REPORT OF THE COTTONSEED TREATMENT COMMITTEE FOR 1997

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Introduction

The 1997 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. Thirteen fungicide seed treatments were nominated by chemical industry representatives for evaluation in the 1997 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 soil populations of selected soilborne fungi also were included in the 1997 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 the College Station and Lubbock sites in 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

Twenty field experiments were conducted by 17 cooperators across the U.S. Cotton belt (Table 2). However, the South Carolina and Hope, Arkansas, sites were abandoned, cooperators J. D. Mueller and T. L. Kirkpatrick, respectively. 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 9 (Table 2). The stand counts used in the analyses were taken from 28 to 52 days after planting, average 32 days, depending on the location. A soil sample and seedling samples from plots containing nontreated seed were taken from 28 to 52 days after planting, average 32 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.

Seedling Disease and Pathogen Isolation

Approximately 49 seedlings (range 41 to 53 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 (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 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* 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 P_5ARP (Jeffers and Martin, 1986), 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.

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Results and Discussion

Seed germination for nontreated seed was 90% and 82% for Deltapine 50 and Paymaster HS26, respectively. After the seed were treated with the fungicide treatments, seed germination ranged from 85% to 96% for Deltapine 50, with an average germination of 91%. Seed germination ranged from 78% to 90% for Paymaster HS26, with an average germination of 83%, after the seed were treated with the fungicide treatments. There were no significant differences among the treatment combinations for 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. The treatment Apron XL + Maxim 4FS + Dividend was not applied to Paymaster HS26 seed and thus stand data is not presented. In addition, not all treatments were planted at the TX3b site as a result of a lack of space. A significant difference between treatments was found for 12 of the 18 experiments (Table 4). In all of these experiments at least one of the fungicide treatments performed better than the nontreated control. In 8 of these 12 experiments (FL2, LA1, OK1, OK2, OK3, TX1, TX2 and TX3b), the Apron FL treatment increased stands compared to the nontreated control. In 5 of the 12 experiments (FL2, MS1, OK1, OK3, TN), the PCNB treatment increased stands over the nontreated control. The Vitavax-PCNB + Apron FL standard fungicide treatment increased stands compared to the nontreated control in 9 of the 12 experiments where significant stand differences were found among treatments (FL2, LA1, MS1, 0K1, OK3, TN, TX1, TX2, and TX3b). The nominated treatments increased stands over the nontreated control for 73% of the sites (8 of 11 sites) to all of the sites (11 or 12 sites) depending on the treatment. Only four of the nominated treatments did not give significant stand increases at all sites where stand responses were found; Apron XL + Maxim + Dividend (6 of 7 sites), Apron XL + Maxim + Nu-Flow M (11 of 12 sites), Captan + Nu-Flow M + Apron XL (11 of 12 sites), and HM9703 (8 of 11 sites). Specific nominated fungicide treatments performed significantly better than the standard fungicide treatment, Vitavax-PCNB + Apron FL, at nine locations (AR3, FL2, LA2, MS1, OK1, OK2, OK3, TN, and TX3b). The Baytan + Thiram + Apron FL treatment increased stands above the standard fungicide treatment for 6 of the 12 sites a response was found. Other treatments frequently giving stand responses over the standard fungicide treatment included; LS140 + Apron FL (5 of 12 sites), HM 9542A (4 of 11 sites), Baytan + LS001 + Apron FL (4 of 12 sites), Nusan + Maxim + Nu-Flow M + Apron XL (4 of 12 sites), and Apron XL + Maxim + Dividend (2 of 7 sites). The number of fungicide treatments significantly increasing stands over the nontreated control ranged from 9 of the 13 nominated treatments for the AR3 site to all the nominated treatments tested for 9 sites (FL2, LA1, LA2, MS1, 0K1, OK3, TX1, TX2, and TX3b). The mean stand for a location was not related to locations where stands were increased by fungicide treatments.

Hypocotyl disease indices ranged from 1.8 at GA to 3.0 at OK3, average 2.4 (Table 5). Root disease indices ranged from 2.0 at TX3b to 4.8 at AL, average 3.0. Some discoloration of seedlings appeared to have occurred during shipping for the FL1 location. *R. solani* was isolated from seedlings in the nontreated plots at 16 of 17 locations (Table 5). *R. solani* was isolated from 58% of the seedlings at the AR3 site, and 5 locations had isolation frequencies greater than 25% (AR3, FL2, LA2, MS1, TN). *Pythium* spp. were isolated from seedlings at 16 of 17 locations (Table 5). Isolation frequencies for *Pythium* spp. were less than 25% for all sites. *T. basicola* was isolated from seedlings at seven locations on the modified TB-CEN medium (Table 5). Five sites had isolation frequencies above 75% (AR3, LA2, OK1, OK2, and TX2). *Fusarium* spp. were isolated from seedlings at all locations (Table 5). Isolation frequencies for *Fusarium* spp. ranged from 52% to 100%. *Macrophomina phaseolina* was isolated from seedlings at seven locations. Only the AL site had an isolation frequency above 10%.

Soil populations of *R. solani* were detected at 7 of the 17 sites assayed, range 6 to 26 CFU/100 g of soil (Table 6). *Pythium* spp. were detected in soils from 14 of 17 sites assayed, range 17 to 733 CFU/g of soil. *T. basicola* was detected in 7 of the 17 soils assayed, range 4 to 92 CFU/g soil.

The mean stand for the locations was negatively correlated with Pythium soil populations, -0.54 ($\underline{P}$=0.02). Hypocotyl disease severity was positively correlated with Pythium soil populations. These correlation support the data from the Apron only treatment which gave substantial increases in stands for 6 sites (OK1, OK2, OK3, TX1, TX2, TX3b). Soil populations and isolation frequency of $\underline{T}$. *basicola* were positively correlated, 0.50 ($\underline{P}$=0.04).

Conclusions

The results from 18 locations in the 1997 National Cottonseed Treatment Program indicated that seed treatment fungicides consistently improved stands of cotton compared to a nontreated control. Most of the nominated fungicide combinations improved stands over the nontreated seed at all sites where a response was found. Apron alone increased stands for 8 sites, indicating an important role for *Pythium* spp. in these tests. This is supported by the negative correlations between mean stand and soil Pythium populations. PCNB increased stands at 5 sites indicating a role for *R. solani* in seedling disease at these sites. 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 from seedlings over all or most locations. *Thielaviopsis basicola* was isolated from seedlings from several locations and soil populations and isolation frequency were positively correlated.

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 1997 National Cottonseed Treatment Program.

Common or registered name[1]	Formulation	Active ingredient (%)
APRON FL (Metalaxyl)	Flowable	28.35% *N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl) alanine methyl ester
APRON XL (Mefenoxam)	Liquid	32.3% (R,S)-2-[(2,6-dimethylphenyl)-methoxyacetylamino]-propionic acid methyl ester
BAYTAN 30 (Triadimenol)	Flowable	30% Beta-(4-chlorophenoxy)-alpha-(1,1-dimethylethyl)-1*H*-1,2,4-triazole-1-ethanol
Captan 4000	Flowable	38.7% N-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide
DIVIDEND (Difenoconazole)	Flowable	32.8% 1-{2-[4-(chlorophenoxy)-2-chlorophenyl-(4-methyl-1,3-dioxolan-2-yl) -methyl]}-1*H*-1,2,4-triazole
HM-9542-A		Helena Chemical Company
HM-9703		Helena Chemical Company
LS001		Gustafson Incorporated
LS140		Gustafson Incorporated
MAXIM 4FS	Flowable	42% 4-(2,2-difluoro-1,3-benzdioxol-4-yl)-1*H*-pyrrole-3-carbonitrile
NU-FLOW M (Myclobutanil)	Emusifiable conc.	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, 9.0% 2-(thiocyanomethylthio)benzothiazole
NUSAN 30 (TCMTB)	Emusifiable conc.	30% 2-(thiocyanomethylthio)benzothiazole
RTU-PCNB	Flowable	24% Pentachloronitrobenzene
RTU-BAYTAN-Thiram	Flowable	5% Beta-(4-chlorophenoxy)-alpha-(1,1-dimethylethyl)-1*H*-1,2,4-triazole-1-ethanol, 15.3% Tetramethylthiuram disulfide
Thiram 42-S	Flowable	42% Tetramethylthiuram disulfide
VITAVAX (Carboxin) - PCNB	Flowable	17% 5,6-dihydro-2-methyl-N-phenyl-1,4-oxathiin-3-carboxamide, 17% Pentachloronitrobenzene
WE 120C		Wilbur-Ellis Company

[1] Registered chemical name, all capital letters.

Table 2. List of cooperators and procedures used in the 1997 National Cottonseed Treatment Program.

Cooperator	Location		Date Planted	Date Sampled	Date Counted	Reps.	Row length counted (ft)	Seed planted
W. S. Gazaway	Auburn, AL	(AL)	4/9	5/12	5/12	6	10	67
G. Palmer	Keiser, AR	(AR2)	4/18	6/9	6/9	9	25	150
C. S. Rothrock	Clarkedale, AR	(AR3)	5/6	6/4	6/4	7	50	250
F. M. Shokes	Quincy, FL	(FL1)	4/25	5/27	5/27	6	23	100
	Marianna, FL	(FL2)	5/7	6/7	6/7	6	23	100
D. R. Sumner	Tifton, GA	(GA)	3/25	4/25	4/25	5	40	100
P. D. Colyer	Bossier City, LA	(LA1)	4/17	5/19	5/19	6	25	100
K. S. McLean	Monroe, LA	(LA2)	4/18	5/19	5/16	5	40	200
W. E. Batson	Mississippi State, MS	(MS1)	5/12	6/9	6/9	5	83	240
G. L. Sciumbato	Stoneville, MS	(MS2)	4/18	5/19	5/19	5	40	200
L. Verhalen &	Tipton, OK	(OK1)	5/5	6/5	6/5	4	22	100
B. E. Greenhagen	Altus, OK	(OK2)	5/5	6/5	6/5	4	22	100
	Perkins, OK	(OK3)	5/6	6/5	6/5	4	20	100
A. Y. Chambers	Jackson, TN	(TN)	5/2	6/4	6/3	9	20	100
P. M. Thaxton	College StationTX	(TX1)	4/10	5/13	5/8	8	30	100
H. W. Kaufman	Lubbock, TX	(TX2)	5/2	6/4	5/30	4	35.5	178
T. S. Isakeit	Weslaco, TX	(TX3a)	3/3	4/2	4/2	4	20	100
	Weslaco, TX	(TX3b)	3/23	4/24	4/24	4	20	100

Table 3. Mean squares for combined analysis of variance across locations, 1997 National Cottonseed Treatment Program.

Source	Degrees of freedom	Mean squares
Location	17	35996*[1]
Replication(Location)	86	255*
Treatment	16	3,674*
Location*treatment	257	326*
Error	1300	100

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

Table 4. Cotton seedling stands for locations of the 1997 National Cottonseed Treatment Program

Treatment	Rate (fl oz/cwt)	Plant Stand (%) AL	AR2	AR3	FL1	FL2	GA	LA1	LA2	MS1	MS2	OK1	OK2	OK3	TN	TX1	TX2	TX3a	TX3b
APRON XL + MAXIM 4FS + DIVIDEND	0.43 + 0.08 + 1.0	47.3	24.7	65.0	14.2	59.7	50.6	44.7	69.9	81.0	75.4	----	----	----	23.7	----	----	86.8	81.2
APRON XL + MAXIM 4FS + NU-FLOW M	0.43 + 0.08 + 1.31	48.3	23.3	65.5	8.2	66.8	42.4	52.0	62.0	79.1	63.8	58.2	61.5	40.5	26.4	59.6	61.0	83.2	84.5
BAYTAN 30 + LS001 + APRON FL	0.5 + 2.0 + 0.75	46.8	32.3	70.7	13.8	60.8	47.6	46.2	70.3	80.2	79.3	69.8	53.5	60.5	29.3	69.6	73.4	89.5	82.8
BAYTAN 30 + THIRAM 42S + APRON FL	1.0 + 2.0 + 0.75	42.5	25.7	67.4	15.2	65.7	41.8	46.2	71.4	78.6	77.8	59.5	65.5	63.5	27.6	63.8	71.1	87.2	83.5
Captan 4000 + NU-FLOW M + APRON XL	2.5 + 1.25 + 0.32	56.2	26.9	67.0	10.2	58.2	60.8	46.7	70.9	79.7	74.6	63.5	53.8	66.2	24.8	66.9	69.3	90.2	81.8
HM 9542A	10	55.0	24.7	68.8	9.5	58.0	54.0	44.3	71.0	78.1	73.9	69.0	59.5	71.2	25.4	73.2	62.5	83.2	----
HM 9703	12	56.0	27.4	58.9	11.3	55.3	59.6	39.5	68.4	74.8	76.4	50.8	50.8	45.2	16.0	63.2	63.5	82.2	----
LS140 + APRON FL	10.25 + 0.75	57.0	21.9	69.2	8.8	60.5	43.2	42.3	68.4	79.0	74.0	62.0	64.0	67.8	28.4	69.6	71.5	90.0	85.2
NU-FLOW ND + NU-FLOW M + APRON XL	7.5 + 1.75 + 0.32	52.0	23.0	72.2	16.0	64.5	52.4	48.8	68.8	73.5	77.2	64.0	55.0	70.0	32.9	68.1	66.8	85.0	----
NUSAN 30 + MAXIM 4FS + NU-FLOW M + APRON XL	2.0 + 0.08 + 1.75 + 0.32	45.3	26.9	70.7	8.8	71.8	47.6	50.2	69.9	79.5	75.9	57.5	62.5	62.0	31.0	65.1	68.2	85.2	81.8
NUSAN 30 + NU-FLOW M + APRON XL	4.0 + 1.75 + 0.32	55.5	18.8	68.2	12.7	62.8	55.6	46.8	75.8	76.9	72.3	56.2	54.2	33.2	27.1	56.5	62.1	80.2	81.0
RTU BAYTAN-THIRAM + APRON FL + THIRAM 42S	3.0 + 0.75 + 1.0	51.2	28.1	71.8	13.0	61.3	55.4	44.7	63.5	68.1	68.1	54.2	64.8	64.7	25.2	69.1	67.6	82.0	79.2
WE 120C + NU-FLOW M + APRON XL	0.48 + 1.25 + 0.32	61.7	24.8	68.1	10.5	64.0	41.4	50.2	71.8	74.8	75.5	60.0	53.5	54.8	25.0	70.1	64.2	91.5	----
VITAVAX-PCNB + APRON FL	6.0 + 0.75	51.5	22.1	59.5	12.5	55.8	41.8	48.3	53.8	71.9	75.0	52.0	48.2	56.2	19.1	62.3	65.9	83.0	72.0
APRON FL	1.5	46.0	20.1	63.3	8.5	47.0	48.2	38.8	55.5	57.5	68.9	59.8	59.0	63.8	16.8	65.6	65.1	73.2	67.5
RTU-PCNB	14.5	45.0	12.8	62.3	11.3	69.2	52.0	30.0	46.0	74.2	69.9	48.8	49.0	44.2	21.8	29.6	33.5	86.8	49.0
Nontreated	---	51.0	19.0	59.8	8.3	37.5	34.2	23.2	45.3	58.8	69.3	16.8	35.5	13.8	10.0	19.1	31.0	76.8	41.8
Location Average		51.1	23.7	66.3	11.3	48.7	51.4	43.7	64.9	74.4	73.4	56.4	55.6	54.9	24.2	61.1	62.3	84.5	74.7
LSD (*P*=0.05)		NS	NS	7.3	NS	8.7	NS	9.8	12.1	8.6	NS	12.7	15.3	14.0	7.2	13.3	11.5	NS	11.2
Coefficient of Variation, %		24	48	10	59	18	31	19	15	9	11	16	20	19	32	22	13	10	10

Table 5. Disease ratings and isolation frequency of seedling pathogen groups for the 1997 National Cottonseed Treatment Program locations.

			Isolation frequency (%)[1]			
	Disease Index		*R*	*Pythium*	*T.*	*Fusarium*
Location	Hypo.[2]	Root[3]	solani	spp.	basicola	spp.
AL	2.1	4.8	4	8	0	85
AR2	2.3	2.6	8	14	0	100
AR3	2.5	4.1	58	10	96	52
FL1	2.4	3.8[4]	16	6	0	54
FL2	2.4	2.2	48	18	4	82
GA	1.8	2.4	0	6	0	90
LA1	2.6	3.7	2	10	0	76
LA2	2.5	2.8	26	8	100	54
MS1	2.3	2.3	56	6	0	76
MS2	2.5	2.8	16	6	0	80
OK1	2.4	3.0	9	9	100	69
OK2	2.7	3.7	14	0	100	78
OK3	3.0	3.5	2	6	62	90
TN	2.9	3.1	43	6	0	71
TX1	2.2	2.1	4	16	0	86
TX2	1.9	2.3	2	8	88	73
TX3b	2.0	2.0	18	16	0	54

[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.

[4] Discoloration associated with shipping.

Table 6. Soil populations of selected soilborne fungi from sites in the 1997 National Cottonseed Treatment Program.

Location	*Rhizoctonia* solani	*Pythium* spp.	*Thielaviopsis* basicola
	CFU[1]/100g	CFU/g	CFU/g
AL	ND[2]	17	0
AR2	6	100	0
AR3	ND	33	19
FL1	ND	150	0
FL2	26	167	0
GA	ND	117	0
LA1	19	183	0
LA2	13	50	8
MS1	ND	33	45
MS2	19	ND	0
OK1	ND	ND	92
OK2	13	83	4
OK3	ND	350	27
TN	6	733	0
TX1	ND	67	0
TX2	ND	133	20
TX3b	ND	ND	0

[1] Colony forming units.

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