

REPORT OF THE COTTONSEED TREATMENT COMMITTEE FOR 1995

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Introduction

The 1995 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 1995 National Cottonseed Treatment Program. A standard fungicide treatment Vitavax-PCNB + Apron and a nontreated control were included to assess efficacy of the nominations and seedling disease pressure. In addition, the fungicide treatments Apron 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 populations of selected soilborne fungi in soil also were included in the 1995 National Cottonseed Treatment Program by collecting seedlings and soil from the nontreated control plots at each location.

Materials and Methods

Fungicide Treatment

Acid-delinted seed of *Gossypium hirsutum* L., 'Deltapine 50' or 'Paymaster HS26' (Delta and Pine Land Company, Scott, MS), were planted at all locations. Deltapine 50 was planted at all locations, with the exception of locations in Oklahoma and Texas where the cultivar Paymaster HS26 was planted. Fungicide treatments were mixed with water at a rate of 2% water to seed weight (v/w). Water also was 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.

Field Experiments

Nineteen field experiments were conducted by 18 cooperators across the U.S. Cotton belt. However, data was collected from only 17 sites in 1995 (Table 2). Sites in South Carolina and Oklahoma (OK2) were abandoned. Soils were naturally infested with seedling disease pathogens for all experiments. Each location utilized a randomized complete block experimental design, with the

number of replications ranging from 4 to 10 (Table 2). The stand counts used in the analyses were taken from 27 to 47 days after planting, average 31 days, depending on the location. A soil sample, approximately 250 grams, and seedling samples from plots containing nontreated seed were taken from 27 to 58 days after planting, average 33 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.

Seedling Disease and Pathogen Isolation

Approximately 50 seedlings (range 42 to 61 seedlings) per location were 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 on 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 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 (4), 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 multiple-pellet soil method (1), and *Rhizoctonia* populations were quantified on a modified Ko and Hora medium (3). Soil populations of *Pythium* species 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 (2), 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). Analysis of percent stand over locations indicated a significant location by treatment interaction (Table 3), thus subsequent analyses were done by location. Treatment means for a location were separated by using a protected LSD at $P=0.05$. The Pearson-product correlation method was used to examine the relationship among stand, disease, pathogen isolation frequency, and soil populations over locations.

Results and Discussions

Seed germination for nontreated seed was 94% and 95% for Deltapine 50 and Paymaster HS26, respectively. After the

seed were treated with the fungicide treatments, seed germination ranged from 90% to 99% for Deltapine 50, with an average germination of 94%. Seed germination ranged from 88% to 97% for Paymaster HS26, with an average germination of 93%, after the seed were treated with the fungicide treatments. There was no consistent effect of any of the treatment combinations on germination.

There was a significant location, treatment, and location x treatment effect when cotton stands were analyzed over locations (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 11 of the 17 experiments (Table 4). In all of these experiments at least one of the fungicide treatments performed better than the nontreated control. In 5 of these 11 experiments (AR1, AR2, OK3, TN, and TX1) the Apron treatment increased stands compared to the nontreated control. In 6 of the 11 experiments (AR1, AR3, OK1, OK3, TN, TX1) the PCNB treatment increased stands over the nontreated control. The number of fungicide treatments significantly increasing stands over the nontreated control ranged from 1 of the 16 treatments for the CA site to 16 of the 16 nominated treatments for six sites (AR1, OK1, OK3, TN, TX1, TX2). The Vitavax-PCNB + Apron FL standard fungicide treatment increased stands compared to the nontreated control in 8 of the 11 experiments where significant stand differences were found among treatments. All nominated treatments increased stands over the nontreated control at 8 or more of these 11 experiments, with the exception of the Busan 30AU + Baytan treatment which increased stands at only 7 sites. The treatment Nuflow ND + Apron TL (7.5 + 2.0) increased stands at all 11 sites, while the treatment Apron 350F-A + Dividend + Maxim 4FS (0.9 + 1.25 + 0.08) increased stands at 10 of the 11 sites. Most of the nominated treatments increased stands at 9 of the 11 sites where significant stand differences were found. Several of the nominated fungicide treatments performed significantly better than the standard fungicide treatment for at least one location. The mean stand for a location was not related to locations where stands were increased by fungicide treatments.

Hypocotyl disease indices ranged from 1.5 at MS1 to 3.3 at FL, average 2.4 (Table 5). Root disease indices ranged from 1.3 at MS1 and CA to 4.2 at OK3, average 2.5. Some discoloration of seedlings appeared to have occurred during shipping for the GA, LA1, and TX2 locations. *R. solani* was isolated from seedlings in the nontreated plots at 13 of 17 locations (Table 5). *R. solani* was isolated from 78% of the seedlings at the AR1 site, and 8 locations had isolation frequencies greater than 25%. *Pythium* spp. were isolated from seedlings at 15 of 17 locations (Table 5). Four locations had isolation frequencies for *Pythium* spp. of 25% or greater. *Thielaviopsis basicola* was isolated from seedlings at eight locations on the modified TB-CEN medium (Table 5). The greatest frequency of isolation of

this pathogen from seedlings was 100% and 94% at LA2 and TN, respectively. Four locations had isolation frequencies for *T. basicola* of 25% or greater. *Fusarium* spp. were isolated from seedlings at all locations (Table 5). Isolation frequencies for *Fusarium* spp. ranged from 42% to 96%. *Macrophomina phaseolina* was isolated from seedlings at 10 locations, only CA, MS1, and OK3 had isolation frequencies above 5%.

Soil populations of *R. solani* were detected at 4 of the 12 sites assayed, range 5 to 31 CFU/100 g of soil (Table 6). *Pythium* was detected in 12 of the 14 soils assayed, range 8 to 192 CFU/g of soil. *T. basicola* was detected at 6 of the 16 soils assayed, range 1 to 160 CFU/g soil.

The nontreated seed stand for the locations was negatively correlated with the hypocotyl and root disease indices, -0.71 ($P=0.01$) and -0.51 ($P=0.05$), respectively. The hypocotyl and root disease indices were positively correlated with each other, 0.60 ($P=0.01$). The hypocotyl disease index also was positively correlated with isolation frequency of *R. solani*, 0.61 ($P=0.01$). The population and isolation frequency of *T. basicola* were positively correlated, 0.88 ($P=0.0001$). Similarly, the population and isolation frequency of *Pythium* spp. were positively correlated, 0.59 ($P=0.05$).

Conclusions

The results from 17 locations in the 1995 National Cottonseed Treatment Program indicated that seed treatment fungicides consistently improved stands of cotton compared to a nontreated control. A number of fungicide combinations gave increased plant stands, however, some fungicide combinations gave more consistent stand responses than other fungicide combinations among the locations. Differences in disease severity and the frequency of pathogen isolation over locations may partially explain the variation in cotton seedling survival observed among the fungicide treatments. *Rhizoctonia solani*, *Pythium* spp., and *Fusarium* spp. were isolated frequently from seedlings over all or most locations. *Thielaviopsis basicola* was isolated from seedlings from approximately half of the locations. Disease indices were negatively correlated with plant stand over locations, and the hypocotyl disease index was positively correlated with isolation frequency of *R. solani*.

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 1995 National Cottonseed Treatment Program.

Common or registered name ¹	Formulation	Active ingredient (%)
APRON FL (Metalaxyl)	Flowable	28.4%
<u>N</u> -(2,6-dimethylphenyl)- <u>N</u> -(methoxyacetyl) alanine methyl ester		
APRON 350F-A (Metalaxyl)	Flowable	33.3%
<u>N</u> -(2,6-dimethylphenyl)- <u>N</u> -(methoxyacetyl) alanine methyl ester		
APRON TL (Metalaxyl)	Liquid	11.5%
<u>N</u> -(2,6-dimethylphenyl)- <u>N</u> -(methoxyacetyl) alanine methyl ester		
BAYTAN 30 (Triadimenol)	Flowable	3 0 %
Beta-(4-Chlorophenoxy)-alpha-(1,1-dimethylethyl)-1 <u>H</u> -1,2,4-triazole-1-ethanol		
BUSAN 30AU (TCMTB)	Emusifiable conc.	30% 2-
(thiocyanomethylthio)benzothiazole		
DIVIDEND (Difenoconazole)	Flowable	35.6%
1-{2-[4-(4-chlorophenoxy)-2-chlorophenyl-(4-methyl-1,3-dioxolan-2-yl)-methyl]}-1 <u>H</u> -1,2,4-triazole		
FR-19 (Triadimenol + Thiram)	Flowable	5 %
Beta-(4-Chlorophenoxy)-alpha-(1,1-dimethylethyl)-1 <u>H</u> -1,2,4-triazole-1-ethanol,		1 5 %
Tetramethylthiuram disulfide		
MAXIM4FS	Flowable	42% 4-
(2,2-difluoro-1,3-benzodioxol-4-yl)-1 <u>H</u> -pyrrole-3-carbonitrile		
NU-FLOW AD (Chloroneb & 1,4-dichloro-2,5-dimethoxybenzene, (Metalaxyl)	Flowable	3 0 %
<u>N</u> -(2,6-dimethylphenyl)- <u>N</u> -(methoxyacetyl) alanine methyl ester		3.5%
NU-FLOW M (Myclobutanil)	Emusifiable conc.	2 5 %
alpha-n-Butyl-alpha-(4-chlorophenyl)-1 <u>H</u> -1,2,4-triazole-1-propanenitrile		
NU-FLOW ND (Chloroneb & 1,4-dichloro-2,5-dimethoxybenzene, 2-(thiocyanomethylthio)benzothiazole TCMTB)	Flowable	23.5%
		9.0%
NUSAN 30 (TCMTB)	Emusifiable conc.	30% 2-
(thiocyanomethylthio)benzothiazole		
RTU-PCNB (FR-12)	Flowable	2 4 %
Pentachloronitrobenzene		
RTU-BAYTAN-THIRAM	Flowable	5 %
Beta-(4-Chlorophenoxy)-alpha-(1,1-dimethylethyl)-1 <u>H</u> -1,2,4-triazole-1-ethanol,		1 5 %
Tetramethylthiuram disulfide		
Thiram 42-S	Flowable	4 2 %
Tetramethylthiuram disulfide		
Thiram 75WDG	Wettable granule	7 5 %
Tetramethylthiuram disulfide		
VITAVAX (Carboxin) - PCNB	Flowable	1 7 %
5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide, Pentachloronitrobenzene		1 7 %

¹Registered chemical name, all capital letters.

Table 2. List of cooperators and procedures used in the 1995 National Cottonseed Treatment Program.

Cooperator	Location		Planted	Date		Counted	Reps.	Row length counted (ft)	Seed planted
				Sampled					
W. S. Gazaway	Auburn, AL	(AL)	4/14	5/15	5/15	6	10	70	
T. L. Kirkpatrick	Hope, AR	(AR1)	4/19	5/16	5/16	5	40	200	
G. Palmer	Keiser, AR	(AR2)	4/19	6/7	6/5	8	20	120	
C. S. Rothrock	Clarkedale, AR	(AR3)	5/16	6/15	6/15	6	50	250	
M. R. Davis	Shafter, CA	(CA)	5/2	5/31	5/30	6	25	120	
F. M. Shokes	Quincy, FL	(FL)	3/22	4/22	4/21	6	23	100	
D. R. Sumner	Tifton, GA	(GA)	3/27	4/26	4/26	5	40	100	
P. D. Colyer	Bossier City, LA	(LA1)	4/27	5/24	5/24	6	25	100	
K. S. McLean	Monroe, LA	(LA2)	4/18	5/17	5/17	5	40	240	
W. E. Batson	Mississippi State, MS	(MS1)	5/8	6/12	6/12	5	80	240	
G. L. Sciumbato	Stoneville, MS	(MS2)	4/19	5/18	5/18	4	40	200	
L. Verhalen &	Tipton, OK	(OK1)	5/17	6/21	6/21	4	20	100	
B. E. Greenhagen	Perkins, OK	(OK3)	5/15	6/19	6/19	4	20	100	
A. Y. Chambers	Jackson, TN	(TN)	4/28	5/30	5/29	10	20	100	
P. M. Thaxton	College Station, TX	(TX1)	4/11	5/15	5/11	8	32	96	
H. W. Kaufman	Lubbock, TX	(TX2)	4/12	6/9	5/10	6	37	222	
T. S. Isakeit	Weslaco, TX	(TX3)	2/23	3/22	3/22	4	20	100	

Table 3. Mean squares for combined analysis of variance across locations, 1995 National Cottonseed Treatment Program.

Source	Mean squares	Degrees of freedom
Location	31,515 ¹	16
Replication(Location)	496 [*]	80
Treatment	1,449 [*]	19
Location*treatment	176 [*]	304
Error	103	1517

¹* = significant F-test, P=0.0001.

Table 4. Cotton seedling stands for locations of the 1995 National Cottonseed Treatment Program.

Treatment	Rate (fl.oz. form/cwt)	Plant stand (%)																
		AL	AR1	AR2	AR3	CA	FL	GA	LA1	LA2	MS1	MS2	OK1	OK3	TN	TX1	TX2	TX3
Apron 350F-A + Dividend + Maxim 4FS	0.9 + 1.0 + 0.08	40	52	48	57	81	23	66	76	50	85	56	74	60	68	82	51	84
Apron 350F-A + Dividend + Maxim 4FS	0.9 + 1.25 + 0.08	36	56	41	58	74	32	76	77	49	82	51	72	57	69	82	55	75
Baytan 30 + Apron FL + Thiram 42S	0.5 + 0.75 + 2.0	33	46	38	58	76	34	66	75	46	82	54	77	63	71	84	55	80
Baytan 30 + Apron FL + Thiram 42S	1.0 + 0.75 + 2.0	40	61	40	61	80	23	73	76	66	81	53	74	65	72	82	51	80
Busan 30AU + Baytan	2.5 + 0.5	42	58	18	60	75	16	71	71	66	84	53	80	68	61	80	63	80
Busan 30AU + Baytan + Apron FL	2.5 + 0.5 + 1.0	42	58	44	63	83	38	70	81	67	83	49	82	68	67	83	59	85
FR 19 + FR 12 + Apron FL	2.0 + 3.0 + 0.75	42	62	35	52	82	28	76	72	68	85	53	74	58	72	78	56	74
Nuflow AD + Nuflow M + Nusan 30	5.75 + 2.5 + 2.0	43	61	42	54	84	23	64	72	40	83	56	75	58	69	83	55	79
Nuflow ND + Apron TL	5.0 + 2.0	47	58	41	51	69	32	67	78	53	83	48	74	72	66	78	62	84
Nuflow ND + Apron TL	7.5 + 2.0	45	58	41	58	87	28	78	76	59	85	49	80	70	59	81	59	68
Nusan 30 + Nuflow M + Apron TL	2.5 + 1.75 + 2.0	41	63	36	50	72	25	66	74	46	80	60	70	64	70	83	61	85
Nusan 30 + Nuflow M + Apron TL	2.0 + 1.25 + 2.0	48	58	42	52	81	32	68	78	81	83	59	78	70	69	79	62	74
Nusan 30 + Nuflow M + Apron TL	2.0 + 2.5 + 2.0	38	57	34	60	80	24	71	72	55	83	53	73	68	69	85	53	76
RTU Baytan-Thiram + Apron FL	3.0 + 0.75	40	64	40	59	76	33	70	81	62	84	53	71	62	66	82	47	75
Thiram 75WDG + Nuflow M + Apron TL	1.35 + 1.75 + 2.0	40	64	43	54	78	24	67	72	64	83	57	71	61	70	83	53	82
Thiram 75WDG + Nuflow M + Apron TL	1.35 + 1.25 + 2.0	41	54	42	57	80	27	70	74	67	81	53	74	65	67	80	56	67
Nontreated	---	31	41	15	42	76	19	61	61	57	81	53	58	32	41	52	34	65
Apron FL	1.5	34	47	46	40	76	30	65	59	60	80	55	66	51	63	67	44	72
RTU-PCNB	14.5	41	59	12	55	69	17	54	71	47	84	45	74	49	50	71	42	78
Vitavax-PCNB + Apron FL	6.0 + 0.75	45	58	40	52	82	24	51	73	68	84	50	76	64	66	80	53	72
Location average		41	57	37	55	78	27	68	73	58	83	53	74	61	65	79	54	77
LSD 0.05		NS ¹	5	11	11	10	NS	14	10	NS	NS	NS	10	8	9	6	12	NS
Coefficient of Variation, %		25	12	31	17	11	43	17	12	36	5	16	10	9	15	7	16	19

¹F test not significant (P=0.05)

Table 5. Disease ratings and isolation frequency of seedling pathogen groups for the 1995 National Cottonseed Treatment Program locations.

Location	Disease Index		Isolation frequency (%) ¹			
	Hypocotyl ²	Root ³	R. Pythium solani	Pythium spp.	T. Fusarium basicola	Fusarium spp.
AL	2.6	3.0	40	6	0	78
AR1	3.2	3.1	78	2	0	42
AR2	2.2	2.0	0	8	0	94
AR3	3.1	2.8	48	27	46	65
CA	1.8	1.3	6	10	2	90
FL	3.3	3.2	31	28	0	57
GA	2.0	2.9	10	22	0	80
LA1	2.0	3.3	10	4	0	80
LA2	2.3	2.9	18	2	100	56
MS1	1.5	1.3	26	14	12	78
MS2	2.2	2.3	38	2	33	75
OK1	2.3	2.1	8	0	19	96
OK3	2.5	4.2	0	21	0	92
TN	3.0	3.0	26	10	94	70
TX1	2.3	1.6	26	26	6	90
TX2	2.4	2.1	0	67	0	83
TX3	1.6	1.6	0	0	0	46

¹ Isolation frequency is based on approximately 50 seedlings per location.

² Hypocotyl disease severity index; 1=no symptoms, 2=few pinpoint lesions or diffuse discolored areas, 3=distinct necrotic lesion, 4=girdling lesion, and 5=seedling dead.

³ Root disease 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.

Table 6. Soil populations of selected soilborne fungi from sites in the 1995 National Cottonseed Treatment Program.

Location	Rhizoctonia solani	Pythium spp.	Thielaviopsis basicola
	CFU ¹ /100g	CFU/g	CFU/g
AL	-- ²	17	0
AR1	--	67	0
AR2	ND ³	33	0
AR3	5	157	51
CA	ND	--	0
FL	6	192	0
GA	ND	50	0
LA1	ND	8	0
LA2	31	42	62
MS1	--	150	7
MS2	--	--	--
OK1	ND	ND	1
OK3	ND	17	0
TN	ND	100	160
TX1	7	33	0
TX2	ND	--	2
TX3	--	ND	0

¹ Colony forming units.

² Soil samples were not assayed.

³ Populations were not detected in the soil sample.