REPORT OF THE COTTONSEED TREATMENT COMMITTEE FOR 2012

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Abstract

The 2012 National Cottonseed Treatment Program evaluated cotton seedling survival for a number of fungicide seed treatment combinations over diverse environmental conditions and populations of cotton seedling pathogens. Nine fungicide seed treatments were nominated by chemical industry representatives for evaluation in 2012. The results from the 17 locations where stand data were collected for the 2012 National Cottonseed Treatment Program indicated that seed treatment fungicides improved stands of cotton compared to a nontreated control for 29% of the locations (5 locations). Three of the nine nominated seed treatments increased stand compared to the nontreated control at all five locations a stand response was observed. All nominated fungicide combinations improved stands over the nontreated seed at a majority of the locations where a stand improvement was observed. In addition, all but one of the nominated treatment combinations improved stands over the historical standard fungicide seed treatment

at one of these five locations. Number of nodes for seedlings for the nontreated control was negatively correlated with the hypocotyl disease index, -0.89 (*P*=0.0012) and root disease index, -0.82 (*P*=0.0067) and the root disease index was negatively correlated with the mean soil temperature, -0.60 (*P*=0.0229). Soil populations and isolation frequency of *T. basicola* were positively correlated 0.83 (0.0007). Use of broad-spectrum fungicide cottonseed treatments is an important practice for obtaining uniform vigorous cotton stands.

Introduction

The 2012 National Cottonseed Treatment Program evaluated cotton seedling survival for a number of fungicide seed treatment combinations over diverse environmental conditions and populations of cotton seedling pathogens. Nine fungicide seed treatments were nominated by chemical industry representatives for evaluation in the 2012 National Cottonseed Treatment Program. Two standard fungicide treatments, Vitavax-PCNB + Allegiance, the historical standard, and RTU Baytan-Thiram + Allegiance FL, and a nontreated control were included to assess efficacy of the nominations and seedling disease pressure. In addition, the fungicide treatments Allegiance and PCNB were included to aid in determining the importance of *Pythium*s spp. and *Rhizoctonia solani*, respectively. Disease ratings and pathogen isolations for seedlings and soil populations of selected soilborne genera were conducted by collecting seedlings and soil from the nontreated control plots at each location. Soil temperature and water and plant development data also were collected for sites for the 2012 National Cottonseed Treatment Program.

Materials and Methods

Fungicide treatment

Acid-delinted seed of *Gossypium hirsutum* L. cv 'DP 1044 B2RF' were provided by Delta and Pine Land Company, Scott, MS. Fungicide treatments were mixed with $CaCO_3$ (7 oz/cwt), polymer (Secure 1 oz/cwt), Gaucho 600 (12.8oz/cwt), and dye (Color Coat Red, 1 oz/cwt) (Syngenta Inc.) in water at a rate of 2.75% (RTU-PCNB 2.86%) liquid to seed weight (w/w). Water, $CaCO_3$, polymer, Gaucho 600 and dye also were applied to the nontreated seed treatment at the same rate. Treatments were applied to the cottonseed while the seed mixed in a Hege 11 Liquid Seed Treater. 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 for 3 days at 30°C.

Field experiments

Seventeen field experiments were conducted by 14 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 3 to 8. The stand counts used in the analyses were taken from18 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 18 to 35 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 Dr. T. L. Kirkpatrick, Southwest Research and Extension Center, Hope, Arkansas, for determination of populations of plant parasitic nematodes. Soil temperature and moisture was monitored by burying a temperature sensor and a Watermark soil moisture sensor connected to a data logger (Spectrum Technologies, Inc., Plainfield, IL) 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. Seedlings were then rinsed for 20 minutes in running tap water. Approximately 50 seedlings were rated for disease symptoms, 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. 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. 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 was plated on the selective medium P_5ARP (Jeffers and Martin, 1986) to examine the isolation frequency for *Pythium* species. Soil samples were assayed for

Table 1. Fungicides, formulations and active ingredients included in the 2012 National Cottonseed Treatment Program.

Common or registered name[1]	Formulation	Active ingredient (%)
A16148C		Syngenta Crop Protection
ALLEGIANCE FL (Metalaxyl)	Flowable	28.35% *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
BAYTAN 30 (Triadimenol)	Flowable	30% Beta-(4-Chlorophenoxy)-alpha-(1,1-dimethylethyl)-1*H*-1,2,4-triazole-1-ethanol
BION (Acibenzolar-S-methyl)	Flowable	42% 1,2,3-Benzothiadiazole-7-carbothioic acid S-methyl ester
DYNASTY 100FS (Azoxystrobin)	Flowable	9.6% Methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate
DYNASTY CST (Azoxystrobin)	Flowable	6.64% Methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate
(Fludioxonil)		1.11% 4-(2,2-difluoro- 1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile
(Mefenoxam)		3.32% (R,S)-2-{(2,6-dimethylphenyl)-methoxyacetylamino}-propionic acid methyl ester
EVERGOL PRIME (Penflufen)	Flowable	22.7% N-[2-(1,3-dimethylbutyl)phenyl]-5-fluoro-1,3-dimethyl-1Hpyrazole-4-carboxamide
MAXIM 4FS (Fludioxonil)	Liquid	40.3% 4-(2,2-difluoro- 1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile
NUSAN 30EC (TCMTB)	Liquid	30% 2-(Thiocyanomethylthio) benzothiazole
RTU BAYTAN-Thiram	Flowable	15.3% Tetramethylthiuram disulfide
(Triadimenol)		5% Beta-(4-Chlorophenoxy)-alpha-(1,1-dimethylethyl)-1*H*-1,2,4-triazole-1-ethanol,
RTU PCNB	Flowable	24% Pentachloronitrobenzene
SPERA 240FS (Myclobutanil)	Flowable	22.37% A-butyl-a-(4-chlorophenyl)-1*H*-1,2,4-triazole-1-propanenitrile
SYSTHANE WSP (Myclobutanil)	Powder	40% A-butyl-a-(4-chlorophenyl)-1*H*-1,2,4-triazole-1-propanenitrile
TRILEX 2000 (Trifloxystrobin)	Flowable	7.12% methyl (E)-methoxyimino-{(E)-α-[1-(α,α,α-trifluoro-m-tolyl)ethylideneaminooxy]-o-tolyl}acetate
(Metalaxyl)		5.69% methyl N-(methoxyacetyl)-N-(2,6-xylyl)-DL-alaninate
VITAVAX (Carboxin) – PCNB	Flowable	17% 5,6-dihydro-2-methyl-N-phenyl-1,4-oxathiin-3-carboxamide
		17% Pentachloronitrobenzene
VORTEX (Ipconazole)	Flowable	40.7% 2-[(4-chlorophenyl)methyl]-5-(1-methylethyl)-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol

[1] Registered chemical name, all capital letters.

Table 2.List of cooperators and procedures for locations in the 2012 National Cottonseed Treatment Program.

Cooperator	Location		Date Planted	Date Sampled	Date Counted	Reps.	Row feet counted	Seed planted	Soil temperature[1]
K. Lawrence	Auburn, AL	(AL)	4/17	5/15	5/15	5	10	40	19(14)
J. Barham	Rohwer, AR	(AR1)	4/18	5/17	5/17	4	50	176	19(12)
A. Beach	Keiser,AR	(AR2)	4/2	5/2	5/2	6	18	100	22(18)
C. Rothrock	Judd Hill, AR	(AR4)	4/27	5/25	5/25	6	50	250	24(20)
R. Kemerait	Tifton, GA	(GA)	4/12	5/8	5/8	3	50	150	21 (NA)
P. Colyer	Bossier City, LA	(LA1)	4/10	5/10	5/14	8	25	100	22(19)
B. Padgett	Winnsboro, LA	(LA2)	4/13	5/15	5/14	5	25	100	23(21)[2]
G. Lawrence	Mississippi State, MS	(MS1)	4/11	5/14	5/14	5	40	160	18(8)
G. L. Sciumbato	Stoneville, MS	(MS2C)	6/19	7/17	7/17	4	45	180	30(26)
G. L. Sciumbato	Stoneville, MS	(MS2D)	4/12	4/30	4/30	4	45	180	22(15)
T. Kelly	Perkins, OK	(OK1)	5/2	6/3	6/3	4	20	100	28(24)
R. Thacker	Perkins, OK	(OK2)	5/2	6/3	6/3	4	20	100	28(23)
M. Bayles	Perkins, OK	(OK3)	5/8	6/8	6/8	4	20	100	23(17)
J. Woodward	Halfway, TX	(TX2)	4/30	6/4	5/25	4	35	140	21(20)
J. Woodward	New Deal, TX	(TX11)	5/10	6/4	6/4	4	35	140	19(19)
J. Woodward	Quaker, TX	(TX10)	5/3	6/4	5/30	4	35	140	26(25)
P. Phipps	Suffolk, VA	(VA)	4/27	5/29	5/29	4	60	180	16(14)

[1] Mean (Minimum) 4" soil temperature; 3-day average following planting. NA=not available.
[2] Mean (Minimum) 2" soil temperature; 3-day average following planting.

populations of *Rhizoctonia* species by using the toothpick-baiting-method (Paulitz and Schroeder, 2005) using 9 toothpicks per sample and Rhizoctonia populations were quantified on the Rhizoctonia selective medium TS (Spurlock et al., 2011). Soil populations of *Pythium* spp. and *T. basicola* were detected by diluting 25 g (oven dry weight) 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 modified 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, disease, pathogen isolation frequency, and soil populations over locations.

Results and Discussion

Seed germination after seed treatment ranged from 86% to 94% for the cultivar DP 1044 B2RF, with an average germination of 90%. No differences in seed germination in the rolled germination paper assay were found among seed treatments. For the 17trials in the 2012 National Cottonseed Treatment Program reporting stand data, there were significant location, treatment, and location x treatment effects (Table 3), indicating that the treatment response was dependent on the environment or pathogen pressures for a particular location.

A significant difference among treatments was found for 7 of the 17 locations (Table 4). However, significant increases in stands for a fungicide treatment compared to the nontreated control were only found for 5 of these locations. This frequency of response, 29%, is slightly lower than most years when stands from over 50% of the locations responded to fungicide use. The mean stand for a location was not related to locations where stands were increased by fungicide treatments, suggesting factors other than seedling diseases were important at some locations in 2012 in stand establishment. The Allegiance treatment increased stands compared to the nontreated control in 0 of these 5 locations having a significant response, indicating *Pythium* spp. as a group were not limiting stand establishment in 2012. At 3 of these 5 locations, the PCNB treatment increased stands over the nontreated control (AR2, AR4, and OK2), indicating *Rhizoctonia solani* was a major factor in stand establishment at these locations in 2012. The Vitavax-PCNB + Allegiance historical standard fungicide treatment increased stands compared to the nontreated control at 3 of 5 locations (AR2, AR4, and OK2). The RTU Baytan Thiram + Allegiance FL standard treatment increased stand at 4 of the 5 locations having a fungicide response (AR1, AR2, AR4, and OK2). Three of

Table 3. Mean squares for the combined analysis of variance across locations, 2012 National Cottonseed Treatment Program.

Source	Degrees of freedom	Mean squares
Location	16	17872**[1]
Replication(Location)	59	111
Treatment	13	406**
Location*treatment	208	117*
Error	765	84

[1] Significant *F*-test; * P=0.0009, ** P<0.0001.

Table 4. Cotton seedling stands for locations of the 2012 National Cottonseed Treatment Program

		Plant stand (%)																	
Treatment	Rate (oz/cwt)	AL	AR1	AR2	AR4	GA	LA1	LA2	MS1	MS2c	MS2d	OK1	OK2	OK3	TX2	TX10	TX11	VA	Mean
Vortex + Allegiance FL + Spera 240 FS + Evergol Prime + Trilex 2000	0.08+1.13+1.8 +0.32+1.0	74	87	78	87	94	77	52	87	56	22	88	76	73	80	76	83	84	75
Apron XL 3LS + Maxim 4FS + Systhane WSP + Dynasty 100FS	0.32+0.08 +0.84+1.53	88	77	71	85	92	76	50	88	44	23	84	70	84	86	75	82	86	74
Baytan 30 + Allegiance FL + Vortex + Nusan 30EC	0.5+0.75 +0.08	74	91	74	87	95	76	48	84	54	13	79	77	80	88	78	82	78	74
Baytan 30 + Allegiance FL + Vortex + Evergol Prime	0.5+0.75 +0.08+0.32	74	80	79	90	88	74	45	81	53	24	80	69	84	79	79	82	79	73
Vortex + Allegiance FL + Spera 240 FS + Evergol Prime	0.08+0.75 +1.8+0.32	81	77	69	86	92	74	47	84	51	16	81	75	82	81	77	79	87	73
Apron XL 3LS + Maxim 4FS + SysthaneWSP + Dynasty CST + Nusan 30EC	0.32+0.08+0.84 +4.13+2.0	70	70	75	97	85	76	47	84	52	19	77	72	77	86	79	80	73	72
Apron XL 3LS + Maxim 4FS+ Systhane WSP + Dynasty 100FS + A16148C	0.32+0.08+0.84 +1.53+0.32	76	76	61	86	90	76	48	79	50	25	83	68	73	89	75	82	80	72
Apron XL 3LS + Maxim 4FS + Systhane WSP + Dynasty100FS + Bion	0.32+0.08 +0.84+1.53+0.03	80	81	68	89	89	74	42	82	46	28	77	65	72	85	77	81	80	71
Baytan 30 + Allegiance FL + Vortex	0.5+0.75+0.08	73	79	66	86	86	77	45	85	40	31	81	68	57	87	76	79	76	70
RTU Baytan Thiram + Allegiance FL	3.0+0.75	67	76	71	87	86	71	41	85	48	25	84	74	72	83	74	82	80	71
Vitavax-PCNB + Allegiance FL	6.0+0.75	62	68	78	87	91	76	44	82	60	14	75	71	65	86	78	81	64	70
RTU-PCNB	14.5	58	68	71	86	90	76	38	81	47	23	81	77	84	85	77	82	67	70
Allegiance FL	1.5	58	66	65	82	92	73	40	77	59	13	69	59	73	80	79	82	86	68
Nontreated	---	54	63	58	78	87	75	43	79	51	12	72	54	86	86	82	80	77	66
Location average		70	76	70	87	90	75	45	83	51	21	80	70	76	84	77	81	78	
Coefficient of Variation (%)		20.5	9.5	14.7	7.0	5.8	3.9	21.9	6.6	22.5	77.5	10.1	12.9	17.1	5.0	6.3	5.3	13.9	
LSD (P=0.05)		18.4	10.3	11.9	7.0	NS	3.4	NS	NS	NS	NS	NS	13.2	NS	6.1	NS	NS	NS	

the nominated treatments increased stands over the nontreated control for all of the 5locations where a significant stand response was observed. These treatments were Vortex + Allegiance FL + Spera 240 FS + Evergol Prime + Trilex 2000, Baytan 30 + Allegiance FL + Vortex + Nusan 30EC, and Baytan 30 + Allegiance FL + Vortex + Evergol Prime. All of the nominated products increased stand at 4 of these 5 locations, except for the treatment Apron XL 3LS + Maxim 4FS + Systhane WSP + Dynasty CST + Nusan 30EC which increased stands at 3of the 5 locations. At 3 of the 5 locations where a response was found (AL, AR1 and AR4) one or more of the nominated fungicide treatments performed significantly better than the historical standard fungicide treatment Vitavax-PCNB + Allegiance. All nominated products increased stand over the historical standard fungicide at one of the sites except Apron XL 3LS + Maxim 4FS+ Systhane WSP + Dynasty 100FS + A16148C which did not increase stand over the standard fungicide treatment at any of the 5 locations.

Seedling development across the locations at the time of disease assessment and isolation ranged from 2.0 nodes to 9.3 nodes (Table 5). Hypocotyl disease indices ranged from 2.0 at the OK1, TS10, and TX11locations to 2.8 at the LA1 location, average 2.3 (Table 5). Root disease indices ranged from 2.0 for the MS2C, OK1, TX10, and TX11 locations to 3.5for the AL location, average 2.6. *Rhizoctonia solani* was isolated from seedlings from the nontreated plots for 9 of the 11 locations (Table 5). *Rhizoctonia solani* was isolated from 20% or greater of the seedlings at 5 locations (AR2, AR4, MS1, TX10, and VA). *Pythium* spp. were isolated from seedlings at all locations (Table 5). Isolation frequencies for *Pythium* spp. on amended water agar were greater than 20% for one location (AL). Isolation frequencies increased dramatically by plating roots without surface disinfestation on the selective medium P_5ARP (Table 5). *Thielaviopsis basicola* was isolated from seedlings at 6 of the 15 locations on the modified TB-CEN medium (Table 5). *Thielaviopsis basicola* was isolated from 20% or greater of the seedlings for the AL, AR4, LA2 locations. *Fusarium* spp. were isolated from seedlings at all 11 locations (Table 5). Isolation frequencies for *Fusarium* spp. ranged from 20 to 98%.

Table 5. Disease ratings and isolation frequencies of seedling pathogen groups for the 2012 National Cottonseed Treatment Program locations.

				Isolation frequency (%)[1]			
		Disease index		*Rhizoctonia*	*Pythium*	*Thielaviopsis*	*Fusarium*
Location	Nodes[2]	Hyp.[3]	Root[4]	*solani*	spp.	*basicola*	spp.
AL	3.3	2.6	3.5	12	24	98	82
AR1	3.3	2.6	3.2	NA[5]	NA	0	NA
AR2	2.0	NA	NA	46	2	0	50
AR4	NA	2.2	2.3	62	4(51)[6]	24	62
GA	5.7	2.3	2.4	NA	NA	0	NA
LA1	5.0	2.8	3.0	10	12(34)	0	80
LA2	4.0	2.7	3.4	NA	NA	42	NA
MS1	3.7	2.7	2.3	24	8(18)	8	20
MS2C	9.3	2.1	2.0	NA	NA	0	NA
OK1	8.3	2.0	2.0	0	8(46)	0	88
OK2	8.3	2.1	2.1	18	6(21)	0	92
OK3	6.3	NA	NA	NA	NA	NA	NA
TX2	NA	2.2	2.6	10	4	2	90
TX10	NA	2.0	2.0	24	18	2	88
TX11	NA	2.0	2.0	0	18	0	98
VA	NA	2.4	3.4	62	0(24)	0	88

[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] Data not available.
[6] Isolation frequency in parentheses from P_5ARP.

Rhizoctonia solani was detected in 7 of the soils assayed, range 4 to 18 CFU/100 cm^3 of soil. *Pythium* spp. were detected in soil at all but two locations for the soils assayed, range 8 to 283 CFU/g of soil. *T. basicola* was detected in 6 of the 15 soils assayed, range 4 to 1122 CFU/g soil. Number of nodes for seedlings for the nontreated control was negatively correlated with the hypocotyl disease index, -0.89 (*P*=0.0012) and root disease index, -0.82 (0.0067). The root disease index was negatively correlated with the mean temperature, -0.60 (*P*=0.0229) and associated with isolation frequency of *T. basicola*, 0.052 (*P*=0.0829). Soil populations and isolation frequency of *T. basicola* were positively correlated 0.83 (0.0007).

Table 6. Soil populations of selected soilborne genera from sites in the 2012 National Cottonseed Treatment Program.

Location	*Rhizoctonia solani*	*Pythium* spp.	*Thielaviopsis basicola*
	CFU[1]/100 cm^3	CFU/g	CFU/g
AL	17.7	67	1122
AR1	ND[3]	283	ND
AR2	NA[2]	NA	NA
AR4	13.3	100	649
GA	ND	100	ND
LA1	4.4	67	ND
LA2	8.8	50	ND
MS1	4.4	67	ND
MS2C	ND	NA	116
OK1	ND	NA	ND
OK2	ND	NA	ND
OK3	ND	33	ND
TX2	8.8	ND	146
TX10	ND	8	4
TX11	ND	ND	171
VA	4.4	NA	ND

[1] Colony forming units.
[2] Information not available.
[3] Populations not detected in soil sample; less than approximately 2 CFU/100 cm^3 of soil for *R. solani*, assay volume of 2.5 cm^3/toothpick, 4 CFU/g of soil for *Pythium* spp. and 0.5 CFU/g of soil for *T. basicola.*

Summary

The results from the 17 locations where stand data were collected for the 2012 National Cottonseed Treatment Program indicated that seed treatment fungicides improved stands of cotton compared to a nontreated control for 29% of the locations (5 locations). Three of the nine nominated seed treatments increased stand compared to the nontreated control at all five locations a stand response was observed. All nominated fungicide combinations improved stands over the nontreated seed at a majority of the locations where a stand improvement was observed. In addition, all but one of the nominated treatment combinations improved stands over the historical standard fungicide seed treatment at one of these five locations. Number of nodes for seedlings for the nontreated control was negatively correlated with the hypocotyl disease index, -0.89 (*P*=0.0012) and root disease index, -0.82 (*P*=0.0067) and the root disease index was negatively correlated with the mean soil temperature, -0.60 (*P*=0.0229). Soil populations and isolation frequency of *T. basicola* were positively correlated 0.83 (0.0007). Use of broad-spectrum fungicide cottonseed treatments is an important practice for obtaining uniform vigorous cotton stands.

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 Division of Agriculture nor does it imply registration under FIFRA.

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