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The 2017 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 2017. The results from the 13 locations where stand data were collected for the 2017 National Cottonseed Treatment Program indicated that seed treatment fungicides improved stands of cotton compared to a nontreated control for 54% of the locations (7 locations). Three of the 7 nominated seed treatments increased stand compared to the nontreated control at 6 of the 7 locations where a stand response was observed. All of the nominated treatment combinations improved stands at 4 or more of the 7 locations where a stand response was found. In addition, all of the nominated treatments increased stand for at least 1 location compared to the historical standard fungicide seed treatment Vitavax-PCNB + Allegiance. Selective fungicide treatments provided a positive response compared to the nontreated control at only 2 locations for control of *Pythium* spp. and 0 locations for control of *Rhizoctonia solani*, suggesting more than one pathogen was frequently responsible for the fungicide responses. Early-season growth (nodes) was negatively

correlated with the root disease index, -0.66 ($P=0.0202$) and positively correlated with mean soil temperature the first 3 days after planting, 0.80 ($P=0.0033$). Soil populations of *Thielaviopsis basicola* were positively correlated with the hypocotyl disease index, 0.85 ($P=0.0004$). These regional studies confirm the importance of seedling diseases, the value of fungicide seed treatments, and the continued improvement of seed treatment chemistries.

Introduction

The 2017 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 2017 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 EverGol Prime 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 nontreated control plots at each location. Soil temperature and moisture and plant development data were also collected for locations included in the 2017 National Cottonseed Treatment Program.

Materials and Methods

Fungicide treatment

Acid-delinted seed of *Gossypium hirsutum* L. cv 'DP 1522 B2XF' 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 Crop Science) in water at a rate of 2.75% 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

Fourteen field trials 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 3 to 8. The stand counts used in the analyses were taken from 28 to 52 days after planting, average 33 days, depending on the location. A soil sample and seedling sample from plots containing nontreated seed were taken from 28 to 35 days after planting, average 30 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) 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 $0.5 \mu\text{l}$ 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 2017 National Cottonseed Treatment Program.

Common or registered name ¹	Formulation	Active ingredient (%)
A21606B (Syngenta)		
AB0271473 (Bayer Crop Science)		
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
EVERGOL PRIME (Penflufen)	Flowable	22.7% <i>N</i> -[2-(1,3-dimethylbutyl)phenyl]-5-fluoro-1,3-dimethyl-1H-pyrazole-4-carboxamide
EVERGOL XTEND (Penflufen) (Trifloxystrobin)	Flowable	14.26% <i>N</i> -[2-(1,3-dimethylbutyl)phenyl]-5-fluoro-1,3-dimethyl-1H-pyrazole-4-carboxamide 14.26% 1-[3-(trifluoromethyl)phenyl]ethylideneamino]oxymethyl]phenyl]acetate
Fluopyram 600FS (Fluopyram)	Flowable	48.4% 2-(trifluoromethyl)benzamide; <i>N</i> -{2-(3-chloro-5-(trifluoromethyl)-2-pyridyl)ethyl}- alpha,alpha,alpha-trifluoro- <i>o</i> -toluamide
Fungicide base (Albaugh LLC) (Myclobutanil)	Flowable	63.34% <i>A</i> -butyl- <i>a</i> -(4-chlorophenyl)-1 <i>H</i> -1,2,4-triazole-1-propanenitrile
(Metalaxyl)		30.25% <i>N</i> -(2,6-dimethylphenyl)- <i>N</i> -(methoxyacetyl) alanine methyl ester
(Fludioxonil)		3.78% 4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1 <i>H</i> -pyrrole-3-carbonitrile
MAXIM 4FS (Fludioxonil) Liquid	Flowable	40.3% 4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1 <i>H</i> -pyrrole-3-carbonitrile
Premium fungicide overtreatment (Albaugh LLC)	Flowable	
RTU BAYTAN-Thiram (Triadimenol)	Flowable	15.3% Tetramethylthiuram disulfide 5% Beta-(4-Chlorophenoxy)-alpha-(1,1-dimethylethyl)-1 <i>H</i> -1,2,4-triazole-1-ethanol,
SP102000026368 (Bayer)	Flowable	
SPERA 240FS (Myclobutanil)	Flowable	22.37% <i>A</i> -butyl- <i>a</i> -(4-chlorophenyl)-1 <i>H</i> -1,2,4-triazole-1-propanenitrile
SYSTHANE 40WSP (Myclobutanil)	Powder	40% <i>A</i> -butyl- <i>a</i> -(4-chlorophenyl)-1 <i>H</i> -1,2,4-triazole-1-propanenitrile
VITAVAX (Carboxin) – PCNB	Flowable	17% 5,6-dihydro-2-methyl- <i>N</i> -phenyl-1,4-oxathiin-3-carboxamide 17% Pentachloronitrobenzene
VORTEX FL (Ipconazole)	Flowable	40.7% 2-[(4-chlorophenyl)methyl]-5-(1-methylethyl)-1-(1 <i>H</i> -1,2,4-triazol-1-yl)methyl)cyclopentanol

¹ Registered chemical name, all capital letters.

Table 2. List of cooperators and procedures for locations in the 2017 National Cottonseed Treatment Program.

Cooperator	Location		Date			Reps.	Row feet counted	Seed planted	Soil temperature ¹
			Planted	Sampled	Counted				
K. Lawrence	Auburn, AL	(AL)	4/20	5/25	5/25	4	25	75	23(18)
T. Spurlock	Rowher, AR	(AR1)	5/16	6/14	7/7	8	20	98	27(17)
W. Barnett	Keiser, AR	(AR2)	4/10	5/9	5/19	6	20	100	20(14)
C. Rothrock	Judd Hill, AR	(AR4)	5/17	6/14	6/14	6	25	125	26(22)
R. Kemerait	Tifton, GA	(GA)	4/18	5/16	5/16	3	50	186	--- ²
P. Colyer	Bossier City, LA	(LA1)	4/17	5/18	5/18	8	20	100	23(19)
P. Price	Winnsboro, LA	(LA2)	4/7	5/8	5/8	5	25	100	21(13)
G. Lawrence	Mississippi State, MS	(MS1)	4/12	5/10	5/10	5	40	165	24(18)
T. Allen	Stoneville, MS	(MS2)	5/8	6/12	6/12	4	70	308	26(21)
M. Bayles	Perkins, OK	(OK3)	6/15	7/14	7/14	4	20	100	29(24)
H. Kelly	Jackson, TN	(TN)	4/19	5/22	5/22	4	60	240	20(16)
J. Woodward	Lubbock, TX	(TXF)	5/22	6/20	---	4	70	280	22(20)
J. Woodward	Halfway, TX	(TXH)	5/17	6/16	---	4	70	280	24(21)
H. Mehl	Suffolk, VA	(VA)	5/15	6/12	6/13	4	60	225	20(16)

¹ Mean (Minimum) 10 cm (4 in.) soil temperature (°C); 3-day average following planting.

² Not Available

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 equivalent) 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 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

For the 13 locations in the 2017 National Cottonseed Treatment Program reporting stand data, there were significant location and treatment effects (Table 3). There was also a significant treatment x location effect suggesting that treatment response was dependent on the environment or pathogen pressures for a particular location.

There was a significant treatment response for 7 of the 13 locations reporting data where a significant increase in stands for a fungicide treatment compared to the nontreated control were found, a frequency of response of 54% (Table 4). 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 2017 in stand establishment. The Allegiance treatment increased stands compared to the nontreated control in 2 (AL, AR1) of these 7 locations having a significant response, indicating *Pythium* spp. as a group was limiting stand establishment at these locations in 2017. The EverGol Prime treatment did not increase stands over the nontreated control at any location in 2017 indicating *Rhizoctonia solani* was not a major factor in stand establishment. Vitavax-PCNB + Allegiance, the historical standard fungicide treatment, increased stands compared to the nontreated control at 3 of the 7 locations having a fungicide seed treatment response, while the RTU BaytanThiram + Allegiance FL standard treatment increased stands at 4 of these 7 locations. The range of stand responses for nominated products was 4 of the 7 locations to 6 of the 7 locations having a fungicide seed treatment response. The nominated treatments that

Table 3. Mean squares for the combined analysis of variance across locations, 2017 National Cottonseed Treatment Program.

Source	Degrees of freedom	Mean squares ¹
Location	12	17871*
Replication(Location)	52	264*
Treatment	11	979*
Location*treatment	132	183*
Error	564	86

¹ Significant *F*-test; * *P*<0.0001

Table 4. Cotton seedling stands for locations of the 2017 National Cottonseed Treatment Program.

Treatment	Rate (oz/cwt)	Plant stand (%)													
		AL	AR1	AR2	AR4	GA	LA1	MS1	MS2	OK3	TN	TXF	TXH	VA	Mean
Allegiance + EverGol Prime + Spera + EverGol XTend + AB0271473	0.75 + 0.33 + 1.8 + 1.0 + 0.16	48	48	72	72	16	76	88	85	56	53	74	86	66	64
Allegiance + EverGol Prime + Spera + AB0271473 + SP102000026368	0.75 + 0.33 + 1.8 + 0.16 + 0.16	48	59	62	70	30	73	89	91	57	52	75	81	65	63
Albaugh Base + Premium Fungicide Overtreatment	2.2 + 4.8	44	61	59	72	17	71	90	88	57	52	73	82	60	62
Apron XL + Maxim + Systhane + A21606B	0.31 + 0.08 + 0.84 + 3.33	49	60	59	72	25	77	93	78	52	55	74	79	62	64
Allegiance + EverGol Prime + Spera + Vortex	0.75 + 0.33 + 1.8 + 0.08	48	54	66	71	16	75	80	80	61	52	78	84	72	65
Apron XL + Maxim + Systhane + A21606B	0.31 + 0.08 + 0.84 + 4.08	40	53	64	69	30	75	83	80	56	52	70	86	82	67
Allegiance + EverGol Prime + Fluopyram + AB0271473	0.75 + 0.33 + 5.6 + 0.16	26	46	66	73	30	72	90	84	48	59	77	81	63	64
RTU-Baytan-Thiram + Allegiance	3.0 + 0.75	33	48	59	70	31	84	86	86	52	49	75	77	66	63
Vitavax-PCNB + Allegiance	6.0 + 0.75	38	32	51	71	29	67	88	82	60	47	75	76	74	61
EverGol Prime	0.64	25	30	51	70	23	75	92	86	49	30	72	64	59	56
Allegiance	1.5	37	33	51	67	19	73	90	79	42	44	59	60	63	55
Nontreated	---	23	24	45	68	27	72	88	79	49	42	65	65	67	55
Location average		38	46	59	70	24	74	88	83	53	49	72	77	67	62
Coefficient of Variation (%)		21.1	18.7	19.8	6.1	28.2	14.0	10.0	5.8	32.7	18.3	7.9	8.3	16.0	15.0
LSD (P=0.05)		11.6	8.5	13.5	NS	NS	NS	NS	7.0	NS	12.9	8.1	9.1	NS	3.2

increased stands over the nontreated control at 6 locations were Allegiance + EverGol Prime + Spera + AB0271473 + SP1020000263698, Albaugh Base + Premium Fungicide Overtreatment, and Apron XL + Maxim + Systhane + A21606B. All 7 of the nominated treatments significantly increased stands compared to the historical standard fungicide treatment Vitavax-PCNB + Allegiance for at least 1 location. The seed treatment Allegiance + EverGol Prime + Spera + EverGol XTend + AB0271473 increased stands over the historical standard fungicide treatment at 3 of the 7 locations.

Seedling development across the locations at the time of disease assessment and isolation ranged from 2.0 nodes to 9.0 nodes (Table 5). Hypocotyl disease indices ranged from 2.1 at GA, MS2, OK3 and VA to 2.8 at the AL location, average 2.2 (Table 5). Root disease indices ranged from 2.1 for the LA1 and MS1 locations to 4.7 for the AR2 location, average 2.7. *Rhizoctonia solani* was isolated from seedlings from the nontreated plots for 9 of the 11 locations (Table 5). *Rhizoctonia solani* was isolated from greater than 15% of the seedlings for the AR1, MS1, OK3, and TN locations. *Pythium* spp. were isolated from seedlings from 9 of 11 locations (Table 5). *Pythium* was isolated from greater than 15% of the seedlings for the AR2 and LA1 locations. Isolation frequencies for *Pythium* spp. increased by plating roots without surface disinfestation on the selective medium P₅ARP (Table 5). *Thielaviopsis basicola* was isolated from seedlings at 3 of the 11 locations using the modified TB-CEN medium (Table 5). *Thielaviopsis basicola* was isolated from greater than 75% of the seedlings at all 3 locations. *Fusarium* spp. were isolated from seedlings at all 11 locations (Table 5). Isolation frequencies for *Fusarium* spp. ranged from 49 to 97%.

Table 5. Disease ratings and isolation frequencies of seedling pathogen groups for the 2017 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	4.0	2.8	3.1	0	2 (6) ⁵	78	49
AR1	4.7	2.3	3.0	26	0 (70)	94	82
AR2	2.0	2.4	4.7	4	38 (---) ⁶	0	82
AR4	5.3	2.2	2.8	12	4 (28)	96	92
GA	5.0	2.1	2.2	0	6 (---)	0	97
LA1	4.7	2.2	2.1	8	18 (74)	0	80
MS1	6.0	2.2	2.1	22	2 (---)	0	72
MS2	7.3	2.1	2.9	12	10 (36)	0	88
OK3	9.0	2.1	2.2	36	12 (26)	0	82
TN	4.0	2.2	2.7	18	6 (66)	0	63
TXF	---	---	---	---	---	---	---
TXH	---	---	---	---	---	---	---
VA	4.7	2.2	2.4	4	0 (38)	0	80

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 P₅ARP.

⁶ Information not available.

Rhizoctonia solani was detected in soil for 2 of the 11 soils assayed, range 0.7 to 1.4 propagules/100 cm³ of soil (Table 6). *Pythium* spp. were detected in soil assays at all 11 locations, range 17 to 435 CFU/g of soil (Table 6). *Thielaviopsis basicola* was detected in 5 of the 11 soils assayed, range 1 to 266 CFU/g soil (Table 6).

Early-season growth (nodes) was negatively correlated with the root disease index, -0.66 ($P=0.0202$) and positively correlated with mean soil temperature the first 3 days after planting 0.80 ($P=0.0033$). Soil populations of *Thielaviopsis basicola* were positively correlated with the hypocotyl disease index, 0.85 ($P=0.0004$). There was a trend for *Pythium* isolation to be associated with an increase in root disease index, 0.53 ($P=0.780$).

Table 6. Soil populations of selected soil borne genera from sites in the 2017 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 ²	17.0	266
AR1	0	31.2	52
AR2	0	76.4	0
AR4	0	76.4	23
GA	0	43.6	0
LA1	1.4	109.1	0
MS1	0	48.2	0
MS2	0	63.8	1
OK3	0.7	435.3	0
TN	0	144.4	4
TX2	--- ³	--- ³	--- ³
TXF	--- ³	--- ³	--- ³
VA	0	133.7	0

¹ Colony forming units.

² Populations not detected in soil sample; less than approximately 0.4 CFU/100 cm³ 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.

Summary

The results from the 13 locations where stand data were collected for the 2017 National Cottonseed Treatment Program indicated that seed treatment fungicides improved stands of cotton compared to a nontreated control for 54% of the locations (7 locations). Three of the 7 nominated seed treatments increased stand compared to the nontreated control at 6 of the 7 locations where a stand response was observed. All of the nominated treatment combinations improved stands at 4 or more of the 7 locations where a stand response was found. In addition, all of the nominated treatments increased stand for at least 1 location compared to the historical standard fungicide seed treatment Vitavax-PCNB + Allegiance. Selective fungicide treatments provided a positive response compared to the nontreated control at only 2 locations for control of *Pythium* spp. and 0 locations for control of *Rhizoctonia solani*, suggesting more than one pathogen was frequently responsible for the fungicide responses. Early-season growth (nodes) was negatively correlated with the root disease index, -0.66 ($P=0.0202$) and positively correlated with mean soil temperature the first 3 days after planting 0.80 ($P=0.0033$). Soil populations of *Thielaviopsis basicola* were positively correlated with the hypocotyl disease index, 0.85 ($P=0.0004$).

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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