REPORT OF THE COTTONSEED TREATMENT COMMITTEE FOR 2016

C. S. Rothrock
S. A. Winters
Dept. of Plant Pathology - University of Arkansas
Fayetteville, AR
T. W. Allen
Delta Research and Extension Center - Mississippi State University
Stoneville, MS
J.D. Barham
SWREC - University of Arkansas Division of Agriculture
Hope, AR
W. Barnett
NEREC - University of Arkansas Division of Agriculture
Keiser, AR
M. B. Bayles
Oklahoma State University
Stillwater, OK
P. D. Colyer
LSU AgCenter
Bossier City, LA
H. M. Kelly
WTREC - The University of Tennessee Institute of Agriculture
Jackson, TN
R. C. Kemerait
Department of Plant Pathology - University of Georgia
Tifton, GA
G. W. Lawrence
Mississippi State University
Mississippi State, MS
K. S. Lawrence
Auburn University
Auburn, AL
H. L. Mehl
Tidewater Ag Res & Ext Ctr - Virginia Tech
Suffolk, VA
P. Price
LSU AgCenter - Northeast Region
Winnsboro, LA
J. E. Woodward
Texas AgriLife Extension Service
Lubbock, TX

Abstract

The 2016 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. Eight fungicide seed treatments were nominated by chemical industry representatives for evaluation in 2016. The results from the 13 locations where stand data were collected for the 2016 National Cottonseed Treatment Program indicated that seed treatment fungicides improved stands of cotton compared to a nontreated control for 54% of the locations (7 locations), with an additional 1 location being significant at P=0.10. Two of the 8 nominated seed treatments increased stand compared to the nontreated control at 7 of the 7 locations where a stand response was observed. All of the nominated treatment combinations improved stands at 4 or more of the 7 locations where a stand response was found. In addition, all of the nominated treatments increased stand for at least 2 locations compared to the historical standard fungicide seed treatment Vitavax-PCNB + Allegiance. Selective fungicide treatments provided a positive response compared to the nontreated control at 3 locations indicating *Pythium* spp. or *Rhizoctonia solani* were important at these locations. Stand for the nontreated seed was negatively correlated with

the hypocotyl disease index, -0.64 (*P*=0.0175), and root disease index, -0.63 (*P*=0.0204). Early-season growth (nodes) also was negatively correlated with the hypocotyl disease index, -0.72 (*P*=0.0051), and root disease index, -0.78 (*P*=0.0016). Pythium isolation was positively correlated with hypocotyl disease index, 0.82 (*P*=0.00051), and root disease index, 0.73 (*P*=0.0045). Isolation of *Thielaviopsis basicola* was positively correlated with soil populations of *Thielaviopsis basicola,* 0.72 (*P*=0.0052).These regional studies confirm the importance of seedling diseases, the value of fungicide seed treatments, and the continued improvement of seed treatment chemistries.

Introduction

The 2016 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. Eight fungicide seed treatments were nominated by chemical industry representatives for evaluation in the 2016 National Cottonseed Treatment Program. Two historical standard fungicide treatments, Vitavax-PCNB + Allegiance and RTU Baytan-Thiram + Allegiance, and a nontreated control were included to assess efficacy of the nominations and seedling disease pressure. In addition, the fungicide treatments Allegiance and EverGol Prime were 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 nontreated control plots at each location. Soil temperature and water and plant development data also were collected for locations for the 2016 National Cottonseed Treatment Program.

Materials and Methods

Fungicide treatment

Acid-delinted seed of *Gossypium hirsutum* L. cv 'DP 1522 B2XF' 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), and dye (Color Coat Red, 1 oz/cwt) (Syngenta Crop Protection) and Gaucho 600 (12.8 oz/cwt) (Bayer CropScience) in water at a rate of 2.75% 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

Data from the 13 field experiments reported were conducted by 13 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 8. The stand counts used in the analyses were taken from 13 to 42 days after planting, average 30 days, depending on the location. A soil sample and seedling sample from plots containing nontreated seed were taken from 28 to 48 days after planting, average 34 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) 10 cm (4 in.) 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, 5=51-75% of the root system discolored, and 6>75% of the root system

Table 1. Fungicides, formulations and active ingredients included in the 2016 National Cottonseed Treatment Program.

Common or registered name[1]	Formulation	Active ingredient (%)
A21606B Syngenta		
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
EVERGOL ENERGY (Penflufen)	Flowable	3.59% N-[2-(1,3-dimethylbutyl)phenyl]-5-fluoro-1,3-dimethyl-1Hpyrazole-4-carboxamide
(Prothioconazole)		7.18% 2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1H-1,2,4-triazole-3-thione
(Metalaxyl)		5.74% *N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl) alanine methyl ester
EVERGOL PRIME (Penflufen)	Flowable	22.7% N-[2-(1,3-dimethylbutyl)phenyl]-5-fluoro-1,3-dimethyl-1Hpyrazole-4-carboxamide
Fungicide Base (Albaugh LLC)	Flowable	
(Myclobutanil)		63.34% A-butyl-a-(4-chlorophenyl)-1*H*-1,2,4-triazole-1-propanenitrile
(Metalaxyl)		30.25% *N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl) alanine methyl ester
(Fludioxonil)		3.78% 4-(2,2-difluoro- 1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile
L1979-A (Bayer CropScience)		
MAXIM 4FS (Fludioxonil) Liquid		40.3% 4-(2,2-difluoro- 1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile
Premium Fungicide (Albaugh LLC)		
Premium Fungicide/Nematicide (Albaugh LLC)		
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,
SPERA 240FS (Myclobutanil)	Flowable	22.37% A-butyl-a-(4-chlorophenyl)-1*H*-1,2,4-triazole-1-propanenitrile
SYSTHANE 40WSP (Myclobutanil)	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
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 2016 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)	4/19	5/18	5/18	4	25	100	20(14)
J. Barham	Hope, AR	(AR1)	4/26	5/24	5/24	4	80	240	23(21)
W. Barnett	Keiser, AR	(AR2)	4/13	5/16	5/16	6	23	100	18(12)
C. Rothrock	Judd Hill, AR	(AR4)	5/9	6/6	6/6	6	50	250	26(15)
R. Kemerait	Tifton, GA	(GA)	5/5	6/13	5/18	4	50	150	---[2]
P. Colyer	Bossier City, LA	(LA1)	4/6	5/10	5/5	8	20	100	19(13)
P. Price	Winnsboro, LA	(LA2)	4/5	5/4	5/6	4	20	100	19(14)
G. Lawrence	Mississippi State, MS	(MS1)	4/20	5/20	5/20	5	40	160	21(18)
T. Allen	Stoneville, MS	(MS2)	4/26	6/13	5/26	4	70	308	23(20)
M. Bayles	Perkins, OK	(OK3)	5/13	6/24	6/24	4	20	100	20(16)
H. Kelly	Jackson, TN	(TN)	4/26	6/2	6/2	4	60	240	20(18)
J. Woodward	Quaker, TX	(TX10)	5/5	6/12	6/2	4	70	320	---
H. Mehl	Suffolk, VA	(VA)	5/20	6/20	6/20	4	25	270	18(15)

[1] Mean (Minimum) 10 cm (4 in.) soil temperature; 3-day average following planting.
[2] Not Available

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.*

Soil samples were assayed for 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 *Thielaviopsis basicola* were detected by diluting 25 g (oven dry weight equivalent) 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 (Jeffers and Martin, 1986) and *Thielaviopsis basicola* populations were quantified using the pour-plate method with the modified selective medium TB-CEN.

Statistics

Data were analyzed by the Mixed 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

For the 13 locations in the 2016 National Cottonseed Treatment Program reporting stand data, there were significant location and treatment effects (Table 3). There was a significant treatment x location effect suggesting that treatment response was dependent on the environment or pathogen pressures for a particular location.

Significant increases in stands for a fungicide treatment compared to the nontreated control were found for 7 of the 13 locations, a frequency of response of 54% (Table 4). In addition, 1 location, MS1 had a *P*-value of less than 0.10. 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 2016 in stand establishment. The Allegiance treatment increased stands compared to the nontreated control in 3 (AR1, LA1, and OK3) of these 7 locations having a significant response, indicating *Pythium* spp. as a group was limiting stand establishment in 2016. At 3 of these 7 locations (AR1, LA1 and TXQ), the EverGol Prime treatment increased stands over the nontreated control, indicating *Rhizoctonia solani* was a major factor in stand establishment at these locations in 2016. Vitavax-PCNB + Allegiance, the historical standard fungicide treatment, increased stands compared to the nontreated control at 5 of the 7 locations having a fungicide seed treatment response, while the RTU BaytanThiram + Allegiance FL standard treatment increased stands at 6 of these 7 locations. The range of stand responses for nominated products was 4 of the 7 locations to all 7 locations. The nominated treatments that increased stands over the nontreated control at all 7 locations were Albaugh nomination Base + Prem. Fung. Overtreatment and Apron Xl 3 LS + Maxim

Table 3. Mean squares for the combined analysis of variance across locations, 2016 National Cottonseed Treatment Program.

Source	Degrees of freedom	Mean squares[1]
Location	12	17168*
Replication(Location)	48	148*
Treatment	12	1012*
Location*treatment	144	193*
Error	575	70

[1] Significant *F*-test; * $P<0.0001$

Table 4. Cotton seedling stands for locations of the 2016 National Cottonseed Treatment Program.

		Plant stand (%)													
Treatment	Rate (oz/cwt)	AL	AR1	AR2	AR4	GA	LA1	LA2	MS1	MS2	OK3	TN	TXQ	VA	Mean
Albaugh Base + Prem. Fung.	1.9 + 5.25	51	81	72	89	87	73	58	92	87	68	69	91	67	76
Apron XL + Maxim 4FS + Systhane 40WPS + A21606B	0.31 + 0.08 + 0.84 + 4.08	52	82	76	88	84	78	21	94	88	71	71	88	73	74
Allegiance FL + EverGol Prime + Spera 240FS + L1979-A	0.75 + 0.33 + 1.8 + 0.15	40	83	68	86	84	75	25	94	88	77	77	90	81	74
Allegiance FL+ EverGol Prime + Spera 240FS + L1979-A + EverGol Energy	0.75 + 0.33 + 1.8 +0.3 + 1.0	52	74	71	89	88	76	36	89	90	70	65	93	71	74
Albaugh Base + Prem. Fung./Nema	1.9 + 11.3	58	81	68	85	87	76	40	91	90	63	60	86	63	73
Allegiance FL + EverGol Prime + Spera 240FS + Vortex	0.75 + 0.33 + 1.8 + 0.08	58	75	77	89	81	72	17	90	89	53	76	89	63	71
Allegiance FL + EverGol Prime + Spera 240FS + L1979-A	0.75 + 0.33 + 1.8 + 0.3	54	70	62	85	84	77	20	86	88	63	70	89	76	71
Apron XL + Maxim 4FS Systhane 40WPS + A21606B	0.31 + 0.08 + 0.84 + 3.33	60	78	74	87	81	81	4	88	77	53	69	87	71	70
RTU-Baytan-Thiram + Allegiance FL	3.0 + 0.75	62	77	72	87	81	75	42	88	86	57	66	83	73	73
Vitavax-PCNB + Allegiance FL	6.0 + 0.75	50	60	68	88	90	66	37	89	84	74	71	85	56	71
EverGol Prime	0.64	48	79	62	83	87	67	13	92	84	37	58	85	61	66
Allegiance FL	1.5	44	79	62	83	81	69	22	87	80	61	70	75	72	68
Nontreated	---	50	64	55	84	85	59	6	89	74	35	68	77	43	61
Location average		52	76	68	86	85	72	26	90	85	60	68	86	67	72
Coefficient of Variation (%)		22.4	11.6	10.6	3.8	6.5	9.1	22.2	4.5	8.2	24.7	12.9	3.0	24.7	
LSD (P=0.05)		NS	12.6	8.4	3.8	NS	6.6	8.3	NS	10.0	21.2	NS	3.7	NS	

4 FS + Systhane WPS + A21606B. All 8 of the nominated treatments significantly increased stands compared to the historical standard fungicide treatment Vitavax-PCNB + Allegiance for at least 2 locations. Nominations increasing stands over the historical standard fungicide treatment at 3 of the 7 locations were Albaugh Base + Prem. Fung. Overtreatment, Allegiance + EverGol Prime + Spera + L1979-A, Allegiance + EverGol Prime + Spera + L1979-A + Evergol Energy, and Allegiance + EverGol Prime + Spera + Vortex.

Seedling development across the locations at the time of disease assessment and isolation ranged from 2.0 nodes to 11.3 nodes (Table 5). Hypocotyl disease indices ranged from 2.0 at GA, MS2, OK3 and TXQ to 2.6 at the AR2 location, average 2.3 (Table 5). Root disease indices ranged from 2.0 for the TXQ location to 5.9 for the LA2 location, average 3.3. *Rhizoctonia solani* was isolated from seedlings from the nontreated plots for 12 of the 13 locations (Table 5). *Rhizoctonia solani* was isolated from greater than 15% of the seedlings for the AL and TN locations. *Pythium* spp. were isolated from seedlings from 11 of 13 locations (Table 5). Pythium was isolated from greater than 30% of the seedlings for the LA2 site. Isolation frequencies for *Pythium* spp. increased dramatically by plating roots without surface disinfestation on the selective medium P_5ARP (Table 5). *Thielaviopsis basicola* was isolated from seedlings at 4 of the 13 locations using the modified TB-CEN medium (Table 5). *Thielaviopsis basicola* was isolated from greater than 80% of the seedlings for the AR4 and TN locations. *Fusarium* spp. were isolated from seedlings at all 13 locations (Table 5). Isolation frequencies for *Fusarium* spp. ranged from 71 to 100%.

Table 5. Disease ratings and isolation frequencies of seedling pathogen groups for the 2016 National Cottonseed Treatment Program locations.

				Isolation frequency (%)[1]			
		Disease index		*Rhizoctonia*	*Pythium*	*Thielaviopsis*	F*usarium*
Location	Nodes[2]	Hyp.[3]	Root[4]	*solani*	spp.	*basicola*	spp.
AL	3.0	2.3	4.2	20	2 (61)[5]	0	88
AR1	3.0	2.4	4.2	4	16 (36)	26	96
AR2	3.0	2.6	4.4	2	14 (52)	0	98
AR4	4.0	2.4	3.6	2	28 (67)	100	74
GA	8.3	2.0	2.0	10	2 (27)	0	88
LA1	2.7	2.5	4.1	0	6 (24)	0	94
LA2	2.0	3.2	5.9	13	43 (---[6])	0	91
MS1	3.3	2.3	2.9	10	0 (22)	0	71
MS2	11.3	2.0	2.1	8	2 (20)	0	76
OK3	5.3	2.0	2.1	12	4 (22)	0	88
TN	5.3	2.1	2.1	18	0 (34)	86	76
TXQ	9.0	2.0	2.0	4	2 (12)	8	80
VA	4.3	2.4	3.9	12	4 (37)	0	100

[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, 5=51-75% of the root system discolored, and 6>75% of the root system discolored.
[5] Isolation frequency in parentheses from P_5ARP.
[6] Information not available.

Rhizoctonia solani was detection in soil for 5 of the 13 soils assayed, range 0.7 to 5.8 propagules/100 cm^3 of soil (Table 6). *Pythium* spp. were detected in soil at 10 locations for the 12 soils assayed, range 15 to 248 CFU/g of soil (Table 6). *Thielaviopsis basicola* was detected in 6 of the 13 soils assayed, range 1 to 166 CFU/g soil (Table 6).

Stand for the nontreated seed was negatively correlated with the hypocotyl disease index, -0.64 (P=0.0175), and root disease index, -0.63 (P=0.0204) Early-season growth (nodes) also was negatively correlated with the hypocotyl disease index, -0.72 (P=0.0051), and root disease index, -0.78 (P=0.0016). Pythium isolation was positively correlated with hypocotyl disease index, 0.82 (P=0.0005), and root disease index, 0.73 (P=0.0045), as was isolation on the Pythium selective medium; hypocotyl disease index, 0.58 (P=0.0497), and root disease index, 0.64

(P=0.0242). Fusarium isolation frequency was positively correlated with the root disease index, 0.56 (*P*=0.0457). Isolation of *Thielaviopsis basicola* was positively correlated with soil populations of *Thielaviopsis basicola,* 0.72 (*P*=0.0052).

Table 6. Soil populations of selected soilborne genera from sites in the 2016 National Cottonseed Treatment Program.

Location	*Rhizoctonia solani*	*Pythium* spp.	*Thielaviopsis basicola*
	CFU[1]/100cm^3	CFU/g	CFU/g
AL	5.8[2]	15.1	2
AR1	0	138.6	4
AR2	0.7	76.4	0
AR4	0	0	166
GA	1.4	0	0
LA1	0	78.7	0
LA2	0	174.9	0
MS1	0	73.8	0
MS2	2.2	---[3]	2
OK3	0	142.3	0
TN	0	247.9	1
TX-Q	5.0	15.4	8
VA	0	15.1	0

[1] Colony forming units.
[2] Populations not detected in soil sample; less than approximately 0.4 CFU/100 cm^3 of soil for *Rhizoctonia solani*, 8 CFU/g of soil for *Pythium* spp. and 0.5 CFU/g of soil for *Thielaviopsis basicola.*
[3] Information not available.

Summary

The results from the 13 locations where stand data were collected for the 2016 National Cottonseed Treatment Program indicated that seed treatment fungicides improved stands of cotton compared to a nontreated control for 54% of the locations (7 locations), with an additional 1 location being significant at *P*=0.10. Two of the 9 nominated seed treatments increased stand compared to the nontreated control at 7 of the 7 locations where a stand response was observed. All of the nominated treatment combinations improved stands at 4 or more of the 7 locations where a stand response was found. In addition, all of the nominated treatments increased stand for at least 2 locations compared to the historical standard fungicide seed treatment Vitavax-PCNB + Allegiance. Selective fungicide treatments provided a positive response compared to the nontreated control at 3 locations indicating *Pythium* spp. or *Rhizoctonia solani* were important at these locations. Stand for the nontreated seed was negatively correlated with the hypocotyl disease index, -0.64 (*P*=0.0175), and root disease index, -0.63 (P=0.0204). Early-season growth (nodes) also was negatively correlated with the hypocotyl disease index, -0.72 (*P*=0.0051), and root disease index, -0.78 (P=0.0016). *Pythium* isolation was positively correlated with the hypocotyl disease index, 0.82 (*P*=0.00051), and root disease index, 0.73 (P=0.0045). Isolation of *Thielaviopsis basicola* was positively correlated with soil populations of *Thielaviopsis basicola,* 0.72 (*P*=0.0052).

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 System Division of Agriculture nor does it imply registration under FIFRA. This work is supported in part by a USDA National Institute of Food and Agriculture Hatch project.

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