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The 2018 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. Eight fungicide seed treatments were nominated by chemical industry representatives for evaluation in 2018. The results from the 12 locations where stand data were collected indicated that seed treatment fungicides improved stands of cotton compared to a non-treated control, on average, across all locations. Although, due to low disease pressure at only 25% of the locations (3 locations: AR, OK, TX1) did treatments significantly improve stand over the non-treated control. Furthermore, only the nominated treatment of Metalaxyl, Maxim, Systhane, and Vibrance CST (higher rate) increased stand over the non-treated at all 3 locations. Five of the 8 nominated seed treatments increased stand compared to the non-treated control at 2 of the 3 locations where a stand response was observed. Selective fungicide treatments provided a positive response compared to the non-treated control at only 1 location (OK) for control of *Pythium* spp. and 2 locations (OK and AR) for control of *Rhizoctonia solani*, suggesting these pathogens were limiting stand establishment at those locations, but other pathogens were responsible for the fungicide response at TX1 location. While isolation frequencies were high in 2018, the levels of pathogens quantified from soil samples was relatively low for *Rhizoctonia solani* and *Thielaviopsis basicola*, which could further support why only 25% of locations saw improved stands from fungicide treatments. Furthermore, the high isolation frequencies from seedlings and high levels of CFU/g soil of *Pythium* spp. in 2018 could be due to misidentifying colonies of *Pythium* spp. and/or high levels nonpathogenic *Pythium* spp. being present. These regional studies confirm the importance of IPM strategies for seedling diseases, the value of fungicide seed treatments, and the continued improvement of seed treatment chemistries and knowledge about soilborne pathogens.

Introduction

The National Cottonseed Treatment Program evaluates cotton seedling survival for a number of fungicide seed treatment combinations that are nominated by chemical industry representatives over diverse environmental conditions and populations of cotton seedling pathogens. Unfortunately, none of the historical standard fungicide treatments, Vitavax-PCNB + Allegiance or RTU Baytan-Thiram + Allegiance, were able to be included in the 2018 trials, but a new standard was tested, which was also a nomination, that included Alleigance + EverGol Prime + Spera + Proline. A non-treated control was included to assess efficacy of the nominations and seedling disease pressure, as well as, the fungicide treatments Allegiance and EverGol Prime 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 non-treated control plots at each location. Soil temperature and plant development data also were collected for locations for the 2018 National Cottonseed Treatment Program.

Materials and Methods

Fungicide Treatment

Acid-delinted seed of *Gossypium hirsutum* L. cv 'DP 1522 B2XF' were provided by Monsanto, St. Louis. Fungicide treatments were mixed with Color Coat Red (1 oz/cwt) and Gaucho 600 (16 oz/cwt) (Bayer Crop Science) in a total slurry rate of 30 oz/cwt (i.e. the amount of water added to each treatment was adjusted to have a 30 oz/cwt slurry). Water, Gaucho 600, and dye also were applied to the nontreated check at the same rate. Treatments were applied to the cottonseed while the seed mixed in a Kobalt 4-cu ft 0.5-HP Cement Mixer (model # SGY-CM1) used as a 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 fungicide treatments 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

Twelve field trials were conducted by 10 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 11 to 41 days after planting, averaging 27 days after planting. A soil sample and ~100 seedlings were collected from plots containing non-treated seed and were collected from 14 to 33 days after planting, averaging 27 days after planting. Soil and seedlings were placed in insulated packages with refrigerated cool packs and mailed overnight to the West Tennessee Research and Education Center 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 data were obtained from the nearest weather station.

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. 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.

Half of seedlings were surface disinfested by immersion for 1.5 min in 0.5% NaClO, blotted dry in a paper towel. Half of the seedlings (H50) were plated on water agar 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 and the other half of the seedlings were plated on the *Pythium* selective media P₅ARP (Jeffers and Martin, 1986). After incubation for 3-5 days at H23°C, resulting colonies from water agar plates were transferred to PDA, incubated another 2-5 days, and then identified to genus. Seedlings originally plated on water agar 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 media TS (Spurlock et al., 2011). Soil populations of *Pythium* spp. and *Thielaviopsis basicola* were detected

by diluting 30 g (wet 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 modified selective medium TB-CEN.

Statistics

Data were analyzed with JMP 12.0.1 (SAS Institute Inc., Cary NC). Percent stand was analyzed over locations and by location by the Fit Model – Standard Least Squares procedure. Treatment means were separated by using Tukey HSD at $P=0.05$. The Pearson product-moment correlation method was used to examine the relationship among soil temperature, early season growth (nodes), percent stand, disease hypocotyl and root ratings, pathogen isolation frequency, and soil populations over locations.

Table 1. Fungicides, formulations, and active ingredients included in the National Cottonseed Treatment Program¹

#	Common or registered name ²	Formulation	Active ingredient (%)	rate (oz/cwt)
2	ALLEGIANCE FL	Flowable	Metalaxyl (28.35)	1.5
3	EVERGOL PRIME	Flowable	Penflufen (22.7)	0.64
4	ALLEGIANCE FL	Flowable	Metalaxyl (28.35)	0.75
	EVERGOL PRIME	Flowable	Penflufen (22.7)	0.33
	SPERA 240FS	Flowable	Myclobutanil (22.37)	1.8
	PROLINE 480 SC	Flowable	Prothioconazole (41.0)	0.16
	SP102000026368 (Bayer)	Flowable		0.16
5	ALLEGIANCE FL	Flowable	Metalaxyl (28.35)	0.75
	EVERGOL PRIME	Flowable	Penflufen (22.7)	0.33
	SPERA 240FS	Flowable	Myclobutanil (22.37)	1.8
	PROLINE 480 SC	Flowable	Prothioconazole (41.0)	0.32
	SP102000026368 (Bayer)	Flowable		0.32
6	EVERGOL XTEND	Flowable	Penflufen (14.26), Trifloxystrobin (14.26)	1.0
7	ALLEGIANCE FL	Flowable	Metalaxyl (28.35)	0.75
	EVERGOL PRIME	Flowable	Penflufen (22.7)	0.33
	SPERA 240FS	Flowable	Myclobutanil (22.37)	1.8
	PROLINE 480 SC	Flowable	Prothioconazole (41.0)	0.16
8	Base fungicide (Albaugh LLC)	Flowable	Myclobutanil (63.34), Metalaxyl (30.25), Fludioxonil (3.78)	2.15
9	Premium fungicide (Albaugh LLC)	Flowable		5
10	METALAXYL 4.0 ST	Emulsion concentration	Metalaxyl (44.08)	0.5
	MAXIM 4 FS	Flowable	Fludioxonil (40.3)	0.08
	SYSTHANE WSP	Wettable Powder	Myclobutanil (40)	0.84
	VIBRANCE CST	Flowable	Mefenoxam (6.71), Azoxystrobin (6.71), Sedaxane (3.13), Fludioxonil (1.12)	3.06
11	METALAXYL 4.0 ST	Emulsion concentration	Metalaxyl (44.08)	0.5
	MAXIM 4 FS	Flowable	Fludioxonil (40.3)	0.08
	SYSTHANE WSP	Wettable Powder	Myclobutanil (40)	0.84
	VIBRANCE CST	Flowable	Mefenoxam (6.71), Azoxystrobin (6.71), Sedaxane (3.13), Fludioxonil (1.12)	4.08

¹ All treatments included GAUCHO 600, Flowable, Imidacloprid (48.7%), at 16 oz/cwt, including nontreated check

² Registered chemical name are in all capital letters

Table 2. List of cooperators and procedures for locations in the 2018 National Cottonseed Treatment Program

Cooperator	Location		Date			Reps.	Row ft counted	Seed planted	Soil temp. ¹
			Planted	Sampled	Counted				
Terry Spurlock	Monticello, AR	AR	5/1	5/31	5/31	8	20	160	75 (65)
Kathy Lawrence	Auburn, AL	AL	4/13	5/14	5/14	4	25	100	73 (71)
Trey Price	Winnsboro, LA	LA1	4/13	5/14	5/14	5	25	125	68 (56)
Tessie Wilkerson and Tom Allen	Stoneville, MS	MS1	4/20	5/23	5/1	4	70	280	67 (62)
Tessie Wilkerson and Tom Allen	Stoneville, MS	MS2	4/20	5/23	5/1	4	70	280	67 (62)
Melanie B. Bayles	Perkins, OK	OK	5/30	7/1	7/1	4	20	80	83 (70)
P. D. Colyer	Bossier City, LA	LA2	4/16	5/16	5/16	7	25	175	65 (51)
Jason Woodward	Halfway, TX	TX1	5/16	5/30	6/18	4	60	240	70 (33)
Jason Woodward	Lubbock, TX (Quaker Farm)	TX2	5/15	5/29	6/14	4	60	240	39 (39)
Jason Woodward	Lubbock, TX (FBRI)	TX3	5/5	5/19	6/15	4	60	240	75 (63)
Heather Kelly	Jackson, TN	TN	5/2	6/1	5/14	5	60	300	68 (62)
Hillary Mehl	Suffolk, VA	VA	5/14	6/13	6/13	4	60	225	79 (71)

¹ Mean (Minimum) soil temp. (°C); 3-day average following planting

Results and Discussion

For the 12 locations in the 2018 National Cottonseed Treatment Program both location, treatment, and their interaction (location x treatment) significantly affected stand data ($P < 0.0001$). Hence, the significant interaction suggests treatment response was dependent on the environment and/or pathogen pressure for a particular location, which is expected. All treated and non-treated seed had a 90% germination, ranging from 90 to 97%.

There was a significant treatment response for 3 of the 12 locations (Table 3). The Allegiance treatment increased stands compared to the non-treated control in 1 (OK) of these 3 locations having a significant response, indicating *Pythium* spp. as a group was limiting stand establishment at this location in 2018. The EverGol Prime treatment also increased stands over the non-treated control at 2 (AR and OK) of the 3 locations indicating *Rhizoctonia solani* was a limiting factor in stand establishment at these locations in 2018. The new standard tested in the 2018 program, Allegiance + EverGol Prime + Spera + Proline, only increased stand over the non-treated check at 1 of the locations (AR). The nominated treatment of Metalaxyl, Maxim, Systhane, and greater rate of Vibrance CST (treatment 11) was the only treatment that increased stand over the non-treated at all 3 locations. Other nominated treatments that increased stand over the non-treated check at 2 locations included treatments 4, 5, 8, and 10; and treatments that increased stand over the non-treated check at 1 location included treatments 6 and 9 (Table 3).

Seedling development across the locations at the time of disease assessment and isolation ranged from 2.2 nodes to 10.0 nodes (Table 4). Hypocotyl disease indices ranged from 1.4 at OK and VA locations to 2.7 at the TN location, averaging 2.0 across all locations (Table 4). Root disease indices ranged from 2.0 at OK location to 5.7 for the LA2 location, averaging 3.8 across all locations. *Rhizoctonia solani* was isolated from seedlings from the non-treated plots

for 9 of the 12 locations (Table 4). *Rhizoctonia solani* was isolated from 20% or more of the seedlings for the AL, LA1, MS1, and TX3 locations. *Pythium* spp. were isolated from seedlings from all 12 locations (Table 4). *Pythium* spp. isolation frequencies ranged from 20 to 100%. This high level of isolation, yet only 1 locations had significant effect on stand from the Allegiance treatment, suggests some colonies may have been misidentified as *Pythium* and/or were not pathogenic *Pythium* species, hence a molecular analysis to further aid in specific pathogen identification would be beneficial. *Thielaviopsis basicola* was isolated from seedlings at 11 of the 12 locations using the modified TB-CEN medium (Table 4). *Thielaviopsis basicola* was isolated from greater than 70% of the seedlings at 7 locations. *Fusarium* spp. were isolated from seedlings at all 12 locations (Table 4). Isolation frequencies for *Fusarium* spp. ranged from 16 to 90%. Similarly, to the *Pythium* spp. identification, the high level of isolation frequencies and yet low stand response to fungicide seed treatments suggest that many of the *Fusarium* identifications were of nonpathogenic species and/or misidentified; hence a more specific identification protocol, either selective *Fusarium* media and/or molecular identification of pathogenic species would more accurately identify pathogenic species of *Fusarium*.

Rhizoctonia solani was detection in 7 of the 7 soils assayed, and ranged 2.2 to 15.8 propagules/100 cm³ of soil (Table 5). *Pythium* spp. were detected in soil assays at all 12 locations, ranging from 61 to 422 CFU/g of soil (Table 5). *Thielaviopsis basicola* was detected in 5 of the 6 soils assayed, ranging from 3.8 to 13.9 CFU/g soil (Table 5).

Summary

The results from the 12 locations where stand data were collected for the 2018 National Cottonseed Treatment Program indicated that seed treatment fungicides improved stands of cotton compared to a non-treated control for 25% of the locations (3 locations), indicating a lower than average disease pressure year. While isolation frequencies were high in 2018, the levels of pathogens quantified from soil samples was relatively low for *Rhizoctonia solani* and *Thielaviopsis basicola*, which could further support why only 25% of locations saw improved stands from fungicide treatments.

Selective fungicide treatments provided a positive response compared to the non-treated control at only 1 location (OK) for control of *Pythium* spp. and 2 locations (OK and AR) for control of *Rhizoctonia solani*, suggesting these pathogens could have limited stand establishment at these locations, but that other pathogens were responsible for the fungicide response at the TX1 location. Only 1 nominated seed treatment (Metalaxyl, Maxim, Systhane, and higher rate of Vibrance CST) increased stand compared to the non-treated control at all 3 locations where a stand response was observed. Five of the 8 nominated treatment combinations improved stands over the non-treated control at 2 of the 3 locations where a stand response was found and all nominated treatments improved stands over the non-treated control at least 1 location. Only 1 nominated treatment (Allegiance, EverGol Prime, Spera, and higher rates of Proline and SP102000026368) increased stand over the new standard fungicide seed treatment (Allegiance + EverGol Prime + Spera + Proline).

Even though high isolation frequencies from seedlings and high levels of CFU/g soil of *Pythium* spp. was report in 2018, this could be due to misidentifying colonies of *Pythium* spp. and/or high levels nonpathogenic *Pythium* spp. being present in locations in 2018. Further supporting evidence that nonpathogenic *Pythium* spp. are being identified is the positive correlation between early season growth (nodes) from seedlings. This same statement about reporting on nonpathogenic species can be said about the other pathogen genera evaluated in the National Cottonseed Treatment program, especially *Fusarium* as indicated by high levels of seedling isolation frequencies for *Fusarium* spp. yet low stand response to fungicide seed treatments. Hence, to better evaluate pathogenic species in future trials molecular techniques and additional selective media will be evaluated to improve pathogenic species identification.

Selective media being used for *Pythium* spp. and *T. basicola* isolations from seedlings do improve identification and analysis of these pathogens. For example in 2018, the trend for *Pythium* spp. population frequencies from seedlings on selective P₅ARP to be associated with increased root and hypocotyl disease indices and *T. basicola* population frequencies from seedlings on TB-CEN decreasing on older or more vigorous seedlings (based on early season growth measure by node counts) illustrate the usefulness of selective media. These regional studies confirm the importance of IPM strategies for seedling diseases, the value of fungicide seed treatments, and the continued improvement of seed treatment chemistries and knowledge about soilborne pathogens.

Table 3. Cotton seedling stands for locations of the 2018 National Cottonseed Treatment Program

Table 3. Cotton-seedling stands for treatments of the EverGol + Allegiance + Proline + Spera + SP102000026368															
No	Treatment	Rate (oz/cwt)	Plant Stand % ¹												
			AR	AL	LA1	MS1	MS2	OK	LA2	TX1	TX2	TX3	TN	VA	Mean
11	Metalaxyl + Maxim 4 FS + Rally 40 + Vibrance CST	0.5 + 0.08 + 0.84 + 4.08	48 a	53	44	86	77	55 b	64	66 a	63	50	73	74	63 a
10	Metalaxyl + Maxim 4 FS + Rally 40 + Vibrance CST	0.5 + 0.08 + 0.84 + 3.06	52 a	54	48	81	74	57 b	70	68 ab	66	55	62	73	63 a
9	Albaugh Premium fungicide	5.0	45 a	50	50	85	78	50 bc	66	60 ab	65	53	66	78	62 a
8	Albaugh base fungicide blend	2.15	49 a	48	50	81	78	62 b	69	63 ab	63	57	59	76	63 a
7	Allegiance + EverGol Prime + Spera + Proline 480	0.75 + 0.33 + 1.8 + 0.16	44 a	52	49	81	76	41 bc	72	64 ab	68	58	58	73	61 a
6	Allegiance + EverGol Prime + Spera + Proline 480 + Evergol Extend	0.75 + 0.33 + 1.8 + 0.16 + 1	49 a	55	34	83	75	40 bc	70	66 ab	63	56	61	75	61 a
5	Allegiance + EverGol Prime + Spera + Proline 480 + SP102000026368	0.75 + 0.33 + 1.8 + 0.32 + 0.32	48 a	57	51	83	73	67 a	65	66 ab	66	56	61	71	64 a
4	Allegiance + EverGol Prime + Spera + Proline 480 + SP102000026368	0.75 + 0.33 + 1.8 + 0.16 + 0.16	51 a	60	46	84	76	56 ab	75	62 ab	62	57	59	73	63 a
3	Evergol Prime	0.64	44 a	53	43	83	77	61 ab	60	56 ab	66	59	53	75	61 ab
2	Allegiance	1.5	19 b	56	39	86	77	64 ab	64	56 ab	61	50	50	68	58 ab
1	Non-treated check (just gaucho)	16.0	13 b	49	47	79	71	25 c	60	51 b	55	49	50	75	52 b
Location average			42	53	45	83	76	53	67	62	64	55	59	74	61
Coefficient of Variation (%)			31.4	6.7	11.5	2.7	2.9	24	7.2	8.7	5.5	6.4	11.4	3.6	5.6
LSD (P=0.05)			12.2	NS	NS	NS	NS	25.1	NS	16.2	NS	NS	NS	NS	9.7

¹ Data were analyzed with JMP 12.0.1 (SAS Institute Inc., Cary NC). Percent stand was analyzed over locations and by location by the Fit Model – Standard Least Squares procedure. Treatment means were separated by using Tukey HSD at $P=0.05$. Treatments with the same column followed by the same letter are not significantly different ($Pd0.05$).

Table 4. Disease ratings and isolation frequencies of seedling pathogen groups for the 2018 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.
AR	--- ⁵	---	---	0	100 (96) ⁶	72 ⁷	16
AL	2.2	1.6	4.0	43	24 (54)	100	61
LA1	3.4	2.0	3.0	26	10 (56)	96	82
MS1	8	2.5	5.1	20	26.8 (98)	0	44
MS2	7.4	2.5	4.8	8	55 (93)	3	28
OK	10	1.4	2.0	0	82 (78)	33	43
LA2	3.6	2.6	5.7	4	38.8 (88)	100	90
TX1	5.2	1.6	4.5	0	30 (86)	100	53
TX2	7.6	1.6	3.6	4	92 (86)	18	51
TX3	---	---	2.9	20	20 (88)	100	69
TN	8.4	2.7	4.4	6	86 (84)	92	30
VA	6.6	1.4	2.3	2	88 (56)	56	69

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 ---Information not available. ⁶ Isolation frequency in parentheses from P₅ARP and outside of parentheses from water agar. ⁷ Only based on 25 seedlings assayed for AR location. Early-season growth (nodes) was negatively correlated with both the seedling population frequency of *T. basicola* and *Fusarium* spp., -0.69 ($P=0.0269$) and -0.73 ($P=0.0172$), respectively; and positively correlated with seedling population frequency of *Pythium* spp. from water agar plating, -0.71 ($P=0.0224$). Soil of populations of *T. basicola* were positively correlated with the minimum soil temperature the first 3 days after planting, 0.80 ($P=0.0562$). As could be expected the hypocotyl and root disease ratings were positively correlated, 0.76 ($P=0.0101$). There was a trend for *Pythium* spp. population frequencies from the selective media (P₅ARP) to be associated with increase in root and hypocotyl disease indices, 0.55 ($P=0.0795$) and 0.56 ($P=0.0924$), respectively.

Table 5. Soil populations of selected soilborne genera from sites in the 2018 National Cottonseed Treatment Program

Location	<i>Rhizoctonia solani</i> propagules/100 cm ³	<i>Pythium</i> spp. CFU ¹ /g	<i>Thielaviopsis basicola</i> CFU/g
AR	3.6	61	12.8
AU	-- ²	347	--
MR	--	228	--
MS1	--	131	--
MS2	--	278	--
OU	2.2	231	7.3
R3	--	422	--
TX1	3.6	67	0 ³
TX2	2.9	72	3.8
TX3	15.8	111	4.7
UT	10.1	308	--
VA	15.8	103	13.9

¹ Colony forming units

² Information not available

³ Populations not detected in soil sample; less than approximately 0.4 propagules/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*.

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 Tennessee Institute of Agriculture nor does it imply registration under FIFRA.

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