National Institute of Food and Agriculture Hatch project.

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