

REPORT OF THE COTTONSEED TREATMENT COMMITTEE FOR 2001

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Introduction

The 2001 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. Sixteen fungicide seed treatments were nominated by chemical industry representatives for evaluation in the 2001 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 fungi were conducted by collecting seedlings and soil from the nontreated control plots at each location. Soil temperature and plant development data also were collected for sites for the 2001 National Cottonseed Treatment Program.

Materials and Methods

Fungicide Treatment

Acid-delinted seed of *Gossypium hirsutum* L., 'Sure-Grow 747' or 'Paymaster 2326 RR' (PM 2326 RR), were provided by Delta and Pine Land Company, Scott, MS. Sure-Grow 747 was planted at all locations, with the exception of locations in Oklahoma and the College Station and Lubbock sites in Texas, where the cultivar PM 2326 RR was planted. Fungicide treatments and dye (DayGlo EPX seed colorant, Gustafson Inc.) were mixed with water at a rate of 2.5% water to seed weight (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 rotating drum. 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 at 30°C.

Field Experiments

Eighteen field experiments were conducted by 17 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 23 to 34 days after planting, average 29 days, depending on the location. A soil sample and seedling sample from plots containing nontreated seed were taken from 27 to 37 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 T. L. Kirkpatrick, Southeast Research and Extension Center, Hope, Arkansas, for determination of populations of plant parasitic nematodes. Soil temperature was monitored by burying a temperature sensor, tidbit (Onset Computer Corp, Pocasset MA), 10 cm. 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. Approximately 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 (2%) amended with 10 mg and 250 mg of the antibiotics rifampicin and ampicillin, respectively, and 0.5 ml 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 were plated on the selective medium P₅ARP (Jeffers and Martin, 1986) following the 20 minute water rinse for some locations 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 0.1% 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, 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 soil temperature, percent stand, plant growth, 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 88% to 98% for Sure-Grow 747, with an average germination of 94%. Six of the fungicide treatments increased germination slightly but significantly, >96% germination, compared to the nontreated seed treatment, 91% germination. Seed germination ranged from 85% to 94% for PM 2326 RR, with an average germination of 89%, after the seed were treated with the fungicide treatments.

For the 2001 National Cottonseed Treatment Program, 3 of the 18 sites were lost due to weather related problems. For the 15 locations reporting data for 2001, 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 between treatments was found for 8 of the 15 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 4 of the 8 experiments having a significant response compared to the nontreated control (CA, LA1, MS2, and VA), indicating the importance of *Pythium* spp. in stand establishment at these sites. In 2 of these 8 experiments (CA and GA), 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 7 of the 8 experiments (AR3, CA, GA, LA1, MS2, TN, and VA). The nominated treatments increased stands over the nontreated control from 12% of the sites (1 of 8 sites) to 100% of the sites (8 of 8 sites) depending on the treatment. Treatments giving increases in stand compared to the nontreated control at all 8 sites where a stand response was found were Apron XL-LS + Maxim 4FS + Nu-flow M and HM 9906. Seven treatments gave increased stands compared to the nontreated control in 7 of the 8 sites where a stand response was found. At 3 of the 8 sites where a response was found (CA, LA2, and TN), some of the nominated fungicide treatments performed significantly better than the historical standard fungicide treatment, Vitavax-PCNB + Allegiance. Nu-Flow ND + Apron XL-TL + Nu-Flow M increased stand over the historical standard fungicide treatment at all 3 sites. Baytan 30 + Ascend 30 + Allegiance LS and RTU Baytan Thiram + Allegiance FL + L1080 + L1072 increased stand over the historical standard fungicide treatment for 2 of the 3 sites that increases were found. The number of fungicide treatments significantly increasing stands over the nontreated control ranged from 6 of the 16 nominated treatments for the MS2 site to all of the nominated treatments tested for the VA site. The mean stand for a location was not related to locations where stands were increased by fungicide treatments.

Hypocotyl disease indices ranged from 2.1 at TN to 3.3 at OK1, average 2.5 (Table 5). Root disease indices ranged from 2.0 at TX1 and VA to 3.8 at MS2, average 2.8. *R. solani* was isolated from seedlings from the nontreated plots at 12 of 15 locations (Table 5). *R. solani* was isolated from over 20% of the seedlings at 6 locations (AL, AR3, LA1, LA2, MS2, and TN). *Pythium* spp. were isolated from seedlings at all locations (Table 5). Isolation frequencies for *Pythium* spp. were 20% or greater for 2 sites (LA1 and TX1). Isolation frequencies were increased dramatically by plating roots without surface disinfestation on the selective medium P₅ARP (Table 5). *Thielaviopsis basicola* was isolated from seedlings at 8 of the 15 locations on the modified TB-CEN medium (Table 5). *T. basicola* was isolated from over 50% of the seedlings for the AL, AR3, MS1, OK1, and TX2 sites. *Fusarium* spp. were isolated from seedlings at all locations (Table 5). Isolation frequencies for *Fusarium* spp. ranged from 42% to 96%.

Soil populations of *R. solani* were detected at 2 of the 15 sites, range 6 to 18 CFU/100 g of soil (Table 6). *Pythium* spp. were detected in soils from 14 of 15 sites, range 18 to 483 CFU/g of soil. *T. basicola* was detected in 6 of the 15 soils assayed, range 2 to 493 CFU/g soil. *Meloidogyne incognita*, the root-knot nematode, was detected in the soil sample from the LA2 site and *Rotylenchulus reniformis*, the reniform nematode, was detected in the soil sample from the MS2 site.

The percent stand for the nontreated seed treatment for the locations was negatively correlated, -0.52 ($P=0.05$), with soil populations of *Pythium* spp. The hypocotyl disease index and the root disease index were positively correlated, 0.65 ($P=0.01$). *T. basicola* recovery from seedlings was positively correlated with soil populations of *T. basicola*, 0.85 ($P=0.0001$). Isolation frequency of *R. solani* and *Pythium* spp. were weakly positively correlated with soil assays for populations for each genus, 0.49 ($P=0.06$) and 0.50 ($P=0.07$), respectively.

Conclusions

The results from the 15 locations where data was collected for the 2001 National Cottonseed Treatment Program indicated that seed treatment fungicides improved stands of cotton compared to a nontreated control for the majority of the sites. All of the nominated fungicide combinations improved stands over the nontreated seed at one or more of the sites where a response was found. Allegiance alone increased stands for 4 of the 8 sites where a stand response was found, indicating an important role for *Pythium* spp. in these tests. This is supported by the negative correlation between mean stand for the nontreated seed treatment and soil populations of *Pythium* spp. Isolation frequencies of *T. basicola*, *R. solani*, and *Pythium* spp. from seedlings were positively correlation with soil populations for each of these genera.

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 2001 National Cottonseed Treatment Program.

Common or registered name¹	Formulation	Active ingredient (%)
ALLEGIANCE -FL(Metalaxyl)	Flowable	28.35% <i>N</i> -(2,6-dimethylphenyl)- <i>N</i> -(methoxyacetyl) alanine methyl ester
ALLEGIANCE -LS(Metalaxyl)	Liquid	17.7% <i>N</i> -(2,6-dimethylphenyl)- <i>N</i> -(methoxyacetyl) alanine methyl ester
APRON XL-LS (Mefenoxam)	Liquid	33.3% (R)-[(2,6-dimethylphenyl)-methoxyacetyl-amino]-propionic acid methyl ester
APRON XL-TL (Mefenoxam)	Liquid	33.3% (R)-[(2,6-dimethylphenyl)-methoxyacetyl-amino]-propionic acid methyl ester
ASCEND 30 (TCMTB)	Liquid	30% 2-(thiocyanomethylthio)benzothiazole
BAYTAN 30 (Triadimenol)	Flowable	30% Beta-(4-Chlorophenoxy)-alpha-(1,1-dimethylethyl)-1 <i>H</i> -1,2,4-triazole-1-ethanol
CGA301940		Syngenta Crop Protection, Inc.
DIVIDEND XL (Difenoconazole & CGA-329351)	Liquid	16.5% 1 <i>H</i> -1,2,4-Triazole, 1[[2-[2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-yl]methyl]-, 1.38% (R)-[(2,6-dimethylphenyl)-methoxyacetyl-amino]-propionic acid methyl ester
HM 011501-A		Helena Chemical Company
HM 2049		Helena Chemical Company
HM 9906		Helena Chemical Company
HM 9703		Helena Chemical Company
L1072		Gustafson Incorporated
L1080		Gustafson Incorporated
MAXIM 4FS (Fludioxonil)	Liquid	42% 4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1 <i>H</i> -pyrrole-3-carbonitrile
NU-FLOW M (Myclobutanil)	Wettable powder	40% <i>A</i> -butyl- <i>a</i> -(4-chlorophenyl)-1 <i>H</i> -1,2,4-triazole-1-propanenitrile
NU-FLOW ND (Chloroneb & TCMTB)	Flowable	23.5% 1,4-dichloro-2,5-dimethoxy-benzene 9.0% 2-(thiocyanomethylthio)benzothiazole
PROTÉGÉ 70WP (Azoxystrobin)	Wettable powder	70% methyl (<i>E</i>)-2-{2-[6-(2-cyanophenoxy) pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate
RTU BAYTAN-Thiram	Flowable	5% Beta-(4-Chlorophenoxy)-alpha-(1,1-dimethylethyl)-1 <i>H</i> -1,2,4-triazole-1-ethanol, 15.3% Tetramethylthiuram disulfide
RTU PCNB	Flowable	24% Pentachloronitrobenzene
VITAVAX (Carboxin) - PCNB	Flowable	17% 5,6-dihydro-2-methyl- <i>N</i> -phenyl-1,4-oxathiin-3-carboxamide, 17% Pentachloronitrobenzene
WECO 0118		Wilbur-Ellis Company
WECO 01B50		Wilbur-Ellis Company

¹ Registered chemical name, all capital letters.

Table 2. List of cooperators and procedures used in the 2001 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)	4/11	5/9	5/9	5	25	125	25(21)
T. L. Kirkpatrick	Hope, AR	(AR1)	4/27	- ²		-	-	-	-
R. Benson	Keiser, AR	(AR2)	4/17	5/14	5/15	4	80	300	17(7)
C. S. Rothrock	Clarkedale, AR	(AR3)	5/1	5/30	5/30	6	50	250	23(19)
R. B. Hutmacher	Shafter, CA	(CA)	4/6	5/10	5/10	8	25	125	15(13)
K. Seebold	Tifton, GA	(GA)	4/12	5/11	5/11	4	20	100	24(21)
P. D. Colyer	Bossier City, LA	(LA1)	4/6	5/8	5/4	5	25	100	21(19)
B. Padgett	Winnsboro, LA	(LA2)	4/17	5/17	5/17	4	25	100	17(13)
W. E. Batson Jr.	Mississippi State, MS	(MS1)	4/10	5/10	5/10	4	40	120	22(20)
G. L. Sciumbato	Stoneville, MS	(MS2)	4/6	5/9	5/4	5	40	200	23(20)
L. Verhalen &	Tipton, OK	(OK1)	5/10	6/11	6/11	4	22	100	24(19)
B. E. Greenhagen	Altus, OK	(OK2)	5/11	-		-	-	-	-
	Perkins, OK	(OK3)	5/10			-	-	-	-
A. Y. Chambers	Jackson, TN	(TN)	4/26	5/29	5/29	10	24	100	21(16)
P. M. Thaxton	College Station, TX	(TX1)	4/10	5/8	5/3	8	30	100	-
H. W. Kaufman	Lubbock, TX	(TX2)	5/1	5/29	5/29	4	35.5	178	-
T. S. Isakeit	Weslaco, TX	(TX3)	4/17	5/24	5/15	4	18	100	18(16)
P. M. Phipps	Suffolk, VA	(VA)	4/26	5/24	5/24	4	60	180	16(10)

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

²Information not available.

Table 3. Mean squares for combined analysis of variance across locations, 2001 National Cottonseed Treatment Program.

Source	Degrees of freedom	Mean squares
Location		14
48417 ^{**1}		
Replication(Location)	64	220 ^{**}
Treatment	19	1654 ^{**}
Location*treatment	266	238 ^{**}
Error	1215	85

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

Table 4. Cotton seedling stands for locations of the 2001 National Cottonseed Treatment Program.

Treatment	Rate (oz/cwt)	Plant stand (%)															
		AL	AR2	AR3	CA	GA	LA1	LA2	MS1	MS2	OK1	TN	TX1	TX2	TX3	VA	Mean
Nu-Flow ND + Apron XL-TL + Nu-Flow M	7.5 + 1.0 + 0.84	41	39	77	65	56	66	27	80	56	66	86	15	8	48	73	54
Baytan 30 + Ascend 30 + Allegiance LS	0.5 + 1.5 + 1.2	36	40	75	69	69	61	24	84	44	56	77	20	10	34	72	51
RTU Baytan Thiram + Allegiance FL	3.0 + 0.75	42	44	72	64	65	65	18	82	44	55	78	14	12	45	70	51
Protégé 70 WP + Ascend 30 + Allegiance LS + Baytan 30	0.07 + 1.5 + 1.2 + 0.25	45	30	76	61	62	59	8	84	56	57	82	18	16	40	65	51
Apron XL-TL + WECO 01B50 + Nu-Flow M	1 + 0.5 + 0.84	40	35	72	66	62	59	20	84	44	63	81	17	6	45	66	51
Apron XL-LS + Maxim 4FS + Nu-Flow M	0.32 + 0.08 + 0.84	43	23	76	61	68	60	18	74	47	60	83	15	13	42	67	50
RTU Baytan Thiram + Allegiance FL + L1080 + L1072	3.0 + 0.75 + 0.5 + 9.1	40	39	74	63	38	57	30	66	52	61	78	24	13	40	70	50
Protégé 70 WP + Ascend 30 + Allegiance LS	0.07 + 1.5 + 1.2	34	48	72	47	58	64	13	79	43	59	81	22	6	43	71	49
Apron XL-LS + Maxim 4FS + CGA301940	0.32 + 0.08 + 3.1	40	34	68	52	64	59	17	83	43	54	74	23	10	38	63	48
HM 9906	12.0	44	34	72	48	71	63	16	70	50	57	74	15	9	40	64	48
Apron XL-LS + Maxim 4FS + Dividend XL	0.32 + 0.08 + 1.0	30	34	68	48	60	53	8	81	43	60	76	24	7	45	68	47
Apron XL-LS + Maxim 4FS + CGA301940	0.32 + 0.08 + 1.5	37	29	71	43	56	65	16	75	48	64	73	21	7	38	63	47
HM 9703	12.0	35	35	65	50	69	54	11	81	44	61	70	18	14	35	61	47
WECO 0118 + WECO 01B50	2.0 + 0.5	38	30	68	36	60	33	20	69	34	58	72	21	9	38	58	43
HM 2049 + 011501-A	12.0 + 12.0	35	40	60	42	56	25	16	83	28	60	69	19	8	41	56	43
HM 2049	12.0	36	33	62	35	54	17	4	72	42	61	72	20	4	42	56	41
Vitavax-PCNB + Allegiance FL	6.0 + 0.75	40	30	70	50	64	65	11	77	56	61	78	16	12	37	68	49
Allegiance FL	1.5	39	31	60	46	43	40	8	74	48	50	66	19	3	35	65	42
RTU-PCNB	14.5	41	28	61	38	61	12	6	68	30	58	72	20	11	46	45	40
Nontreated	---	34	42	55	27	43	12	2	71	34	44	66	20	3	25	47	35
Location average			38	35	69	51	59	49	15	77	44	58	75	19	9	40	63
Coefficient of Variation (%)		19	34	10	18	19	21	62	14	21	16	11	57	76	22	10	
LSD ($P=0.05$)	00	NS	NS	8.3	9.2	15.9	13.3	12.7	NS	11.6	NS	7.3	NS	NS	NS	8.7	

Table 5. Disease ratings and isolation frequency of seedling pathogen groups for the 2001 National Cottonseed Treatment Program locations.

Location	Nodes ²	Disease Hyp. ³	Index Root ⁴	Isolation frequency (%)			
				<i>Rhizoctonia solan</i>	<i>Pythium</i> spp.	<i>Thielaviopsis Basicola</i>	<i>Fusarium</i> spp
AL	- ⁵	2.2	3.6	28	10	54	82
AR2	-	2.7	3.6	0	4	0	92
AR3	3.4	2.5	2.4	43	2 (57) ⁶	71	80
CA	3.2	-	-	8	2 (61)	18 ⁷	94
GA	2.2	2.3	2.9	2	13 (83)	0	77
LA1	3.8	2.6	2.9	29	29 (100)	16	84
LA2	3.4	2.3	2.2	34	2	0	80
MS1	2.4	2.4	2.4	0	12 (78)	60	42
MS2	3.4	2.8	3.8	26	14 (33)	0	82
OK1	-	3.3	3.3	0	4 (26)	69	90
TN	4.6	2.1	2.1	22	10	2	84
TX1	5.8	2.2	2.0	2	34 (90)	0	84
TX2	1.8	2.9	3.7	6	10	82	80
TX3	-	2.6	3.0	2	12	0	92
VA	-	2.2	2.0	4	2	0	96

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 water agar.

Table 6. Soil populations of selected soilborne fungi from sites in the 2001 National Cottonseed Treatment Program.

Location	<i>Rhizoctonia solan</i> CFU ¹ /100g	<i>Pythium</i> spp. CFU/g	<i>Thielaviopsis basicola</i> CFU/g
AL	ND ²	37	2
AR2	ND	15	0
AR3	18	108	60
CA	ND	293	493
GA	ND	166	0
LA1	ND	298	0
LA2	ND	350	0
MS1	ND	18	8
MS2	ND	92	0
OK1	ND	- ³	0
TN	ND	88	4
TX1	6	483	0
TX2	ND	ND	64
TX3	ND	247	0
VA	ND	50	0

¹ Colony forming units.

² Populations not detected in soil sample; less than approximately 3 CFU/100 g of soil for *Rhizoctonia solani*, and 8 CFU/g of soil for *Pythium* spp.

³ Information not available.