REPORT OF THE COTTONSEED TREATMENT COMMITTEE FOR 2021

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Abstract

The 2021 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. Eleven fungicide seed treatments were nominated by chemical industry representatives for evaluation in 2021, along with 4 control-check treatments.

Introduction

The National Cottonseed Treatment Program evaluates cotton seedling survival for a number of fungicide seed treatment combinations that are nominated by chemical industry representatives over diverse environmental conditions and populations of cotton seedling pathogens. The standard treatment evaluated, since 2018, was Allegiance + EverGol Prime + Spera + Proline. A non-treated control (insecticide only) was included to assess efficacy of the fungicide treatments and seedling disease pressure. Allegiance (metalaxyl-only) and EverGol Prime (penflufen-only) were also included to aid in determining the importance of *Pythium* 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 non-treated control plots at each location. Soil temperature and plant development data were also collected for each location included in the 2021 National Cottonseed Treatment Program.

Materials and Methods

Fungicide Treatment

Acid-delinted seed of *Gossypium hirsutum* L. cv 'DP 1646 B2XF' were provided by Bayer, St. Louis. Fungicide treatments were mixed with Color Coat Red (1 oz/cwt) and Gaucho 600 (12.8 oz/cwt) (Bayer Crop Science) in a total slurry rate of 28 oz/cwt (i.e., the amount of water added to each treatment was adjusted to have a 28 oz/cwt slurry). Water, Gaucho 600, and dye also were applied to the non-treated check at the same rate. Treatments were applied to the cottonseed while the seed mixed in a Kobalt 4-cu ft 0.5-HP Cement Mixer (model # SGY-CM1) used as a 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 fungicide treatments is provided in Table 1. Seed germination was evaluated for all treated and non-treated seed by rolling seed in a moistened paper towel and incubating for 7 days at 21°C inside a large plastic container covered with a lid and misted with water each day.

Field Experiments

Thirteen field trials were conducted by 12 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 three to six. The stand counts used in the analyses were taken from 27 to 48 days after planting, averaging 32 days after planting. A soil sample and ≈100 seedlings were collected from plots containing non-treated seed and were collected from 27 to 35 days after planting, averaging 31 days after planting. Soil and seedlings were placed in insulated packages with refrigerated cool packs and mailed overnight to the West Tennessee Research and Education Center for processing. A subsample of soil from each location was sent to the Southwest Research and Extension Center in Hope, Arkansas to screen for plant-parasitic nematodes. Soil moisture and temperature data were obtained through the use of WaterScout SM100 soil moisture and external temperature sensors connected to WatchDog 1200 micro stations (Spectrum Technologies, Aurora, IL), or the nearest weather station (National Weather Service) (see Table 2).

Isolations

A total of 100 seedlings were sampled from non-treated plots at each location. Seedlings were evaluated for growth by recording the number of nodes from five arbitrarily selected seedlings and 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. 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, 5=51-75% of the root system discolored, and 6>75% of the root system discolored.

Following rating, two subsets of 25 seedlings each were dipped three times in sterile water, blotted dry with a sterile paper towel, and plated onto *Pythium*-selective media, P_5ARP (Jeffers and Martin, 1986), and *Rhizoctonia solani*-selective media, TSM (Spurlock et al., 2011), respectively. The remaining seedlings were divided into two subsets of 25 seedlings, surface-sterilized by immersion for 60 secs in 1% NaClO, blotted dry with a sterile paper towel, and plated onto *Fusarium*-selective media, MGA (Castellá et al., 1997), and *Thielaviopsis basicola*-selective media, TB-CEN (Specht and Griffin, 1985), respectively. Plates were incubated at ≈27°C for 3-5 days (MGA and TSM) or 7-14 days (P_5ARP and TB-CEN) prior to screening for colonies.

Soil samples evaluated for populations of *R. solani* were screened using the toothpick-baiting method (Paulitz and Schroeder, 2005). Nine toothpicks were baited per sample and populations were quantified on the *Rhizoctonia*-selective media, TSM (Spurlock et al., 2011).

Pathogenicity Assays

A pathogenicity assay for *Pythium* isolates was not possible as none were recovered from seedlings across all locations due to mis-identification of saved isolates.

Two assays were completed for *Fusarium* isolates collected from 10 of the 13 locations: the plate method with germinated seeds and a root dip method with seedlings. While *Fusarium* spp. were recovered from all locations, isolates that were saved from three locations (AL1, TN, and TX2) either were unable to be revived or did not sporulate following frozen storage. The *Pythium* pathogenicity assay developed by Zhang and Yang (2000) for soybean and corn was modified for *Fusarium* and cotton as the host as follows: *Fusarium* isolates were taken from initial seedling plantings onto MGA and saved in long term storage via freezing on filter paper. One isolate per location was then

selected at random to be screened for pathogenicity. Two replications were completed for each isolate. Isolates were plated onto 1% water agar amended with streptomycin (0.075 g/L) and ampicillin (0.02 g/L) to incubate at 27°C. After 3 days, 10 black cottonseeds were placed towards the outer edge of each plate containing a *Fusarium* isolate and incubated at 27°C for 7 days. Seeds were sterilized before plating by rinsing in tap water, submerging in 1% bleach solution for 60 secs, and dipping in sterile purified water before placing on sterile paper towels to dry. During each pathogenicity assay, 10 sterilized seeds were plated on 1% water agar and incubated at 27°C as a germination control. After the completion of each assay, data were collected on the seedling germination rate for each isolate. A scale of 0-4 was used to determine the pathogenicity of each isolate, where 0=seed germinated without visible infection, 1=germinated with light discoloration on roots, 2=germinated with short severely discolored roots, 3=died after germination, and 4=died before germination.

The *Fusarium* root dip method developed by Wang and Gottwald (2017) for wheat was modified for cotton in a greenhouse setting. The same set of isolates selected for the plate method were grown out from frozen storage onto PDA for 3 days at 27°C. Isolates were then replicated onto SNA media and grown out under fluorescent light (24 hr. cycle, 550 uMol) at 20°C for 9 days (Conviron PGR15 growth chamber, Canada). Plates were washed with a 0.02% Tween-20 solution and the resulting suspension passed through 4 layers of sterile cheesecloth. Spore concentration was determined with a hemocytometer, adjusted to 1×10^6 spores/ml, and stored in Falcon vials at -80°C. The greenhouse trial included nine isolates plus a non-treated check, totaling 10 treatments with 2 replications. Seed was surface-disinfected with 2% bleach for 3 minutes, dipped in sterile water three times, and blotted dry with a sterile paper towel prior to sowing at a rate of 4 seeds/pot, culled to 3 seeds/pot prior to inoculation. Emergence occurred approximately 3 days after planting and the trial was inoculated 12 days after planting. Seedlings at the V3 growth stage were gently uprooted with a hand trowel, washed under running water until clean of substrate, and dipped into individual spore suspensions for 5 minutes. They were replanted and watered daily. At 10 days post-inoculation seedlings were uprooted for a final time, rinsed under running water, and rated for root discoloration using the same 1-6 scale used to evaluate the seedlings received from cooperators earlier in the season (explained above).

Nematode Screening

Subsamples of soil (150 cc) from field sites were sent to Cathy Howard at the Arkansas Nematode Diagnostic Laboratory for analysis. Fourteen parasitic nematodes (soybean cyst, dagger, lance, lesion, ring, reniform, spiral, sting, stubby-root, stunt, root-knot, needle, sheath, and pin) were included in the screening process and the results are reported in number of nematodes per 100 cm^3 of soil (Table 5).

Statistics

Data were analyzed with JMP 15 Pro (SAS Institute Inc., Cary NC). Percent emergence was analyzed across and by locations using Mixed Model – Tukey HSD means separation with $\alpha = 0.05$. A multivariate pairwise correlation method was used to examine the relationship among soil temperature, early season growth (nodes), percent stand, disease hypocotyl and root ratings, pathogen isolation frequency, and soil populations over locations.

Table 1. Fungicides, formulations, and active ingredients in the 2021 National Cottonseed Treatment Program

No.[z]	Common or registered name	Active ingredient (%)	Rate oz/cwt	Target Pathogen
1	Gaucho 600	Imidacloprid (47.8)	12.8	No target
2	Allegiance FL	Metalaxyl (28.35)	1.5	*Pythium*
3	Evergol Prime	Penflufen (22.7)	0.64	*R. solani*
4	Spera 240 FS	Myclobutanil (22.37)	1.85	*R.solani, T. basicola*
	Proline 480 SC	Prothioconazole (41.0)	0.16	*R.solani, Fusarium*
	Evergol Prime	Penflufen (22.7)	0.32	*R. solani*
	Allegiance FL	Metalaxyl (28.35)	0.75	*Pythium*
5	Apron XL LS	Mefanoxam (33.3)	0.3	*Pythium*
	Maxim 4FS	Fludioxonil (40.3)	0.1	*Fusarium, R. solani*
	A7568D	Myclobutanil (40)	0.8	*T. basicola*
	Vibrance CST	Mefenoxam (6.71), Azoxystrobin (6.71), Sedaxane (3.13), Fludioxonil (1.12)	4.1	*Pythium, R. solani, Fusarium*
6	Apron XL LS	Mefanoxam (33.3)	0.3	*Pythium*
	Maxim 4FS	Fludioxonil (40.3)	0.1	*Fusarium,R. solani*
	A7568D	Myclobutanil (40)	0.8	*T. basicola*
	Vibrance CST	Mefenoxam (6.71), Azoxystrobin (6.71), Sedaxane (3.13), Fludioxonil (1.12)	4.1	*Pythium, R. solani, Fusarium*
	A20597B	-	0.2	-
7	Apron XL LS	Mefanoxam (33.3)	0.3	*Pythium*
	Maxim 4FS	Fludioxonil (40.3)	0.1	*Fusarium,R. solani*
	Rally 40 WSP	Myclobutanil (40)	0.8	*T. basicola*
	Vibrance CST	Mefenoxam (6.71), Azoxystrobin (6.71), Sedaxane (3.13), Fludioxonil (1.12)	4.1	*Pythium, R. solani, Fusarium*
	Saltro	Adepidyn/Pydiflumetofen (41.7)	10.6	*Fusarium*
	A20597B	-	0.2	-
8	Spera 240 FS	Myclobutanil (22.37)	1.3	*T. basicola*
	Stamina	Pyraclostrobin (20.4)	1.7	*Fusarium, R. solani, Pythium*
	Systiva	Fluxapyroxad (28.46)	0.9	*R. solani, T. basicola, Fusarium*
	Allegiance FL	Metalaxyl (28.35)	0.8	*Pythium*
9	Spera 240 FS	Myclobutanil (22.37)	1.3	*T. basicola*
	Proline 480 SC	Prothioconazole (41.0)	0.2	*R. solani, Fusarium*
	Fluoxastrobin FS480	Fluoxastrobin (40.3)	0.3	*Pythium, R. solani*
	Evergol Prime	Penflufen (22.7)	0.3	*R. solani*
	Allegiance FL	Metalaxyl (28.35)	0.8	*Pythium*
10	Spera 240 FS	Myclobutanil (22.37)	1.3	*T. basicola*
	Proline 480 SC	Prothioconazole (41.0)	0.2	*R. solani, Fusarium*
	Fluoxastrobin FS480	Fluoxastrobin (40.3)	0.3	*Pythium, R. solani*
	Evergol Prime	Penflufen (22.7)	0.3	*R. solani*
	Allegiance FL	Metalaxyl (28.35)	0.8	*Pythium*
	Evergol Xtend	Penflufen (14.26), Tryfloxystrobin (14.26)	1.0	*Pythium, R. solani*

11	Spera 240 FS	Myclobutanil (22.37)	1.3	*T. basicola*
	Proline 480 SC	Prothioconazole (41.0)	0.2	*R. solani, Fusarium*
	Fluoxastrobin FS480	Fluoxastrobin (40.3)	0.3	*Pythium, R. solani*
	Evergol Prime	Penflufen (22.7)	0.6	*R. solani*
	Evergol Xtend	Penflufen (14.26), Tryfloxystrobin (14.26)	1.0	*Pythium, R. solani*
12	Kabina ST	Penthiopyrad (40)	0.7	*R. solani*
	Rally 40 WSP	Myclobutanil (40)	0.8	*T. basicola*
	Allegiance FL	Metalaxyl (28.35)	1.5	*Pythium*
	Maxim 4FS	Fludioxonil (40.3)	0.2	*Fusarium, R solani*
13	Kabina ST	Penthiopyrad (40)	0.4	*R. solani*
	Vibrance CST	Mefenoxam (6.71), Azoxystrobin (6.71), Sedaxane (3.13), Fludioxonil (1.12)	3.1	*Pythium, R. solani, Fusarium*
	Maxim 4FS	Fludioxonil (40.3)	0.2	*Fusarium, R solani*
	Allegiance FL	Metalaxyl (28.35)	0.8	*Pythium*
	Rally 40 WSP	Myclobutanil (40)	0.8	*T. basicola*
14	Kabina ST	Penthiopyrad (40)	0.4	*R. solani*
	Evergol Xtend	Penflufen (14.26), Tryfloxystrobin (14.26)	1.0	*Pythium, R. solani*
	Thiram	Thiram (42), 1,2-Propanediol (10)	2.5	*Fusarium, Pythium*
	Allegiance FL	Metalaxyl (28.35)	0.8	*Pythium*
	Rally 40 WSP	Myclobutanil (40)	0.8	*T. basicola*
	Maxim 4FS	Fludioxonil (40.3)	0.2	*Fusarium, R solani*
15	Mefenoxam	Mefenoxam	0.6	*Pythium*
	Ipconazole	Ipconazole	0.1	*Fusarium*
	Difenoconazole	Difenoconazole (32.8)	0.3	*Fusarium, R. solani*
	Azoxystrobin	Azoxystrobin	3.5	*Pythium, R solani*
	Myclobutanil	Myclobutanil (40)	2.3	*T. basicola*
	BioSt VPH	Nitrogen (5% water soluble nitrogen, 2% urea)	1.0	-

[Z]All treatments included GAUCHO 600, Flowable, Imidacloprid (48.7%), 12.8 oz/cwt.

Table 2. List of cooperators and procedures for each location in the 2021 National Cottonseed Treatment Program

Cooperator	Location	Site	Date		Replicates	Seed planted/ft	Length row counted/plot	Soil temp.[V]	Soil moisture[X]	Avg. % Emergence[Z]
			Planted	Sampled						
Kathy Lawrence	Auburn, AL	AL1	4/20/2021	5/23/2021	4	4	25	15(11)	6(4)	43.5
Amanda Strayer-Scherer	Auburn, AL	AL2	4/26/2021	5/15/2021	4	4	25	-[Y]	-	84.8
Alejandro Rojas	Fayetteville, AR	ARF1	5/14/2021	6/14/2021	6	4	30	21(16)	4(4)	92.1
Alejandro Rojas	Fayetteville, AR	ARF2	5/24/2021	6/28/2021	5	4	30	-	-	91.6
Terry Spurlock	Kelso, AR	ARM	5/15/2021	6/15/2021	4	3	10	21(13)[W]	-	37.3
Ian Small	Quincy, FL	FL	4/28/2021	6/1/2021	4	4	13.12	24(20)	-	82.0
Robert Kemerait	Tifton, GA	GA				-	-	-	-	-
Trey Price	Winnsboro, LA	LA1	4/20/2021	5/18/2021	5	4	25	16(10)	6(4)	58.2
Tessie Wilkerson, Tom Allen	Stoneville, MS	MS1	4/22/2021	5/24/2021	4	4	70	17(8)	30(17)	70.5
Tessie Wilkerson, Tom Allen	Stoneville, MS	MS2	6/4/2021	7/6/2021	4	4	70	24(20)	12(9)	75.4
Akhtar Ali	Tulsa, OK	OK2	5/26/2021	6/27/2021	3	4	30	24(20)	6(5)	90.0
Heather Kelly	Jackson, TN	TN	4/27/2021	5/25/2021	4	4	60	20 (15)	-	58.0
Cecilia Monclova-Santana	Lubbock, TX	TX2	5/18/2021	6/17/2021	4	4.5	70	19(14)	-	22.4

[V]Mean (Minimum) soil temp. (°C); 3-day average following planting.
[W]Weather data collected from local weather station.
[X]Mean (Minimum) soil moisture (%VWC).
[Y]Information not available.
[Z]Average percent emergence based on plants counted approximately 30 days after planting across all treatments.

Results and Discussion

For the 12 locations in the 2020 National Cottonseed Treatment Program, that emergence data was collected, location and treatment significantly affected seedling emergence ($P < 0.0001$); as did their interaction (location × treatment, $P < 0.0001$). Half of the sites had treatment significantly affect % emergence (Table 3). Seedling emergence ranged from 7.7 to 98.4% across locations, with an average of 64.5% across all locations (Table 3). Across all locations, all treatments significantly protected seedling emergence (compared to the non-treated check), except treatment 3 (Evergol Prime only). This is most likely due to high *Pythium* pressure observed at some locations (MS1 and TN, in particular). The rolled towel method determined that all treatments had >83% germination; however, treatment 2 (Allegiance-only) had statistically lower germination compared to all other treatments except treatment 1 (Table 3). In general, this reduced germination did not result in reduced emergence in field locations. Only at ARF1 location, was treatment 2 statistically reduced than treatments 4 – 15. The additional five locations (AL1, ARF2, LA1, MS1, and TN) that had statistically significant emergence data, treatment 1 (black seed) had the lowest emergence. Additionally, at MS1 and TN locations, treatment 3 (Evergol Prime only) had low emergence, likely as a result of *Pythium* pressure which may be assumed due to the lack of other pathogen pressure reported.

Seedling development across the locations at the time of disease assessment and isolation ranged from 1.4 nodes to 6 nodes with an average of 4 nodes (Table 4). Hypocotyl disease indices ranged from 2.1 (TN) to 3.0 (TX2), averaging 3 across all locations. Root disease indices ranged from 2.9 (FL) to 5.9 (TX2), averaging 5 across all locations. *Thielaviopsis basicola* was isolated from seedlings at 5 of the 13 locations with isolation frequencies ranging from 1 to 24%, averaging 4%. *Fusarium* spp. were detected at all sites using selective media with an average isolation frequency of 22% (Table 4). Even though *Fusarium* had a relatively high isolation frequency, results from the pathogenicity screening suggests they may all have been non-pathogenic (Figure 1). From the 'Dip' pathogenicity assay none of *Fusarium* isolates differed from the non-treated check, where all treatments (including check) had high root discoloration ratings. While the germination pathogenicity assay did result in statistical differences among isolates, a non-treated check was not included. Without the check in the germination assay and the overall lower ratings, it is uncertain whether the isolates evaluated had pathogenicity (Figure 1). *Rhizoctonia solani* was detected in soil screened from 4 of 13 sites, ranging from 0.7 to 23.8 propagules/100 cm^3 of soil, averaging 4. *R. solani* was detected from seedlings from 9 of the 13 sites (which included the same 4 sites it was detected from soil), with isolation frequency ranging from 1 to 23%, averaging 9% (Table 4).

Potential *Pythium* spp. were isolated on selective media from seedlings from all locations, but when going back to these isolates for pathogenicity testing none were properly identified as members of the genus *Pythium*. Hence, all *Pythium* isolation data were excluded due to lack of confidence in its quality.

Isolation frequency of pathogens did not have significant correlations with any of the parameters. This may be due to the lack of *Pythium* isolation data in 2021, since in previous years *Pythium* isolations have had correlations with additional pathogen isolations, mainly *Fusarium.* Specifically, in 2020, *Pythium* spp. and *Fusarium* spp. were positively correlated with a correlation coefficient of 0.72 (P=0.019), which could indicate co-infection/colonization at some locations. This was the opposite as what was observed in 2019, where isolation frequency of *Pythium* spp. and *Fusarium* spp. were negatively correlated with a correlation coefficient of -0.70 (P=0.01), which could indicate competition between these pathogens in colonizing seedlings. The only significant correlations observed from the 2021 data include hypocotyl and root ratings being negatively correlated with the number of nodes, as can be expected and observed in previous years, larger plants display lower disease ratings.

Table 3. Cotton seedling stands for locations of the 2021 National Cottonseed Treatment Program

No[X]	Germ Results[Y]	Emergence %[Z]												
		AL1	AL2	ARF1	ARF2	ARM	FL	LA1	MS1	MS2	OK2	TN	TX2	Average
1	91.8 ab	31.5 b	88.4	84.9 bc	82.3 b	14.3	72.9	44.6 b	54.0 c	75.3	60.7	14.0 b	16.7	53.6 f
2	84.3 b	35.3 ab	85.6	84.2 c	87.5 ab	20.8	80.0	57.8 ab	68.3 abc	75.8	69.0	59.8 a	13.5	60.8 de
3	96.0 a	33.3 ab	87.1	89.9 abc	94.4 ab	17.3	78.6	47.0 ab	57.5 bc	75.3	72.8	14.8 b	15.9	56.0 ef
4	99.0 a	43.3 ab	75.9	93.2 ab	92.3 ab	10.0	81.5	59.2 ab	74.3 abc	72.0	72.7	65.8 a	14.8	63.2 bcd
5	98.3 a	47.5 ab	83.3	94.2 a	92.3 ab	17.3	80.5	60.4 ab	69.3 abc	79.8	76.3	68.8 a	21.8	65.7 a-d
6	99.3 a	48.0 ab	85.6	94.8 a	92.0 ab	15.0	83.8	61.0 ab	71.0 abc	78.0	85.5	80.5 a	43.5	69.7 a
7	97.0 a	50.0 ab	84.3	95.6 a	88.2 ab	12.5	82.4	63.8 ab	75.3 abc	75.0	91.4	73.0 a	32.5	68.7 ab
8	99.5 a	43.5 ab	86.4	94.5 a	91.0 ab	7.7	84.8	65.2 a	77.0 ab	80.5	88.1	67.8 a	30.7	68.6 ab
9	98.8 a	44.5 ab	83.8	92.1 abc	86.9 ab	16.3	81.0	62.2 ab	81.5 a	75.0	87.8	57.8 a	22.2	66.0 a-d
10	97.3 a	43.0 ab	86.0	92.7 abc	97.0 a	13.3	81.5	60.4 ab	73.5 abc	77.8	73.8	69.0 a	18.9	65.8 a-d
11	97.3 a	48.3 ab	85.3	90.5 abc	92.9 ab	12.8	81.0	52.0 ab	66.3 abc	71.5	88.1	23.3 b	28.7	62.2 cd
12	94.3 a	42.3 ab	87.1	91.1 abc	93.6 ab	12.3	87.7	45.6 ab	70.3 abc	77.3	83.9	69.3 a	24.8	65.3 a-d
13	97.5 a	43.8 ab	82.6	91.6 abc	89.8 ab	13.8	89.6	62.2 ab	75.0 abc	76.3	94.0	77.0 a	14.6	67.5 abc
14	98.5 a	52.0 a	83.6	96.9 a	93.9 ab	13.5	85.8	65.6 a	69.0 abc	66.3	86.5	62.0 a	18.4	66.5 a-d
15	98.0 a	46.5 ab	86.4	94.9 a	98.4 a	12.0	78.6	64.0 ab	75.0 abc	75.5	89.9	69.0 a	27.2	68.2 ab
Avg	96.4	43.5	84.8	92.1	91.5	13.9	82.0	58.1	70.5	75.4	81.4	58.1	22.9	64.5
P>F	0.0001	0.0339	ns	<0.0001	0.0028	ns	ns	0.0214	0.0079	ns	0.1	<0.0001	ns	<0.0001

[X]See Table 1 for treatment details.

[Y]Germination results from lab moist paper towel roll on subsample of each treatment

[Z]Data were analyzed with JMP 15 Pro (SAS Institute Inc., Cary NC), values with the same letter within a column are not significantly different, where percent stand was analyzed across locations using Mixed Model – Tukey HSD means separation with $\alpha = 0.05$ and by location using the Fit Model – Standard Least Squares procedure – Tukey HSD means separation with $\alpha = 0.1$.

Table 4. Disease ratings and isolation frequencies of seedling pathogen groups for the 2021 National Cottonseed Treatment Program locations

		Dis. index		Isolation frequency (%)				Avg. %
Location	Nodes[S]	Hyp[T]	Root[U]	*T. basicola*[V]	*Fusarium spp.*[V]	*R. solani*		Emergence[X]
AL1	2	3.7	5.6	24	22	0W	7V	43.5
AL2	2	3.0	5.4	0	21	0.0	5	84.8
ARF1	4	2.5	5.3	0	24	11.5	22	92.1
ARF2	5	2.7	5.4	1	22	0.0	14	91.6
ARM	4	2.7	5.8	0	25	10.8	23	37.3
FL	6	2.4	2.9	0	20	0.0	0	82.0
GA[Y]	3	3.5	5.6	3	12	0.0	1	--[Z]
LA1	2	2.9	5.2	0	21	23.8	4	58.2
MS1	4	2.7	5.5	0	24	0.0	0	70.5
MS2	5	2.6	3.7	0	22	0.0	15	75.4
OK2	6	2.8	5.5	0	25	0.0	0	90.0
TN	3	2.1	5.7	17	24	0.0	0	58.0
TX2	1.4	3.9	5.9	11	23	0.7	22	22.4
Avg	4	3	5	4	22	4	9	67

[S]Nodes based on five seedlings per location.

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

[U]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, 5=51-75% of the root system discolored, and 6>75% of the root system discolored.

[V]Isolation frequency from selective media is based on 25 seedlings per location and reported as a percentage.

[W]Soil populations from toothpick baiting method reported in no. propagules/100 cm3.

[X]Percent stand based on plants counted 30 days after planting across all treatments.

[Y]Isolation frequency from selective media is based on 39 seedlings from this location

[Z]Information not available.

Table 5. Number of nematodes/100 cm^3 soil[Z]

Location	Lance	Lesion	Ring	Reniform	Spiral	Stunt	Root-knot
AL1	0	0	0	0	115	0	0
AL2	0	0	23	0	0	0	8
ARF1	0	8	8	0	0	0	0
ARF2	0	0	0	0	0	8	0
ARM	0	0	0	0	0	23	0
FL	0	8	0	69	131	0	0
GA	--[Y]	--[Y]	--[Y]	--[Y]	--[Y]	--[Y]	--[Y]
LA1	0	0	0	0	15	0	0
MS1	0	0	0	200	0	0	0
MS2	0	0	0	0	0	0	0
OK2	0	0	0	0	0	8	0
TN	0	0	0	223	0	0	0
TX2	0	0	0	0	0	38	0

[Y]Information not available.

[Z]Nematodes screened for but not found include soybean cyst, dagger, sting, stubby-root, needle, sheath, and pin.

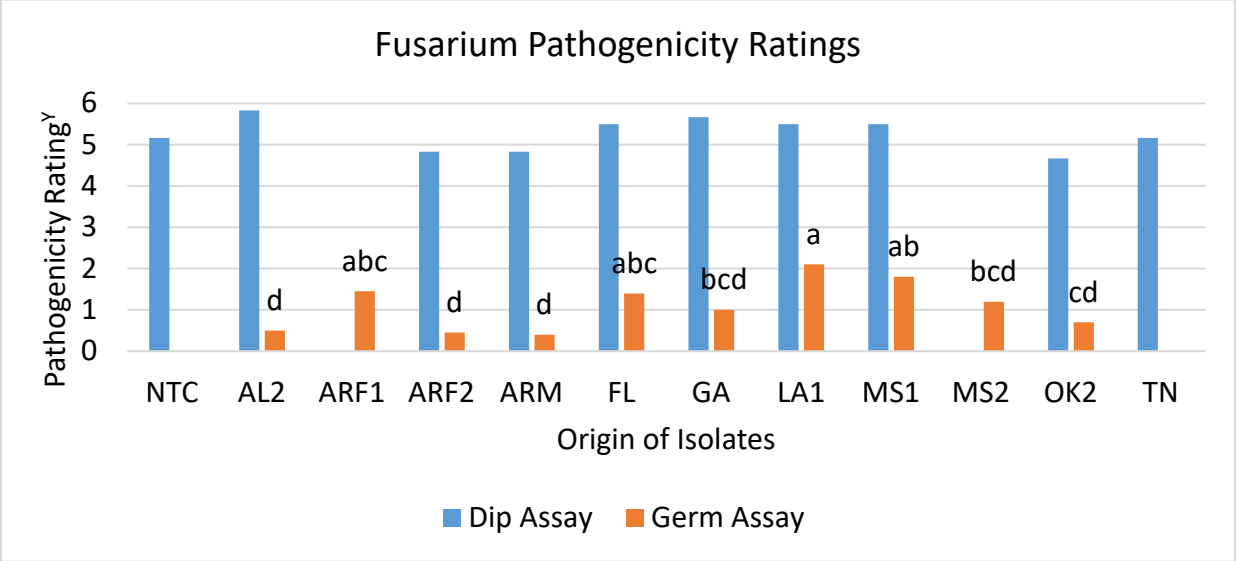

Figure 1. Pathogenicity results from randomly selected isolates of *Fusarium* spp. from locations in 2021.
[Z]Bars with the same letter are not significantly different at αd0.05.
[Y]Pathogenicity ratings for the 'Dip Assay' were as follows using a scale of 0-4, where 0=seed germinated without visible infection, 1=germinated with light discoloration on roots, 2=germinated with short severely discolored roots, 3=died after germination, and 4=died before germination; for the 'Germ Assay' a scale of 1-6 was used, where 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, 5=51-75% of the root system discolored, and 6>75% of the root system discolored.

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 Tennessee Institute of Agriculture or additional academic institutions represented herein, nor does it imply registration under FIFRA.

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