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The 2013 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 2013. The results from the 15 locations where stand data were collected for the 2013 National Cottonseed Treatment Program indicated that seed treatment fungicides improved stands of cotton compared to the non-treated control for 47% of the locations (7 locations). Three of the ten nominated seed treatments increased stand compared to the non-treated control at four or five of the seven 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 or more of these seven locations. Average stand for a location was negatively correlated with isolation of *Pythium* spp., -0.75 ($p=0.0120$), and *Fusarium* spp., -0.71 ($p=0.0208$). Isolation of *Thielaviopsis basicola* was positively correlated with the root disease index, 0.83 ($p=0.0028$), and hypocotyl disease index, 0.71 ($p=0.0208$).

Introduction

The 2013 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 2013 National Cottonseed Treatment Program. Two historical standard fungicide treatments, Vitavax-PCNB + Allegiance 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. Disease ratings and pathogen isolations for seedlings and soil populations of selected soil borne genera were conducted by collecting seedlings and soil from the non-treated control plots at each location. Soil temperature and water and plant development data also were collected for sites for the 2013 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), Cruiser 5FS (9 oz/cwt), and dye (Color Coat Red, 1 oz/cwt)(Syngenta Crop Protection) 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 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 non-treated seed by rolling seed in moistened germination paper and incubating for 3 days at 30°C.

Field experiments

Data from the 15 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 6. The stand counts used in the analyses were taken from 27 to 51 days after planting, average 34 days, depending on the location. A soil sample and seedling sample from plots containing non-treated seed were taken from 27 to 51 days after planting, average 34 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 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*.

Table 1. Fungicides, formulations and active ingredients included in the 2013 National Cottonseed Treatment Program.

Common or registered name ¹	Formulation	Active ingredient (%)
A16148C		Syngenta Crop Protection
A9625C		Syngenta Crop Protection
ALLEGIANCE FL (Metalaxyl)	Flowable	28.35% <i>N</i> -(2,6-dimethylphenyl)- <i>N</i> -(methoxyacetyl) alanine methyl ester
APRON XL 3LS (Mefenoxam)	Liquid	33.3% (R,S)-2-[(2,6-dimethylphenyl)methoxyacetyl]amino}-propionic acid methyl ester
DYNASTY CST (Azoxystrobin)	Flowable	6.64% Methyl (E)-2-[[6-(2-cyanophenoxy)pyrimidin-4-yl]oxy]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)methoxyacetyl]amino}-propionic acid methyl ester
EVERGOL EXTEND (Penflufen)	Flowable	14.26% N-[2-(1,3-dimethylbutyl)phenyl]-5-fluoro-1,3-dimethyl-1H-pyrazole-4-carboxamide
(Trifloxystrobin)		14.26% methyl (E)-methoxyimino-[(E)-α-[1-(α,α,α-trifluoro-m-tolyl)ethylideneamino]oxy]-o-tolyl} acetate
EVERGOL PRIME (Penflufen)	Flowable	22.7% N-[2-(1,3-dimethylbutyl)phenyl]-5-fluoro-1,3-dimethyl-1H-pyrazole-4-carboxamide
MAXIM 4FS (Fludioxonil)	Liquid	40.3% 4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile
METLOCK (Metconazole)	Flowable	38-43% 5-[(4-chlorophenyl)methyl]-2,2-dimethyl-1-(1H-1,2,4-triazole-1-ylmethyl)cyclopentanol
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)-1H-1,2,4-triazole-1-ethanol,
RTU PCNB	Flowable	24% Pentachloronitrobenzene
SPERA 240FS (Myclobutanil)	Flowable	22.37% A-butyl-a-(4-chlorophenyl)-1H-1,2,4-triazole-1-propanenitrile
SYSTHANE WSP (Myclobutanil)	Powder	40% A-butyl-a-(4-chlorophenyl)-1H-1,2,4-triazole-1-propanenitrile
TRILEX 2000 (Trifloxystrobin)	Flowable	7.12% methyl (E)-methoxyimino-[(E)-α-[1-(α,α,α-trifluoro-m-tolyl)ethylideneamino]oxy]-o-tolyl} acetate
(Metalaxyl)		5.69% methyl N-(methoxyacetyl)-N-(2,6-xylyl)-DL-alaninate
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
WECO-BOOST		Wilbur-Ellis
WECO-mix		Wilbur-Ellis

¹ Registered chemical name, all capital letters.

Table 2. List of cooperators and procedures for locations in the 2013 National Cottonseed Treatment Program.

Cooperator	Location		Date			Reps.	Row feet counted	Seed planted	Soil temperature ²
			Planted	Sampled	Counted				
K. Lawrence	Auburn, AL	(AL)	4/16	5/21	5/21	5	25	100	20(15)
J. Barham	Rohwer, AR	(AR1)	4/23	5/21	5/21	4	40	120	16(10)
A. Beach	Keiser, AR	(AR2)	5/29	6/25	5/22	6	19.5	100	25(23)
C. Rothrock	Judd Hill, AR	(AR4)	5/16	6/14	6/14	6	50	250	25(20)
R. Kemeraite	Tifton, GA	(GA)	5/7	---	6/4	4	50	150	---
P. Colyer	Bossier City, LA	(LA1)	4/8	5/8	5/8	5	25	100	18(12)
T. Price	Winnsboro, LA	(LA2)	4/16	5/14	5/14	5	25	100	20(13)
G. Lawrence	Mississippi State, MS	(MS1)	4/18	5/22	5/20	5	40	160	15(8)
G. L. Sciumbato	Stoneville, MS	(MS2a)	4/23	5/26	5/28	4	45	180	16(10)
G. L. Sciumbato	Stoneville, MS	(MS2b)	5/8	---	6/4	4	45	180	21(18)
G. L. Sciumbato	Stoneville, MS	(MS2c)	5/15	---	6/12	4	45	180	23(20)
T. Kelly	Perkins, OK	(OK1)	6/11	7/24	7/24	4	20	100	31(26)
R. Thacker	Perkins, OK	(OK2)	6/3	7/24	7/24	4	20	100	28(24)
M. Bayles	Perkins, OK	(OK3)	6/14	7/25	7/24	4	20	100	26(22)
H. Mehl	Suffolk, VA	(VA)	4/18	5/18	5/20	4	60	180	17(10)

¹ Not Available² Mean (Minimum) 4" soil temperature; 3-day average following planting.

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 wrist action shaker for 20 minutes. *Pythium* spp. were quantified by the spread-plate method on the selective medium P₅ARP (Jeffers and Martin, 1986) and *Thielaviopsis 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 89% to 97% for the cultivar DP 1044 B2RF, with an average germination of 93%. For the 15 trials in the 2013 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 8 of the 15 locations (Table 4). However, significant increases in stands for a fungicide treatment compared to the non-treated control were only found for 7 of these locations. This frequency of response, 47%, is slightly higher than recent 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 2013 in stand establishment. The Allegiance treatment increased stands compared to the non-treated control in 1 of these 7 locations (VA) having a significant response, indicating *Pythium* spp. as a group were limiting stand establishment at this location in 2013. At 1 of these 7 locations (AR4), the PCNB treatment increased stands over the non-treated control, indicating *Rhizoctonia solani* was a major factor in stand establishment at this location in 2013. The Vitavax-PCNB + Allegiance historical standard fungicide treatment increased stands compared to the non-treated control at 4 of 7 locations (LA1, MS2a, MS2b, VA). The RTU BaytanThiram + Allegiance FL standard treatment increased stands at 4 of the 7 locations having a fungicide response (AR4, MS2a, MS2b, VA). The nominated products increased stand from 0 to 5 locations where a response was found. The nominated treatment that increased stands over the non-treated control for 5 of the 7 locations where a significant stand response was observed was Apron XL + Maxim 4FS + Systhane WSP + Dynasty CST. Two of

Table 3. Mean squares for the combined analysis of variance across locations, 2013 National Cottonseed Treatment Program.

Source	Degrees of freedom	Mean squares
Location	14	22752 ¹
Replication(Location)	53	497*
Treatment	14	553*
Location*treatment	196	207*
Error	730	112

¹ Significant *F*-test; * *P*<0.0001.

Table 4. Cotton seedling stands for locations of the 2013 National Cottonseed Treatment Program.

Treatment	Rate (oz/cwt)	Plant stand (%)															Mean
		AL	AR1	AR2	AR4	GA	LA1	LA2	MS1	MS2a	MS2b	MS2c	OK1	OK2	OK3	VA	
Apron XL + Maxim 4FS + Systhane WSP + Dynasty CST	7.5+2.5+21.0gai/100kg+0.03mgai/seed	36	17	66	82	87	70	50	61	54	76	86	71	54	53	74	62
Vortex + Allegiance FL + Spera 240 FS + Evergol Prime + Trilex 2000	0.08+0.75+1.8+0.32+1.0	36	12	54	82	87	49	47	77	43	65	87	72	55	60	73	60
Vortex + Allegiance FL + Spera 240 FS	0.08+0.75+1.8	35	38	57	84	84	69	39	65	36	53	80	70	56	60	67	59
Vortex + Allegiance FL + Spera 240 FS + Evergol Prime + Evergol Extend	0.08+0.75+1.8+0.32 + 1.0	39	24	60	85	88	62	46	65	38	63	80	71	51	41	73	59
WECO mix + BOOST	4.15 + 2.0	24	28	66	82	89	64	27	58	25	64	88	68	50	64	67	57
Vortex + Allegiance FL + Spera 240 FS + Evergol Prime	0.08+0.75+1.8+0.32	35	24	56	85	83	54	41	45	37	70	85	66	56	61	64	57
WECO mix	4.15	31	31	59	82	86	65	39	58	32	60	76	66	51	59	63	57
Apron XL+ Maxim 4FS + Systhane WSP + Dynasty CST + A9625C + A16148C	7.5+2.5+21.0 gai/100kg+0.03 mgai/seed+1.0+5.0 gai/100kg	32	31	64	85	86	56	31	61	33	63	84	66	55	53	61	57
Apron XL+Maxim 4FS +Systhane WSP + Dynasty CST + A9625C + A16148C	7.5+2.5+21.0 gai/100kg+0.03mgai/seed+1.0+2.5gai/100kg	34	25	66	79	87	45	46	43	35	53	83	71	58	47	56	55
Vortex + Allegiance FL + Spera 240 FS + Metlock + Rizolex	0.08+0.75+1.8+0.36+0.30	29	21	60	84	85	49	37	40	28	64	86	75	43	51	65	54
RTU BaytanThiram + Allegiance FL	3.0+0.75	32	13	64	86	83	62	46	57	42	83	88	70	54	55	74	60
Vitavax-PCNB + Allegiance FL	6.0+0.75	25	15	57	78	89	70	47	60	43	71	88	60	51	40	65	57
RTU-PCNB	14.5	22	28	51	82	87	54	23	47	39	66	82	70	54	57	36	53
Allegiance FL	1.5	28	17	64	74	84	63	41	44	27	63	71	76	61	54	67	56
Nontreated	---	14	19	47	75	85	51	37	64	24	54	84	70	52	54	44	52
Location average		30	23	59	82	86	59	40	56	36	65	83	69	53	54	63	
Coefficient of Variation (%)		49.6	45.0	18.9	5.9	5.2	22.8	31.9	17.7	31.6	15.8	10.6	10.8	11.8	26.7	15.1	
LSD (P=0.05)		NS	14.7	NS	5.9	NS	17.0	16.0	12.6	16.1	14.6	NS	NS	NS	NS	13.6	

the treatments increased stands over the non-treated seed at 4 of the locations where a stand increase was found; Vortex + Allegiance FL + Spera 240FS + Evergol Prime + Trilex 2000 and Vortex + Allegiance FL + Spera 240 FS. At 3 of the 5 locations where a response was found (AR1, AR4, and MS1) one or more of the nominated fungicide treatments performed significantly better than the historical standard fungicide treatment Vitavax-PCNB + Allegiance. All nominated products increased stand over the historical standard fungicide at one of the sites, except Apron XL + Maxim 4FS + Systhane WSP + Dynasty CST and Apron XL + Maxim 4FS + Systhane WSP + Dynasty CST + A9625C + A16148C (low rate). The treatments increasing stands at 2 of the 7 locations over Vitavax-PCNB + Allegiance were Vortex + Allegiance FL + Spera 240 FS and Apron XL + Maxim 4FS + Systhane WSP + Dynasty CST + A9625C + A16148C (high rate).

Seedling development across the locations at the time of disease assessment and isolation ranged from 2.0 nodes to 9.3 nodes (Table 5). Hypocotyl disease indices ranged from 2.0 at OK1 and OK2 to 3.1 at the AL location, average 2.5 (Table 5). Root disease indices ranged from 2.1 for the OK1 and VA locations to 5.0 for the AL location, average 3.2. *Rhizoctonia solani* was isolated from seedlings from the non-treated plots for 8 of the 10 locations with isolation data (Table 5). *Rhizoctonia solani* was isolated from greater than 20% of the seedlings for the AR2, AR4, and MS2a locations. *Pythium* spp. were isolated from seedlings from 9 of 10 locations with isolation data (Table 5). *Thielaviopsis basicola* was isolated from seedlings at 4 of the 11 locations with isolation data on the modified TB-CEN medium (Table 5). *Thielaviopsis basicola* was isolated from 20% or greater of the seedlings for the AL, AR1, and AR4 locations. *Fusarium* spp. were isolated from seedlings at all 10 locations with isolation data (Table 5). Isolation frequencies for *Fusarium* spp. ranged from 50 to 96%.

Table 5. Disease ratings and isolation frequencies of seedling pathogen groups for the 2013 National Cottonseed Treatment Program locations.

Location	Nodes ²	Disease index		Isolation frequency (%) ¹			
		Hyp. ³	Root ⁴	<i>Rhizoctonia solani</i>	<i>Pythium</i> spp.	<i>Thielaviopsis basicola</i>	<i>Fusarium</i> spp.
AL	3.3	3.1	5.0	---	---	54	---
AR1	3.3	2.9	4.6	0	12	54	96
AR2	2.0	2.8	2.4	52	4	0	64
AR4	5.7	---	---	28	2	96	48
GA	---	---	---	---	---	---	---
LA1	5.0	2.6	3.5	6	8	0	84
LA2	4.0	2.2	3.2	4	10	0	82
MS1	3.7	2.5	3.5	---	---	---	---
MS2a	9.3	2.2	2.5	22	4	12	86
OK1	8.3	2.0	3.5	2	0	0	86
OK2	8.3	2.0	2.1	2	8	0	82
OK3	6.3	2.3	2.5	0	6	0	82
VA	2.1	2.5	2.1	4	4	0	50

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 Data not available.

Rhizoctonia solani was detected in soil at 5 of the 9 soils assayed, range 0.7 to 3.5 propagules/100 cm³ of soil (Table 6). *Pythium* spp. were detected in soil at all but one location for the soils assayed, range 17 to 100 CFU/g of soil (Table 6). *Thielaviopsis basicola* was detected in 3 of the 13 soils assayed, range 7 to 695 CFU/g soil (Table 6). Average stand for a location was negatively correlated with isolation of *Pythium* spp., -0.75 ($p=0.0120$), and *Fusarium* spp., -0.71 ($p=0.0208$). Isolation of *Thielaviopsis basicola* was positively correlated with the root disease index, 0.83 ($p=0.0028$), and hypocotyl disease index, 0.71 ($p=0.0208$).

Table 6. Soil populations of selected soil borne genera from sites in the 2013 National Cottonseed Treatment Program.

Location	<i>Rhizoctonia solani</i> CFU ¹ /100cm ³	<i>Pythium</i> spp. CFU/g	<i>Thielaviopsis basicola</i> CFU/g
AL	0.7	67	695
AR1	---	---	0 ³
AR2	---	28	0
AR4	---	83	156
GA	---	---	---
LA1	2.1	0	0
LA2	0	50	0
MS1	3.5	17	0
MS2a	1.4	100	7
OK1	0	---	0
OK2	2.1	---	0
OK3	0	---	0
VA	0	17	0

¹ Colony forming units.² Information not available.³ Populations not detected in soil sample; less than approximately 4 CFU/g of soil for *Pythium* spp. and 0.5 CFU/g of soil for *Thielaviopsis basicola*.

Summary

The results from the 15 locations where stand data were collected for the 2013 National Cottonseed Treatment Program indicated that seed treatment fungicides improved stands of cotton compared to a non-treated control for 47% of the locations (7 locations). Three of the ten nominated seed treatments increased stand compared to the non-treated control at four or five of the seven 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 or more of these seven locations. Average stand for a location was negatively correlated with isolation of *Pythium* spp. -0.75 ($p=0.0120$) and *Fusarium* spp. -0.71 ($p=0.0208$). Isolation of *Thielaviopsis basicola* was positively correlated with the root disease index, 0.83 ($p=0.0028$), and hypocotyl disease index, 0.71 ($p=0.0208$).

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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