REPORT OF THE COTTONSEED TREATMENT COMMITTEE FOR 1999

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Introduction

The 1999 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 1999 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 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 1999 National Cottonseed Treatment Program.

Materials and Methods

Fungicide Treatment

Acid-delinted seed of *Gossypium hirsutum* L., 'Deltapine 50' or 'Paymaster HS26', were provided by Delta and Pine Land Company, Scott, MS. 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 and dye (Pro-Ized seed colorant, Gustafson Inc.) were mixed with water at a rate of 2% 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.

Field Experiments

Ninteen field experiments were conducted by 17 cooperators across the U.S. Cotton belt (Table 2). However, data were not collected at the South Carolina site, cooperator J. D. Mueller, and one site in Oklahoma. 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 38 days after planting, average 30 days, depending on the location. A soil sample and seedling samples from plots containing nontreated seed were taken from 28 to 38 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 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 (mean 48, range 24 to 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 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 ml of the miticide Danitol (Valent Chemical Co.) per liter. Resulting colonies were transferred to potato dextrose agar 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*. In 1999, a similar number of seedlings, range 24 to 50, were not surface disinfested prior to plating on water agar to obtain a second indication of prevalence of *Pythium* spp. on cotton seedlings.

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.

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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

For the 17 locations reporting data for 1999 there was a significant location and treatment effect, but no significant location x treatment effect, when cotton stands were analyzed over locations (Table 3). Thus comparing means for treatments across locations was appropriate. The lack of a significant location x treatment interaction indicated that the treatment response was consistent over the environments and pathogen pressures in 1999. This consistency in response was unexpected as it had not been observed in previous years. When analyzed by location, a significant difference between treatments was found for 10 of the 17 sites, 59% of the sites (Table 4).

All of the nominated treatments increased stands significantly compared to the nontreated seed treatment. The range of stand improvement over the nontreated control for treatments ranged from 12.3% to 18.8%. The nominated treatments gave similar stand improvements when compared to each other with the exception of the treatment HM9907, which had significantly lower stands than 10 of the 15 other nominated treatments. The historic standard fungicide treatment, Vitavax-PCNB + Allegiance, performed similarly to all the nominated treatments. *Pythium* spp. and *Rhizoctonia solani* were both demonstrated to be important across the locations by a significant increase in stand over the nontreated seed for the Allegiance and PCNB treatments, respectively. For specific locations, PCNB increased stands over the nontreated control at AR3, OK2, and TN.

Hypocotyl disease indices ranged from 1.5 at CA to 2.6 at AR3 and TN, average 2.2 (Table 5). Root disease indices ranged from 1.4 at TX1 to 3.2 at TN, average 2.5. *R. solani* was isolated from seedlings from the nontreated plots at 14 of 17 locations (Table 5). *R. solani* was isolated from 58% of the seedlings at the AR1 site, and 5 locations had isolation frequencies of 30% or greater (AR1, MS1, MS2, OK2, TN). *Pythium* spp. were isolated from seedlings at 16 of 17 locations (Table 5). Isolation frequencies for *Pythium* spp. were greater than 25% for four sites depending on the method of isolation (GA, LA2, TN, and VA). Isolation for *Pythium* spp. without using a disinfestation step of NaOCl increased the mean isolation percentage across all sites from 8.6% to 20.9%. However, this increase was in large part due to dramatic increases in isolation frequencies for a few sites. These results do indicate that a more critical evaluation of isolation methods used to assess prevalence of seedling disease pathogens is warranted. *Thielaviopsis basicola* was isolated from seedlings at 10 of the 17 locations on the modified TB-CEN medium (Table 5). Five sites had isolation frequencies of 50% or greater (AR1, CA, MS1, OK2, and TN). *Fusarium* spp. were isolated from seedlings at all locations (Table 5). Isolation frequencies for *Fusarium* spp. ranged from 26% to 94%. *Macrophomina phaseolina* was isolated from seedlings at 10 locations, with only the AR2 site having an isolation frequency above 10%.

Soil populations of *R. solani* were detected at 12 of the 17 sites assayed, range 2 to 38 CFU/100 g of soil (Table 6). *Pythium* spp. were detected in soils from 15 of 17 sites assayed, range 17 to 175 CFU/g of soil. *T. basicola* was detected in 9 of the 17 soils assayed, range 1 to 83 CFU/g soil.

The percent stand for the nontreated seed treatment for the locations was negatively correlated, –0.48 (*P*=0.05), with *T. basicola* soil populations and positively correlated, 0.50 (*P*=0.04), with Pythium soil populations. The hypocotyl disease index was positively correlated, 0.57 (*P*=0.02), with isolation frequency of *R. solani* and weakly positively correlated, 0.42 (*P*=0.09), with soil populations of *R. solani.* Frequencies of isolation of *R. solani, Pythium* spp. and *T. basicola* from seedlings were positively correlated with soil populations of these pathogens; 0.49 (*P*=0.4), 0.54 (*P*=0.02), and 0.53 (*P*=0.03), respectively.

Conclusions

The results from 17 locations in the 1999 National Cottonseed Treatment Program indicated that seed treatment fungicides consistently improved stands of cotton compared to a nontreated control. No interaction between treatments and locations was found in 1999. PCNB and metalaxyl alone also increased plant stands compared to nontreated seed, indicating the importance of *R. solani* and *Pythium* spp. in stand establishment. This is supported by the positive correlation between hypocotyl disease and *R. solani* isolation frequency. *Rhizoctonia solani*, *Pythium* spp., and *Thielaviopsis basicola* isolation frequencies from seedlings were positively correlation with their respective soil populations.

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 1999 National Cottonseed Treatment Program.

Common or registered name[1]	Formulation	Active ingredient (%)
ALLEGIANCE (Metalaxyl)	Flowable	28.35% *N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl) alanine methyl ester
APRON FL (Metalaxyl)	Flowable	28.35% *N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl) alanine methyl ester
APRON TL (Metalaxyl)	Liquid	11.55% *N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl) alanine methyl ester
APRON XL 3LS (Mefenoxam)	Liquid	33.3% (R,S)-2-[(2,6-dimethylphenyl)-methoxyacetylamino]-propionic acid methyl ester
ASCEND (TCMTB)	Flowable	30% 2-(thiocyanomethylthio)benzothiazole
Azoxystrobin 100FS	Flowable	9.2% Methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate
BAYTAN 30 (Triadimenol)	Flowable	30% Beta-(4-Chlorophenoxy)-alpha-(1,1-dimethylethyl)-1*H*-1,2,4-triazole-1-ethanol
DIVIDEND 3FS (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-9906		Helena Chemical Company
HM-9907		Helena Chemical Company
LS288		Gustafson Incorporated
MAXIM 4FS (Fludioxonil)	Flowable	42% 4-(2,2-difluoro-1,3-benzdioxol-4-yl)-1*H*-pyrrole-3-carbonitrile
NU-FLOW D (Chloroneb)	Flowable	30.0% 1,4-dichloro-2,5-dimethoxy-benzene
NU-FLOW M (Myclobutanil)	Emusifiable conc.	25.1% 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
NU-FLOW T (TCMTB)	Liquid	30% 2-(thiocyanomethylthio)benzothiazole
PROTÉGÉ		Gustafson Incorporated
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
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 1999 National Cottonseed Treatment Program.

			Date				Row length		
Cooperator	Location		Planted	Sampled	Counted	Reps.	counted (ft)	Seed planted	Soil temperature
W. S. Gazaway	Auburn, AL	(AL)	4/12	5/12	5/12	6	10	50	20
T. L. Kirkpatrick	Hope, AR	(AR1)	4/30	6/1	5/27	5	40	180	-
R. Benson	Keiser, AR	(AR2)	4/21	5/23	5/23	5	20	150	-
C S. Rothrock	Clarkedale, AR	(AR3)	5/3	5/31	5/31	8	50	250	17
R. H. Garber	Shafer, CA	(CA)	3/29	4/29	4/27	8	25	130	12
D. R. Sumner	Tifton, GA	(GA)	4/1	5/6	5/6	5	35	105	20
P. D. Colyer	Bossier City, LA	(LA1)	4/14	5/13	5/12	5	25	100	14
B. Padgett	Winnsboro, LA	(LA2)	4/20	5/19	5/19	4	21	99	22
W. E. Batson	Mississippi State, MS	(MS1)	4/13	5/17	5/13	5	80	240	-
G. L. Sciumbato	Stoneville, MS	(MS2)	4/21	5/20	5/17	4	40	200	22
L. Verhalen &	Altus, OK	(OK2)	5/7	6/8	6/8	4	20	100	18
B. E. Greenhagen	Perkins, OK	(OK3)	5/24	6/28	6/28	4	20	100	19
A. Y. Chambers	Jackson, TN	(TN)	4/30	5/28	5/28	10	25	100	15
P. M. Thaxton	College Station, TX	(TX1)	4/9	5/17	5/17	8	30	100	11
H. W. Kaufman	Lubbock, TX	(TX2)	4/21	5/19	5/19	4	36	178	-
T. S. Isakeit	Weslaco, TX	(TX3)	3/31	5/5	4/30	4	20	100	-
P. M. Phipps	Suffolk, VA	(VA)	4/28	5/27	5/27	4	60	240	10

Table 3. Mean squares for combined analysis of variance across locations, 1999 National Cottonseed Treatment Program.

Source	Degrees of freedom	Mean squares
Location	16	19076**[1]
Replication (Location)	77	303**
Treatment	19	715**
Location* treatment	304	112
Error	1456	101

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

Table 4. Cotton seedling stands for locations of the 1999 National Cottonseed Treatment Program

		Plant stand (%)																	
Treatment	Rate (oz/cwt)	AL	AR1	AR2	AR3	CA	GA	LA 1	LA 2	MS 1	MS 2	OK 2	OK 3	TN	TX 1	TX 2	TX 3	VA	Mean
WE-120C + NU-FLOW M + APRON TL	0.24 + 1.75 + 2.0	60	74	84	72	75	76	84	70	70	70	82	82	59	79	35	72	45	70.3 a
APRON XL 3LS + MAXIM 4FS + AZOXYSTROBIN	0.32 + 0.08 + 0.77	61	76	95	67	75	74	84	63	65	69	80	79	59	78	32	79	49	69.9 a
NU-FLOW ND + NU-FLOW M + APRON TL	5.0 + 1.75 +2.0	63	75	95	64	76	78	85	82	64	77	81	78	47	78	32	78	48	69.8 a
APRON XL 3LS + MAXIM 4FS + NU-FLOW M	0.32 + 0.08 + 1.25	68	58	97	73	77	76	84	72	63	70	78	76	57	80	34	72	40	69.7 a
BAYTAN + ASCEND + ALLEGIANCE	0.5 + 1.5 + 0.75	66	74	84	72	72	62	84	72	67	70	84	80	56	79	46	74	44	69.6 a
PROTEGE + ALLEGIANCE + ASCEND	0.256 + 0.75 + 1.5	63	76	86	68	75	80	88	76	68	66	74	82	45	81	38	75	50	69.6 a
RTU BAYTAN-THIRAM + ALLEGIANCE	3.0 + 0.75	55	79	75	74	73	69	84	82	70	70	80	83	56	80	38	73	42	69.5 a
HM9906	12.0	63	78	84	65	74	77	87	78	64	66	82	78	52	79	36	76	46	69.5 a
NU-FLOW T + NU-FLOW M + APRON TL	2.25 + 1.25 + 2.0	57	74	88	66	77	75	87	78	62	70	75	84	50	78	51	78	43	69.5 a
NU-FLOW D + APRON FL + NU-FLOW M + NU-FLOW T	11.75 + 1.25 + 2.25	59	68	90	72	74	74	84	78	62	66	76	83	53	79	45	73	50	69.5 a
LS288	0.5	63	72	86	69	69	72	84	72	66	72	79	88	59	75	32	66	49	69.0 ab
VITAVAX-PCNB + ALLEGIANCE + BAYTAN	6.0 + 0.75 + 0.25	60	75	72	68	75	73	85	69	65	70	82	78	54	81	37	73	49	68.4 ab
APRON XL 3LS + MAXIM 4FS + DIVIDEND 3FS	0.32 + 0.08 + 1.00	52	72	98	70	74	71	82	76	62	71	75	82	56	80	34	74	47	69.2 ab
NU-FLOW D + APRON FL + NU-FLOW M	11.75 + 1.25	54	79	95	68	70	76	84	60	68	72	83	76	56	72	36	77	47	68.8 ab
APRON XL 3LS + MAXIM 4FS + NU-FLOW M	0.32 + 0.08 + 1.75	57	70	88	68	76	75	83	63	69	75	78	80	50	76	29	65	43	67.5 ab
HM9907	12.0	58	68	84	75	69	63	81	73	64	66	77	87	42	82	38	65	43	66.5 bc
VITAVAX-PCNB + ALLEGIANCE	6.0 + 0.75	69	67	93	72	72	75	85	72	58	67	73	76	44	78	35	74	46	67.9 ab
RTU-PCNB	14.5	52	78	86	67	60	59	84	72	61	70	76	73	55	70	26	75	33	64.5 cd
ALLEGIANCE	1.5	53	71	83	62	68	66	83	67	52	70	73	74	38	73	27	68	34	62.1 d
NONTREATED		68	56	77	56	66	70	78	62	56	63	65	62	37	68	23	70	30	59.2 e
Location average		60	72	87	68	72	72	84	72	64	70	78	79	51	77	35	73	44	68.0
LSD (*P*=0.05)		NS	NS	NS	7.6	6.2	NS	NS	13.3	9.6	NS	9.2	NS	13.8	7.8	11.1	8.0	7.7	2.9
Coefficient of Variation (%)		23	17	16	11	9	16	7	13	12	11	8	12	31	10	22	7	12	15

Table 5. Disease ratings and isolation frequency of seedling pathogen groups for the 1999 NationalCottonseed Treatment Program locations.

	Disease Index			Isoolation frequency (%)[1]			
Location	**Nodes[2]**	**Hyp.[3]**	**Root[4]**	***Rhizoctonia solani***	***Pythium* spp.**	***Thielaviopsis basicola***	***Fusarium* spp.**
AL	3.0	2.4	2.9	10	0(16)[5]	0	86
AR1	5.6	2.4	2.2	58	10(12)	92	62
AR2	3.2	2.5	2.8	6	8(10)	36	82
AR3	5.0	2.6	2.8	22	4(0)	29	57
CA	-	1.5	2.4	0	2(14)	84	26
GA	4.4	2.1	2.1	0	56(83)	0	50
LA1	2.8	1.8	2.7	12	4(8)	0	38
LA2	2.8	2.3	2.9	24	2(68)	0	76
MS1	-	2.3	2.0	34	6(3)	52	32
MS2	2.8	2.3	1.9	50	2(5)	0	66
OK2	-	2.3	2.5	30	0	64	37
OK3	6.4	2.1	2.1	12	4(12)	34	92
TN	4.0	2.6	3.2	48	8(32)	50	34
TX1	-	2.1	1.4	0	2(10)	40	94
TX2	1.6	1.7	2.5	2	4(0)	36	74
TX3	-	2.2	2.6	14	14(12)	0	82
VA	1.8	2.1	2.9	6	12(49)	0	76

[1] Isolation frequency is based on approximately 50 seedlings per location.
[2] Nodes based on five seedlings per location.
[3] 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.
[4] 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.
[5] Pythium isolation frequency in parentheses based on seedlings not disinfested in NaOCl.

Table 6. Soil populations of selected soilborne fungi from sites in the 1999 National Cottonseed Treatment Program.

Location	***Rhizoctonia solani***	***Pythium* spp.**	***Thielaviopsis basicola***
	CFU[1]/100g	CFU/g	CFU/g
AL	2	24	ND
AR1	ND[2]	77	83
AR2	21	165	2
AR3	8	46	7
CA	ND	117	6
GA	7	175	ND
LA1a	7	125	ND
LA1b	12	88	ND
MS1	8	67	2
MS2	38	17	ND
OK2	ND	ND	13
OK3	5	35	ND
TN	33	104	13
TX1	ND	83	ND
TX2	ND	ND	69
TX3	8	89	ND
VA	12	17	1

[1] Colony forming units.
[2] Populations were not detected in the soil sample; less than approximately 3 CFU/100 g, 8 CFU/g, and 0.5 CFU/g of soil for *Rhizoctonia solani, Pythium* spp., and *Thielaviopsis basicola,* respectively.