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REPORT OF THE COTTONSEED TREATMENT COMMITTEE FOR 2003

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Introduction

The 2003 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. Fourteen fungicide seed treatments were nominated by chemical industry representatives for evaluation in the 2003 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* species or *Rhizoctonia solani*, respectively. Disease ratings and pathogen isolations for seedlings and soil populations of selected soilborne pathogens 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 2003 National Cottonseed Treatment Program.

Materials and Methods

Fungicide Treatment

Acid-delinted neutralized seed of *Gossypium hirsutum* L., 'DP 451 B/RR' or 'PM 2326 RR', were provided by Delta and Pine Land Company, Scott, MS. DP 451 B/RR was planted at all locations, with the exception of locations in Oklahoma and Texas, where the cultivar PM 2326 RR was planted. Fungicide treatments and dye (Color Coat Red, Syngenta Inc.) were mixed with water and applied to seed at a rate of 2.5%, RTU-PCNB 2.6%, (v/w). Water and dye also were applied to the nontreated seed treatment at the same rate. Treatments were applied to the cottonseed while the seed tumbled 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 of all treatments for DP 451 B/RR was evaluated by the Mississippi State Seed Testing Laboratory (Mississippi State, MS). Seed germination of treatments for PM 2326 RR was evaluated by rolling seed in moistened germination paper and incubating at 30°C.

Field Experiments

Nineteen 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 24 to 45 days after planting, average 30 days, depending on the location. A soil sample and seedlings from plots containing nontreated seed were taken from 25 to 45 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 48 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 μl 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 for 15 sites, average 47 seedlings (range 7 to 50), 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, plant development, disease, pathogen isolation frequency, and soil populations over locations.

Results and Discussion

After the seed were treated, seed germination for the cultivar DP 451 B/RR over all treatments averaged 90%, with an average cool test germination of 86%. Seed germination for the cultivar PM 2326 RR averaged 88% and did not differ significantly among treatments.

For the 2003 National Cottonseed Treatment Program, 17 of 19 sites had data reported. For these 17 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 8 of the 17 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 2 of the 8 experiments having a significant response compared to the nontreated control (TN and TX4), indicating the importance of *Pythium* spp. in stand establishment at these sites. In 2 of these 8 experiments (LA1 and LA2), the PCNB treatment increased stands over the nontreated control, indicating the importance of *R. solani* in stand establishment at these sites. The Vitavax-PCNB + Allegiance standard fungicide treatment increased stands compared to the nontreated control in 6 of these 8 experiments (AR1, CA, LA1, LA2, TN and TX2). Nominated treatments increased stands over the nontreated control for 25% of the sites (2 of 8 sites) to 88% of the sites (7 of 8 sites) depending on the treatment. Treatments giving increases in stand compared to the nontreated control at 7 of the 8 sites where a stand response was found were Apron XL + Nu-Flow M + Nu-Flow ND, Dynasty + Systhane 40 WP, Apron XL + WECO 0257 + Nu-Flow ND, Baytan 30 + Argent 30 + Allegiance LS, RTU Baytan-Thiram + Allegiance FL, Apron XL + Nu-Flow M + Nusan 30 + WECO 0257, L0020 + L0288 + L0189, and L1194 + L0030 + Allegiance LS. At 2 of the 8 sites where a response was found (TN and TX4), some of the nominated fungicide treatments performed significantly better than the historical standard fungicide treatment, Vitavax-PCNB + Allegiance. The number of fungicide treatments significantly increasing stands over the nontreated control ranged from 1 of the 14 nominated treatments for the GA site to all of the nominated treatments tested for the LA1 site. The mean stand for a location was not related to locations where stands were increased by fungicide treatments.

Plant development at the time seedlings were sampled ranged from 2.0 to 4.6 nodes, average 3.5 nodes. Hypocotyl disease indices ranged from1.7 at the TX5 site to 3.2 at the AR3 site, average 2.3 (Table 5). Root disease indices ranged from 1.6 at MS2 to 4.2 at TX2, average 2.8. *R. 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 6 locations (AL, AR2, AR3, CA, MS1, and MS2). *Pythium* spp. were isolated from seedlings at 14 of 16 locations (Table 5). Isolation frequencies for *Pythium* spp. were 20% or greater for 2 sites (OK1 and TX3). Isolation frequencies of *Pythium* spp. were increased dramatically by plating roots without surface disinfestation on the selective medium P_5ARP, with all sites with seedlings plated on P_5ARP having 20% or greater recovery of *Pythium* spp. (Table 5). *T. 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 30% of the seedlings for the AL, AR3, CA, and TX2 sites. *Fusarium* spp. were isolated from seedlings at all locations (Table 5). Isolation frequencies for *Fusarium* spp. ranged from 62% to 100%.

Soil populations of *R. solani* were detected at 10 of the 17 sites, range 2.4 to 24.0 CFU/100 g of soil (Table 6). *Pythium* spp. were detected in soils at 15 of the 17 sites, range 3 to 740 CFU/g of soil. *T. basicola* was detected in 6 of the 17 soils assayed, range 4 to 117 CFU/g soil. Nematode counts were conducted for 12 of the 17 sites. The root-knot nematode was detected for the VA site. The reniform nematode was detected for the MS1 and MS2 sites.

The percent stand for the nontreated seed treatment for the locations was negatively correlated with Pythium population, -0.71 (*P*=0.001). The hypocotyl disease index and the root disease index were positively correlated with isolation of *T. basicola* on TB-CEN, 0.73 (*P*=0.001) and 0.54 (*P*=0.03), respectively. Isolation frequency of *Pythium* spp. on water agar and P_5ARP were positively correlated, 0.72 (*P*=0.009)

Conclusions

The results from the 17 locations where data was collected for the 2003 National Cottonseed Treatment Program found that seed treatment fungicides improved stands of cotton compared to the nontreated control for 47% of the sites. All of the nominated fungicide combinations improved stands over nontreated seed at some of the sites where a response was found, with 8 of the 14 nominations increasing stands at 7 of the 8 sites where a response was observed. The percent stand for the nontreated seed treatment for the locations was negatively correlated with Pythium population and Allegiance improved stand for 2 of the locations. PCNB increased stands at 2 of the locations, indicating the importance of *R. solani* for these sites. The hypocotyl disease index and the root disease index were positively correlated with isolation of *T. basicola* on TB-CEN.

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 2003 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
ALLEGIANCE LS (Metalaxyl)	Liquid	17.7% *N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl) alanine methyl ester
APRON XL TL (Mefenoxam)	Liquid	33.3% (R)-[(2,6-dimethylphenyl)-methoxyacetylamino]-propionic acid methyl ester
ARGENT 30 FL (TCMTB)	Liquid	30% 2-(Thiocyanomethylthio) benzothioazole
BAYTAN 30 (Triadimenol)	Flowable	30% Beta-(4-Chlorophenoxy)-alpha-(1,1-dimethylethyl)-1*H*-1,2,4-triazole-1-ethanol
DYNASTY (Azoxystrobin, Fludioxonil, Mefenoxam)	Flowable	6.64% Methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate, 1.11% 1H-Pyrrole-3-carbonitrile, 4-(2,2-difluoro-1,3-benzodioxol-4-yl) 3.32% (R)-[(2,6-dimethylphenyl)-methoxyacetylamino]-propionic acid methyl ester
HM 0233		Helena Chemical Company
HM 0301		Helena Chemical Company
L0020		Gustafson LLC
L0030		Gustafson LLC
L0189		Gustafson LLC
L0288		Gustafson LLC
L1194		Gustafson LLC
L1226		Gustafson LLC
NU-FLOW M HF (Myclobutanil)	Liquid	25% A-butyl-a-(4-chlorophenyl)-1*H*-1,2,4-triazole-1-propanenitrile
NU-FLOW ND (Chloroneb, TCMTB)	Flowable	23.5% 1,4-Dichloro-2, 5-dimethoxy-benzene, 9% 2-(Thiocyanomethylthio) benzothioazole
NUSAN 30 EC (TCMTB)	Liquid	30% 2-(Thiocyanomethylthio) benzothioazole
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
SYSTANE 40WP (Myclobutanil)	Wettable 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
WECO 0257		Wilbur-Ellis Company

[1] Registered chemical name, all capital letters.

Table 2. List of cooperators and procedures used in the 2003 National Cottonseed Treatment Program.

Cooperator	Location		Date			Reps	Row length counted (ft)	Seed planted	Soil temperature[1]
			Planted	Sampled	Counted				
K. McLean	Auburn, AL	(AL)	4/14	5/29	5/29	5	25	125	21(17)
T. L. Kirkpatrick	Hope, AR	(AR1)	4/29	5/28	5/28	5	42	189	24(21)
F. Bourland	Keiser, AR	(AR2)	4/15	5/15	5/15	6	25	100	19(17)
C. S. Rothrock	Clarkedale, AR	(AR3)	4/30	5/28	5/28	8	50	250	21(19)
R. Hutmacher	Shafer, CA	(CA)	4/7	5/13	5/13	8	25	150	20(14)
K. W. Seebold Jr.	Tifton, GA	(GA)	4/14	5/15	5/13	5	25	100	22(17)
P. D. Colyer	Bossier City, LA	(LA1)	4/11	5/12	5/9	5	25	100	19(13)
B. Padgett	Winnsboro, LA	(LA2)	4/14	5/14	5/12	4	25	100	20(15)
W. E. Batson Jr.	Mississippi State, MS	(MS1)	4/14	5/19	5/14	4	83	240	22(16)
G. L. Sciumbato	Stoneville, MS	(MS2)	4/28	5/23	5/22	4	40	224	24(20)
L. Verhalen	Tipton, OK	(OK1)	5/1	6/4	6/4	4	20	100	22(19)
M.A. Newman	Jackson, TN	(TN)	4/15	5/15	5/15	10	20	100	18(14)
H. W. Kaufman	Lubbock, TX	(TX2)	5/2	5/30	5/30	4	35.5	178	22(18)
T. S. Isakeit	Victoria, TX	(TX3)	3/31	4/28	4/28	4	17	100	19(13)
	Beaumont, TX	(TX4)	4/2	4/29	4/29	4	20	100	20(15)
	College Station, TX	(TX5)	4/3	5/1	5/1	4	20	100	21(19)
P. M. Phipps	Suffolk, VA	(VA)	4/15	5/14	5/14	4	60	180	16(12)

[1]Mean (Minimum) soil temperature; 3-day average following planting.

Table 3. Mean squares for combined analysis of variance across locations, 2003 National Cottonseed Treatment Program.

Source	Degrees of freedom	Mean squares
Location	16	34668[**1]
Replication(Location)	71	1369[**]
Treatment	17	1113[**]
Location*treatment	272	185[**]
Error	1204	88

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

Table 4. Cotton seedling stands for locations of the 2003 National Cottonseed Treatment Program.

Treatment	Rate (oz/cwt)	Plant stand (%)																	
		AL	AR1	AR2	AR3	CA	GA	LA1	LA2	MS1	MS2	OK1	TN	TX2	TX3	TX4	TX5	VA	Mean
Apron XL + Nu-Flow M + Nu-Flow ND	1.0+2.5+14.5	71	84	74	74	84	87	60	81	31	64	70	77	85	75	37	15	42	65
Dynasty + Systhane 40 WP	3.1+0.84	65	76	74	67	78	87	65	82	32	58	80	72	81	80	41	15	41	64
Apron XL + WECO 0257 + Nu-Flow ND	1.0+0.6 3+7.5	62	79	74	79	86	89	46	86	36	60	69	78	80	75	34	10	41	64
Baytan 30 + Argent 30 + Allegiance LS	0.5+1.5+1.2	75	79	73	70	80	84	50	83	36	57	71	82	83	63	39	7	39	63
Dynasty	3.1	64	72	73	70	75	86	64	80	30	55	75	74	87	67	41	16	40	63
Apron XL + Nu-Flow M + Nusan 30	1.0+2.5+2.0	68	73[1]	74	74	82	86	67	85	33	62	72	71	79	69	25	10	40	63
RTU Baytan-Thiram + Allegiance FL	3.0+0.75	62	79	70	73	80	85	56	80	32	59	76	76	85	72	33	8	41	63
Apron XL + Nu-Flow M + Nusan 30 + WECO 0257	1.0+2.5+2.0+0.63	73	78	75	71	80	85	52	82	26	61	69	74	82	69	39	6	42	63
Dynasty	3.9	70	79	67	73	80	85	49	74	34	67	68	75	79	75	40	8	38	62
L0020 + L0288 + L0189	0.75+0.2+3.0	57	79	69	71	79	83	68	78	37	66	68	80	81	66	34	9	38	62
Vitavax-PCNB + Allegiance FL	6.0+0.75	61	80	70	73	82	86	60	80	34	70	66	73	82	74	20	6	41	62
L1194 + L0030 + Allegiance LS	6.1+1.5+1.2	61	84	72	73	84	85	59	82	23	51	72	77	81	62	42	11	38	62
L1226 + L0030 + Allegiance LS	0.64+1.5+1.2	59	75	73	74	77[1]	82	53	73	37	46	74	77	83	69	29	15	40	61
RTU PCNB	14.5	63	66	67	67	69	88	66	77	30	61	61	53	79	77	12	23	40	59
HM 0233 + HM 0301	1.5+12	60	66	77	69	71	87	55	79	34	52	74	47	80	74	7	15	43	58
HM 0233	1.5	68	67	78	67	73	72	51	77	31	59	55	44	79	69	3	10	44	56
Allegiance FL	1.5	56	66	64	66	67	75	36	74	25	53	65	68	78	62	26	4	35	54
Nontreated	---	65	63	65	68	66	82	23	67	31	40	62	55	74	66	7	12	37	52
Location average		64	75	72	71	77	84	54	79	32	58	69	70	81	70	28	11	40	63
Coefficient of Variation (%)		19	11	11	12	15	7	23	8	22	25	15	13	6	16	33	80	10	15
LSD (*P*=0.05)		NS	10.0	NS	NS	11.1	7.0	15.7	9.0	NS	NS	NS	7.8	6.4	NS	13.2	NS	NS	

[1] Treatment mean is significantly different from the nontreated control, even though the mean does not appear to be different as a result of rounding.

Table 5. Disease ratings and isolation frequency of seedling pathogen groups for the 2003 National Cottonseed Treatment Program locations.

				Isolation frequency (%)[1]			
		Disease Index		*Rhizoctonia*	*Pythium*	*Thielaviopsis*	*Fusarium*
Location	Nodes[2]	Hyp.[3]	Root[4]	*solani*	spp.	*basicola*	spp.
AL	4.6	2.3	3.8	20	0 (43[6])	32	100
AR1	-[5]	2.0	2.3	14	8 (67)	0	90
AR2	4.0	2.1	2.0	54	8 (70)	2	75
AR3	3.8	3.2	3.9	20	18 (98)	100	86
CA	2.0	2.4	2.5	20	12 (70)	78	92
GA	4.6	2.2	3.7	14	10 (40)	0	84
LA1	4.6	2.0	2.7	2	4 (62)	0	98
LA2	4.2	2.1	2.9	6	6 (66)	0	80
MS1	4.6	2.5	2.3	24	10	2	62
MS2	4.0	2.1	1.6	56	12	0	92
OK1	-	2.4	2.3	0	34 (92)	0	78
TN	2.8	2.6	2.9	6	19 (57)	0	91
TX2	2.0	2.7	4.2	2	2 (28)	58	86
TX3	3.0	2.0	2.1	0	48 (94)	0	84
TX4	2.0	2.1	2.6	16	0	0	92
TX5	2.4	1.7	2.9	6	6	0	88
VA	-	2.4	2.9	-	-	-	-

[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]Information not available.
[6]Isolation frequency from P_5ARP.

Table 6. Soil populations of selected soilborne fungi from sites in the 2003 National Cottonseed Treatment Program.

	Rhizoctonia solani	*Pythium* spp.	*Thielaviopsis basicola*
Location	CFU[1]/100g	CFU/g	CFU/g
AL	ND1[2]	ND2	112
AR1	4.8	130	0
AR2	ND1	15	0
AR3	2.8	99	25
CA	24.0	21	36
GA	ND1	17	0
LA1	2.7	342	0
LA2	8.1	78	0
MS1	10.6	49	0
MS2	4.7	90	4
OK1	ND1	62	0
TN	2.4	152	117
TX2	4.2	ND3	21
TX3	ND1	109	0
TX4	ND1	740	0
TX5	7.0	210	0
VA	ND1		0

[1]Colony forming units.
[2]Populations not detected in soil sample; less than approximately 1 (ND1) CFU/100 g of soil for *Rhizoctonia solani,* and 8 (ND2) or 2 (ND3) CFU/g of soil for *Pythium* spp.