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RESULTS FROM THE NATIONAL COTTONSEED TREATMENT PROGRAM

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Introduction

The 2005 National Cottonseed Treatment Program evaluated cotton seedling survival for a number of fungicide seed treatment combinations over diverse environmental conditions and levels and types of cotton seedling pathogens. Twelve fungicide seed treatments were nominated by chemical industry representatives for evaluation in the 2005 National Cottonseed Treatment Program. A standard fungicide treatment, Vitavax-PCNB + Allegiance, and a nontreated control were included to assess efficacy of the nominations and seedling disease pressure. In addition, the fungicide treatments Allegiance or PCNB were included to aid in determining the importance of *Pythium* spp. or *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 2005 National Cottonseed Treatment Program.

Materials and Methods

Fungicide treatment

Acid-delinted seed of *Gossypium hirsutum* L. cv 'DP 451 B/RR' were provided by Delta and Pine Land Company, Scott, MS. DP451 B/RR was planted at all locations. Fungicide treatments were mixed with $CaCO_3$ (7 oz/cwt), polymer (Secure 1 oz/cwt,, Syngenta Inc.), Cruiser 7.5 oz/cwt, and dye (Color Coat Red 1 oz/cwt, Syngenta Inc) in water at a rate of 2.75% (RTU-PCNB 2.86%) water to seed weight (w/w). Water, $CaCO_3$, polymer, Cruiser, 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

Sixteen field experiments were conducted by 15 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 10. The stand counts used in the analyses were taken from 26 to 46 days after planting, average 32 days, depending on the location. A soil sample and seedling sample from plots containing nontreated seed were taken from 26 to 46 days after planting, average 31 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 T. L. Kirkpatrick, Southeast 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. An average of 50 seedlings per location were then rinsed for 20 minutes in running tap water and 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, and 5>50% of the root system discolored. Seedlings were 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 Φl of the miticide Danitol (Valent Chemical Co.) per liter. 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*. An additional set of seedlings was plated on the selective medium P_5ARP (Jeffers and Martin, 1986) following a 20 minute water rinse as another method to examine the isolation frequency for *Pythium* species.

Soil samples were assayed for populations of *Rhizoctonia* species by using the multiple-pellet soil method (Henis et. al., 1978),

and Rhizoctonia populations were quantified on a modified Ko and Hora medium (Ko and Hora, 1971). Soil populations of *Pythium* spp. and *T. basicola* were detected by diluting 25 g 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, and *T. basicola* populations were quantified using the pour-plate method with the selective medium 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 percent stand, disease, pathogen isolation frequency, and soil populations over locations.

Results and Discussion

After the seed were treated with the fungicide treatments, seed germination ranged from 86.5 to 95.5% for the cultivar DP 451 B/RR, with an average germination of 92%. No differences were found among treatments for seed germination.

For the 2005 National Cottonseed Treatment Program, 16 sites reported data. For these 16 locations, there was a significant location, treatment, and location x treatment effect (Table 3), indicating that the treatment response was dependent on the environmental or pathogen pressures for a particular location. A significant difference among treatments was found for 14 of the 16 locations (Table 4). In all of these experiments, at least one of the fungicide treatments performed better than the nontreated control. The Allegiance treatment increased stands compared to the nontreated control in 5 of the 14 experiments having a significant response (AR2, GA, OK1, OK2, TX4), indicating the importance of *Pythium* spp. in stand establishment at these sites. In 5 of these 14 experiments (AR1, AR4, GA, OK1, OK2), the PCNB treatment increased stands over the nontreated control, indicating the importance of *Rhizoctonia solani* in stand establishment at these sites. The Vitavax-PCNB + Allegiance standard fungicide treatment increased stands compared to the nontreated control in 10 of the 14 experiments. The nominated treatments increased stands over the nontreated control from 57% of the sites (8 of 14 sites) to 100% of the sites (14 of 14 sites) depending on the treatment. The treatment giving an increase in stand compared to the nontreated control at all 14 sites where a stand response was found was Apron XL + WECO 0257 + Nu-Flow ND. Two treatments gave increased stands compared to the nontreated control in 13 of the 14 sites were a stand response was found; RTU Baytan Thiram + Allegiance FL + Dynasty CST + Syn 214T and L1480 + L0020 + L1028. At 5 of the 14 sites where a response was found (AR1, AR2, LA2, TX2, VA), some of the nominated fungicide treatments performed significantly better than the historical standard fungicide treatment, Vitavax-PCNB + Allegiance. Baytan 30 + Argent 30 + Allegiance FL performed better that the historical standard at three of the fourteen sites. The number of fungicide treatments significantly increasing stands over the nontreated control ranged from 2 of 12 nominated treatments for VA to 12 of the 12 nominated treatments for the AR2, GA, OK1, OK2 and TX2 sites. The mean stand for a location was not related to locations where stands were increased by fungicide treatments.

Hypocotyl disease indices ranged from 2.0 at the CA site to 2.9 at the LA2 sites, average 2.4 (Table 5). Root disease indices ranged from 2.1 for the CA site to 4.7 for the TN site, average 3.2. *Rhizoctonia solani* was isolated from seedlings from the nontreated plots at 14 of 16 locations (Table 5). *R. solani* was isolated from 20% or greater of the seedlings at 5 locations (AR4, LA2, MS2, OK2, and TN). *Pythium* spp. were isolated from seedlings at all locations (Table 5). Isolation frequencies for *Pythium* spp. were 20% or greater for 6 sites (AR1, AR2, LA2, OK1, TX2 and TX4). Isolation frequencies were increased dramatically by plating roots without surface disinfestation on the selective medium P_5ARP, with all sites having 20% or greater recovery of *Pythium* spp., except AR4 and TX2 (Table 5). *Thielaviopsis basicola* was isolated from seedlings at 6 of the 16 locations on the modified TB-CEN medium (Table 5). *T. basicola* was isolated from over 20% of the seedlings for the AR4, CA, TN and TX2 sites. *Fusarium* spp. were isolated from seedlings at all locations (Table 5). Isolation frequencies for *Fusarium* spp. ranged from 26% to 100%.

Soil populations of *R. solani* were detected at 11 of the 16 sites, range 1.6 to 30.7 CFU/100 g of soil (Table 6). *Pythium* spp. were detected in soils at 14 of the 15 sites assayed, range 17 to 383 CFU/g of soil. *T. basicola* was detected in 6 of the 16 soils assayed, range 1 to 77 CFU/g soil.

The percent stand for the nontreated seed treatment for the locations was negatively correlated with the hypocotyl disease index,, –0.51 (P=0.05). The hypocotyl disease index and the root disease index were positively correlated, 0.65 (P=0.005). The hypocotyl disease index was positively correlated with isolation of *Pythium* spp on P_5ARP, 0.51 (P=0.04). *T. basicola* recovery from seedlings was positively correlated with soil populations of *T. basicola*, 0.53 (P=0.04).

Conclusions

The results from the 16 locations where data was collected for the 2005 National Cottonseed Treatment Program indicated that seed treatment fungicides improved stands of cotton compared to a nontreated control for 88% of the sites. All of the nominated fungicide combinations improved stands over the nontreated seed at most of the sites where a response was found. The percent stand for the nontreated seed treatment for the locations was negatively correlated with the hypocotyl disease index. The hypocotyl disease index was positively correlated with isolation of *Pythium* spp on P_5ARP. *T.* b*asicola* recovery from seedlings was positively correlated with soil populations of *T. 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 Arkansas, Department of Plant Pathology, nor does it imply registration under FIFRA.

References

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Table 1. Fungicides, formulations and the active ingredients included in the 2005 National Cottonseed Treatment Program

Common or registered name[1]	Formulation	Active ingredient (%)
ALLEGIANCE –FL (Metalaxyl)	Flowable	28.35% *N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl) alanine methyl ester
APRON XL (Mefenoxam)	Liquid	33.3% (R)-[(2,6-dimethylphenyl)-methoxyacetylamino]-propionic acid methyl ester
ARGENT 30 (TCMTB)	Liquid	30.0% 2-(Thiocyanomethylthio)benzothiazol
BAYTAN 30 (Triadimenol)	Flowable	30% Beta-(4-Chlorophenoxy)-alpha-(1,1-dimethylethyl)-1*H*-1,2,4-triazole-1-ethanol
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)-2-{(2,6-dimethylphenyl)-methoxyacetylamino}-propionic acid methyl ester
L0020		Gustafson LLC
L0037		Gustafson LLC
L1028		Gustafson LLC
L1226		Gustafson LLC
L1480		Gustafson LLC
NU-FLOW M (Myclobutanil)	Liquid	25% A-butyl-a-(4-chlorophenyl)-1*H*-1,2,4-triazole-1-propanenitrile
NU-FLOW ND (Chloroneb)	Flowable	23.5% 1,4-Dichloro-2, 5-dimethoxy-benzene
RTU BAYTAN-Thiram	Flowable	5% Beta-(4-Chlorophenoxy)-alpha-(1,1-dimethylethyl)-1*H*-1,2,4-triazole-1-ethanol, 15.3% Tetramethylthiuram disulfide
RTU PCNB	Flowable	24% Pentachloronitrobenzene
SYN214D		Syngenta Crop Protection Inc.
SYN214T		Syngenta Crop Protection Inc.
SYSTHANE 40WP (Myclobutanil)	Wettable powder	40-42% A-butyl-a-(4-chlorophenyl)-1*H*-1,2,4-triazole-1-propanenitrile
TRILEX (Trifloxystrobin)	Flowable	22% Methyl (E)-methoxyimino-{(E)-alpha-[1-(alpha, alpha, alpha-trifluoro-m-tolyl) ethylideneaminooxyl]-o-tolyl}acetate
VITAVAX (Carboxin) - PCNB	Flowable	17% 5,6-dihydro-2-methyl-N-phenyl-1,4-oxathiin-3-carboxamide, 17% Pentachloronitrobenzene
WECO 0257		Wilbur-Ellis Company
WECO 4004		Wilbur-Ellis Company

[1] Registered chemical name, all capital letters.

Table 2. List of cooperators and procedures used in the 2005 National Cottonseed Treatment Program

Cooperator	Location		Date Planted	Date Sampled	Date Counted	Reps.	Row length counted (ft)	Seed planted	Soil temperature[1]
K. Lawrence	Auburn, AL	(AL)	4/10	5/11	5/11	5	25	75	18(17)
J. D. Barham	Hope, AR	(AR1)	4/20	5/18	5/18	5	40	200	20(17)[2]
F. Bourland	Keiser, AR	(AR2)	4/18	5/18	5/18	8	25	100	21(17)
C. S. Rothrock	Judd Hill, AR	(AR4)	5/13	6/10	6/10	8	50	250	22(16)
R. Hutmacher	Shafter, CA	(CA)	3/31	5/23	5/4	8	30	125	18(14)
Bob Kemerait	Tifton, GA	(GA)	4/14	5/13	5/13	5	60	300	16(11)
P. D. Colyer	Bossier City, LA	(LA1)	4/28	5/24	5/24	6	25	100	20(13)
B. Padgett	Winnsboro, LA	(LA2)	4/15	5/16	5/16	5	25	100	---
W. E. Batson Jr.	Mississippi State, MS	(MS1)	4/19	5/18	5/18	5	83	250	22(15)
G. Sciumbato	Stoneville, MS	(MS2)	4/4	5/2	5/2	4	45	180	17(16)
L. Verhalen	Tipton, OK	(OK1)	4/21	6/1	6/6	4	20	100	19(14)
L. Verhalen	Altus, OK	(OK2)	4/28	6/1	6/6	4	20	100	17(13)
Melvin Newman	Jackson, TN	(TN)	4/18	5/18	5/18	10	20	100	20(16)
H. W. Kaufman	Lubbock, TX	(TX2)	4/24	6/3	5/30	4	20	100	14(9)[3]
T. S. Isakeit	Beaumont, TX	(TX4)	4/14	5/12	5/12	4	20	100	21(17)
P. M. Phipps	Suffolk, VA	(VA)	4/18	5/16	5/16	4	60	240	19(12)

[1] Mean (Minimum) soil temperature; 3-day average following planting.
[2] Soil temperatures for 4/23/05 only.
[3] Soil temperature at 6 inches.

Table 3. Mean squares for combined analysis of variance across locations, 2002 National Cottonseed Treatment Program

Source	Degrees of freedom	Mean squares
Location	15	24869**[1]
Replication(Location)	74	440**
Treatment	15	1523**
Location*treatment	225	173**
Error	1095	96

[1] ** = significant *F*-test, *P*=0.0001.

Table 4. Cotton seedling stands for locations of the 2005 National Cottonseed Treatment Program.

Treatment	Rate (oz/cwt)	Plant stand (%)																
		AL	AR1	AR2	AR4	CA	GA	LA1	LA2	MS1	MS2	OK1	OK2	TN	TX2		TX4	
VA Mean																		
Baytan 30 + Argent 30 + Allegiance FL	0.5 + 1.5 + 0.75	83	74	77	74	70	84	87	70	55	81	89	89	45	72	79	22	72
L0037 + L1226 + L0020 + L1028	0.5 + 0.64 + 0.75 + 0.086	92	69	77	77	69	85	88	48	64	73	92	89	47	69	88	22	72
Apron XL + WECO 0257 + Nu-Flow ND	0.32 + 0.65 + 8.0	86	72	78	78	63	85	88	48	69	67	90	84	48	68	83	33	71
RTU Baytan Thiram + Allegiance FL + Dynasty CST + Syn 214T	3.0 + 0.75 + 3.95 + 0.5	85	70	81	77	74	80	87	51	68	65	89	86	50	73	82	22	71
L1480 + L0020 + L1028	1.7 + 0.75 + 0.086	90	67	79	78	76	83	83	48	59	73	87	84	42	71	87	31	71
Apron XL + WECO 0257 + Nu-Flow M + Nu-Flow ND	0.32 + 0.65 + 2.5 + 8.0	83	65	71	76	75	84	87	48	70	70	90	90	46	72[2]	85	26	71
RTU Baytan Thiram + Allegiance FL + Dynasty CST + Syn 214D	3.0 + 0.75 + 3.95 + 0.5	94	69	76	75	72	86	86	50	62	75	80	86	48	71	82	23	71
RTU Baytan Thiram + Allegiance FL + Dynasty CST + Synthane 40 WP	3.0 + 0.75 + 3.95 + 0.84	79	69	77	77	76	83	83	41	66	71	90	89	50	69	70	26	70
Apron XL + Nu-Flow ND + Nu-Flow M	0.32 + 14.5 + 2.5	89	71	73	75	62	82	83	65	56	60	91	87	45	74	88	19	70
RTU Baytan Thiram + Allegiance FL	3.0 + 0.75	75	71	77	77	71	84	85	33	62	81	88	85	43	71	84	22	69
WECO 4004 + WECO 0257 + NuFlow ND	0.6 + 0.7 + 8.0	79	68	72	72	76	80	85	49	63	75	76	90	39	69	80	29	69
Vitavax-PCNB + Allegiance FL	6.0 + 0.75	67	67	71	77	72	80	84	30	60	79	87	88	44	62	82	26	67
RTU Baytan Thiram + Trilex FL + Allegiance FL	3.0 + 0.64 + 0.75	79	71	77	74	68	82	82	25	58	59	90	88	46	71	74	27	67
RTU-PCNB	14.5	86	67	63	80	61	72	82	45	66[1]	57	74	84	26	58	73	26	64
Allegiance FL	1.5	85	65	73	65	63	70	79	27	49	50	79	82	39	50	78	25	61
Nontreated	---	102	59	58	66	63	58	76	24	54	43	58	72	31	53	67	24	57
Location average		85	68	74	75	69	79	84	44	61	67	84	86	43	67	80	25	
Coefficient of Variation (%)		16	8	9	9	18	8	6	41	15	21	12	5	26	10	9	19	
LSD (P=0.05)		NS	7	7	7	NS	8	6	23	12	20	15	6	10	10	10	7	

[1] Treatment not significantly different from the nontreated control even though rounding indicates significance.

[2] Treatment not significantly different from the historical fungicide control even though rounding indicates significance.

Table 5. Disease ratings and isolation frequency of seedling pathogen groups for the 2005 National Cottonseed Treatment Program locations

		Disease Index		Isolation frequency (%)[1]			
Location	Nodes[2]	Hyp.[3]	Root[4]	*Rhizoctonia solani*	*Pythium* spp.[5]	*Thielaviopsis basicola*	*Fusarium* spp.
AL	2.0	2.3	4.4	8	18 (86)	16	76
AR1	3.8	2.3	3.4	4	22 (90)	0	72
AR2	2.6	2.8	4.5	14	36 (92)	0	84
AR4	4.8	2.1	2.5	50	8 (14)	33	90
CA	6.4	2.0	2.1	4	12 (67)	40	100
GA	2.2	2.3	4.0	12	12 (61)	0	76
LA1	3.6	2.3	2.8	0	16 (90)	0	100
LA2	3.8	2.9	3.9	20	24 (94)	0	60
MS1	2.6	2.5	3.9	16	8 (60)	2	26
MS2	3.2	2.8	2.4	46	6 (100)	0	80
OK1	6.2	2.4	2.7	0	82 (80)	0	94
OK2	5.5	2.1	2.4	74	14 (36)	0	94
TN	1.8	2.8	4.7	20	10 (82)	100	82
TX2	3.2	2.2	2.4	2	38 (12)	38	86
TX4	3.6	2.2	2.2	16	36 (100)	0	90
VA	1.8	2.2	2.9	12	4 (36)	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, and 5>50% of the root system discolored.

[5] Isolation frequency in parentheses from P_5ARP.

Table 6. Soil populations of selected soilborne genera from sites in the 2005 National Cottonseed Treatment Program

Location	*Rhizoctonia solani*	*Pythium* spp.	*Thielaviopsis basicola*
	CFU[1]/100g	CFU/g	CFU/g
AL	1.6	67	1
AR1	ND2[2]	67	0
AR2	1.6	183	0
AR4	2.5	217	77
CA	ND1	67	0
GA	4.4	ND	0
LA1	ND2	117	0
LA2	15.6	117	0
MS1	8.6	-[3]	0
MS2	30.7	67	4
OK1	2.3	150	0
OK2	ND2	17	5
TN	5.4	183	29
TX2	ND2	50	24
TX4	4.5	383	0
VA	12.5	17	0

[1] Colony forming units.

[2] Populations not detected in soil sample; less than approximately 2.1 (ND1) or 1.4 (ND2) CFU/100 g of soil for *Rhizoctonia solani*, and 8 CFU/g of soil for *Pythium* spp.

[3] Information not available.