Enhanced Pest Control Systems for Mid-South Soybean Production

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Check for updates

Evaluating Cercospora leaf blight resistance in soybean accessions using an improved categorical disease-evaluation scale

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Enhanced Pest Control Systems for Midsouth Soybean Production: Stink Bug Resistance Update 2021

- Previous USB/MSSB funded projects to LA and TX supported redbanded stink bug sampling, seasonal movement, control tactics, and screening of soybean plant introductions for resistance
- Part of this work resulted in the creation of 6 stink bug breeding lines: TX12-1033 TX12-1034 TX12-1035 TX12-1039 TX12-1041 TX12-1061







Enhanced Pest Control Systems for Midsouth Soybean Production: Stink Bug Resistance Update 2021

- 13 new lines with a stink bug resistant parent in 2021 Missouri preliminary yield tests grown in 4 locations.
- R2Y ranging from MG4E-5E and need to be screened to confirm stink bug resistance.
- 5 populations (approx. 500 F4:5 lines) from crosses with stink bug resistant parents in our 2021 progeny row nursery.
- Screening of new Dr. Chen populations ongoing in the field at Baton Rouge under Dr. Jeff Davis along with collaborators in the Mid-South.
- Five new stink bug resistant crosses were also made in 2020 and are now in our winter nursery for generation advancement.









Plant Introduction (PI) Screenings for CLB resistance

PI (Plant Introduction) Locations

Cooperator	Year	Location (s)	Status
Allen	2016/2017/2018	Stoneville, MS	Completed
Buckley	2016/2017/2018	Bossier City, LA	Completed
Chen	2017/2018	Portageville, MO	Completed
Price	2017/2018	Alexandria, LA	Completed
Rupe	2016/2018 2017/2018 2017/2018 2017/2018	Fayetteville, AR Stuttgart, AR Keiser, AR Rohwer, AR	Completed Completed Completed Completed

- 18 location-years (~9,000 individual plots rated)
- Very important data set...
 - Breeders need breeding material in order to breed soybeans!

New, Simplified CLB Rating Scales

CLB Categorical Rating Scale

- 0 No disease symptoms
- 1 Light purple/bronzing, few petiole lesions, no blight
- 2 Moderate purple/bronzing and/or petiole lesions, light blight
- 3 Heavy purple/bronzing and/or petiole lesions, light blight
- 4 Heavy purple/bronzing and/or petiole lesions, moderate blight
- 5 Severe blight, <50% defoliation
- 6 Severe blight, >50% defoliation

CLB Incidence Rating Scale

- 1 < 25% 2 25 - 50%
- 3 50 75%
- 4 > 75%







Breeding Line Resistance

- 2019 & 2020 increased seed (MO) of resistant lines...
- Increased at winter nursery...
- Attempted to ID QTLs with this data (Koebernick, Shrestha, Richards)
- Seed increase of <u>susceptible</u> lines 2020/2021 (MO/LA)

Plant Introduction	Maturity Group
PI423731	IV
PI424360	VI
PI85490	VI
PI593653	V
PI157488	VI
PI398809	V
PI548477	VI
PI592756	VI
PI399044	V
PI398382	IV

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🖲 Check for updates

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 Manuscript detailing resistant PIs published in Journal of Crop Improvement

Susceptible Pls

2021 Breeder Trial



2021 CLB Breeding Effort

Advanced Yield Test (AYT):

- 98 advanced lines in 2021 AYTs with CLB resistant parental lines in the pedigree.
- 6 Missouri locations and off-site locations with 2 reps in Arkansas, Illinois, Tennessee,
 Mississippi, Louisiana, and Virginia.
- Lines with competitive yields will be selected for 2022 USDA uniform trials and regional tests.

Preliminary Yield Test (PYT):

- 271 preliminary lines from CLB resistant crosses in our 2021 preliminary tests (PYT) that were selected from the 2020 progeny row nursery based on maturity and phenotypic appearance.
- 4 Missouri locations with single replication.
- High yielding lines will be selected for the 2022 AYT.

Progeny Row Nursery:

- 19 populations (approx. 1900 F4:5 lines) derived from crosses with CLB resistant parents.
- Allows us to incorporate conventional, RR1, R2Y, and STS herbicide tolerances with highyielding potential along with CLB resistance.
- Will select the top 10-15% new lines for 2022 PYT.
- 4 CLB resistant populations being advanced from F1 to F4 (currently in F2 stage) in our winter nursery to be planted in our progeny row nursery for new line selection in 2022.





Breeder Variety Trials

Commercial/Public Cultivar Resistance preliminary data

		CUN	И		AL			AR			LA		мо		_	MS			TN			TX					
Cultivar	MG°	AVG [†]	N ^e	# ^h	AVG	N	#	AVG	N		#	AVG	N		#	AVG	N		#	AVG	N	#	AVG	N	#	AVG	N
AG46X6	4.6	0.035	50	1	0.005	2	2	0.003	12		4	0.026	24			N/A	0		5	0.136	8	1	0.000	4		N/A	0
AG53X6	5.3	0.043	50	4	0.017	3	19	0.019	12		5	0.028	24			N/A	0		17	0.173	7	3	0.003	4		N/A	0
R11-2354	6.0	0.057	92	9	0.026	6	8	0.009	24		б	0.033	24		21	0.022	6		1	0.092	16	5	0.015	8	55	0.281	8
LA560512	5.0	0.062	86	18	0.056	5	18	0.017	20		1	0.020	24		6	0.006	6		2	0.115	16	10	0.027	7	50	0.265	5 8
S12-2418	4.0	0.067	90	17	0.055	5	14	0.014	24	1	1	0.052	24		1	0.000	6		12	0.165	16	14	0.036	7	41	0.163	8
R10-197RY	5.6	0.070	92	14	0.052	6	11	0.011	24		2	0.024	24		16	0.016	6		9	0.154	16	22	0.053	8	54	0.285	5 8
AG47X6	4.7	0.073	51	6	0.020	3	13	0.013	12	1	9	0.088	24			N/A	0		15	0.171	8	2	0.003	4		N/A	0
UA5814HP	5.8	0.074	90	8	0.023	5	15	0.015	24		3	0.026	24		1	0.000	6		10	0.154	16	20	0.048	7	63	0.343	8
S11-20124	5.0	0.074	92	21	0.069	7	5	0.005	24		8	0.041	24		20	0.021	6		19	0.174	16	11	0.027	7	51	0.268	8
DG5580	5.5	0.075	49	2	0.010	3	16	0.015	12	2	3	0.105	22			N/A	0		6	0.137	8	4	0.014	4		N/A	0
Osage	5.6	0.075	93	26	0.085	7	12	0.011	24		9	0.047	24		31	0.039	6		4	0.133	16	28	0.071	8	49	0.260	8
REV 56R63	5.6	0.080	50	7	0.020	3	28	0.038	12	2	1	0.088	24			N/A	0		11	0.163	8	9	0.025	3		N/A	0
DG4967LL	4.9	0.080	136	11	0.043	8	22	0.024	36	2	4	0.105	39		2	0.001	9		13	0.166	24	23	0.054	8	23	0.103	12
S12-3782	4.0	0.081	88	12	0.047	6	7	0.008	23	1	4	0.074	24		1	0.000	б		24	0.193	16	19	0.043	5	43	0.203	8
R11-1192	5.7	0.082	41	13	0.050	3	36	0.063	20			N/A	0		8	0.009	3		7	0.146	12	24	0.063	3		N/A	0
4670RR2Y	4.6	0.083	40	20	0.067	4	3	0.005	12			N/A	0		3	0.001	6		28	0.205	8	30	0.078	2	38	0.149	8
R09-4798	5.7	0.088	43	32	0.107	4	24	0.027	23			N/A	0			N/A	0		32	0.213	12	16	0.041	4		N/A	0
UA5715GT	5.7	0.091	91	16	0.054	7	46	0.085	24		7	0.037	23		4	0.002	6		3	0.126	16	32	0.083	7	58	0.303	; 8
Croplan5265	5.2	0.092	51	5	0.018	3	23	0.027	12	_2	5	0.115	24			N/A	0		20	0.178	8	12	0.029	4		N/A	0
48A76	4.8	0.094	38	38	0.157	3	1	0.002	12			N/A	0		17	0.017	6		31	0.213	8		N/A	1	40	0.156	i 8
UA5213C	5.2	0.097	91	15	0.053	6	21	0.023	24	1	7	0.083	23		18	0.019	б		30	0.208	16	21	0.049	8	52	0.273	8
UA5612	5.6	0.102	94	22	0.079	8	26	0.031	24	_2	0	0.088	24		19	0.019	б		22	0.184	16	15	0.039	8	61	0.334	1 8
55R68	5.5	0.104	42	30	0.100	4	9	0.010	12			N/A	0		7	0.008	6		18	0.174	8	37	0.113	4	47	0.242	8
R09-430	5.2	0.106	88	29	0.099	6	25	0.030	24	1	6	0.080	24		26	0.030	6		21	0.183	16	44	0.175	8	61	0.390	4
S11- 20337GT	4.0	0.109	105	24	0.083	7	_20	0.021	29	1	0	0.051	22		28	0.033	6		39	0.243	24	8	0.022	5	48	0.254	12
R13-1019	4.9	0.110	32	3	0.015	2		N/A	0	1	3	0.074	19		29	0.033	3			N/A	0	27	0.068	4	69	0.429	4

Variety Trial Summary

- Many Location-Years (where CLB was present)
- Many Varieties/Entries
- Thousands of individual plots evaluated
- Dr. Brian Ward, Dr. Thanos Gentimis, Dr. Bishnu Shrestha compiled and analyzed data
- Working on manuscript now...
- Hundreds of *Cercospora* spp. isolates!

- Very important collection for future research...

Location Summary – Variety Trial

Year	# of entries	Success Rate	Sampled?
2016	30	12/14	Ν
2017	30	11/14	Ν
2018	45	13/13	Y
2019	40	12/12	Y
2020	56	12/12	Y
2021	25	?/12	Υ

Screening for Qol resistance (Shrestha)

- SDHI
- MBC
- Others MOAs in the future
 Genetic Characterization (Doyle, grad
 student)
- Opportunity to define species composition of pathogen population geographically



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Identification of the isolates using PCR-RFLP

S No	Location	Stata	Cercospora	Cercospora	Unknown	Total	Percentage		
3. NO.	Location	Sidle	flagellaris	sps. (?)	species	TOLAI	Cf*	Cs	
1	Klibler	Arkansas	24	2	0	26	<mark>92</mark>	<mark>8</mark>	
2	Tolbert	Arkansas	22	0	0	22	<mark>100</mark>	<mark>0</mark>	
3	Fairhope	Alabama	20	0	0	20	<mark>100</mark>	0	
4	Alexandria	Louisiana	10	14	0	24	<mark>38</mark>	<mark>54</mark>	
5	Benhur	Louisiana	21	2	3	27	<mark>81</mark>	<mark>8</mark>	
6	St. Joe	Louisiana	24	3	2	29	<mark>83</mark>	<mark>10</mark>	
7	Stoneville	Mississippi	20	3	0	23	<mark>87</mark>	<mark>13</mark>	
8	Portageville	Missouri	12	3	3	18	<mark>67</mark>	<mark>17</mark>	
9	Milan	Tennessee	1	7	0	8	<mark>13</mark>	<mark>87</mark>	
10	Beaumont	Texas	19	6	2	27	<mark>70</mark>	<mark>22</mark>	

*Cf = Cercospora flagellaris; Cs = Cercospora sps.

Total

- ~81% of isolates were found to be Cercospora flagellaris
- 19% were other than C. flagellaris, may be C. sigesbeckiae(?)

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• We are still on the process of identifying species of the isolates other than *C. flagellaris*

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- Primers: DML 172 and DML 150
- Expected PCR product: 473 bp
- Restriction enzyme: MluCl
- Restriction site: AATT overhanged 3'... T T A A ... 5'
- Two different patterns produced after restriction digestion

5'.... A A T T 3'

- i. (190 + 148 + 143)bp for *C. flagellaris*
- ii. (287+ 190) bp for C. sigesbeckiae (?)
- Two bands at 148 and 143 bp are only 5 bp apart and which makes us difficult to separate visually.

PCR-RFLP assay to characterize fungicide sensitivity of *Cercospora* spp.

S No	Location	Stata	Provintant	Sanaitiva	Total	Percentage		
5. NO.	Location	State	Resistant	Sensitive	Total	R*	S	
1	Klibler	Arkansas	1	25	26	<mark>4</mark>	<mark>96</mark>	
2	Rhower	Arkansas	22	0	22	<mark>100</mark>	<mark>0</mark>	
3	Fairhope	Alabama	20	0	20	<mark>100</mark>	<mark>0</mark>	
4	Alexandria	Louisiana	24	0	24	<mark>100</mark>	<mark>0</mark>	
5	Benhur	Louisiana	8	14	23	<mark>35</mark>	<mark>61</mark>	
6	St. Joe	Louisiana	23	4	27	<mark>85</mark>	<mark>15</mark>	
7	Stoneville	Mississippi	23	0	23	<mark>100</mark>	<mark>0</mark>	
8	Portageville	Missouri	14	1	15	<mark>93</mark>	<mark>7</mark>	
9	Milan	Tennessee	7	1	8	<mark>87</mark>	<mark>13</mark>	
10	Beaumont	Texas	15	10	25	<mark>60</mark>	<mark>40</mark>	

*R = Resistant isolates; S = Sensitive isolates

Total

 PCR-RFLP assay using cytochrome b (cyt b) gene showed that 73% of total isolates were resistant and 26% were sensitive to fungicides



- Primers: DML 327 and DML 328
- Expected PCR product: 267 bp
- Restriction enzyme: Alul
- Restriction site: AG'CT
- Two different patterns produced after restriction digestion
 - i. 267 bp (uncut) for Sensitive isolates
 - ii. (144 + 113 +10) bp for Resistant isolates
- DNA band with the size of 10 bp is so small we were unable to visualize in the gel

LAMP assay to characterize fungicide sensitivity of *Cercospora* spp.

- In the figure, DNA from seven different isolates and water control was used.
- After 1-hour incubation at 65°C for amplification and 5 minutes incubation at 80°C for stopping reaction, color change from violet to sky blue in the tube labelled as, BN₁, CN₁, DN₁ and FN₁, and no color change in the tube labelled as AN₁, EN₁, GN₁ and HN₁ was observed
- Color change from violet to sky blue represents the positive reaction whereas no change in color represents negative reaction
- In this study positive reaction indicates resistant isolates and negative reaction indicates sensitive isolates to fungicide
- So, isolates DMCC-3607, -3790, -3724 and -3609 are resistant isolates and isolates DMCC-3612, -3608, -3640 are sensitive isolates. Water was used as control.



Poison plate assays to characterize fungicide sensitivity of *Cercospora* spp.



- Isolates were grown in PDA, either amended with 1 µg/ml of Azoxystrobin or without it.
- Observed after 7 days of incubation at room temperature
- Radial growth inhibition was calculated and observed in the graph
- Poison plate assay was conducted with three replications per isolate



LAMP, PCR-RFLP and Poison plate assays correlate each other with corresponding isolates



Sensitive	Resitant							
DMCC 3612	DMCC 3607							
DMCC 3608	DMCC 3790							
DMCC 3640	DMCC 3724							
	DMCC 3609							

Isolate Summary – 2020 Preliminary Data

Location	State	Pure Culture	Glycerol Stock	Cercospora flagellaris	Cercospora spp.	Other genera	Resistant	Sensitive	Cercospora flagellaris (%)	Cercospora spp. (%)	Other genera (%)	Resistant (%)	Sensitive (%)
Winnsboro	Louisiana	59	54	25	0	2	25	1	<mark>92.59</mark>	<mark>0.00</mark>	7.41	<mark>96.15</mark>	<mark>3.85</mark>
Alexandria	Louisiana	32	30	17	1	0	18	0	<mark>94.44</mark>	<mark>5.56</mark>	0.00	<mark>100.00</mark>	<mark>0.00</mark>
Portageville	Missouri	25	24	13	1	1	14	1	<mark>86.67</mark>	<mark>6.67</mark>	6.67	<mark>93.33</mark>	<mark>6.67</mark>
Kibler	Arkansas	4	4	0	2	2	0	4	<mark>0.00</mark>	<mark>50.00</mark>	50.00	<mark>0.00</mark>	<mark>100.00</mark>
Rhower	Arkansas	25	25	11	0	0	11	0	100.00	0.00	0.00	100.00	0.00
Benhur	Louisiana	39	39	18	3	0	9	12	<mark>85.71</mark>	<mark>14.29</mark>	0.00	<mark>42.86</mark>	<mark>57.14</mark>
Beaumont	Texas	15	15	8	0	1	1	8	<mark>88.89</mark>	0.00	11.11	11.11	<mark>88.89</mark>
Stoneville	Mississippi	27	27	18	1	0	19	0	<mark>94.74</mark>	<mark>5.26</mark>	0.00	<mark>100.00</mark>	<mark>0.00</mark>
Jackson	Tennessee	45	45	15	3	0	14	4	<mark>83.33</mark>	<mark>16.67</mark>	0.00	77.78	<mark>22.22</mark>
Verona	Mississippi	24	24	14	1	0	8	7	<mark>93.33</mark>	<mark>6.67</mark>	0.00	<mark>53.33</mark>	<mark>46.67</mark>
Montecello	Arkansas	8	8	6	0	0	6	0	100.00	0.00	0.00	100.00	0.00
Auburn	Alabama	19	19	12	3	0	6	9	<mark>80.00</mark>	20.00	0.00	40.00	<mark>60.00</mark>
	Total	334	326	157	15	6	131	46					
	Percentage			88.20	8.43	3.37	74.01	25.99					

Expected Outputs/Deliverables

- Commercial variety resistance information
- Disease resistance data for PIs and selections
- ✓ ID CLB resistance (use as sources for future)
- ID QTL/markers for CLB (Shrestha, Koebernick, Richards)...in progress (delayed by field disasters)
- Confirm QTL/markers for CLB and regional evaluation of breeding lines for resistance
- ✓ ID stinkbug resistance (tolerance)
- Cross stinkbug resistant lines with current cultivars
- Use MAS to pyramid genes into adapted cultivars

Bonus Accomplishments (Pathology Angle...)

- ID fungicide resistance (working on 1st report)
- Determine Cercospora species composition at each location
- Compare ratios of pathogens across locations
- ID correlations between pathogen ratio, fungicide resistance, and disease severity
- Refine cercosporin screening assay
- Further define host/pathogen relationship
- Ultimately develop rapid screening protocol!
- Continue to refine management strategies.

Thanks for your support!









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Louisiana Soybean and Grains Research and Promotion Board