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REPORT OF THE COTTONSEED TREATMENT COMMITTEE FOR 2002

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Introduction

The 2002 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. Twelve fungicide seed treatments were nominated by chemical industry representatives for evaluation in the 2002 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 2002 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. DP451 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 (DayGlo EPX seed colorant, Gustafson Inc.) were mixed with water at a rate of 2.6% 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

Nineteen field experiments were conducted by 16 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 26 to 50 days after planting, average 30 days, depending on the location. A soil sample and seedling sample from plots containing nontreated seed were taken from 26 to 50 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 (Onset Computer Corp, Pocasset MA) 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 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 (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 16 sites, average 44 seedlings (range 9 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, 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 93 to 98% for the cultivar DP 451 B/RR, with an average germination of 95%. Seed germination for the cultivar PM 2326 RR ranged from 94 to 98% after seed treatment, with an average germination of 96%. No differences were found among treatments for seed germination for either cultivar.

For the 2002 National Cottonseed Treatment Program, 18 of 19 sites had data reported. For these 18 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 9 of the 18 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 1 of the 9 experiments having a significant response compared to the nontreated control (OK2), indicating the importance of *Pythium* spp. in stand establishment at this site. In 4 of these 9 experiments (CA, GA, OK2 and VA), 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 9 experiments (AR1, AR3, CA, GA, LA2, OK2, and TN). The nominated treatments increased stands over the nontreated control from 56% of the sites (5 of 9 sites) to 100% of the sites (9 of 9 sites) depending on the treatment. Treatments giving increases in stand compared to the nontreated control at all 9 sites where a stand response was found were A13012B + Systhane and Apron XL-TL + Nu-Coat + WECO 0257. Three treatments gave increased stands compared to the nontreated control in 8 of the 9 sites were a stand response was found. At 4 of the 9 sites where a response was found (AR3, OK2, TN, and VA), some of the nominated fungicide treatments performed significantly better than the historical standard fungicide treatment, Vitavax-PCNB + Allegiance. None of the treatments performed better that the historical standard at more than two of the four sites. The number of fungicide treatments significantly increasing stands over the nontreated control ranged from 6 of the 12 nominated treatments for the AR1 and AR2 sites to all of the nominated treatments tested for the AR3, CA, GA, OK2, and TN sites. The mean stand for a location was not related to locations where stands were increased by fungicide treatments.

Hypocotyl disease indices ranged from 2.0 at the OK1 and TX3 sites to 3.4 at the AR3 site, average 2.4 (Table 5). Root disease indices ranged from 2.0 at OK1 to 4.8 at AR3 and TX2, average 3.0. *Rhizoctonia solani* was isolated from seedlings from the nontreated plots at 15 of 18 locations (Table 5). *R. solani* was isolated from 20% or greater of the seedlings at 6 locations (AR2, AR3, LA2, MS1, MS2, and TN). *Pythium* spp. were isolated from seedlings at all locations (Table 5). Isolation frequencies for *Pythium* spp. were 20% or greater for 6 sites (AR1, AR2, CA, OK1, OK2 and TX5). Isolation frequencies were increased dramatically by plating roots without surface disinfestation on the selective medium P_5ARP, with all 15 sites with seedlings plated on P_5ARP having 20% or greater recovery of *Pythium* spp. (Table 5). *Thielaviopsis basicola* was isolated from seedlings at 8 of the 18 locations on the modified TB-CEN medium (Table 5). *T. basicola* was isolated from over 40% of the seedlings for the AL, AR1, AR3, TN, and TX2 sites. *Fusarium* spp. were isolated from seedlings at all locations (Table 5). Isolation frequencies for *Fusarium* spp. ranged from 60% to 98%.

Soil populations of *R. solani* were detected at 9 of the 17 sites, range 1.6 to 9.7 CFU/100 g of soil (Table 6). *Pythium* spp. were detected in soils at 15 of the 16 sites assayed, range 17 to 300 CFU/g of soil. *T. basicola* was detected in 6 of the 17 soils assayed, range 1 to 127 CFU/g soil. The root-knot nematode was not detected for any of the sites in 2002. The reniform nematode was detected for the MS2 site and the lance nematode, *Hoplolaimus* sp., was detected at the GA site.

The percent stand for the nontreated seed treatment for the locations was negatively correlated with recovery of *Pythium* spp. on the medium P_5ARP, -0.72 (*P*=0.002) and weakly negatively correlated with the hypocotyl disease index, –0.45 (*P*=0.08). The hypocotyl disease index and the root disease index were positively correlated, 0.64 (*P*=0.008). The hypocotyl disease index was positively correlated with isolation of *Rhizoctonia solani*, 0.64 (*P*=0.007) and weakly positively correlated with isolation of *Pythium* spp. on P_5ARP, 0.44 (*P*=0.10) and *T. basicola* on TB-CEN, 0.43 (*P=0.09)*. *T.* b*asicola* recovery from seedlings was positively correlated with soil populations of *T. basicola,* 0.58 (*P*=0.02). Isolation frequency of *Pythium* spp. on water agar and P_5ARP were positively correlated, 0.72 (*P*=0.002)

Conclusions

The results from the 18 locations where data was collected for the 2002 National Cottonseed Treatment Program indicated that seed treatment fungicides improved stands of cotton compared to a nontreated control for 50% of the sites. All of the nominated

fungicide combinations improved stands over the nontreated seed at most of the sites where a response was found. The hypocotyl disease index was positively correlated with isolation of *R. solani* and PCNB increased stand for 4 of the 9 sites where a fungicide response was found, indicating the importance of *R. solani* in the 2002 tests. Stands were negatively correlated with recovery of *Pythium* spp. on the medium P_5ARP, indicating the importance of Pythium spp. in stand establishment in these tests. Isolation frequencies of *T. basicola* from seedlings were positively correlation with soil populations of this pathogen.

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 2002 National Cottonseed Treatment Program.

Common or registered name[1]	Formulation	Active ingredient (%)
A13012B		Syngenta Crop Protection, Inc.
A13012C		Syngenta Crop Protection, Inc.
A13012D		Syngenta Crop Protection, Inc.
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
ASCEND 30 (TCMTB)	Liquid	30% 2-(thiocyanomethylthio)benzothiazole
BAYTAN 30 (Triadimenol)	Flowable	30% Beta-(4-Chlorophenoxy)-alpha-(1,1-dimethylethyl)-1*H*-1,2,4-triazole-1-ethanol
HM 0207		Helena Chemical Company
L1006		Gustafson Incorporated
L1072		Gustafson Incorporated
L1080		Gustafson Incorporated
NU-COAT (Chloroneb & TCMTB)	Flowable	23.5% 1,4-dichloro-2,5-dimethoxy-benzene, 9.0% 2-(thiocyanomethylthio)benzothiazole
NU-FLOW M (Myclobutanil)	Wettable powder	40% A-butyl-a-(4-chlorophenyl)-1*H*-1,2,4-triazole-1-propanenitrile
PROTÉGÉ FL (Azoxystrobin)	Flowable	21% methyl (*E*)-2-{2-[6-(2-cyanophenoxy) pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate
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 WSP (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 2002 National Cottonseed Treatment Program

Cooperator	Location		Date Planted	Date Sampled	Date Counted	Reps.	Row length counted (ft)	Seed planted	Soil temperature[1]
K. McLean	Auburn, AL	(AL)	4/15	5/13	5/13	5	25	125	-[2]
T. L. Kirkpatrick	Hope, AR	(AR1)	4/22	5/22	5/22	5	40	200	23(20)
F. Bourland	Keiser, AR	(AR2)	4/15	5/15	5/15	6	25	100	22(20)
C. S. Rothrock	Clarkedale, AR	(AR3)	4/29	5/27	5/27	6	50	250	19(17)
R. Hutmacher	Shafer, CA	(CA)	3/28	4/23	4/23	8	25	140	19(17)
K. W. Seebold Jr.	Tifton, GA	(GA)	4/15	5/13	5/13	5	25	125	25(20)
P. D. Colyer	Bossier City, LA	(LA1)							
B. Padgett	Winnsboro, LA	(LA2)	4/23	5/21	5/21	4	25	113	22(20)
W. E. Batson Jr.	Mississippi State, MS	(MS1)	4/24	5/22	5/22	4	83	249	19(16)
G. L. Sciumbato	Stoneville, MS	(MS2)	4/12	5/8	5/8	4	40	200	23(20)
L. Verhalen &	Tipton, OK	(OK1)	5/3	6/3	6/3	4	20	100	22(19)
B. E. Greenhagen	Altus, OK	(OK2)	5/3	6/3	6/3	4	20	100	22(19)
	Perkins, OK	(OK3)	5/13	6/12	6/12	4	20	100	19(16)
A. Y. Chambers	Jackson, TN	(TN)	4/24	6/3	5/31	10	25	100	16(14)
H. W. Kaufman	Lubbock, TX	(TX2)	5/1	5/29	5/29	4	37.5	188	17(14)
T. S. Isakeit	Victoria, TX	(TX3)	4/3	5/2	5/2	4	17	100	18(16)
	Brazos, TX	(TX4)	3/26	4/23	4/29	4	17	100	18(13)
	Burleson, TX	(TX5)	3/5	4/24	4/24	4	17	100	15(13)
P. M. Phipps	Suffolk, VA	(VA)	4/16	5/14	5/14	4	60	180	25(20)

[1] Mean (Minimum) soil temperature; 3-day average following planting.
[2] Data not recorded.

Table 3. Mean squares for combined analysis of variance across locations, 2002 National Cottonseed Treatment Program.

Source	Degrees of Freedom	Mean squares
Location	17	34518**[1]
Replication(Location)	71	62**
Treatment	15	1429**
Location*treatment	254	140**
Error	1057	88

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

Table 4. Cotton seedling stands for locations of the 2002 National Cottonseed Treatment Program.

Treatment	Rate (oz/cwt)	Plant stand (%)																	
		AL	AR1	AR2	AR3	CA	GA	LA2	MS1	MS2	OK1	OK2	OK3	TN	TX2	TX3	TX4	TX5	VA	Mean
A13012B + Systhane	3.07 + 0.84	76	74	64	37	73	38	39	79	71	92	92	52	68	78	81	24	21	69	62
Apron XL TL + Nu-Coat + Nu-Flow M	1.0 + 7.5 + 1.0	82	72	59	43	70	41	31	74	75	90	91	47	70	83	86	30	24	63	62
RTU Baytan Thiram + Allegiance LS + L1006 + L1080	3.0 + 1.2 + 0.6 + 0.5	79	72	54	53	72	36	30	78	78	88	89	64	69	78	85	16	23	65	62
A13012B	3.07	78	75	52	41	71	38	32	82	66	88	89	64	66	83	86	20	20	67[*1]	61
Protégé FL + Ascend 30 + Allegiance LS	0.2 + 1.0 + 1.2	79	73	61	29	73	40	18	80	55	93	87	57	62	78	85	40	29	74	61
Apron XL TL + Nu-Coat + WECO 0257	1.0 + 7.5 + 0.5	81	73	66	33	70	42	29	75	64	88	91	57	65	79	87	25	20	69	61
Baytan 30 + Ascend 30 + Allegiance LS	0.5 + 1.0 + 1.2	85	68	60	27	70	37	25	78	73	94	91	57	72	84	84	15	19	67	60
A13012D	3.07	81	69	51	43	68	35	30	76	70	88	90	45	67	79	81	34	20	70	60
RTU Baytan Thiram + Allegiance FL	3.0 + 0.75	78	66	55	46	66	35	32	79	67	87	90	50	71	79	88	22	22	66	60
A13012C	3.07	73	70	57	32	68	40	31	72	73	88	88	52	69	78	82	41	20	65	60
RTU Baytan Thiram + Allegiance FL + L1080 + L1072	3.0 + 0.75 + 0.5 + 9.1	80	70	44	32	65	36	29	79	77	91	88	33	67	84	81	32	21	71	59
HM 0207	16.0	76	64	50	39	71	36	19	73	63	82	83	50	55	75	75	15	16	57	54
Vitavax-PCNB + Allegiance FL	6.0 + 0.75	-	76	56	37	69	38	30	77	69	90	84	60	57	79	83	38	26	67	61
RTU-PCNB	14.5	74	68	49	21	68	34	25	73	63	84	84	39	48	75	84	19	22	67[*]	54
Allegiance FL	1.5	84	60	46	23	57	15	14	73	61	85	82	44	39	79	72	21	13	56	49
Nontreated	---	79	62	47	13	58	10	14	65	60	84	75	42	41	75	78	16	17	61	48
Location average		79	69	55	34	68	34	27	76	68	88	87	51	62	79	82	25	21	66	58
Coefficient of Variation (%)		14	10	15	33	9	27	32	8	20	7	4	39	15	6	9	57	33	7	16
LSD (P=0.05)		NS	8.6	9.1	13.0	6.0	11.9	12.2	NS	NS	NS	5.1	NS	8.0	NS	NS	NS	NS	6.4	

[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 2002 National Cottonseed Treatment Program locations.

		Disease Index		Isolation frequency (%)[1]			
Location	**Nodes[2]**	**Hyp.[3]**	**Root[4]**	***Rhizoctonia solani***	***Pythium* spp.**	***Thielaviopsis basicola***	**F*usarium* spp.**
AL	-[5]	2.6	3.6	19	12	67	71
AR1	3.8	2.7	2.8	18	28 (88[6])	82	88
AR2	3.8	2.4	3.1	56	20 (68)	0	66
AR3	2.0	3.4	4.8	48	12 (96)	86	80
CA	-	2.1	2.2	14	26 (66)	0	88
GA	3.4	2.1	2.6	4	2 (44)	0	96
LA2	-	2.9	3.3	44	8 (31)	0	70
MS1	-	2.1	2.1	30	14 (48)	2	62
MS2	4.0	2.4	2.8	20	6 (64)	6	60
OK1	5.0	2.0	2.0	0	22 (62)	0	90
OK2	5.6	2.2	3.5	16	59 (82)	8	98
OK3	-	2.1	2.4	10	4 (20)	0	94
TN	5.0	2.5	2.2	20	6 (55)	100	70
TX2	2.0	2.4	4.8	0	8 (22)	42	98
TX3	-	2.0	2.1	0	2	0	94
TX4	-	2.4	3.5	10	8(42)	0	80
TX5	-	2.3	2.4	2	24 (97)	0	76
VA	2.0	2.3	4.4	4	2 (31)	0	92

[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 2002 National Cottonseed Treatment Program.

Location	***Rhizoctonia solani* CFU[1]/100g**	***Pythium* spp. CFU/g**	***Thielaviopsis basicola* CFU/g**
AR1	4.3	117	24
AR2	9.7	133	0
AR3	8.0	75	127
CA	ND2[2]	25	0
GA	ND1	217	0
LA2	4.8	94	0
MS1	4.8	17	0
MS2	1.8	17	6
OK1	ND1	-[3]	0
OK2	ND1	100	1
OK3	ND2	50	0
TN	ND1	142	20
TX2	ND1	22	20
TX3	ND2	217	0
TX4	7.2	67	0
TX5	7.2	300	0
VA	1.6	ND	0

[1] Colony forming units.
[2] Populations not detected in soil sample; less than approximately 2.4 (ND1) or 1.2 (ND2) CFU/ 100 g of soil for *Rhizoctonia solani,* and 8 CFU/g of soil for *Pythium* spp.
[3] Information not available.